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Michael G. Rinaldi
Thomas J. Walsh
Editors

Diagnosis and Treatment of Fungal Infections



Third Edition

Springer

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ISBN 978-3-031-35802-9 ISBN 978-3-031-35803-6 (eBook)
https://doi.org/10.1007/978-3-031-35803-6

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The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Since the publication of *Diagnosis and Treatment of Fungal Infections, 2nd Edition* in 2015, fungi continue to emerge as important agents of human infection. Fungal infections (mycoses) continue to plague humankind as the at-risk population continues to expand with more immunosuppressive therapies, enlarging populations receiving cancer therapy, and continued support of our most critically ill in intensive care units and with broad-spectrum antibacterial agents. *Diagnosis and Treatment of Fungal Infections, 3rd Edition* again brings together globally recognized experts to guide readers in the use of our current knowledge to diagnose and treat patients with fungal infections.

In addition to basic and directed culturing techniques, histopathology, serological methods, and radiological studies, molecular biology methods continue to improve our ability to diagnose fungal infections and identify the offending fungi. Genotypic identification has led to an expansion of our understanding of fungal pathogens and has led to many new fungi being identified as the cause of human infection. This, and recent changes in nomenclature and molecular taxonomy, can lead to confusion in keeping up with the most proper name for any recovered fungus and difficulty in identifying the appropriate medical literature to review. A new chapter on molecular taxonomy examines these changes.

We currently have three major classes of antifungal agents from which to choose for systemic treatment of fungal infections: the polyenes (amphotericin B), echinocandins, and triazoles. Selecting which drug to use can be difficult in the empirical setting and targeted therapy typically requires identification of the pathogen to species level. Antifungal susceptibility testing can assist in selecting the best antifungal drug to use, but clinical correlation of this testing with treatment success remains largely limited to the *Candida* and *Aspergillus* species.

Diagnosis and Treatment of Fungal Infection, 3rd Edition is meant to be a concise text that will provide the busy infectious diseases, hematology-oncology, pulmonology, hospitalist, critical care, and transplant specialists a practical tool for diagnosis and management of fungal infections. In addition, the depth of the material in the text will provide these and other medical specialists and trainees an excellent reference and learning resource.

The text is divided into four parts to guide the reader. Part I provides a general introduction to the epidemiology of fungal infections and presents practical approaches for using patient risk factors, exposures, and site of infection to direct diagnostic evaluations. Part II introduces the science of medical mycology and the current tools available to diagnose fungal infections using basic clinical mycology laboratory techniques, molecular biology, histopathology, immunology, and imaging technologies. Part III provides a review of the available systemic antifungal agents, their use, and discussion of resistance and antifungal susceptibility testing. Part IV reviews fungal infections (mycoses) in seventeen uniform, easy-to-read chapters, with accompanying tables and figures.

San Antonio, TX, USA — Duane R. Hospenthal, MD, PhD
San Antonio, TX, USA — Michael G. Rinaldi, PhD
Baltimore, MD, USA — Thomas J. Walsh, MD, PhD (hon)

In Memoriam: Michael G. Rinaldi

It is with great sadness that we memorialize our friend, colleague, and co-editor, Dr. Michael Rinaldi, who passed away on January 1, 2022.

Michael was a bold pioneer of the field of modern medical mycology. Educated at the University of the Pacific and University of California Davis, Michael served as Adjunct Assistant Professor at Montana State University, Bozeman, from 1980 to 1985. He then moved to the University of Texas Health Science Center—San Antonio, where he dedicated the remainder of his highly productive career to the fledgling, but rapidly growing field of medical mycology. Rising to full Professor of Pathology, Medicine, Microbiology, and Clinical Laboratory Sciences, he established the Fungus Testing Laboratory (FTL), which became internationally recognized as an exemplary model for a state-of-the-art diagnostic reference laboratory for medically important fungi. The FTL provided a wide range of critical clinical laboratory services, including fungal identification, using phenotypic and advanced molecular taxonomic tools, antifungal susceptibility testing, and therapeutic antifungal drug monitoring. As a founding member of the Antifungal Subcommittee, of the National Committee for Clinical Laboratory Standards (NCCLS), which later became the Clinical Laboratory Standards Institute (CLSI), Michael demonstrated exemplary leadership in the field of antifungal susceptibility testing with assistance from Annette Fothergill of the FTL.

Michael also distinguished himself as an excellent medical mycological taxonomist, working particularly in collaboration with Dr. Michael McGuinness and Dr. Ira Salkin in developing and refining nomenclature of medically important fungi. With assistance from Deanna Sutton, Michael pioneered important advances in fungal nomenclature, as well as being instrumental in providing accurate clinical diagnoses and for expanding awareness of less common and rare fungi as medically important pathogens.

Michael was a prolific contributor to the field of Medical Mycology, having written 340 papers in the peer review literature, as well as having written multiple chapters in a wide range of books on medical mycology, clinical microbiology, and infectious diseases. He was a founding editor of *Diagnosis and Treatment of Human Mycoses*, which was retitled *Diagnosis and Treatment of Fungal Infections,* for the 2nd, and now this 3rd edition.

At a time when medical mycology was considered a relatively minor field, Michael was at the forefront of education and training of our vital field. His lecturing style was internationally acclaimed as enthusiastic, understandable, entertaining, and clinically relevant. Michael was an ardent proponent of the importance of educational awareness of fungal diseases in clinical medicine. His compelling support for the field was instrumental in helping clinicians understand the importance of invasive fungal diseases, inspiring trainees to enter the field, and to bring new resources for education and training.

Foremost among the venues for training in medical mycology, was the highly successful "Focus on Fungal Infections," which became the premiere national program for training and education in medical mycology. With his co-director, Dr. Elias Anaissie, Michael's efforts built "Focus on Fungal Infections" into a stellar success in providing new information and comprehensive annual reviews in medical mycology.

Michael's kindness, compassion, and sincerity fostered friendships throughout the world. His simple, kind, country-western humor veiled a brilliant, energetic, and thoughtful medical

mycological scientist. Michael leaves an extraordinary legacy of trainees, many of whom trained directly with him and others who were inspired by him. Michael will be missed profoundly by his friends and colleagues but his work and spirit of humanity will continue to thrive.

Thomas J. Walsh
Duane R. Hospenthal

In Memoriam: Deanna Sutton

It is with great sadness that we memorialize our friend, colleague, and author, Dr. Deanna Sutton, who passed away on July 4, 2017. Deanna Sutton, PhD, was a wonderful friend, esteemed colleague, brilliant medical mycologist, devoted mother, and loving grandmother who will be greatly missed.

Deanna graduated in 1964 from the University of Oregon Health Science Center with a Bachelor of Science degree in medical technology. She joined Dr. Michael Rinaldi in the Fungus Testing Laboratory in the Department of Pathology at the University of Texas Health Science Center at San Antonio in 1987. She, Michael, and Annette Fothergill would subsequently build the Fungus Testing Laboratory as a world class, preeminent reference laboratory that made outstanding contributions to patients with invasive fungal diseases and to the field of medical mycology. During the next three decades, Deanna made numerous important contributions to patient care and diagnostic medical mycology. While serving as the Administrative Director of the Fungus Testing Laboratory, Deanna attained her Master of Science degree (M.S.) and her doctoral degree (PhD) and achieved promotions from Research Associate to Professor of Pathology. In addition to overseeing the missions of patient care and research, Deanna trained numerous students and fellows from the USA and abroad. Many of these students are now contributing members of the medical mycology community.

Deanna's scholarly work was formidable and groundbreaking. As the author or coauthor of more than 300 publications, Deanna contributed greatly to our understanding of the clinical diseases, laboratory diagnosis, antifungal susceptibility testing, therapeutics, and fungal taxonomy of a wide range of medically important and emerging fungal pathogens. Her expertise was further reflected by her serving as an Associate Editor for *Medical Mycology* and on the editorial boards of *Antimicrobial Agents and Chemotherapy* and the *Journal of Clinical Microbiology*.

Deanna was a highly dedicated teacher who imparted her excitement for medical mycology in illuminating regional, national, and international lectures and workshops. She had developed an especially masterful command of fungal taxonomy, which she passionately imparted in her presentations. Reflecting her contributions in this area, her colleagues Drs. Josep Guarro and Josepa Gené from the Universitat Rovira i Virgili in Spain named a new species, *Acremoniopsis suttonii*, in 2014 in honor of her contributions to Medical Mycology.

Further illustrating her distinguished accomplishments in Medical Mycology, Deanna received the prestigious Billy H. Cooper Award from the Medical Mycological Society of the Americas in 2008. She was further honored in 2012 as the recipient of the Maxwell L. Littman Award from the Medical Mycology Society of New York for her sterling achievements in diagnostic clinical mycology.

Deanna was also very devoted to her family and her church. She greatly enjoyed traveling with her family, which included her daughter, two granddaughters, and son-in-law. Deanna also would plan and travel in pilgrimages with her church, the St. Elizabeth Ann Seton Catholic Church in San Antonio. Beyond her travels, Deanna also was a highly accomplished gardener who created floral beauty around her home.

In summary, Deanna was a brilliant medical mycologist, caring friend, inspiring teacher, and devoted mother and grandmother, who will be profoundly missed.

Thomas J. Walsh
Duane R. Hospenthal

In Memoriam: Ángela Restrepo Moreno

On February 3, 2022, we said goodbye with deep sorrow to our beloved Dr. Restrepo, "la Doctora" as all of her alumni, colleagues, and peers called her with respect, affection, and admiration. The importance of the scientific, academic, pedagogic, and humane work of Dr. Restrepo and her legacy have made her one of the principal figures in the history of science in Colombia, and her contributions to medical mycology made her a pioneer and a renowned expert in Latin America and worldwide.

Dr. Restrepo was born in Medellín in 1931. Already as a young girl, her curiosity to know more about the small microorganisms that cause diseases, and that could be observed with her grandfather's microscope, opened the way to science as an axis of her life. She belonged to the first graduating group of the clinical laboratory techniques at the Colegio Mayor de Antioquia, in 1954. Her professional practice began at the School of Medicine of the University of Antioquia (UdeA) (1958–1960). She obtained her master's degree and shortly afterwards her PhD (1960–1965) at Tulane University (New Orleans, United States).

During this period, she founded the laboratory of Medical Mycology at the School of Medicine, UdeA. Upon her return to Colombia her Medical Mycology Laboratory, with the support of her students and professors from other disciplines, became a Reference Center of diagnostics, research, and teaching for diseases caused by fungi. Her main line of research was paracoccidioidomycosis (PCM), caused by *Paracoccidioides brasiliensis*. She described the first cases of PCM in Colombia, and then made major contributions to it study in all its aspects—epidemiological, etiologic, pathogenic, diagnostic, and therapeutic. She was the main pillar and played a central role in the creation of a master's program in Medical Microbiology at the UdeA, which was reinforced by a Latin American Professorship program of the American Society for Microbiology (ASM) allowing an important exchange with distinguished microbiologists from the United States and Colombia.

Dr. Restrepo remained Full Professor at the UdeA until she left in 1976, and then served for 2 years as subdirector of the Departmental Laboratory of Antioquia.

In 1978 she relocated to the laboratories of the Corporación para Investigaciones Biológicas (CIB), an independent research institute of which she was co-founder in 1970 and Scientific Director between 1978 and 2015. Principally in collaboration with Dr. William Rojas, she succeeded in making the CIB one of the leading biological research centers not only in Colombia but in all of Latin America. At the CIB Dr. Restrepo reached the summit of her scientific career, as a leading expert on PCM and *P. brasiliensis*: searching for the natural habitat of the fungus, investigating its biology, its molecular structure and the immune response it generates in humans and in animal models, and contributing to the development of new diagnostic methods and the pioneering of clinical studies for a range of antimycotics for treatment. When the application of molecular epidemiology and population genetics recently revealed that *Paracoccidioides* is composed of at least five different species, one of the new species of the fungus was named *Paracoccidiodes restrepiensis* in her honor.

Although most of her work was centered on PCM, Dr. Restrepo also made outstanding contributions in diagnostics and in the study of other mycotic and bacterial infections.

Her scientific work includes over 400 national and international publications: original articles and over 40 book chapters, many of them in the most distinguished books on Medical

Microbiology. During her long scientific career, Dr. Restrepo received more than fifty recognitions, prizes/awards, distinctions and tributes from universities, national and international societies, and public institutions. Among them, three are particularly noteworthy, and had not been received by any Latin American scientist before: the Lucille K. George Award given to her by the International Society for Human and Animal Mycology (ISHAM) in 1979, the Rodha Benham Award bestowed by the Medical Mycology Society of the Americas, in 1990, and the Medical Mycology Medal awarded by the Canadian Society of Mycology in 1991. She has also two "Honoris causa" honorary doctorate titles, awarded by the Universidad Pontificia Bolivariana in Medellín in 1994 and by the University of Antioquia in 1996.

La Doctora chose science and education as center of her life. Throughout her scientific career and even after retiring, she taught hundreds of young students in mycology and related disciplines. Her alumni, now working in institutions in Colombia and in other countries, will always bear the indelible mark of having been her disciples. She used to say with much pride that the alumni/former students she mentored were her favorite awards, and that they were her family. She devoted much of her last years to continue supporting the Education Institution Ángela Restrepo Moreno, named in her honor.

Doctora Ángela had great familiarity with art, literature, and music, was modest and generous, had a wonderful sense of humor, and had an immense love of her country. She was wonderful company; with her sensitivity and her immense warmth and kindness she was without equal. In good as well as in difficult times she always had an encouraging message or helpful advice, with affection and sympathy or solidarity for each person and in each moment.

For her legacy of science and humanity, her students, colleagues, companions in projects, and contemporaries, and all of us who had the privilege and enormous fortune to have been close to her, felt and expressed together our eternal gratitude, affection, and admiration for her, as great scientist, as maestra par excellence, a woman with such warmth, so exemplary, who will remain an inspiration and a model for present and future generations.

Beatriz L. Gomez
Juan G. McEwen
Angela Tobon
Gil Benard

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Part I

Approach to Patients

Approach to Patients with Suspected Fungal Diseases

1

Duane R. Hospenthal and Thomas J. Walsh

Introduction

Fungal infections (mycoses) are increasing in incidence throughout the world as a result of modern medical practice and rise in the population of those at risk. Supporting this increase is the expanding use of immunosuppressive therapies, broad-spectrum antibiotics, central venous access, other medical devices, and emergence of resistance to licensed antifungal agents. Technology has led to the improved survival of persons with malignancies, with transplanted organs, with HIV infection, following trauma, and at the extremes of age. The medical scientific community has met this challenge with the introduction of new antifungal agents, often with less toxicity and improved spectrums of activity. Additionally, newer, more sensitive, and specific diagnostic strategies such as improved radiographic imaging and biomarkers have provided clinicians with better tools for detection of fungal infections earlier and potentially influencing disease outcomes. Modern molecular techniques can now allow more precise identification of recovered and unrecovered fungal pathogens with the potential to rapidly improve diagnosis of fungal infections. Despite these advances, the approach to the diagnosis and management of fungal infections often still relies on recognizing the interaction between the pathogen and host. Although some fungal diseases have classic presentations, many of these occur so rarely that clinicians may not initially include them in their differential diagnoses. In the setting of immunosuppression, mycoses may produce nonspecific signs and symptoms, making their diagnosis a challenge. Early recognition and treatment are fundamental to modifying disease outcomes in many fungal infections, especially those in immunocompromised individuals. Increased awareness of key risk factors and clinical presentations of the human mycoses may enable clinicians to develop an inclusive approach to the diagnosis of these diseases.

Epidemiology

Deaths associated with mycoses worldwide have been estimated at greater than 1.6 million per year, with the burden of disease estimated to affect more than a billion [1]. By comparison, the annual global mortality of tuberculosis is estimated to be 1.5 million [2] and that of malaria to be 409,000 in 2019 [3]. In the USA, fungal mortality increased from the tenth most common infectious disease cause of death in 1980 to the seventh in 1997 [4]. Sepsis due to fungal infection increased over 200% in the USA between 1979 and 2000 [5]. Fungal sepsis is chiefly secondary to candidemia. *Candida* continues to be the fourth most common organism recovered from bloodstream infections in the USA, associated with an attributable mortality of approximately 49% [6, 7]. Candidemia and disseminated (also termed systemic or invasive) candidiasis continue to be the most common causes of healthcare-associated fungal infections, responsible for more than 80% of these infections and up to 15% of nosocomial infections overall. Worldwide, the burden of invasive candidiasis is estimated to affect 700,000 individuals annually [1]. Infections with *Candida* have declined in patients with cancer and undergoing hematopoietic stem cell transplantation (HSCT), likely in association with antifungal prophylaxis. Candidemia, after surging in numbers in the 1980s, appears to have declined, at least in the intensive care setting [8]. This overall decline is chiefly due to fewer infections with *C. albicans*, as non-*albicans Candida* (NAC) candidemia has increased over this same period (1989–1999).

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_1

New, drug-resistant species including *Candida auris* have emerged in the hospital (healthcare-associated) setting [9].

Opportunistic mold infections, most commonly caused by the *Aspergillus* species, continue to expand their range of hosts from severely neutropenic cancer patients to patients with other risk factors, including prolonged immunosuppressive therapies with corticosteroids and newer biological therapeutic agents, including those that inhibit tumor necrosis factor alpha (TNF-α) [10]. Severe influenza and COVID-19 have emerged as increasingly recognized risks for the development of pulmonary aspergillosis [11]. *Aspergillus* is the second most common cause of nosocomial fungal infection and the most common mold to cause invasive mycosis. Other rare opportunistic molds (e.g., the Mucorales, *Fusarium* spp., and *Scedosporium* spp.) and yeasts (e.g., *Trichosporon* spp. and *Malassezia* spp.) have emerged as more frequent causes of disease in patients with a wide range of risks [12–18].

Outbreaks of endemic mycoses, including coccidioidomycosis in association with the growing urbanization of the US Southwest, and on a smaller scale, histoplasmosis, continue to be reported more frequently, often affecting greater numbers of persons. Outbreaks of endemic disease are occasionally diagnosed outside their known geographical areas, occurring in travelers to those locales. An outbreak of infection with the non-*neoformans Cryptococcus*, *C. gattii*, in mostly immunocompetent patients, has been going on in the US Northwest and Southwest Canada (Vancouver Island) over the past several decades [19, 20]. A new threat of dimorphic fungi, *Emergomyces* spp., has emerged globally as a serious infection in HIV-infected patients and other host populations [21].

Suspicion Based on Risk Factors

The risks for fungal infections are highly dependent on the combination of host immune competency and the specific exposures people have both within the healthcare system and in their communities.

Immunocompromise

Host immune status is probably the most important underlying factor determining whether people develop life-threatening, self-limiting, or no infection following exposure to fungi in their environment. Defense against invasive mycoses depends chiefly on intact mucosal barriers, innate immunity provided by phagocytic cells, and cell-mediated immunity (CMI). The impact of humoral immunity is limited and remains poorly defined in defense against the fungi.

Neutropenia and Altered Phagocytic Function

Classically, neutropenia has been associated with candidemia and invasive candidiasis. With prolonged neutropenia, *Aspergillus* species become more common causes of infection. Infection with the Mucorales, *Fusarium, Scedosporium, Trichosporon*, and other rare species can also be seen with prolonged loss of neutrophils. The incidence of candidiasis in the highest risk populations appears to have declined over the past decade in association with antifungal prophylaxis of these patients. This decrease has been associated with an increase in aspergillosis and other invasive mold infections. In addition to insufficient numbers of neutrophils, declination in phagocytic function also raises the risk of mycoses. The phagocytic dysfunction seen in chronic granulomatous disease (CGD) is associated with fungal infections, especially aspergillosis.

Immunomodulation with Monoclonal Antibodies and Chimeric Antigen Receptor (CAR) T-Cell Therapies

Hematological and other malignancies are increasingly being treated with monoclonal antibodies to surface antigens (CD) of the lymphocyte and other targeted immune therapies. These include immune checkpoint inhibitors and CAR T-cell therapies. These novel therapies constitute evolving risk factors in patients with hematological malignancies, particularly leukemia and lymphoma, where inhibition of a wide range of immune regulatory pathways results in increased risk for invasive fungal diseases [22]. The most common mycoses associated with these therapies include candidemia, aspergillosis, and mucormycosis. As these agents continue to be more widely employed, maintaining a high index of diagnostic suspicion will increase the probability of early intervention.

Impaired Cell-Mediated Immunity

Impaired CMI occurs in patients infected with HIV and those receiving many of the currently used immunosuppressive therapies. Impairment of CMI is associated with mucocutaneous candidiasis, *Pneumocystis* pneumonia, infection with *Cryptococcus*, and more severe and/or disseminated endemic mycoses. The specific mycoses associated with $CD4^+$ T lymphocyte decline as seen in HIV/AIDS have been carefully documented, allowing the clinician to increase their level of suspicion for particular fungal infections based on $CD4^+$ T lymphocyte counts of their patients (Table 1.1).

Organ Transplantation

Solid organ and HSCT recipients are at great risk for fungal infections [23–25]. In addition to immunosuppressive therapies, the mucosal damage and intensive therapy associated with these procedures place the persons who receive them at risk for the entire spectrum of fungal disease. Transplant

Table 1.1 Mycoses commonly associated with HIV infection

$CD4^+$ T lymphocyte cell count (cells/μl)	Fungal infections
>500	Candidal vaginitis
200–500	Thrush (oropharyngeal candidiasis)
<200	PCP, disseminated histoplasmosis, disseminated coccidioidomycosis
<100	Cryptococcosis, candidal esophagitis, talaromycosis

PCP *Pneumocystis* pneumonia

medicine has seen substantial advancements in tailoring regimens to minimize the duration of neutropenia and to reduce immunosuppressive treatments used to control rejection. Unfortunately, most of these still place patients at a substantial risk for opportunistic infections. In solid-organ transplantation, the risk of fungal infection is associated with risk surrounding the initial surgery and the use of immunosuppression to prevent rejection. This risk varies greatly based on the organ transplanted and underlying condition of the recipient. As an example, in liver transplantation, the substantial risk of *Candida* infection in the first month is mostly associated with surgical manipulation of the gastrointestinal tract and need for intensive care monitoring, as well as initial immunosuppressive agents given to control rejection (Table 1.2). Lung transplants are at high risk for invasive pulmonary aspergillosis, likely secondary to the route of inoculation and immunosuppression. Although a similar sequence of occurrence of fungal infection is seen in HSCT, the underlying factors creating risk differ from those of solid-organ transplant (Table 1.3). In HSCT, initial conditioning commonly leads to neutropenia and breakdown of the mucosal surfaces. This neutropenia can be prolonged and associated with life-threatening mold infections. In allogeneic HSCT, graft versus host disease (GvHD) and its treatment may put the patient at risk for fungal infection for a prolonged period of time following engraftment.

Healthcare Exposure (Nosocomial)

A multitude of risk factors for nosocomial fungal infections have been identified (Table 1.4) [10, 26, 27]. Unfortunately, many of these healthcare-associated risk factors overlap with those associated with bacterial infections or are risks that are common to many or most hospitalized patients. This is especially true for those patients hospitalized in intensive care units, the majority of whom have central venous catheters and are receiving broad-spectrum antibiotics [28, 29]. In addition to the use of vascular catheters, other procedures including urinary catheterization and intubation establish portals of entry for fungal pathogens. Other risk factors include immunosuppression seen with the use of corticosteroids and chemotherapy, and with malnutrition and malignancy. Infusion of contaminated infusates, inclusion of lipids in parenteral nutrition, and construction within the hospital are additional exposures that can lead to fungal infections. A few specific risks allow the clinician to suspect certain fungi. Ketoacidosis and deferoxamine therapy has been clearly shown to be a risk for mucormycosis (formerly zygomycosis). More recently, rhinocerebral mucormycosis has emerged in patients with type 2 diabetes mellitus suffering from COVID-19, especially with concomitant use of corticosteroids for control of the inflammatory response to SARS CoV-2 infection [30]. Unfortunately, given the overlapping nature of most of these risk factors with those associated with bacterial infections, it is often difficult to apply these risk factors to differentiate patients at higher risk of fungal versus bacterial infection.

Table 1.2 Fungi associated with hematopoietic stem cell transplantation

Time period	Common fungi	Other fungi
Pre-engraftment (<30 days)	*Candida*	*Aspergillus*
Post-engraftment (30–100 days)	*Aspergillus*, *Candida*, *Pneumocystis*	Mucorales, *Fusarium*, *Pseudallescheria* (*Scedosporium*)
Late (>100 days)	*Aspergillus*, *Pneumocystis*	

Table produced from data in reference [23]

Table 1.3 Fungi associated with solid-organ transplantation

Time period	Common fungi	Other fungi
First month	*Candida*	
1–6 months	*Aspergillus*, *Pneumocystis*, *Cryptococcus*	Endemic fungi[a]
>6 months	Endemic fungi[a]	*Cryptococcus*

Table produced from data in reference [24]
[a] Chiefly, *Coccidioides* and *Histoplasma*

Table 1.4 Risk factors commonly associated with healthcare-associated invasive mycoses

Mycosis	Risk factors
Candidiasis	*Candida* colonization, surgery (especially abdominal), acute renal failure, parenteral nutrition, central venous catheters, neutropenia, broad-spectrum antibacterial antimicrobials, mucosal surface disruption
Aspergillosis	Prolonged neutropenia, corticosteroids, neutrophil dysfunction, hematologic malignancy, cytotoxic drugs, AIDS, HSCT (highest in allogeneic), solid-organ transplantation (highest heart-lung), underlying lung disease, GvHD, GvHD therapies (TNF-α blockers), checkpoint inhibitors, COVID-19

HSCT hematopoietic stem cell transplantation, *GvHD* graft versus host disease, *TNF-α* tumor necrosis factor alpha

Community Exposure

The fungi that cause community-acquired infections commonly originate in the environment and are "true pathogens" (i.e., cause disease in persons with normal immune status). Most are restricted to certain geographic environments or exposure risks (Table 1.5). The source of disease includes inhalation, ingestion, or traumatic inoculation of the fungi. Diseases most commonly afflict the lungs, paranasal sinuses, skin, and soft tissues. Rarely, disseminated, central nervous system or osteoarticular disease occurs. The most commonly recognized community-acquired infections are the endemic mycoses, each with their limited geographical areas of exposure. With the extensive use of antibiotics, corticosteroids, and other immune modulators in the community, as well as the increased number of elderly, and population of immunocompromised persons receiving their care outside of the hospital, the boundaries between community-acquired and healthcare-associated infection have become blurred.

Other Risks

Other risks or probable risks associated with immune competency or genetic disposition include gender and race. The role of gender and potentially inhibitory effect of estrogen have been postulated to be important in the risk of clinical paracoccidioidomycosis. A clear risk exists for disseminated coccidioidomycosis in women when disease is acquired in pregnancy. Disseminated and severe coccidioidomycosis has also been associated with Filipino and African descent.

The use of antifungal therapy or prophylaxis in populations at risk should also be kept in mind when evaluating patients for potential fungal infections [31]. We continue to observe the emergence of NAC, non-*fumigatus Aspergillus* infection, and increased numbers of infections with the more rare yeasts and molds. This shift appears to reflect our greater usage of antifungals and use of the newer agents. Included in this change in epidemiology is the emergence of fluconazole-resistant *Candida* spp., such as *C. krusei* and

Table 1.5 Geographic areas in which the endemic mycoses reside

Mycosis	Region	Specific countries/areas with increased prevalence	Associated exposure risks[a]
Blastomycosis	North America[b]	Southeastern and South Central USA, Canada	Soil exposure near fresh water (fishing, hunting, farming, construction)
Coccidioidomycosis	Western hemisphere	Southwestern USA, Central and South America	Soil/dust exposure (construction, archeology)
Emergomycosis	Africa, Asia, Europe, North America	South Africa	Likely soil
Histoplasmosis	Worldwide	Mississippi and Ohio River valleys, South America, Western Africa	Soil or organic material associated with bird or bat guano (construction, demolition, spelunking)
Paracoccidioidomycosis	Latin America	Brazil, Columbia, Venezuela, Ecuador, Argentina	Farming or other outdoor employment
Sporotrichosis	Worldwide	North America, Japan	Gardening, sphagnum moss, hay, roses/thorns
Talaromycosis (formerly penicilliosis)[c]	Southeast Asia	China, Northeast India, Taiwan, Thailand, Vietnam	Rice farming, rodent burrows

[a] Not all well-proven
[b]Rare reports from Africa, Central and South America, India, and the Middle East
[c]Restricted almost exclusively to persons with AIDS

C. glabrata, as well as the recent increase in non-*Aspergillus* molds (e.g., the Mucorales, *Fusarium*, *Scedosporium,* and *Lomentospora prolificans*) which have decreased susceptibility or resistance to many of the currently available antifungal agents.

Suspicion Based on Organs Involved

Although the fungi may and often do cause disease in more than one organ system, many of these are associated with certain organ system infections. The presentation of disease (e.g., prolonged or chronic pneumonia with lymphadenopathy on chest radiography) can guide the clinician to the diagnosis. Disease localization and presentation can be altered based upon the host immune system, route of pathogen inoculation (e.g., inhalation, cutaneous inoculation, ingestion), and quantity of inoculum. The most common presentations are pulmonary, cutaneous/subcutaneous, and disseminated diseases (Table 1.6). Other presentations include those localized or involving the central nervous system, bones, joints, genitourinary tract, oral cavity, eyes, or gastrointestinal tract. Fungal infection can affect any organ or system, often following asymptomatic respiratory system colonization and dissemination. The fungus recovered at a specific site may portend varying diagnoses based on the combination of fungus and site, often modified by patient immune status. Oral lesions in histoplasmosis or paracoccidioidomycosis typically indicate the presence of disseminated disease. Oral lesions from *Candida* in a patient recently given a short course of corticosteroids likely only indicate mild, transient, localized disease.

Table 1.6 Mycosis by the organ system chiefly affected

Focus of disease on presentation	Community-associated fungi	Healthcare-associated fungi
Pulmonary	*Blastomyces*, *Coccidioides*, *Histoplasma*, *Paracoccidioides*	*Aspergillus*, Mucorales, *Pseudallescheria* (*Scedosporium*), *Fusarium*, *Cryptococcus*, *Pneumocystis*
Superficial/ cutaneous/ subcutaneous	Dermatophytes (*Trichophyton*, *Microsporum*, *Epidermophyton*), *Candida*, *Malassezia*, agents of mycetoma, agents of chromoblastomycosis, *Blastomyces*, *Paracoccidioides*, *Cryptococcus*, *Sporothrix*, Mucorales, phaeohyphomycetes, *Lacazia*	*Candida*, *Fusarium*, *Trichosporon*
Bone and joint	*Blastomyces*, *Coccidioides*, *Histoplasma*, *Paracoccidioides*, *Sporothrix*	*Candida*, *Cryptococcus*
Central nervous system	*Cryptococcus*, *Coccidioides*, *Blastomyces*, *Histoplasma*, phaeohyphomycetes, *Pseudallescheria* (*Scedosporium*)	*Aspergillus*, *Candida*
Genitourinary	*Blastomyces*, *Coccidioides*, *Histoplasma*	*Candida*, *Trichosporon*
Oral	*Histoplasma*, *Paracoccidioides*, *Candida*	*Candida*
Eye	Keratitis—*Candida*, *Aspergillus*, *Fusarium*, phaeohyphomycetes, other hyalohyphomycetes	Endophthalmitis/retinitis—*Candida*
Disseminated disease	*Coccidioides*, *Histoplasma*, *Paracoccidioides*, *Talaromyces marneffei*	*Candida*, *Aspergillus*, *Fusarium*, Mucorales, *Cryptococcus*, *Trichosporon*, other rare yeasts

Summary

Approaching patients with a wide range of infections, including respiratory, CNS, bloodstream, musculoskeletal, abdominal, and cutaneous processes, warrants inclusion of fungal diseases within the differential diagnosis. As fungal infections are increasingly recognized globally as causes of life-threatening and debilitating diseases, clinicians are incorporating mycoses within their diagnostic plans. Recognition of impaired host factors affecting cutaneous barriers, neutrophils, CMI, and cytokines will narrow the differential diagnosis to specific pathogens. While patients with hematological malignancies, solid-organ transplantation, HSCT, and primary immunodeficiencies have clearly increased risk of invasive fungal diseases, new types of immunomodulatory therapy and chemotherapy may also increase the risk of these mycoses.

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www.drfungus.org is an excellent internet resource for information about current taxonomy and other quick reference material.

Part II

Laboratory and Radiological Diagnosis

Basic Mycology

2

Sarah A. Ahmed and Sybren de Hoog

Introduction

Currently, over 700 species of fungi have been reported to cause infections in humans, and this number continues to increase over time [1]. Fungi are eukaryotic and heterotrophic organisms, which may be unicellular or filamentous, and have cells surrounded by cell walls containing glucan, chitin, or both [2]. In the past, medical problems attributed to these organisms, in comparison to those caused by bacteria, viruses, and parasites, have been relatively few and included allergic symptoms, mushroom poisoning, mycotoxicoses from ingested fungal toxins, and occasional fungal infections [3]. However, with the advent of modern medical advances, fungal infections (mycoses) have increased significantly over the past couple of decades. Human and/or animal pathogens historically considered to be fungal are now placed in several kingdoms. The *Chromista* contains the oomycete genera *Pythium* and *Lagenidium* [4], while *Prototheca* is a member of the algae order Chlorellales, Trebouxiophyceae, and Chlorophyta. The bulk of the human pathogens are in the kingdom *Fungi* [3]. Within this kingdom, main phylogenetic groups are recognized, viz. Ascomycota, Basidiomycota, and numerous clades of lower fungi such as Chytridiomycota, Mucoromycotina, and Entomophthoromycotina. Clinically significant fungi are found at several locations within these groups, and of them only around 150 taxa are known to be regularly involved in human and animal mycoses. That is because most fungi are evolutionarily not adapted to infect humans, but when accidentally introduced to the human host, some have the capacity to cause infections, which tends to disseminate when the host is immunologically impaired [5]. Alteration in the host immunity either by the use of immunosuppressive agents or due to underlying diseases is therefore a main risk factor for acquiring deep fungal infections. With the increasing population at risk in recent decades, the frequency of severe mycotic diseases has increased, and as a result, the mycology laboratories have gained great importance, not only in the diagnosis of these diseases, but also in the development of our knowledge on the diversity of fungal pathogens and their epidemiology.

The role of the mycology laboratory is to diagnose fungal infections by accurately identifying the cause of the disease in a prompt manner. These laboratories should have a minimum capacity to optimally recover and accurately identify all the possible causes of mycotic infections, and if not all, at least the most common ones [6]. Laboratory diagnosis of mycoses starts with the collection of high-quality specimens from appropriate sites of infection, followed by proper transporting, and ends with optimal processing of samples and reporting of the final results [7]. The laboratory request form should include the complete clinical data, travel history, and the patient's preparation steps. When necessary, communication between physicians and the lab personnel is advised to guide the diagnosis and to ensure safety measures for handling the clinical samples [8, 9]. Collection, transportation, and storage might differ between specimens as well as

This chapter is a revision of Chapter 02 written by Deanna A. Sutton in the second edition of this book. It contains material published by D. A. Sutton.

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_2

based on the suspected etiology of the infection. For sample processing, the selection of suitable fungal stains for preliminary direct microscopy and the use of appropriate media and incubation conditions are all crucial [7]. Although recovery of the causative organisms from host tissue and their identification is paramount for diagnosis and treatment of mycotic disease, and remains the classic way for documentation of pathogenicity, current methods to achieve this goal are often not suitable for point-of-care diagnosis. Current research is focused on developing more innovative tools for rapid, simple, and accurate diagnosis of fungal infections [10]. This includes antigen or antibody assays, molecular techniques, and detection of volatile markers. These will be addressed elsewhere in this book.

This chapter provides an introductory review of laboratory fundamentals as they relate to medical mycology. It also reviews basic taxonomy and classification regarding the kingdom of Fungi and includes descriptions of mycologic terms/features common to the most frequently recovered etiologic agents in Ascomycota, Basidiomycota, and lower fungi.

Specimen Collection, Transportation, and Pre-treatment

The likelihood of recovering a fungal etiologic agent is directly proportional to the quality of methods employed in the collection, transport, and processing of clinical specimens. For all mycotic diseases, recovery is highest when sufficient sample is collected from the active site of infection. Specimens peripheral to the site of infection may also be diagnostic in disseminated disease or when foci are not easily accessible. Guidelines are now available for diagnosis and treatment of nearly all common fungal conditions, to assist in selecting appropriate specimens and to ensure optimal diagnostic methods [11]. In addition, standardized guidelines such as the *Principles and Procedures for Detection of Fungi in Clinical Specimens, M54,* by the Clinical and Laboratory Standards Institute (CLSI) provide a user-friendly document addressing all aspects of handling and processes of specimens for mycological investigations [12]. Specimen types include those from superficial lesions such as skin, hair, and nail or deep locations such as body fluids and tissue biopsies (Table 2.1).

The hair and skin samples should be collected from the margin of the lesions, while for the nails, a small piece from the nail or the nail bed should be obtained. Samples may be collected and transported in a clean paper envelope placed into a secured plastic bag [7]. Corneal scraping or swabbing is collected in the suspected cases of fungal keratitis [13]. In general, swabs are not recommended for mycological investigations, but can be used in some circumstances such as sampling from eye, oral, vaginal, ear canal, nasopharynx, or throat. Swabs from open wounds or grains collected from open sinuses are usually contaminated and might not be useful for fungal isolation [14].

Specimens from deep body sites, including those from the respiratory tract, should be handled with extra care, particularly when collected from patients with proven or suspected COVID-19 infection; numerous cases of pulmonary fungal infections associated with COVID-19 have been reported [15]. Sputum samples might require pre-treatment with lysis solutions, while bronchoalveolar lavage (BAL),

Table 2.1 Common specimen collection sites for fungal cultures[a]

Collection site	Comments
Abscesses, subcutaneous sites	Aspirate abscess; sample base of subcutaneous lesions
Blood	Use maximum amount of blood recommended for the system being used
Bone marrow	Pediatric Isolator™ recommended[b]
Cerebrospinal fluid (CSF)	Do not refrigerate. Require concentration step
Draining sinus tracts	Search for granules of eumycotic mycetoma; wash several times with saline-containing antibiotics
Ear	Rotate swab firmly in outer ear
Eye	Use needle aspiration for the vitreous fluid. Inoculate corneal scrapings directly onto plates in a "C" shape
Hair	Use forceps to collect several hairs with shaft intact and sample any active lesions; Wood's lamp can be used
Intravenous catheters	Use Maki ("roll") method
Lower respiratory	Process promptly for dimorphic pathogens (BAL, brush, aspirate, wash, sputum)
Medical devices (valves, hardware, etc.)	Dislodge any biofilms before inoculation into liquid medium
Nails	For dermatophytes, agents of dermatomycoses, and *Candida* spp., clean with 70% alcohol; collect subungual debris; and clip affected nails
Nasal sinus	Surgical collection, commonly ethmoid and maxillary sinuses
Open wound	Aspirate or swab vigorously
Prostatic fluid	Primarily for blastomycosis
Skin	For dermatophytes, *Candida*, and *Malassezia*, clean with 70% alcohol and scrape vigorously
Sterile body fluids	May be concentrated by centrifugation or syringe filtration
Tissue	Surgical collection; use punch biopsies for skin lesions
Urine	Early-morning midstream collection
Vagina	Primarily for refractory vaginal candidiasis
Upper respiratory (oral)	Swab lesions, use selective or chromogenic media for yeasts

[a] This list is not all inclusive
[b] Wampole Laboratories, Princeton, NJ

bronchial aspirate, or wash is processed directly [16]. Lung biopsies or tissue samples from other parts of the body may be minced and homogenized to enhance the recovery of fungal pathogens. However, when the patient's history suggests infection with a member of the *Mucorales*, extensive homogenization or grinding should be avoided as it may be deleterious to the growth in culture of fragile fungal hyphae [7]. Samples from sterile body fluids such as urine, CSF, aspirated abscess fluids, and draining sites can be processed directly or centrifuged first to increase the concentration of fungal elements [17].

Blood culture is requested for the diagnosis of fungemia and disseminated fungal infections. Although several routine blood culture systems reliably recover yeast pathogens, a dedicated fungal blood culture is recommended for the diagnosis of fungemia [18, 19]. If manual blood cultures are used, consider a broth/agar biphasic system, a minimum of blood:broth ratio of 1:10, and 2–3 culture sets collected at different time points [20, 21]. Automated, continuously monitored blood cultures are also available with instruments designed and scalable for both high- and small-volume laboratories. These include the VersaTREK (Trek Diagnostic Systems/Thermo Scientific, Cleveland, Ohio), BacT/Alert (bioMérieux, Durham, NC), and BD Bactec (BD Diagnostic Systems, Sparks, MD) systems [22–24]. Always follow the manufacturer's recommendations for the specific system and use the maximum recommended amount of blood samples. The ratio of blood to broth is the most critical factor in fungal recovery [20]. Lysis centrifugation methods, either commercially available as the Isolator™ system (Alere, Waltham, MA) or manual methods, are recommended for dimorphic fungal pathogens and filamentous fungi [25, 26]. In cases of suspected catheter-related septicemia, blood cultures should be drawn at the time of catheter removal to correlate catheter colony counts and organisms recovered. Approximately, 4 cm of the catheter tip should be collected and cultured according to the semiquantitative method of Maki roll [27]. Catheter colony counts 3-folds higher or more than 15 CFU of the same pathogen recovered from the blood sample are indicative of catheter-related fungemia, whereas counts of less than 15 are less likely to be associated with infection.

Following proper collection, specimens for mycological investigations should be maintained at room temperature and transported to the laboratory as soon as possible, ideally within 2 h. Exceptions include storage of central nervous system specimens at 30 °C, and 4 °C extended storage for specimens likely to have bacterial contamination. Specimens should be kept moist and dispatched in sterile leakproof containers, but hair, skin, and nail samples must be kept dry [7, 12].

Processing of Specimens for Mycological Investigations

When sufficient specimen is submitted to the mycology laboratory, a portion of it should be examined directly by microscopy, typically, with the use of a potassium hydroxide (KOH) preparation, gram stain, calcofluor white fluorescent stain, India ink stain (limited to cerebrospinal fluid examination for *Cryptococcus* spp.), or some other method (Table 2.2). Solid specimens are digested in 20% KOH by gentle heating or incubation for 2 h and up to 24 h depending on the type of the specimen. If the clinical material is of low quantity, the priority is given to the culture over the direct examination with the exception of dermatological samples and when Mucorales are suspected [28]. Observation of fungal structures in direct microscopy and/or histology is essential to corroborate organism recovery in culture (rule out contamination). Histopathologic examination of tissue with appropriate stains is usually necessary to document fungal invasion. Stains used for detection of fungal element include the routine hematoxylin and eosin stain (H&E), Gomori methenamine silver stain (GMS), periodic acid-Schiff stain (PAS), Fontana-Masson, and others (see Chap. 5).

Different types of media are available for primary isolation of fungi from clinical samples. Selection should be based upon the type of specimen and the suspected fungal pathogen. With the availability of multiple choices, selection may vary according to personal preferences; however, certain basic tenets apply to all media used for primary recovery. Material from non-sterile sites should be cultured on media that will support fungal growth but also inhibit

Table 2.2 Useful direct microscopy methods for the routine mycology laboratory[a]

Method	Comments
Calcofluor white	Requires fluorescent microscope; can be used with KOH to detect all fungi, including *Pneumocystis*
Gram stain	Detects most fungi present; however, *Cryptococcus* spp. may exhibit only faint staining
Giemsa stain	Several modifications; detects intracellular *Histoplasma capsulatum* and intracystic bodies and trophozoites of *Pneumocystis*
India ink	Commonly used for demonstration of capsular material of *Cryptococcus neoformans* in CSF
Methylene blue stain	For staining the thin scales from cases of pityriasis versicolor
Potassium hydroxide	Clears debris, so fungi are more readily observed; stains may be added for better visualization of fungal elements
Toluidine blue O	Stains the intracystic bodies of *Pneumocystis*
Wright stain	Useful to detect intracellular *Histoplasma capsulatum* in bone marrow and peripheral smears

[a] Additional fungal stains are available through the histopathology laboratory. This list is not all inclusive

bacteria. Antibacterial agents, single or in combinations, are added for this purpose. Common choices include chloramphenicol (40–50 μg/ml or <16 μg/ml when higher bacteria are suspected), gentamicin (5–100 μg/ml), penicillin (20 U/ml), streptomycin (40 μg/ml), and ciprofloxacin (5 μg/ml). These agents should not be included when actinomycetes are suspected. Media may also be made selective by the addition of the eukaryotic protein synthesis inhibitor cycloheximide at 0.5 μg/ml. This may be useful in the detection of dimorphic fungi and dermatophytes; however, many clinically significant saprophytes may be suppressed, leading to failure in recovering opportunistic etiologic agents in compromised hosts. Therefore, media with and without this agent should routinely be employed. The fungicidal agent benomyl (10 μg/ml) can be added to inhibit fast-growing fungi and promote the recovery of slower growing ones, when environmental samples are examined. Enriched media with 5–10% sheep blood may be incorporated into the battery for fastidious thermally dimorphic fungi such as *Histoplasma* and *Blastomyces* [28]. Peptone-based versus plant-based media may also be a consideration. Many of the opportunistic filamentous fungi prefer plant-based media, producing more typical colony morphologies and more diagnostic structures, thus increasing the potential to make identification possible from primary plates. However, in many clinical laboratories, the Sabouraud's glucose agar remains the commonly used media for primary isolation, while for identification, addition of a plant-based media is used. Both peptone- and plant-based media can be made selective with antibacterial agents or cycloheximide [1, 12, 28]. Table 2.3 lists several types of media that may be used for both primary isolation and identification.

The choice of tubed versus plated media is made based upon space constraints, personal preference, and safety. Tubes provide maximum safety when dealing with BSL2/3 clinical pathogens and also prevent dehydration and contamination in cases of slow-growing fungi. Nevertheless, the greater surface area provided by plates is preferred by many laboratorians (and always preferred by the fungi!), as manipulation of cultures, isolation procedures, etc. are more easily performed on plates. When used, plate lids should be firmly attached with an air-permeable material, parafilm-sealed, or sealed in air-permeable bags to avoid cross-contamination or laboratory worker exposure [15, 28].

Optimally, cultures should be incubated at a temperature between 25 and 30 °C. If incubators with such temperature are not available, room temperature can be used (must be near 25 °C). For specimens from deep body sites, incubation at higher temperature (37 °C) in addition to lower temperature should be used to promote fast growth of the etiologic fungus. Seven days' incubation is generally adequate when screening for yeasts from oropharyngeal or vaginal sites. Although 4-week incubation time has traditionally been recommended, studies suggest that 3 weeks is adequate to detect fungal growth from most other specimens, excluding those from skin, hair, and nails, and in cultures requested specifically to recover dimorphic pathogens [29]. The time required for the development of diagnostic structures, particularly for some coelomycetes and ascomycetes, may be considerably longer, up to several weeks [30]. To obtain fruiting bodies of these fungi, firm natural material such as sterilized lupine or tomato stems may be required.

Table 2.3 Media useful for primary isolation and identification[a]

Medium	Uses/comments
Sabouraud's glucose agar (SGA)	For yeasts and filamentous fungi
	Usually adequate for aspergilli
	Poor conidiation for black molds
	Classic morphologic descriptions for dermatophytes
Chromogenic media	Several commercially available media contain chromogenic substrates and antimicrobial agents, for isolation and identification of yeasts
Potato dextrose agar (PDA)	Useful for all mold recovery/identification
Potato flake agar (PFA)	
Birdseed agar	For *Cryptococcus* spp.
Brain-heart infusion agar (BHI)	
Inhibitory mold agar (IMA)	
Yeast extract phosphate medium	
Sabhi agar	
Modified Leeming-Notman media	For isolation of *Malassezia*
Mycosel agar™[b] or Mycobiotic agar	SGA with chloramphenicol and cycloheximide
Dermatophyte test medium (DTM)	
Dermatophyte identification medium (DIM)	

[a] This list is not all inclusive
[b] BD Diagnostic Systems, Sparks, MD

Examining Cultures

One recommendation for culture examination is every day for the first 3 days and twice a week thereafter. Some safety precautions common to both yeasts and molds include the careful handling of plates and tubes, so as not to create aerosols of infectious material and to prevent contamination of patient cultures from the surroundings. Interpretation of fungal growth should be made carefully as many of the pathogenic fungi are ubiquitous in the environment and contamination may easily occur during sampling or transportation. Growth of any clinically significant fungus at different inoculation points from clinical material is usually diagnostic with the exception of the most common contaminants *Aspergillus*, *Penicillium*, and *Mucorales*. For these fungi, a confirmation with direct examination or histology and patient clinical

Table 2.4 Common laboratory contaminants and their significance when recovered from clinical specimen[a]

Specimen	*Aspergillus*	*Penicillium*	*Mucorales*
Skin	Significant in disseminated infections or when repeatedly isolated from the same lesion. Confirmation by direct microscopy, histology, or in situ hybridization is required	Probably contaminant	Probably significant. Confirmation with histology or direct microscopy is needed
Blood	Rarely significant, usually contaminant	Usually contaminant	Usually contaminant
Tissue	Significant when seen in direct microscopy or histology. Others might be contaminant	Rarely significant. Confirmation with direct microscopy or histology is required	Significant when seen in direct microscopy or histology. Others might be contaminant
Pus or fluid aspiration	Significant	Rarely significant	Probably significant when the patient is at risk
CSF	Significant when confirmed with antigen test	Usually contaminant	Probably significant when the patient is at risk
Urine	Rarely significant	Usually contaminant	Usually contaminant
Sputum/ respiratory samples	Significant when the patient is at risk; allergic chronic or invasive disease	Rarely significant	Significant when the patient is at risk
Nails	Only significant if isolated in pure culture and confirmed by direct microscopy	Contaminant	Contaminant
Ear swab	Significant when confirmed with direct microscopy. Mostly *Aspergillus* section Nigri. Other Aspergilli consider invasive otitis	Contaminant	Usually contaminant
Cornea scraping	Significant if confirmed by direct microscopy. Growth on inoculation site is also significant	Rarely significant	Contaminant

[a]Table adapted from http://www.life-worldwide.org/ and ref. [31]

symptoms should be made. Also, growth of certain saprophytes from non-sterile clinical samples might not always be considered significant [31] (Table 2.4). For definite proof of deep infection by fungi that are usually found as contaminants or saprophytes, advanced methods such as in situ hybridization or immunohistochemistry may be necessary [32]. On cultures, yeasts grow typically as creamy to waxy, while molds appear velvety to woolly to cottony.

Phenotypic Yeast and Yeast-Like Organism Identification

Yeast cultures, containing unicellular organisms that replicate by budding, may be handled on the open bench, adhering to the same safety precautions as for bacteria. Yeast and yeast-like fungi should be examined for their colony color, growth rate, temperature requirements (or preferences), and macro- and microscopic morphology. On SGA, yeasts grow as creamy white to pink, or brownish-black in cases of the *Chaetothyriales* black yeast species. On CHROMagar Candida™ (CHROMagar Microbiology, Paris, France), *Candida* species appear blue to green or pink. Yeast colonies can be smooth, wrinkled, glabrous, moist, or dry, and the species can be differentiated microscopically based on the cell size and shape, presence of blastoconidia, capsules, germ tubes, pseudohyphae, true hyphae, and chlamydoconidia [33]. The dimorphic fungal taxa *Blastomyces*, *Coccidioides*, *Emergomyces*, *Histoplasma*, *Paracoccidioides*, and *Talaromyces* produce yeast in vivo or at 37 °C but grow as molds at routinely used incubation temperature and thus can easily be differentiated from the typical yeast. Yeast morphology is most reliably observed on a cornmeal agar plate using the Dalmau method [34]. This technique involves streaking a very small amount of yeast onto a plate in two or three parallel lines, streaking back and forth over these lines for better isolation, and covering the area with a flame-sterilized coverslip. The plate is incubated at room temperature for 18–24 h and then examined microscopically for diagnostic structures. Tease mounts may also provide useful information. Additional procedures that may be required for the specific identification of yeasts include the reduction of nitrate to nitrite, urease activity, ability of the organism to grow on media containing cycloheximide, and assimilation or fermentation patterns. Many commercial systems, both manual and automated, are available to assist in yeast identification by assessing assimilation pattern; these include API ID32C (bioMérieux), AuxaColor (Bio-Rad), and Vitek 2 (bioMérieux) [35].

Phenotypic Identification of Filamentous Fungi

Any filamentous organisms recovered on culture should be examined and manipulated in a biological safety cabinet. While molds can be recovered on a variety of media, conidiation/sporulation is generally enhanced on plant-based

media or on low-nutrient media. If not used in primary isolation, such media should be employed during the identification process. Molds should be examined for their growth rate, temperature requirements, and macroscopic morphology to include color (hyaline to brightly colored or brownish to blackish), texture (velvety, woolly, granular, cottony, etc.), and observation of any diagnostic features visible to the naked eye. The large structures such as fruiting bodies, synnemata, or conidial chains can also be observed using stereomicroscopes, and that will help in selecting the diagnostic part of the colony for microscopic preparations [1]. The microscopic detail may be studied using tease mounts or temporary tape mounts (clear tape only) in lactophenol cotton blue or lactic acid. The preferred technique to demonstrate diagnostic structures and methods of conidiogenesis for most filamentous fungi is the slide culture method. Additionally, this method can provide a permanent mount that can be preserved in a slide collection for future studies and is extremely useful for comparison with other similar isolates or atypical strains [36]. Members of the order *Mucorales* may rapidly overgrow slide cultures, making this method less than optimal for studying these fungi. Slide cultures should also not be set up for molds where the clinical history suggests a dimorphic pathogen such as *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides* species, *Paracoccidioides brasiliensis*, *Talaromyces* (*Penicillium*) *marneffei* (usually restricted to HIV-infected individuals from endemic areas of Southeast Asia), or dangerous opportunists such as *Cladophialophora bantiana* [37]. Tease mounts should be prepared for these isolates in a mounting fluid known to kill the fungus, such as lactophenol cotton blue. *Sporothrix schenckii* and other *Sporothrix* spp. [38], also dimorphic organisms, pose less of an exposure risk and may be examined by slide culture. Cultures of *Histoplasma capsulatum* and *B. dermatitidis* may be definitively identified using the DNA Gen-Probe® (AccuProbe, San Diego, CA), a chemiluminescent DNA-labeled probe that binds to rRNA of target species [39]. This method, which confirms a *Coccidioides* species, does, however, showed cross-reactivity with isolates of non-targeted species, and therefore, results should be interpreted carefully [40].

Molecular Fungal Identification

While this work contains a chapter on diagnostic molecular biology (Chap. 4), information about fungal identification would be incomplete without brief mention here of the most commonly used molecular method for the identification of clinically significant fungi. Molecular methods can be used to identify the fungal etiology directly from clinical material, which includes in situ hybridization, real-time and conventional PCR-based assays, and isothermal amplification assays. However, it has often been used for the identification of isolated fungi with the most cited method being PCR and sequencing [41, 42]. For the identification by sequencing, the genomic DNA is extracted, an optimal gene target (barcode) is amplified and sequenced, and then the data are aligned and analyzed [1]. Although there is no single barcode marker suitable for identification of all clinically important fungi, the internal transcribed spacer regions (ITS1 and ITS2) and the D1/D2 regions of the large ribosomal subunit were found to be useful for a wide range of species [43]. Additional targets that may be required for identification of some exceptional fungi include beta-tubulin (TUB), calmodulin (CAL), translation elongation factor 1-α (TEF1), and others [44]. The obtained sequence results are then compared against type strains or other credible deposits in a reference database using the BLASTn algorithm. The GenBank database is currently curated, and information on sequences derived from type materials is provided [45]. However, for taxa of which the sequences of the type are not available, there is no assurance that the deposited sequence is from a correctly identified isolate. Other curated databases include ISHAM barcoding database, CBS database of Westerdijk Institute, and MycoBank database among others. Of note, these databases might be interconnected.

In light of cost containment and dwindling number of individuals trained in classical morphologic identification, more laboratories are considering adding molecular methods in conjunction with phenotypic methods, or as a standalone method. The mass spectrometry-based assay, MALDI-TOF, is now being used in many laboratories for specific identification of clinical isolates providing rapid and reliable identification of yeast species, whereas for filamentous fungi, the method is still under evaluation [46]. It should be noted that molecular-alone identification without a comparison of the phenotypic features has the potential for misidentification of isolates that could negatively impact patient management [47]. On the other hand, when the clinical isolate did not show sporulation or diagnostic structures, molecular methods remain the only way to properly identify such isolates [48].

Taxonomy, Classification, and Nomenclature

The term classification, in the fungal sense, refers to the application of names for the categories into which the taxa (taxonomic groups) may be grouped, with some subdivisions regarding their relative order. "Taxonomy" refers to this classification in a very systematic way, and nomenclature is the assigning of names to fungi. Naming of fungi is regulated by the International Code of Nomenclature for algae, fungi, and

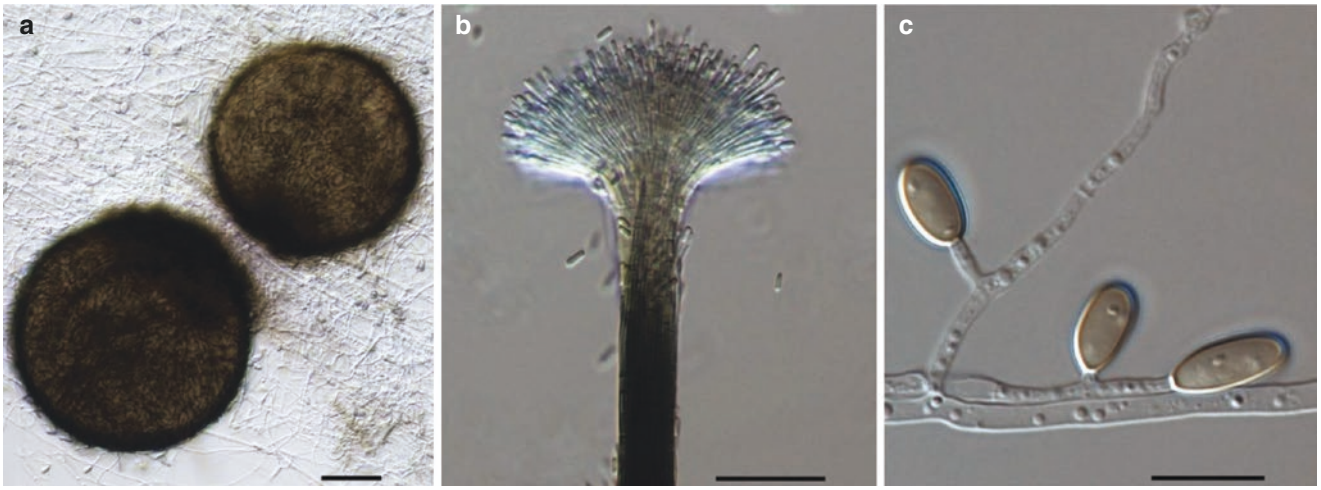


Fig. 2.1 (**a**) Ascoma (cleistothecium); (**b**) synnemata (Graphium-like morph); (**c**) conidia (Scedosporium-like). Species *Scedosporium minutisporum*

plants. The Melbourne Code, adopted in 2011, and published in 2012 [49, 50], resulted in major changes in fungal taxonomy and nomenclature, which became effective on January 1, 2013. Some of the most significant are as follows: a) the abolishment of Article 59 of the previous ICBN permitting multiple names for the same organism, i.e., the use of both *Aspergillus fumigatus* and *Neosartorya fumigata* (one fungus = one name); b) the need to determine *which* name will be used (now resolved for a large number of taxa); and c) the deposition of isolate information into a recognized repository for the valid publication of a fungal name (such as MycoBank and Index Fungorum). As consequences, many names were reduced to synonym, and the decision of choosing and selecting taxa names has created a lot of conflicts. For example, *Microascus cinereus*, a sexual fungus (teleomorph) that produces perithecia, asci, and ascospores in culture, also simultaneously produces an asexual form that is microscopically quite different. Asexual fungi are traditionally known as "mitosporic" or "anamorphic" fungi, i.e., those reproducing by mitosis rather than meiosis. The asexual form of *Microascus cinereus* is the phaeoid fungus *Scopulariopsis cinereus* [51]. Anamorphic fungi are identified mostly on the basis of their method of conidiogenesis (how they form their reproductive structures). Asexual reproductive propagules are referred to as conidia, hence the term conidiogenesis. Sexual fungi are mostly identified based on the method they use to form their sexual reproductive propagules (e.g., ascospores, basidiospores). As mentioned above, multiple names will no longer be permitted, and the determinations as to which name will be used is now more or less resolved for the most common groups. Priority was given to the earliest published name, with some exceptions. Some fungi produce multiple anamorph forms, such as is seen with *Scedosporium boydii* (syn. *Pseudallescheria boydii*), *Scedosporium apiospermum*, and *Scedosporium minutisporum* (Fig. 2.1). *Pseudallescheria boydii* is the teleomorph, while *Scedosporium boydii* is the anamorph [52], and they may also produce a *Graphium* synanamorph, or another anamorphic form of the "whole fungus." Although many mitosporic fungi have known teleomorphs, most require two mating strains to produce the sexual form. These are referred to as heterothallic. A few clinically significant fungi require only one strain to produce the teleomorph, and these are considered homothallic. *Microascus cinereus* and *Scedosporium boydii*, cited above, are examples of homothallic fungi.

Practically speaking, most etiologic agents are identified in the laboratory on the basis of structures formed by the asexual form of the fungus. Despite the fact that routine identification of clinical fungi mostly relies on their morphological characters, the taxonomy and classification are now based on a combination of approaches with the emphases on molecular and phylogeny. The phylogenetic studies of eukaryotes have placed the fungi into the domain *Amorphea*, subgroup *Opisthokonta*, and the clade *Nucletmycea* (Holomycota) [53]. At least, seven phyla are recognized in the kingdom *Fungi* with human and animal pathogens being in the phyla *Ascomycota* and *Basidiomycota*, and the formerly known phylum *Zygomycota*. The latter was found to be polyphyletic and divided between *Mucoromycota* and *Zoopagomycota*. The *Chytridiomycota* comprises pathogens of cold-blooded vertebrates only.

The following is an abbreviated classification scheme traditionally used for the kingdom Fungi:

Group	Group ending
Kingdom	None
Subkingdom	None
Phylum	-mycota
Subphylum	-mycotina
Class	-mycetes

Group	Group ending
Order	-ales
Family	-aceae
Genus	No specific ending
Species	No specific ending

Although large numbers of fungi can still be organized and placed within this hierarchical system, molecular phylogeny has shown a remarkable instability in this system, especially at the higher taxonomic ranks. In addition, the application of phylogenetic approaches for taxonomy of fungi has resulted in a dramatic change in many clinically important taxa and requires regular updates with recent literature. Chap. 3 of this book will discuss this issue in detail. Below are the common identifying characteristics for the main phylogenetic groups of the kingdom *Fungi* (see Table 2.5).

Table 2.5 Simplified schematic of the kingdom *Fungi* for some human/animal pathogens

Phylum Ascomycota
Subphylum Saccharomycotina—ascomycetous yeasts
Subphylum Taphrinomycotina
Contains the unculturable genus *Pneumocystis*
Subphylum Pezizomycotina—molds; produce ascospores in a variety of sexual structures known
As ascomata (plural), ascoma (singular). The asexual reproduction results in the formation of conidia
Ascomycetes producing fruit bodies
Cleistothecium—round, closed ascoma
Example: *Scedosporium minutisporum* (Fig. 2.1)
Perithecium—pear-shaped ascoma, with an opening or ostiole
Example: *Chaetomium perlucidum* (Fig. 2.2)
Gymnothecium—ascoma with a loose network of hyphae
Example: *Myxotrichum deflexum* (Fig. 2.3)
Asci (*plural*), ascus (*singular*)—within the ascoma and containing ascospores
Ascospores, various sizes, shapes, colors, ornamentation
Ascomycetes producing conidia
Methods of conidiogenesis
Blastic—conidia blown out
Phialidic conidiogenous cells—often have discernible collarettes and produce conidia in cluster or chain
Example: *Phialophora americana* (Fig. 2.4) and *Aspergillus alabamensis* (Fig. 2.5)
Annellidic conidiogenous cells—have rings or annellations and become longer and narrower with the production of annelloconidia
Example: *Lomentospora prolificans* (Fig. 2.6)
Some species blow out conidia through pores on geniculate conidiophores
Example: *Curvularia australiensis* (Fig. 2.7)
Thallic—conidia formed from preexisting hypha
Arthroconidia produced that may or may not have intervening disjunctor cells
Example: *Coccidioides immitis* (Fig. 2.8) and *Trichophyton rubrum* (Fig. 2.9)
Hyphomycetes—bear their conidia free and display various colors, methods of conidiogenesis, growth rates, etc.
Example: *Aspergillus alabamensis* (Fig. 2.5)
Coelomycetes—bear their conidia within some type of asexual structure known as a conidioma and display various colors, methods of conidiogenesis, growth rates, etc.
Pycnidium—round conidioma with an opening (ostiole) and conidia contained within for example *Phoma herbarum* (Fig. 2.10)
Acervulus—flat, cup-shaped conidioma, with conidia more or less exposed; for example: *Colletotrichum* species
Phylum Basidiomycota
Subphylum Wallemiomycotina
Contains the halophilic genus *Wallemia*
Subphylum Pucciniomycotina
Primarily contains the rusts and few red yeasts
Subphylum Ustilaginomycotina
Contains yeast-like members of smut fungi. *Malassezia* is a member of this group
Subphylum Agaricomycotina
Contains basidiomycetes producing basidiocarps (mushrooms) with yeast anamorphs (*Cryptococcus* species) or filamentous anamorphs that are frequently sterile or may produce arthroconidia
Example: *Schizophyllum commune*, a human etiologic agent, produces fruit bodies and clamp connections
Basidiospores sometimes seen from basidiocarps of *S. commune* (Fig. 2.11)

Table 2.5 (continued)

Phylum Mucoromycota
Subphylum Mucoromycotina
Mucorales—asexual reproduction by multi-spored or few- (to one) spored sporangia (sporangiola)
Heterothallic genera (require two mating strains) include some spp. of *Rhizopus, Lichtheimia, Mucor,* and others; produce sporangiospores
Example: *Rhizopus microsporus* (Fig. 2.12), and *Mucor hiemalis* (Fig. 2.13)
Homothallic genera/species (one mating strain required) produce zygospores
Subphylum *Mortierellomycotina*
Sporangia without columella
Example: *Mortierella wolfii* (Fig. 2.14)
Phylum Zoopagomycota
Entomophthorales—characterized by forcibly discharged conidia. Produce asexual primary conidia and smaller secondary conidia
Example: *Conidiobolus incongruus* (Fig. 2.15)
Example: *Basidiobolus ranarum*, produces zygospores

Ascomycota

Under the phylum *Ascomycota*, the ascomycetous yeasts are usually identified by yeast methods, while the molds are identified based upon the structures they produce. Some of the filamentous homothallic ascomycetes produce ascomata in vitro, usually after mating, with the products being known as cleistothecia, perithecia, or gymnothecia in which the asci and ascospores are contained (Figs. 2.1, 2.2, and 2.3). Ascomycetes without known sexual forms comprise the most common etiologic agents of human and animal disease. These fungi are highly diverse both genetically and phenotypically. Taxa belonging to this group are identified on the basis of their asexual reproductive propagules, conidia (method of conidial formation or conidiogenesis). Two main groups exist: the hyphomycetes bear their conidia free to the air, while the coelomycetes have their conidia contained within some type of enclosed to semi-enclosed structure [30]. The hyphomycetes contain numerous common hyaline and dematiaceous (dark) genera and generally produce their conidia by either blastic or thallic methods. Blastic conidia are "blown out" of some type of conidiogenous cell. These include those produced from phialides, as in *Phialophora* (Fig. 2.4) species or *Aspergillus* (Fig. 2.5) species, or from annellides, as in *Lomentospora prolificans* (formerly *Scedosporium prolificans*) (Fig. 2.6). Conidia can be solitary, catenate, or non-catenate and arranged in basipetal or acropetal order. In some species, multiple conidia can be produced at the same time from a single conidiogenous cell (synchronous), while in other species, a single conidium is produced, and then the conidiogenous cell grows out laterally to form a new conidium (sympodially), such as in *Curvularia* (Fig. 2.7). Thallic conidia are formed from preexisting hyphae, as in *Coccidioides* species (Fig. 2.8), *Malbranchea* species, and dermatophytes [1] (Fig. 2.9). The structures produced by coelomycetes to contain their conidia are known as conidioma (*singular*) or conidiomata (*plural*). They may be round structures with an opening or ostiole known as a pycnidium, as in *Phoma* and *Phoma*-like species (Fig. 2.10), or a flat, cup-shaped, semi-enclosed structure known as an acervulus. The conidiogenous cells within both of these conidiomata may be either phialidic or annellidic. Because different species might produce phenotypically similar conidioma, identification of coelomycetes based on these structures proved to be of a limited value. Molecular tools remain the recommended method for identification and revealed dramatic changes in the taxonomy and nomenclature of coelomycetes. A recent review provides updated taxonomy of clinically important coelomycetes and their molecular identification [54].

Basidiomycota

Similar to the ascomycetes yeast, the red and white yeasts within the phylum *Basidiomycota* are commonly identified by yeast methodologies. The filamentous basidiomycetes are rarely implicated in human infection, and when isolated from clinical specimens, they pose identification dilemmas, as they frequently remain sterile in culture, producing no unique reproductive structures [55]. In nature, filamentous basidiomycetes produce mushroom-like fruit bodies or present as crust or shelf fungi. In the lab, they can occasionally be recognized by the presence of clamp connections, basidiocarps, or basidiospores when dikaryons (compartments of a hypha that contain two nuclei, each derived from a different parent) are present [1] (Fig. 2.11).

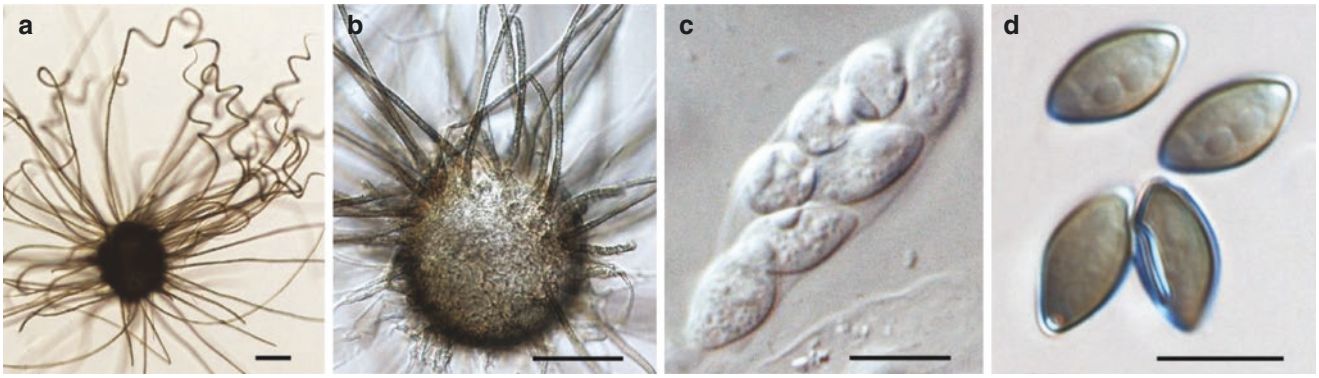
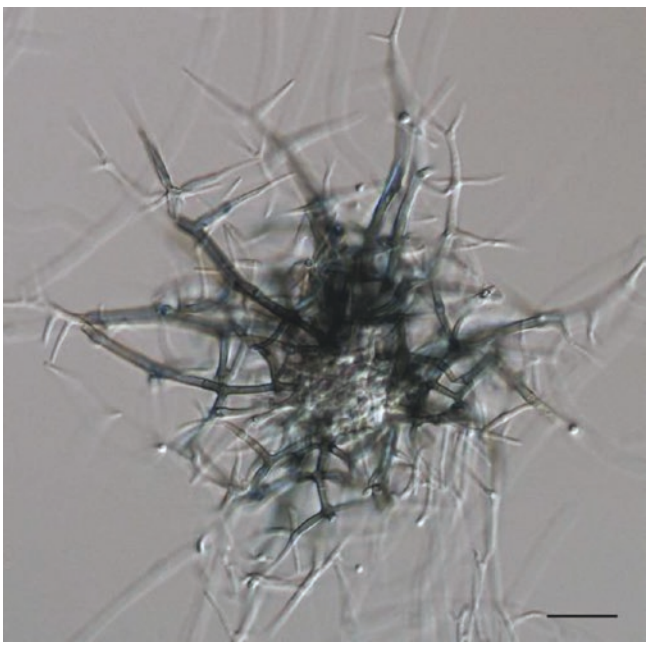


Fig. 2.2 (**a**, **b**) Ascoma (perithecium), scale bars = 40 μm; (**c**) asci (contain eight ascospores), scale bar = 10 μm; (**d**) ascospores, scale bar = 10 μm. Species *Chaetomium perlucidum*

Fig. 2.3 Gymnothecium (ascoma with a loose hyphal network surrounding the ascospores) of *Myxotrichum deflexum*, scale bar = 20 μm. (Reproduced with permission from the Foundation Atlas of Clinical Fungi)



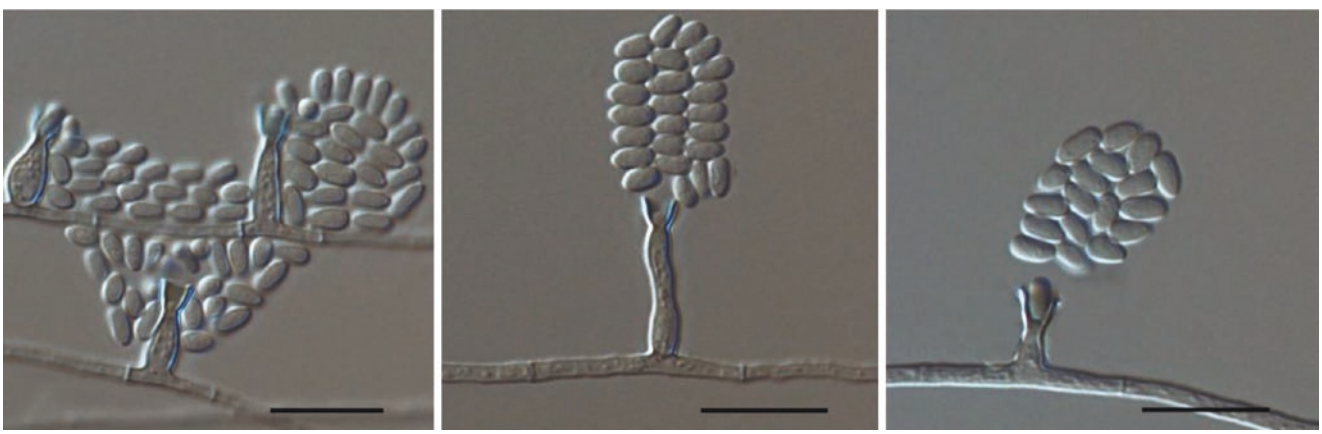


Fig. 2.4 Phialides of *Phialophora americana* with deep collarettes, scale bars = 10 μm

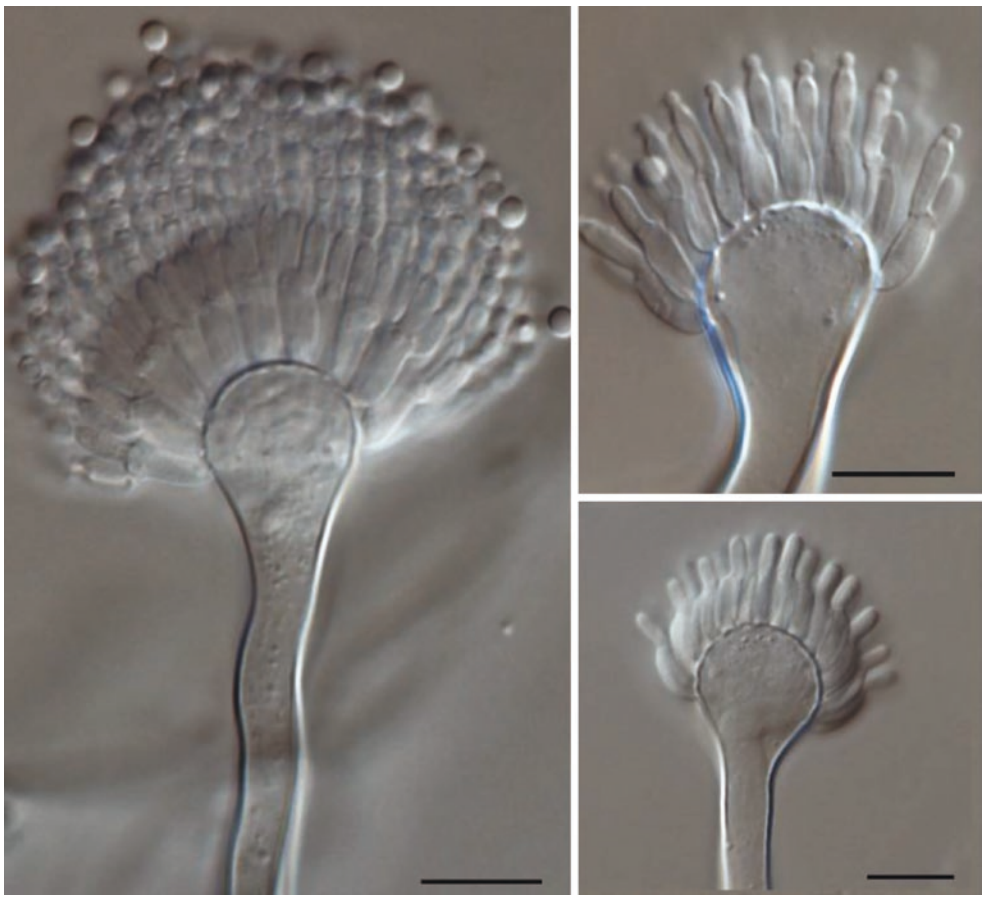


Fig. 2.5 Phialides bearing conidia of *Aspergillus alabamensis*, scale bars = 10 μm. (Reproduced with permission from the Foundation Atlas of Clinical Fungi)



Fig. 2.6 Conidiophore (annellides) bearing conidia of *Lomentospora prolificans*, scale bars = 10 μm. (Reproduced with permission from the Foundation Atlas of Clinical Fungi)

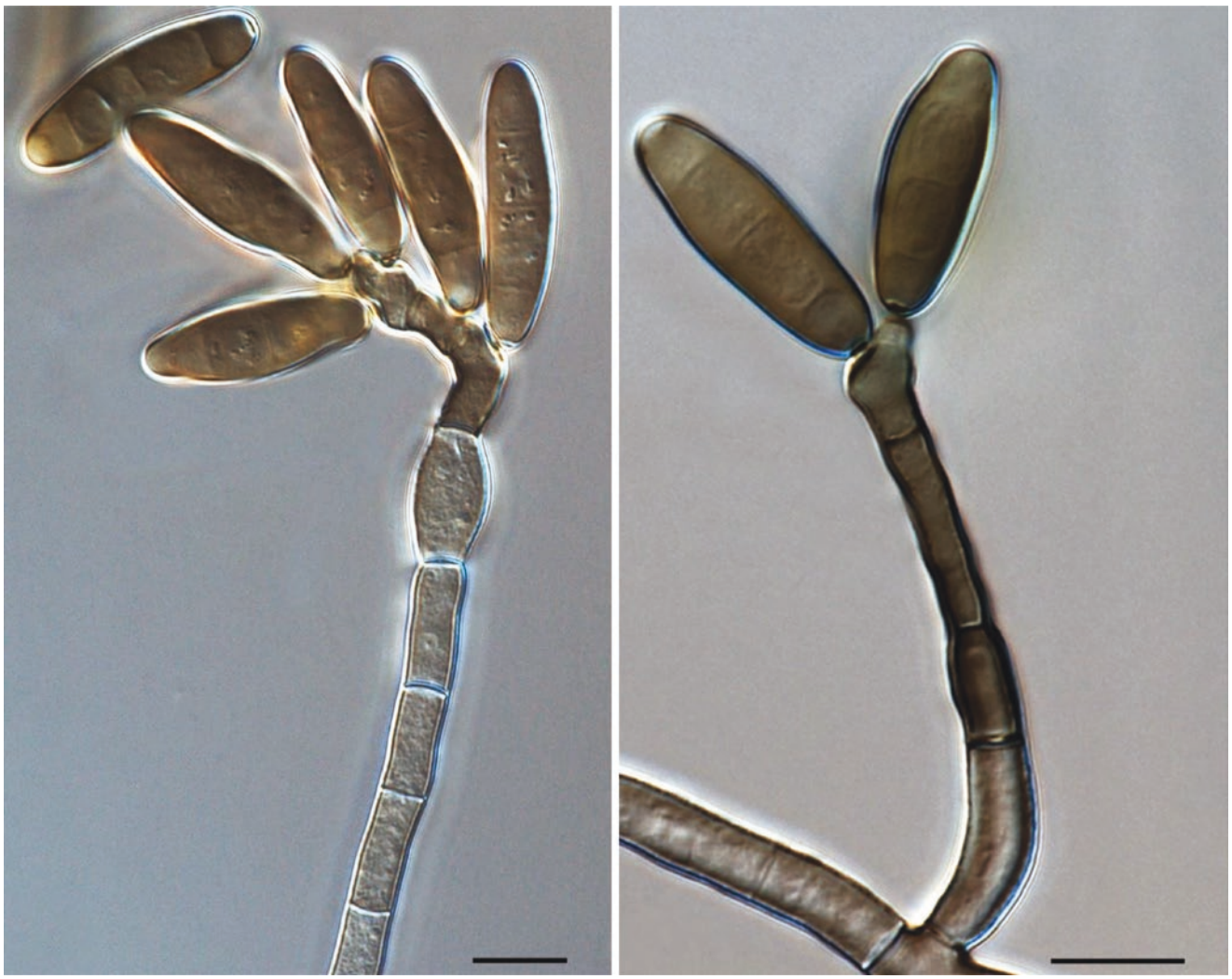


Fig. 2.7 Geniculate conidiophores (sympodial) with pores through which the conidia of *Curvularia australiensis* are blown out, scale bars = 10 μm. (Reproduced with permission from the Foundation Atlas of Clinical Fungi)



Fig. 2.8 Hyphae and arthroconidia of *Coccidioides* species, scale bar = 10 μm. (Reproduced with permission from the Foundation Atlas of Clinical Fungi)

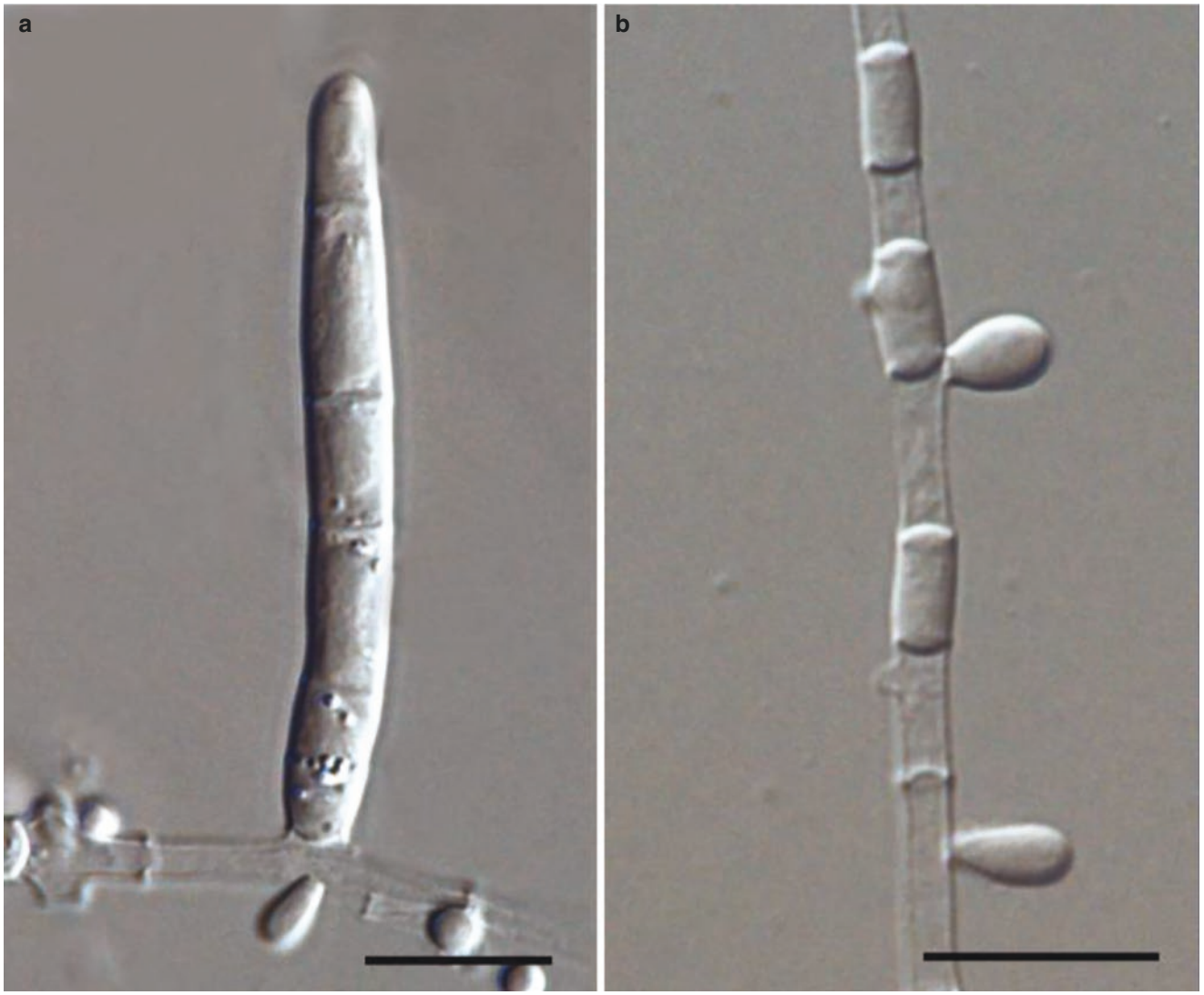


Fig. 2.9 Macro- and microconidia of *Trichophyton rubrum* (thallic conidiogenesis **a** and **b**), scale bars = 10 μm. (Reproduced with permission from the Foundation Atlas of Clinical Fungi)

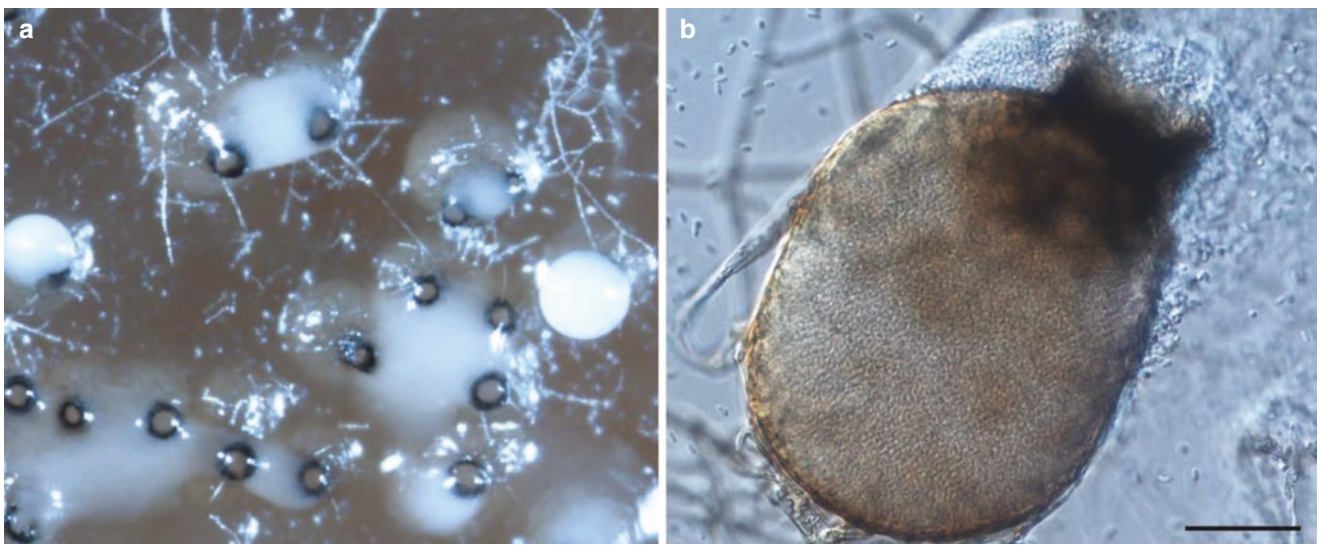


Fig. 2.10 Conidiomata of *Phoma herbarum* containing ostiole or opening. (**a**) On the gar surface, (**b**) under the microscope, scale bar = 40 μm

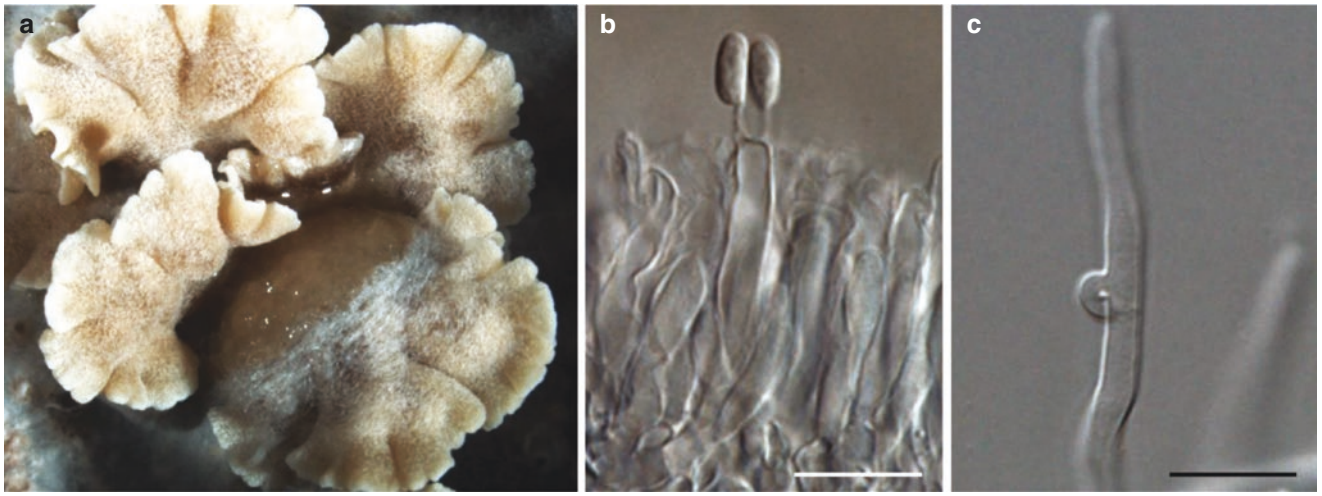


Fig. 2.11 Basidiocarp (**a**), basidia and basidiospores (**b**), and clamp connections (**c**) of *Schizophyllum commune*. Scale bars = 10 μm

Mucoromycota and *Zoopagomycota*

The phylum Zygomycota was abandoned based on phylogenetic studies of the fungal kingdom; the taxa belonging to it were accommodated into *Mucoromycota* (contains *Glomeromycotina*, *Mortierellomycotina*, and *Mucoromycotina*) and *Zoopagomycota* (comprises *Entomophthoromycotina*, *Kickxellomycotina*, and *Zoopagomycotina*) [56]. Members of *Mucoromycotina* contain the largest number and the most common clinical mucoralean genera, viz. *Actinomucor*, *Apophysomyces*, *Cokeromyces, Cunninghamella, Lichtheimia* (formerly *Absidia*), *Mucor* (Fig. 2.13), *Rhizomucor, Rhizopus* (Fig. 2.12), *Saksenaea, Syncephalastrum*, and *Thamnostylum*. These genera are characterized by production of asexual spores (sporangiospores) carried mostly inside sporangia, while the sexual reproduction involves the production of zygospores (Fig. 2.13). *Mortierellomycotina* contains only one clinically significant genus *Mortierella* (Fig. 2.14), and the *Entomophthoromycotina* encompasses the less frequently seen genera *Conidiobolus* (Fig. 2.15) and *Basidiobolus* (both characterized by forcibly discharged conidia) [1].



Fig. 2.12 Ramified rhizoids; short, dark sporangiophores; collapsed columellae; and sporangiospores of *Rhizopus microsporus*

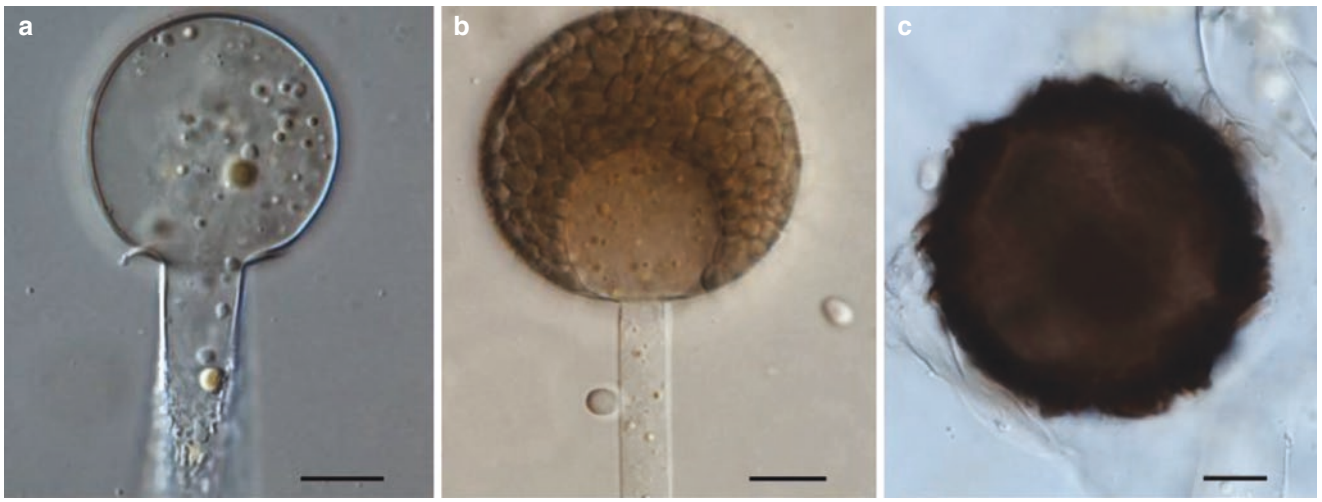


Fig. 2.13 Columellae (**a**), sporangia containing sporangiospores (**b**), and thick-walled zygospores (**c**) of *Mucor hiemalis*. Scale bars = 10 μm

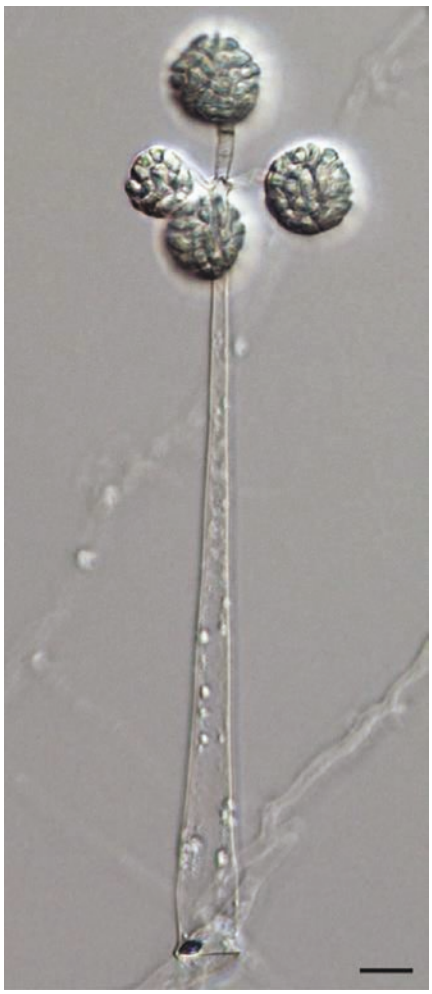


Fig. 2.14 Long sporangiophore, and sporangia of *Mortierella wolfii*, scale bar = 10 μm. (Reproduced with permission from the Foundation Atlas of Clinical Fungi)

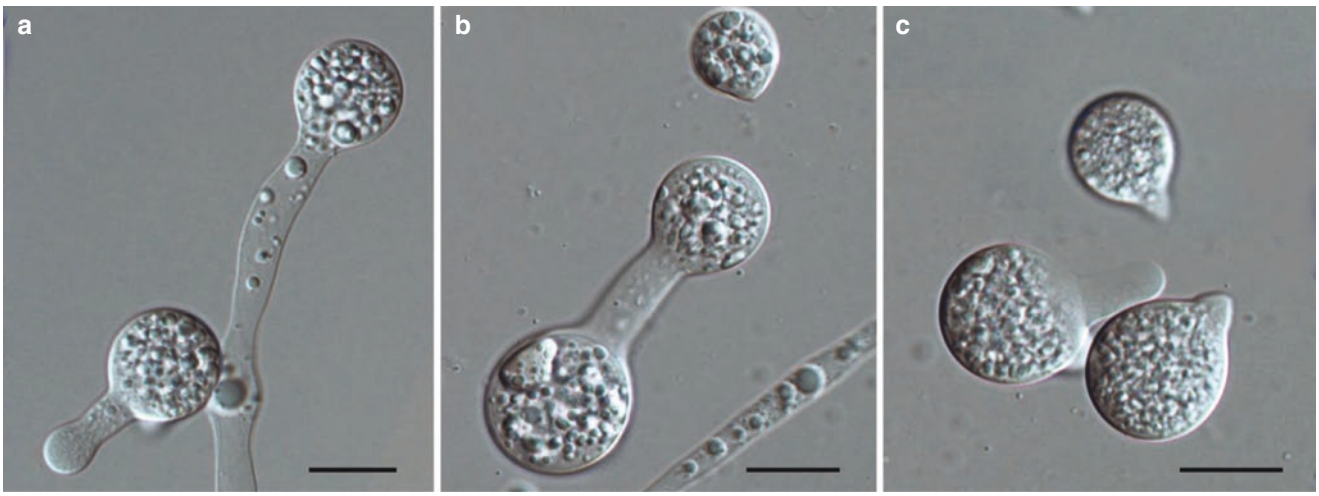


Fig. 2.15 Primary sporangiole giving rise to secondary sporangiole (**a**, **b**), sporangiole (**c**) of *Conidiobolus coronatus*. Scale bars = 20 μm

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Further Reading

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Molecular Taxonomy

3

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Introduction

The wide-scale use of molecular phylogenetic studies in mycology has significantly changed our understanding of the relationships between fungal taxa at all levels, including species, genera, families, and beyond. The use of molecular phylogenies in mycology initially focused on parts of the ribosomal RNA encoding genes, such as the D1/D2 domains of the large subunit ribosomal DNA (LSU rDNA), the small subunit rDNA (SSU rDNA), and the internal transcribed spacer regions 1 and 2 (ITS1, ITS2) [1–6]. After extensive evaluation in a large international barcoding consortium, the ITS region was selected as the universal fungal barcode [7], despite the general knowledge that this marker does not distinguish between all fungal species (e.g., species of *Aspergillus* and *Fusarium* cannot be reliably recognized using this barcode) [8–10]. Consequently, people have been investigating other markers, such as *TEF1*α, to identify unknown fungal (including yeast) isolates [11]. Notwithstanding the drawbacks to using the ITS as a fungal barcode, some large-scale studies using strains preserved in the Westerdijk Fungal Biodiversity Institute have further investigated the application of those barcodes, including evaluations of various thresholds for separation of species, genera, and families [12, 13].

A major step forward was the use of multiple molecular markers to better understand phylogenetic relationships of fungi. A hallmark paper used six loci to infer phylogenetic relationships across the fungal kingdom [14], and this has resulted in the first modern phylogeny-based taxonomy supported by molecular data [15]. Later, multiple genes were used to improve phylogenetic resolution among ascomycetous and basidiomycetous yeasts [16–20], dermatophytes [21], dimorphic fungi [22, 23], *Aspergillus*, *Penicillium*, and *Fusarium* [24, 25], among others. The most recent development is the use of whole genome-based data in phylogenomic studies [26–32].

With respect to clinically important fungi, these studies have yielded several important new insights. For instance, *Pneumocystis*, long thought to be a protozoan, turned out to be a yeast-like fungus belonging to Taphrinomycotina, the basal lineage of Ascomycota [33–35]. *Malassezia*, yeasts that are known to occur on the skin of warm-blooded animals, including humans, turned out to be relatively closely related to plant pathogens in the Ustilaginomycotina [36–38], and now reside in their own class Malasseziomycetes [19]. The genus *Candida*, well known for including several clinically important species, turned out to be largely polyphyletic [39, 40], and many species have recently been reclassified in other genera that were originally defined based on sexually reproducing states (Table 3.1). A clinically important observation is that *Candida* species that can cause disease belong to different clades and differ in their susceptibility to commonly used antifungals [44, 45].

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_3

Table 3.1 Overview of recent taxonomic changes made for ascomycetous and basidiomycetous yeasts as presented in theyeasts.org database

Genus	Species	Taxonomy / Genus update[a]	Clade affinity[b]
Candida			
	Candida aaseri		*Yamadazyma*
	Candida abiesophila	*Martiniozyma*	
	Candida adriatica		*Cyberlindnera*
	Candida aechmeae		*Metschnikowia*
	Candida aglyptinia	*Teunomyces*	
	Candida albicans		*Lodderomyces - Spathaspora*
	Candida alimentaria		*Yarrowia*
	Candida alishanica		*Kodamaea*
	Candida allociferrii		*Sugiyamaella*
	Candida alocasiicola	*Wickerhamiella*	
	Candida amapae	*Saccharomycopsis*	
	Candida amazonensis	*Scheffersomyces*	
	Candida ambrosiae	*Suhomyces*	
	Candida anatomiae	*Nakazawaea*	
	Candida anglica		*Kurtzmaniella*
	Candida anutae		unaffiliated
	Candida apicola	*Starmerella*	
	Candida arabinofermentans		*Ogataea*
	Candida asiatica	*Martiniozyma*	
	Candida asparagi		*Metschnikowia - Clavispora*
	Candida athensensis		*Meyerozyma*
	Candida auringiensis	*Groenewaldozyma*	
	Candida aurita		unaffiliated
	Candida awuaii		*Pichia*
	Candida bentonensis		*Yarrowia*
	Candida berthetii		sister *Starmera*
	Candida bituminiphila	*Zygoascus*	
	Candida blackwellae		*Lodderomyces - Spathaspora*
	Candida blankii		*Candida blankii*
	Candida blattae		*Metschikowia*
	Candida blattariae		*Yamadazyma*
	Candida bombiphila	*Wickerhamiella*	
	Candida boreocaroliniensis	*Sugiyamaella*	
	Candida borneana	*Kuraishia*	
	Candida bracarensis		*Nakaseomyces*
	Candida broadrunensis		*Scheffersomyces*
	Candida bromeliacearum		*Phaffomyces - Komagataella*

[a]Column "Taxonomy/genus update" indicates recent taxonomic changes for species that have been proposed in literature (light gray background)
[b]Column "Clade affinity" gives the clade to which those species that have not yet been properly placed taxonomically belong
[c]Taxonomic change needs to be corrected as a wrong reference strain has been used (dark gray background). Clade affiliations are taken from www.theyeasts.org and references [39–43]

Tab. 3.1 (continued)

	Candida bullrunensis		*Sugiyamaella*
	Candida caryicola		*Suhomyces?*
	Candida caseinolytica	*Tortispora*	
	Candida catenulata	*Diutina*	
	Candida cellulosicola	*Spensermartinsiella*	
	Candida chilensis		*Nakazawaea*
	Candida chiropterum	*Blastobotrys*	
	Candida chrysomelidarum	*Metschnikowia*	
	Candida coquimbonensis		*Phaffomyces*
	Candida cylindracea		basal *Ogataea*
	Candida dajiaensis		*Wickerhamomyces*
	Candida deformans	*Yarrowia*	
	Candida dendrica		*Starmera*
	Candida digboiensis		*Candida blankii*
	Candida diversa	*Saturnispora*	
	Candida ergatensis	*Scheffersomyces*	
	Candida ernobii	*Nakazawaea*	
	Candida ethanolica		*Pichia*
	Candida fennica	*Hyphopichia*	
	Candida ficus		unaffiliated *Pichia* - *Saturnispora* - *Kregervanrija*
	Candida freyschussii		near *Starmera*
	Candida galis		near *Barnettozyma*
	Candida ghanaensis		unaffiliated
	Candida glaebosa		*Candida glaebosa*
	Candida humilis	*Kazachstania*	
	Candida insectamans	*Hemisphaericaspora*	
	Candida kashinagacola	*Ambrosiozyma*	
	Candida lassenensis	*Saccharomycopsis*	
	Candida linzhiensis		unaffiliated
	Candida litsaeae	*Danielozyma*	
	Candida lundiana		*Zygoascus*
	Candida nonsorbophila		unaffiliated *Yamadazyma*
	Candida psychrophila		*Debaryomyces*
	Candida restingae		near *Kodamaea*
	Candida silvatica		unaffiliated, basal to *Pichia*
	Candida sinolaborantium		unaffiliated *Yamadazyma*
	Candida transvaalensis	*Diddensiella*	
	Candida tunisiensis		*Dipodascaceae basal*
	Candida zeylanoides		*Kurtzmaniella*
Cryptococcus			
	Cryptococcus aciditolerans	*Goffeauzyma*	
	Cryptococcus adeliensis	*Naganishia*	
	Cryptococcus aerius	*Solicoccozyma*	

(continued)

Tab. 3.1 (continued)

	Cryptococcus albidus var. ovalis	*Naganishia*	
	Cryptococcus allantoinivorans	*Pseudotremella*	
	Cryptococcus amylolentus		
	Cryptococcus amylolyticus	*Genolevuria*	
	Cryptococcus anemochoreius	*Papiliotrema*	
	Cryptococcus aquaticus	*Mrakiia*	
	Cryptococcus arboriformis	*Cutaneotrichosporon*	
	Cryptococcus arrabidensis	*Heterocephalacria*	
	Cryptococcus aureus	*Papiliotrema*	
	Cryptococcus bacillisporus		
	Cryptococcus baii	*Papiliotrema*	
	Cryptococcus bestiolae	*Kwoniella*	
	Cryptococcus carnescens	*Vishniacozyma*	
	Cryptococcus chernovii	*Filobasidium*	
	Cryptococcus cistialbidi	*Dimennazyma*	
	Cryptococcus cuniculi	*Teunia*	*Tremella* clade I
	Cryptococcus curvatus	*Cutaneotrichosporon*	
	Cryptococcus cylindricus	*Piskurozyma*	
	Cryptococcus ferigula	*Cystofilobasidium*	
	Cryptococcus festucosus	*Holtermavniella*	
	Cryptococcus flavus	*Saitozyma*	
	Cryptococcus fragicola	*Vanrija > Prillingera*	
	Cryptococcus huempii	*Krasilnikovozyma*	
	Cryptococcus humicolus	*Vanrija*	
	Cryptococcus luteolus	*Hannaella*	
	Cryptococcus luteus		
	Cryptococcus magnus	*Filobasidium*	
	Cryptococcus marinus	*Bandonia*	
	Cryptococcus mujuensis	*Fonsecazyma*	
	Cryptococcus nanyagensis	*Rhynchogastrema*	
	Cryptococcus neoformans		
	Cryptococcus pseudolongus	*Vanrija*	
	Cryptococcus spencermartinsiae	*Geladitrema*	
	Cryptococcus tepidarius	*Takashimella*	
	Cryptococcus yokohamensis	*Tremella*	
Filobasidiella		*Cryptococcus*	
	Filobasidiella bacillispora	*Cryptococcus*	
	Filobasidiella neoformans	*Cryptococcus*	
	Filobasidiella depauperata	*Cryptococcus*	
	Filobasidiella lutea	*Cryptococcus*	
Pseudozyma			
	Pseudozyma abaconensis	*Ustilago*	
	Pseudozyma aphidis	*Moesziomyces*	
	Pseudozyma brasiliensis	*Kalmanozyma*	
	Pseudozyma churashimaensis	*Dirkmeia*	
	Pseudozyma crassa	*Triodomyces*	
	Pseudozyma flocculosa	misclassified in *Antracocystis*[c]	
	Pseudozyma fusiformata	*Kalmanozyma*	

Tab. 3.1 (continued)

	Pseudozyma graminicola	*Sporisorium*	
	Pseudozyma hubeiensis	*unknown*	
	Pseudozyma jejuensis	*Langdonia*	
	Pseudozyma prolifica	*Ustilago*	*Ustilago maydis*
	Pseudozyma pruni	*unknown*	
Rhodosporidium			
	Rhodosporidium azoricum	*Rhodosporidiobolus*	
	Rhodosporidium babjevae	*Rhodotorula*	
Rhodotorula			
	Rhodotorula acheniorum	*Farysia*	
	Rhodotorula auriculariae	*Yunzhangia*	
	Rhodotorula arctica	*Bannozyma*	
	Rhodotorula armeniaca	*Buckleyzyma*	
	Rhodotorula bacarum	*Microstroma album*	
	Rhodotorula benthica	*Cystobasidium*	
	Rhodotorula bloemfonteinensis	*Microsporomyces*	
	Rhodotorula bogoriensis	*Pseudohyphozyma*	
	Rhodotorula cladiensis	*Sakaguchia*	
	Rhodotorula colostri	*Rhodosporidiobolus*	
	Rhodotorula cresolica	*Slooffia*	
	Rhodotorula crocea	*Spencerozyma*	
	Rhodotorula cycloclastica	*Colacogloea*	
	Rhodotorula ferulica	*Udeniozyma*	
	Rhodotorula glacialis	*Phenoliferia*	
	Rhodotorula hinnulea	*Microstroma*	
	Rhodotorula hordea	*Ustilentyloma*	
	Rhodotorula hylophila	*Trigonosporomyces*	
	Rhodotorula ingeniosa	*Sampaiozyma*	
	Rhodotorula javanica	*Vonarxula*	
	Rhodotorula lactosa	*Hasegawazyma*	
	Rhodotorula lignophila	*Hamamotoa*	
	Rhodotorula marina	*Symmetrospora*	
	Rhodotorula nothofagi	*> Curvibasidium*	
	Rhodotorula pilati	*Sloofia*	
	Rhodotorula psychrophila	*Phenoliferia*	
	Rhodotorula pustula	*Peudohyphozyma*	
	Rhodotorula rosulata	*Yamadamyces*	
	Rhodotorula silvestris	*Oberwinklerozyma*	
Saprochaete			
	Saprochaete capitata	*Magnusiomyces capitatus*	
	Saprochaete chiloensis		
	Saprochaete clavata		
	Saprochaete fungicola		
	Saprochaete gigas		
	Saprochaete ingens		
	Saprochaete japonica		
	Saprochaete ludwigii	*Magnusiomyces magnusii*	
	Saprochaete psychrophila		

(continued)

Tab. 3.1 (continued)

	Saprochaete quercus	
	Saprochaete saccharophila	
	Saprochaete sericea	*Magnusiomyces ovetensis*
	Saprochaete suaveolens	
Sporidiobolus		
	Sporidiobolus johnsonii	*Sporobolomyces*
	Sporidiobolus longiusculus	*Sporobolomyces*
	Sporidiobolus metaroseus	*Sporobolomyces*
	Sporidiobolus microsporus	*Rhodosporidiobolus*
	Sporidiobolus pararoseus	*Sporobolomyces*
	Sporidiobolus ruineniae	*Rhodosporidiobolus*
	Sporidiobolus salmonicolor	*Sporobolomyces*
Sporobolomyces		
	Sporobolomyces albo-rubescens	*Rhodotorula*
	Sporobolomyces bannaensis	
	Sporobolomyces bischofiae	*Bannoa*
	Sporobolomyces clavatus	*Ruinenia*
	Sporobolomyces coprosmae	*Symmetrospora*
	Sporobolomyces coprosmicola	*Phyllozyma*
	Sporobolomyces elongatus	*Erythrobasidium*
	Sporobolomyces falcatus	*Colacogloea*
	Sporobolomyces fushanensis	*Chrysozyma*
	Sporobolomyces inositophilus	*Fellozyma*
	Sporobolomyces kluyveri-nielii	*Buckleyzyma*
	Sporobolomyces lactophilus	*Cystobasidiopsis*
	Sporobolomyces lactosus	*Hasegawazyma*
	Sporobolomyces magnisporus	*Microsporomyces*
	Sporobolomyces odoratus	*Rhodosporidiobolus*
	Sporobolomyces sasicola	*Ballistosporomyces*
	Sporobolomyces singularis	*Hamamotoa*
	Sporobolomyces tsugae	*Slooffia*
Tilletiopsis		
	Tilletiopsis albescens	*Robbauera*
	Tilletiopsis cremea	
	Tilletiopsis derxii	*Phragmotaenium*
	Tilletiopsis pallescens	*Golubevia*
	Tilletiopsis penniseti	*Gjaerumia*
Trichosporon		
	Trichosporon aquatile	
	Trichosporon cacaoliposimilis	*Apiotrichum*
	Trichosporon chiarellii	*Haglerozyma*
	Trichosporon cutaneum	*Cutaneotrichosporon*

The application of molecular phylogenies not only has impacted our understanding of the broader phylogenetic relationships between fungi, e.g., at the ordinal level or above, but has also been used to revise generic concepts that have prevailed for a long time. A major consequence of such revisions for the user of fungal names, including the clinical field, is that the names of many fungal species have changed. Examples are species that were classified before in broadly defined genera, such as *Cryptococcus, Rhodotorula, Trichosporon* [17, 19], *Fusarium* [27], and dermatophytes [21]. Lists of name changes of fungal species have been published to increase awareness by the end users, such as clinicians [41–43]. Note that some of the proposed changes have been extensively discussed, and it has been suggested that the profusion of generic and whole-binomial name changes may impact a clinician's decisions, since a specific recently introduced name may not alert the clinician [21, 46, 47]. On the other hand, the evolution of the classification system of fungi towards a more natural system soundly based on reliable molecular phylogenies will need to continue.

In this chapter, we will present and discuss the impact of molecular phylogeny on the classification of the fungi causing candidiasis, aspergillosis, hyalohyphomycosis, phaeohyphomycosis, mucormycosis, cryptococcosis (and other basidiomycetous yeast infections), endemic mycosis, and dermatophytosis, with emphasis on those species that cause human fungal infections.

Candidiasis

Several yeast species classified in the form genus *Candida* (asexual Saccharomycetales, Saccharomycotina) [40] are a major source of systemic infections, such as bloodstream and intra-abdominal infections, but also cause infections of the skin, vagina, and oral cavity. *Candida* infections are a serious threat to high-risk patients, such as HIV-infected patients, oncology patients, pediatric patients, transplant patients, neonates, newborns, diabetic patients, intensive care patients, and chronically bedridden patients, and are often associated with high rates of morbidity and mortality. Crude mortality is ca. 35%, with the highest values observed in the elderly, patients suffering from fungemia caused by *C. tropicalis*, and patients with solid tumors. Five species are responsible for most infections, namely *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis,* and *Pichia kudriavzevii* (a.k.a. *C. krusei*) [48]. In recent years, an increase has been noticed in the clinic of the so-called non-*C. albicans Candida* species. [48]. A literature analysis reported 23 *Candida* species that occur relatively commonly in the clinic (Table 3.2, [45]). The genus *Candida* as currently defined contains >300 species belonging to >30 clades. Recently, several *Candida* species have been renamed in >20 other genera (Table 3.1; [39, 40, 49]). Due to the application of molecular phylogenies, many *Candida* species have been transferred to other genera, and several new genera have been created, e.g., *Diutina* [50], *Danielozyma* [51], *Groenewaldozyma* [52], and *Teunomyces* [53]. We present in Table 3.1 the most up-to-date overview of taxonomic changes in clinically relevant *Candida* yeasts based on information from the online database theyeasts.org [49]. For species that have not yet been renamed, the clade names are indicated. Very likely, these species will be recombined in the future, and several, if not all, that currently comprise only "*Candida*" species may form the basis of new genera or be used in the resurrection of older, long synonymized genera. It is important to realize, in reference to the biological/taxonomic recognition of such species and genera, that the naming of these taxa is governed by the "*International Code of Nomenclature for Algae, Fungi, and Plants*" (ICNafp, https://www.iapt-taxon.org/nomen/main.php; [54–58]). As many yeast names are not validly described due to problems with typification, a formal validation action to correct these cases is required [58]. Note that some critical comments have been made on this issue in

Table 3.2 Incidence of most common yeast pathogens

Species	Incidence[a]
Candida albicans	32–70%
Nakaseomyces glabratus (=*Candida glabrata*)	2.0–29%
Candida parapsilosis	2–46%
Candida tropicalis	4–42%
Pichia kudriavzevii (=*Candida krusei*)	1–6.0%
Meyerozyma guilliermondii (=*Candida guilliermondii*)	<1–7.0%
Candida orthopsilosis	~4%
Candida dubliniensis	<1–4.0%
Yarrowia lipolytica (=*Candida lipolytica*)	<1%
Clavispora lusitaniae (=*Candida lusitaniae*)	<1–3.0
Wickerhamiella pararugosa (=*Candida pararugosa*)	<1%
Kluyveromyces marxianus (=*Candida kefyr*)	<1–1.5%
Debaryomyces hansenii (=*Candida famata*)	<1%
Diutina rugosa (=*Candida rugosa*)	Up to 3.0%
Cyberlindnera jadinii (=*Candida utilis*)	<1%
Wickerhamomyces anomalus (=*Candida pelliculosa*)	<1%
Meyerozyma caribbica (=*Candida fermentati*)	<1%
Pichia norvegensis (=*Candida norvegensis*)	<1%
Nakaseomyces bracarensis (=*Candida bracarensis*)	<1%
Candida inconspicua	<1%
Candida intermedia	<1%
Nakaseomyces nivariensis (=*Candida nivariensis*)	<1%

[a]Based on a literature meta-analysis [45]. Other clinically rare yeast species are *Candida metapsilosis*, *Saccharomyces cerevisiae*, *Candida sake*, *Kluyveromyces lactis* (=*Candida sphaerica*), *Geotrichum capitatum*, *Candida melibiosica*, *Cyberlindnera fabianii*, *Lodderomyces elongisporus*, *Trichomonascus ciferrii* (=*Candida ciferrii*), *Torulaspora delbrueckii* (=*Candida colliculosa*), *Kazachstania exigua* (=*Candida holmii*), *Pichia fermentans* (=*Candida lambica*), *Candida magnoliae*, *Metschnikowia pulcherrima* (=*Candida pulcherrima*), *Pichia membranifaciens* (=*Candida valida*), *Diutina catenulata* (=*Candida catenulata*), *Candida auris*, *Candida haemulonii*, *Candida palmioleophila*, and *Candida quercitrusa*

an effort to minimize the number of name changes due to past nomenclatural errors [58].

Several new, clinically relevant *Candida* species have been described, namely *C. auris*, *C. dubliniensis, C. nivariensis, C. bracarensis, C, orthopsilosis, C. metapsilosis*, *C. duobushaemulonii*, *C. pseudohaemulonii,* and *C. vulturna* (Tables 3.1 and 3.2; [59–64]). *Candida auris* was described from a single isolate from ear discharge of a hospital inpatient in Japan [62] and since then has evolved into a major pathogen that is causing infections on a global scale [65–70]. The species belongs to the Metschnikowia clade where it forms a small subclade with other clinically relevant species, such as *C. haemulonii, C. duobushaemulonii,* and *C. vulturna*. *Candida dubliniensis* is a sibling species of *C. albicans* causing oral and oropharyngeal infections in HIV-infected AIDS patients; in people suffering from dental stomatitis, diabetes, and cystic fibrosis; as well as in neutropenic patients [71, 72]. Recently, two sibling species of *C. glabrata*, *C. nivariensis*, and *C. bracarensis* have been described [59, 61, 73–75]. Two sibling species of *C. parapsilosis, C. orthopsilosis,* and *C. metapsilosis* were described to replace the informal designations *C. parapsilosis* groups II and III [64, 76, 77], but comparative genomic studies revealed that both *C. orthopsilosis* and *C. metapsilosis* are hybrids [78, 79]. *Candida rugosa, C. mesorugosa,* and *C. neorugosa*, together with some other species, have been renamed within the genus *Diutina* [50]. Other so-called *Candida* species have already been renamed in other genera, and we now see *C. guilliermondii* listed as *Meyerozyma guilliermondii*, *C. lipolytica* as *Yarrowia lipolytica*, *C. kefyr* as *Kluyveromyces marxianus*, and *C. krusei* as *Pichia kudriavzevii*. Nonetheless, there seems to be a strong tendency in the clinical field to retain the use of old *Candida* names for those species. As addressed by Stavrou et al. (2019), species belonging to the various clades in Saccharomycotina, including the *Candida* budding yeasts, widely differ in their susceptibility to antifungal drugs [45]. Hence, we strongly favor the use of the proper generic names in clinical mycology.

Aspergillosis

The genus *Aspergillus* classified in the family *Aspergillaceae*, order *Eurotiales* (class *Eurotiomycetes*) [15], includes more than 450 species [9]. In the dual-nomenclature era, several sexual (teleomorph) genera were associated with *Aspergillus*, such as *Emericella*, *Eurotium*, *Neosartorya*, and *Petromyces*. With the introduction of the single-name nomenclature, the optimal naming of the traditionally delimited *Aspergillus* species became a subject of discussion. The main discussion point was whether to split *Aspergillus* into multiple genera or to maintain it as a broadly circumscribed genus [80–82]. The International Commission on *Penicillium* and *Aspergillus* (IPCA) supported the broad concept, maintaining the historical delimitation of *Aspergillus* based on characters such as vesiculate, aseptate conidiophores with phialidic conidiogenous cells, and basipetal, connected chains of dry, one-celled conidia [82]. The use of a broad concept resulted in various name changes for well-known species (e.g., *Aspergillus nidulans*/*Emericella nidulans*, *Aspergillus chevalieri*/*Eurotium chevalieri*), but fortunately, many of the clinically relevant species were already known under their current *Aspergillus* name.

Aspergillus has a long history of having a formal infrageneric classification system with subgenera, sections, and series. This classification system was initially based on macro- and micromorphological characters but is nowadays based on molecular data. The use of an infrageneric classification system makes working with the large, speciose genus *Aspergillus* easier. Currently, *Aspergillus* is subdivided into six subgenera, 27 sections and 75 series [9]. The sections that include most of the clinically relevant species are *Flavi*, *Fumigati*, *Nidulantes*, *Nigri*, *Terrei*, and *Usti*.

More than 60 species cause infections in humans [83]. Correct species demarcation is important, as it may affect the choice of therapy and may warrant interventions to prevent invasive fungal infection in immunocompromised persons or to identify sources of nosocomial spreads. In the past, identification of *Aspergillus* primarily relied on morphological criteria, while nowadays, it relies on sequence data, used in conjunction with traditional phenotype-based methods. The ITS barcode is not suitable for species identification due to insufficient resolution; therefore, sequencing of the calmodulin gene is recommended with β-tubulin as an alternative [84].

Section *Fumigati* ("cryptic *A. fumigatus*") encompasses 61 species, of which the best known member is *A. fumigatus*. The majority of section *Fumigati* species (42/61) can produce a *Neosartorya* morph either homo- or heterothallically, depending on the species [9]. *A. fumigatus* is the most common cause of invasive aspergillosis, a condition associated with substantial severity and significant mortality rates. Azole-resistant *A. fumigatus*, selected for by the extensive use of azoles in agriculture and the clinic, can complicate treatment [85, 86]. Molecular studies have revealed that more or less atypical clinical isolates phenotypically identified as *A. fumigatus* and exhibiting altered antifungal susceptibility can belong to closely related but distinct species [87]. Other clinically relevant sections of *Fumigati* species include *A. felis*, *A. fumigatiaffinis, A. hiratsukae*, *A. lentulus*, *A. thermomutatus* (syn. *N. pseudofischeri*), and *A. udagawae* [87].

Species classified in section *Nigri* ("the black Aspergilli" or "cryptic *A. niger*") are phenotypically similar to one another, and characters for phenotypic species identification are lacking. Sequence-based identification of clinical strains indicates that besides *A. niger*, other species that may occur

as human pathogens include *A. luchuensis, A. tubingensis*, and *A. welwitschiae*. Indeed, *A. tubingensis* and *A. welwitschiae* are sometimes more frequently detected than *A. niger* in connection with human disease [88, 89].

A. calidoustus is the most frequently occurring clinical species of section *Usti*, followed by *A. pseudodeflectus* and *A. ustus* [90]. Relatively low susceptibilities to several antifungal drugs including triazoles are frequent in *A. calidoustus*, a status that complicates treatment [91].

Invasive infections caused by *Aspergillus nidulans* (section *Nidulantes*, syn. *Emericella nidulans*) are uncommon in animals and humans. In humans, this species appears to occur predominantly in patients with chronic granulomatous disease (CGD). Reidentification of clinical *A. nidulans* isolates showed some to be *A. sublatus*, an allodiploid hybrid species formed by the fusion of two closely related section *Nidulantes* species, one of them being *A. spinulosporus* [92]. This observation has an impact on the patient management: *Aspergillus nidulans* is less susceptible than *A. sublatus* to amphotericin B, but more susceptible to caspofungin. Also, *A. sublatus* is less susceptible than *A. spinulosporus* to being killed by neutrophils and differs from *A. quadrilineatus* and *A. nidulans* in its susceptibility to high doses of oxidative stress [92].

Aspergillus terreus (section *Terrei*, "cryptic *A. terreus*"), another important human pathogen obtained from invasive aspergillosis in medical centers, has a relatively low susceptibility to AmB. Of the 17 accepted species in section *Terrei* [9], 5 are human pathogens, namely *A. alabamensis*, *A. citrinoterreus*, *A. floccosus*, *A. hortae* (=*A. hortai*), and *A. neoafricanus* [83].

Several species of section *Flavi* occur as etiologic agents in clinical specimens, e.g., *A. flavus, A. tamarii, A. nomius,* and *A. pseudonomius*. *A. flavus* is the most commonly occurring species in the section, and this species is the leading cause of aspergillosis in areas of arid climate. *A. alliaceus*, which is a member of section *Flavi* with conidia in shades of yellow, can also cause aspergillosis. It has relatively low susceptibilities to amphotericin B and caspofungin.

Hyalohyphomycosis

The phylogenetic nomenclature of *Fusarium* is currently unsettled. A major debate is ongoing on the demarcation of genera within *Fusarium sensu lato* (ss. lat., "in the broad sense"). Two schools are apparent, namely one that prefers to keep all species in a predominantly morphologically defined genus *Fusarium* [47, 93, 94], and another that has defined smaller monophyletic lineages within the complex as separate genera [27, 95–97]. Crous and coauthors (2021) recognized 7 genera, including *Neocosmospora* for the *F. solani* species complex [27]. This last taxonomic decision is being intensely debated [47], and the future will determine which generic names will be used eventually.

Fusarium ss. lat. is a clinically important genus with at least 300 phylogenetically defined species that are classified in the order Hypocreales (class Sordariomycetes) [15, 47]. The genus as defined most broadly contains over 70 species belonging to 12 species complexes that are known from human patients [47]. In the phylogenetic re-evaluations that have suggested dividing *Fusarium* into ten genera, three, *Fusarium* sensu stricto (ss. str., in the restricted sense), *Neocosmospora*, and *Bifusarium*, are causes of human opportunistic infection [27]. Numerous species are able to cause infections ranging from relatively benign conditions like onychomycosis in immunocompetent individuals to life-threatening disseminated infections with a high rate of mortality in highly immunocompromised humans. Immunocompromised patients may be affected by skin infections and are vulnerable to nosocomial outbreaks of life-threatening systemic infection that have been linked to water supply systems in hospitals [98]. Major contact lens-associated keratitis outbreaks in the USA during 2005 and 2006 were caused by members of the *Neocosmospora solani* complex [99, 100]. In the environment, *Fusarium* and related species are commonly found as saprobes in soil, as endophytes, or as plant pathogens. Many isolates of *Fusarium* are intrinsically resistant to many classes of antifungal drugs, including azoles. Increasingly, multidrug resistance is seen [8].

The clinically most important species belong to the *N. solani* and *F. oxysporum* species complexes. Traditionally, members of these species complexes were referred to as the single morphospecies *Fusarium solani* and *F. oxysporum*. Because of the importance of these species in agriculture, a subspecific naming system based on host specificity in plant disease was adopted. Recent molecular analyses have shown that these so-called formae speciales delineated by plant pathogenicity do not always correspond to "natural" groups and that these species complexes can include multiple phylogenetically distinct species [100–102]. Medically important isolates generally could not be typed as belonging to any forma specialis. Initially, once the phylogenetic analysis of the known pathogenic types had been completed, a nomenclature based on numbered multi-locus haplotypes was used to facilitate communication about these phylogenetically defined species [101].

The *N. solani* species complex contains at least 45 phylogenetic species distributed among 3 major clades [47, 101] with 23 species that are clinically relevant [101]. Until recently, only four of them had a formal name, namely *Neocosmospora* (*Fusarium*) *falciforme*, an agent of white-grain mycetoma and opportunistic infections in patients with transplants [103]; *Neocosmospora* (*Fusarium*) *lichenicola*, an agent of keratitis and cutaneous to disseminated infec-

tions [103]; *Neocosmospora cyanescens*, known from a single case of mycetoma [104]; and *Neocosmospora vasinfecta*, an agent of systemic infections in patients with transplants. Names have now been elaborated for most members of this species complex [97]. Alternative names in *Fusarium* ss. lat. have also been produced [94].

Species of the *F. oxysporum* are increasingly reported from disseminated infections of immunocompromised patients. It is estimated that 2/3 of all infections by fusarioid fungi are caused by species belonging to this complex [47]. Early molecular studies demonstrated that this morphospecies includes several phylogenetically distinct species. A study that used the analysis of IGS and *TEF1*α gene sequences of 850 isolates of the *F. oxysporum* species complex demonstrated that these isolates are grouped into 257 sequence types; 25 of them are clinically relevant [104]. A recently dispersed and geographically widespread clonal lineage was the agent of nosocomial infections [101].

Fusarium-ID (http://isolate.fusariumdb.org/) is a multistate haplotype nomenclature system based on molecular data for 20 markers, including β-tubulin, calmodulin, *TEF1*α, IGS, ITS, *RPB1*, and *RPB2*, based on sequence data of >35,000 *Fusarium* ss. lat. isolates attributed to seven species complexes, labeled as the *F. solani* species complex, the *F. oxysporum* species complex, the *F. incarnatum-equiseti* species complex, the *F. chlamydosporum* species complex, the *F. tricinctum* species complex, the *F. dimerum* species complex, and the "*Gibberella fujikuroi*" species complex (a historical name no longer formally used). The system is a major tool for the identification of unknown isolates [105, 106].

Phaeohyphomycosis

The melanized fungi, also known as dematiaceous or brown-pigmented fungi, mainly belong to the order Chaetothyriales, while few of the pathogenic genera belong to another six orders [107]. Chromoblastomycosis is one of the two major groups of diseases caused by melanized fungi and is also the major fungal implantation disease caused by distantly related species belonging to the genera *Cladophialophora*, *Fonsecaea*, *Phialophora*, and *Rhinocladiella* [108, 109]. The genus *Cladophialophora* contains close to 30 species of which *C. carrionii* and *C. bantiana* are the two pathogens that can be identified by ITS sequencing using ITS4 and ITS5 primers [108–112]. *Cladophialophora bantiana* is known as the cause of phaeohyphomycosis, and not chromoblastomycosis, and is feared as a neurotropic pathogen causing brain infections which have, with some exceptions, a fatal outcome [113].

Fonsecaea is the main agent of chromoblastomycosis, with approximately 80% of the globally identified cases being one of the members of this genus [108, 109]. The most recent addition to the genus *Fonsecaea* was *F. pugnacius* that was described as a novel species based on multigene phylogeny (ITS, β-tubulin, and *CDC42*) [114]. Five years before, *F. nubica* and *F. multimorphosa* were recognized as new species, next to the two best known species *F. monophora* and *F. pedrosoi* [115, 116]. Schneider and co-workers developed a molecular identification tool based on the *CBF5* gene to discriminate between these five pathogenic species [117].

The genus *Phialophora* is known as the cause of chromoblastomycosis and phaeohyphomycosis, with *Phialophora verrucosa* sensu stricto as the most frequent cause of disease [118]. Phylogenetic analysis using ITS, β-tubulin, and *TEF1*α gene sequences underlined the existence of other species in the *P. verrucosa* species complex, namely *P. americana*, *P. ellipsoidea, P. expanda*, and *P. tarda* that are less frequent to even extremely rare causes of disease, while *P. chinensis* and *P. macrospora* are only known from environmental sources [118]. *Phialophora ambigua*, *P. europaea*, *P. oxyspora*, and *P. reptans* are closely related to *Cyphellophora*, and they belong to the "europaea-clade" within the Chaetothyriales and can be discriminated from each other by ITS sequencing [119].

Rhinocladiella is rarely described as the cause of chromoblastomycosis, and the involved species are *R. aquaspersa* and *R. similis* [109, 120]. This genus also houses a species, *R. mackenziei*, known to cause cerebral phaeohyphomycosis, which often has a fatal outcome [121].

Phaeohyphomycosis refers to infections, diagnosed by histopathology, in which brown-pigmented hyphae or other fungal structures are noted. The etiology of phaeohyphomycosis is commonly due to fungi belonging to the diverse genera of the order Chaetothyriales. Most fungi mentioned above that cause chromoblastomycosis have also been reported to cause phaeohyphomycosis. However, *Exophiala* is a notorious culprit of the latter and comprises around 40 species [122, 123]. Numerous *Exophiala* species have been identified from infections in humans and animals; most commonly seen are *E. asiatica*, *E. dermatitidis*, *E. lecanii-corni*, *E. oligosperma*, *E. phaeomuriformis*, *E. spinifera*, and *E. xenobiotica* [122]. The ITS barcoding marker can be used to differentiate the *Exophiala* species, but also MALDI-TOF MS has been proven to be a rapid and reliable method to distinguish the clinically relevant species [122]. Recent additions to the clinical spectrum of *Exophiala* species are *E. arunalokei*, *E. campbellii*, and *E. polymorpha* [122–124].

Scedosporium and *Lomentospora* belong to the family *Microascaceae* and order *Microascales*. The *S. apiospermum* species complex comprises five species: *S. angustum*, *S. apiospermum*, *S. boydii*, *S. ellipsoideus*, and *S. fusoideum* [125]. Other clinically relevant species are *S. aurantiacum*, *S. dehoogii*, and *S. minutisporum* [126]. *Scedosporium americanum* has been recently described from a clinical case in Argentina [127]. *Lomentospora prolificans*, previously

known as *Scedosporium prolificans*, was reinstated as it was found to be relatively distantly related to the genus *Scedosporium* [126]. These species can be reliably identified using ITS barcoding, eventually supplemented with β-tubulin sequencing [127].

Mucormycosis

The phylum Zygomycota comprises fungi that reproduce sexually by thick-walled and often pigmented and ornamented cells (zygospores) developing after the fusion of two differentiated hyphal tips. Several multi-locus phylogenies and a genome-based analysis of 192 protein sequences including 46 fungal species and 25 zygomycetes did not find support for the monophyly of the phylum [15]. Consequently, the phylum Zygomycota was abandoned and replaced by the phyla Mucoromycota (including the clinically important Mucorales) and Zoopagomycota (including the Entomophthoromycotina with the clinically important genera *Basidiobolus* and *Conidiobolus*) [128]. This classification resulted also in the replacement of the term "zygomycosis" for infections caused by Zygomycota by "mucormycosis" and "entomophthoromycosis" [129]. However, recent more comprehensive genome analyses including 290 genes of 1644 fungal species, including 71 zygomycetes, provided support for the Zygomycota [130]. In this analysis, the Basidiobolales with the opportunistic genus *Basidiobolus* formed a separate clade apart from the Entomophthorales that includes the opportunistic genus *Conidiobolus*.

Mucorales

In the Mucorales, the number of species that are reported to cause infections has increased due to numerous molecularly based taxonomic revisions [131–133]. Currently, 38 species in 11 genera are known as causative agents of mucormycosis [134]. The clinically most important mucoralean genera are *Apophysomyces*, *Lichtheimia*, *Mucor*, *Rhizomucor*, and *Rhizopus* [134–137].

Apophysomyces spp. are important opportunists in tropical and subtropical countries such as India and Mexico [136]. Multi-locus analyses detected five new species of which four are known as etiological agents of mucormycosis: *A. mexicanus*, *A. ossiformis*, *A. trapeziformis*, and *A. variabilis* [132, 138]. For *A. elegans*, which was formerly regarded as the only species in *Apophysomyces*, no case report could be found that was based on molecular identification of the mold.

Based on molecular phylogenetic analyses and phenotypic features, *Absidia*-like fungi were divided into three genera: *Absidia sensu stricto*, *Lentamyces*, and *Lichtheimia* [139–141]. Only the latter is of medical importance. Molecular phylogenetic analyses based on three markers recognized *L. ramosa* as a discrete species distinct from *L. corymbifera* [142]. A molecular revision of the genus revealed three species involved in infections: *L. corymbifera*, *L. ornata*, and *L. ramosa* [143].

Mucor is the largest genus within the Mucorales with currently 76 accepted species [134]. Sequence analyses based on ITS and LSU revealed several subgroups within the genus with different morphologies that are intermingled with those of other, morphologically deviating genera [144]. Several *Mucor* species were transferred to *Backusella* because they formed a supported clade with existing *Backusella* species and also showed the characteristic transitorily recurved sporangiophores of *Backusella*. All *Zygorhynchus* spp. were recombined into *Mucor* because the genus was polyphyletic, with members positioned in several subgroups of *Mucor* [144].

On the basis of ITS and LSU analyses, the medically important *M. velutinosus* was described and *Rhizomucor variabilis* was transferred to *M. irregularis* [145]. A five-locus study of the *M. circinelloides* complex revealed five new species. The earlier formae of *M. circinelloides* were recognized as distinct species, viz. *M. janssenii, M. lusitanicus,* and *M. griseocyanus* [146]. Several opportunistic *Mucor* species show specific antifungal susceptibility profiles for azoles including the therapeutically important posaconazole [146].

The genus *Rhizomucor* includes only thermophilic species. *Rhizomucor* species able to grow at relatively low temperatures, such as the medically important *R. variabilis*, were recombined into *Mucor* based on molecular data [145]. This disposition is of importance for treatment, because true *Rhizomucor* species have lower minimum inhibitory concentrations for posaconazole, itraconazole, and isavuconazole than *Mucor* species [147].

The opportunistic *Rhizopus* species are—arranged according to their clinical importance—*R. arrhizus* (syn. *R. oryzae)* [148], *R. microsporus* [149], *R. homothallicus* [150], and *R. schipperae* [151].

There is an ongoing debate on the taxonomic status of *R. delemar*, which is considered as either discrete sibling species of *R. arrhizus* or its variety. In single-locus [144, 152] and multi-locus studies [148, 153], *R. arrhizus* and *R. delemar* formed separate clades evincing no evidence of recombination. Genome analyses of the genus *Rhizopus* including *R. arrhizus* and *R. delemar* provided ambiguous results. An analysis of 192 orthologous proteins resulted in two well-supported clades [154]. In contrast, an analysis of 76 orthologous genes did not resolve these clades, and a phylogeny based on single-nucleotide polymorphisms (SNPs) revealed three clades in this relationship, one of *R. arrhizus*, one of *R. delemar*, and a third clade with mixed ancestry sug-

gestive of an incomplete mating barrier consistent with varietal status [155].

The morphologically different varieties of *Rhizopus microsporus*, namely *R. microsporus* var. *azygosporus*, *R. microsporus* var. *chinensis*, *R. microsporus* var. *oligosporus*, *R. microsporus* var. *rhizopodiformis*, and *R. microsporus* var. *tuberosus*, previously considered to be separate species by some authors, were reduced to synonyms [149]. Strains previously assigned to these varieties did not form monophyletic clades in uni- and multi-locus phylogenies [144, 149, 152, 156].

Conidiobolus (Entomophthoromycotina)

In sequence analyses using four markers (LSU, SSU, *RPB2*, mtSSU), the genus *Conidiobolus* was considered paraphyletic [157]. A molecular revision based on four phylogenetic markers (nucLSU, nucSSU, mtSSU, and *TEF1*α) resulted in the division of the genus into four genera, *Conidiobolus*, *Capillidium*, *Microconidiobolus*, and *Neoconidiobolus*. A recombination of *Macrobiotophthora vermicola* into *Neoconidiobolus* was also done [158].

Basidiobolus (Basidiobolales)

In a recent genome analysis, the genus *Basidiobolus* formed a clade positioned basally to the Mucoromycotina and separate from the Entomophthorales [130]. Other than *B. ranarum, B. meristosporus* and a so far undescribed species were shown to cause infections. *B. meristosporus* was distinguished in ITS sequence analysis, while the undescribed species was evident in LSU analysis [159, 160].

Cryptococcosis and Trichosporonosis

The taxonomy of basidiomycetous yeasts strongly changed recently due to the implementation of the "one fungus = one name" principle, but most changes were supported by molecular phylogenetic evidence [17–20, 161]. The genus *Cryptococcus* is now limited to *C. neoformans*/*C. gattii* species complexes, along with some phylogenetically related but nonpathogenic species [17, 162]. Hence, the majority of species formerly classified in the genus *Cryptococcus* [163] belong presently to other genera (Table 3.1). The *C. neoformans*/*C. gattii* species complexes contain at least seven species based on inferences from multiple gene sequences [164, 165]; that finding is also supported by comparative genomics studies [166, 167]. This view is somewhat debated as other researchers prefer to refer to so-called molecular types and species complexes [46]. The recent discovery of yet another genotype from Zambia [166] may hint at the presence of more species in the complex. Molecular studies also corroborated the presence of several distinct hybrids within the complex [168–171].

Basidiomycetous yeasts that form hyphae and that reproduce with arthroconidia have long been classified in the genus *Trichosporon* [172] in the order *Trichosporonales*, a basal lineage within Tremellomycetes and phylogenetically closely related to the jelly fungi (*Tremellales*) [2, 173]. However, recent taxonomic changes due to the use of multiple molecular markers have resulted in the recognition among these yeasts of *Apiotrichum*, *Cryptotrichosporon*, *Cutaneotrichosporon*, *Effuseotrichosporon*, *Haglerozyma*, *Pascua*, *Prillingera*, *Takashimella*, *Tausonia*, *Trichosporon*, and *Vanrija* (Table 3.1; [17, 18, 130, 174–176]). Taxonomic heterogeneity within the former genus *Trichosporon* had been known for a long time and five clades (viz. cutaneum, ovoides, brassicae, gracile, porosum) had been [173, 174, 177–182]. Based on the use of nine marker genes, including LSU, ITS, SSU, *RBP1, RPB2*, the two subunits of RNA polymerase II, *TEF1*α, and *CYTB* (a fragment of cytochrome B), a reclassification of the species in *Trichosporon sensu lato* was proposed [17, 18]. This study resulted in the recognition of the genera *Apiotrichum*, *Cryptotrichosporon*, *Cutaneotrichosporon*, *Effuseotrichosporon*, *Haglerozyma*, *Takashimella*, *Trichosporon*, and *Vanrija* [18]. Takashima and co-workers, using genome data, largely corroborated the taxonomy proposed by Liu et al. (2015) [18], but, in addition, they proposed two new genera, *Pascua* typified by *P. guehoae*, a species that had been classified in *Cutaneotrichosporon* by Liu et al. (2015) [18] and that earlier had been originally described in *Trichosporon* [183], as well as *Prillengera* to accommodate *P. fagicola*, a species that originally had been described as *Cryptococcus fagicola* [182] and later placed into *Vanrija* by Liu et al. (2015) [18].

Several clinically relevant *Trichosporon* species, viz. *T. asahii*, *T. asteroides*, *T. coremiiforme*, *T. dohaense*, *T. faecale*, *T. japonicum*, *T. inkin*, and *T. ovoides*, remain classified in the genus *Trichosporon*. However, other clinically relevant species, viz. *T. cutaneum*, *T. jirovecii*, *T. dermatis*, *T. mucoides*, and *T. debeurmannianum*, are now classified in the genus *Cutaneotrichosporon*, while *T. domesticum*, *T. loubieri*, *T. montevideense*, and *T. mycotoxinivorans* are in *Apiotrichum* [18, 175]. *T. pullulans*, a trichosporonoid species that belongs to *Cystofilobasidiales* and not in *Trichosporonales*, was reclassified as *Guehomyces pullulans* [184] and recently as *Tausonia pullulans* [18]. Interestingly, *C. mucoides, T. coremiiforme, and T. ovoides* were found to be hybrids [175].

Endemics/Dimorphic Fungi

The ascomycetous order Onygenales accommodates various genera of dimorphic fungi. Most of them are part of the family *Ajellomycetaceae*, including *Blastomyces*, *Emergomyces*, *Emergomyces*, *Histoplasma*, *Lacazia*, and *Paracoccidioides*, while the notorious pathogenic *Coccidioides* genus is placed within the family Onygenaceae [185–187]. Another, distantly related pathogen is the species belonging to the genus *Sporothrix* (order *Ophiostomatales*, family *Ophiostomataceae*). Here, too, the emergence of molecular methods over the past two decades has resulted in the discovery of new species in nearly all of the genera listed above.

The most recent addition to the *Ajellomycetaceae* was the genus *Emergomyces*, which was described by Dukik et al. (2017) [185], based on phenotypic characteristics and multigene phylogenetic analysis using the (partial) sequences of the ribosomal DNA loci ITS and LSU, plus translation elongation factor 3α (*TEF3*), β-tubulin (*TUB2*), and 60S ribosomal protein L10. Additionally, genome-based sequencing of representative isolates was performed. The newly defined genus included two species that were previously identified as *Emmonsia*, *Emergomyces africanus* (previously *Emmonsia* "species 5") and *Emergomyces pasteurianus* (previously *Emmonsia pasteuriana*). Shortly afterwards, three peculiar species were proposed: *Emergomyces orientalis* was described based on ITS-based phylogenetic analysis [188], while *Emergomyces canadensis* and *Emergomyces europaeus* were established based on multigene phylogeny [186, 189]. Eventually, after changes noted below had removed the type species from *Emmonsia*, it was proposed to transfer the two remaining representatives of the genus, *E. crescens* and *E. sola*, into the genus *Emergomyces* while retaining their epithets "*crescens*" and "*sola*" [186, 187]. Among the new *Emergomyces* species, *E. africanus* is the most prominent member, as it is a frequent cause of systemic infections among South African HIV-positive subjects [190], and it has also been molecularly detected in soil samples across South Africa [191].

The dismantling of the genus *Emmonsia* began with the transfer of type species *Emmonsia parva* into the genus *Blastomyces* as *B. parvus*. This move was supported by multigene analysis that included ITS, LSU, *TEF3*, *TUB2*, and RNA polymerase II (*RPB2*) sequences [186]. A few years earlier, *Blastomyces dermatitidis* had been found to harbor a cryptic species that was designated *Blastomyces gilchristii* [192]. The description of *B. gilchristii* was based on multilocus sequence typing using the seven loci *CHS2*, *DRK1*, *FADS*, *pyrF*, *TUB1*, *ARF6*, and ITS2, using mostly North American *Blastomyces* isolates and few isolates from Africa. Soon thereafter, *Blastomyces percursus* was described based on comparative genomics and phenotypic characteristics [185]. Not only was *Emmonsia parva* transferred to *Blastomyces*, but also *Emmonsia helica* was transferred as *Blastomyces helices*, while an atypical strain was described as the new species *Blastomyces silverae* [193]. Blastomycosis is endemic not only on the North American continent, but also in Africa and the Middle East, as can be discerned by a review of reported cases and molecular analysis of strains preserved in culture collections [194, 195]. The majority of these Old World strains were found to be *B. percursus* in an ITS-based phylogenetic analysis. A more in-depth genetic analysis using ITS, LSA, actin, β-tubulin, and intein *PRP8* sequences revealed the additional presence of the recently described *Blastomyces emzantsi*, only reported so far from South Africa [194]. An updated map of the global occurrence of blastomycosis showed that the focus of this endemic mycosis is on the Eastern part of Canada and the USA, while case report-based studies provide some evidence for its presence in Africa, the Middle East, and India [195, 196].

Histoplasmosis was known as an endemic mycosis of the Americas and Africa, but extensive literature search revealed that it is endemic in large parts of North America, South America, sub-Saharan Africa, Southeast Asia, the east coast of Australia, the Philippines, and even Central Europe [196]. Until 2017, *Histoplasma capsulatum* was the single species in the genus and was thought to comprise three pathogenic varieties with different clinical characteristics: *H. capsulatum* var. *capsulatum*, agent of New World Histoplasmosis; *H. capsulatum* var. *duboisii*, agent of African Histoplasmosis; and *H. capsulatum* var. *farciminosum*, agent of Equine Histoplasmosis [197]. The first steps in the molecular taxonomy of *Histoplasma* were made by Kasuga and co-workers who investigated 46 isolates using ITS, ADP-ribosylation factor (*ARF*), H-antigen precursor (H-anti), Δ9-fatty acid desaturase (*OLE*), and α-tubulin (*TUB1*), and they observed five clades within *H. capsulatum* var. *capsulatum* [198]. A subsequent study concluded, based on phylogenetic analysis of 137 isolates, that at least seven phylogenetic species were present within *H. capsulatum sensu lato* and that another seven single-isolate genotypes represent potential new species [23]. Teixeira and colleagues (2016) [199] mined *ARF*, H-anti, *OLE*, and *TUB1* sequences of 234 isolates from public databases to perform phylogenetic analysis and concluded that there are at least 17 cryptic phylogenetic species [199]. A comparative genomics approach resulted in the recognition of four clades representing the (novel) species: *H. capsulatum* sensu stricto (Panama lineage), *H. mississippiense* (NAm1 lineage), *H. ohiense* (NAm2 lineage), and *H. suramericanum* (LAmA lineage) [29]. In this taxonomic proposal from Sepulveda and colleagues, two lineages remained untouched, *H. capsulatum* var. *duboisii* and *H. capsulatum* var. *farciminosum*. When a combined approach of multigene and AFLP fingerprint analyses was used, even more genetic

diversity was observed, leading to close to 30 genetic groups [197]. This, in combination with the largely unknown genetic diversity from under-sampled regions in Africa, Asia, Australia, and Europe [196], potentially led to the recognition of a vast number of *Histoplasma*. It remains enigmatic what the clinical importance of this diversity may be.

Paracoccidioidomycosis, a fungal infection that can be acquired in certain parts of South America [196], was for nearly eight decades believed to be caused by a single species named *Paracoccidioides brasiliensis*, but this gradually changed with the advent of molecular epidemiological studies in which extensive genetic diversity was revealed by a variety of molecular tools [200]. *Paracoccidioides lutzii*, also known as lineage "Pb01-like," was found to be distantly related to the *P. brasiliensis* species complex, and based on phylogenetic analysis of the *ARF* and *GP43* genes and morphological characteristics, it was described as a separate species [201]. Thereafter, a proposal was made to recognize the four major lineages within *P. brasiliensis* species complex as species, with genotype PS1 as *P. brasiliensis* sensu stricto, PS2 as *P. americana*, PS3 as *P. restrepiensis*, and PS4 as *P. venezuelensis* [200]. At present, *P. brasiliensis sensu stricto* is split into two lineages named S1a and S1b; the latter has a broad geographic range, while the S1a and the other species are geographically restricted [32, 202]. Not only is *P. lutzii* genetically distant from the other four species, but also, it is geographically restricted to Midwestern and Northern Brazil, and most importantly from a clinical point of view, it cannot be detected with the serological diagnostics initially developed for *P. brasiliensis* sensu lato [203].

Coccidioidomycosis, also known as Valley Fever, is caused by *Coccidioides* species and is geographically restricted to the west coast of the USA, Mexico, Central America, Colombia, Venezuela, and central and northeastern areas of South America [196]. Until two decades ago, *Coccidioides immitis* was the single species, including a divergent "non-California *C. immitis*" lineage. Microsatellite typing and morphological differences led to the description of the latter lineage as *C. posadasii* [204]. In whole-genome sequence analyses, it was determined that *C. immitis* contains two subpopulations (designated Central and Southern California), and *C. posadasii* harbors three subpopulations (Arizona, Mexico/Texas/South American, and Caribbean) [205, 206].

Sporotrichosis is an implantation mycosis known as "rose gardener's disease", a fungal infection caused by traumatic cutaneous inoculation of *Sporothrix*-infected plant or soil material. The genus *Sporothrix* accommodates 53 species of which *Sporothrix schenckii*, *Sporothrix globosa*, and *Sporothrix brasiliensis* are the major pathogenic representatives [207, 208]. Other species are rarely encountered in the clinic: *S. chilensis*, *S. humicola*, *S. mexicana*, and *S. pallida* that belong to the *S. pallida* species complex, and *S. luriei*, that is the basal lineage to the three major pathogenic species [207, 209–211]. Global molecular *Sporothrix* epidemiology identified that *S. schenckii* occurs on all inhabited continents, followed by *S. globosa*. *Sporothrix brasiliensis*, known from the feline sporotrichosis (cat-to-cat and cat-to-human) outbreaks in southern parts of Brazil, is nowadays expanding further to northern Brazil and to Argentina [207, 212]. For species differentiation, the ITS marker can be used as primary barcoding gene, while calmodulin, β-tubulin, and *TEF1*α can be used in addition to obtaining higher phylogenetic resolution [207, 211]. The use of an additional marker, besides ITS, is mandatory to differentiate the species that belong to the *S. pallida* species complex (see Fig. 7 in [211]).

Tinea (Dermatophytes)

The phylogenetic taxonomy of the dermatophytes represents a branch of natural history that appears to have no equivalent elsewhere in biology. A clade of saprobic sexual species with unifactorial mating systems has diversified in contact with various animal species to yield a profusion of pathogenic species, some facultative and some obligate, many of which are clonal lineages derived from just one of the two ancestral mating types [213, 214]. At least one of these clonal lineages, the *Trichophyton rubrum* clade, appears to have defied "Müller's ratchet," the tendency of clones to lose fitness due to accumulating locked-in deleterious mutations, for a prolonged period of evolutionary time as a pathogen of humans and has spawned substituent clonal lineages, such as *Trichophyton violaceum*, that have changed habitat and have formed ecologically convincing facsimiles of separate species. The separateness of these subclone species may be significantly less evident when typical phylogenetic evaluations are used to assess the species than it is in the clinical laboratory.

Most medically important dermatophyte types were well known in the clinical laboratory by 1990 and were being seen dozens to thousands of times per year in each lab active in identification. Some species within the three recognized genera, *Trichophyton*, *Microsporum*, and *Epidermophyton*, were highly variable, and the identification of many had been facilitated by the development of special physiological tests for characters such as vitamin auxotrophy and urease enzyme expression [215]. Other pathogen types, such as "*Microsporum gypseum*" ss. lat., were stable in morphology, but were known from *in vitro* mating experiments to consist of morphologically indistinguishable sibling species complexes [216]. Some of these species underwent incomplete mating reactions with related pathogens that were then revealed to consist of just a single ancestral mating type [214]. In general, fully sexual types were either saprobes or associates of animals dwelling in soil habitats, while asexual

types were associates of animals, including humans, not sleeping on or within soil-dwelling places [214].

Early developments in sequence-based phylogeny were biologically revelatory, but also occasionally caused some confusion about clinical identifications. The first sequence-based phylogeny of the group, by Nishio et al. (1992) [217], based on mitochondrial DNA, laid out the major relationships that all later studies would follow, but also announced that there were two species within the name *Trichophyton rubrum* among the 92 stored clinical isolates examined. Subsequent studies found *T. rubrum* to be remarkably genetically uniform; Nishio et al.'s second group stated not to produce red pigment and can be deduced as cottony *T. interdigitale* isolates that had been misidentified [217]. Later studies using the chitin synthase 1 gene (*CHS1*) and ITS were free of such errors but showed unexpectedly close relationships between soil-associated and non-soil-associated species that lacked morphologically evident signs of relatedness, such as the mouse-associated dermatophyte *Trichophyton quinckeanum* and the agent of human favus, *T. schoenleinii* [218–222]. The taxon, now known as *T. interdigitale*, was shown to be remarkably uniform worldwide and of a single mating type [223], suggesting clonality. Similar uniformity was shown in the much more ancient *Trichophyton rubrum*, and several morphologically divergent species described as probably related to it, such as *Trichophyton raubitschekii*, were revealed as similar enough to be considered synonymous [224].

Confusion caused by the close similarities of phylogenetically used sequences among clinically divergent entities reached a maximum circa 1999–2005. The antipodal horse-infecting dermatophyte *Trichophyton equinum* var. *autotrophicum* was declared a synonym of the human scalp ringworm fungus *T. tonsurans*, and the suggestion was made that the more common cosmopolitan *T. equinum* var. *equinum* was merely a metabolic mutant [225]. The last assertion overlooked that *T. equinum* had been known since the 1980s to be of positive mating type, while *T. tonsurans* was negative [226]. The two species also differed in population host (horse, human), and, in type-variety, in vitamin requirements. The conclusions of the synonymizing authors may have been influenced by a sequencing kit with resolution issues in some templates, and later studies redoing some of the key sequences clarified the slender but consistent distinction between *T. tonsurans* and all forms of *T. equinum* [227]. Evidently, however, a clash of species concepts was involved in whether or not the separation of the two entities was to be accepted.

A more difficult muddle was created when the *T. rubrum* lineage was phylogenetically analyzed in detail. *Trichophyton megninii* (mating type +, requiring exogenous L-histidine) was first accepted as a well-supported "cohesion species" by Summerbell et al. (1999) [224], despite relatively sparse divergences in ITS sequence, but then synonymized with *T. rubrum* (mating type −, autotrophic) by Gräser et al. (2000) [228]. At the same time, *Trichophyton soudanense* and the relatively rare and nomenclaturally invalid *T. yaoundei* were synonymized with *T. violaceum* [228]. Clinically, *T. soudanense* was easily distinguished from *T. violaceum* by several criteria and was obtained almost entirely from sub-Saharan African sources, while *T. violaceum* was endemic in the north and east of the Sahara from Morocco to Pakistan, with some ingress down the East African coastline. A more in-depth look at *T. soudanense* by the Gräser group, using microsatellite typing and additional genes, redirected it into synonymy with *T. rubrum* [229]. The same study distinguished the Afro-Asiatic "*T. raubitschekii*" subgroup of *T. rubrum* isolates that were phenotypically plesiomorphic and mostly caused tinea corporis and tinea cruris but submerged the clinically and phenotypically distinguished *T. soudanense* and *T. megninii* into the group containing these isolates.

Reaction in the clinical community to this mixture of stimulating biological discovery and roughshod taxonomic reductionism culminated in a protest review by Woodgyer (2004) calling the suggested phylogenetically based taxonomic changes a "modern fairy tale" [230]. Only recently have studies using expanded databases of isolates and sequences clarified the distinctness of *T. soudanense* [231]. At this point, the identity of isolates belonging to this taxon is so confused by synonymy in culture collection databases and sequence repositories that only the painstaking tracing of strain numbers among multiple publications, or the assembly of a sequence alignment, can refind the mostly obliterated information. Numerous isolates labeled *T. rubrum* or *T. violaceum* in culture and sequence repositories conform with *T. soudanense*, *T. megninii*, "*T. yaoundei*," and another rare African species distinguished by Packeu and colleagues (2020), *T. kuryangei* [231].

Phylogenetic re-evaluation of taxa held a potential source of confusion other than unexpected shifts in perceived relationships. It also required that species with missing or unanalyzable type material be re-typified. Interpreting nineteenth-century concepts for selection of the most appropriate *de novo*-type material was challenging, and one decision in particular, that of using CBS 318.56, a member of the uncommon and regional, mouse-associated "*quinckeanum*" clade, as a neotype of one of the most commonly used names in medical mycology, *T. mentagrophytes* [225], caused considerable debate. Almost none of the isolates reported to clinicians worldwide under this name belonged to the clade in question, and the historical basis of the neotypification was questionable. Ultimately, the problem was solved by designating an alternate neotype, IHEM 4268, which had both plausible consistency with the historical concept of *T. mentagrophytes* and membership in one of the clades long

designated by this name [21]. That clade thus became *T. mentagrophytes* ss. str. and, under revised nomenclatural rules, lost its parallel designation under the teleomorph name *Arthroderma vanbreuseghemii*.

A thorough phylogenetically based revision of generic concepts in the dermatophytes and related non-pathogens by de Hoog et al. (2017) has been generally accepted, even though clinical reporting practices are only gradually being adapted to it [21]. The only ubiquitous and commonly reported pathogen group that has acquired a generic name change is the *Microsporum gypseum* species complex, now the *Nannizzia gypsea* species complex. Two well-known groups of dermatophytoid non-pathogens, *Paraphyton* (*Microsporum*) *cookei* and *Arthroderma* (*Trichophyton*) *terrestre* complex, gained newly applied or delineated generic names, while the uncommon poultry pathogen *Lophophyton gallinae*, occasionally infecting humans, was also renamed. The last, incidentally, is the final remaining "sore spot" in the discrepancy between sexual species with soil-associated phenology (e.g., heavy and dimorphic conidiation) in contrast to non-mating, derived apomorphs with transformed phenotype. The name *L. gallinae* synonymizes a heavily micro- and macroconidial soil fungus with mating ability, formerly called *Microsporum vanbreuseghemii*, with a non-mating, aberrantly conidial chicken pathogen, formerly called *Microsporum gallinae*. Though these biotypes share common ITS barcodes, it is safe to predict that further investigations, such as mating gene studies, will evince significant differences between types. Whether species status will then be evaluated purely by some metric of sequence similarity, or instead will take phenotypically transformative unifactorial habitat saltations (jumps by one mating type to a novel host relationship) into account, remains to be seen.

Apart from studies mentioned above, extensive phylogenetic biosystematics overviews have been produced recently for the *Trichophyton benhamiae* complex [213, 232], the *T. rubrum* complex [231, 233], *Nannizzia* including the former *Microsporum gypseum* complex [234], the *Trichophyton mentagrophytes/T. interdigitale* clade [235–237], the *Microsporum canis* complex [238], and the redefined genus *Arthroderma* [239]. The emergence of the recently phylogenetically distinguished *Trichophyton indotineae* as a potential worldwide pandemic strain of terbinafine-resistant dermatophytes causing inflammatory tinea corporis and tinea cruris [235, 237, 240] is a particularly noteworthy development.

Whole-genome and other genomic explorations have recently made significant contributions to the biosystematic picture of dermatophytes. The elucidation of the mating-type idiomorphs *MAT1-1* and *MAT1-2* in a group of dermatophytes and Onygenalean dimorphic pathogens by Li et al. (2010) [241] paved the way for more broad-ranging genomic mapping of the distributions of mating-type idiomorphs in dermatophytes [242–246]. Various interesting distinctions among species have been disclosed, such as the simultaneous presence of multiple polymorphisms of the ribosomal intergenic spacer within individual clonal isolates of *T. violaceum*, as compared to stable strain-specific markers in *T. rubrum* [247]. The overall difference among genomes in dermatophyte species is small [248], indicating a relatively recent common ancestor; to what extent niche differentiation depends on differential expression patterns rather than basic differences among genes is unclear. Except among the possibly small number of genes that account for niche differentiation, the highly rappellative and interrappellative nature of the functional parts of coding genes [104] will tend to make the study of these genes suggest synonymy among closely related species, while a focus on noncoding regions high in arbitration will tend to distinguish diverging forms. Following the historical structure of saltatory host switch events by giving due attention to mating-type gene regularities among species will be a major aid to understanding taxonomic structure within this strongly interrelated group.

Clinical laboratories may still hold phylogenetic taxonomy at arm's length because of the history of instability brought about by early investigations. Table 3.3 summarizes the information needed to correctly identify and report the most commonly occurring dermatophytes infecting humans, as understood according to current phylogenetic taxonomy.

Table 3.3 Overview of the common (or historically well-known) human-infecting dermatophytes as distinguished by phylogenetic taxonomy

Current name	Previous name	Reference sequence	Mating type	Population host/main human body site	Simplest definitive recognition character for current species	Currently reported name (if no sequence data)
Trichophyton benhamiae var. *benhamiae*	*Trichophyton mentagrophytes*—zoophilic	LR794129	MAT1-1 and MAT1-2	North American porcupine, chinchilla	ITS sequence	*Trichophyton mentagrophytes* complex—zoophilic
Trichophyton benhamiae var. *luteum*	*Trichophyton mentagrophytes*—zoophilic	LR794131	MAT1-1	Guinea pig	ITS sequence + yellow colony reverse	*Trichophyton mentagrophytes* complex—zoophilic
Trichophyton concentricum	*Trichophyton concentricum*	LR794126	MAT1-1	Human—ALB[a]	Isolated from tinea circinata in member of a known genetically susceptible population; no conidiation	*Trichophyton concentricum*
Trichophyton europaeum	*Trichophyton mentagrophytes*—zoophilic	LR794126	MAT1-2; one MAT1-1 strain known	Guinea pig	ITS sequence	*Trichophyton mentagrophytes* complex—zoophilic
Trichophyton japonicum	*Trichophyton mentagrophytes*—zoophilic	LR794132	MAT1-1	Rabbit, guinea pig	ITS sequence	*Trichophyton mentagrophytes* complex—zoophilic
Trichophyton erinacei	*Trichophyton erinacei* or *T. mentagrophytes* var. *erinacei*	LR794136	MAT1-2 (*Erinaceus* strains); MAT1-1 and MAT1-2 (*Atelerix* strains)	European hedgehog (*Erinaceus*), African hedgehog (*Atelerix*)	*Erinaceus* strains: (note: yellow colony reverse + urease negative) *Atelerix* strains: ITS sequence	*Trichophyton erinacei* (*Erinaceus* strains); *Trichophyton mentagrophytes* complex—zoophilic (*Atelerix* strains)
Trichophyton eriotrephon	*Trichophyton mentagrophytes*	FM992674	MAT1-1 (small sample)	unknown	ITS sequence	*Trichophyton mentagrophytes* complex
Trichophyton verrucosum	*Trichophyton verrucosum*	LR890161	MAT1-2	Bovine	Pale colonies, slow, dense growth; long chains of symmetrical chlamydospores at 37 °C on BCP-MSG[b], thiamine requirement, sometimes with inositol requirement	*Trichophyton verrucosum*
Trichophyton africanum	*Trichophyton mentagrophytes*—zoophilic	LR794140	MAT1-1 and MAT1-2	Unknown	ITS sequence	*Trichophyton mentagrophytes* complex—zoophilic
Trichophyton bullosum	Rarely encountered, no established report	LR794143	MAT1-1	Equine (horse, donkey)	ITS sequence No reliable phenotypic distinction from *T. verrucosum* recorded (similar chlamydospore chains and vitamin responses) but usually has equine link	Sequence probably required to resolve identity
Trichophyton mentagrophytes ss. De Hoog et al. (2016), genotypes III, III*, IV, V, VI, VII, IX	*Trichophyton mentagrophytes*—zoophilic	MF926358	MAT1-1 and MAT1-2	Rabbit, guinea pig, snow leopard	ITS sequence for species ID, but for zoophilic species complex member ID, rapid growth, pustulate conidiophore masses, rounded microconidia, spirals common, urease +, BCP-MSG + alkalinity	*Trichophyton mentagrophytes* complex—zoophilic
Trichophyton interdigitale	*Trichophyton mentagrophytes*	JX122216	MAT1-2	Human—ALB	Velvety to cottony colonies, usually rounded conidia, rapid growth, urease-+, BCP-MSG + alkalinity; spirals possible	*Trichophyton mentagrophytes* complex

(continued)

Table 3.3 (continued)

Current name	Previous name	Reference sequence	Mating type	Population host/main human body site	Simplest definitive recognition character for current species	Currently reported name (if no sequence data)
Trichophyton indotineae	*Trichophyton mentagrophytes*	LC508024	MAT1-2	Human—ALB	Resembling *T. interdigitale* velvety type, but urease (− or very weak), hair perforation (−)	*Trichophyton indotineae*
Trichophyton tonsurans	*Trichophyton tonsurans*	KT155650	MAT1-1	Human—CS[c]	Broadly pedicellate conidia, thiamine requirement	*Trichophyton tonsurans*
Trichophyton equinum	*Trichophyton equinum*	KT155643	MAT1-2	Horse	Nicotinic acid requirement; for *T. e.* var. *autotrophicum* in Australia, New Zealand, Timor, and Indonesia, ITS sequence	*Trichophyton equinum*
Trichophyton quinckeanum	*Trichophyton mentagrophytes*—zoophilic	NR 144899	MAT1-1 and MAT1-2	Mouse	ITS sequence	*Trichophyton mentagrophytes* complex—zoophilic
Trichophyton schoenleinii	*Trichophyton schoenleinii*	LC375549	MAT1-2	Human—LBS[d]	Lack of conidiation, vitamin independent, nailhead hyphae, clinical scutula	*Trichophyton schoenleinii*
Trichophyton simii	*Trichophyton simii*	KT155890	MAT1-1 and MAT1-2	Unclear	Copious macroconidia give index of suspicion, confirm by mating or sequence	*Trichophyton simii*
Trichophyton rubrum	*Trichophyton rubrum*	JX122311	MAT1-1	Human—ALB	Common but not universal: blood-red pigment, cottony colony, clavate microconidia, BCP-MSG (−) neutral pH for 7 days at 25 °C, urease (−). Also "raubitschekii" pattern from endemic area tinea corporis/cruris with velvety colony, unusually copious macroconidia, urease +	*Trichophyton rubrum*
Trichophyton violaceum	*Trichophyton violaceum*	JX122372	MAT1-1	Human—CS	Slow, dense, deep-red colonies (or whitish from East Africa), thiamine requirement, endothrix hairs	*Trichophyton violaceum*
Trichophyton soudanense	*Trichophyton soudanense*	MK299097[e]	MAT1-1	Human—CS	Stellate growth, reflexive branching, endothrix hairs	*Trichophyton soudanense*
Trichophyton megninii	*Trichophyton megninii*	AJ270800	MAT1-2	Human—LBS	Ectothrix hairs if scalp involved; requires L-histidine	*Trichophyton megninii;* some labs accept synonymy with *T. rubrum*
Epidermophyton floccosum	*Epidermophyton floccosum*	KT155837	MAT1-2	Human—ALB	Smooth-walled macroconidia, no microconidia	*Epidermophyton floccosum*
Nannizzia duboisii	*Microsporum gypseum* (ss. lat.)	MF926380	Unknown	Possible saprobe	ITS sequence	*Nannizzia gypsea* complex, formerly *Microsporum gypseum* complex
Nannizzia fulva	*Microsporum gypseum* (ss. lat.)	MF926376	MAT1-1 and MAT1-2	Possible saprobe	ITS sequence	*Nannizzia gypsea* complex, formerly *Microsporum gypseum* complex
Nannizzia gypsea	*Microsporum gypseum* (ss. lat.)	KT155845	MAT1-1 and MAT1-2	Possible saprobe	ITS sequence for species ID, but for zoophilic species complex member ID, copious fusoid, rough-walled macroconidia, plus microconidia; sandy-brown colony	*Nannizzia gypsea* complex, formerly *Microsporum gypseum* complex

Table 3.3 (continued)

Current name	Previous name	Reference sequence	Mating type	Population host/main human body site	Simplest definitive recognition character for current species	Currently reported name (if no sequence data)
Nannizzia incurvata	*Microsporum gypseum* (ss. lat.)	KT155816	MAT1-1 and MAT1-2	Possible saprobe	ITS sequence	*Nannizzia gypsea* complex, formerly *Microsporum gypseum* complex
Nannizzia nana	*Microsporum nanum*	KT155868	MAT1-1 and MAT1-2	Pigs	Mostly two-celled, rough-walled macroconidia; sandy-brown colony	*Nannizzia nana*, former *Microsporum nanum*
Nannizzia persicolor	*Microsporum persicolor*	KT155656	MAT1-1 and MAT1-2	Unknown	Resemblance to *T. mentagrophytes* complex except pedicellate microconidia, rough-walled macroconidia on Sabouraud + 3% NaCl, negative for alkalinity on BCP-MSG, weak growth at 37 °C	*Nannizzia persicolor,* former *Microsporum persicolor*
Nannizzia praecox	*Microsporum praecox*	MH378243	Unknown	Horses (or their immediate environment)	Resembles *N. gypsea* complex but reverse yellow; hair perforation negative	*Nannizzia praecox*, former *Microsporum praecox*
Microsporum audouinii	*Microsporum audouinii*	KT155940	MAT1-2	Human—CS	From patient <20 years old; pale to pale-brown colony reverse; often few deformed beaked rough-walled macroconidia; microconidia common; no growth on autoclaved polished rice	*Microsporum audouinii*
Microsporum canis	*Microsporum canis*	MH861991	MAT1-1; one genetically divergent MAT1-2 mating strain known, clusters with *M. audouinii*	Cats	Yellow colony reverse; usually copious rough-walled, beaked microconidia, e.g., on Leonian's agar; negative for alkalinity on BCP-MSG	*Microsporum canis*
Microsporum ferrugineum	*Microsporum ferrugineum*	MH855227	MAT1-2	Human—ALB	ITS sequence; index of suspicion via no conidiation; "bamboo hyphae," isolation from Wood's light positive lesion	*Microsporum ferrugineum*
Lophophyton gallinae	*Microsporum gallinae, Trichophyton gallinae*	KT155862	MAT1-1 and MAT1-2 in ancestral form; unknown in derived form	Chickens in derived form; saprobe in ancestral form	Aberrant, short macroconidia sometimes with curved end, yellow diffusing soluble pigment that turns red over time, urease negative, hair perforation negative, connection with chickens in derived form; for ancestral form, index of suspicion from long, rough-walled macroconidia 6–12 cells long borne on long stalks	*Lophophyton gallinae*

[a]ALB = mostly adult lower body dermatophytosis
[b]Bromocresol purple milk solids glucose agar
[c]CS = mostly childhood scalp ringworm
[d]LBS = lower body and scalp infection
[e]Many *T. soudanense* sequences do not have the 37 bp deletion of conserved ITS1 sequence shown by this sequence from neotype isolate IHEM 19751 [231]

Conclusions and Recommendations

From the above, it is clear that our understanding of fungal pathogens is changing considerably due to the use of molecular phylogenies. Initially, the resulting name changes may be an annoyance for the user, but as in many cases, significant biological differences that might be clinically relevant correspond between the various clades. For example, susceptibility to antifungal drugs may regularly differ, and in these cases at least, it is expected that the field will quickly adapt to the name changes. This state of affairs also yields an important role for databases, such as MycoBank, the Atlas of Clinical Fungi, and theyeasts.org, in keeping such name changes updated in order to help the clinicians, e.g., with the proper interpretation of fungal names that have been received from automated identification systems, which may not always contain the most updated names.

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Diagnostic Molecular Mycology

4

Brian L. Wickes

Introduction

The demand for faster, cheaper, and more accurate diagnosis of fungal etiologic agents continues to grow, putting pressure on clinical microbiology laboratories that may be constrained by budget and technical expertise. Fungal infections can be difficult to diagnose, particularly systemic infections, because symptoms and certain clinical tests, such as imaging, can be nonspecific or insensitive. Because fungi are not the most common infectious agent, a specific clinical suspicion may be required before the appropriate test can be ordered and, even if correct, a specific test may lack sensitivity. For example, culture, often referred to as the gold standard of diagnostic microbiology, can be negative depending on specimen type or fungal species. Diagnosing candidemia by blood culture has low sensitivity (50%) and can even be negative despite disseminated infection [1–3]. For some cultures, even if positive, days can go by before an identity can be made, because additional tests need to be performed after the primary culture grows out. For molds, this time frame can be longer as hyphal elements may need to produce spores before an identification can be made due to the role of specific spore and hyphal characteristics in making a conclusive identification. Some fungi, such as *Histoplasma capsulatum*, can take weeks to grow, and then additional time may be required to induce an alternate morphology before identification is conclusive, since *H. capsulatum* is dimorphic.

Perhaps, the most pressing problem in diagnostic mycology is the continually expanding spectrum of fungi that infect patients. The spectrum reflects the severe immunosuppression that today's patients present with, either through underlying disease or aggressive treatment of these diseases. Importantly, the number of fungi that infect humans as well as the frequencies of individual fungi that show up as clinical samples are not static. Two well know examples are *Exserohilum rostratum*, a fungus associated with an outbreak of fungal meningitis in patients receiving injections of contaminated steroid preparations, and *Apophysomyces trapeziformis*, a fungus associated with an outbreak in trauma patients injured by the Joplin Missouri tornado [4, 5]. Very little was known about these fungi, including how to treat them, identify them, or what made them pathogenic, prior to these reported outbreaks, as they are rarely seen in the clinical microbiology laboratory. However, in our own studies of combat trauma patients, *Apophysomyces* sp. are common in these patients and can be extremely challenging to treat, because they can be hard to identify [6]. In a more recent example, COVID-19 patients with severe respiratory distress can be at risk for infection by both yeasts (*Candida* sp.) and molds (*Aspergillus* sp.), resulting in a significant increase in mortality (53% infected vs. 31% uninfected) [7]. While it is cost-effective to develop diagnostic assays for the most common fungal infections, there will always be a need for pan fungal diagnostic capability.

There is an excess of 1500 fungi that have been recovered from human specimens [8], the vast majority of which are rarely seen and may only have a few or no published cases. In fact, humans with a normal immune system are extremely resistant to fungal infection with only a few fungi being able to cause systemic infection in healthy individuals. This resistance to fungal infection can be attributed to a complex immune system and a body temperature of 37 °C, which is too high to grow at for most fungi [9, 10]. Furthermore, there is legitimate debate about whether a recovered fungus from a patient is a colonizer or invader, which makes classifying a specific fungus as a pathogen difficult and may argue for diagnostic assays being able to provide some indication of fungal burden. In fact, several fungi are human commensals, including *Candida* sp., which are the most common human fungal pathogens, and many fungal species

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_4

are constantly inhaled and can show up in sputum samples. In the case of a presumed fungal infection, it can be challenging to ascertain if the fungi identified in clinical specimens are the causative agents of disease. This problem can add an additional layer of complication to diagnostic assays, because in the absence of determining fungal burden in a specific specimen, and perhaps more importantly, what level of fungus in a specimen is conclusively shown to be associated with a given mycosis, positive specimens from nonsterile sites may be challenging to interpret.

Identifying fungi, even if they can be recovered as pure cultures, can be problematic even for trained mycologists and impossible for routine clinical microbiology personnel. Reference laboratories, which can use a variety of identification methods that are not available to clinical microbiology laboratories, fill this gap. However, the turnaround time required to grow a fungus in pure culture (if it can be grown), and then ship it to a reference laboratory, can lead to extremely long delays in patient treatment, not to mention the additional costs associated with making an identification. This difficulty in identification can lead to challenging management issues that can be limited to reversal of immunosuppressive conditions (not always possible), surgical debridement (which can further complicate a sick patient's recovery), or empirical treatment with antifungal drugs with varying efficacy (some of which can have severe side effects) [11].

Current Challenges to Fungal Diagnostic Assay Development

Frequency of Human Mycoses

Despite the current and growing fungal morbidity and mortality, some of which have reached catastrophic levels, diagnostic assay development for fungal infections has lagged other diseases. Research support for human fungal pathogens is substantially lower than for other pathogens that cause similar mortality [12].

While there are concerted efforts to increase the visibility of the seriousness of fungal disease by different fungal societies (i.e., International Society for Human and Animal Mycology), government agencies (i.e., Centers for Disease Control), and international organizations (Global Action Fund for Fungal Infections; GAFFI), fungal diseases are still clinically neglected, which gets translated into a lack of commercial investment into diagnostics and, more importantly, antifungal drugs. In fact, there are no licensed vaccines or immunotherapies for any fungal disease in spite of a number of fungi in the USA being endemic and capable of causing serious, life-threatening infections [13].

Expertise in Medical Mycology

A looming crisis in medical mycology caused by the decreasing number of classically trained mycologists was predicted almost 20 years ago [14]. This crisis has been precipitated by the lack of formal training programs in medical mycology, retirements of key people, and lack of financial support for workshop or short training programs. The number of fungi that cause disease in humans continues to increase rapidly. Trying to keep track of these new species as they appear and as they are named, renamed, or reassigned after being discovered in clinical specimens or classified by sequence-based analysis has created enormous stress on clinicians and clinical microbiology laboratories.

Fungal Nomenclature

In contrast to bacterial, viral, or parasite identification, fungal identification has an additional layer of complexity that has always been challenging to microbiologists and clinicians who did not have formal training in mycology. The rules governing the scientific naming of fungi are determined by the *International Code of Nomenclature for Algae, Fungi, and Plants*. Previously, under these rules, fungi have a dual naming system that includes a name for an anamorph and a separate name for the teleomorph. The anamorphic phase is the asexual phase of the fungus and often is the first discovered. Upon demonstration of a sexual phase, the anamorph is given a new name. Both names follow the binomial naming system. For example, *Histoplasma capsulatum* was discovered in 1904 by Samuel Taylor Darling [15]. In 1972, the teleomorph was discovered, which displayed two mating types (+) and (−), and was named *Emmonsiella capsulata* [16]. While the dual nomenclature system was useful to classical mycologists, it became increasingly cumbersome and unwieldy for two main reasons. With the arrival of the AIDS epidemic and medical advances that led to increasingly immunosuppressed patients, more fungal infections caused by an increasing variety of fungi were being observed. With a larger number of fungi to keep track of clinically, it became impossible for clinicians to keep track of anamorphs and teleomorphs especially with no incentive to do so, because infections were treated the same way regardless of the name. Basic researchers had a similar disincentive to use both names; however, the problem accelerated with the application of DNA sequencing techniques to medical mycology. As sequencing, and by extension, PCR, became more important for fungal identification, the same sequences were being assigned to fungi with two different names. In the case of rarer fungi, when database searches of DNA deposits return two apparently unrelated fungi with the exact same sequence, this outcome creates a chaotic situation for molecular-based identification. Addressing this problem became even more

Table 4.1 Recent proposed fungal taxonomic revisions

Previous name	New name
Bipolaris australiensis	*Curvularia australiensis*
Bipolaris hawaiiensis	*Curvularia hawaiiensis*
Bipolaris spicifera	*Curvularia spicifera*
Candida glabrata	*Nakaseomyces glabrata*
Candida guilliermondii	*Meyerozyma guilliermondii*
Candida haemulonii	*Candida duobushaemulonii*
Candida kefyr	*Kluveromyces marxianus*
Candida krusei	*Pichia kudriavzevii*
Candida lipolytica	*Yarrowia lipolytica*
Candida lusitaniae	*Clavispora lusitaniae*
Cryptococcus gattii (serotype B, VGI)	*Cryptococcus gattii*
Cryptococcus gattii (serotype C, VGIII)	*Cryptococcus bacillisporus*
Cryptococcus gattii (serotype B, VGII)	*Cryptococcus deuterogattii*
Cryptococcus gattii (serotype C, VGIV)	*Cryptococcus tetragattii*
Cryptococcus gattii (serotype B, VGIV)	*Cryptococcus decagattii*
Cryptococcus neoformans var *grubii*	*Cryptococcus neoformans*
Cryptococcus neoformans var *neoformans*	*Cryptococcus deneofomans*
Cryptococcus laurentii	*Papiliotrema laurentii*
Emmonisia crescens	*Emergomyces crescens*
Emmonisia parva	*Blastomyces parvus*
Fusarium solani species complex 9	*Neocosmospora tonkinensis*
Fusarium solani species complex 7	*Neocosmospora gamsii*
Fusarium solani species complex 20	*Neocosmospora suttoniana*
Fusarium solani species complex 43	*Neocosmospora catenata*
Geosmithia argillacea	*Rasamsonia argillacea*
Microsporum gypseum	*Nannizzia gypsea*
Phialemonium curvatum	*Phialemoniopsis curvata*
Rhodotorula slooffiae	*Papiliotrema ciferrii*
Trichsporon cutaneum	*Cutaneotrichosporon cutaneum*
Trichosporon mycotoxinivorans	*Apiotrichem mycotoxinivorans*

critical as sequence-based identification identified preferred, or standardized, sequences (universal barcoding) as a molecular gold standard for molecular-based fungal identification. In essence, a newer genotype-based identification method was replacing older phenotype-based identification methods. A resolution to the dual naming problem was proposed under the phrase, "One Fungus = One Name" [17]. At the 18th International Botanical Congress held in Melbourne Australia, in 2011, this change was accepted and implemented beginning on January 1, 2012 [18]. As this approach becomes increasingly integrated into fungal names, among the downstream advantages are a clearer output for molecular assays that result in an unambiguous name.

While the dual naming system seems to be somewhat resolved, the role of sequencing in fungal taxonomy has accelerated reassignment of fungi to new taxonomic positions, usually with an accompanying name change. Examples of recent name changes are shown in Table 4.1 [19–21]. In most cases, these changes make sense as there can be clear phenotypic differences in a misplaced species from other members of the genus. For example, *Candida glabrata* has extensive differences from other members in the *Candida* genus, particularly for investigators studying molecular characteristics of this fungus. However, mitigating this clearly warranted reassignment is the fact that *C. glabrata*, like other members of the genus, inhabits the intestinal tract of warm-blooded mammals. It also, like *C. albicans*, can be associated with oropharyngeal candidiasis oral thrush and is, as are other *Candida* sp., an important bloodstream pathogen. Perhaps one argument in favor of realigning fungi as needed is that it should only be done once, if in fact, sequence-based taxonomy is the best tool for taxonomic assignment.

Finally, a growing problem in fungal taxonomy that may not be readily apparent to clinical microbiologists is the increasing use of next-generation sequencing. For single isolates, this technology is not a major issue, because the entire sequence is used for identification and yields the highest molecular precision. Where the technology becomes problematic is when it is used to sample different ecosystems of specimens that are mixed with many isolates. These types of ecosystems, often referred to as a microbiome, can be environmental samples, but also samples from different locations within and on the human body. Fungal "mycobiomes" have been used for many investigations. Unfortunately, because next-generation sequencing utilizes short reads and frequently targets the ribosomal loci with conserved primers, specificity is lost due to this strategy and the resulting output can be genus level only, or higher. For this reason, using operational taxonomic units (OTUs) for identification, which results in only higher taxa (genus, family, order) being identified, should be discouraged [22]. Unfortunately, most massively parallel systems use short to very short reads and while longer reads approaching 1000 bp may be possible, they are not routinely used. However, newer sequencing platforms such as nanopore sequencing allow longer reads, which can be on the order of a megabase, as one of the advantages.

Template Preparation

Template preparation, particularly in the case of nucleic acid assays, encompasses three major processes with variation as needed: isolation, purification, and concentration [23]. Ideally, extraction methods should be fast and easy without complicated equipment or the need for specialized skills. While it would be a major challenge, if the extraction method worked on a broad range of substrates, from pure culture to body fluids of various consistencies (sputum, blood, urine), to solid tissue, this flexibility would be a major asset. Since many clinical microbiology laboratories use some type of commercial system, flexibility is extremely important. This

aspect could be as simple as varying the type of kit that may be required for presample preparation, or adding additional upstream steps, such as reduction of solid tissue bulk. Extraction methods or systems should be versatile so that they can be applied to a variety of organisms or substrates.

While fungal extraction methods have worked their way into commercial kits and even instruments, fungi are different from all other human microbial pathogens with regard to cellular organization. Because the fungal cell has similarity to plant cells, disrupting yeast cells can be challenging and even more difficult for fungi growing as hyphae. Fungal cell walls are composed of chitin and/or cellulose and, like plants, are resistant to rupture by simple osmotic pressure or detergent treatment. While enzymatic treatment does weaken fungal cell walls, this approach is often not desirable for diagnostic assay extractions as these enzymes are typically specific for a narrow spectrum of fungi and using them can add additional time to the extraction process. Additionally, lysing enzymes can be contaminated with fungal DNA [24]. In fact, it is possible to have contamination in virtually all aspects of a fungal molecular diagnostic assay [25, 26]. Chemical methods can work on fungal cells and several commercial extraction kits utilize Proteinase K to weaken the cell wall; however, fungi can vary in their sensitivity to this approach, particularly if this approach is used on molds or fungi that are not from fresh cultures. The final downstream assay method also can influence extraction choice. PCR-based or other nucleic acid detection methods need template nucleic acid that is relatively free of inhibitors, particularly if an amplification step (i.e., PCR) will follow. On the other hand, MALDI-TOF may use chemical extraction exclusively with a combination of acids (alpha-cyano-4-hydroxycinnamic acid, trifluoroacetic acid, formic acid) and/or organic solvents (acetonitrile). With regard to a universal method, breakage of yeast cells and hyphae, usually by bead beating, is probably the most common method [27]. However, even this method can require numerous variations or additional downstream steps as fungi often have large amounts of polysaccharide associated with their cell walls. Unfortunately, polysaccharide behaves chemically similar to DNA, so simple precipitations may not remove it. Column-based methods that many kits are based on work very well in removing polysaccharide and other impurities, and some use simple chemical extractions without a physical breakage step. However, columns can lead to sheared DNA that can be smaller in size than noncolumn-based methods. The same problem can occur if physical breakage is used if the actual breakage continues too long. Together, these methods all have the same weakness in that it is difficult to standardize them, particularly for microbiology laboratories that must identify both yeasts and molds.

Yeasts, in general, are much easier to lyse than molds and are more amenable to simpler methods. The morphology of the fungus, yeast or mold, and the type of extraction method as well as the target (DNA, protein, etc.), can be further complicated by the sample specimen. When fungi are available as pure cultures, extraction is greatly simplified mainly because there is unlimited material, no background host material contamination, and the ability to manipulate growth conditions to make cell lysis easier. However, for diagnostic assays, pure culture may be less preferred, because it requires growing the fungus out from the original clinical specimen, such as blood, tissue, or sputum, which adds significant delays to turnaround time. However, if there is little or no suspicion of a fungus in the specimen, culture may be preferable to first demonstrate the presence of fungi as a potential etiologic agent before running a fungus-specific diagnostic test. Alternate preliminary identification methods, such as microscopy or serology, may supplant culture due to turnaround time, as these methods can yield instant or same day results. Nonetheless, if a fungus is a possibility or suspected in the specimen, analysis directly from the clinical specimen is the goal of virtually all diagnostic assays because of the savings in turnaround time and reduction in specimen manipulation. However, in the case of direct analysis on a clinical specimen, extraction may take place in a background of host and/or other microbial material, which is often present in massive excess compared to the fungal template target. For some assays, such as MALDI-TOF, pure culture is generally required due to interference from host material if direct analysis is performed on clinical specimens. For assays that use an amplification step with primers, a gross excess of nonfungal nucleic acids or other substances becomes less of a problem if the background material is not inhibitory or present in such an excess as to make fungal templates undetectable after dilution to meet assay standard starting requirements. Unfortunately, swamping fungal elements with an excess of host material is not unusual and can happen in tissue samples. One way around this issue is to selectively enrich for fungal elements such as by laser capture microdissection as long as it is possible to recover enough fungal elements to meet the limits of detection.

For many, if not most molecular-based assays, combining template preparation with the assay to yield a fully automated diagnostic platform is a major goal and arguably should be a gold standard for molecular assays. Unfortunately, for fungi, this goal is especially challenging due to the difficulty in breaking the cell walls of fungal elements (yeast and/or hyphae). The specimen type also can limit a sample-to-answer approach. However, for arguably one of the most important specimens for fungal diagnosis (blood), commercial assays are appearing that have successfully incorporated extraction with analysis in a single assay. Two examples are the BioFire® Blood Culture Identification 2 (BCID2) (BioFire Diagnostics, Salt Lake City, Utah, which is an

FDA-cleared device, and the T2Candida® Panel (T2 Biosystems, Lexington, MA), also an FDA-cleared device.

The BioFire system is a pouch-based, multiplex PCR system that works by injecting the specimen and a hydration solution into a pouch, which is then loaded onto the instrument to start the run. Template nucleic acid is extracted from the sample followed by the first of two PCR reactions. The first is a multiplex reaction and the second consists of single PCR reactions targeted towards individual species. Analysis is by endpoint melting curves and data are outputted after screening by the software for the presence of individual species. The BCID2 is for blood specimens and detects *Candida albicans*, *C. auris, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis,* and *Cryptococcus neoformans/gattii*, all of which are yeasts, in addition to select Gram-positive and Gram-negative bacteria. Selected bacterial antimicrobial resistance gene markers can also be detected in the same assay, for total of 43 bacteria, fungi, and drug resistance targets [28–32]. This system has a reported sensitivity and specificity of 99.2% and 98.7% respectively [33]. Turnaround time is approximately 1 h, and the automated nature of the system combined with an extraction component enhances utility of this system.

The T2Candida system, in contrast, works by a completely different mechanism that is dependent on magnetic resonance for pathogen identification. It is an automated nonculture system that uses whole blood as a specimen and can detect *C. albicans, C. tropicalis, C. krusei, C. glabrata*, and *C. parapsilosis*. Template extraction is integrated within the assay and utilizes physical breakage of cells by bead beating. The sample is cleaned by magnetic beads followed by a PCR step that targets the ITS region of the fungal ribosomal repeat. Amplicons anneal to nanoparticles carrying complementary DNA sequences, which then aggregate into microclusters that can then be excited by the magnetic resonance signal that allows species-specific patterns to be identified. Identities can be determined in 3–5 h with 91.1% sensitivity and 99.4% specificity [34–37]. The high negative predictive value of the T2Candida assay can be viewed as an important asset, since it can eliminate the need for empiric treatment with antifungals and their associated side effects. Because the assay detects a panel of *Candida* spp., non-albicans *Candida,* which are increasingly common in clinical specimens, can be detected with this assay. Additionally, although approved for research use only, recent modifications of the assay have expanded the target organisms to include *Candida auris* [38]. The combination of an expandable platform, rapid turnaround time, and the sample-to-answer nature of the assay, which includes an extraction module, makes this assay an important clinical microbiology laboratory asset for rapid fungal detection.

Because both assays use liquid samples, integration of an extraction module into the assay is easier than for other types of samples, such as tissue or viscous samples (i.e., sputum). Nevertheless, these two diagnostic platforms have succeeded in creating load and walk-away assays for fungi. Unfortunately, in addition to the specimen type, not all fungi will be as amenable to the template extraction methods employed by the FilmArray and T2 systems. Organisms must be in the yeast form for identification in these systems, a form which is easier to extract DNA from because cells are usually unicellular and round or ovoid in contrast to hyphae, which are long and filamentous. A potential solution to this problem may be using cell-free fungal DNA as a target template. This strategy has been developed in the last few years and targets fungal DNA in the plasma, usually by PCR, that is not associated with hyphae or cells [39]. Although DNA can be short and fragmented, in addition to only transiently present, since it has been liberated by immune system attack, further development of this strategy can alleviate a major burden of trying to recover DNA from intact hyphae.

Mass Spectrometry-Based Molecular Diagnostic Assays

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS)

There are several mass spectrometry systems available for microbial identification, including fungi. Presently, the most widely used mass spectrometry diagnostic system for fungi is MALDI-TOF-MS. MALDI-TOF-MS allows the identification of microbes by sampling specific cellular biomolecules, which can include carbohydrates, DNA, lipids, and proteins. For fungal identification, proteins are targeted. Fungal elements are generally prepared for analysis from pure cultures and require minimal extraction. This advantage makes this type of diagnostic platform popular for fungal identification, due to the avoidance of complex and lengthy extraction techniques. The sample is mixed with a matrix material (the "matrix" component of MALDI-TOF-MS) that is capable of absorbing energy from a laser on the sample slide or screen. Upon exposure to a laser beam, the sample is fragmented and ionized as a vapor (the "laser desorption ionization" component of MALDI-TOF-MS). Acceleration of the charged ions through a detector results in separation of the molecules by weight. Travel along the ion flight path varies with mass and charge (the "time of flight" component of MALDI-TOF-MS), which can be detected and used to prepare a spectrum characteristic of a particular species. A plot of the mass-to-charge ratio of individual proteins is used to generate a spectrum (the "mass spectrometry" component of

MALDI-TOF-MS), which serves as a species-specific signature that can be used to interrogate a curated library of spectra to screen for matches to known species. For detailed reviews see [40–43].

For fungi, there are two FDA-approved systems, although other commercial systems are available. The first system is by bioMérieux (Marcy l'Etoile, France), which is called Vitek MS and was approved in 2013. Bruker (Billerica, MA) has a system, also approved in 2013, called the MALDI Biotyper CA System. Both systems rely on their own internal validated database of fungal spectra, and have expanded Research Use Only (RUO) databases of additional organisms [41]. Both systems perform similarly with regard to accuracy [41]. For microbiology laboratories that encounter more diverse fungi in clinical samples, these spectra can be added to a user upgradeable library regardless of which system is used, although identities must be validated depending on regulatory requirements. The major attractiveness of MALDI-TOF-MS is the ease of use since there is minimal sample preparation required. Results can also be obtained quickly, sometimes in a matter of minutes, and do not need to be interpreted nor manipulated with any sort of postprocessing steps. Weaknesses include the lack of a public database of spectra, the requirement to perform analysis on pure cultures, and the need to test isolates under conditions that the reference library was prepared under (growth conditions, sample preparation).

Raman Spectroscopy

MALDI-TOF-MS is not the only mass spectroscopy system that can be used for fungal diagnostics. Other methods, which are not nearly as mature with regard to development, include Raman spectroscopy, Electrospray ionization PCR mass spectroscopy, and Fourier transform infrared spectroscopy. Raman spectroscopy is a type of vibrational spectroscopy that detects molecular vibrations after exposure to a laser, and then measures the scattered light, which is characteristic of the chemical composition of the substrate. It is nondestructive in nature and has been useful for analyzing blood and blood components [44]. It has been used to identify and discriminate various fungi based on melanin content of spores [45], phagocytized *C. albicans* from blood specimens [46], and discrimination of *C. neoformans* from *C. gattii* [47]. Another advantage of this technique is that, because it is nondestructive, samples can be analyzed multiple times. There is no sample preparation as the method only requires focusing a Raman microscope on the region of interest. Analysis can be performed through sealed containers such as vials or tubes, and samples can be analyzed in a variety of liquids, eliminating the need for extraction, and it can be combined with a variety of microscopic methods such as scanning electron microscopy and confocal microscopy [48].

Fourier Transform Infrared Spectroscopy (FTIR)

(FTIR) is another vibrational type of mass spectroscopy that is rapid and nondestructive, similar to Raman spectroscopy. It can detect minute changes in bond length and angles of multiple biomolecules including nucleic acids, lipids, carbohydrates, and proteins [49]. The output from these spectra can be used to establish an organism fingerprint, which can be used for species identification. FTIR has been used to identify mycotoxin-producing molds [49], fungi causing chromoblastomycosis [50], *C. neoformans*, and multiple *Candida* spp. [51], as well as the general identification of multiple yeasts and filamentous fungi [52]. There are some commercialized variations of this method. For example, Renishaw Diagnostics (West Dundee, IL) has developed the CE-IVD certified RenDx Multiplex Assay platform that runs the Fungiplex assay for the detection of *Candida* and *Aspergillus* spp., which targets PCR amplicons prepared from blood.

PCR/Electrospray Ionization Mass Spectroscopy (PCR/ESI-MS)

PCR/Electrospray ionization mass spectroscopy (PCR/ESI-MS) is a mass spectroscopy method that uses PCR amplicons as target molecules. The method works by measuring the charge/mass ratio of amplicons and then calculating the mass. This information is used to determine the base composition of the amplicon, which is then used to interrogate a curated database of reference sequences to derive an identity. An advantage of this method is that it can be multiplexed to run on multiple samples in a single run, and it is highly accurate [53]. For example, using a database grounded by 60 ATCC reference strains, Massire et al. [54] used PCR/ESI-MS to analyze a total of 394 clinical fungal isolates. In comparison to phenotypic identification, PCR/ESI-MS identified 81.4% of 264 molds to either the genus or species level, with concordance rates of 89.7% and 87.4%. PCR/ESI-MS also identified 98.4% of 130 yeasts to either the genus or species level, with concordance rates of 100% with phenotypic results. PCR/ESI-MS performed best with *Aspergillus* and *Candida* isolates, generating species-level identification in 94.4% and 99.2% of isolates, respectively.

PCR/ESI-MS was commercialized by Ibis Biosciences, which was incorporated into Abbott, and upgraded as PLEX-ID [55]. PLEX-ID was then redesigned as a system called IRIDICA, which became CE-marked, however, the

system was eventually discontinued [55]. While it potentially could serve as one of the few pan fungal diagnostic assays to be commercialized, it is PCR dependent, so all the caveats of using PCR as a diagnostic tool apply to PCR/ESI-MS, including the first step of template DNA extraction. Because spectra are used for the identification, an entire database of reference spectra would need to be generated, which could further encumber development. It would be intriguing if it were possible to build a database by working backwards from publicly available fungal DNA sequences in GenBank, particularly since the NCBI now has separate databases of highly accurate reference sequences and genomes that are highly curated and derived from well characterized cultures.

Hybridization Probes

Fluorescence In Situ Hybridization (Fish)

Fluorescence in situ Hybridization (FISH) is a diagnostic method that uses a nucleotide probe, which has been labeled with a fluorescent reporter molecule, to target a specific nucleic acid sequence, usually rDNA. FISH is used as a diagnostic tool for microbial pathogens, including fungi, with great success. There is minimal sample preparation, unlike template nucleic acid extractions required for other molecular diagnostic methods, and FISH assays can be performed directly on clinical specimens such as blood. Turnaround time can be less than 2 h and assay manipulations are minimal. Additionally, because the target is usually ribosomal RNA molecules, probe sequences can be varied to be specific at the genus or species level. Disadvantages include a requirement for a trained technician who can operate a fluorescent microscope and interpret the results. It is less sensitive than PCR and because of the specificity of the probes, there needs to be an index of suspicion that the specimen contains fungi (or other target organism) to avoid wasting reagents [56]. Despite some of the weaknesses, there are several FDA-approved FISH assays for fungi.

There are three general steps for FISH analysis: sample preparation, hybridization, and visualization. Sample preparation is done by treating cells with a fixative (methanol, ethanol, paraformaldehyde, etc.) to preserve cellular morphology and stabilize target sequences [57]. This step is followed by permeabilizing the cells with dilute acids, xylene, or enzymes [57], but that may not always be required for yeast cells if the assay is not quantitative [56]. After fixation, hybridization with the probe occurs, with conditions dependent on probe type and anticipated target (i.e., mismatches). Because probes can be labelled with a variety of different fluorescent dyes and can be labeled in a variety of ways (5′ and/or 3′ end), conditions need to take probe chemistry into account since FISH has numerous modifications to choose from. Peptide Nucleic Acid FISH (PNA-FISH) is a nucleic acid mimic, which contains a pseudo-peptide backbone composed of charge neutral and achiral *N*-(2-aminoethyl) glycine units to which the nucleotides are attached via a methylene carbonyl linker [58]. The uncharged neutral backbone of PNA-FISH probes leads to stronger binding [8]. CARD-FISH is a FISH modification used for detecting environmental organisms, which can require greater sensitivity due to lower ribosome content. CARD-FISH functions by incorporating horse radish peroxidase into the FISH reporter [59]. DOPE-FISH also amplifies the FISH signal but does so by using a 5′ and 3′ double labelled probe [60].

Although FISH requires an index of suspicion prior to deployment, it is easily adaptable to a broad spectrum of fungal targets. Importantly, both yeasts and molds can be targets in FISH assays. This flexibility is derived from the sequence-based nature of the assay strategy, which is only limited by how difficult it is to permeabilize target cells and how specific the probe target sequence is. Because ribosomal DNA sequences can be species-specific depending on the region of the ribosomal locus that is being targeted, in theory, any fungus could be identified by FISH. In fact, while *Candida* spp. are the most common targets, FISH has been used to identify *Aspergillus* spp., *Fusarium* spp., and *Scedosporium* spp. [8]. The utility of FISH assays has led to several commercial assays. The yeast Traffic Light PNA-FISH assay (AdvanDX, Woburn, MA) is performed on blood and can be completed in 90 min. The assay is CE-marked and can detect *C. albicans*. *C. parapsilosis*, *C. tropicalis*, *C. glabrata,* and *C. krusei* although it cannot distinguish between *C. albicans* from *C. parapsilosis* or *C. glabrata* from *C. krusei* [61]. A second generation of this assay, the QuickFish assay (AdvanDX), can yield results in 20–30 min and can differentiate *C. albicans*, *C. glabrata*, and *C. parapsilosis* from each other [61]. This assay is FDA approved.

Ribosomal RNA Hybridization Probes

The Accuprobe system (Hologic, San Diego, CA) first marketed by Gen-Probe Inc., (San Diego, CA) uses a DNA probe targeted to ribosomal RNA. It is mechanistically similar to FISH in that the target is the multicopy ribosomal sequences with a fluorescent probe that can be made to specifically target a single species with high specificity and can be easily detected. It differs from FISH in that instead of permeabilizing cells and scoring positives and negatives by visualizing cells with a fluorescent microscope after the reaction, Accuprobe uses extracted RNA as a target, with cell lysis and nucleic acid liberation being a required first step. After nucleic acid extraction, the Accuprobe reagent binds to free ribosomal RNA molecules to form a stable DNA:RNA

hybrid. Additional downstream processing steps are needed before the chemiluminescent acridinium ester moiety bound to the probe can be detected [62]. Detection is by way of a luminometer. This assay has been in existence for more than 25 years and is available for the detection of *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum* (Hologic, Inc.). The advantages of the assay are that it is relatively simple to perform and does not require expensive instrumentation. It also takes advantage of a rRNA target, which can be present in thousands of copies per cell, providing an amplified signal in the absence of a PCR amplification step. The Accuprobe assays for all three fungi were FDA cleared in 1990 and display a sensitivity range 98.1–100% and specificity range of 99.7–100%.

PCR

Standard PCR

Polymerase chain reaction (PCR) is one of the oldest molecular diagnostic methods. Depending on the format, it is relatively easy, fast, and inexpensive. It also has the capability of detecting non culturable organisms that may be dead or hard to grow in vitro, or present in numbers too low to detect conventionally. Because it utilizes nucleic acid as a target, it can detect a fraction of a genome if the DNA target is multi copy or if RNA is the target nucleic acid and if the target is still intact as a fragment. Cell-free DNA can also be detected from lysed cells, such as those that have been phagocytized. For molecular-based diagnostic assays, PCR and its variations currently have the largest number of assays available commercially (Table 4.2)

In its simplest form, PCR is a nucleic acid amplification reaction conducted with two primers complementary to a target sequence, which are extended by a heat-stable polymerase in a cyclic reaction of denaturation, annealing, and extension. The primers are generally no more than 2.0–2.5 kb apart, and the reaction is run in a thermocycler that cycles through a temperature profile that takes into account primer annealing temperature (dependent on primer length and base composition, but usually ~60 °C), optimum polymerase activity (usually 68–72 °C), denaturing temperature (~94 °C), extension time (0.5–1.0 min/kb), and a final holding temperature (~4 °C) after a final 5 min extension time at the optimum polymerase temperature to finish off all products. PCR products are detected after resolving on an agarose gel, which is ethidium bromide (or some other DNA-binding dye) stained, and then photographed for record keeping. This general protocol, sometimes called standard PCR, is used routinely in virtually all basic molecular laboratories. However, every step of this protocol has been modified, from equipment, to reagents, to cycling parameters, to detection, with many of these modifications resulting in an FDA-approved assay for some microbial target. Although the newer variations of standard PCR are much more advanced and complicated, standard PCR is crucial to one of the few panfungal diagnostic tests that is commonly used for fungal identification. This diagnostic test involves sequencing the standard PCR product and is extremely important for identifying rare fungi.

PCR Sequencing

Sequence-based fungal identification has been in use routinely for 15–20 years and while too labor intensive for most microbiology laboratories, it is an important tool for fungal reference laboratories. Sequence-based fungal identification arguably is the gold standard of fungal identification and works well due to universal PCR primers that target a region of the ribosomal locus, which contains conserved primer binding sites that flank a sufficiently variable region that is useful for species-level identification of many fungi. The region, generally referred to as the ITS region (internal transcribed spacer), has more than 500,000 deposits in GenBank and is a requirement for new fungal species identification [8]. It has also been proposed as the preferred fungal barcode due to the probability that it will detect the largest amount of fungi at the species level [63] and it can be easily amplified using a standard PCR reaction. Following confirmation of amplification, the PCR product can be cleaned using simple column purification kits and sequenced. Importantly, because the ITS amplicon is relatively short (~500–750 bp), forward and reverse sequencing runs can usually cover the region in a single sequencing reaction. However, because the ITS region is not always sufficiently sensitive for species identification of all fungi, other target regions have been developed that can lead to a species level identification once the genus has been determined by the ITS sequence, or classical identification methods. Although somewhat labor intensive due to the number of manipulations and the need to interpret data after the assay is complete, there is at least one commercial sequence-based system on the market (MicroSEQ™ D2 rDNA Fungal PCR Kit, Thermo Fisher, Grand Island, NY). Importantly, this system, as well as any future system that uses sequence-based identifications, requires a biocurated database of confirmed reference sequences, since GenBank is an open database that allows anyone to deposit sequences. While there is some oversight of GenBank, it has extensive errors in sequence quality, names, and nomenclature, which make it unsuitable for an FDA-approved assay based on sequence searches of this database [64].

Table 4.2 Commercialized PCR-based diagnostic assays

Assay name	Manufacturer	Platform	Species detected
Candida assays			
AusDiagnostics Sepsis panel	AusDiagnostics Pty Ltd., Mascot, NSW, Australia	Multiplex tandem PCR	*Candida* spp.
CandID and AurisID	OlmDiagnostics, Newcastle, United Kingdom	Multiplex real-time PCR	*Candida* spp.
FilmArray Blood Culture	BioFire Diagnostics, Salt Lake city, Utah, USA	Multiplex real-time PCR	*Candida* spp.
FungiPlex R Candida	Bruker Daltonik GmbH, Bremen, Germany	Multiplex real-time PCR	*Candida* spp.
Magicplex Sepsis	Seegne, Seoul, South Korea	Multiplex real-time PCR	*Candida* spp. and *A. fumigatus*
MycoReal Candida	Ingenetix, Vienna, Austria	Real-time PCR	*Candida* spp.
SeptiFast Real-Time PCR	Roche Diagnostics, Mannheim, Germany	Multiplex real-time PCR	*Candida* spp.
SepsiTest-UMD	Molzym Molecular Diagnostics, Bremen, Germany	PCR/Sanger sequencing	All fungal species
T2Candida	T2 Biosystems, Lexington, MA, United States	Magnetic resonance	*Candida* spp.
Sepsis Flow Chip	Master Diagnostica, Granada, Spain	Multiplex PCR/ hybridization	*Candida* spp.
Aspergillus assays			
Affigene Aspergillus tracer	Cepheid, Rolling Meadows, IL, United States	Real-time PCR	*Aspergillus* spp.
A. fumigatus Bio-Evolution	Bio-Evolution, Bry-sur-Marne, France	Real-time PCR	*A. fumigatus*
Artus Aspergillus diff. RG PCR	Qiagen, Düsseldorf, Germany	Multiplex real-time PCR	*A. fumigatus, A. terreus, A. flavus*
AsperGenius	PathoNostics B.V., Maastricht, Netherlands	Multiplex real-time PCR	*Aspergillus* spp.
Aspergillus spp. ELITe MGBR Kit	ELITechGroup S.p.A, Turin, Italy	Quantitative real-time PCR	*Aspergillus* spp.
AspID	OlmDiagnostics, Newcastle, United Kingdom	Multiplex real-time PCR	*Aspergillus* spp.
FungiPlex R Aspergillus	Bruker Daltonik GmbH, Bremen, Germany	Multiplex real-time PCR	*Aspergillus* spp.
LightCycler Septifast	Roche Diagnostics, Mannheim, Germany	Multiplex real-time PCR	*A. fumigatus*, *Candida* spp.
Magicplex sepsis	Seegne, Seoul, South Korea	Multiplex real-time PCR	*A. fumigatus*, *Candida* spp.
MycoReal Aspergillus	Ingenetix GmbH, Vienna, Austria	Real-time PCR	*Aspergillus* spp.
MycoGENIE R	Ademtech, Pessac, France	Duplex real-time PCR assay	*Aspergillus* spp.
Mucorales assays			
MucorGenius	PathoNostics B.V., Maastricht, Netherlands	Multiplex real-time PCR	Mucorales spp.
Pneumocyctis assays			
AmpliSens Pneumocystis jiroveci (carinii)-FRT	AmpliSens, Bratislava, Slovak Republic	Real-time PCR	*Pneumocystis jiroveci*
AusDiagnostics Pneumonia and atypical Pneumonia panels	AusDiagnostics Pty Ltd., Mascot, NSW, Australia	Multiplex tandem PCR	*Pneumocystis jiroveci, Cryptococcus* spp., *Aspergillus fumigatus*
PneumoGenius	PathoNostics B.V., Maastricht, Netherlands	Multiplex real-time PCR	*Pneumocystis jiroveci*
Pneumocysistis jiorovecii Bio-Evolution	Bio-Evolution, Bry-sur-Marne, France	Real-time PCR	*Pneumocystis jiroveci*
PneumoID	OlmDiagnostics, Newcastle, United Kingdom	Multiplex real-time PCR	*Pneumocystis jiroveci*
Real Star Pneumocystis jiroveci PCR kit 1.0	Altona Diagnostics, Hamburg, Germany	Real-time PCR assay	*Pneumocystis jirovecii*

Real-Time PCR

While PCR and sequencing are extremely powerful, particularly for rarer fungi, employing them in a clinical microbiology laboratory is difficult due to the number of manipulations, the need to interpret database searches, and the inaccuracy of GenBank. A common variation of the standard PCR assay is real-time PCR. This assay differs from the standard PCR strategy in that it monitors and detects nucleic acid amplification as it occurs in real time using fluorescent dyes. These dyes can be specific for double-stranded PCR products but not specific for a particular target sequence, or they can be incorporated into a sequence-specific probe that binds to the amplification product as the PCR reaction proceeds through its cycles to yield an amplification curve with specific characteristics. Because fluorescence must be detected to be measured, specific instrumentation must be used that is capable of thermocycling as in a standard PCR reaction, but also measuring the fluorescence of the dye. While this capability adds to instrumentation cost, the use of dyes with different emission spectra allows multiplexing of reactions in which multiple targets can be detected. Real-time PCR is also quantitative in nature, which is a hallmark of this strategy, so it is possible to use the method to obtain some estimate of fungal burden. These capabilities have resulted in many commercially available assays for fungi, although most are targeted to the common fungal pathogens, which are *Candida* spp. and *Aspergillus* spp. However, some clinically important members of the Mucorales can also be detected by commercially available assays [65]. An absolutely crucial aspect of any real-time PCR assay is that it needs an index of suspicion for the assay target.

Loop-Mediated Isothermal Amplification (LAMP)

Some of the weaknesses of standard and real-time PCR have led to the development of new strategies that avoid the temperature cycling requirement. These assays use a single amplification temperature and are called isothermal PCR. Isothermal PCR reactions are attractive, in part, because the cost of thermocyclers can be avoided. There are multiple isothermal PCR strategies with LAMP being one of the most popular for fungi. LAMP is named for the dumbbell or loop-shaped intermediate that is formed by the product of the four or more primers used in the amplification reaction [66]. Instead of using a *Taq* polymerase, the reaction uses *Bst*, a polymerase that has high strand displacement activity and a temperature optimum of 60–65 °C. Because there are so many primers in the reaction, LAMP has great specificity. It is also relatively easy to perform, sensitive, and rapid, with reactions yielding product in as little as 10 min and, depending on detection method, products being visible with the naked eye. LAMP is also relatively resistant to common PCR inhibitors, which makes it a good assay for pathogens in blood specimens. The drawbacks include a ladder-like pattern on agarose electrophoresis if this detection method is used, which precludes size-based identification of target species, although there are a multitude of other detection methods, including fluorescence and turbidity [67, 68]. Multiplexing is possible; however, primer design requires an online algorithm and is more complicated than standard PCR or real-time PCR due to the large number of compatible primers that must be designed and integrated into the reaction. Numerous fungal species have been successfully targeted by LAMP assays including *Fonsecaea* spp., *Cladophialophora carrionii*, *Candida* spp., *Paracoccidioides brasiliensis*, *Cryptococcus* spp., *Pneumocystis jiroveci*, *Aspergillus fumigatus*, and *Histoplasma capsulatum* [69].

Rolling Circle Amplification (RCA)

RCA uses a circular DNA molecule as template in a mechanism similar to bacterial plasmid amplification [70]. The circular molecule is produced by the annealing of specialized probes called padlock probes to a linear molecular, usually a PCR product. The complex is then circularized at the ends of the probes by ligase. Amplification and strand displacement occurs through the polymerase activity of *Bst*, which is incubated at 60–65 °C and ultimately can yield a potentially massive amount of DNA. RCA is highly specific and sensitive to single mutations, which is valuable for distinguishing closely related species of fungi or different genotypes of the same species. The reaction is robust and requires little optimization [71]. Drawbacks include a more laborious assay when compared to other amplification strategies, since the template is a PCR product, which requires an additional step and handling to produce. The additional ligation step necessitates inclusion of ligase, which with the polymerase to produce the initial template, can increase cost. Fungi that have been investigated with RCA include agents of chromoblastomycosis and dermatophytosis, *Candida* spp., *Cryptococcus* spp., *Aspergillus* spp., and *Penicillium marneffei* [69].

Nucleic Acid Sequence–Based Amplification (NASBA)

NASBA is a third PCR method frequently used for fungal identification. NASBA was developed in 1991 and can be distinguished from other PCR methods by template difference. NASBA can use RNA or DNA as a template instead of just DNA alone [72]. Depending on target, if the ribosomal region is targeted, these sequences are multicopy

DNA sequences, which can enhance sensitivity. By inclusion of RNA templates as targets, target copy numbers can be increased further. By targeting the ribosomal locus, a sensitivity of 0.01 CFU/mL was reported for *C. albicans* using NASBA [73], an outcome that is possible because of the multicopy nature of ribosomal genes, which can be as high as 100 copies or more per single cell [74]. Reaction temperature is typically 41 °C, although a second temperature can be used. However, for each step of the reaction, temperature is constant. Reactions are complete in 1.5–3 h and usually use a fluorescent probe such as Molecular Beacons. Despite outstanding sensitivity, specificity is reduced, likely because of the low reaction temperature, which can allow primer mismatches. Furthermore, since RNA can be a target, care must be taken to avoid degradation of RNA templates during and after extraction. NASBA has been used to successfully detect *Candida* spp., *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Fusarium* spp., and *Alternaria* spp. [69, 73, 75].

Whole-Genome Sequencing

Clinically, the most common application presently of whole-genome sequencing (WGS) is in cancer therapy and diagnosing Mendelian diseases [76]. However, few platforms are as eagerly anticipated as WGS for microbial diagnostics. WGS is attractive, because it is possible to obtain all the genetic information that an organism has in a single assay. This information potentially provides multiple important assets that typically would need separate assays to produce. These include organism identification, drug susceptibility, genotyping, and epidemiology. As an example, in a single sequencing run, multiple drug resistance markers were detected in *C. glabrata*, which would have taken numerous separate PCR and/or sequencing experiments to detect [77].

WGS information can be obtained even from a background of other organisms and host cells, reducing the purification or enrichment burden of other assays, which can be swamped with nontarget nucleic acids. In fact, microbiome analysis includes sequence from all organisms in a local ecosystem. While mainly focused on bacteria, microbiome analysis has been extended to fungi. For example, Hager and Ghannoum [78] found a diverse set of fungi in the intestinal mycobiome covering the four major phyla (Ascomycota, Basidiomycota, Glomeromycota, Zygomycota) and 22 different orders, and have established a potential link between *Candida tropicalis* and Crohn's disease [79]. In the oral mycobiome, 74 different genera of fungi were recovered including human fungal pathogens in four different genera (*Candida, Aspergillus, Cryptococcus, and Fusarium*) [80]. Keum et al., investigated a potential microbial association with sensitive skin syndrome and found increased levels of *Mucor racemosus* and decreased levels of *Malassezia restricta*, a normal skin commensal [81]. However, because the GI tract, respiratory tract, and skin are nonsterile environments, nonpathogenic fungi will be found in these samples as environmental contaminants. Determining which of these fungi is of pathogenic significance will almost certainly involve the clinical microbiologist. An even more pressing issue will be determining which fungi are colonizers and which are truly invading, even when potential fungal pathogens are found, which is an extremely important challenge as it could affect antifungal selection. Normally sterile sites, such as blood, will not be affected by this issue, which can make data analysis easier. In the case of cell-free specimens of fungal DNA, such as plasma, these samples can serve as "liquid biopsies" when combined with WGS. Hong et al. used this strategy to screen for invasive fungal infections and successfully identified a number of molds associated with invasive fungal infections, which were confirmed by other assays [82]. The turnaround time for this strategy is a little more than 24 h and some of the advantages of the WGS of cell-free specimens approach is that molds can be detected as easily as yeasts [83], and taxonomic resolution to the species or even variety level, in addition to other benefits of WGS (determination antifungal susceptibility, epidemiology, etc.), is possible. However, to harvest this information, WGS must include a second component, which is the bioinformatics platform.

Because of the massive data that is generated by WGS, it is not possible to inspect output manually by eye. Instead, programs need to be linked to the data in order to identify and select relevant information from a massive background of information that would be of little diagnostic use otherwise. Some of these programs are transferrable from other applications such as SNP analysis, which is an important tool for investigating human genome information. In fungi, SNP analysis after WGS proved to be crucial for linking *Exserohilum rostratum* to contaminated methylprednisolone injections introduced during pharmaceutical compounding [84]. This technique also was used to link bloodstream infections caused by an outbreak of *Sarocladium kiliense* to contaminated antinausea medication in cancer patients [85]. Alternatively, custom programs can be written to perform specific tasks; however, this strategy requires good bioinformatics skills. Once WGS is mainstream, these programs will almost certainly be attached to specific platforms and can be run automatically to yield results in a sample to answer fashion.

While WGS has great promise as a diagnostic method, there are drawbacks. These include high capital costs, which will include a service contract with an annual fee, high run costs, a requirement for an experienced technician to do the sequencing, a heavy downstream analysis burden that requires sufficient computation resources, data storage, and

bioinformatics expertise, and long turnaround times compared to current assays or classical diagnostic methods. Because of the massive throughput of WGS, runs may be delayed until instrument run capacity is maximized to make runs more economical, which can delay results even further. In addition to these issues is a requirement for previously sequenced and annotated fungal genomes that can be used as reference sequences for comparative analysis of clinical sequence data. While virtually of all the common major systemic fungal genomes have been sequenced, less common and rarely encountered genome sequences remain a work in progress, particularly the annotation. Despite these challenges, WGS is a promising technology that should soon find a place in the clinical microbiology laboratory.

Summary

Molecular diagnostics can be defined as diagnostic assays directed toward the detection or identification of genomic variants that can be used to facilitate detection, diagnosis, epidemiology, or prognosis, in addition to being an important tool to monitor the response to therapy [86]. Molecular diagnosis of fungal diseases has lagged behind other microbes due mainly to the low frequency of mycoses compared to bacterial infections. However, the peculiarities of fungi have also inhibited the application of many molecular diagnostic platforms to fungi. However, as fungal infections continued to increase in frequency and severity, better and more efficient ways to identify the causative agents of fungal infections were, and continue to be, urgently needed. A particularly pressing reason for new assay development has been the increasing spectrum of fungal pathogens, which puts pressure on clinical microbiologists who likely have never seen rare fungi. Finally, the background data to build molecular diagnostic assays was slow to be developed for fungi compared to other microbes. The size, complexity, and variability of the fungal genome delayed the generation of genome sequences and the lack of researchers who could assist in annotating and developing genome sequences into useful tools also slowed the maturation of this resource. However, as genome sequences became available, other -omics data soon followed. Together, these resources are forming the bases for new diagnostic assays. The common fungi have been targeted first, and this approach has yielded assays with FDA approval. Secondary fungal pathogens are now being included in commercial assays, which are entering the market at increasing frequencies. It remains to be seen whether molecular diagnostics will supplant conventional fungal diagnostic and identification assays; however, these platforms have the capability to reduce human error, increase throughput and accuracy, and reduce turnaround time. When these attributes are properly balanced against cost, they are welcome and important additions to the clinical microbiology laboratory.

Acknowledgments Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number R01AI148641 to BLW. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

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Diagnostic Histopathology

5

John E. Bennett

Introduction

Diagnosis of mycoses using microscopy has the advantage of rapidity and often confers the advantage of showing that the fungus is invading tissue, not colonizing bronchi, pulmonary cavities, paranasal sinuses or surfaces of the skin or mucous membranes. Fungal contaminants that grow on culture are often too sparse to be seen microscopically, so the problem of contamination is less with microscopy. Some fungi are difficult (e.g., mucormycosis, histoplasmosis) or impossible to grow (e.g., lobomycosis) and are best identified by microscopy. However, microscopic diagnosis depends on the skill of the observer and the fungal species. Yeasts are easier to identify on smear or histopathology than molds.

Specimen Preparation

Wet Mounts and Touch Preparations

Wet mount is a smear of liquid specimen or, for ringworm, scrapings of skin or nail. Digestion with potassium hydroxide is the oldest method but calcofluor white staining with UV microscopy is far superior. Touch preparations are done by touching a clean microscope slide with fresh tissue, usually a biopsy specimen, and staining with calcofluor (Fig. 5.1)

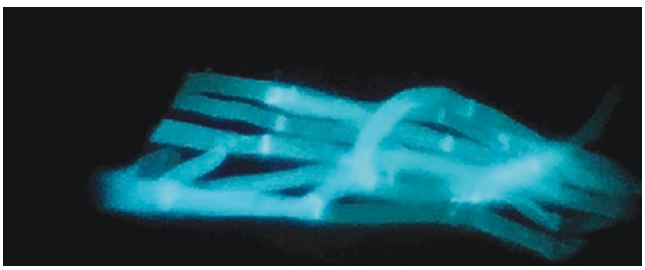


Fig. 5.1 Touch preparation of skin biopsy tissue showing septated hyphae of Fusarium solani stained with calcofluor-white and viewed with UV light

Cytopathology

Needle biopsies and aspirates, as well as bronchoalveolar lavage, sputum, and urine may be sent for cytopathology. The cells to be examined may be cytocentrifuged and placed onto a glass slide or centrifuged into a pellet, fixed, mounted in paraffin, sectioned, and placed on a slide. With cytocentrifuge specimens, only one or two slides may be available, limiting the number of stains.

Histopathology

Pieces of tissue are fixed in formalin, embedded in paraffin, and cut sections mounted on slides and stained. If the tissue is large enough, multiple sections may be stained and sent for review outside of the institution. Histopathology also provides the opportunity for assessment of the associated inflammatory response.

Host Inflammatory Response

The inflammatory response varies between tissues of the same patient and also between patients. What are listed below are general characteristics that often help confirm the identity of the fungus seen on special stains. Nothing about the inflammation distinguishes mycoses from other pathogens such as tuberculosis or noninfectious diseases.

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_5

Pyogenic

Some fungi typically elicit a neutrophilic response around the fungus, provided that the patient is not granulocytopenic. Histopathology may show neutrophils around the fungus even when the surrounding response is granulomatous, forming a "pyogranuloma." Clusters of neutrophils are usually in a necrotic area (i.e., an abscess). Pus seen on clinical examination is an indication of a neutrophilic response and is more likely to be seen in blastomycosis and coccidioidomycosis, for example, than in histoplasmosis or cryptococcosis.

Granulomatous

This word is not clearly defined among pathologists. In cytopathology, it may mean only clumps of macrophages. In histopathology, "granuloma" usually indicates multinucleated giant cells, lymphocytes and macrophages, particularly the long slender macrophages termed "epithelioid cells." A "well-organized" or "tuberculoid" granuloma means a round cluster of epithelioid cells in whorls, usually with multinucleated giant cells. The most distinctive granuloma, a "caseous granuloma," is a rounded lesion with a central core devoid of cellular detail, ringed by columnar epithelioid cells, and surrounded by lymphocytes, plasma cells, macrophages, and multinucleated giant cells. Caseous granulomas should suggest histoplasmosis or tuberculosis, though they are not unique to these.

Hoeppli-Splendore Phenomenon

Hoeppli-Splendore phenomenon is amorphous eosinophilic coating around organisms. It is characteristicly seen with hyphae in basidiobolomycosis and conidiobolomycosis, but also occasionally seen around yeast cells in sporotrichosis. Schistosomiasis and other chronic nonmycotic infections may also show this feature.

Grains

Grains are tight clusters of organisms, characteristic of actinomycosis and mycetoma, including those caused by fungi (i.e., eumycetoma) and higher bacteria (i.e., actinomycetoma). Deep dermatophyte lesions may also produce grains. Loose clusters of bacteria in very chronic infections are called "botryomycosis."

Stains

Hematoxylin and Eosin (H&E)

H&E is a standard stain used in histopathology, which is useful in identifying the inflammatory response and the presence of malignant cells, but is much less sensitive for visualizing fungal cells. Although fungi are not easily identified on H&E, sometimes, the purple of hematoxylin will stain hyphae, and sometimes *Blastomyces* species or other yeast cells. The golden color of heavily melanized hyphae or "copper penny" chromoblastomycosis cells is better seen on H&E than on GMS or PAS.

Gomori Methenamine Silver (GMS)

GMS is the best stain for finding fungi and seeing septae in hyphae, which appear black or dark brown. GMS can stain other structures, which may be misidentified as fungi. Overstaining is common and GMS stains numerous artifacts, reticulum fibers, and neutrophils. If a structure is not stained by GMS, it is rarely a fungus. As a rare exception, hyphae of mucormycosis may not stain with GMS. The inflammatory response is difficult to discern on GMS.

Periodic Acid Schiff (PAS)

PAS stains fungal cell wall polysaccharides red. Fungi are often more difficult to locate in many tissues with PAS when compared with GMS, but this stain typically allows the evaluation of the inflammatory response. Lipid droplets, glycogen storage vacuoles, and some artifacts also stain with PAS and may resemble yeasts.

Mayer's Mucicarmine Stain

Mucicarmine stain used in identifying mucinous adenocarcinoma can also help identify yeast cells as *Cryptococcus neoformans* or *Cryptococcus gattii*. Staining is not uniform among cryptococci in the same section and sometimes, cells are not visibly stained at all. The process of tissue fixation and paraffin removal shrinks the polysaccharide capsule around the cryptococcal cell, providing a reddish rim but the wall itself is not stained.

Masson-Fontana

Agents of phaeohyphomycosis may stain brown with Masson-Fontana stain, allowing the differentiation of their hyphae from those of the hyalohyphomycosis, such as *Aspergillus*, *Fusarium*, and *Scedosporium* spp. Cryptococci may also stain brown with this stain. The contrast is generally not sufficient to make this a useful stain for finding fungi in histopathology sections. The inflammatory response is also not well seen with this stain.

Brown and Brenn Stain (Brown and Hopps, Tissue Gram Stain)

Brown and Brenn is a poor stain for fungi, which may appear gram positive or gram negative in tissue. Candida yeast and pseudohyphae are usually gram positive.

Histopathological Characteristics of Deep Fungal Pathogens

Yeast-Like Cells in Tissue

Blastomycosis

In the skin, *Blastomyces* species are often in small subcutaneous abscesses, surrounded by neutrophils. Differentiation between *Blastomyces*, *Cryptococcus* and *Histoplasma* spp. can be difficult if only a few cells are seen. *Blastomyces* yeast cells have a thick, refractile cell wall and have initially a broad pore between mother and daughter cells (Fig. 5.2). The daughter cells remain attached until the daughter is nearly as large as the mother cells, with the pore narrowing and disappearing as the attached daughter cell enlarges. A pseudoepitheliomatous response is prominent in the dermis and may be mistaken for squamous cell carcinoma. In the lung, a granulomatous response predominates, but collections of neutrophils are also present, producing a "pyogranulomatous response." Cavitation and fibrosis are common in chronic lung lesions, but calcification in lung or lymph nodes is rare, if it occurs.

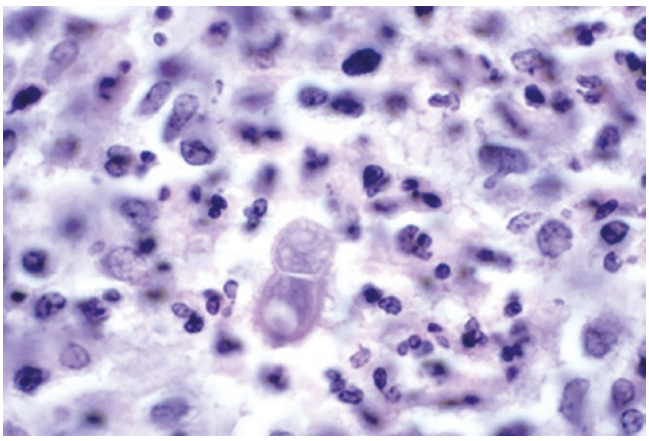


Fig. 5.2 Budding cell of *Blastomyces dermatitidis*. Note that the daughter cell is almost as large as the mother cell and still connected. H&E. From MB Smith, MR McGinnis. Diagnostic histopathology. *In* Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer

Candidiasis

Pseudohyphae and yeast cells are seen in invasive candidiasis. Pseudohyphae differ from true hyphae in having constrictions at the septae and showing branching only at points of septation. Yeast cells, also called blastospores or blastoconidia, may bud off pseudohyphae at septae (Fig. 5.3). Yeast cells are also seen separately in the tissue, along with pseudohyphae. Yeast cells are round or elliptical, smaller than *Blastomyces*, and may show budding or even chains of budding cells. Pores between mother and daughter yeast cells are too small to be seen readily. Yeast cells are rarely seen without pseudohyphae, with the exception that *Candida glabrata* produces only yeast cells (Fig. 5.4). If patients are not granulocytopenic, the predominant inflammation is neutrophilic. *Magnusiomyces*, *Saprochaeta*, and *Trichosporon* have elongated, arthroconidia-like structures in tissue that can be mistaken for mold hyphae or *Candida* pseudohyphae.

Chromoblastomycosis

The most distinctive structures are thick-walled golden hued cells in the dermis, called "copper penny" cells, often surrounded by neutrophils and a granulomatous response (Fig. 5.5). The hyperplastic epidermis sometimes contains short, wavy "fumigoid" hyphae.

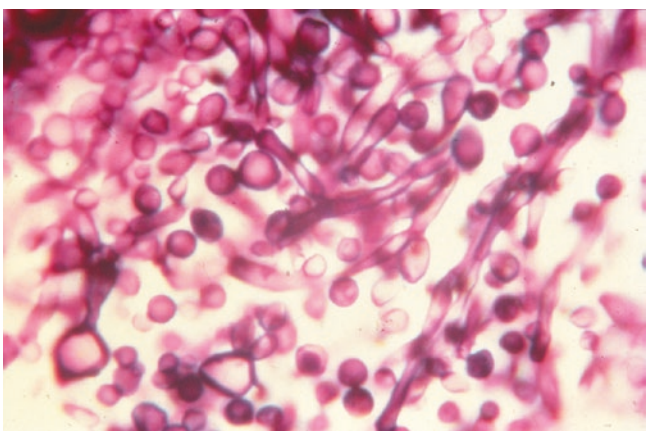


Fig. 5.3 *Candida albicans* pseudohyphae and blastospores. Note constrictions along the pseudohyphae. GMS

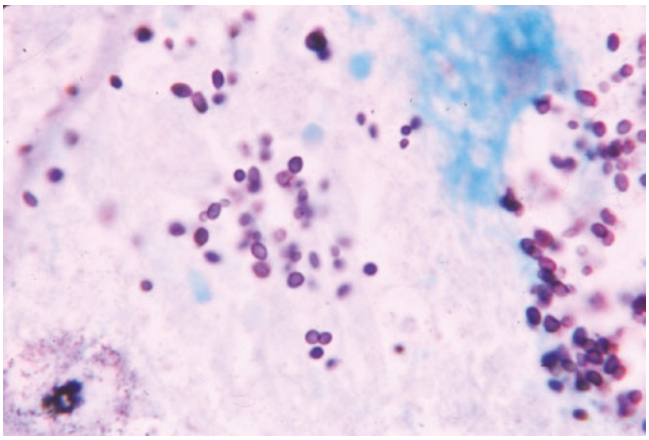


Fig. 5.4 *Candida glabrata* in a cardiac valve vegetation. Note the absence of pseudohyphae. GMS

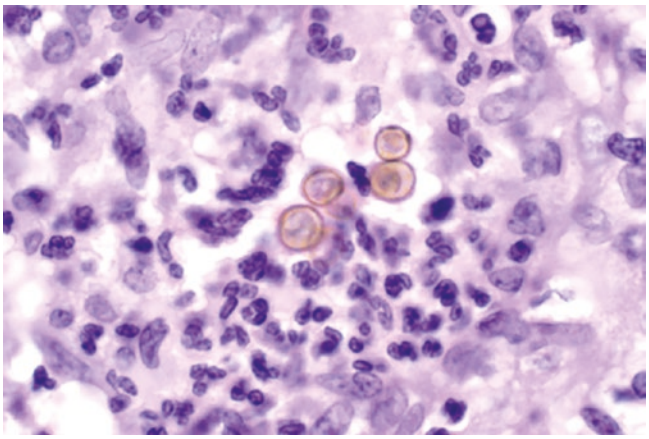


Fig. 5.5 Brown thick-walled "copper penny" cells of chromoblastomycosis in the dermis. H&E

Coccidioidomycosis

Spherules are all round, nonbudding, and range in size from that of *Blastomyces* or *Cryptococcus* to several times that large. Rounded endospores may be seen inside the larger spherules (Fig. 5.6). Neutrophils clusters around endospores released from ruptured spherules. Large pockets of pus may form in the tissue. Granulomatous inflammation or pyogranulomas are usual, similar to that seen with blastomycosis. Tissue eosinophilia is often present on H&E stain but easily missed. Caseous necrosis may be seen in chronic lesions, resembling tuberculosis, but calcification is not seen. Short hyphae are occasionally seen in *Coccidioides* lesions. Rounded structures containing endospores also can be seen with *Prototheca* species, *Rhinosporidium seeberi*, a protistan parasite of mucous membranes (Fig. 5.7), and with *Emergomyces crescens*, the fungus causing adiaspiromycosis of the lung.

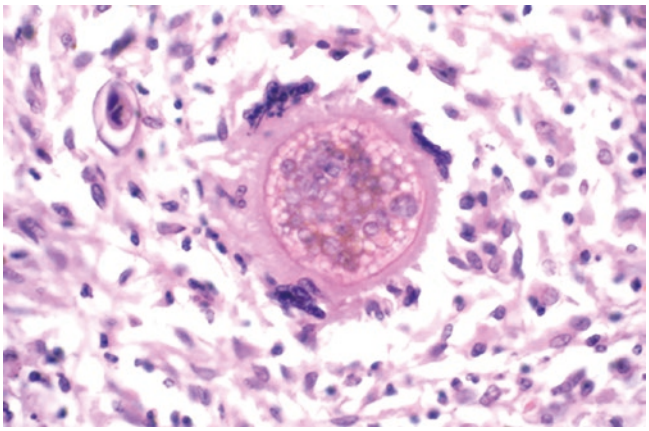


Fig. 5.6 Large spherule of *Coccidioides* species containing endospores. H&E. From MB Smith, MR McGinnis. Diagnostic histopathology. *In* Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer

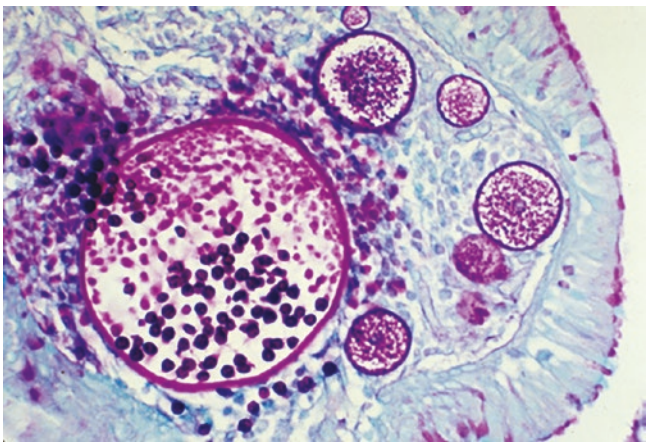


Fig. 5.7 Large cyst of *Rhinosporidium seeberi* containing spores. PAS. From MB Smith, MR McGinnis. Diagnostic histopathology. *In* Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer

Cryptococcosis

The yeast cells sometimes are obvious from the space around the cell, previously occupied by capsule that shrank around the cell during fixation (Fig. 5.8). The capsule is easiest to detect in cells within the cytoplasm of phagocytes and in the giant "bubbles" of cryptococci in brain tissue. The yeast size overlaps *Blastomyces*, *Candida*, small *Coccidioides* spherules, *Paracoccidioides*, and larger *Histoplasma* cells. Definite staining with Mayer's mucicarmine can be very helpful. If mucicarmine is negative, Masson Fontana staining can be useful. In the lung, dense granuloma formation is usual, sometimes with clusters of cryptococci resembling caseous necrosis. Pyogranulomas, calcification, and clusters of neutrophils are not seen.

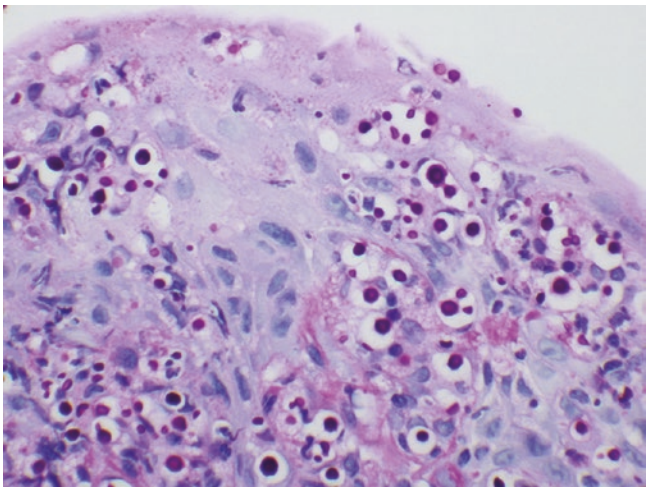


Fig. 5.8 Cryptococcal cells in a laryngeal biopsy stained red with PAS. Note the clear spaces around the yeast where the capsule had been before shrinkage during fixation

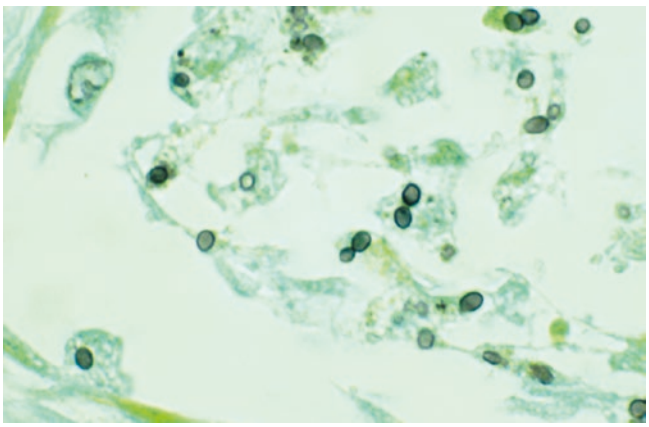


Fig. 5.9 *Histoplasma capsulatum* with an unusually large number of budding cells. GMS. From MB Smith, MR McGinnis. Diagnostic histopathology. *In* Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer

Histoplasmosis

The yeast cells of *Histoplasma* are smaller than those of *Blastomyces* or *Cryptococcus*, but similar in size to *Pneumocystis jirovecii*, *Candida glabrata*, *Talaromyces marneffei*, *Emergomyces species*, *Malasezzia furfur*, and the protozoa, *Leishmania* and *Trypanosoma cruzi* (Figs. 5.9 and 5.10). Even though *Histoplasma* is a budding yeast, unlike *Pneumocystis* or protozoa, pores between mother and daughter cells are rarely visible. Distinction between budding cells and cells simply touching one another may be difficult to discern in clustered cells. Location outside the lung is rare for *Pneumocystis*, where the cells appear as clusters in alveloli. *Malasezzia* is rarely visualized outside the skin. *C. glabrata* elicits a pyogenic inflammation, not seen with histoplasmosis. *Leishmania* and the amastigotes of *T. cruzi* stain with PAS but don't stain with GMS and contain a distinctive bar-shaped structure, a kinetoplast, that is visible on H&E, but better seen on Giemsa stain. *P. marneffei* has a distinctive geographic distribution in Southeast Asia and multiplies by fission, not by budding (Fig. 5.11). *Emergomyces species* are dimorphic fungi that closely resemble *H. capsulatum* in tissue. Valvular vegetations in *Histoplasma* endocarditis may show only short hyphae, incorrectly suggesting a mold endocarditis. The inflammatory response in histoplasmosis resembles tuberculosis and runs the gamut from macrophages packed with yeast cells to epithelioid granulomas or caseation necrosis with rare organisms. Calcification of old granulomas in the lung, hilum, or mediastinum is common. Fibrosis can be extensive in the lung or mediastinum.

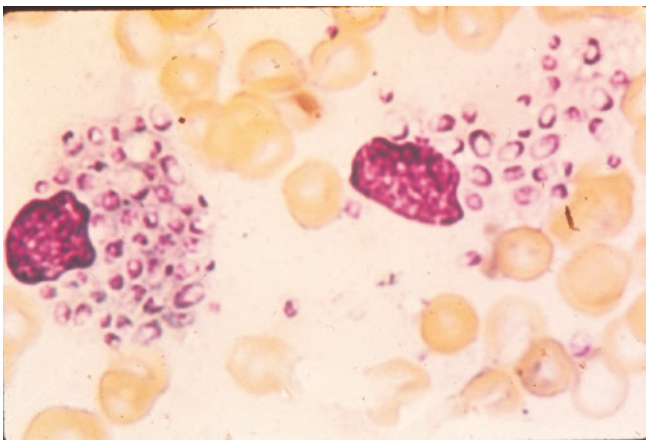


Fig. 5.10 *Histoplasma capsulatum* cells in cytoplasm of macrophages. Giemsa stain

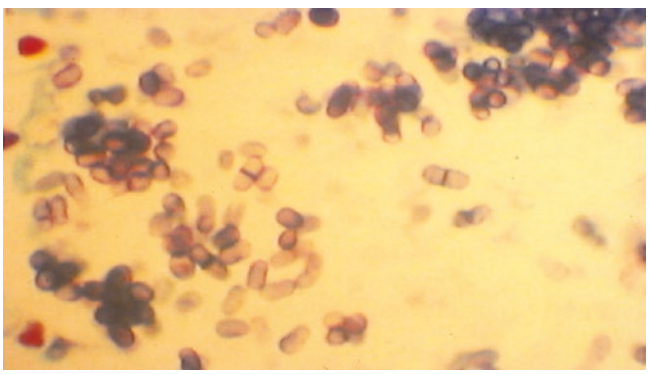


Fig. 5.11 *Talaromyces marneffei*. Note cells dividing by fission with septum between mother and daughter cell. GMS

Paracoccidiodomycosis

The appearance and associated inflammation seen with *Paracoccidioides* in tissue is very similar to that observed in blastomycosis, the single difference being that a single cell may be budding from more than one site on the cell wall of the *Paracoccidioides* yeast. The pore between mother and daughter cell is tiny and the daughter cell separates while still small (Fig. 5.12). When one cell has multiple buds, the cell may resemble the "pilot wheel" of a ship, though such structures are rare in tissue.

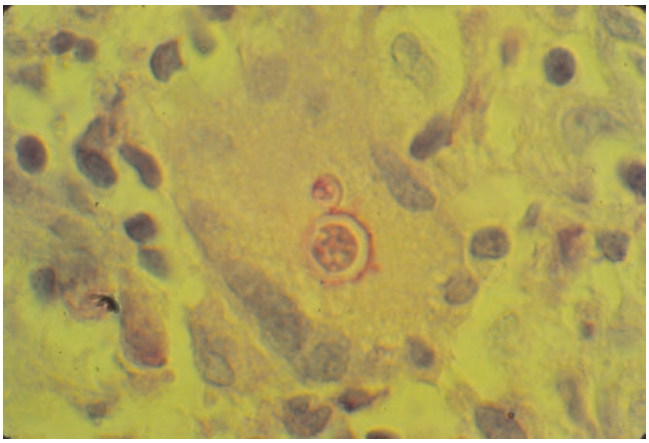


Fig. 5.12 *Paracoccidioides brasiliensis* budding cell. Note narrow attachment of the small daughter cell. PAS

Sporotrichosis

Budding yeast cells of *Sporothrix* species are elliptical (cigar-shaped) or spherical (Fig. 5.13). Organisms are abundant in extracutaneous sites but so scant in skin lesions that multiple GMS-stained sections may be needed to locate them. The usual inflammatory response is a pyogranuloma.

Hyphae in Tissue

Identifying molds in tissue is fraught with errors. Air-containing structures, such as lung cavities, paranasal sinuses, and the external auditory canal, may permit sporulation, allowing identification of *Aspergillus* or *Scedosporium* hyphae by their spore-bearing structures. Bundled hyphae, called synnemata, may appear in *Scedosporium* and related molds when growing in air-containing structures. The broad, aseptate hyphae of basidiobolomycosis and conidiobolomycosis may be distinguished by the tissue eosinophilia and Hoeppli-Spendore phenomenon. In theory, hyphae of mucormycosis should be distinguished by being pauciseptate, broad, of variable width, lacking Y-shaped (less than 90°) branching, and their ability to make sharper bends in tissue than many other molds (Fig. 5.14). In practice, mucormycosis is commonly mistaken for other molds and vice versa. Hyphae of phaeohyphomycosis often contain bulbous swellings and stain brown with Masson Fontana (Fig. 5.15). Other fungi appearing as septate hyphae include *Aspergillus*, *Fusarium*, *Scedosporium*, *Lomentospora*, *Alternaria*, and in skin biopsies, dermatophytes (Fig. 5.16). Most commonly, hyphae in tissue cannot be identified with certainty by morphology alone. Immunocytochemistry has not proven to have adequate spec-

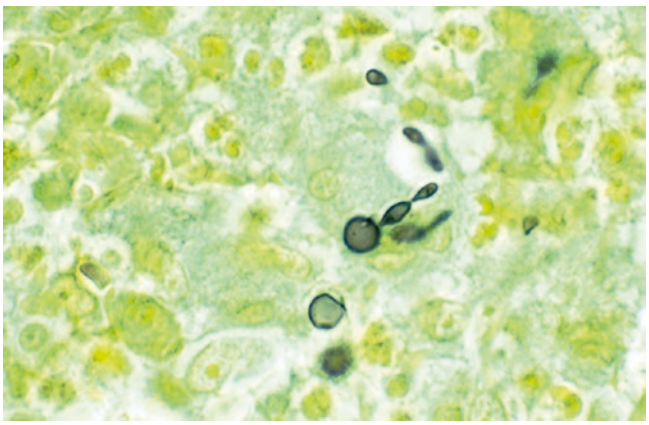


Fig. 5.13 *Sporothrix* species. Both spherical and elongated cells are seen here but either shape may occur alone. GMS. From MB Smith, MR McGinnis. Diagnostic histopathology. *In* Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer

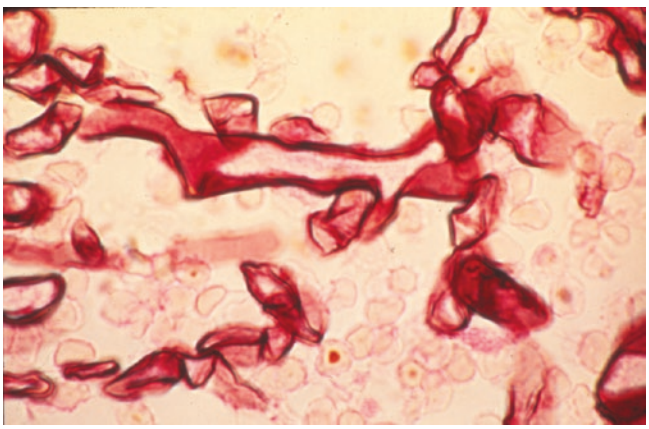


Fig. 5.14 Mucormycosis. Note variable hyphal width, absence of septae, and sharp turns. GMS

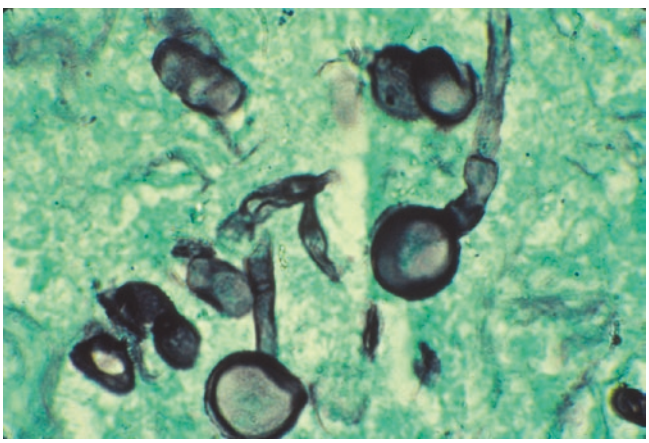


Fig. 5.15 Phaeohyphomycosis. *Curvularia lunata*. Note bulbous swellings of hyphae. GMS

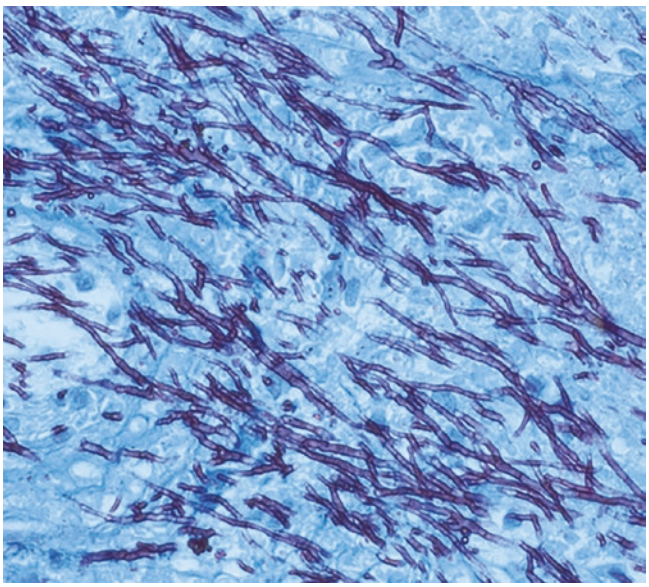


Fig. 5.16 *Aspergillus fumigatus*. Note straight hyphae of even diameter. GMS. From MB Smith, MR McGinnis. Diagnostic histopathology. *In* Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer

ificity. Molecular techniques on fresh or fixed tissue are the best option for species identification of molds in tissue (see Chap. 4).

Acknowledgment This chapter was supported by the Division of Intramural Research, NIAID, NIH, Bethesda, Maryland.

Suggested Readings

Connor DH, Chandler FW, Manz RJ, Schwartz DS, Lack EE, editors. Pathology of infectious diseases. Stanford: Appleton & Lange; 1997.

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Diagnostic Immunology

6

Michael Conte, Christopher D. Pfeiffer, and Brian Wong

Introduction

The frequency of invasive fungal infections has risen dramatically in recent decades, mostly because of a larger population of at-risk patients who are immunocompromised, neutropenic, or critically ill. For clinicians evaluating these patients, it has become increasingly important to make the diagnosis early so that timely antifungal therapy can be instituted. Although culture of body fluids or tissue for the causative fungus continues to be the gold standard for definitive diagnosis, this process can sometimes take several weeks for results and often lacks sufficient sensitivity. For example, blood cultures are positive in only ~50% of cases of invasive candidiasis (IC) and very rarely in cases of invasive aspergillosis (IA) [1, 2]. A presumptive diagnosis can also be made on the basis of characteristic histopathology and special tissue stains. However, obtaining adequate samples from protected anatomical sites is often not feasible in the populations at highest risk for such infections. Non-culture-based diagnostic tests are classified into four groups based on what component of the invading pathogen or host immune response they target. These include detection of host antibody, fungal antigen, fungal metabolites, or fungal nucleic acid. Overall, despite these multiple potential targets and extensive efforts toward development, relatively few non-culture-based tests have proven clinically useful, and even fewer have reached commercial availability. As current diagnostic techniques are less than ideal, development of new methods is a priority in medical mycology. This chapter outlines the available immunologic tests based on what component of the invading pathogen or host immune response they target and provides some discussion of their strengths and weaknesses. Given the growing interest in this field, there is also some introduction to newer assays that are currently being investigated. Specific recommendations for utilizing the currently available tests in conjunction with the culture and histopathology are discussed for individual fungal species and specific disease manifestations.

Host Antibody Testing

Many tests in current use have been developed to detect host antibodies against specific fungal antigens. These require identification of one or more distinctive antigens to which host antibodies are directed, sufficient immunocompetence on the part of the host to mount a specific antibody response, and the use of a variety of techniques to detect the antibody. Tube precipitin (TP) assays, immunodiffusion (ID) assays, complement fixation (CF) assays, radioimmunoassays (RIAs), and enzyme-linked immunosorbent assays (ELISAs/EIAs) are some such techniques. One major limitation of this general approach is that many immunocompromised patients have impaired abilities to mount specific antibody responses. Moreover, these responses may be delayed and antibodies do not necessarily distinguish acute from chronic or a past history of infection. Finally, antibodies to some fungal antigens may be demonstrable in uninfected people, thereby reducing the diagnostic specificity of positive tests.

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_6

Fungal Antigen Testing

A second common method to diagnose fungal infection includes tests that use immunologic reagents to identify antigenic components of the fungus. These require the presence of unique antigens in a body fluid or tissue specimen that is available for testing and use of a variety of techniques to detect these antigens. Some such techniques include latex agglutination (LA) assays, dot immuno-binding assays, ELISA/EIA, RIA, and lateral flow immunoassays/devices (LFAs/LFDs). Monoclonal or polyclonal antibody is often needed in these assays to help detect the antigen of interest. The major limitations of this general approach are the low level and transient nature of antigenemia in some hosts, cross-reactions between antigens derived from different fungal species, and lack of specificity for a particular antigen when polyclonal antibodies are used.

Fungal Metabolite Testing

Another methodology includes the direct detection of fungal metabolites in patient serum or other samples. These are usually by-products of a specific fungus, which are detected either by gas chromatography, mass spectrometry, or an enzymatic reaction. One limitation of these tests is that the metabolic products are not unique to individual fungal species and may be present in small amounts in uninfected individuals. Furthermore, the level of metabolite may not be present in sufficient quantity outside of the local tissue being invaded, making detection difficult.

Fungal Nucleic Acid Testing

There has been a rapid expansion of diagnostic tests targeting fungal nucleic acid, which is now discussed elsewhere in this book (see Chap. 4).

Pan-Fungal Testing: 1,3-β-D-glucan

1,3-β-D-glucan (BG) is a major component of the cell wall of many different fungi. The ability of fungal BG to activate Factor G, an enzyme in the clotting cascade of the horseshoe crab, has led to the development of assays capable of detecting very small amounts of BG, to be utilized as a biomarker of infection. Five separate assays are commercially available and each assay uses individualized cutoff values to define positivity. Available assays include Fungitell and Glucatell (Associates of Cape Cod, East Falmouth, MA), Fungitec-G (Seikagaku, Tokyo, Japan), Wako test (Wako Pure Chemical Industries, Osaka, Japan), and the Dynamiker Fungus assay (Dynamiker Biotechnology Ltd., Tianjin, China) [3]. Fungitell is the current assay available for patient care in the USA. The primary clinical scenarios where the accuracy of BG has been evaluated are discussed below and summarized in Table 6.1.

Overall, diagnostic accuracy BG for invasive fungal disease (IFD) has been shown to have moderate sensitivity and specificity, that is, 75–83% and 81–87%, respectively [4–8]. The utility of BG was the subject of a Cochrane systematic review of BG diagnosing IFD in immunocompromised or critically ill people. However, due to the variation of the studies, no conclusions could be drawn on BG diagnostic accuracy, with the exception for the subgroup of 10 studies evaluating IC as discussed below [3]. Because of the low pretest probability for IFI (5–15% in many clinical settings), the BG test has a high negative predictive value (i.e., if the test is negative, the likelihood of IFD is quite low). A major limitation of the BG is its inability to differentiate between different species of fungi. This is because BG is a component of the cell walls of many but not all clinically relevant fungi, including *Candida*, *Aspergillus*, *Pneumocystis*, *Trichosporon*, *Fusarium*, and *Saccharomyces* species, Importantly, BG is not found in *Cryptococcus*, the Mucorales, or the yeast form of *Blastomyces*. False-positive results are associated with receipt of hemodialysis, recent surgery, exposure to immunoglobulin or albumin products, receipt of certain medications (e.g., amoxicillin-clavulanic acid), and bacteremia [12, 17–23].

Diagnosis of IFD in High-Risk Hemato-Oncological Patients

In the hematologic malignancy population, BG evaluation in case-control studies has demonstrated a wide range of sensitivity (50–90%) and specificity (70–100%) [24–26]. A previous meta-analysis that included six cohort studies found the diagnostic performance of two consecutive BG assays was superior to the performance of one test alone for the diagnosis of proven or probable IFD; the sensitivity and specificity of two consecutive positive tests were 49.6% (95% CI, 34.0–65.3%) and 98.9% (95% CI, 97.4–99.5%), respectively [9]. When a threshold of only one positive test was applied, the BG sensitivity and specificity were 61.5% and 90.8%, respectively. A more recent meta-analysis including a total of 19 cohort and case-control studies with a mixture of single and multiple positive BG testing thresholds aimed to reduce heterogeneity by evaluating studies with a single type of assay (Fungitell) and only patients with malignancy. The authors concluded the BG assay diagnostic accuracy in hemato-oncologic patients is moderate with an overall sensitivity of 83% (95% CI, 74–89%) and specificity of 79% (95% CI, 64–88%); when restricting to low bias studies only, sensitivity remained

Table 6.1 Studies of serum 1,3-β-D-glucan (BG) for invasive fungal infection (IFI) and *Pneumocystis jirovecii* pneumonia (PJP)

Population	Assay	Study design	Sen. (%)	Spe. (%)	Comments[a]	References
All	Any	Meta-analysis of 16 studies: 2979 patients; 594 with proven/probable IFI. Excluded diagnosis of PJP	77	85	1 positive test required; study population = 11 hemato-oncologic; 5 at risk for IC; 1 transplant; 1 miscellaneous; test performance for detection of IC and IA was similar.	[4]
	Any	Systematic review and meta-analysis of 31 studies: uncertain number of patients	80	82	1 positive test using the test cutoff that offered the best test performance in each individual study; diagnostic accuracy was similar for IC vs. IA; sensitivity and specificity were lower (72% and 78%, respectively) when analysis was restricted to 17 cohort studies.	[5]
	Any	Meta-analysis of 11 prospective cohort studies with 1068 patients	75	87	Subgroup analysis with Fungitell[b] only (6 studies; Sn 82%, Sp 86%), IC only (4 studies; Sn 80% and Sp 77%), and IA only (6 studies; Sn 73% and Sp 81%).	[6]
	Any	Systematic review and meta-analysis of 28 studies, 896 IFI positive out of 4214 patients	78	81	1 positive test required.	[7]
	Any	Meta-analysis of 37 case control or cohort studies: uncertain number of patients	83	81	Significant heterogeneity found for sensitivity (I^2 = 83.5%) and Specificity (I^2 = 95.5%).	[8]
High-risk hemato-oncologic	Any	Systematic review and meta-analysis of 6 cohort studies: 1771 patients analyzed; 215 had proven or probable IFI	62	91	1 positive test required.	[9]
			50	99	2 consecutive positive tests required.	
	Fungitell[b]	Systematic review and meta-analysis of 19 studies with 3040 patients, 22% positive for IFI	83	79	When excluding studies of potential high risk of bias, sensitivity = 80% and specificity 63%.	[10]
High-risk intensive care unit (ICU)	Fungitell[b]	Prospective cohort: 95 ICU patients with length of stay >5 days; 16 with IFI (IC = 14)	93	94	1 positive test required; analysis performed for proven IC only.	[11]
	Fungitell[b]	Prospective, randomized: 64 ICU patients, 6 developed proven/probable IC	100	75	2 sequential positive tests required; those with positive tests received empiric antifungal (anidulafungin) therapy.	[12]
At risk for PJP	Any	Meta-analysis of 14 studies: 1723 controls (with other medical conditions) and 357 PJP cases	95	86	1 positive test using test cutoffs according to (or closest to) the manufacturers' instructions required; HIV status did not impact accuracy.	[13]
	Any	Systematic review and meta-analysis of 23 studies with a total 997 patients. 7 studies were exclusively PLWH	91	79	PLWH had sensitivity of 94% and specificity of 83%. Non-HIV patients had sensitivity of 86% and specificity of 83%.	[14]
	Any	Meta-analysis of 13 studies, 5 were exclusively PLWH only	91	75	PLWH had sensitivity of 92% and specificity of 78%. Non-HIV patients had sensitivity of 85% and specificity of 73%.	[15]
At risk for PCM	Fungitell	Prospective cohort of 29 patients with PCM (17 active, 12 chronic)	97	n/a	Collected at baseline, 3 months, and 6 months. Considered positive if baseline test >60 pg/ml. Did not have control group to detect specificity.	[16]

IC invasive candidiasis, *IA* invasive aspergillosis, *IFI* invasive fungal infection, *PJP Pneumocystis jirovecii* pneumonia, *PCM* Paracoccidioidomycosis, *Sen.* sensitivity, *Spe.* specificity

[a] Positive test cutoff according to the manufacturer's instructions used unless otherwise noted

[b] Associates of Cape Cod, East Falmouth, MA

at 80% yet specificity decreased to 63% [10]. The use and interpretation of BG must be mindfully considered in context of local factors specific to a hemato-oncologic program's fungal prophylaxis and detection strategy.

Diagnosis of Invasive Candidiasis (IC) in High-Risk Intensive Care Unit (ICU) Patients

Several reports have evaluated BG for diagnosis of IFD, primarily invasive candidiasis (IC), in the ICU setting. As mentioned above, in the Cochrane systematic review, a subgroup analysis of IC (10 studies, 8 of which involved at risk ICU patients) was determined BG to have a sensitivity of 81.3% (95% CI, 75.3–86.0%) and specificity of 64.1% (55.6–71.8%) [3].

In a prospective study of 95 nonneutropenic ICU patients with signs of sepsis and a length of stay >5 days, 16 (17%) were diagnosed with IFD (14 IC, 1 aspergillosis, 1 fusariosis). In this setting, a single BG test demonstrated 94% sensitivity and 93% specificity; moreover, for all 13 candidemia cases, the BG assay was positive 24–72 h before a positive blood culture result [11]. Additionally, a prospective randomized pilot study was performed and involved 64 ICU patients, 6 of whom developed proven or probable IC [12]. Optimal assay performance in that study was found to use two sequential positive BG tests; the sensitivity for IC was 100% and specificity 75%.

Using a different approach, several studies have explored the application of single or consecutive BG testing as a tool to discontinue empiric antifungal therapy in the ICU setting [27–29]. In the most recent IDSA guidelines for management of candidiasis, the patients where one should consider stopping empiric antifungal therapy are those not responding to antifungal therapy at 4–5 days and either do not have evidence of IC or have a negative BG [30].

BG also has been evaluated in concert with other *Candida*-specific immunoassays targeted at IC diagnosis—refer to the IC section below for further discussion. Finally, in the 2020 EORTC/MSG update, two consecutive positive serum BG (Fungitell) assays are recognized as a mycologic criterion to support a diagnosis of IC. One positive BG of >80 pg/ml alone might be suitable for diagnosing probable IC in particular groups at >10% risk (upper GI perforation, recurrent anastomotic leaks, and necrotizing pancreatitis) [31].

Diagnosis of *Pneumocystis jirovecii* Pneumonia (PJP)

In the setting of highly active antiretroviral therapy, the epidemiology of PJP has shifted from patients living with HIV (PLWH) toward immunocompromised individuals of other etiologies, such as transplant recipients, malignancies, and autoimmune conditions requiring exogenous immunosuppression. Building upon many individual studies, there have been two recent systematic reviews evaluating BG diagnostic accuracy stratified by PLWH versus otherwise immunocompromised. The first determined a pooled BG sensitivity for those with HIV to be 92% and specificity of 78%; those without HIV recorded a sensitivity of 85% and specificity of 73% [15]. The second meta-analysis included 23 studies and resulted in a similar conclusion, calculating a BG diagnostic sensitivity of 94% in PLWH versus 86% in those without HIV. Specificity was similar at 83% for both groups [14]. Given the higher sensitivity, BG testing may be sufficiently sensitive to effectively exclude a diagnosis of PJP without bronchoscopy in PLWH but should be interpreted more cautiously in immunocompromised individuals of other causes.

In the 2020 EORTC/MSG update, a serum BG (Fungitell) ≥80 pg/ml in two consecutive serum samples, again in the right clinical context as with IC, fulfills mycological criterion to support the diagnosis of PJP pneumonia [31].

Conclusions and Recommendations

Overall BG has some degree of clinical utility in three major clinical scenarios: (a) monitoring for general IFD in hematologic malignancy, often in concert with *Aspergillus* GM, (b) screening ICU patients for evidence of IC, and (c) as a non-invasive diagnostic test for PJP.

The latter 2 scenarios are included as acceptable mycologic criterion to support diagnoses of IC and PJP as per the updated EORTC/MSG clinical diagnostic criteria, noting that these criteria are consensus criteria developed to enable comparison of epidemiologic, diagnostic, and interventional study findings and not to directly guide clinical care.

See Box 6.1 for recommendations on how test use is recommended.

Box 6.1 Recommendations for Using the Serum 1,3-β-D-glucan (BG) Assay

1. A positive test generally indicates IFI but is not pathogen specific; always interpret the results in the context of other clinical and laboratory findings.
2. Serum BG can be employed as part of IFI surveillance in a similar fashion to serum GM (e.g., perform1–2/week on at-risk hemato-oncologic patients). The test has moderate accuracy in this setting.
3. Serum BG appears to be a sensitive test when incorporated into a screening protocol for ICU

patients at high risk for invasive candidiasis. However, one positive test alone, particularly early in the ICU course, may be falsely positive and should ideally be confirmed with a second test. Conversely, two negative tests may aid in stopping empiric antifungals in the ICU setting.

4. Serum BG has excellent performance characteristics for the diagnosis of PJP in PLWH and may be particularly helpful in ruling out disease for patients suspected of PJP when BAL fluid cannot be readily obtained. Note that sensitivity is slightly lower for PJP in non-HIV immunocompromised populations.

Invasive Candidiasis (IC)

Candida species are commonly isolated from the bloodstream of hospitalized patients in the United States, an estimated 7.0 cases per 100,000 persons in 2017 for a total of ~23,000 leading to a high degree of morbidity and mortality [32]. Rapid detection of IC is critical and warrants the development of culture-independent diagnostic approaches. Recent attention has focused primarily on the pan-fungal BG assay (discussed above), while other *Candida*-specific immunodiagnostics (discussed below) have garnered less success.

Antibody Detection

Antibody detection assays have focused on mannan (combined with antigen, see below discussion) and, more recently, the *C. albicans* germ tube antibody assays (CAGTA), which target *Candida* hyphal protein (Hwp1), which is most sensitive for *C. albicans* but detects the other commonly encountered species. Initial studies have demonstrated promising but mixed results. In a 2020 publication of 70 at-risk patients for IC in the ICU undergoing monitoring, positive CAGTA results were found in 4/4 with proven IC (candidemia), 17/19 with probable IC, and 15/47 without IC. In a 2017 study comparing 2 versions of the CAGTA assay (one automated and one manual), sensitivity for IC overall was 69–77% and specificity was 76–80% in at-risk ICU patients [33]. However, in a 2015 publication assessing CAGTA in combination with BD in a prospective cohort of 107 ICU patients at risk for IC, BD was found to be predictive of IC, whereas CAGTA was not able to distinguish IC from *Candida* colonization [34]. Further study on the CAGTA tests is required prior to routine clinical adoption.

Antigen Detection

Several tests that target a variety of cell-wall and cytoplasmic components have been developed to detect macromolecular *Candida* antigens (Table 6.2). Some of these are no longer available, such as an assay to detect enolase antigen. Of the available tests, the earliest was the Cand-Tec LA assay (Ramco Laboratories, Stafford, TX), which was designed to detect circulating *Candida* antigen in patients with serious, disseminated infection. Unfortunately, there are conflicting reports on its overall sensitivity and specificity, especially in patients with renal failure or rheumatoid factor positivity, making it difficult to confirm the diagnosis of candidiasis by the Cand-Tec assay alone [35, 36].

The mannan component of the *Candida* cell wall is a major antigen and the target of many serum detection assays. These assays vary in the laboratory method and type of antibody used for antigen detection. Two tests that use the same monoclonal antibody are the no longer available Pastorex *Candida* LA test (Bio-Rad, Marnes-la-Coquette, France) and the CE-marked Platelia *Candida* Antigen EIA test (Bio-Rad, Marnes-la-Coquette, France). Although the EIA test is more sensitive than the LA test, they are both limited by the rapid clearance of mannan antigenemia. In an effort to overcome this, an anti-mannan antibody EIA was developed and marketed individually (as the Platelia *Candida* Antibody test) and combined with the Platelia *Candida* Antigen (as the Platelia *Candida* Antibody and Antigen test) [37]. In a review of 14 studies that evaluated the three Platelia *Candida* assays, the pooled per-patient sensitivity of the Platelia *Candida* Antigen, Platelia *Candida* Antibody, and both tests combined was 58%, 59%, and 83%, respectively, while the corresponding specificities were 93%, 83%, and 86%, respectively [38]. Both Platelia *Candida* tests were refined and are now CE-marked as *Candida* Ag Plus and *Candida* Ab Plus. In one evaluation of their performance using a case-control design, their sensitivity and specificity were, respectively, 59% and 98% for *Candida* Ag Plus, 63% and 65% for *Candida* Ab Plus, and 89% and 63.0% for the tests combined (*Candida* Ag/Ab Plus) [39]. However, in a second study comparing the Plus versus the original test, while the authors found the Plus was able to detect mannan at a lower level, the per-patient sensitivity was not improved and specificity suffered by a 50% drop hypothesized as secondary to release of mannan due to superficial colonization [42].

In a recently published multimodal diagnostic study of 234 nonimmunocompromised ICU patients at high risk for IC, the prognostic value of repeated measures of Fungitell BG and the *Candida* Ag and Ab Plus assays was evaluated. Of the 234 subjects, 27 (11.5%) developed disease overall; when stratified by BG over versus under 80 pg/mL, the risk of IC was 19.8% versus 5.2%, respectively. So while BG pre-

Table 6.2 Studies of serum immunological assays for *Candida* infection (excluding BG)

Target	Assay	Population tested	Sensitivity (%)	Specificity (%)	Comments	References
Antigens as detected by						
Unknown	Cand-Tec LA[a]	Retrospective case control: 39 candidemia cases; 40 controls (20 healthy volunteers, 10 patients with *Candida* colonization only, 10 patients with other deep mycoses)	77	88	Titer of 1:4 considered positive.	[35]
		Prospective cohort of patients at risk for IC: 202 patients; 23 developed IC	70	69	Titer of 1:4 considered positive.	[36]
Mannan	Pastorex LA[b]	As above	26	100		[35]
		Retrospective case control: 43 cases of proven IC; 150 controls included ICU patients, patients with other deep mycoses, and healthy volunteers	28	100		[37]
	Platelia *Candida* antigen EIA[c]	Per-patient analysis of 14 studies (13 retrospective) including 453 patients (proven/probable IC) and 767 controls (healthy volunteers and high-risk patients without IC). The population studied was split (7 studies mainly hemato-oncologic; 7 studies mainly ICU/surgery)	58	93	Cutoffs used were adopted from the primary study and varied. Combined testing performance was superior.	[38]
	Platelia *Candida* antibody EIA		59	83		
	Platelia Candida antigen and antibody EIA[b]		83	86		
	Platelia *Candida* Ag plus EIA	Retrospective case control: 56 candidemia cases; 200 controls (100 bacteremic, 100 nonbacteremic)	59	98	Cutoffs used per manufacturer's recommendations *C. parapsilosis* and *C. guilliermondii* were not detected; by Platelia *Candida* Ag Plus BG in comparison: sensitivity 88%, specificity 86%.	[39]
	Platelia *Candida* Ab plus EIA		63	65		
	Platelia *Candida* Ag/Ab plus EIA		89	63		
Metabolites as detected by						
D-arabinitol	Enzymatic-chromogenic	Prospective analysis of high risk oncology patients and control patients that included those with fever, neutropenia, and mucosal colonization with *Candida* but no culture evidence of IC, and those without these and also without culture evidence of IC	74 (31/42)	86 (178/206)	Candidemia patients	[40]
			83 (25/30)		Persistent candidemia patients	
			40 (4/10)		IC patients w/o candidemia	
			44 (7/16)		Deep mucosal candidiasis patients	
	Enzymatic-fluorometric	Retrospective evaluation of patients with candidemia and healthy control patients	76 (63/83)	89 (89/100)	Candidemia patients	[41]
			83 (25/30)		Persistent candidemia patients	

BG 1,3-β-D-glucan
[a] Ramco Laboratories, Stafford, TX
[b] Bio-Rad, Marnes-la-Coquette, France
[c] Bio-Rad, Munich, Germany

dicted IC, unfortunately, the Candida Ag Plus, Candida Ab Plus, or a combination thereof was not predictive of IC [43].

Detection of Fungal Metabolites

D-arabinitol (DA) is a five-carbon polyol metabolite that is produced by several pathogenic *Candida* species (except for *C. krusei* and *C. glabrata*). It has been shown to be present in higher serum concentrations in humans and animals with IC than in uninfected or colonized controls, making it potentially useful as a diagnostic marker for IC. There are two general methods to measure DA: gas chromatography or an enzymatic method. The former is labor intensive and not readily available in most hospital laboratories, while the latter is more suited to a commercial test kit, as is currently marketed in Japan as Arabinitec-Auto (Marukin Diagnostics, Osaka, Japan). This assay is also available for DA testing on urine samples. Several studies have shown that DA can be detected earlier than the presence of *Candida* in blood cultures and that serial measurements correlate well with clinical response to therapy [40, 41].

Conclusions and Recommendations

Immunodiagnostic tests are promising additional diagnostic strategies in the detection of IC, but they have yet to supplant traditional methods. In general, there appears to be reasonable utility for serum BG (see BG section) in the right clinical scenario and much less enthusiasm regarding CAGTA, mannan antigen/antibody, and serum DA. In the 2020 EORTC/MSG consensus definition update, BG (but no other immunodiagnostic test) was included in the mycologic criterion as evidence to support IC diagnosis [31]. For all of these tests, the greatest value appears to be in serial testing of high-risk populations where the trend (rather than a single value) will allow for accurate diagnosis and initiation of early antifungal therapy, or to use a negative test (or two) to discontinue already initiated empiric therapy. An emerging strategy is to use BG and other immunodiagnostic tests in combination with one another and/or molecular assays to optimize a diagnostic approach to these complex patients. Further, rigorous, prospective clinical trials are needed to determine which tests or combinations thereof will offer the greatest clinical utility.

Invasive Aspergillosis (IA)

The main clinical manifestations of *Aspergillus* infection include aspergillomas, allergic bronchopulmonary aspergillosis (ABPA), chronic invasive aspergillosis, and IA. Different types of immunologic tests have shown different utility for this spectrum of disease. We primarily focus on IA in this section.

Antibody Detection

The diagnosis of aspergilloma is made by combined radiologic and serologic testing, where IgG antibodies are usually positive. Similarly, for ABPA, a combination of routine blood tests, radiographic findings, skin testing for *Aspergillus* sensitivity, and both IgG and IgE antibody positivity are used for diagnosis. Conversely, antibody detection is less useful and not recommended for invasive disease, since the immunocompromised patients most at risk are less likely to mount a sufficient response.

Antigen Detection

Galactomannan (GM) is a polysaccharide component of the *Aspergillus* cell wall. It has been demonstrated in the serum of some patients with IA and thus has been the target of several serum detection assays. An earlier test called the Pastorex *Aspergillus* (Bio-Rad, Hercules, CA) utilized an LA method with a monoclonal antibody. This test yielded disappointing results with low sensitivity unless multiple samples were used and also yielded false-positive reactions due to the cross-reactivity of the antibody with several other fungal species.[44, 45] In 2003, the Platelia *Aspergillus* Ag EIA (Bio-Rad, Hercules, CA) was approved by the FDA for serum diagnosis of IA. This sandwich ELISA uses the rat EBA-2 monoclonal antibody to detect GM at much lower limits than the previous LA test, thereby improving the test's sensitivity. Results are reported as an optical density index (ODI) comparing the patient's sample to two standardized controls in the test kit [46]. In an early retrospective review of stored serum specimens on heme-malignancy patients, the Platelia EIA had a sensitivity of 81% and specificity of 89% [47]. However, subsequent investigations have shown a lower sensitivity; for example, a meta-analysis of 27 studies of the Platelia *Aspergillus* assay in serum, performed in the setting of repeated surveillance in high-risk patients, demonstrated an overall sensitivity of 61% and specificity of 93% in proven or probable IA cases (Table 6.3) [48]. The sensitivity of the test, when limited to proven cases only, was 71%. The analysis also showed that sensitivity and specificity varied according to ODI cutoff used for suspected IA diagnosis, with the general trend that increasing the cutoff value decreased sensitivity but increased specificity. A Cochrane Review supported this concept by examining 54 studies with 586 cases of proven or probable IA in a mixed immunocompromised population. Serum GM at an ODI of 0.5 resulted in a sensitivity of 78% and specificity of 85%; at an ODI of 1.0,

Table 6.3 Studies of *Aspergillus* galactomannan antigen detection

Assay/body fluid	Study design and population	Sensitivity (proven/probable IA) (%)	Specificity (not IA) (%)	Comments	References
Platelia[a] ELISA/serum	Meta-analysis of 27 studies – 21 prospective; 6 retrospective – 23 hemato-oncologic; 3 SOT;1 not specified – GM sampled in at-risk patients 1–2/week in all studies	61	93	Positive tests required: 1 (11 studies); 2 (16 studies) Proven cases only: sensitivity 71%, specificity 89% SOT subgroup: sensitivity 41% Sensitivity by OD cutoff for positivity: 79% for 0.5; 65% for 1.0; 48% for 1.5	[48]
Platelia ELISA/BAL	Meta-analysis of 13 studies – 5 prospective; 6 retrospective – 9 hemato-oncologic; 2 SOT, 1 pediatric IC; 1 bronchogenic carcinoma – GM typically performed as part of diagnostic BAL	90	94	Positive tests required: 1 (10 studies); 2 (3 studies) Sensitivity by OD cutoff for positivity: 86% for 0.5; 85% for 1.0; 70% for 1.5; 61% for 2.0	[49]
Platelia ELISA/BAL	Meta-analysis of 30 studies – 14 prospective; 16 retrospective – 12 hemato-oncologic; 4 SOT; 14 other – GM typically performed as part of a diagnostic BAL	87	89	1 positive BAL GM required OD ≥0.5 available in 24 studies and used for primary analysis OD ≥1 (21 studies) performance: sensitivity 86%; specificity 95% Serum GM: 65% sensitive; 95% specific	[50]
Platelia ELISA/BAL	Systematic review of 19 studies for PCR – 7 compared PCR to GM (OD ≥0.5) – 75% of population was hemato-oncologic	82	96	Reference standard imperfect: positive GM or PCR	[51]
LFD[b]/serum, BAL	Meta-analysis of 7 studies – 3 serum, 4 BAL – 5 prospective, 1 retrospective, 1 case-control – 650 total cases, 278 hemato-oncologic; 68 SOT; 233 other respiratory disease – 115 proven/probable IA (19%)	68 (serum) 86 (BAL)	87 (serum) 93 (BAL)	Hemato-oncologic subgroup: BAL Sn 100%, Sp100% (20 cases); Serum Sn 69%, Sp 86% (162 cases) SOT subgroup: BAL Sn 94% (45 cases) Respiratory diseases subgroup: BAL Sn 77% (242 cases)	[52]

(continued)

Table 6.3 (continued)

Assay/body fluid	Study design and population	Sensitivity (proven/probable IA) (%)	Specificity (not IA) (%)	Comments	References
Platelia ELISA/serum	Systematic review of 54 studies, meta-analysis of 50 studies – 197 patients with proven IA; 573 with probable IA; 980 with possible IA, 5284 with no IA	73	89	Positive tests required: 1 (30 studies) 2 (24 studies) Sensitivity by OD cutoff for positivity: 78% for 0.5; 71% for 1.0; 63% for 1.5	[53]
Platelia ELISA/BAL	Systematic Review and Meta-analysis of 17 studies – 1634 total patients, 366 with proven or probable IA	83	88	Sensitivity by OD cutoff for positivity: 88% for 0.5; 78% for 1.0 Specificity by OD cutoff for positivity: 81% for 0.5; 93% for 1.0	[54]

SOT solid organ transplant, *OD* optical density
[a] Bio-Rad, Hercules, CA
[b] OLM diagnostics, Newcastle Upon Tyne, UK

a sensitivity of 71% and specificity of 90%; and at 1.5, a sensitivity of 63% and specificity of 93% [53].

Serum GM performs best in the heme-malignancy populations with profound neutropenia and is less sensitive in solid organ transplant patients or other nonneutropenic hosts [48, 55]. As it is often present 5–8 days prior to symptoms, serum GM has been utilized as surveillance in high-risk populations; several reports have demonstrated that the trend of GM during therapy predicts survival outcome and treatment response [56–59]. False-negative results can occur due to limited angioinvasion, low fungal load, high antibody titers, or the use of prophylactic or preemptive antifungals. Alternatively, false positives occur due to cross-reactivity of the assay with other fungal species such as *Fusarium*, *Histoplasma*, *Talaromyces*, and *Penicillium* [55]. Previously, coadministration of pipercillin-tazobactam was found to produce false positives as large-scale production of the antibiotic involved fermentation from *Aspergillus* species; however, this is thought to no longer be a significant source of false positivity [60].

The Platelia *Aspergillus* assay was FDA-cleared for use on bronchoalveolar lavage (BAL) fluid in 2011, and overall demonstrates a higher sensitivity (generally >80%) as compared to serum across various populations including nonneutropenic patients [61–64]. A meta-analysis of 30 studies demonstrated a sensitivity of 87% and specificity of 89% for the BAL-GM.[50] As with serum GM, test performance depends on the selected OD cutoff, but in contrast to serum, BAL GM sacrifices less sensitivity when increasing the cutoff threshold [49, 50, 65].

Though it can be detected in other body fluids, the GM assay is validated only for serum and BAL samples at this time. However, a single CSF GM result ODI >1.0 is present in the most revised criteria for probable IA in the immunocompromised patient population [31]. A study of 17 individuals predominantly with underlying heme-malignancy diagnosed with proven or probable demonstrated GM CSF sensitivity and specificity of 88% and 96%, respectively, regardless of using an ODI cutoff of 0.5, 1.0, or 1.5 [66].

A Lateral Flow Device (LFD) targeting the JF5 antigen first developed in 2008 has been studied as a point-of-care assay that produces results in 15 min. Performance of the LFD in serum has shown unsatisfactory sensitivity, however improved accuracy in BAL samples. For instance, a meta-analysis in a mixed immunocompromised population with 115 proven or probable cases of IA demonstrated a pooled serum sensitivity of 68% versus a pooled BAL sensitivity of 86% [52]. An updated version of the LFD is commercially available from England as the AspLFD assay (OLM Diagnostics, Newcastle Upon Tyne, UK). Also available is the IMMY sona Aspergillus GM LFA (IMMY, Norman, OK), a sandwich assay utilizing two monoclonal antibodies to target *Aspergillus* GM; as of this writing, both assays are CE-marked but not FDA-cleared, and the studies have thus far evaluated only hematological patients, thus limiting application to this population. A review of performance suggests the AspLFD has an overall sensitivity and specificity of 64% and 87% in BAL fluid and 68% and 87% in the serum, while the sona LFA sensitivity and specificity was calculated at 77% and 81% in BAL fluid and 70% and 96% in the serum

[67]. Development of accurate LFA/LFD assays would certainly offer patient care advantages with the ability for more laboratories to offer onsite, rapid *Aspergillus* GM testing.

Detection of Fungal Metabolites

Mannitol, a six-carbon acyclic polyol, is produced in large amounts by many different fungi including several *Aspergillus* species in culture and by *Aspergillus fumigatus* in infected animals. Unfortunately, available data do not support the usefulness of mannitol as a diagnostic marker in aspergillosis. *Aspergillus* produces a number of volatile organic compounds (VOCs) that display an array of physiologic properties. Research has been conducted around detecting VOC profiles in patients with suspected IA via in vivo breath samples compared with in vitro reference profiles. In a prospective cohort of 34 patients with proven or probable IA and 30 patients with pneumonia other than *Aspergillus*, volatile metabolite detection displayed a sensitivity of 94% and specificity of 93% in diagnosing IA [68]. Future studies are needed to establish a role for secondary metabolite detection in assisting with IA diagnosis.

Conclusions and Recommendations

Despite the broad spectrum of disease caused by *Aspergillus* organisms, it is the invasive disease that is most important and most challenging diagnostically. The Platelia *Aspergillus* assay supports a diagnosis of IA in the appropriate clinical setting. All clinicians should keep in mind the potential for false-positive and false-negative results and incorporate the GM results into the general clinical assessment of the patient, rather than as the sole basis on which to change management. For instance, a change in the assay from negative to positive in an immunosuppressed patient under surveillance should prompt a more thorough investigation for IA, while a change from positive to negative should lend support to other evidence that proper therapy has been instituted. Additionally, it is important to remember that the positive predictive value of this test is highest in populations with a high pretest probability; using it for routine diagnosis in lower-risk populations will likely increase the chance that a positive result is a false positive.

For optimal accuracy, newer recommendations suggest an ODI cutoff of 1.0 on a single GM serum, BAL, GM sample in order to make a diagnosis of probable IA in the immunocompromised population. This will lead to less false positives at the expense of slightly more false negatives compared to an ODI cutoff of 0.5, although more so for serum than BAL samples. When both samples are available, a combination of serum ODI >0.7 and BAL >0.8 is also suggestive of probable IA [31]. The emergence of new LFA/LFD assays, if found to be as or more accurate as the traditional Platelia GM, could transform the laboratory immunodiagnosis landscape as it has for cryptococcus. Despite its limitations, the GM EIA assay is a suitable, noninvasive adjunct for diagnosing and managing IA (see Box 6.2 for recommendations).

Box 6.2 Recommendations for Using the Platelia *Aspergillus* Galactomannan (GM) ELISA Assay

1. Serum GM is ideally used as a part of IA surveillance and performed 1–2/week on at-risk hemato-oncologic patients. The test has moderate accuracy in this setting.
2. BAL GM is a useful adjunctive diagnostic test for at-risk patients presenting with compatible clinical illness. The test has moderate-to-high accuracy in this setting.
3. Positivity for ODI cutoff is recommended at >1.0 for serum or BAL GM samples alone, while combination of serum >0.7 and BAL >0.8 is also suggestive of probable IA.
4. Consider using serum GM testing to monitor treatment response.
5. Always interpret the results in the context of other clinical and laboratory findings.

Cryptococcosis

The worldwide burden of cryptococcosis, primarily due to *Cryptococcus neoformans,* increased in parallel with the HIV epidemic and subsequently other types of immunosuppression including organ transplantation. Additionally, *C. gattii*, a pathogen historically limited to tropical and subtropical regions, has emerged to cause invasive cryptococcosis cases and outbreaks in the Pacific Northwest, USA, and British Columbia, Canada [69]. While culture and histopathology remain the gold standards for diagnosing cryptococcal disease, detection of cryptococcal polysaccharide antigen is a critically important test, which affords a more rapid diagnosis and allows for earlier disease treatment.

Antibody Detection

Tests for cryptococcal antibodies are not useful and are not widely available for clinical use, because they have high false-positive and false-negative rates.

Antigen Detection

Cryptococcal antigen (CrAg) testing has become the foundation for evaluation of a patient with suspected cryptococcosis. CrAg is composed of the capsular polysaccharide glucuronoxylomannan (GXM), which is shed by the organism during infection and can be detected in various body fluids. Traditionally, EIA and LA including the CrAg LA System (CALAS; Meridian Bioscience, Cincinnati, OH] have been primarily used to detect CrAg.

In 2011, the FDA approved a novel semiquantitative lateral flow assay (LFA) CrAg test (IMMY, Immuno-Mycologics, Norman, OK). This dipstick sandwich immunochromatographic assay has several technical advantages over LA and EIA in that it can be stored at room temperature, is cheaper, and enjoys quicker turnaround time as no reagent preparation is required [70]. Titers may be obtained by serial dilution of the sample. The LFA can be employed as a point-of-care fingerstick test without sacrificing sensitivity compared with venipuncture, a particularly valuable tool for resource limited settings [71].

Furthermore, beyond the technical advantages, studies comparing the assays demonstrate LFA to have either equal or superior sensitivity (often >99%) as compared to the LA and EIA methods (Table 6.4) [70, 72, 74–78, 82]. As a summary of overall LFA performance, a meta-analysis including 12 studies was performed evaluating 4622 total specimens of primarily PLWH individuals. CrAg LFA was determined to

Table 6.4 Studies of Cryptococcus antigen detection

Study design	Assay	Fluid	Sensitivity (%)	Specificity (%)	References
Retrospective cohort: 704 serum samples, all HIV+, compared with EIA	LFA[a]	Serum	90	99	[72]
		Urine	70		
Retrospective cohort: 62 samples of serum, plasma, and urine. All HIV+ patients	LFA	Serum/plasma	100		[73]
		Urine	98		
Prospective cohort for serum (634 samples, 9 positive). Retrospective for CSF (51 samples, 18 cases)	LFA	Serum	100	100	[74]
		CSF	100	100	
Retrospective cohort: 25 positive cases (4 HIV+, 12 immunocompetent, 9 other immunocompromised)	LFA	Serum	100	100	[75]
		CSF	100	100	
		Urine	94		
Prospective cohort: 589 serum (41 positive) and 411 (15 positive) CSF specimens tested at reference laboratory. Varied immune status.	LFA	Serum	100	96	[70]
		CSF	100	100	
Mixture of prospective and retrospective cohorts: 666 CSF samples of HIV+ patients.	LFA	CSF	99	99	[76]
Two prospective cohorts: 112 total HIV patients with 47 suspected *Cryptococcus meningitis*.	LFA	CSF	100	100	[77]
Prospective cohort: 207 HIV+ patients with suspected *Cryptococcus meningitis*. Fingerstick whole blood compared with serum LFA.	LFA	Whole Blood	100	100	[71]
Retrospective cohort: All non-HIV patients. 36 serum samples (26 positive) and 23 CSF samples (13 positive).	LFA	Serum	100		[78]
		CSF	100		
Retrospective cohort: 227 patients with serum testing, 141 PLWH	LFA	Serum	HIV−, localized: 90.9		[79]
			HIV−, disseminated: 82.6		
			HIV+, disseminated: 100		

(continued)

Table 6.4 (continued)

Study design	Assay	Fluid	Sensitivity (%)	Specificity (%)	References
Retrospective cohort: 4650 BAL fluid samples tested, 9 positive cases Sn 44% if including possible colonization, Sn 80% if do not include	EIA[b]	BAL	44–80	99	[80]
Retrospective cohort: 429 non-HIV patients with 23 positive cases	LA[c]	BAL	83	98	[81]
	LA	Serum	74	99	

[a] LFA = Immuno-Mycologics, Norman, OK
[b] EIA = Meridian Diagnostics, Cincinnati, OH
[c] LA = Eiken, Kagaku, Tokyo

have a pooled sensitivity and specificity of 97.6% and 98.1% in the serum, 98.9% and 98.9% in the CSF, and 85.0% sensitivity in the urine with specificity not able to be calculated [83]. LFA has also been shown, in a handful of small studies, to have a higher sensitivity than LA in capturing *C. gattii* infections [70, 75, 84], an important issue in regions such as the pacific northwest where this organism has emerged as a major clinical consideration.

The performance characteristics of the cryptococcal antigen assays to diagnose cryptococcosis depend on both the disease status (localized vs. disseminated) and host status (HIV, transplant, otherwise immunosuppressed, or immune competent). Because of the multiple possible combinations, the ability to tease out these performance differences is difficult. In general, a higher fungal burden (as is typical for CNS disease) is associated with higher antigen titers. For example, the sensitivity of serum cryptococcal antigen is lower for isolated pulmonary disease than that for meningitis. Indeed, in immunosuppressed patient, a positive serum cryptococcal antigen from a patient who may only appear to have localized pulmonary disease should prompt the clinician to strongly consider adding blood cultures and obtaining CSF fluid to rule out dissemination [85]. The argument follows that for HIV-positive patients who are unable to undergo lumbar puncture (LP) or with vague central nervous system (CNS) symptoms not warranting an LP, a serum antigen test may be a reasonable surrogate for meningitis screening [86, 87]. On the other hand, in immune competent patients with meningitis symptoms, the utility of a serum antigen is less clear (i.e., CSF antigen is required) [88, 89].

The prognostic value of antigen titers is controversial given the above-mentioned issues of disease and host factors. In the majority of studies, higher initial titers are associated with increased mortality and relapsed disease; however, other studies have not confirmed this association [69, 86, 90–94]. Also controversial is the use of sequential antigen titers obtained during therapy. This practice is best studied in the HIV patient population, in which setting the use of titers to monitor treatment response is probably unreliable and therefore not recommended [85, 95]. LFA CrAg titer may remain elevated during effective treatment, because the clearance of cryptococcal antigen is thought to be slower than antifungal activity [77]. In certain situations, sites other than serum and CSF may be useful in detection of cryptococcal disease including the pleural fluid and urine [72, 96]. The use of cryptococcal antigen, particularly in the setting of BAL fluid studies, has typically shown suboptimal sensitivity especially for isolated pulmonary disease. However, all of the published studies have utilized LA or EIA for CrAg detection and therefore, it is unknown how LFA would perform in this setting [80].

The limitations of the cryptococcal antigen assays are the occasional false-negative results in patients with extremely low or high *Cryptococcus* organism burden and infrequent false-positive results, generally resulting in low titers, in patients with other infections including disseminated trichosporonosis, *Capnocytophaga canimorsus* sepsis, and *Stomatococcus* infection [97–99].

Detection of Fungal Metabolites

Cryptococcus species, like *Aspergillus* species, also produce large amounts of mannitol, but it has not proven useful as a diagnostic marker for this disease either.

Conclusions and Recommendations

Cryptococcal infection is the rare condition where a serodiagnostic test has extremely high accuracy. A positive cryptococcal antigen result is highly suggestive of infection and can be the sole basis for initiating targeted therapy. However, definitive proof of disease still requires culture or histopathology and efforts to prove the diagnosis by these means are always warranted. The clinical utility of the anti-

gen test depends on the extent of disease and host immune status (see Box 6.3 for recommendations). The LFA has emerged as an easy-to-use, accurate, point-of-care test alternative, which may provide added benefit of improved sensitivity for *C. gattii* infection as compared to LA and EIA. Care must be taken not to compare titers derived from different kits given the lack of standardization among manufacturers.

Box 6.3 Recommendations for Using Cryptococcal Antigen Tests in Different Host Populations

HIV-positive patients
1. For meningeal symptoms, check both serum and CSF antigens. If unable to do LP or the neurologic symptoms are vague, one can use the serum antigen (which has a high sensitivity for meningitis) as a surrogate screening test 2. For nonmeningeal symptoms, check serum antigens. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid). 3. A positive serum antigen is associated with disseminated disease and warrants blood cultures and CSF evaluation (cultures and antigen testing). 4. Initial antigen titers correlate with disease burden and likely provide prognostic information; serial antigen titers probably do not provide prognostic information during therapy (and therefore are not recommended).
Solid organ transplant patients
1. For meningeal symptoms, check both serum and CSF antigens. The sensitivity of a serum test to screen for CNS disease also appears to be high in this population; however, an LP is mandatory to evaluate for *Cryptococcus* and other diseases. 2. For nonmeningeal symptoms, check serum antigens. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid). 3. A positive serum antigen is associated with disseminated disease and warrants blood cultures and CSF evaluation (cultures and antigen testing). 4. Initial antigen titers correlate with disease burden and likely provide prognostic information; it is unknown whether serial titers provide prognostic information during therapy (and therefore are not specifically recommended).
Immune competent patients
1. For meningeal symptoms, check both CSF and serum antigens; serum antigen alone may be insufficient to rule out meningitis. 2. For nonmeningeal symptoms, check serum antigens. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid). 3. Initial antigen titers correlate with disease burden and likely provide prognostic information; some experienced clinicians monitor serial titers during therapy to document therapeutic response and predict relapse, but others do not. 4. If *C. gattii* infection is a concern (immunocompetent or other patients), consider preferentially using LFA CrAg platform.

Histoplasmosis

Clinical histoplasmosis is divided into progressive disseminated histoplasmosis (PDH) and pulmonary histoplasmosis, which is further classified as either acute, subacute, or chronic forms. The gold standard for the diagnosis of histoplasmosis remains isolation and specific identification of the causative organism from histopathology or fungal culture from the blood or at the site of infection. Unfortunately, the fungal culture is labor intensive and can take 2–6 weeks for a result. Immunologic tests offer a more rapid alternative and in some disease manifestations are the preferred means of establishing a diagnosis. Molecular testing including PCR has been evaluated for rapid diagnosis of histoplasmosis; however, data is limited and further investigation is required before widespread implementation.

The following provides a general review of immunologic diagnostic options available to the clinician; please refer to Table 6.5 for a review of pertinent studies and Box 6.4 for diagnostic recommendations relating to histoplasmosis.

Box 6.4 Recommendations for Immunologic Test Selection Stratified by Clinical Syndrome for Histoplasmosis, Blastomycosis, and Coccidioidomycosis

Disease/clinical syndrome	Antigen sensitivity	Antibody (ID and/or CF) sensitivity	Recommendations
Histoplasmosis			
Progressive disseminated disease	90–95%	50–75%	Antigen testing of serum and urine is recommended.
Acute pulmonary disease	35–80% in urine	>80% (at 4–6 weeks)[a]	Antibody (ID plus CF) testing is recommended. Also consider antigen testing of serum and urine, particularly early in disease.
Subacute pulmonary disease	~30% in urine	90–95%	Antibody (ID plus CF) testing is recommended.
Chronic pulmonary histoplasmosis	~20% in urine (note 88% in 1 study)	80–100%	Sputum or BAL fluid for culture, antigen testing (BA preferred over serum, urine) as well as antibody (ID plus CF) are recommended.

Disease/clinical syndrome	Antigen sensitivity	Antibody (ID and/or CF) sensitivity	Recommendations
Fibrosing mediastinitis Broncho lithiasis; Asymptomatic lung granuloma; Chronic mediastinal lymphadenopathy	Antigen test usually negative	50–65%	Antibody (ID and CF) testing is recommended.
Meningitis	~20–80% in CSF ~40–70% in urine ~20–50% in serum	0–80% in CSF 0–80% in serum	Antigen testing in CSF, serum, and urine plus antibody testing in CSF (EIA or ID) is recommended.
Blastomycosis			
All forms of disease	76–93% in urine 57% in serum	Poor for ID, CF (0–80%) EIA—87.8% (1 study)	Obtain culture and histopathology; perform urine antigen testing in cases where serologic diagnosis is needed.
Coccidioidomycosis			
Acute pulmonary disease: immune competent	41% in serum or urine	80–95% (ID + CF)	Send serum antibodies (ID or EIA); if positive, obtain CF.
Acute disease: immunosuppressed	50–70% in urine ~70% in serum	50–70% (ID + CF)	Send serum antibodies (ID + CF ± EIA) and consider sending serum and urine antigen.
Meningitis	93% (single study)	~40–80% in CSF ~75–95% in serum	Send serum antibodies (ID + CF ± EIA). Culture CSF (alert the laboratory) and send for antigen, EIA, and/or CF.

[a] Data and recommendations from references [100–104, 106, 109–121]; Histoplasmosis: also from [105, 122]; blastomycosis: Also from [123, 124]; coccidioidomycosis: also from [125, 126]

Table 6.5 Studies of *Histoplasma* antibody and antigen detection

Assay	Sn in PDH PLWH	Sn in PDH without HIV	Sn in limited disease	Specificity	Specimen	References
Antibodies as detected by						
ID[a]	62% (32/52)	67% (14/21)	80% (65/81)	n/a	Serum	[100]
		81% (17/21)	82% (210/255)	99% (763/767)	Serum	[101]
			17% (5/29; acute)	n/a	Serum	[102]
ID plus CF	79% (15/19)	71% (37/53; other IC) 89% (8/9; non-IC)	67% (4/6; acute) 95% (39/41; subacute) 83% (5/6; chronic)	n/a	Serum	[103]
CF[b]	63% (29/46)	64% (14/22)	90% (75/83)	n/a	Serum	[100]
			64% (18/28)	n/a	Serum	[102]
		57% (12/21)	83% (212/255)	96% (342/357)	Serum[c]	[104]
		81% (17/21)	77% (197/255)	98% (349/357)	Serum[d]	
EIA[e]			68% (15/22; CNS IC) 96% (22/23; CNS non-IC)	93% (142/153)	CSF	[105]
Antigens as detected by						
EIA[e]	95% (38/40)	75% (12/16)	37% (11/30)	99% (95/96)	Urine	[106]
			65% (84/130; acute)	n/a	Urine	[102]
			68% (24/35; acute)	n/a	Serum	
	95% (53/56)	93% (81/87; other IC) 73% (11/15; non-IC)	83% (5/6; acute) 30% (14/46; subacute) 88% (7/8; chronic)	n/a	Urine	[103]
	98% (62/63)			97% (496/510)		[107]
	88% (22/25 all IC)	64% (17/25; non-IC)		n/a	CSF	[105]
LFA[e]	90% (94/104)			93% (265/287)	Urine	[108]

Sn sensitivity, *PDH* progressive disseminated histoplasmosis, *PLWH* people living with HIV, *IC* immunocompromised, *EIA* enzyme immunoassay, *ID* immunodiffusion, *CF* complement fixation

[a] Meridian Diagnostics, Cincinnati, OH

[b] Immuno-Mycologics, Norman, OK; 1:8 or greater titer considered positive for CF testing

[c] Antibody to yeast phase antigen

[d] Antibody to mycelial phase antigen

[e] Mira Vista Diagnostics, Indianapolis, IN

Antibody Detection

ID, CF, and, less commonly, ELISA are employed for antibody detection. For ID, the antigenic targets are comprised of the H and M antigens of the mycelial phase protein histoplasmin. Antibodies against H antigen, a β-glucosidase, form in a subset of patients during acute disease and more commonly in severe histoplasmosis, whereas antibodies against M antigen, a catalase, are formed in most patients during both acute and chronic diseases. The ID test is a qualitative test that provides both H and M band results. Antibodies against M antigen are detected six to eight weeks after exposure in 80% of patients but can persist for years in patients who have recovered from infection; therefore, their presence does not distinguish remote infection from current disease. On the other hand, antibodies against H antigen are detected in only 20% of exposed patients, but their presence signifies an active infection.

CF is quantitative assay and includes assays to both mycelial phase (histoplasmin) and yeast phase antigens (a suspension of antigens from killed yeast phase cells). The inclusion of the yeast phase antigens renders CF more sensitive, but less specific than ID, that is, this test adds sensitivity for histoplasmosis detection to ID, but is more likely to cross-react with other fungal infections, particularly coccidioidomycosis and blastomycosis. The anti-yeast antibodies are detectable early, about 4 weeks after exposure, and can remain detectable over a long time period (years). The anti-mycelial Abs appear later, with less robust titers except for in cases of chronic histoplasmosis, and for a shorter period. In general, titers ≥1:32 or rising titers indicate strong evidence of disease, whereas ≥1:8 indicates presumptive evidence of disease but can represent cross-reactivity with other mycoses.

In general, asymptomatic patients are less likely to have detectable antibody levels, and if present, they are usually in lower titers. This is evidenced by the low levels of antibody detected in ~10% of healthy patients residing in an endemic area. Antibody titers generally decline over several months following exposure but may remain positive for years in some chronic forms of the disease. False-negative tests occur during the early stages of infection and are more common in immunocompromised patients. False-positive results occur in ~15% of patients mainly due to cross-reaction with the agents of coccidioidomycosis or blastomycosis. EIA is a third method of antibody detection that has been described but not as frequently utilized nor as widely available in practice compared with CF and ID.

Antigen Detection

One of the major developments in diagnostic strategies for histoplasmosis was the introduction of assays able to recognize a histoplasmosis polysaccharide antigen (Table 6.5). Depending on the disease manifestation, this antigen can be present in urine, serum, CSF, or bronchoalveolar lavage (BAL) fluid. The original assay was an RIA; this is no longer available due to the development of nonradioactive newer tests. The first modern test was developed by MiraVista Diagnostics, the current version of which is the MVista *Histoplasma* quantitative antigen (MiraVista Diagnostics, Indianapolis, IN). This assay is a quantitative sandwich EIA that uses polyclonal rabbit *anti-Histoplasma* antibodies to bind antigen and has demonstrated favorable results compared to its earlier generations and to the original RIA test [106, 127]. A second FDA-approved polyclonal antibody in-vitro diagnostic EIA was developed by IMMY in 2007 and subsequently replaced by the manufacturer in 2013 in favor of a monoclonal antigen EIA using analyte-specific reagents (ASRs) (IMMY, Norman, OK) [128, 129]. Unlike the MVista antigen, this assay is qualitative and validated for urine samples only.

Generally, the urine (and serum) antigen detection assays are the best tests to quickly establish a diagnosis of PDH in immunosuppressed patients (i.e., those with higher fungal burden), while they are less sensitive in detection of disease on the other end of the clinical spectrum, that is, isolated pulmonary histoplasmosis in immunocompetent patients. Though urinary and serum antigen detection is the most sensitive specimen for these patients, CSF and BAL fluid testing may prove more valuable in patients with disease at those specific sites. Urinary samples are typically favored over serum antigen due to ease of collection and familiarity; however, combining both urine and serum antigen tests has been shown to increase overall sensitivity [102].

Two meta-analyses have been performed evaluating antigen diagnostic accuracy. The first incorporated 9 total studies all utilizing EIA except for one RIA diagnostic study, and it found an overall antigen sensitivity of 81.4% and specificity of 98.3% but no significant differences between urine and serum antigen accuracy [130]. A second meta-analysis included 30 studies with a mixture of EIA, RIA, and LFA testing of PDH in advanced HIV and determined an antigen sensitivity of 95% and specificity of 97% [131].

Furthermore, the quantitative antigen test is useful for monitoring antigen levels during treatment; levels decrease with appropriate therapy and increase with disease relapse [132]. Cross-reactivity of the assay occurs commonly with penicilliosis, paracoccidioidomycosis, and blastomycosis; less frequently in coccidioidomycosis; rarely in aspergillosis; and possibly in sporotrichosis [133].

A novel lateral flow assay (LFA) also produced by MiraVista, which is CE marked but not yet FDA approved at time of this writing, has been developed as a dipstick sandwich immunochromatographic assay with polyclonal rabbit antibodies that recognize *H. capsulatum* galactomannan antigen. Initial results are promising with a multicenter pro-

Table 6.6 Studies of *Coccidioides* antibody and antigen detection

Population	Assay	Sensitivity	Specificity	Comments	Ref.
Retrospective analysis of antibody detection in CSF of a group of patients with various forms of coccidioidomycosis and control patients without coccidioidomycosis	CF	0% (0/9)	100% (13/13)	Pulmonary disease (CSF)	[112]
		0% (0/2)	n/a	Disseminated disease (CSF)	
		42% (14/33)	n/a	Meningeal disease (CSF)	
	LA	89% (8/9)	n/a	Pulmonary disease (CSF)	
		100% (3/3)	n/a	Disseminated disease (CSF)	
Retrospective analysis of 47 patients with proven coccidioidomycosis; 362 control patients with noncoccidioidal pulmonary illness, other fungal illness, HIV disease, and no illness	Meridian EIA Ab	92% (43/47)	97% (352/362)	IgG alone	[139]
		77% (36/47)	98% (354/362)	IgM alone	
		100% (47/47)	96% (347/362)	IgG and IgM together	
	IDCF	100% (47/47)	100% (362/262)		
Retrospective review of 298 immunocompetent and 62 immunocompromised patients diagnosed with coccidioidomycosis	Meridian EIA Ab	67% (38/57)	n/a	Immunocompromised, IgG and IgM together	[113]
		87% (212/244)		Immunocompetent, IgG and IgM together	
	CF	67% (35/52)	n/a	Immunocompromised	
		75% (188/252)		Immunocompetent	
	ID	53% (21/40)	n/a	Immunocompromised	
		73% (180/248)		Immunocompetent	
Retrospective analysis of mostly immunosuppressed/HIV-positive patients with 24 cases of proven coccidioidomycosis and 188 controls	Mvista EIA Ag	71% (17/24)	98% (184/188)	Urine Ag samples only	[114]
Combined retrospective and prospective analysis of 42 patients with coccidioidomycosis meningitis	MVista EIA Antigen	93%	100%	Meningeal disease (CSF); 56% of patients in study PLWH. Antibody testing done at university laboratories	[140]
	ID	67%	99%		
	CF	70%	100%		
	EIA IgM Ab	8%	100%		
	EIA IgG Ab	85%	99%		
	ID, CF, and EIA Ag	98%	99%		
Comparison of 103 cases of pulmonary and disseminated coccidioidomycosis tested with EIA and ID along with 373 controls	Mvista EIA Ab	88% (91/103)	90%	IgG and IgM together	[138]
	Mvista IDCF + IDTP	60% (62/103)	99%	IgG and IgM together	
	CF	65% (40/62)	n/a		
Comparison of EIA assays against a composite reference standard of 49 positive cases	Meridian EIA Ab	84% (41/49)	68%	IgG and IgM together	[141]
	IMMY EIA Ab	74% (36/49)	82%	IgG and IgM together	
Retrospective analysis of antibody, antigen, as well as combined antibody and antigen detection with 158 cases and 487 controls	ID (At UC Davis)	84% (123/146)	97%	IgG and IgM together	[125]
	CF (At UC Davis)	68% (86/126)	100%		
	MVista EIA Ag	57% (90/158)	99%	Serum or urine	
	ID Ab Plus EIA Ag	93 (147/158)	97%	ID, serum Ag, or urine Ag	

EIA enzyme immunoassay, *IDCF* immunodiffusion using complement fixation antigen, *IDTP* immunodiffusion using tube precipitin, *CF* complement fixation, *PLWH* patients living with HIV

spective double-blinded study testing the MiraVista urine LFA on 391 samples of PLWH with 108 confirmed PDH cases demonstrating a sensitivity of 90% and specificity of 92% [108]. With rapid turnaround time and minimal equipment required, this could prove a valuable tool especially for resource limited settings.

Detection of Fungal Metabolites

No tests of this nature are currently available.

Skin Testing

Skin testing with histoplasmin antigen has been a useful epidemiologic tool to document past exposure and to investigate histoplasmosis outbreaks. It is of little use in the diagnosis of individual cases. Prior skin test positivity can be lost with disseminated disease or immunosuppression.

Conclusions and Recommendations

Immunodiagnostic tests for histoplasmosis are invaluable diagnostic adjuncts to culture and histopathology. Due to the wide disease spectrum, different approaches are recommended to guide the appropriate use of immunologic tests (see Box 6.4). Patients with acute localized disease and a low burden of organisms or patients with chronic sequelae of a prior histoplasmosis infection should predominantly be diagnosed via antibody testing. Conversely, antigen testing is preferred for patients with a high burden of organisms such as PDH. Combining antigen with antibody testing likely adds to overall diagnostic sensitivity in certain clinical situations (i.e., acute pulmonary histoplasmosis). Additionally, quantitative antigen testing is recommended during and after completion of therapy for PDH to monitor response to therapy and for relapse, respectively [132].

Blastomycosis

A high level of suspicion for *Blastomyces dermatitidis* infection is important to its successful diagnosis, since no clinical syndrome is characteristic for infection with this organism. While definitive diagnosis requires the growth of the organism from clinical specimens, a presumptive diagnosis can be made by histological characteristics and further supportive evidence can be gained from immunologic tests.

Antibody Detection

Early serologic tests for blastomycosis were directed toward detecting host antibodies against *B. dermatitidis* A antigen. These tests utilized different laboratory techniques including ID, CF, and EIA. Although the ID test has reported a higher sensitivity than CF (10–80% versus 0–57%), neither test had adequate sensitivity or specificity to be clinically useful [109, 134, 135]. An updated EIA directed at the BAD-1 adhesion antigen (formerly WI-1 antigen) of the outer cell wall of *B. dermatitidis* demonstrated 87.8% sensitivity in a study with 39 proven and 2 probable Blastomycosis cases whereas specificity was 99.2% in controls without fungal disease and 94% in patients with Histoplasmosis, demonstrating little cross-reactivity [123]. However, the overall data for the BAD-1 antibody EIA is limited and the test is not widely available; commercially available reference antibody testing is primarily limited to ID.

Antigen Detection

A quantitative antigen detection assay for use on urine, serum, plasma, CSF, and BAL fluid specimens of patients with suspected blastomycosis is available (MVista *Blastomyces* Antigen EIA; MiraVista Diagnostics, Indianapolis, IN). This assay targets a glycoprotein antigen that unfortunately is not genus-specific. The test has high sensitivity in the urine, ranging from 76% to 93% across five studies [110, 111, 124, 134, 136]. However, the sensitivity is much lower in serum (in contrast to histoplasmosis). While the specificity is high for patients without fungal disease, the cross-reactivity is similar to the MVista *Histoplasma* Antigen EIA (common cross-reaction with penicilliosis, paracoccidioidomycosis, and histoplasmosis; less frequent with coccidioidomycosis; rarely in aspergillosis; and possibly in sporotrichosis) [133]. While antigen positivity can remain up to 200 days on average while on antifungals, some authors suggest antigen monitoring can be useful to guide treatment [124, 137].

Detection of Fungal Metabolites

No tests of this nature are currently available.

Conclusions and Recommendations

The standard to diagnose blastomycosis remains identification through culture or histopathology. Due to long lead times to culture positivity, there is a role for noninvasive antigen testing with sensitivity favoring urine over serum, but BAL or CSF specimens should also be tested in the correct clinical scenario. However, caution is advised given the high degree of test cross-reactivity with other fungal diseases, especially *Histoplasma*, which is difficult to differentiate in overlapping endemic areas. Ultimately, most authors would suggest urine antigen testing as a means to provide preliminary diagnosis and initiate antifungal therapy until definitive

diagnosis is made on culture or histopathology. Unfortunately, the poor accuracy of currently available *Blastomyces* antibody testing is generally unhelpful for diagnosing blastomycosis. Therefore, a negative antibody test should not be used to rule out disease, nor should a positive test be an indication to start treatment (see Box 6.4 for recommendations).

Coccidioidomycosis

Culture and histopathology remain the gold standards for positive diagnosis of coccidioidomycosis; however, antibody and antigen testing has become critically important in routine clinical care. Culture is estimated to be positive in only around half of coccidioidomycosis and growth is relatively slow [138]. In addition, Biosafety Level 3 conditions are required as they pose a risk of acquisition to laboratory personnel. Direct examination by histopathology has even lower sensitivity because of the small number of *Coccidioides* organisms present in most clinical specimens. The mycelial form of growth rarely allows microscopic identification of *Coccidioides* species, requiring further testing, often in a reference laboratory, to confirm the diagnosis. These limitations are why most diagnoses of coccidioidomycosis are made by immunologic testing.

Antibody Detection

The cornerstone of serologic diagnosis is based on the detection of anti-coccidioidal antibodies via several different laboratory methods (Table 6.6). The original assays are the tube precipitin (TP) and complement fixation (CF) tests. Subsequently, two immunodiffusion tests, the IDTP and IDCF, were developed, which detect the same TP and CF antibodies by an alternative, more straightforward procedure. The TP test detects primarily IgM antibodies directed against the TP antigen, a heat-stable carbohydrate in the fungal cell wall. In contrast, the CF assay detects primarily IgG antibodies directed against the chitinase antigen, a protein enzyme of the fungal cell wall, which is generally detectable while the disease is active.

EIAs have subsequently been developed, which measure IgM and IgG antibodies directed against the TP and chitinase (of the CF test) antigens, respectively.

Three tests are widely employed today: EIA, ID (both IDTP and IDCF), and CF. EIAs are relatively simple to perform at local laboratories, can be performed on serum and CSF, and are generally quite accurate. However, IgM antibody testing may have significant false-negative rate early disease, and immunocompromised patients require serial testing [142, 143]. In addition, an isolated positive IgM (when IgG is negative) may be falsely positive. This relatively common finding (~10% of positive tests) has been the subject of several publications with quite discrepant conclusions (false-positive rate range 0–82%) [144]. In particular, one should question the validity of an isolated positive IgM test in an asymptomatic patient or one without classic symptoms.[115, 143]. In general, EIAs should be confirmed with ID and/or CF.

ID and CF testing is performed by reference laboratories as they are more labor intensive and require a higher level of skill. ID assays are sensitive (although perhaps not as sensitive as EIA) and highly specific; they are often used to confirm the results of other serologic tests. The CF assay is quantitative, expressed as titers, and generally reflect the extent of infection. Use of serial testing can help the clinician gauge disease progression, remission, or cure. In contrast to many other infectious diseases, CF (and IgG) becomes undetectable with treatment over time [145].

Notably, these assays are less reliable in the immunosuppressed population. In a publication evaluating 27 solid organ transplant recipients with newly acquired coccidioidomycosis, the sensitivity of all assays (EIA, ID, and CF) used in conjunction was only 77% (20/26), whereas the positivity of any single assay was limited (see below for discussion of utility of antigen testing in this population) [146]. For patients being evaluated for transplantation residing or spent significant time in an endemic area, serology and chest imaging screening is recommended to determine potential treatment prior to transplant [147].

Coccidioidal meningitis is a notoriously difficult diagnosis to establish; CSF testing should generally include CF (or EIA) testing in addition to fungal culture (plus/minus antigen and/or PCR testing). When EIA and CF tests are generally less sensitive than serum, when positive, these tests are highly specific for meningitis [148]. In contrast with serum, CF titer levels do not necessarily correspond to extent of disease [149].

Antigen Detection

Research laboratories have demonstrated the ability to detect coccidioidomycosis antigen in both acute and chronic disease [150]. This sparked the development of the MVista *Coccidioides* antigen EIA (MiraVista Diagnostics, Indianapolis, IN). In the initial report of patients who were mostly immunosuppressed and who had moderate-to-severe disease, the test in urine demonstrated a 71% (17/24) sensitivity and 99% (159/160) specificity in healthy volunteers; however, cross-reactivity was seen in 11% (3/28) with other endemic mycoses (2 histoplasmosis, 1 paracoccidioidomycosis) [114]. A second study evaluated the same test in serum (pretreated with EDTA at 100 °C) and urine in 28 patients with milder disease; in this limited dataset, the sensitivity in

serum and urine were 73% and 50%, respectively [116]. A more recent retrospective study demonstrated higher sensitivity when combining antigen and ID antibody testing in an endemic area. ID sensitivity alone was 84.2%, but, when combined with serum or urine antigen testing, sensitivity increased to 93.0%. Importantly, EIA antibody screening was not evaluated, and 52% of the patients were immunocompromised, so the value of antigen testing could be overestimated particularly outside this special population [125].

In the largest study to evaluate CSF antigen testing for coccidioidomycosis meningitis, the test performed well with a sensitivity of 93% and this further increased to 98% when combined with CSF CF and ID [140]. Antigen testing in any specimen is limited by cross-reactivity with histoplasmosis and blastomycosis.

Detection of Fungal Metabolites

No tests of this nature are currently available.

Skin Testing

Skin testing with coccidioidin antigen or spherulin antigen is a useful epidemiologic tool to document past exposure. It may also be useful in patients in whom pulmonary coccidioidomycosis has already been proven by other means. A negative skin test in such a patient may be a bad prognostic sign, suggesting current or impending dissemination. In 2011, the FDA cleared a reformulated test: the *Coccidioides immitis* Spherule Derived Skin Test Antigen (Spherusol; Allermed Laboratories, San Diego, CA). In a published report, this test demonstrated good safety, no histoplasmosis cross-reactivity, sensitivity of 98% in patients with a history of pulmonary coccidioidomycosis, and 98% specificity in adult volunteers living outside an endemic area [151]. The niche for this test in clinical practice remains unclear.

Conclusions and Recommendations

The manifestations of most early coccidioidal infections overlap with those of other respiratory infections; therefore, specific laboratory testing is required to establish a diagnosis of coccidioidomycosis (see Box 6.4 for recommendations). In general, screening for coccidioidomycosis is performed using either an EIA or ID. If EIA is used, it should be confirmed with ID and/or CF. Serum antibodies are important in the diagnosis of this disease and develop in most immune competent patients; however, antibodies are less reliable early in disease or in the immunosuppressed host, particularly transplant, for whom the addition of serum and/or urine antigen tests may help augment disease detection. If initial serology is negative, repeat testing, potentially using all available assays (antibody and possibly antigen) in conjunction, will improve the diagnostic yield. Antigen testing also appears to play an important role in addition to culture and antibody testing in meningitis diagnosis.

For most patients who resolve their infection, the antibody concentrations decrease to undetectable levels during the course of illness, so measurable antibodies are more likely to represent a recent or active illness. The CF assay is primarily used to follow titers, which gauge clinical progression and response to therapy.

Paracoccidioidomycosis

A definitive diagnosis of paracoccidioidomycosis requires either direct visualization of the organism in body fluids or tissues or its isolation and growth in culture; however, immunologic assays are useful and rapid adjuncts for diagnosis, prognosis, and following treatment response. In at least part due to epidemiology, there are no commercially available diagnostic serologic tests in the USA specific to paracoccidioidomycosis; testing may be performed in-house or at reference centers. BG may be an emerging option as a nonspecific surrogate test (see below).

Antibody Detection

Initial efforts at antibody detection caused significant cross-reactivity with other fungal pathogens, leading to the development of antibody tests (ID, LA, counterimmunoelectrophoresis, ELISA) using more specific *P. brasiliensis* antigens. The most commonly used method is ID, which is very specific and has varying sensitivity depending on the antigen preparation used [152]. For example, the sensitivity of ID using the Ag7 antigen preparation was 84% in one multinational study. The 43kDa glycoprotein (gp43) from culture filtrates, now believed to be the dominant immunoantigen of *P. brasiliensis*, has been the target of improved serodiagnosis. A large report on 422 patients being evaluated for paracoccidioidomycosis in Brazil evaluated the use of double immunodiffusion (DID) and Western blot (WB) using a crude exoantigen (primarily consisting of gp43). In this study, the sensitivity and specificity of DID were 80% and 95%, respectively, and the corresponding values for WB were 92% and 64% [153]. DID is considered the test of choice in endemic areas due to familiarity, ease of testing, and cost. Titers correlate with disease severity and can also be followed to determine treatment resolution; however, patients should be tested at their original site of diagnosis as much inter-laboratory variability exists [154]. Additionally, there is some cross-reactivity with other regional diseases

including histoplasmosis and leishmaniasis, and antibody responses are less robust in immunosuppressed patients. In 2014, a novel species of paracoccidioidomycosis, *P. lutzii*, was discovered to be pathogenic in humans. As this species does not express the gp43 antigen and may be missed by traditional methods, there are currently no validated serological techniques to diagnose *P. lutzii*, although DID is still the method used for its detection [155, 156].

Antigen Detection

The same cell wall and cytoplasmic components were also used as targets in early antigen detection assays. Unfortunately, these assays were also limited because of significant cross-reactivity in sera from patients with other mycoses (namely, aspergillosis and histoplasmosis). To improve on this, a new target was sought and gp43 appears promising (see "antibody detection" section above). An immunoblotting assay, performed on urine specimens, has demonstrated good sensitivity and excellent specificity for the detection of this antigen [157]. Also, an ELISA using a monoclonal antibody successfully detected the gp43 antigen in serum, CSF, and BAL fluid of patients with confirmed acute and chronic paracoccidioidomycosis with less cross-reactivity [158, 159]. Furthermore, these antigen levels can be followed as a marker of treatment response [160].

Of interest, given the paucity of available testing in most laboratories, a recent study evaluated Fungitell BG as a screening test for paracoccidiodomycosis. BG showed high sensitivity as a screening test, detecting 28/29 patient serum samples with active infection. This test seems promising in the correct clinical scenario, with the obvious issue that the test would not be specific or play a reliable role in monitoring clinical response (Table 6.1) [16].

Detection of Fungal Metabolites

No tests of this nature are currently available.

Conclusions and Recommendations

Immunologic tests are useful for rapid diagnosis in suspected cases of paracoccidioidomycosis as ~80–90% of patients with clinical disease have specific antibodies at the time of diagnosis. In disseminated disease, antibody titers are high, providing useful prognostic information. Antibody testing however is limited, particularly in immunosuppressed patients, to the caveat that it can have varying sensitivity and miss cases. In contrast, BG and emerging gp-43 antigen–based testing methods have demonstrated high sensitivity. While serum antibody is the standard recommended immunodiagnostic test, emerging data suggest that antigen (BG and/or gp43 where available) also may provide additional diagnostic yield with the caveat that the antigen assays (BG more so than gp43) may detect infection with other mycoses. While most serologic testing is based on the gp43 antigen produced by *P. brasiliensis*, the more recently discovered pathogenic *P. lutzii* has wide antigenic variation, does not contain gp43, and is thus prone to false-negative results by traditional testing [161].

Other Mycoses

Immunodiagnostic tests have also been investigated for several other fungal infections, namely, mycetoma, mucormycosis, penicilliosis, sporotrichosis, and dermatophytoses. While they target a variety of antigens, antibodies, and nucleic acids, they are unfortunately still limited by a lack of prospective trials and commercial availability and cannot yet be recommended for routine clinical use.

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Suggested Reading

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Diagnostic Radiology

7

Maria Angela C. Hospenthal, Christine Nwoke, and Lauren K. Groner

Introduction

Radiologic imaging of the structures of the central nervous system (CNS), upper and lower respiratory tract, abdomen, and musculoskeletal system is integral to the diagnosis and management of most human mycoses. Although there are no pathognomonic radiological findings associated with fungal infections, diagnostic imaging combined with clinical data (including prior endemic exposures, use of invasive devices, coexisting disease or immunodeficiency, surgeries, and duration of illness) can be used to improve diagnostic accuracy and aid in the long-term treatment of many fungal infections.

Central Nervous System Imaging

Magnetic resonance imaging (MRI) is superior to computed tomography (CT) in evaluating fungal infections of the brain and has been shown to be more sensitive than CT for detecting abnormalities. CT commonly underestimates the extent of disease in fungal infection [1]. MRI is especially helpful in the early phases of disease when the brain CT may be nondiagnostic. Magnetic resonance imaging takes advantage of the inherent properties of molecules, especially hydrogen, and manipulates their behavior in an electromagnetic field to generate an image. The composition of tissues and their differences when pathology is present can therefore be distinguished by altering parameters of the electromagnetic field to see the effect on the molecules of the tissue being evaluated. Longitudinal relaxation time (T1) and transverse relaxation time (T2), along with other factors, influence signal intensities, which offer details on specific tissue characteristics. Findings on MRI, such as edema and contrast enhancement, are affected by the inflammatory response, which itself is highly dependent on the competence of the immune system. Nevertheless, patients with MRI images of noncircumscribed, ill-defined areas with little or no contrast enhancement should raise the suspicion for infectious etiologies, especially those with immunodeficiency, as they are predisposed to fungal infections [2]. Computed tomography of the brain with contrast may be normal initially and thus is more helpful in assessing later stages of infection with eventual findings of focal ring enhancing or hemorrhagic lesions. Other brain imaging modalities include proton magnetic resonance spectroscopy (MRS), which has been reported to be useful in the evaluation of fungal abscesses and differentiate these from other intracranial lesions by analyzing metabolic characteristics using echo time (ET) spectra [3]. Diffusion-weighted imaging (DWI) may also be helpful, particularly in diagnosing early infection or differentiating images caused by fungal infections from those associated with neoplastic, vascular, or noninfectious inflammatory processes [1]. A wide variety of radiologic findings may be seen, although intracerebral masses and meningeal enhancement predominate in these infections (Table 7.1).

CNS Mass Lesions

Intracerebral masses are one of the more common findings in fungal brain infections. Predominantly, granulomas or solid enhancing lesions are reported. In *Aspergillus* infections, these have sometimes been referred to as "aspergillomas." Likewise, in patients with cryptococcal infections, the term

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_7

Table 7.1 Abnormalities more commonly seen in central nervous system imaging of fungal infections

Radiological finding	Fungus
Granulomas or solid enhancing lesions	*Aspergillus*, *Cryptococcus*, *Histoplasma*, *Candida*, *Paracoccidioides*
Abscesses	*Aspergillus*, *Blastomyces* (epidural), *Coccidioides*, *Cryptococcus*, *Candida*, dematiaceous fungi, *Pseudallescheria boydii* (currently *Scedosporium apiospermum* species complex)
Parenchymal/ leptomeningeal nodules; pseudocysts	*Cryptococcus*
Hemorrhagic/ infarcted lesions	*Aspergillus*
Meningeal enhancement	*Blastomyces*, *Coccidioides* (chronic granulomatous), *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Aspergillus*
Hydrocephalus	*Cryptococcus*, *Coccidioides*, *Paracoccidioides*

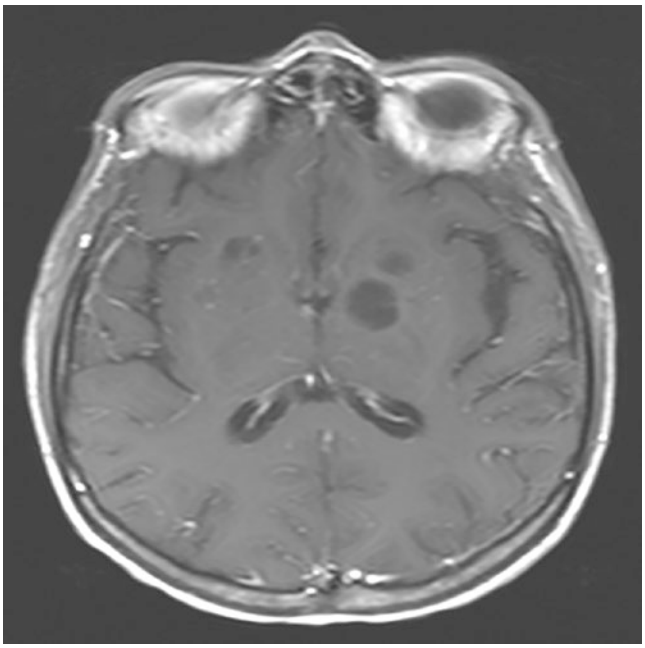


Fig. 7.1 Contrast-enhanced TI transaxial MRI image of the brain demonstrating low signal intensity lesions in the bilateral basal ganglia (left greater than right) associated with no significant enhancement, consistent with gelatinous pseudocysts of cryptococcosis. Also note mild meningeal enhancement

"cryptococcoma" has been used. Cryptococcomas can be single or multiple punctate (i.e., miliary) hyper-intense, small, round lesions on T2-weighted images (T2WI) [4, 5]. Intraparenchymal cryptococcomas show low signal intensity on T1-weighted images (T1WI) and high intensity on T2WI [1, 6, 7]. Immunocompetent patients are more likely to present with cryptococcomas [1] and are more likely to demonstrate enhancement, secondary to ability to mount an immune response [1, 8]. Persistence of cryptococcomas over a prolonged period has been documented and found to be inconsistent with active disease [9]. Future methods, which include bioluminescence imaging (BLI) that allow for demonstration of direct in vivo effects of antifungal therapies on fungal cell growth rates, may enhance traditional MRI surveillance of these lesions.

Pseudocysts are also seen in cryptococcal infection as CSF signal-equivalent (low signal intensity on T1WI and high signal intensity on T2WI) fluid collection that are most commonly seen in the basal ganglia, thalami, midbrain, cerebellum, and periventricular matter (Fig. 7.1). Lesions in the basal ganglia and thalami strongly suggest cryptococcal infection; intraventricular formations are rare but have been reported [1, 8]. Single or multiple enhancing brain lesions have also been reported in *Histoplasma*, *Candida*, and *Paracoccidioides* infections.

Fungal infections in the brain can lead to abscess formation [10]. These lesions can be multiple, hypodense, and may exert little mass effect. They may or may not enhance [11] (Fig. 7.2). Although abscesses occur most commonly in the cerebral hemispheres, they have also been visualized in the cerebellum and brainstem [12]. Organisms reported to cause abscess include *Aspergillus*, *Coccidioides*, *Cryptococcus*, and *Candida*. Candidal organisms tend to cause focal necrosis producing microabscesses [13, 14]. Less commonly, the dematiaceous molds, *Pseudallescheria boydii*, endemic

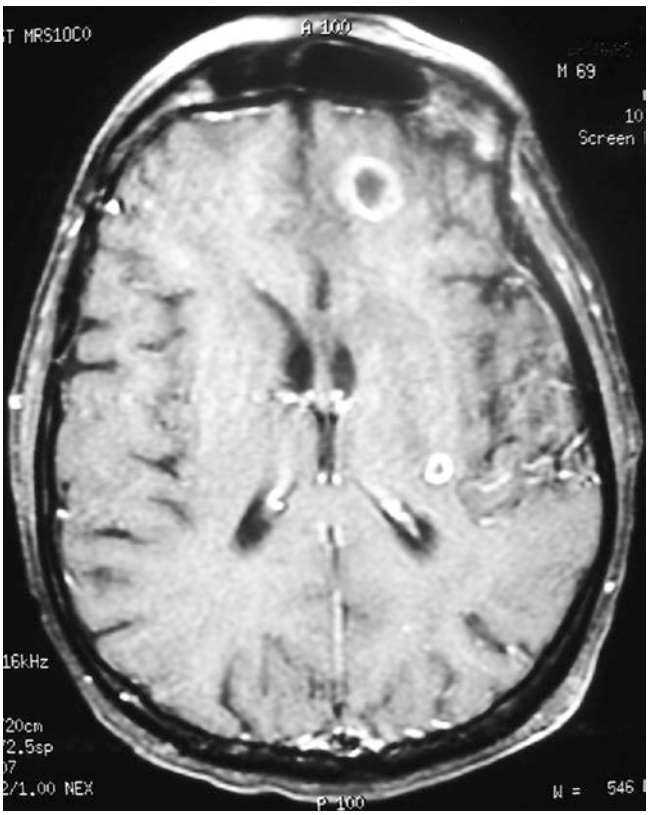


Fig. 7.2 Contrast-enhanced MRI showing multiple ring-enhancing brain abscesses in immunocompromised patient with disseminated aspergillosis. Note the lack of surrounding edema. Courtesy of Dr. D. R. Hospenthal

mycoses such as blastomycosis, paracoccidioidomycosis, talaromycosis, and sporotrichosis, have been reported to cause brain abscesses, and even more rarely meningeal or epidural abscesses [15, 16].

Other intracerebral masses associated with fungal pathogens include edematous, hemorrhagic, or infarcted lesions such as those seen in *Aspergillus* infections [10]. The hemorrhagic lesion, usually a consequence of an area of infarction, is an early radiologic sign owing to the angioinvasive nature of certain fungi [17]. A peripheral ring of isointensity or low signal intensity on T2WI relates to a dense population of fungal hyphae containing paramagnetic elements and small areas of hemorrhage [18]. On cross-sectional imaging, these lesions show little or no enhancement or mass effect [10]. Similar to pyogenic abscesses, fungal abscesses demonstrate decreased diffusion [19].

Meningeal Enhancement

Diffuse enhancement of the meninges on MRI is another common radiological finding of fungal infection of the CNS, thought to be due to active inflammation (meningitis). *Histoplasma*, *Blastomyces*, *Coccidioides*, *Paracoccidioides*, *Cryptococcus*, as well as *Aspergillus* have all been observed to produce meningeal enhancement. *Coccidioides* meningitis early in its course can cause focal or nodular enhancement in the basal cisterns, which represent focal organization of the fungus surrounded by inflammation [20]. Meningeal involvement may be seen secondary to direct extension from fungal infections involving the paranasal sinuses, such as in blastomycosis and mucormycosis [1, 16]. Leptomeningeal enhancement in coccidioidomycosis has been known to extend into the spinal canal as well [20].

Hydrocephalus

Hydrocephalus is usually a communicating hydrocephalus and is a consequence of meningeal involvement (acutely due to meningeal exudate and later because of meningeal adhesions); it is most associated with infections by *Cryptococcus*, *Coccidioides*, and *Paracoccidioides* [1, 8, 21] (Fig. 7.3). While CT is helpful in identifying dilated ventricles, MRI can better assess the patency of the aqueduct of Sylvius. Other nonspecific CNS radiological findings include early vascular enhancement and diffuse cerebral edema.



Fig. 7.3 MRI of brain revealing leptomeningeal enhancement and hydrocephalus in a patient with coccidioidomycosis

Respiratory Tract Imaging

Sinus Imaging

Fungal infections of the nasal cavity and paranasal sinuses are classified as noninvasive (allergic sinusitis or sinus fungus balls) or invasive. Invasive sinonasal fungal infections, characterized by the presence of fungal hyphae within the paranasal sinus mucosa, submucosa, bone, or blood vessels, can be further classified as acute, chronic, or chronic granulomatous fungal sinusitis. Risk factors for fungal sinonasal infections include diabetes mellitus, oral corticosteroid use, human immunodeficiency virus (HIV), and chemotherapy [22].

Computed tomography (CT) is the reference standard and modality of choice to evaluate sinonasal fungal infections [22]. Specifically, CT of the sinuses may demonstrate the presence and extent of soft tissue invasion, necrosis, and early bone erosion. CT can evaluate intraorbital and intracranial involvement. MRI with and without contrast is superior to CT for evaluating intracranial and intra-orbital extension, as MRI can show early changes in major vessels and better demonstrate complications such as cavernous sinus thrombosis [22–24].

Noninvasive Fungal Sinusitis

Allergic fungal sinusitis, the most common form of fungal sinusitis, is an IgE-mediated hypersensitivity reaction to fungal antigens [22]. A history of asthma, allergic rhinitis, or atopy in immunocompetent patients is common. CT will show sinus opacification and expansion by hyperattenuating mucin. MRI will show corresponding mixed signal intensity on T1-weighted imaging (T1WI) and hypointensity (signal voids) on T2WI related to high concentrations of proteins and metal ions and low water content. An inflamed, edematous mucosal lining will appear hyperintense on T2WI and hypo- and hyper-intense on noncontrast and postcontrast T1WI, respectively. Bony remodeling and erosion can occur as mucin increases.

Formation of a fungus ball (sometimes incorrectly termed a mycetoma) is a relatively uncommon presentation of fungal sinusitis, most often caused by *Aspergillus fumigatus* (less often by *Pseudallescheria boydii* (currently named *Scedosporium boydii* in the *S. apiospermun* species complex) and *Alternaria*). A fungus ball is a mass-like accumulation of fungal hyphae, typically due to poor mucociliary clearance [22, 25]. The maxillary sinuses are most commonly involved and older immunocompetent women most affected. Unilateral involvement is more common than bilateral disease [22, 25]. Noncontrast CT (NECT) will show a hyperattenuating mass with intrinsic hyperdensities or calcifications corresponding to metallic byproducts, typically with hypoattenuating mucosal thickening, which enhances on contrast-enhanced CT (CECT), as well as periosteal thickening and sclerosis. Focal areas of periosteal erosion may also be seen. T1- and T2-weighted MRI will show a hypointense fungus ball and a rim of T2 mucosal hyperintensity.

Invasive Fungal Rhinosinusitis (IFR)

IFR is characterized by neurovascular invasion by fungal organisms leading to thrombosis and tissue ischemia and necrosis. Acute IFR is a rare, aggressive form of IFR with mortality rates between 50% and 80%. Acute IFR typically affects immunocompromised and immunosuppressed patients after inhalation of fungal spores, and is usually caused by *Aspergillus* species and fungi of the order Mucorales [26]. Early NECT features include unilateral soft-tissue attenuation in the nasal cavity and thickening of the nasal mucosa, turbinates, septum, and walls, as well as hypoattenuating sinus mucosal thickening and intraluminal soft tissue, most often affecting the ethmoid and sphenoid sinuses. Bone destruction is a relatively late finding, but occurs rapidly and leads to intraorbital and intracranial extension (Fig. 7.4). Extension from the sphenoid sinuses can cause cavernous sinus thrombosis and carotid artery invasion, pseudoaneurysm, and cerebral infarction and hemorrhage [25]. Intraorbital extension can be seen as hyperintense signal within thickened extraocular eye muscles and orbital fat on T2W MRI, indicating edema. Periantral fat obliteration may be one of the earliest signs of intracranial and intraorbital extension. Proptosis on both CT and MRI should also raise suspicion for intracranial and intraorbital involvement, particularly cavernous sinus thrombosis. Lack of enhancement on T1W postcontrast imaging is due to tissue necrosis, although leptomeningeal enhancement can be seen with intracranial involvement and can be subtle. Abscess and granuloma formation can also occur with intracranial extension. Granulomas are hypointense on T1WI and T2WI and enhance minimally.



Fig. 7.4 Transaxial sinus CT of patient with mucormycosis demonstrating osteolysis of the hard palate (arrow) and left maxillary sinus mucosal thickening with surrounding soft tissue air

Chronic IFR has a more indolent course, occurring over months to years, and affects immunocompetent or slightly immunocompromised individuals (i.e., patients on chronic oral corticosteroids). In addition to infection by *Aspergillus* species and the Mucorales, *Bipolaris* (and other dematiaceous fungi) and *Candida* species are also implicated. NECT findings include hyperattenuating soft tissue within the paranasal sinuses, sometimes associated with sinus expansion, sinus wall destruction, and extra-sinus extension, as well as bony mottling and irregularity mimicking malignancy. Osseous sclerosis is also common. Intraluminal soft tissue is hypointense on T1- and T2-weighted MRI. Imaging features of intracranial and intraorbital involvement are similar to acute IFR. Cavernous sinus thrombosis, mycotic aneurysm, cerebral infarction and hemorrhage, cerebral and epidural abscess, meningitis, and osteomyelitis can all be seen.



Fig. 7.5 Transaxial CT image through the sinuses demonstrating high density opacification of the maxillary sinuses consistent with fungal sinusitis

Chronic Granulomatous IFR (CGIFR) is typically due to *Aspergillus flavus* infection of immunocompetent individuals, most often in patients from Sudan and Southeast Asia and rarely the United States [25]. Some consider CGIFR a subset of chronic IFR rather than a distinct entity. Imaging findings are similar to CIFR and malignant neoplasms. A soft tissue mass with sinus opacification and flocculent calcifications are the major findings on NECT [25, 27] (Fig. 7.5).

Pulmonary Imaging

Fungal pneumonia can affect immunocompetent and immunocompromised hosts. Fungal infections affecting immunocompetent hosts include coccidioidomycosis, blastomycosis, and histoplasmosis, whereas pneumocystosis, candidiasis, aspergillosis, and mucormycosis affect immunocompromised individuals. Although pulmonary fungal infections can mimic other infectious and noninfectious processes, various imaging findings may raise suspicion for fungal pneumonia.

Chest radiography (CXR) is the initial modality of choice for evaluating suspected pneumonia in immunocompromised and immunocompetent patients. Findings are usually nonspecific and CXR may be normal early in the disease course, as sensitivity is low for subtle abnormalities. CT with or without contrast is more sensitive than CXR in detecting parenchymal and airways disease, particularly subtle findings, and better characterizes pleural involvement. MRI is less sensitive than CT in detecting parenchymal abnormalities and is not appropriate in the initial work-up of fungal pneumonia. Imaging findings of pulmonary fungal infections are described in Table 7.2.

Table 7.2 Abnormalities more commonly seen in pulmonary imaging of fungal infections

Radiological finding	Fungus
Alveolar infiltrates	*Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Pneumocystis*, Mucorales
Interstitial infiltrates	*Aspergillus*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Talaromyces*, *Pneumocystis*
Nodules	*Aspergillus*/Mucorales (halo sign), *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Pneumocystis*
Masses	*Aspergillus*, *Blastomyces*, *Coccidioides*, *Cryptococcus*, Mucorales
Cavitation	*Aspergillus*/Mucorales (air crescent sign), *Blastomyces*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Pneumocystis*
Abscesses	*Candida*, *Scedosporium*, Mucorales
Lymphadenopathy	*Coccidioides*, *Cryptococcus*, *Histoplasma*
Pleural effusion	*Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Pneumocystis*

Airspace Consolidation

Consolidation is a common, but nonspecific manifestation of fungal pneumonia, seen in invasive and noninvasive aspergillosis, allergic bronchopulmonary aspergillosis (ABPA), candidiasis, mucormycosis, cryptococcosis, pneumocystosis, histoplasmosis, coccidioidomycosis, and blastomycosis. Consolidation may be focal (segmental or lobar), patchy unilateral or bilateral, or peribronchial (bronchopneumonia pattern) [28, 29].

Consolidation is a common acute finding in invasive pulmonary aspergillosis (IPA) and ABPA. Consolidation in angioinvasive IPA (the most common cause of IPA) may be peripheral and wedge-shaped, representing a hemorrhagic infarct [30]. A pulmonary infarct in the setting of invasive fungal pneumonia can also manifest as a complete or incomplete rim of consolidation surrounding central ground-glass opacity, referred to as the reversed halo sign (RHS). Invasive fungal pneumonia is the most common infectious cause of a RHS and while it can be seen in IPA, mucormycosis is the most common infection to manifest as a RHS and will often have reticulation superimposed on central ground-glass ("bird's nest" sign) (Fig. 7.6). Peribronchial and peribronchiolar consolidation is common in airway-invasive pulmonary aspergillosis (the second most common cause of IPA) and typically accompanied by tree-in-bud nodules [31]. Upper lobe consolidation with cavitation has been described in chronic pulmonary aspergillus, as well as coccidioidomy-

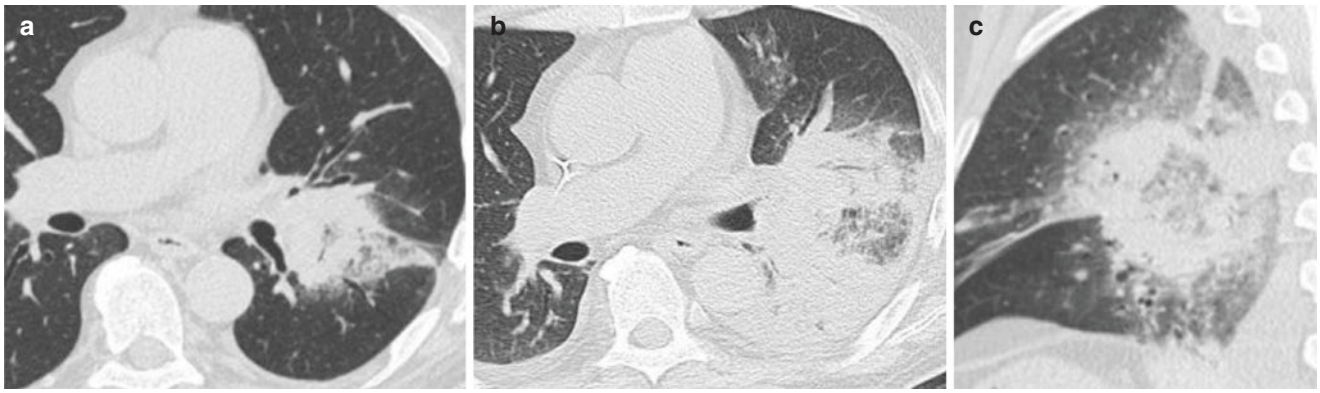


Fig. 7.6 Mucormycosis in a 65-year-old man with acute myeloid leukemia status post stem cell transplant. Initial axial CT image (**a**) shows a consolidation with a reversed halo sign in the left lung. Axial (**b**) and sagittal (**c**) CT images 6 days later show an enlarging consolidation and a reverse halo sign (RHS) resembling a "birds nest" with reticulation in the central ground glass. Increasing ground glass surrounding the consolidation represents hemorrhage

cosis, blastomycosis, and histoplasmosis [32] (Fig. 7.7). Acute histoplasmosis often shows consolidations accompanied by lymphadenopathy on CT. Consolidation is the most common manifestation of blastomycosis (25–75%) and coccidioidomycosis (75%), and occurs in over 65% of patients with candidiasis [31–33]. Consolidation in blastomycosis may be large and confluent or patchy and bilateral. Central consolidation abutting the mediastinum is seen in 31% of patients [31, 34]. In coccidioidomycosis, consolidation may be solitary or multiple (segmental or lobar) and can be seen in any part of the lung, but often has a central (perihilar) and basilar predominance. Multifocal consolidation in coccidioidomycosis can be migratory and are described as phantom consolidations [31, 33, 35].



Fig. 7.7 Axial noncontrast CT image shows a large consolidation in the right upper lobe with central cavitation and surrounding ground-glass opacity secondary to coccidioidomycosis

Interstitial Pattern

An interstitial pattern, defined by the presence of bilateral reticular or linear opacities, most often occurs in pneumocystis and chronic paracoccidioidomycosis (PCM), although cryptococcosis may manifest as an interstitial pattern in immunocompromised hosts with a reticular or reticulonodular pattern [36].

Pneumocystis pneumonia typically manifests as bilateral perihilar hazy (ground-glass) and reticular opacities on CXR. Inter- and intralobular lines (reticulation) superimposed on diffuse bilateral peribronchovascular ground-glass opacity are the most common CT pattern, although thin-walled cysts may be seen in patients with HIV/AIDS, and consolidation may develop if untreated (Fig. 7.8) [37–39].

An interstitial pattern in PCM is associated with chronic infection and characterized by linear opacities, thickening of the interlobular septa and peribronchovascular interstitium, intralobular lines, ground-glass opacity (corresponding to alveolar septal thickening or fibrosis), and cavities associated with architectural distortion, traction bronchiectasis, and paracicatricial emphysema. Concomitant consolidation and nodules or masses are also common [40, 41].

Nodules and Masses

Nodules and masses, defined as round opacities measuring <3 centimeters (cm) and >3 cm in size, respectively, are a common manifestation of fungal pneumonia. Nodules may be single or multiple with well-defined or ill-defined edges. Ill-defined nodular consolidations are common in aspergillosis, mucormycosis, candidiasis, and cryptococcosis, and are typically bilateral, scattered, and >1 cm. Multiple well-defined nodules measuring 1–3 cm in size are a common presentation of IPA on CXR [32, 42]. On CT, solid nodules may demonstrate a halo of ground-glass, as described above, representing focal pulmonary infarction and surrounding hemorrhage. The CT halo sign is highly suggestive of invasive fungal pneumonia and most associated with angioinvasive aspergillosis in immunocompromised hosts [31, 43]

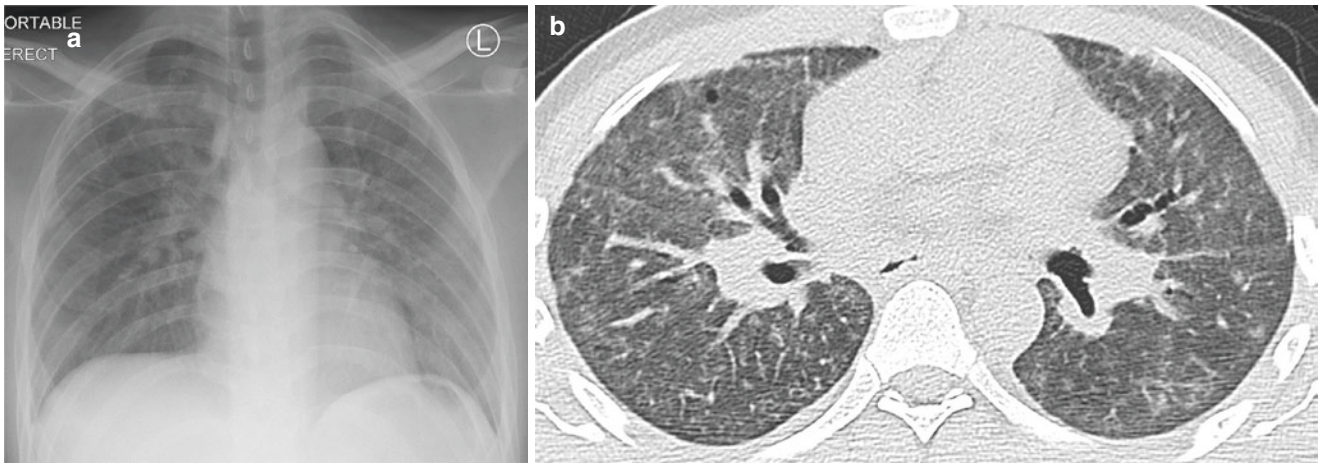


Fig. 7.8 Pneumocystosis in a patient with AIDS. AP chest radiograph demonstrates diffuse bilateral ground-glass opacity (**a**). Axial noncontrast chest CT image shows diffuse bilateral ground glass opacity, septal lines, and a small cyst in the right middle lobe (**b**)



Fig. 7.9 Invasive aspergillosis in a woman with leukemia status post stem cell transplant. Axial contrast-enhanced chest CT image shows a solid nodule in the right upper lobe with a halo of ground glass attenuation representing hemorrhage

(Fig. 7.9); Angioinvasive aspergillosis is the most common cause of IPA. Airway-invasive aspergillosis, accounting for 15–30% of IPA, often manifests as centrilobular and linear, branching (tree-in-bud) nodules and peribronchial and peribronchiolar consolidation on CT, representing necrotizing bronchitis and bronchiolitis [32].

Candidiasis can present with nodules as an isolated finding in 35% of patients. Solitary or multiple nodules are the second most common manifestation (30%) of blastomycosis after consolidation [32]. Nodules are the most common manifestation of cryptococcosis in immunocompetent patients and are typically multiple, small, and well-defined with a predilection for the middle or upper lungs [32, 44, 45]. A random or miliary pattern of nodules, characterized by diffuse, bilateral, innumerable 1–4 millimeters (mm) nodules, can be seen with disseminated coccidiomycosis, blastomycosis, cryptococcosis, and histoplasmosis [31]. Disseminated histoplasmosis is typically limited to immunocompromised hosts or young children, whereas subclinical pulmonary histoplasmosis is common in immunocompetent patients. Pulmonary histoplasmosis can also present with a solitary pulmonary nodule with central calcification ("bulls-eye" calcification) or several nodules measuring up to a few centimeters (cm). A large exposure to *Histoplasma capsulatum* can result in diffuse tiny nodules in a random pattern, which often calcify upon healing (Fig. 7.10).

Nodules are seen in up to 81% of patients with granulomatous *Pneumocystis* pneumonia and represent a granulomatous response to the disease. Nodules are typically multiple and random in distribution, ranging from a few mm to >1 cm in size. In the absence of adequate treatment, nodules will enlarge over weeks to months [46].

An aspergilloma, a subtype of chronic pulmonary aspergillosis, is a mobile round or ovoid nodule or mass that typically colonizes a pre-existing cavity, usually in the setting of sarcoidosis or tuberculosis. The Monod sign is used to describe the crescent of air that surrounds an aspergilloma in a cavity [47].

Nodules and masses that persist on imaging can mimic malignancy, particularly coccidiomycosis, histoplasmosis, aspergillosis, blastomycosis, and cryptococcus. Tissue sampling may be necessary to exclude neoplasm, as fluorodeoxyglucose-positron emission tomography (FDG/PET) CT is usually not helpful to distinguish fungal pneumonia from malignancy due to high false positive rates.

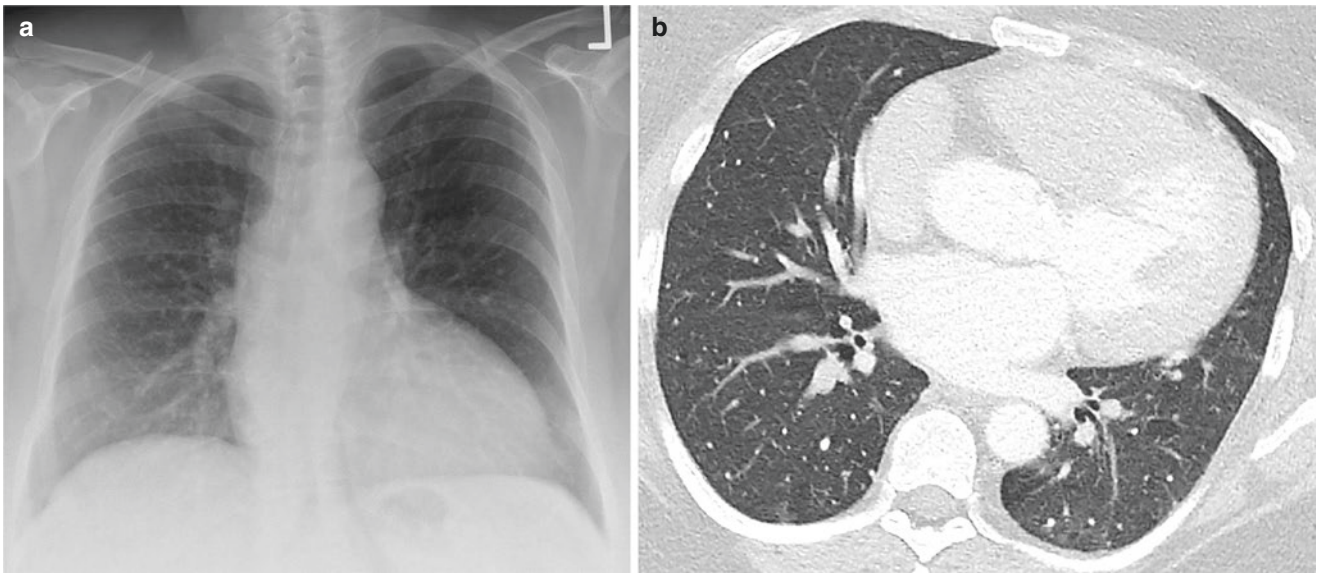


Fig. 7.10 Resolved histoplasmosis. Chest radiograph demonstrates numerous bilateral 2–4 mm nodules (**a**). Axial contrast-enhanced chest CT image shows numerous bilateral 2–4 mm calcified nodules consistent with remote pulmonary histoplasmosis (**b**)

Cavitation

Cavitation of consolidation, nodules, and masses is common in fungal pneumonia. Cavitary consolidation occurs in up to 48% of patients with blastomycosis [32]. Cavitary nodules are less common in cryptococcosis (10–15% of cases) and usually occur in immunocompromised hosts [32].

Nodule or mass cavitation occurs in 40% of patients with IPA [32]. The "air crescent" sign in angioinvasive pulmonary aspergillosis, whereby a crescent of air forms as necrotic lung is resorbed by neutrophils and separates from adjacent viable lung, is typically an indicator of healing, seen 2–3 weeks following treatment [48]. Cavities are typical of chronic pulmonary aspergillosis and the specific imaging features vary by subtype: (1) chronic cavitating pulmonary aspergillosis often shows cavities in the upper lobes with or without aspergillomas; (2) chronic necrotizing pulmonary aspergillosis (semi-invasive) typically shows cavities and consolidation and sometimes an air crescent sign; and (3) chronic fibrosing pulmonary aspergillosis demonstrates fibrocavitary changes and pleural and parenchymal fibrosis, which mimic tuberculosis and actinomycosis [48]. Cavitary consolidation in chronic histoplasmosis also has a predilection for the apical and posterior upper lobes, resembling tuberculosis [32].

Cavities may be an early or late manifestation of coccidioidomycosis (Fig. 7.11). Cavitary consolidation in acute disease occurs in up to 48% of patients and cavitary nodules in coccidioidomycosis often coalesce [32]. Cavitary consolidation or nodules may evolve over time into thick-walled cavities and subsequently thin-walled ("grape-skin") cavities [32, 33, 47]. A majority of cavitary lesions in coccidioidomycosis resolve spontaneously, however, chronic nodules and cavities can persist in a small subset of patients. Grape-skin lesions are suggestive of chronic coccidioidomycosis. A chronic fibrocavitary form of *Coccidioides* pneumonia, seen in 1% of patients, manifests on imaging as slowly progressive fibrocavitary changes characterized by biapical fibronodular lesions with architectural distortion, hilar retraction, and cavitation [33]. Cavities in chronic coccidioidomycosis may be complicated by fungal ball formation or bacterial superinfection and abscess formation. Irregular cavitary lesions in paracoccidioidomycosis may arise from necrotic nodules, masses, and consolidation. Cavitation and abscess formation in mucormycosis is relatively uncommon compared to consolidation and RHS [32]. Cavitation from blastomycosis is highly unusual and also rare in cryptococcosis outside of immunocompromised patients who commonly have cavitation on imaging. Lung cysts varying in size, shape, and wall-thickness occur in up to 30% of patients with PJP and are associated with higher rates of spontaneous pneumothorax [49].

Lymphadenopathy

Lymphadenopathy is common in histoplasmosis, coccidioidomycosis, and cryptococcosis. Most cases of histoplasmosis are subclinical and self-limiting; however, acute histoplasmosis can present with hilar or mediastinal lymphadenopathy and multifocal consolidation. In heavy exposure, lymphadenopathy is usually accompanied by diffuse tiny nodules in a random pattern. Hilar and mediastinal lymphadenopathy can undergo caseous necrosis and dystrophic calcification due to granuloma formation induced by a cell-mediated immune response in the host to *Histoplasma* antigens. A small subset of these patients have a continued

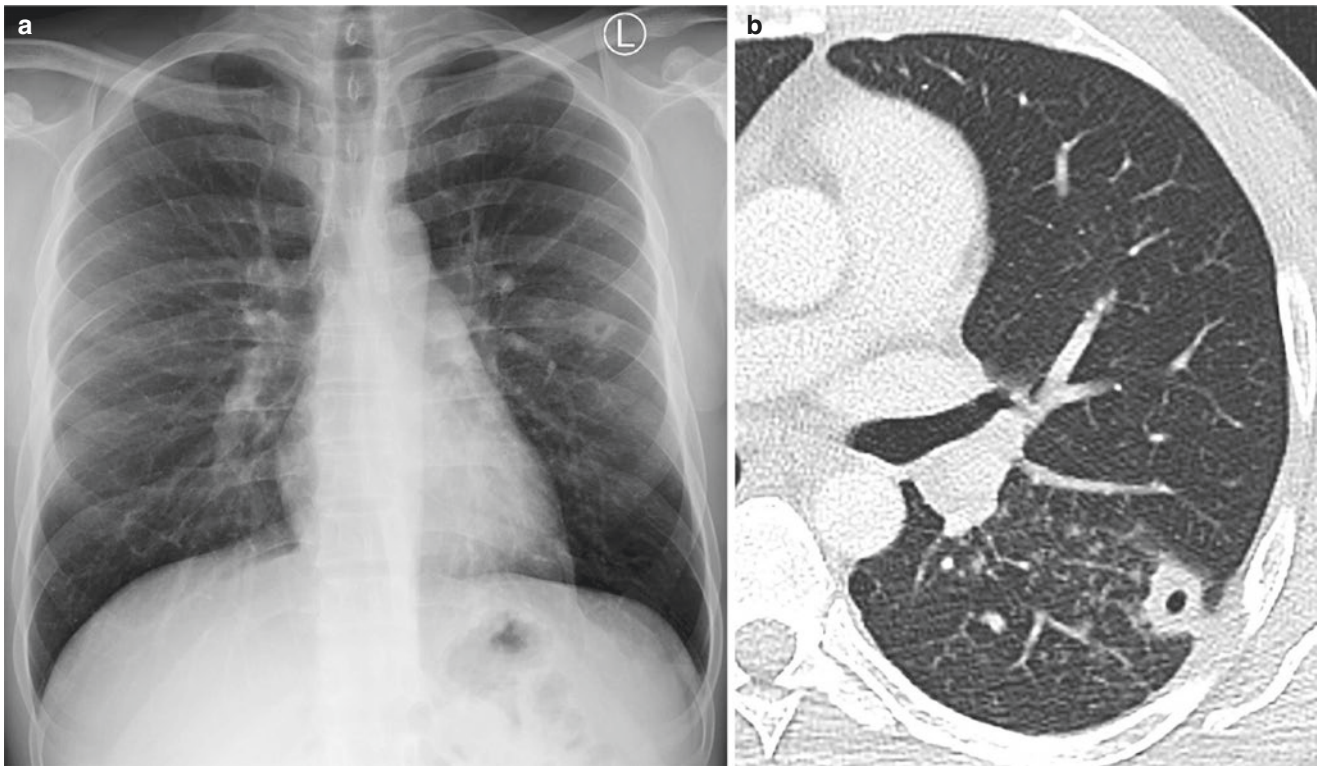


Fig. 7.11 Coccidioidomycosis. Chest radiograph demonstrates a cavitary nodule in the left lower lobe (**a**). Axial contrast-enhanced chest CT image shows the same cavitary nodule and centrilobular nodules (**b**)

fibroinflammatory response whereby fibrous tissue forms and replaces normal mediastinal fat, leading to subsequent narrowing and occlusion of mediastinal and hilar structures, including vasculature and airways, referred to as fibrosing mediastinitis. CT findings of fibrosing mediastinitis include infiltrative or localized soft tissue and calcification causing varying degrees of vascular and tracheobronchial encasement, narrowing, and occlusion [50]. Airway narrowing and occlusion can cause distal bronchial impaction, volume loss, and postobstructive pneumonia. Vascular narrowing or occlusion can cause hypoperfusion and mosaic attenuation of the lung defined as geographic areas of hypoattenuating, hypoperfused lung adjacent to areas of relatively hyperattenuating, hyper- or normally perfused lung. Interlobular septal thickening, ground-glass opacity, and bronchovascular thickening due to pulmonary venous and lymphatic congestion can also occur and lead to pulmonary hypertension. Patients with chronic pulmonary arterial occlusion will develop systemic collaterals, including bronchial artery and intercostal collaterals, that hypertrophy. Hemoptysis can occur as a result of arterial obstruction and systemic collaterals (i.e., bronchial artery collaterals) and chronic venous congestion. Pulmonary infarctions can also result from arterial and venous obstruction and lead to pulmonary hypertension. Histoplasmosis is the most common cause of fibrosing mediastinitis, although cases have also been documented in blastomycosis, cryptococcus, and mucormycosis [50]. Bilateral hilar lymphadenopathy is seen in 20% of patients with coccidioidomycosis [32]. Mediastinal and hilar lymphadenopathy in cryptococcosis is more common in immunocompromised patients than immunocompetent patients who typically have nodules.

Pleural Abnormalities

Pleural involvement is relatively uncommon in fungal infections compared to parenchymal disease. CT, particularly CECT, is helpful to demonstrate pleural enhancement and thickening. Acute coccidioidomycosis may present with transudative or exudative pleural effusions in 15–20% of patients [33], usually ipsilateral to parenchymal abnormality and often resulting from extension of parenchymal infection. Rarely, pneumothorax, pyopneumothorax, or bronchopleural fistula can be seen due to rupture of a peripheral cavity or cavitary nodule into the pleural space, warranting surgical repair and decortication. Culture of a pleural biopsy specimen has the highest diagnostic yield; however, diagnostic yield of pleural fluid is low [33]. Pleural effusions occur in up to 19% of patients with Mucormyocosis and should raise suspicion for pleural infection, as should pleural thickening [51]. Conversely, effusions are rare in IPA. Effusions are also uncommon in histoplasmosis and typically small when present [52]. Immunocompromised patients with cryptococcus

commonly have pleural effusions on imaging, but effusions rarely occur in immunocompetent patients [53]. Pleural effusions in *Pneumocystis* pneumonia are extremely rare, although spontaneous pneumothorax can occur due to ruptured pneumatoceles. Pleural thickening with concomitant upper lobe consolidation potentially progressing to cavitation over weeks to months can be seen in semi-invasive pulmonary aspergillosis [29].

Airway Abnormalities

Allergic bronchopulmonary aspergillosis (ABPA), a type I and III hypersensitivity reaction to inhaled *Aspergillus* conidia, most commonly occurs in patients with asthma, but may also occur in patients with cystic fibrosis, Kartegener's syndrome, or postlung transplantation [32]. CXR may show consolidation, tubular mucoid impaction, and atelectasis in the acute setting and hyperlucent, overinflated areas due to air trapping and hypoxic vasoconstriction in the chronic setting. Other CXR findings of chronic ABPA include atelectasis and tram-track parallel lines and ring-like lucencies in cases of moderate-to-severe bronchiectasis. CT will show cylindrical, varicoid, or cystic bronchiectasis with mucoid impaction described as having a "finger-in-glove" appearance. Hyperattenuating mucus or calcification within mucus plugs are seen in 19–28% of cases and associated with more severe infection and higher rates of recurrence (Fig. 7.12) [36].



Fig. 7.12 Allergic bronchopulmonary aspergillosis (ABPA). Axial contrast-enhanced chest CT image shows hyperattenuating material filling the left lower lobe bronchus and complete left lower lobe atelectasis and consolidation

Invasive aspergillosis can rarely cause isolated acute tracheobronchitis or pseudomembranous or ulcerative tracheobronchitis in immunocompromised individuals. CT findings of acute tracheobronchitis include tracheal and bronchial wall thickening and plaques, which may be hyperattenuating [29, 30, 54]. Mediastinal fat stranding may also be seen. Tracheal *Paracoccidioides* infection may be caused by extension of parenchymal infection or by infected sputum and is seen on CT as irregular tracheal wall thickening and nodularity due to submucosal infection and inflammation. The carina and bronchi are usually spared [41].

Uncommonly, mucormycosis presents with an endotracheal or endobronchial lesion, usually in patients with diabetes, and can cause fatal hemoptysis if the lesion invades the central vasculature [30]. An endobronchial lesion from *Talaromyces marneffei* causing postobstructive pneumonia has also been documented [55].

Cryptococcus infection of the larynx can manifest on CT as vocal cord irregularities and asymmetric enlargement [56].

Miscellaneous

Inhalation of *Aspergillus* conidia can cause an allergic response in the lungs called hypersensitivity pneumonitis, which typically resolves upon removal of the offending antigen, but may progress to pulmonary fibrosis with persistent, chronic exposure. Imaging findings of non-fibrotic (historically classified as acute and subacute) hypersensitivity pneumonitis overlap and may be seen on CXR as hazy (ground-glass) nodular opacities, although CXR may be normal. Typical CT findings include diffuse centrilobular ground-glass nodules and patchy ground-glass opacities often preferential to the upper lobes. Air trapping is usually present in the subacute phase, as mosaic attenuation with geographic, lobular areas of low attenuation that are accentuated on expiratory imaging. Chronic antigen exposure (chronic hypersensitivity pneumonitis) can lead to pulmonary fibrosis manifesting on CXR as peripheral, perihilar, or diffuse reticulation with a mid-upper lung predominance. Pulmonary fibrosis on CT is characterized by reticulation and ground-glass opacity (less pronounced than in non-fibrotic hypersensitivity pneumonitis), architectural distortion, and traction bronchiectasis with areas of lobular air trapping. Fibrosis is typically diffuse in the axial plane with a mid-upper lung predominance. Honeycombing may be present and when fibrosis has a peripheral and lower lung predominance, the pattern can mimic usual interstitial pneumonia.

Vascular changes can also occur in pulmonary fungal infections. Occlusion of a pulmonary artery leading up to a peripheral nodule on CT angiography may be one of the earliest signs of angioinvasive aspergillosis [57]. Pseudoaneurysm

formation of the aorta and pulmonary arteries has been documented, albeit rarely, in the setting of IPA; Pulmonary artery pseudoaneurysm can cause massive hemoptysis [58, 59]. Fungal aortitis may manifest as a saccular aneurysm or aortic wall thickening and enhancement on CT and MR angiography [60].

Abdominal Imaging

Fungal organisms can invade and disseminate to virtually any organ and compartment of the gastrointestinal and hepatobiliary systems. Symptoms are often nonspecific. Similarly, radiologic findings of abdominal fungal infections are also nonspecific with variable lesions, organomegaly, and lymphadenopathy. Computed tomography or MRI should be the initial imaging modality used to evaluate the abdomen for signs of fungal infection. Ultrasonography, a safer and lower-cost imaging modality, can then be used to follow disease progression. Serial ultrasounds every 3–4 weeks may be used to monitor response to therapy, typically observed as decreasing size and number of diffuse hypoechoic lesions or may be useful in detecting new lesions or the evolution of pre-existing lesions [61]. Once the ultrasound is clear, a repeat CT or MRI is suggested.

Target Lesions

Candida is one of the most common fungi to cause abdominal disease. In patients with dysphagia or odynophagia, fulminant *Candida* esophagitis can present as longitudinal plaques along the esophagus. Large filling defects from aggregated plaques can appear as "cobblestoning" in severe disease on fluoroscopic barium esophagram. Barium esophagram can also reveal "foamy" or "feather" appearances of subsequent esophageal stenosis [62]. Involvement of the abdominal organs, particularly the liver, biliary tree, and spleen, has been documented in disseminated disease [63]. With recovery of neutropenia, target lesions seen in the spleen and liver resulting from candidal infection are most commonly detected on CT or MRI [61] (Fig. 7.13). On abdominal CT, chronic disseminated (formerly hepatosplenic) candidiasis is characterized by peripheral, round, low-attenuating microabscesses, scattered in a military pattern throughout the liver and spleen with occasional peripheral enhancement [64]. On ultrasound, four characteristic patterns have been described. The most common pattern is a uniformly "hypoechoic nodule," which can be seen in conjunction with the other three patterns; this nodule indicates fibrotic development around a previously inflamed area. A "wheel within a wheel" pattern represents a central hypoechoic area of fungal or necrotic debris surrounded by a hyperechoic zone of inflammation. Thirdly, a "bull's eye" configuration, measuring from 1 to 4 cm, may evolve from primary lesions: typically seen as a lesion with a hyperechoic center surrounded by a hypoechoic rim. The fourth pattern, commonly seen in the later stages of infection, is "echogenic foci" with various acoustic shadows correlating with fibrosis, calcifications, or both [65]. On MRI, untreated fungal disease may appear as significant hyperintense lesions on T2WI images, which moderately enhance with the administration of contrast; minimal intensity is seen on T1WI. As microabscesses are treated, granulomatas may be seen on MRI. Abdominal MRI provides superior contrast resolution over CT or US; MR cholangiopancreatography (MRCP) is particularly useful in providing detailed assessment of the entire biliary tree in disseminated hepatobiliary candidiasis [65]. Although relatively rare, disseminated *Coccidioides* infections can affect virtually any organ system including the abdominal organs and peritoneum [66]. *Histoplasma* infections, involving the liver and spleen, manifest similarly like other more common fungal infections, typically as microabscesses appearing as target lesions with central areas of low attenuation on CT imaging, which may calcify over time [61, 66].



Fig. 7.13 Contrast-enhanced CT scan through the abdomen showing multiple subcentimeter low attenuation hepatic and splenic lesions in patient with chronic disseminated candidiasis. Courtesy of Dr. D. R. Hospenthal

Organomegaly

Moderate to marked enlargement of the liver and spleen have been noted in disseminated infections from *Histoplasma*, *Candida*, and *Cryptococcus* species. Adrenal enlargement has been reported in disseminated histoplasmosis [67–69].

Lymphadenopathy

Enlarged lymph nodes with or without central or diffuse low attenuation on CT imaging can be seen in the majority of patients with abdominal histoplasmosis [61]. Cryptococcal infection has also demonstrated enlarged lymph nodes on CT imaging [70].

Miscellaneous

Invasive disseminated fungal infections in the abdomen can present with abscesses, colonic wall thickening with adjacent inflammation, and involvement of contiguous structures in the omental and mesenteric peritoneum. Imaging findings may include ascites, peritoneal enhancement, and omental stranding with or without nodularity [61]. Adrenal masses, vascular occlusion, and extensive necrosis have also been noted. Multiple scattered low-attenuation foci can persist from focal scarring and granulomatous change, which may eventually result in calcifications.

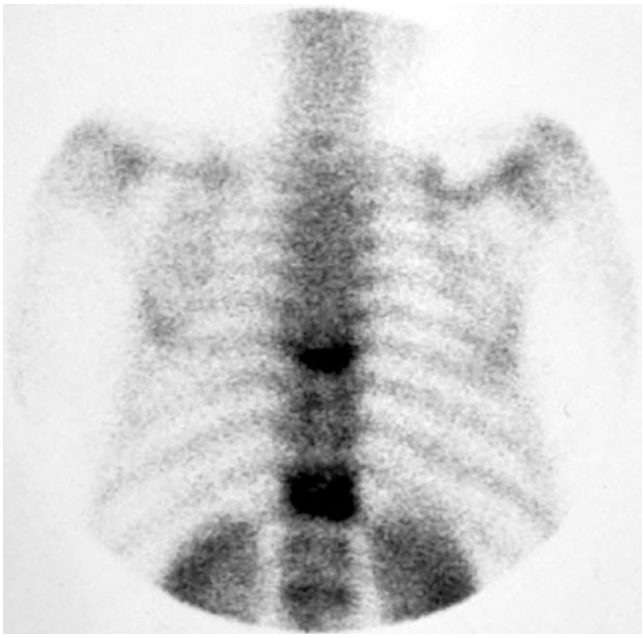


Fig. 7.14 Bone scan demonstrates increased uptake at approximately T8, T11, and T12. Although this patient had coccidioidomycosis, these findings are nonspecific and could represent another infection, inflammatory or neoplastic process

Musculoskeletal Imaging

Pathology involving the musculoskeletal system can be assessed with ultrasound, standard radiography, cross-sectional imaging modalities such as CT and MRI, positron emission tomography (PET), and nuclear scintigraphy (Fig. 7.14). Osteomyelitis is the most frequent consequence of fungal infection, though its radiographic presentation is nonspecific and indistinguishable from other etiologies such as bacterial infections or neoplastic disease. Assessment of metabolic activity using radioisotopes such as technetium depends on perfusion; scintigraphy with gallium is dependent on inflammation. Magnetic resonance imaging (MRI), highly specific and sensitive, is the benchmark method in assessing infectious pathology in the musculoskeletal system. Emerging modalities include a hybrid of PET scanning with CT or MRI imaging systems (PET/CT or PET/MRI), which offer valuable information on the structural and functional characteristics of the infections [71–73].

Osteomyelitis

In blastomycosis, dissemination to the bone is uncommon, but the most frequently affected sites include the lower thoracic and lumbar vertebral column, ribs, skull, and the epiphyseal ends of long bones [74]. Epiphyseal or metaphyseal focal or diffuse osteomyelitis has been reported as have cystic foci or diffuse "moth-eaten" areas. Osseous lesions, with notable eccentric erosions, diffuse lytic areas, periosteal reaction, or sclerotic margins, can be associated, or may even be visible beneath a cutaneous ulcer or abscess [75]. In histoplasmosis, dissemination to the musculoskeletal system is also uncommon but can mimic inflammatory arthritis and can similarly affect the pelvis, ribs, and small tubular bones [76]. In disseminated candidiasis, osteomyelitis primarily affects the thoracic or lumbar spine of adults and the femoral bones of children. Plain radiographs may show vertebral bone destruction. CT scan abnormalities can include soft tissue or spinal canal extension and swelling, intervertebral space narrowing, and epidural abscesses [77]. Common findings on MRI include bone destruction, hypointense T1W1 images, and hyperintense T2WI images [78]. Disseminated coccidioidomycosis can affect the axial skeleton and joints. The most common radiographic finding in *Coccidioides* infection of bones or bony prominences includes osteolytic lesions, which have punched-out, well-circumscribed borders, which can make the area appear "moth-eaten" appearance (Fig. 7.15). In the spine, one or more vertebral bodies may be involved, typically with paraspinal masses and contiguous rib lesions. Imaging by MRI or CT is helpful in evaluating the extent of soft tissue damage and bone erosion and visualizing paraspinal masses or abscesses [79]. The radiological findings of skeletal cryptococcosis present with nonspecific features, including osteolytic lesions with discrete margins, mild or absent surrounding sclerosis, and little or no periosteal reaction [80]. Mucormycosis generally causes osteolytic changes to



Fig. 7.15 Plain radiograph of the right ankle showing a lytic lesion in the medial aspect of the distal tibia secondary to coccidioidomycosis

the skull or face; radiographic features of mucor sinus infections can show irregular diffuse radiolucency consistent with osteomyelitis of the paranasal areas [81]. Fungal mycetoma or 'Madura foot' is a chronic granulomatous infection of the skin and subcutaneous tissues. Early stages of the disease can show bony and soft tissue defects on CT, while standard radiographs can show cortical bone cavities and disuse osteoporosis [82]. Other fungal organisms that can present with radiologic abnormalities of bone and soft tissue include *Scedosporium*, *Paecilomyces*, *Fusarium*, and *Sporothrix schenckii* [83–87].

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Suggested Reading

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Part III

Systemic Antifungal Agents

Systemic Antifungal Agents

8

Russell E. Lewis and Nathan P. Wiederhold

Introduction

Until the 1950s, relatively few drugs were available for the treatment of superficial or invasive mycoses. The era of systemic antifungal chemotherapy effectively began in 1955, with the discovery of the polyene antifungals nystatin and amphotericin B, followed in 1958 by the discovery of the first topical azole antifungal agent, chlormidazole (Fig. 8.1). Although amphotericin B remained the mainstay of systemic antifungal therapy for over 40 years, infusion-related side effects and dose-limiting nephrotoxicity limited the drug's clinical effectiveness, prompting a search for effective but less toxic alternatives. In the 1960s, a synthetic fluorinated pyrimidine analogue originally developed as an antineoplastic agent, flucytosine, was found to have antifungal activity against common yeasts, but resistance developed quickly when used as monotherapy. In 1981, the first orally bioavailable systemic azole, ketoconazole, was introduced into clinical practice. For almost a decade, it would be regarded as the drug of choice for chronic mucocutaneous candidiasis, mild-to-moderate blastomycosis, histoplasmosis, paracoccidioidomycosis and coccidioidomycosis, and occasionally deep-seated infections caused by *Candida* and *Cryptococcus* spp. in patients who could not tolerate amphotericin B. Because ketoconazole was a highly lipophilic weak base, it has many undesirable pharmaceutical characteristics including

- Limited absorption of the drug at elevated gastric pH
- Lack of an intravenous formulation
- Requirement for extensive cytochrome P450 biotransformation before elimination, resulting in a high propensity for drug-drug interactions
- Dose-related gastrointestinal, hepatic, and adrenal toxicities
- Limited penetration into anatomically privileged sites such as cerebrospinal fluid (CSF)

To address these problems, a new chemical group of azoles (i.e., triazoles) was introduced with improved physicochemical characteristics and spectrum of activity. Fluconazole, introduced in the early 1990s, could be administered intravenously or orally, had predictable pharmacokinetics, excellent oral bioavailability, and readily penetrated anatomically restricted sites such as the CSF and vitreous humor. Clinical experience has since confirmed that fluconazole is well tolerated with few serious drug interactions. Its lack of activity against opportunistic molds (e.g., *Aspergillus*, *Fusarium*, and the Mucorales) and intrinsic resistance among some non-albicans species (i.e., *C. glabrata* and *C. krusei*), however, left an unmet medical need for a broader-spectrum oral agent that could be used for prophylaxis and long-term outpatient treatment of invasive molds.

The introduction of itraconazole, and later broader-spectrum triazole derivatives, voriconazole, posaconazole, and isavuconazole, have addressed this need with intravenous and oral formulations and have proven to be effective and safe treatment alternatives. These triazoles have few clinical benefits over fluconazole for the treatment of *Candida* spp. and carry a higher risk for toxicities and CYP3A4-mediated drug interactions. Therefore, new antifungals are still needed, especially with the growing use of targeted oral chemotherapy agents in high-risk hematology patients that are metabolized through CYP3A4.

During the 1990s, new formulations of amphotericin B were also introduced that incorporated amphotericin B into

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_8

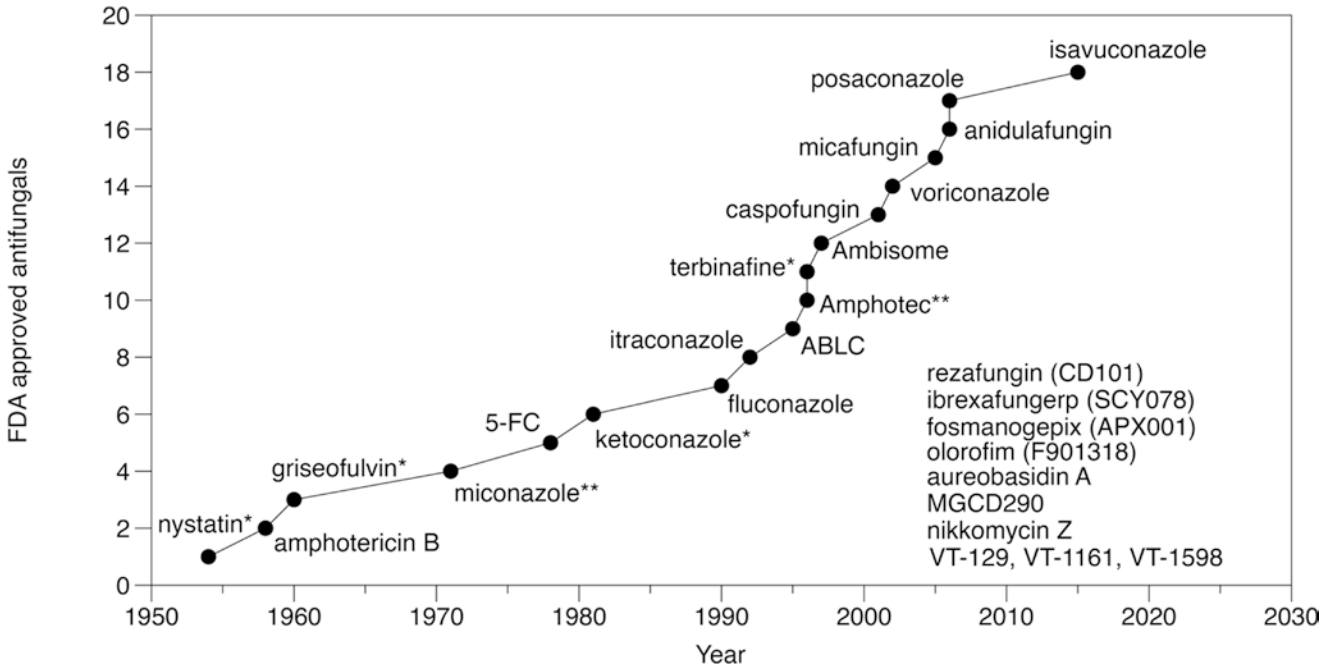


Fig. 8.1 Timeline of antifungals introduced into clinical practice. Antifungals in clinical trials are shown in the bottom right corner

lipid particles or liposomal carriers. These formulations altered the pharmacokinetics of amphotericin B, resulting in reduced accumulation of drug in the kidneys allowing for the delivery of higher drug doses with less nephrotoxicity. However, only the liposomal formulation remains widely used and has largely supplanted conventional amphotericin B in most clinical settings. Liposomal amphotericin B continues to be an important broad-spectrum therapy for empirical therapy and is the initial drug of choice for patients with suspected breakthrough fungal infections on triazole prophylaxis.

The final milestone of antifungal drug discovery during the twentieth century was the identification and development of the echinocandins (caspofungin, micafungin, and anidulafungin)-lipopeptide molecules that inhibit glucan synthesis, leading to damage of the fungal cell wall. Echinocandins are effective drugs for invasive candidiasis and well tolerated by patients, but are only available in intravenous formulation. Echinocandins are the preferred first-line therapy for invasive candidiasis and occasionally are used in combination with other antifungals for the treatment of invasive aspergillosis.

Several other promising drug candidates are currently in clinical trials and, if approved, could have unique advantages in terms of pharmacokinetics and spectrum of activity [1]. Nevertheless, treatment failures, toxicities, and drug interactions are still common. Additionally, pathogens with intrinsic or acquired resistance to multiple antifungal classes are increasing in some regions placing an even greater importance of the clinical microbiology laboratory for early and accurate pathogen identification to the species levels and timely antifungal susceptibility testing results. This chapter will review key components of antifungal pharmacology related to systemic antifungal agents and common patterns of resistance among opportunistic mycoses in humans.

Targets of Antifungal Therapy

Despite differences in the sterol composition of the cell membrane and the presence of a cell wall, fungi are metabolically similar to mammalian cells and offer few pathogen-specific targets for antifungal therapy. Not surprisingly, many of toxicities and drug interactions observed with contemporary antifungal therapies can be attributed to "nonselective" interactions with homologous enzyme or cell membrane systems found in mammalian host cells. Except for flucytosine, currently available systemic antifungals act primarily through direct or indirect interactions with the fungal cell wall or plasma membrane, and the fungal membrane sterol ergosterol and its biosynthetic pathways (Fig. 8.2). The fungal cell envelope has several properties that make it an ideal target for antifungal therapy. In contrast to the cholesterol-rich cell membranes of mammalian cells, the predominant cell membrane sterol in pathogenic fungi is ergosterol. Indirect or direct targeting of ergosterol impairs ion exchange, filtration, and localization of enzymes involved in the metabolism and catabolism of complex nutrients,

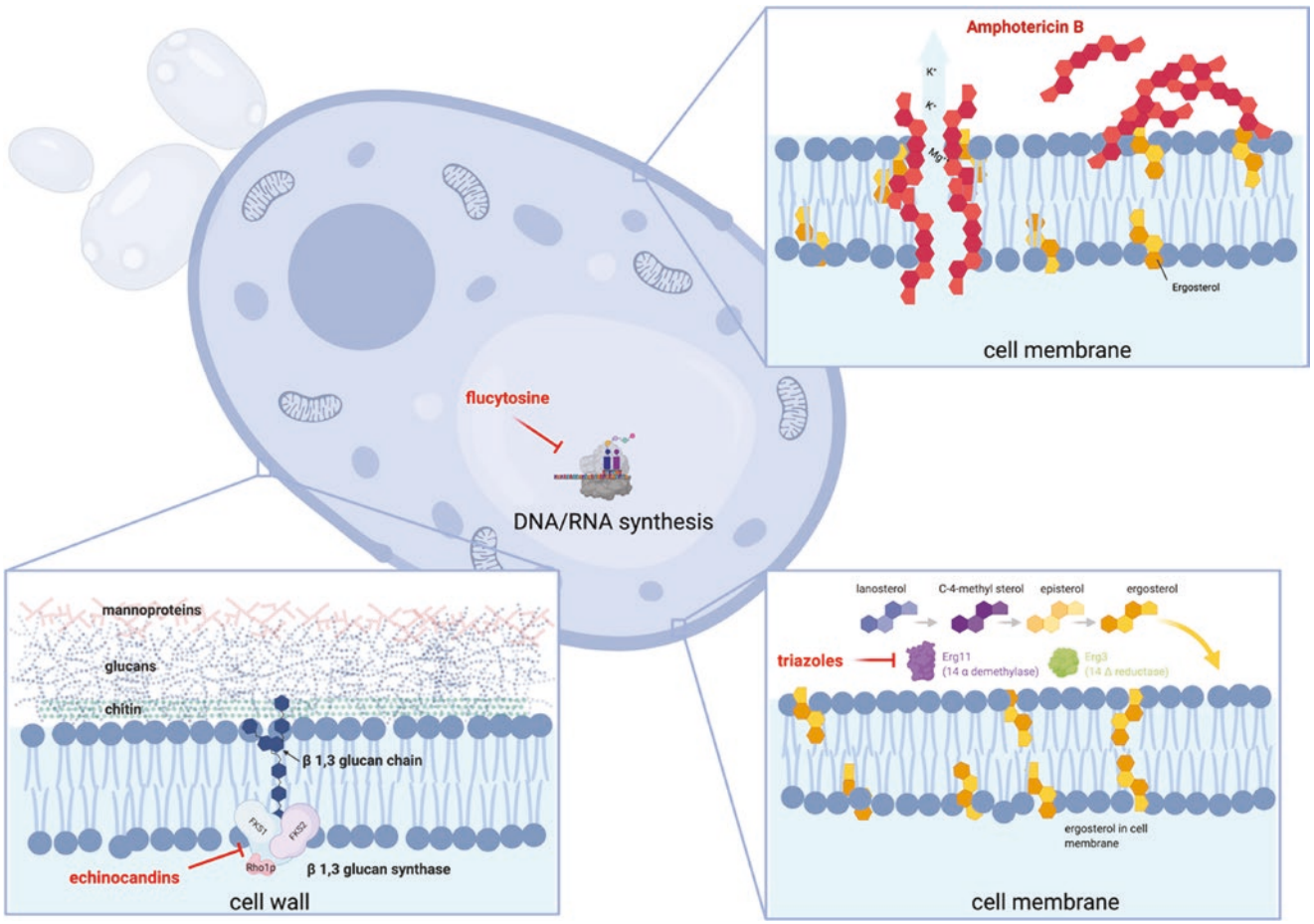


Fig. 8.2 Targets of antifungal therapy. Imaged prepared using biorender.com

resulting in growth arrest. Drugs that disrupt growth of the cell membrane and wall produce pleiotropic effects that can be selectively lethal to fungi.

Polyenes

Amphotericin B (Fig. 8.3) assembles as 4–12 subunit oligomers complexed with ergosterol in the fungal cell membrane to form small membrane-permeabilizing ion channels that allow leakage of K^+, Mg^{++}, and organic substrates. Amphotericin B also has auto-oxidative properties that result in the generation of superoxide, hydrogen peroxide, and hydroxyl radicals that oxidize lipid membranes and lipoprotein receptors, impairing cell membrane function [2]. More recent studies suggest that amphotericin B can also adsorb to and sequester cell membrane ergosterol, causing destabilization of the cell membrane, or can aggregate and act as an ergosterol "sponge" [3]. As amphotericin B concentration increases, the selectively of these mechanisms is reduced with greater binding to lower-affinity cholesterol in mammalian cell membranes: a mechanism that likely accounts for damage of distal tubular membranes of the kidneys.

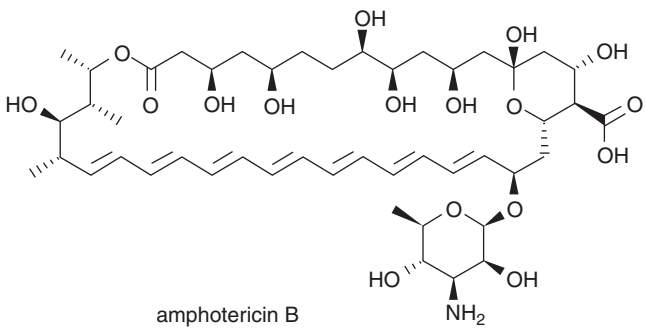


Fig. 8.3 Structure of amphotericin B

Triazoles

Triazole antifungals (Fig. 8.4) block ergosterol biosynthesis in susceptible fungi through inhibition of cytochrome P-450 sterol 14α-demethylase (Erg11p or CYP51p depending on nomenclature). The target enzyme in fungi catalyzes the oxidative removal of a 14α-methyl group of lanosterol in the ergosterol biosynthetic pathway. Inhibition of 14α-demethylase results in an accumulation of 14α-methylated sterols in the cytoplasmic membrane,

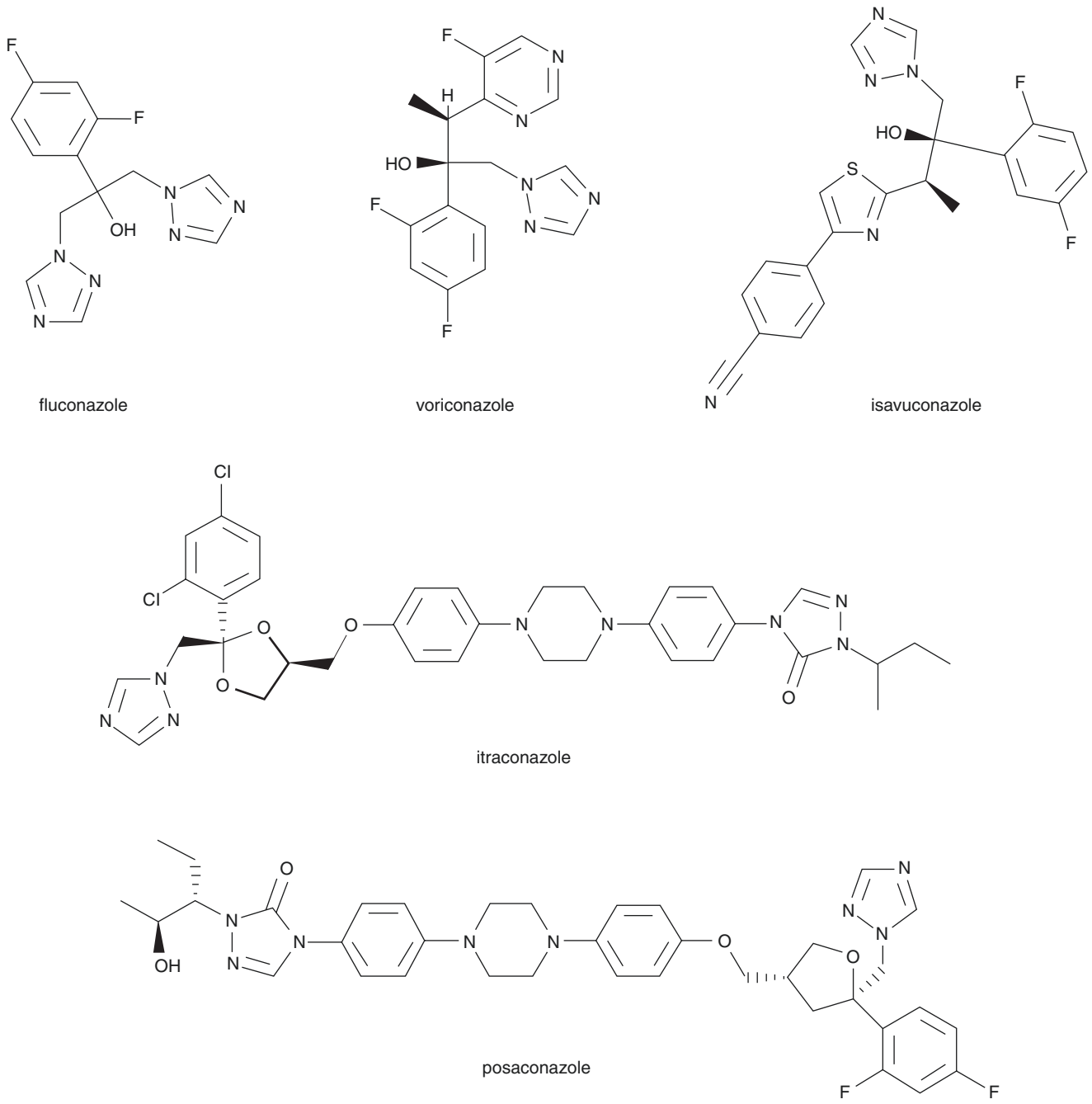


Fig. 8.4 Structures of triazole antifungals

which disrupt phospholipid organization, impair membrane-bound enzyme systems such as ATPase and enzymes of the electron transport system resulting in arrest of cell growth. CYP51p enzyme binding is accomplished through coordination of the triazole N3 or imidazole N4 of the azole ring with the cytochrome P-450 heme target site, while the remainder of the drug molecule binds to the apoprotein in a manner dependent on the individual structure of the azole [4]. Differences in the exact conformation of the active site between fungal species and drug structure largely define the spectrum of each agent. For molecules derived from the ketoconazole pharmacophore (e.g., itraconazole, posaconazole), extension of lipophilic side chain enhances binding of the azole to the P450 apoprotein and expands the potency and spectrum against both yeast and filamentous fungi. For molecules derived from fluconazole (e.g., voriconazole and

isavuconazole), inclusion of an α-O-methyl group confers activity against *Aspergillus* and other filamentous fungi.

There is considerable homology between fungal and human CYP-450 enzymes. As a result, all azoles inhibit with varying degrees of potency and in a dose-dependent fashion mammalian CYP P450 enzymes involved in drug metabolism and steroid biosynthesis. Azole therapy can predispose patients to a number (i.e., > 2000 theoretical) of pharmacokinetic drug-drug interactions when these antifungals are administered concurrently with drugs that are either substrates or inducers of CYP P450 enzymes in humans [5]. Unfortunately, modifications of the azole pharmacophore designed to enhance binding to fungal CYP51p frequently enhance binding of mammalian CYP P450 enzymes. Therefore, improvement in the spectrum of azole antifungals is often accompanied by an increased potential for drug interactions.

Echinocandins

Echinocandins (Fig. 8.5) competitively inhibit the ß-1,3-D glucan synthase complex, which synthesizes branched glucan polymers that serve as key structural components of the cell wall. In *Saccharomyces cerevisiae*, where the enzyme complex has been best studied, the echinocandins are known to bind to the Fks1p component of the two proteins (Fks1p and Fks2p) regulated by the GTP-binding peptide, Rho1p, that comprise the transmembrane ß-1,3-D-glucan synthase complex [6]. Depletion of 1,3-ß-D glucan in susceptible fungi leads to a structurally impaired cell wall, osmotic instability, and lysis in rapidly growing cells.

The density of ß-1,3-D-glucan sugars and expression of the synthase chiefly defines the spectrum and lethality of the echinocandins in pathogenic fungi. In *Candida* spp., the fungal cell wall is rich in ß-1,3-D-glucans and the enzyme complex is highly expressed during rapid cell growth. Hence, echinocandins exhibit fungicidal activity against most rapidly growing *Candida* species. However, echinocandins lack clinically useful activity against *Cryptococcus* spp. and other rare yeasts, such as *Trichosporon* and *Rhodotorula* spp. Among hyaline molds, the cell wall of *Aspergillus* spp. contains ß-1,3- and ß-1,6-D-glucan polymers in actively growing apical tips of the hyphae. Therefore, echinocandins kill only the growing hyphal tips of the fungus, resulting in abnormal, hyperacute branching and aberrant growth, with minimal effects on the viability of subapical components (Fig. 8.6) [7]. Other filamentous fungi such as *Fusarium* spp. and the Mucorales utilize α-1,3-glucans in the cell wall matrix and chitosan polymers. As such, echinocandins lack pronounced activity against these opportunistic fungi.

Pyrimidine Antifungals

Flucytosine (5-fluorocytosine, 5-FC) works as an antifungal agent through conversion to 5-fluorouracil within fungal cells (Fig. 8.2). Once inside cells, fluorouracil inhibits thymidylate synthase, a key enzyme in DNA synthesis and incorporates into RNA, causing premature chain termination. For flucytosine to be effective, it must be internalized and converted to the active fluorouracil form through the activity of two enzymes, cytosine permease and cytosine deaminase. Mammalian cells and many filamentous fungi lack or have very low activity these enzymes thus restricting the activity of the flucytosine to pathogenic yeast [4]. In humans, however, resident intestinal flora may convert flucytosine to fluorouracil, resulting in nausea, vomiting, diarrhea, and bone marrow suppression.

Triterpenoids

Ibrexafungerp is a novel first-in-class member of a new class of antifungals, the triterpenoids. This agent, like the echinocandins, targets glucan synthesis, but through a unique and not yet fully defined mechanism. In contrast to the echinocandins, ibrexafungerp can be administered both orally and intravenously. This agent was recently approved by the U.S. Food and Drug Administration for the treatment of vulvovaginal candidiasis.

Investigational Antifungals

Several new antifungals currently in phase II/III trials at the time of writing may have a future role as treatment or prophylaxis in patients receiving targeted therapies [1]. Rezafungin is analogue of anidulafungin recently approved for the treatment of invasive candidiasis with a prolonged half-life that would allow for once-weekly dosing possibly through the subcutaneous route. Fosmanogepix is a prodrug of the active agent manogepix, a broad-spectrum antifungal compound with activity against common yeasts and molds being developed in IV and oral formulations that interferes with glycosylphosphatidylinositol (GPI)-anchored mannoproteins in the fungal cell wall. Fosmanogepix is reported to have excellent oral bioavailability (>90%), and manogepix has no significant CYP3A4 drug interactions [8]. Olorofim, a novel inhibitor of fungal dihydroorotate dehydrogenase (DHODH), an important enzyme in pyrimidine biosynthesis essential for deoxyribonucleic acid synthesis, is currently being studied for the treatment of invasive aspergillosis and other rare molds but lacks activity against yeast and the Mucorales [9]. The drug is reported to be a weak inhibitor of

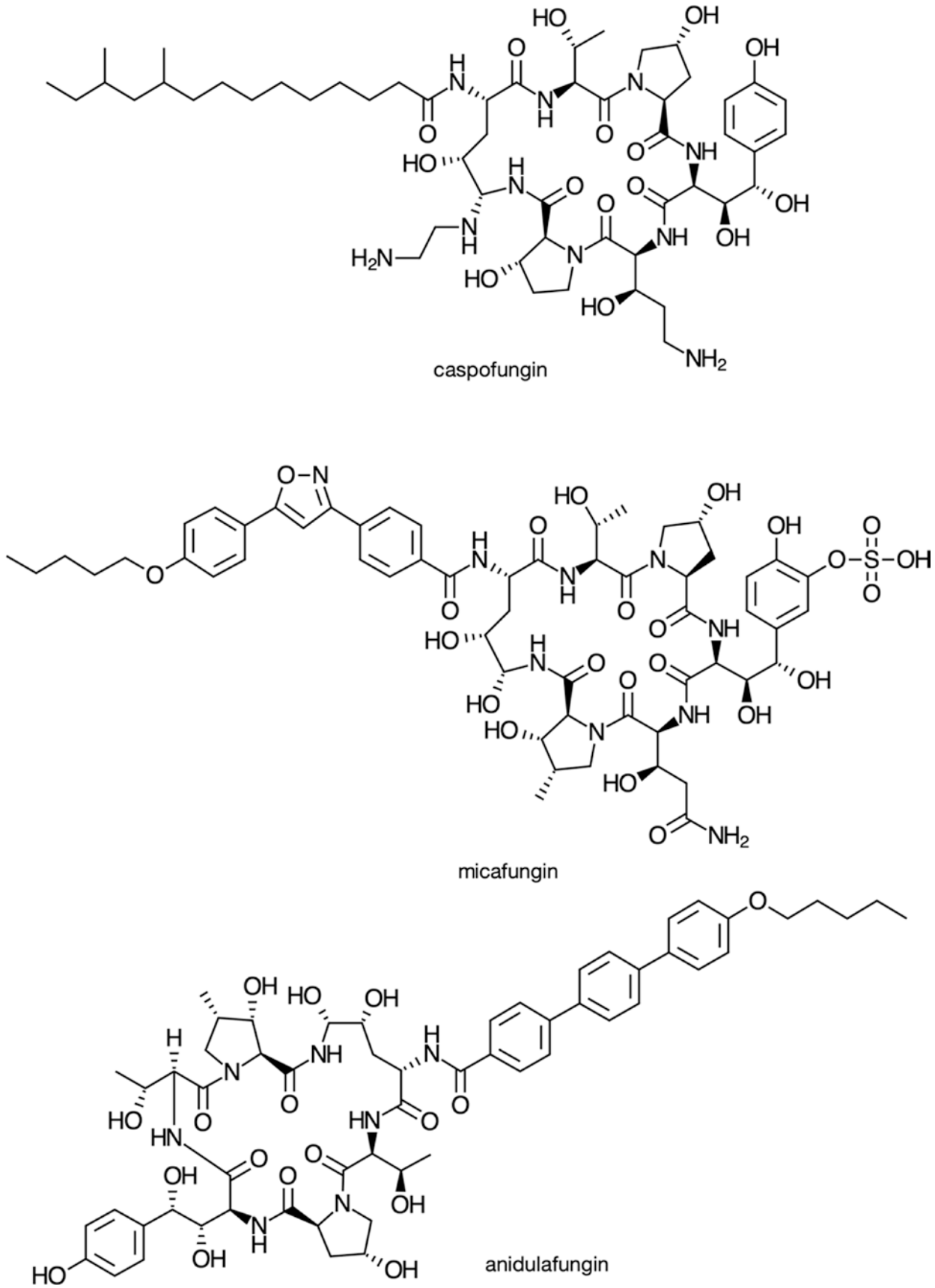


Fig. 8.5 Structures of echinocandin antifungals

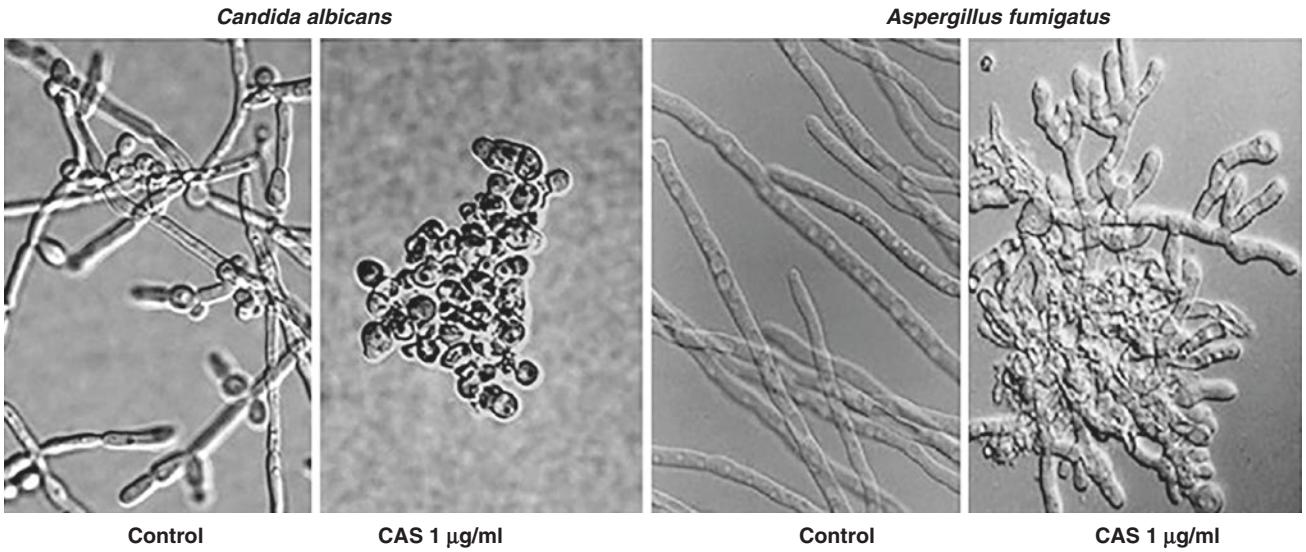


Fig. 8.6 In vitro effects of echinocandins (CAS) on fungal morphology

CYP3A4 and will initially be available as an oral formulation. Finally, two "next-generation" azoles or tetrazoles—VT-1129 and VT-1598—are in earlier stages of clinical development for the study of mucocutaneous candidiasis and cryptococcal meningitis, while one agent, oteseconazole (VT-1161), is in a phase 3 clinical trial for the treatment of recurrent vulvovaginal candidiasis. By replacing the triazole metal-binding group with a tetrazole and making additional modifications to the side chain, these tetrazoles achieve greater selectivity for fungal lanosterol 14α-demethylase (CYP51) versus human CYP3A4, which could theoretically improve antifungal potency and reduce the risk of serious drug-drug interactions with targeted therapies [10].

Antifungal Resistance

Antifungal resistance is a broad concept describing the failure of a fungal infection to respond to antifungal therapy. Resistance has been traditionally classified as either primary (intrinsic) (i.e., present before exposure to antifungal) or secondary (acquired) (i.e., that which develops after antifungal exposure owing to stable or transient genotypic alterations) [11]. A third type of antifungal resistance could be described as "clinical resistance," which encompasses progression or relapse of infection by a pathogenic fungus that appears, by laboratory testing, to be fully susceptible to the antifungal used to treat the infection. Clinical resistance is most commonly a result of persistent and profound immune defects (e.g., AIDS, neutropenia, graft-versus-host disease, and its treatment) or infected prosthetic materials (i.e., central venous catheters), which become encased in protective biofilm, thus limiting drug activity. In some cases, suboptimal drug concentrations at the site of infection resulting from poor drug absorption, drug interactions, or infrequent dosing may contribute to clinical resistance.

Primary or secondary antifungal resistance can arise through several complex mechanisms and may be expressed over a wide phenotypic spectrum [11]. At one extreme, fungi may be susceptible to the effects of an antifungal agent, but growth may not be completely inhibited in vitro. This so-called trailing growth may be observed for antifungals during laboratory testing (particularly azoles and flucytosine) even at high concentrations but is generally considered an artifact of testing methods and not reflective of clinically relevant resistance. Similarly, some echinocandins may exhibit a paradoxical attenuation of activity at higher drug concentrations without clear evidence of diminished drug activity at higher dosages in animal models or patients. Heterogeneous resistance, the presence of subpopulations of fungal cells with varying degrees of resistance to an antifungal agent in a susceptible population, may indicate an increased propensity for the development of antifungal resistance. This type of resistance may not be detected unless specialized testing methods are used in the laboratory. Inducible or transiently expressed (epigenetic) antifungal resistance mechanisms have also been described in fungi, but little is known about the clinical significance of these resistance patterns in human infections [11]. The other extreme in the phenotypic expression of antifungal resistance is represented by isolates with stable and persistent growth even at high antifungal concentrations. It is important to note that most studies of antifungal resistance focus on isolates with a stable resistance phenotype. Molecular mechanisms of resistance have been best described in *C. albicans* isolates recovered from AIDS patients with chronic, recurring fluconazole-refractory oro-

pharyngeal candidiasis. The chronic nature of these mucosal infections allows the longitudinal collection of serial, matched *Candida* isolates that exhibit progressively stable, higher degrees of resistance to antifungals. By contrast, acute bloodstream candidiasis, aspergillosis, or other less common life-threatening mycoses do not typically allow for the study of serial, matched isolates, thus complicating genotypic-phenotypic correlation of resistance development.

Laboratory Detection of Resistance

Susceptibility testing is performed by phenotypic assays performed to determine the in vitro activity of a particular antimicrobial agent against a pathogen of interest. Clinical microbiology laboratories can perform these assays by various methods, including broth microdilution, disk, or gradient diffusion, and by automated systems. In the United States and Europe, two organizations that set methods and standards are the Clinical and Laboratory Standards Institute (CLSI) and the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST), respectively. The methods used by these organizations are primarily broth microdilution methods, although macrodilution testing is also described in the CLSI standards [12, 13]. Although some differences exist between the CLSI and EUCAST methods, notably differences in glucose content, well types (round bottom vs. flat bottom), concentrations of the starting inocula, and endpoint reading methods, efforts have been made to harmonize these methods [14]. Several antifungal susceptibility assays are also available commercially. Examples of these include the YeastOne Sensititre method (TREK Thermofisher), which is a broth microdilution method that incorporates the dye resazurin (alamarBlue) that is converted by metabolically active cells to allow for colorimetric endpoint detection, gradient diffusion assays, which employ a plastic strip containing a concentration gradient of a particular antifungal agent (Etest, bioMerieux, and MTS, Liofilchem), and fully automatic systems, such as the Vitek 2 instrument (bioMerieux).

The readout of these phenotypic assays that are used to gauge in vitro activity is the minimum inhibitory concentration (MIC), defined as the lowest concentration of an antifungal that inhibits the growth of the organism. The endpoint used for antifungal susceptibility testing is dependent on both the antifungal and the organism that is being tested. For example, when tested against yeasts, the endpoint of the azoles is the lowest concentration that results in at least a 50% reduction in growth compared to the growth control. A similar endpoint is also used for the echinocandins against yeast. In contrast, against molds the MIC for the azoles is the lowest concentration that completely inhibits growth (clear well), while for dermatophytes, an endpoint of 80% inhibition of growth is recommended for the azoles, the allylamine terbinafine, and griseofulvin. The different endpoints for dermatophytes compared to other filamentous fungi are used to minimize interlaboratory variability that can occur for fungistatic drugs, such as the azoles and terbinafine, where trailing can occur. For the echinocandins, the endpoint used against filamentous fungi is the minimum effective concentration (MEC), which is the lowest concentration that results in an abnormal morphology (i.e., short, stubby, abnormally branched hyphae). This endpoint is based on the mechanism of action against ß-1,3-D-glucan synthase complex and the location of this enzyme complex at the apical tips and hyphae branch points, as described above. For amphotericin B, against both yeasts, molds, and dermatophytes, the MIC is defined as the lowest concentration that completely inhibits growth. The period of incubation at which the endpoints are read is also dependent upon the antifungal and the species being tested.

Once an MIC for an antifungal is available, it is compared to established clinical breakpoints to determine if the fungal isolate is susceptible or resistant to the antifungals against which it was tested. Clinical breakpoints are not naturally occurring endpoints, but instead are set following consideration of several factors, including MIC distributions of specific antifungal agents against a specific species, pharmacokinetic/pharmacodynamic parameters, and correlations between clinical outcomes and in vitro susceptibility results. Currently, clinical breakpoints have been established by CLSI and EUCAST for the echinocandins and some of the azoles against the more common *Candida* spp. [13, 15], and against a limited number of *Aspergillus* spp. by EUCAST. Recently, CLSI also established a breakpoint for voriconazole against *A. fumigatus*. Interpretive categories set by CLSI for the echinocandins and voriconazole include susceptible (S), intermediate (I), and resistant (R), while those for the fluconazole include susceptible (S), susceptible dose dependent (SDD), and resistant (R). The fluconazole susceptible dose-dependent category is in recognition that *Candida* susceptibility is dependent on achieving maximum blood levels. By maintaining blood levels with higher doses of antifungal, an isolate with an SDD endpoint may be successfully treated with a fluconazole [12] (Table 8.1). This category is not interchangeable with the intermediate category used in the echinocandin and voriconazole breakpoints.

More recently, the EUCAST has changed definitions of susceptibility testing categories of "S, I, and R" to clarify the relationship of drug dosing/exposure with the breakpoint designation. "S" indicates *susceptible-to standard dosing regimen* when there is a high likelihood of therapeutic success using a standard dosing regimen; "I" was changed from *intermediate* to *susceptible increased exposure* when there is a high likelihood of therapeutic success when drug exposure

Table 8.1 Updated CLSI clinical breakpoints for *Candida* spp. and *Aspergillus fumigatus*

Organism/antifungal	Reportable reading conditions	Breakpoint			
		Susceptible	SDD	Intermed.	Resistant
C. albicans					
Anidulafungin	24 h, 50%	≤0.25	–	0.5	≥1
Caspofungin	24 h, 50%	≤0.25	–	0.5	≥1
Micafungin	24 h, 50%	≤0.25	–	0.5	≥1
Fluconazole	24 h, 50%	≤2	4	–	≥8
Voriconazole	24 h, 50%	≤0.12	–	0.025–0.5	≥1
C. glabrata					
Anidulafungin	24 h, 50%	≤0.12	–	0.25	≥0.5
Caspofungin	24 h, 50%	≤0.12	–	0.25	≥0.5
Micafungin	24 h, 50%	≤0.06	–	0.125	≥0.25
Fluconazole	24 h, 50%	–	≤32	–	≥64
Voriconazole	24 h, 50%	–	–	–	–
C. tropicalis					
Anidulafungin	24 h, 50%	≤0.25	–	0.5	≥1
Caspofungin	24 h, 50%	≤0.25	–	0.5	≥1
Micafungin	24 h, 50%	≤0.25	–	0.5	≥1
Fluconazole	24 h, 50%	≤2	4	–	≥8
Voriconazole	24 h, 50%	≤0.12	–	0.025–0.5	≥1
C. krusei					
Anidulafungin	24 h, 50%	≤0.25	–	0.5	≥1
Caspofungin	24 h, 50%	≤0.25	–	0.5	≥1
Micafungin	24 h, 50%	≤0.25	–	0.5	≥1
Fluconazole	24 h, 50%	–	–	–	–
Voriconazole	24 h, 50%	≤0.5	–	1	≥2
C. parapsilosis					
Anidulafungin	24 h, 50%	≤2	–	4	≥8
Caspofungin	24 h, 50%	≤2	–	4	≥8
Micafungin	24 h, 50%	≤2	–	4	≥8
Fluconazole	24 h, 50%	≤2	4	–	≥8
Voriconazole	24 h, 50%	≤0.12	–	0.025–0.5	≥1
C. guilliermondii					
Anidulafungin	24 h, 50%	≤2	–	4	≥8
Caspofungin	24 h, 50%	≤2	–	4	≥8
Micafungin	24 h, 50%	≤2	–	4	≥8
Fluconazole	24 h, 50%	–	–	–	–
Voriconazole	24 h, 50%	–	–	–	–
Aspergillus fumigatus					
Voriconazole	48 h, 100%	≤0.5	–	1	≥2

NOTE: *Candida krusei* is considered intrinsically resistant to fluconazole regardless of the MIC value

is increased by adjusting the dosing regimen or by virtue of high concentration at the site of infection. "R" is *resistant* when there is a high likelihood of clinical failure despite increased drug exposures. A fourth category set by EUCAST is that of *area of technical uncertainty* (ATU) and is assigned when the MIC is in an area where the test results are not interpretable.

An important limitation with any approach toward in vitro susceptibility testing is the correlation of the MIC with patient outcome. Some assumptions can be made, however, about MIC and clinical outcome. Rex and Pfaller proposed the "90-60 Rule" as a general guide for establishing clinically relevant interpretative breakpoints for resistance [16]. This rule states that infections caused by isolates that have MICs considered susceptible respond favorably to appropriate therapy approximately 90% of the time, whereas infections caused by isolates with MICs considered resistant respond favorably in approximately 60% of cases. Results of susceptibility testing are but one aspect that may help to predict a patient's response to a particular antifungal treatment. Other factors may also influence outcomes and may play a larger role than a particular in vitro test result. Such factors include the host's immune response, other comorbidities and the severity of the fungal infection, the overall exposure of the antifungal at the site of infection, timely initiation of appropriate antifungal therapy, as well as drug-drug interac-

tions and adverse effects/toxicities that the patient experiences, which may limit their ability to adhere to a particular treatment.

When clinical breakpoints are not available, some clinical microbiology laboratories are now using epidemiological cut-off values (ECVs or ECOFFs) to provide guidance for therapy. ECVs are statistically derived based on MIC distributions, and for a given species and antifungal combination encompass approximately 97.5% of isolates within a wild-type distribution. This allows for the differentiation between wild-type and non–wild-type isolates. CLSI has begun to publish ECVs for different antifungal/fungal species combinations, and several have also been derived from non-CLSI and non-EUCAST. However, it is important to remember that ECVs are not to be used instead of clinical breakpoints when these are available.

Mechanisms of Resistance

Many aspects of antifungal resistance are still poorly understood, particularly with respect to the regulation and expression of resistance mechanisms following exposure to antifungal agents (secondary resistance). Nevertheless, advances in molecular biology and genome sequencing of pathogenic fungi have yielded progress in our understanding of common mechanisms leading to antifungal resistance. These mechanisms can be grouped into five general categories:

- Decreased drug import or increased drug exportation (efflux pumps)
- Alteration in drug target-binding site
- Changes in biosynthetic pathways (particularly sterol synthesis) that circumvent or attenuate the effects of antifungal inhibition
- Alterations in intracellular drug processing
- Upregulation of homeostatic stress-response pathways to deal with antifungal-associated damage

Multiple resistance mechanisms can be expressed simultaneously following antifungal exposure and that a single mechanism is unlikely to result in a resistant strain. Depending on the mechanisms concurrently expressed, cross-resistance may, or may not, be observed between different antifungals. Whole-genome expression profiles of *C. albicans* have revealed transient upregulation of several resistance mechanisms (e.g., ergosterol biosynthesis—*ERG3*, *ERG11*; efflux pumps—*CDR1*, *CDR2*) following a single exposure to azole antifungals [17]. Development of resistance in longitudinally collected clinical strains in patients who fail progressively higher dosages of antifungals generally demonstrate a gradual accumulation of several alternations that result in a detectable resistance [17].

Changes in drug importation and exportation are probably the most common mechanisms associated with primary and secondary antifungal resistance. Decreased drug importation is consistently associated with primary resistance to flucytosine and azoles antifungals. For example, poor uptake of flucytosine due to alterations in cytosine permease, or decreased availability of this enzyme, largely accounts for the limited spectrum of this agent against opportunistic molds. Similarly, differences in azole susceptibility between fluconazole and itraconazole against *C. krusei* have been reported to be more closely associated with intracellular accumulation than differences in drug binding affinity to the 14 α-demethylase target [18]. Drug importation may also be affected by the sterol composition of the plasma membrane. Several studies have demonstrated that when the ergosterol component of the membrane is altered in favor of other 14 α-methyl sterols, there is a concomitant permeability change in the membrane to drugs and a decrease in membrane fluidity.

Like other eukaryotic cells, fungi are known to contain two types of efflux pumps that contribute to drug resistance: ATP-binding cassette (ABC) transporters and major facilitators (MFs). Overexpression of the ATP-dependent ABC transporters typically confers a multidrug resistance phenotype. In contrast, MF pumps, which expel antifungal though proton motive force (H^+ gradient across membrane), have a much narrower spectrum of substrate specificity. In *Candida albicans*, overexpression of ATP-dependent efflux pumps *CDR1* and *CDR2* confers cross-resistance to all azole antifungals [19]. In contrast, overexpression of MF pump MDR1 effects only the accumulation of fluconazole and does not result in cross-resistance to itraconazole or ketoconazole. Overexpression of ATP-dependent efflux pumps is the most prevalent mechanism of efflux-mediated resistance reported in clinical isolates [19].

Besides drug efflux, the most common mechanism associated with antifungal resistance involves changes in the binding site of the drug. Several genetic alternations in *ERG11*, the gene encoding 14 α-demethylase, have been attributed to decreases in azole activity, including point mutations that result in changes in the active pocket site or overexpression of *ERG11*. Similar alterations in other enzymes of the ergosterol biosynthetic pathway, particularly *ERG3* (C-5-sterol desaturase), which is upregulated with inhibition of 14 α-demethylase, have also been documented in azole-resistant clinical strains. Binding site alteration is also likely to be an important mechanism of echinocandin resistance. Point mutations in the *FKS1* and *FKS2* genes have been linked with clinical failure of echinocandins in the treatment of bloodstream infections and deep-seated candidiasis, especially among *C. glabrata* [20, 21].

Changes in the target expression in the ergosterol biosynthetic pathway alter the fungal cell membrane sterol content. Substitution of alternative sterols for ergosterol, or alterations in the sterol: phospholid ratio in the cell membrane can decrease intracellular accumulation of azoles and reduced the binding of amphotericin B to the cell membrane. Indeed, most polyene resistant yeasts recovered from patients with clear microbiological failure on amphotericin B exhibit decreased ergosterol concentrations in their fungal cell membranes. Several studies have even suggested that pathogenic fungi can scavenge free sterols for the cell membrane, including cholesterol, resulting in resistance to polyene and azole antifungals [22].

Alterations in intracellular drug processing and/or degradation and metabolism are probably the least studied pathways of resistance in fungi, even though these mechanisms are well characterized in other prokaryotic and eukaryotic systems. Resistance to flucytosine has been associated with alterations in cytosine permease and cystosine deaminase, which results in decreased intracellular concentrations and conversion of flucytosine to its active form.

Studies have also assessed homeostatic stress-response pathways in fungi that may be upregulated following exposure to antifungals. Disruption of the evolutionarily conserved protein kinase C (PKC) cell wall integrity and calcineurin pathways enhances azole and echinocandin killing in fungi [23]. Upregulation of these pathways also diminishes the lethal effects of antifungals through upregulation of ergosterol and glucan biosynthesis, increases in chitin content in the fungal cell wall, as well as increased export of cell wall components for cell wall repair. Recently, the molecular chaperone heat shock protein 90 (Hsp90) was reported to play a critical role in regulating resistance to antifungal agents through the calcineurin pathway [24]. Future efforts towards combating antifungal resistance are likely to exploit this important and evolutionarily conserved mechanism for maintaining and expressing resistance mechanisms to antifungals.

Amphotericin B

Conventional amphotericin B has long been the cornerstone of therapy for deeply invasive fungal infections. Toxicity, including infusion-related fever, chills, rigors, headache, and dose-limiting nephrotoxicity, often limits the effectiveness of this agent in severely ill patients. Consequently, three lipid-based formulations (Ambisome®, Abelcet®, and Amphotec®) were developed that offer several advantages over conventional amphotericin B: (1) the ability to administer higher daily dosages of drug, (2) decreased infusion-related side effects (especially for the liposomal formulation), (3) and a reduced rate of nephrotoxicity (Table 8.2). Despite the improved therapeutic index of these formulations, there is still relatively little data from prospective clinical trials to suggest these formulations are more effective than conventional amphotericin B even though they are generally recommended first-line over conventional amphotericin B for most common fungal infections. Ambisome® is the most frequently prescribed formulation, while Amphotec® was subsequently removed from the market.

Spectrum and Susceptibility

Amphotericin B remains one of the broadest-spectrum antifungals available with activity in vitro against almost most major fungal pathogens including *Candida*, *Cryptococcus*, *Aspergillus*, *Blastomyces*, *Histoplasma*, *Coccidioides*, *Sporothrix*, and agents of mucormycosis (including *Rhizopus*, *Mucor*, and *Lichtheimia* spp.), as well as other less frequently recovered yeast and molds.

A few species exhibit elevated MICs when tested against amphotericin B and are known to possess innate resistance to this drug. Resistant species include both members of the *Scedosporium apiospermum* species complex and *Lomentospora* (previously *Scedosporium*) *prolificans*, in addition to *Purpureocillium lilacinum*, *Aspergillus terreus*, and some *Fusarium* spp. (certain isolates with the *F. solani* species complex). Some reports suggest *Candida lusitaniae* resistance to amphotericin B and have shown that this species possesses the ability to develop resistance while on treatment. The first report involved a patient whose initial isolate was susceptible but whose subsequent isolates had developed amphotericin B resistance [25]. Later reports have shown amphotericin B resistance may exist even prior to exposure to amphotericin B [26]. The expected rate of resistance for *C. lusitaniae* is 8–10% of any microbiology lab stock collection.

Pharmacokinetics

Amphotericin B deoxycholate has negligible oral absorption and must be administered intravenously. Following intravenous (IV) administration, the drug is released from the deoxycholate bile salt and is highly bound by plasma proteins (91–95%) including lipoproteins, erythrocytes, and cholesterol in the plasma. Amphotericin B then redistributes from the bloodstream into tissue with an apparent volume of distribution (Vd) of 4 l/kg [27]. In adults, infusion of 0.6 mg/kg of amphotericin B deoxycholate yields peak serum concentrations of approximately 1–3 μg/ml [27]. Concentrations in other body fluids outside the serum are less than 5% of concurrent serum concentrations with poor penetration into bronchial secretions, pleura, peritoneum, synovium, and

Table 8.2 Systemic antifungal therapies

Antifungals	Trade name(s)	Usual adult dose	Mechanism of action	Toxicities	Spectrum/comments
Polyenes					
Amphotericin B Lipid formulations of amphotericin B Liposomal (L)-AMB ABLC	Fungizone Ambisome Abelcet	0.25–1.5 mg/kg IV q24h 3–10 mg/kg/q24h 5 mg/kg/q24h	Binds to ergosterol and intercalates with the fungal cell membrane, resulting in increased membrane permeability to univalent and divalent cations; liposomal amphotericin B passes through fungal cell wall to release amphotericin B at the cell membrane	Acute-fever, chills, rigor, arthralgia with infusion. Thrombophlebitis, dyspnea (rare), arrhythmias (rare) Delayed-azotemia (26%), tubular acidosis, hypokalemia, hypomagnesemia, anemia	Drug of choice for severe infections caused by endemic dimorphic fungi, most *Candida* spp., and common hyalohyphomycetes (including *Aspergillus*) and mucormycosis Nephrotoxicity is the dose-limiting side-effect, reduced with lipid amphotericin B formulations and saline pre- and posthydration Infusion-related reactions: Ambisome < Abelcet < Ambisome® considered the preferred formulation for central nervous system mycoses
Azoles					
Ketoconazole	Nizoral	200–800 mg PO q24h Divided doses recommended ≥400 mg/day)	Inhibition of cytochrome P450 14 alpha-demethylase, decreased production of ergosterol, accumulation of lanosterol, leading to perturbation of fungal cell membrane, fungistatic	Gastrointestinal (20–50%) including nausea and vomiting, anorexia, rash (2%), transient increases in hepatic enzymes, severe hepatotoxicity (rare), alopecia, inhibition of adrenal steroid synthesis (especially at dosages >600 mg/day)	Oral formulation only. Inconsistencies in oral absorption/poor gastrointestinal tolerance limits use for treatment of deep mycoses Potent inhibitor of mammalian cytochrome P450 can lead to potentially severe drug interactions when administered concomitantly with other P450-metabolized drugs
Itraconazole	Sporanox	200–400 mg PO q24h IV 200–400 mg q12h, then q24h[a] Divided doses recommended ≥400 mg/day)	Similar to ketoconazole, but more selective for fungal P450 demethylase	Gastrointestinal (20%) including nausea and vomiting, and diarrhea rash (2%), taste disturbance (oral solution), transient increases in hepatic enzymes, severe hepatotoxicity (rare), alopecia, inhibition of adrenal steroid synthesis (especially at dosages >600 mg/day) Accumulation of hydroxy-propyl-beta-cyclodextran vehicle in patients with CrCl <30 ml/min (intravenous formulation) Congestive heart failure (rare)	Spectrum similar to fluconazole with enhanced activity against *C. krusei* and *Aspergillus*. Not active against *Fusarium* and mucormycosis. Drug of choice for mild-to-moderate infections caused by endemic dimorphic fungi Bioavailability of oral solution is improved over capsules by 30% under fed conditions and 60% in fasting conditions. Potent inhibitor of mammalian cytochrome P450 enzymes. Serum level monitoring is occasionally recommended, trough levels measured by HPLC should exceed 0.5 μg/ml

Table 8.2 (continued)

Antifungals	Trade name(s)	Usual adult dose	Mechanism of action	Toxicities	Spectrum/comments
Fluconazole	Diflucan	100–800 mg PO/IV q24h Dosage adjustment required in renal impairment	Similar to ketoconazole, but more selective inhibitor of 14 alpha-demethylase	Gastrointestinal (5–10%), rash, headache, transient increases in hepatic enzymes, hepatotoxicity (rare), alopecia	Spectrum includes most *Candida* spp., *Cryptococcus neoformans*, and endemic dimorphic fungi. Less active against *Candida glabrata*. *Candida krusei* are intrinsically resistant. Not clinically active for deep mycoses caused by invasive molds Best tolerated of the azoles. Higher daily dosages are recommended (e.g., 12 mg/kg/day) in critically ill patients or in institutions where *Candida glabrata* is common (>10% *Candida* spp.). Fluconazole clearance is more rapid in pediatric patients
Voriconazole	Vfend	6 mg/kg IV q12h × 2 doses, then 4 mg/kg q12h 200 mg PO q12h if ≥40 kg, 100 mg PO q12h if <40 kg	Similar to fluconazole, but higher affinity for fungal 14 alpha-demethylase	Transient visual disturbances (reported up to 30%), rash, hallucinations (2%), transient increases in hepatic enzymes, severe hepatotoxicity (rare) Accumulation of sulfo-butyl ester cyclodextrin vehicle may occur in patients with CrCl <50 ml/min receiving intravenous formulation Long-term therapy associated with risk of squamous cell carcinoma, periostitis (fluoride toxicity) alopecia, neuropathy	Spectrum similar to itraconazole with enhanced activity against *Aspergillus*, *Fusarium*, and *Scedosporium* spp. Retains activity against some fluconazole-resistant *C. glabrata* Inhibitor of mammalian cytochrome P450 enzymes Highly variable pharmacokinetics, especially in pediatric patients. Therapeutic drug monitoring is recommended Trough target, efficacy: >1.0 mg/L Trough target, increased risk of CNS toxicity <6 mg/L
Posaconazole	Noxafil	200 mg PO q6h-or q8h × 7 days, then 400 mg q12h Dose-proportional saturable oral absorption Delayed-release tablet (300 mg twice daily, day 1, then 300 mg daily) Intravenous formulation (300 mg twice daily, day 1, then 300 mg daily)	Similar to voriconazole	Gastrointestinal (5–15%), fever, headache, musculoskeletal pain (5%)	Spectrum similar to voriconazole with enhanced activity against *Fusarium*, mucormycosis, and black molds (phaeohyphomycetes) Inhibitor of mammalian cytochrome P450 3A4 Use of delayed-release tablet improves absorption and avoids gastric pH drug interactions. Tablets can be given without food if necessary Intravenous formulation must be given through central line, as repeated doses through peripheral line associated with phlebitis TDM recommended for posaconazole suspension, may be considered in some patients receiving tablet formulation: Trough (prophylaxis) >0.5–0.7 mg/L Trough (treatment): >1 mg/L

(continued)

Table 8.2 (continued)

Antifungals	Trade name(s)	Usual adult dose	Mechanism of action	Toxicities	Spectrum/comments
Isavuconazole (isaconazonium sulfate)	Cresemba	372 mg IV/PO of prodrug every 8 h × 6 doses, then 372 mg/day (372 mg of prodrug isavuconazonium sulfate = 200 mg of active moiety isavuconazole)	Similar to posaconazole.	Nausea, vomiting, diarrhea, headache, transient increases in hepatic enzymes	Spectrum similar to posaconazole with enhanced activity against Mucorales and black molds (phaeohyphomycetes) Inhibitor of mammalian cytochrome P450 3A4 Therapeutic range of isavuconazole is not established
Echinocandins					
Caspofungin	Cancidas	70 mg IV day 1, then 50 mg q24h	Inhibition of cell wall glucan synthesis, leading to osmotic instability of fugal cell	Fever, chills, phlebitis/ thrombophelibitis (peripheral line), rash. Drug concentrations decreased with P450 3A4 inducers. Decreases tacrolimus blood levels by ~25%	Spectrum includes most *Candida* spp. including fluconazole-resistant *Candida krusei* and *Candida glabrata*. Active against *Aspergillus* spp Not active against *Cryptococcus. Trichosporon*, *Fusarium*, the Mucorales, or dematiaceous molds (phaeohyphomycetes)
Micafungin	Mycamine	50–150 mg IV q24h	Similar to caspofungin	Similar to caspofungin	Similar to caspofungin
Anidulafungin	Eraxis	200 mg IV day 1 then 100 mg/day	Similar to caspofungin	Similar to caspofungin	Similar to caspofungin
Fluoropyrimidines					
Flucytosine (5-FC)	Ancobon	100 mg/kg/daily PO divided q6h Dosage adjustment required in renal impairment	Drug is transported into susceptible fungi by cytosine permease, and then deaminated to active form (5-FU) by cytosine deaminase where the drug interferes with DNA/ RNA synthesis	Increase in serum transaminases (7%), nausea, and vomiting (5%); diarrhea, abdominal pain, rash, entercolitis (rare) Less common- leucopenia, thrombocytopenia, anemia	Narrow spectrum for deep mycoses: *Candida* and *Cryptococcus* spp. only. Resistance is common when used as monotherapy. Typically administered in combination with amphotericin B for cryptococcal meningitis Risk of bone marrow suppression increased with persistent flucytosine levels >100 μg/ml. Careful dosage adjustment is required in patients with renal dysfunction

PO orally, *IV* intravenously, *q6h* every 6 h, *q12h* every 12 h, *q24h* every 24 h, *P450* cytochrome P450
[a] Not available in the United States

aqueous humor. Although amphotericin B is believed to have limited penetration into the CSF, fungal infections of the brain can be successfully treated with amphotericin B [28].

Tissue concentrations of amphotericin B are highest in the kidney followed by the liver, spleen, heart, skeletal, muscle, and brain. However, the limited solubility of the molecule at physiological pH and high protein binding limits the microbiological activity of the drug [29, 30] and is slowly released over time. The formulation of amphotericin B into phospholipid sheets (Abelcet®) or liposome carriers (Ambisome®) changes drug distribution (particularly to the kidney) and the elimination profile of the drug [31] (Table 8.3).

Studies have suggested that amphotericin B undergoes relatively little metabolism, with a terminal elimination half-life of >11–15 days [28]. After 168 h, approximately 60% of a single dose can be recovered from the feces (~40%) and urine (20%) [28]. Less bioactive amphotericin B is excreted in the urine after treatment with lipid formulations making these formulations of amphotericin B less effective for urinary tract infection. Since a relatively lower fraction of the daily dosage is slowly excreted in urine and bile, dosage modification is not necessary to prevent drug accumulation in patients with renal or hepatic failure but may be judicious in patients with organ dysfunction. Amphotericin B behaves as a colloid in aqueous solutions and is highly protein bound, hemodialysis does not remove significant amounts of the drug unless the patients are hyperlipidemic; which enhances amphotericin B binding to some types of dialysis membranes [4].

Table 8.3 Comparative pharmacokinetics of the antifungal agents

	AMB	ABCD	ABLC	L-AMB	Flu	Itra[a]	Vori	Posa	Isa	Anid	Cas	Mica	5FC
Oral bioavailability (%)	<5	<5	<5	<5	95	50	96	60	98	<5	<5	<5	80
Distribution													
Total C_{max} (μg/ml)		4	0.3–1	131	0.7	11	4.6	7.8	7.5	0.83	0.27	0.24	80
AUC (mg h/L)	17	43	14	555	400	29.2	20.3	8.9	121.4	99[b]	119	158[b]	
Protein binding (%)	>95	>95	>95	>95	10	99.8	58	99	>99%	84	97	99	4
CSF (%)	0–4				>60	<10	60		ND	<5	<5	<5	75
Eye (%)	0–38[c,d]	0–38[c,d]	0–38[c,d]	0–38[c,d]	28–75[c,d] (10–70 μg/ml)	10[c] (0.22 μg/ml)	18[c] (0.81 μg/ml)	26 (0.25 μg/ml)	ND	ND	ND	ND	ND
Urine (%)[e]	3–20			4.5	90	1–10	<2	<2	<2	<2	<2	<2	90
Metabolism	Unk	Unk	Unk	Unk	+	++	+++	++	+++	None	Hep	Hep	None
Elimination	Urine/bile	Unk	Unk	Unk	Renal	Hep	Renal	Feces	Urine, feces	Feces	Urine	Feces	Renal
Half-life (h)	50	30	173	100–153	31	24	6	25	130	24	30	15	3–6

AMB amphotericin B deoxycholate, *ABCD* amphotericin B cholesterol dispersion, *ABLC* amphotericin B lipid complex, *L-AMB* liposomal amphotericin B, *Flu* fluconazole, *Itra* itraconazole, *Vori* voriconazole, *Posa* posaconazole, *Isa* isavuconazole, *Anid* anidulafungin, *Cas* caspofungin, *Mica* micafungin, *5FC* 5-fluorocytosine or flucytosine, *ND* no data available, *Unk* unknown, *Hep* hepatic

[a] Data are for oral solution; tablet and intravenous doses produce AUCs of 36.1-38.0 mg L/h

[b] For doses of 100 mg/day

[c] Human

[d] Animal

[e] % of active drug or metabolites

Adverse Effects

The most common acute toxicity of all amphotericin B formulations is infusion-related reactions, which affect more than two-thirds of patients receiving 2–6-h infusions and are characterized by fever, chills, rigors, anorexia, nausea, vomiting, myalgias, arthralgias, and headache. These reactions are the result of direct activation of Toll-like receptor 2 (TLR2) microbial pattern recognition receptors by the amphotericin B molecule through CD14-associated lipid rafts in mononuclear cells, which results in release of proinflammatory cytokines including TNF-α, interleukin (IL)-1β, IL-6, IL-8, and prostaglandin E2 [32, 33]. The onset of symptoms generally occurs after 2–4 h after infusion and is associated with an increase in serum TNF-α, IL-1RA, and IL-6 [32]. Hypotension, flushing, and dizziness are less common, but bronchospasm and true anaphylactic reactions have been reported with both the conventional and lipid formulations of amphotericin B.

Lipid formulations of amphotericin B, particularly liposomal amphotericin B, are associated with a unique type 1 hypersensitivity reaction termed "complement activation-related pseudoallergy" (CARPA) [34]. This reaction results from activation of complement through both classic and alternative pathways, giving rise to C3a and C5a anaphylatoxins that trigger mast cell and basophil secretory responses. Liposomal amphotericin B—triggered CARPA typically presents with a triad of symptoms including: (1) chest pain, dyspnea, hypoxia; (2) abdominal, flank, or leg pain; and (3) flushing and urticaria. Unlike classic amphotericin B infusion reactions that develop over 2–6 h, CARPA develops within the first 5–10 min of the first infusion and spontaneously resolves when the drug is stopped. CARPA symptoms can also be treated with IV administration of diphenhydramine, consistent with the mechanism of this reaction.

Severe hypokalemia and cardiac arrhythmias have also been described in patients with central venous catheters who have received rapid infusions or excessive doses of conventional amphotericin B. Therefore, slower infusion rates (4–6 h or more) and EKG monitoring should be considered in patients with underlying cardiac conduction abnormalities. Thrombophlebitis is a common local side effect with infusion, which often necessitates the placement of a central venous line for therapy >1 week. Slower infusion rates, rotation of infusion sites, application of hot packs, low-dose heparin, and avoidance of concentrations >1 mg/ml can minimize thrombophlebitis.

Acute reactions generally subside over time and with subsequent amphotericin B infusions. In the past, a test dose of amphotericin B deoxycholate (i.e., 1–5 mg) was recommended prior to initiating therapy. This is no longer considered useful for screening patients for hypersensitivity reactions. Pre-medications such as low dose hydrocortisone (1 mg/kg), diphenhydramine, meperidine (0.5 mg/kg), and

nonsteroidal anti-inflammatory agents are often administered prior to amphotericin B infusions to blunt symptoms of acute reactions. Premedication can also be considered prior to infusions of the lipid amphotericin B formulations, despite the reduced rates of infusion reactions seen with these drugs, especially the liposomal formulation.

Nephrotoxicity is the most significant, delayed toxicity of amphotericin B and can be classified into glomerular or tubular mechanisms. Amphotericin B directly constricts the afferent arterioles resulting in decreased renal blood flow and a drop in glomerular filtration (increased serum creatinine), eventually leading to azotemia (increased blood urea nitrogen). Patients with pre-existing decreased intravascular volume, hyponatremia, hypokalemia, and congestive heart failure are more likely to experience initial marked declines in GFR during amphotericin B infusions. Tubuloglomerular feedback (TGF), a normal physiological response that causes afferent arteriolar vasoconstriction because of increased solute concentrations (especially a decreased Na^+/K^+ ratio) in the distal tubule, is also activated during amphotericin B therapy, contributing to reduced GFR. The practice of administering 500–1000 mL of normal saline in adults or 3 mEq/kg in children, referred to as "sodium loading," immediately before and after amphotericin B administration can reduce renal arteriolar vasoconstriction by increasing the solute concentration, especially the Na^+/K^+ ratio, and blunt TGF to maintain the GFR and restore electrolyte homeostasis [35, 36].

Amphotericin B also directly damages the distal tubular membranes of the kidney, presumably through its binding to cholesterol and formation of pores [37]. Pore formation reduces the ability of the tubular membrane to resorb electrolytes, resulting in loss of potassium and bicarbonate. As a result, hypokalemia and hypomagnesemia are common during amphotericin B treatment even before a decrease in GFR and an increase in serum creatinine are evident. The initial tubular toxicity of amphotericin B is most frequently manifested as hypokalemia. Potassium supplementation, often as high as 80–120 mEq/day, is frequently required to reduce the risk of severe hypokalemia (<2.5 mmol/L) [38]. Distal tubular dysfunction also results in impaired resorption of magnesium, which complicates the ability to maintain potassium homeostasis. Magnesium deficiency allows excessive secretion of potassium through maxi-K channels in the distal tubules and collecting duct cells, thereby exacerbating hypokalemia until magnesium stores are replenished [39].

Lipid formulations of amphotericin B reduce but do not eliminate renal toxicity during with amphotericin B therapy. The preferential distribution of these formulations in reticuloendothelial cells and lack of glomerular filtration of liposomal particles reduces drug distribution in the kidney. However, diffusible amphotericin B from lipid carriers can still cause toxicity to distal tubular cells resulting in hypokalemia and decreased glomerular filtration rates, especially when these formulations are administered at higher doses (>5 mg/kg) for prolonged periods.

Patients who receive prolonged courses of amphotericin B can develop normochromic, normocytic anemia due to the inhibitory effects of amphotericin B on renal erythropoietin synthesis. Patients may experience decreases in hemoglobin of 15–35% below baseline that return to normal within several months of discontinuation of the drug. Administration of recombinant erythropoietin has been suggested in patients with symptomatic anemia during amphotericin therapy.

Azoles

The availability of azole antifungals, particularly the oral triazoles itraconazole, fluconazole, voriconazole, posaconazole, and, more recently, isavuconazole, fulfills a critical need for effective and better tolerated alternatives to amphotericin B [40]. Miconazole was the first systemic azole approved for use in humans, but the relatively toxic IV formulation limited its use to severely ill patients. Similarly, ketoconazole was not effective in critically ill patients due to its lack of an intravenous formulation and erratic absorption in patients with relative achlorhydria. Current triazoles are available in both oral and IV formulations (itraconazole IV has been removed from the market in many countries), offering flexibility for long-term treatment. The triazoles have proven to be much more effective in the prevention and treatment of both primary and opportunistic mycoses. As all triazoles are potentially teratogenic, they should be avoided during pregnancy, although a large population-based Danish analysis of short courses of oral fluconazole (150 mg) did not find a significant link with birth defects [41].

Spectrum and Susceptibility

Triazoles are primarily considered fungistatic agents against yeast. The clinical significance of fungistatic versus fungicidal antifungal activity is unclear with the possible exception in the treatment of cryptococcal meningitis where the rate and degree of fungal burden decline in the CSF is predictive of mortality [42, 43]. Although the triazoles have a similar mechanism of action, each agent has a slightly different spectrum of activity.

Fluconazole is principally used for yeast infections including those caused by most *Candida* and *Cryptococcus* spp. and endemic fungal infections caused by *Coccidioides*. Fluconazole has limited activity against molds and no clinical role for the prevention or treatment of these infections.

Acquired resistance in yeasts is a concern during fluconazole therapy. *Candida krusei* is intrinsically resistant to fluconazole; therefore, susceptibility testing is not recommended. Baseline rates of fluconazole resistance among *C. glabrata* range from 15% to 20% of any given population of isolates, but both *C. albicans* and *C. glabrata* are capable of developing resistance following prolonged therapy or with inappropriately low treatment doses dosing. In vitro testing data demonstrate approximately 80% of *C. neoformans* and 91% of *C. albicans* strains are susceptible to fluconazole.

Itraconazole possesses a wide spectrum of activity including activity against both yeasts and molds. While the triazole has some utility for treating aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, and candidiasis, GI adverse effects, hypokalemia, drug interactions, and risk of congestive heart failure often limit long-term use, especially with higher doses. Cross-resistance is of concern between drugs within the azole class. Comparison of resistance patterns between itraconazole and fluconazole reveals similar percentages of resistance among *Candida* spp.

Voriconazole is active against *Aspergillus,* some members of the *S. apiospermum* species complex, and some *Fusarium* spp. This is remarkable, since *Scedosporium* and *Fusarium* spp. are notoriously resistant to other antifungal agents. Susceptibility patterns with the yeasts are like both itraconazole and fluconazole. An important exception is the extremely low incidence of resistance seen with *C. krusei*, in contrast to near 100% resistance of this species to fluconazole and about 10% resistance to itraconazole.

Posaconazole and isavuconazole possess a similar broad spectrum of activity. Both agents show activity against agents of aspergillosis, candidiasis, fusariosis, coccidioidomycosis, and some agents of mucormycosis. Results against certain species within the Mucorales, including *Rhizopus* spp., suggest that these drugs may provide alternative therapy to amphotericin B for infections caused by this group of fungi. Resistance patterns are similar to voriconazole with the exception of the Mucorales, against which they have activity, and *Scedosporium* spp. where in vitro activity may be limited.

Fluconazole

Among the triazole antifungals, fluconazole is the best tolerated agent and has the most desirable pharmacological properties including high bioavailability, high water solubility, low degree of protein binding, linear pharmacokinetics, and a wide volume of distribution including the CSF, eye, and urine. Unlike other azoles, fluconazole is eliminated primarily unchanged through the kidneys and is less susceptible to clinically significant drug interactions through mammalian cytochrome P450 enzymes at standard dosages used to treat superficial (100–200 mg/day) or systemic (400 mg/day) infections.

Itraconazole

Itraconazole was initially introduced in the early 1990s as a capsule formulation that was effective for superficial fungal infections and mild-to-moderate severe endemic mycoses, but erratic absorption in the critically ill patient limited its effectiveness for opportunistic mycoses. The subsequent reformulation of this triazole into an oral solution with hydroxy-beta-propyl cyclodextrin significantly improved the blood levels that could be reliably obtained in critically ill and immunocompromised patients. Itraconazole is a relatively broad-spectrum triazole with activity against many common fungal pathogens including most *Candida*, *Cryptococcus,* endemic dimorphic fungi (*Histoplasma*, *Blastomyces*, and *Coccidioides*), and *Aspergillus*. The drug is lipophilic, highly protein bound and has a long half-life, nonlinear pharmacokinetics, and limited distribution into some body fluids, including the CSF and urine (Table 8.3). The drug is metabolized in the liver and, to a lesser extent, in the gut into more hydrophilic metabolites, one of which retains potent antifungal activity (hydroxyitraconazole). The most common adverse effects associated with itraconazole therapy are gastrointestinal (especially with the oral solution), rash, and transient increases in hepatic transaminases. Prolonged therapy can be associated with metabolic disturbances (suppression of adrenal steroid synthesis) and increased risk of congestive heart failure. Itraconazole is a substrate and potent inhibitor of mammalian cytochrome P450 enzymes and is therefore susceptible to many clinically significant drug interactions (see Azole Drug Interactions Section).

Voriconazole

Voriconazole is a methylated analogue of fluconazole with enhanced activity against yeast as well as important opportunistic molds including *Aspergillus* and *Fusarium* (Table 8.2). Like fluconazole, voriconazole is well absorbed orally, has limited protein binding, and distributes widely throughout the body, including the CSF. Like itraconazole, intravenous voriconazole is formulated in a cyclodextrin solution (sulfobutyl ether cyclodextrin) and has nonlinear pharmacokinetics in adults. Voriconazole is metabolized to inactive metabolites through the liver and is an inhibitor of mammalian cytochrome P450 enzymes (Table 8.3). In addition to the common adverse effects seen with other triazole antifungals (gastrointestinal, rash, increases in hepatic enzymes), vori-

conazole can cause transient visual disturbances in 15–30% of subjects that manifest as photophobia, perception of blinking or flashing lights (even with the eyes closed), and occasionally hazy or blurred vision. Symptoms tend to occur during the first week of therapy and disappear with continued therapy in most patients. Occasionally, visual disturbances are intensified by hallucinations—a separate side effect seen in 2–8% of patients receiving voriconazole (often with concomitant benzodiazepines and narcotic analgesic therapy). Visual disturbances are thought to be a result of temporary alterations in electrical conduction of photoreceptors in the rods and cones of the retina, which revert to normal once therapy is stopped. No permanent damage to the retina has been noted in human or animal studies of voriconazole [44].

Prolonged use of voriconazole has been associated with uncommon, but potentially severe adverse effects. Severe phototoxicity progressing to actinic keratosis and/or squamous cell carcinoma (SCC) has been reported in some patients receiving prolonged voriconazole therapy (i.e., >6 months), which may be accelerated in transplant populations [45–47]. Although the mechanism of voriconazole-induced SCC is unknown, there are multiple retrospective studies associating the use of voriconazole with SCC possibly due to the photosensitizing effects of the voriconazole N-oxide metabolite [48]. Periostitis has also been reported in patients receiving prolonged voriconazole therapy, which may be related to fluoride toxicity [49–51]. Alopecia, cheilitis, and brittle nails have also been noted with prolonged therapy [52]. Like other triazoles, long-term therapy with voriconazole may also be associated with the development of peripheral neuropathies [53, 54].

Posaconazole

Posaconazole is a triazole analogue of itraconazole with enhanced activity against opportunistic molds including *Aspergillus*, and some agents of mucormycosis. Posaconazole is available as an oral suspension, a pH-sensitive delayed-release tablet, and an intravenous formulation. Absorption of posaconazole suspension is dose-limited at 800 mg/day and can be improved if the suspension is administered with a high-fat meal or in divided doses (twice to four times daily) (Table 8.2). A delayed-release tablet formulation uses pH-sensitive polymers to release posaconazole at a controlled rate in the duodenum, thereby circumventing many of the problems associated with poor gastric dissolution of the drug. As a result, following a loading dose (300 mg twice daily on day 1), the average serum concentration achieved with a 300-mg daily dose using delayed-release posaconazole tablets is 1400 ng/mL, which is more than double of that achieved with the oral suspension administered at 200 mg 4 times daily (517 ng/mL) [55]. The tablet formulation also provides the opportunity to administer a loading-dose on the first day of therapy to ensure therapeutic posaconazole plasma levels in the first 24–48 h. This is in contrast with the oral suspension, which typically would not approach steady-state therapeutic levels until 7–10 days of therapy.

An intravenous formulation of posaconazole solubilized in sulfobutyl ether beta cyclodextrin was also introduced in some countries at the time of the new oral formulation. Dosing is similar to the oral delayed-release tablets (300 mg was administered intravenously twice on day 1 followed by 300 mg daily) and achieved trough concentrations greater than 1000 ng/mL within 24 in a majority of patients [56]. The intravenous formulation is preferred in critically ill patients or patients who cannot swallow the tablet formulations, which cannot be crushed.

Once absorbed, posaconazole is widely distributed into tissues throughout the body and is highly protein bound (98%). Elimination of posaconazole occurs predominantly (90%) in the feces as unchanged drug and in the urine (10%) as an inactive metabolite (Table 8.3). Despite its lack of phase I metabolism, posaconazole is a potent inhibitor of mammalian cytochrome P450 3A4 and has a similar potential for drug interactions as itraconazole when coadministered with drugs metabolized through this pathway.

Isavuconazole

Isavuconazole is administered as a prodrug salt, isavuconazonium sulfate, that is rapidly cleaved by plasma esterases following oral or intravenous administration to the active triazole, isavuconazole. The spectrum of isavuconazole activity is similar to posaconazole and is currently approved for the treatment of invasive aspergillosis and mucormycosis in patients who cannot receive amphotericin B–based therapy [57, 58]. Isavuconazole exhibits a uniquely long terminal elimination half-life (mean $t½$ = 130 h), requiring the administration of at loading dose (372 mg isavuconazonium sulfate every 8 h × 6 doses) and then a single maintenance dose 372 mg beginning 12–24 h after the last loading dose (Table 8.2). Isavuconazole has fewer visual, central nervous system and hepatic adverse effects compared to voriconazole and generally pharmacokinetic parameters are more predictable. In contrast with other triazoles, isavuconazole therapy is associated with shortening of the QTc interval rather than prolongation, making it a potentially safe treatment option in patients receiving other drugs associated with QT prolongation. Isavuconazole is a substrate of CYP 3A4/3A5 and a moderate inhibitor of CYP3A4; therefore, pharmacokinetic drug-drug interactions are similar to other triazoles.

Azole Drug Interactions

As mentioned previously, an inherent limitation of azole pharmacology is that the target of antifungal activity in pathogenic fungi, the cytochrome P450 enzyme 14-alpha-demethylase, shares considerable homology with mammalian cytochrome P450 enzymes involved in drug metabolism. As a result, azole antifungals can be both substrates and inhibitors of cytochrome P450 systems in humans [40, 59]. All patients should have their modification profile carefully screened, preferably with a computerized drug-interaction-checking database, when azole antifungals are ignited or stopped. Many of these drug interactions are potentially severe and concomitant use should be avoided. Some azole drug interactions are less predictable and possibly dosage dependent. For example, fluconazole is a weak inhibitor of cytochrome P450 3A4 at dosages of 50–200 mg/day and is excreted primarily (80%) through the urine. However, as daily dosages are increased, fluconazole has a greater potential for inhibition of cytochrome P450 3A4 and a larger percentage of the drug is metabolized via the P450 system [5]. Cytochrome P450 3A4 inducers increase metabolism of all azoles to varying degrees regardless of their primary excretion pathways. Coadministration of rifampin, for example, can reduce fluconazole serum concentrations by ~50% and concentrations of itraconazole, voriconazole, posaconazole, and isavuconazole by >90% [5]. Triazole antifungal therapy should be avoided, whenever possible, during use of high-dose conditioning chemotherapy with busulfan or cyclophosphamide due to an increased risk of acute liver toxicity and accumulation of toxic chemotherapy metabolites [60]. A growing problem is the concomitant use of triazoles with small-molecule kinase inhibitors (SMKIs) used as targeted chemotherapy for hematological malignancies. A vast majority of SMKIs are metabolized through CYP3A4 and the concurrent use of triazoles can result in prolonged cytopenias or toxicities due to increased drug exposure and off-target effects. However, empiric dosage reductions of the targeted chemotherapy may predispose patients to increased risk of malignancy relapse; therefore, the optimal management of these interactions is highly desirable. Although azoles themselves do not appear to exert major effects on cardiac conduction, their combined use with drugs that effect potassium channels and are metabolized through CYP 450 mechanisms (e.g., haloperidol, certain tricyclic antidepressants) have the potential for life-threatening arrhythmias.

Echinocandins

Despite some modest differences in pharmacokinetics and potency, the echinocandins are pharmacologically similar and probably interchangeable. All three currently approved agents, caspofungin, micafungin, and anidulafungin, are large semisynthetic lipopeptides that are available only as intravenous formulations. All have linear pharmacokinetics, are widely distributed (with the possible exception of the CSF and urine), and have prolonged elimination half-lives that permit once daily dosing. Slight differences in the metabolism and excretion are seen between the echinocandins, which may account for some differences in the drug interaction profile of these agents.

Spectrum and Susceptibility

Caspofungin is indicated for candidiasis and for aspergillosis in patients who are refractory to other therapies. Use for infections caused by other molds has not been as extensively studied. Activity is fungicidal against the yeasts while static against the *Aspergilli* and other filamentous fungi. As previously described, the endpoint for the echinocandins against filamentous fungi is determined differently than with other antifungals and is the minimum effective concentration (MEC). While growth is substantial in vitro, it is evident that the growth is grossly abnormal. The MEC is considered the lowest concentration of drug that causes the abnormal growth of hyphae in this species (Fig. 8.6).

Resistance, although previously uncommon, is increasing reported for this class of antifungals with their widespread use, especially among *C. glabrata* [20, 61]. With a few rare exceptions, resistance to one echinocandin often results in cross-resistance to other echinocandins.

Pharmacokinetics

All three echinocandins are available as intravenous formulations only, have (mostly) linear pharmacokinetics, are widely distributed (except for the CSF and urine), have prolonged elimination half-lives, and are metabolized by chemical degradation followed by hepatic metabolism (Table 8.3). Dosage adjustment is recommended for caspofungin in patients with severe chronic hepatic dysfunction (Child Pugh score 7–9), although dosing adjustment may not be warranted in critically ill ICU patients with acute liver dysfunction or hypoalbuminemia [62], but is not required for micafungin or anidulafungin.

Adverse Effects

All three echinocandins are generally well tolerated by patients with the most frequent toxicities of phlebitis/venous irritation, headache, fever, and rash. Infusion-related reactions analogous to the "red person's syndrome" seen with

vancomycin have been described with caspofungin, likely due to its potential to cause histamine release from mast cells but are seen less commonly with micafungin and anidulafungin. The most common laboratory adverse effects reported with echinocandin therapy are transient elevations in serum transaminases and alkaline phosphatase levels. The echinocandins are neither substrates nor inhibitors of cytochrome P450 enzymes of P-glycoprotein enzymes. The coadministration of caspofungin with inducers of P450 3A4 (e.g., rifampin, phenytoin) results in modest (25–50%) decrease in the AUC, which can be overcome with higher caspofungin dosages. P450 inducers do not appear to have as pronounced effect on the clearance of micafungin or anidulafungin. Caspofungin modestly (~20%) decreases the AUC of concomitant tacrolimus therapy. Micafungin modestly increases the AUC of nifedipine and sirolimus. No clinically significant drug interactions have been identified thus far for anidulafungin.

Fluoropyrimidines

Flucytosine (5-fluorocytosine, 5-FC) is the only agent among the fluoropyrimidine class of antifungal agents approved for the treatment of invasive fungal infections. It is available only in oral capsule formulation in the United States. The usefulness of flucytosine for treating invasive mycoses is hampered by its relatively narrow spectrum, high rates of acquired resistance among common pathogens (e.g., *Candida* spp.), and significant potential for toxic effects. For these reasons, flucytosine is not used as monotherapy and has a minimal role in the treatment of most mycoses.

Spectrum and Susceptibility

5-fluorocytosine has activity against both *Candida* and *Cryptococcus* species and is not recommended for the treatment of infections caused by other fungal species. The rate of resistance against *Candida* spp. is expected in about 5% of isolates, while for *Cryptococcus* spp., resistance occurs in about 2% of isolates tested. However, resistance can rapidly develop if this agent is used as monotherapy.

Pharmacokinetics

Because flucytosine widely distributes throughout the body, including the CSF after oral administration, it has an adjuvant use in combination with antifungals that have slow or minimal distribution into anatomically privileged sites. Several randomized prospective studies of cryptococcal meningitis in patients with AIDS have shown that the addition of flucytosine to amphotericin B therapy results in more rapid sterilization of the CSF, decreased early mortality, and fewer relapses after completion of "induction" antifungal therapy.

Adverse Effects

Flucytosine was originally developed as an antitumor chemotherapy before it was discovered to have antifungal activity against common yeast. Not surprisingly, the most common side effects are nausea and vomiting, increases in serum transaminases, and bone marrow suppression. The risk of bone marrow suppression can be reduced if serum levels are maintained <100 μg/ml [63]. Because flucytosine is eliminated unchanged through the kidney, serum level monitoring and dosage adjustments are required in patients receiving flucytosine in combination with amphotericin B or other nephrotoxic agents. Gastrointestinal side effects are seen in up to 6% receiving oral flucytosine including diarrhea, nausea, and vomiting. Reversible elevations in hepatic serum transaminases and alkaline phosphatase have also been reported in 4–10% of patients receiving flucytosine. The most serious toxicity associated with flucytosine, however, is bone marrow suppression, which occurs in 6% of patients. Some evidence has accumulated in the last two decades that marrow toxicity is enhanced if serum concentrations of flucytosine exceed 100 μg/ml.

Combination Antifungal Therapy

Because of their unique mechanism of action, the introduction of the echinocandins has renewed interest in the use of combination antifungal therapy for invasive mycoses. The most common reasons for consideration of combination therapy are to: (1) broaden the spectrum of antifungal coverage of opportunistic mycoses, particularly in severely immunocompromised patients, (2) to enhance the activity of an antifungal regimen through (presumably) synergistic antifungal effects especially in severely immunocompromised patients with progressive disease, and (3) to overcome the pharmacokinetic limitations of a single antifungal agent in the treatment of life-threatening mycoses in an anatomically privileged sites such as the CNS (e.g., combined use of flucytosine and amphotericin B for cryptococcal meningitis). With the exception of cryptococcal meningitis where combination therapy with amphotericin B and flucytosine is associated with improved survival [64], there are few definitive studies supporting the use of combination therapy for deep mycoses. A multicenter prospective randomized study in patients with proven or probable aspergillosis reported significantly improved survival among patients with

galactomannan-diagnosed diseases who received combination of anidulafungin-voriconazole therapy versus voriconazole alone [65]. Nevertheless, the benefits reported in this trial were evident only in a subgroup, post-hoc analysis, raising some lingering questions about the efficacy of combination therapy for invasive aspergillosis. Interest in combination therapy, especially for difficult-to-treat yeasts and molds, is likely to increase in the coming years if new antifungals with novel mechanisms (e.g., olorofim, fosmanogepix) are approved.

Therapeutic Drug Monitoring

Antifungal drug exposures are often difficult with fixed or even weigh-based dosing and there is some evidence, especially among triazole antifungals, that therapeutic drug monitoring (TDM) may be needed to ensure adequate drug exposures and reduce the risk of toxicity [66, 67] (Table 8.2). The majority of patients receiving itraconazole solution or capsules, or voriconazole should undergo routine therapeutic drug monitoring to ensure adequate drug exposures. Evidence supporting the routine use of TDM to preemptively reduce antifungal doses of itraconazole [68] or voriconazole [69, 70] is less clear if the patient does not have evidence of toxicity. Routine TDM was routinely recommended in patients receiving posaconazole suspension, but subtherapeutic exposures are less common in patients receiving the extended-release tablet formulation or intravenous solution. Similarly, a therapeutic range for isavuconazole is not well established and most patients appear to achieve adequate concentrations, although lower weight patients (<50 kg) may have elevated exposures [71]. Therefore, targeted selected TDM may still be indicated if patients have risk factors for inadequate drug absorption (i.e., severe mucositis, diarrhea) or in the cases of drug interactions, unexplained toxicities, or suspected breakthrough fungal infection.

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Part IV

Mycoses

Candidiasis

9

Luis Ostrosky-Zeichner and Jack D. Sobel

Introduction

Candida species are ubiquitous fungi and the most common fungal pathogens affecting humans. The growing problem of skin, mucosal, and systemic candidiasis reflects the enormous increase in the pool of patients at risk and the increased opportunity for *Candida* to invade tissues normally resistant to invasion. *Candida* are true opportunistic pathogens that exploit vulnerable hosts undergoing life-saving surgeries, procedures, and other recent technological advances that prolong life, to gain access to the vascular circulation and deep tissues. *Candida* has a broad range of hosts as it can infect people with seemingly normal immune systems, but it particularly affects high-risk patients who are either immunocompromised or critically ill.

Etiologic Agents

Candida are yeast-like fungi that can form true hyphae and pseudohyphae. These yeasts are typically confined to human and animal reservoirs; however, they are frequently recovered from the hospital environment, including from food, countertops, air conditioning vents, floors, respirators, and the hands and fingernails of health-care workers. They are also normal commensals of diseased skin and mucosal surfaces of the gastrointestinal (GI), genitourinary, and respiratory tracts. Recent outbreaks of *Candida* in wards and intensive care units have shifted the paradigm of thinking infection is mostly related to the host flora and translocation to the current thinking that there is some degree of nosocomial transmission.

More than 100 species of *Candida* exist, but only a few are recognized as causing disease in humans [1]. The medically significant *Candida* species are shown in Table 9.1. *Candida glabrata* and *Candida albicans* account for 70–80% of yeasts isolated from patients with invasive candidiasis. *C. glabrata* has become important because of its increasing worldwide incidence and because it is intrinsically less susceptible to azoles and amphotericin B. Two uncommon *Candida* species, *C. lusitaniae* and *C. guilliermondii*, are important because of their innate and acquired resistance to amphotericin B. *C. krusei*, although not as common as some *Candida* species, is clinically significant because of its intrinsic resistance to fluconazole and decreased susceptibility to all other antifungals, including amphotericin B (Table 9.1) [2]. *C. krusei* is more commonly associated with hematological malignancy and neutropenia than *C. parapsilosis*. *Candida tropicalis* is more frequently recovered in hematological malignancy and neutropenic patients and has been associated with increased mortality. The newest *Candida* species of medical relevance is *Candida auris*, which was initially isolated in 2009 from a patient's ear culture in Japan [3]. This *Candida* species is notable for its ability to spread within hospitals and long-term care settings and for its antifungal resistance. *C. auris* isolates are often resistant to azoles and sometimes resistant to echinocandins and polyenes as well [4]. As seen in Table 9.1, recent changes in fungal taxonomy have prompted the reclassification of *Candida* species into other fungal species; however, the clinical convention is to continue referring to them using the "old nomenclature" for patient care purposes [5, 6].

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_9

Table 9.1 Most frequent *Candida* species with proposed taxonomic classifications and general patterns of susceptibility

Candida species	New taxonomical name	Fluconazole	Voriconazole, posaconaozle, isavuconazole	Flucytosine	Amphotericin B and its lipid compounds	Echinocandins
C. albicans	–	S	S	S	S	S
C. tropicalis	–	S	S	S	S	S
C. parapsilosis	*Candida orthopsilosis* and *Candida metapsilosis* groups	S	S	S	S	S
C. glabrata	*Nakaseomyces glabrataa*	S-DD to R	S to R	S	S to I	S
C. krusei	*Pichia kudriavzevii*	R	S to R	I to R	S to I	S
C. lusitaniae	*Clavispora lusitaniae*	S	S	S	S to R	S
C. kefyr	*Kluyveromyces marxianus*	S	S	S	S	S
C. guilliermondii	*Meyerozyma guilliermondii*	S	S	S	S to R	S
C. dubliniensis	–	S	S	S	S	S
C. auris	–	R	R	S	S to R	S to R

S susceptible, *R* resistant, *S-DD* susceptible-dose dependent

Epidemiology

Candida species are the most common cause of fungal infection, primarily affecting immunocompromised patients. Oropharyngeal colonization is found in 30–55% of healthy young adults, and *Candida* may be detected in 40–65% of normal fecal flora.

Clinical and autopsy studies have confirmed the marked increase in the incidence of disseminated candidiasis, reflecting a parallel increase in the frequency of candidemia. This increase is multifactorial in origin, reflecting an increased recognition as well as a growing population of patients at risk (i.e., patients undergoing highly complex medical and surgical procedures and those with indwelling vascular devices). The increase in disseminated candidiasis also reflects the improved survival of patients with underlying neoplasms, autoimmune disease, transplant, and other forms of immunosuppression. Candidiasis causes more fatalities than any other systemic mycosis [7, 8].

Early studies observed that in febrile neutropenic patients who die of sepsis, there was a 20–40% chance of finding evidence of invasive candidiasis at autopsy. Bodey and colleagues described 21% of fatal infections in leukemic patients as the result of invasive fungal disease, in contrast with 13% and 6% of fatal infections in patients with lymphoma and solid tumors, respectively [9, 10]. Systemic candidiasis has been described in 20–30% of patients undergoing bone marrow transplantation; however, the use of effective antifungal prophylaxis in neutropenic and other high-risk subjects has resulted in reduced occurrence of invasive candidiasis. New risk factors include pancreatitis and left ventricular assist devices. *Candida* species consistently rank as the top one or two most commonly isolated pathogens from blood cultures in hospitals. A dramatic increase in the incidence of candidemia has occurred in the last 4 decades, no longer concentrated in oncology and transplantation wards and now found mainly in nonneutropenic patients. Epidemiologic data indicates that at least 10–12% of all nosocomial infections and 8–15% of all nosocomial bloodstream infections are caused by *Candida* [11–13]. Rates are now highest among adults older than 65 years. Another epidemiological trend is the emergence of community-acquired candidemia, largely driven by intravenous drug abuse and the opioid epidemic as well as the shift to complex home health services [14].

Candidemia and disseminated candidiasis mortality rates have not improved markedly over the past few years and remain in the 30–40% range, resulting in a serious economic impact. Candidemia is associated with considerable prolongation of the length of hospital stay (70 days vs. 40 days in matched patients) [15, 16]. Although mucocutaneous fungal infections such as oropharyngeal candidiasis and *Candida* esophagitis were common in AIDS patients, the frequency of these infections has decreased over the years with the availability of antiretroviral therapy. Candidemia and disseminated candidiasis have always been rare in this population.

Within the hospital setting, areas with the highest rates of candidemia include ICUs, surgical units, trauma units, and neonatal ICUs. In fact, 25–50% of all nosocomial candidemia occurs in critical care units. Neutropenic patients, formerly the highest-risk group, are no longer the most vulnerable subpopulation, likely as a result of the widespread use of fluconazole prophylaxis during neutropenia [17]. In some tertiary care centers, *C. albicans* is no longer the most frequent bloodstream isolate, having been replaced by *C. glabrata*, which has replaced *C. tropicalis* as the most prevalent non-*albicans* species, now causing 3–50% of all candidemias. This is particularly true for some countries like Brazil, where *C. parapsilosis* has displaced other species as the most frequent one. Non-*albicans Candida* have also become an increasing problem in ICUs, attributed to the more widespread use of fluconazole in this population. In addition to the decline of *C.*

albicans as the dominant blood culture isolate, there is a wide global variation in the predominance of particular species, with *C. tropicalis* common in South America and *C. parapsilosis* common in Europe. *C. auris* has been described as the cause of large nosocomial outbreaks in Asia, Europe, Latin America, and most recently in the United States [1]. This trend has been particularly acute during the COVID-19 pandemic, where large volumes of patients and lower standards of care in overwhelmed intensive care units have triggered important outbreaks [18, 19].

Risk factors for *Candida* bloodstream infections include broad-spectrum antibiotic use, mechanical ventilation, chemotherapy, corticosteroids, intravascular catheters, receipt of total parenteral nutrition (TPN), recent surgery, hospitalization in ICU, high disease severity scores, malignancy, pancreatitis, extensive burns, extra corporeal circulation, hemodialysis, neutropenia, and fungal colonization. Perhaps, the most important risk factor for invasive candidiasis is a prolonged stay in the ICU, as the risk increases dramatically after 10 days in the unit [1].

Pathogenesis and Immunology

Host defects play a significant role in the development of candidal infections [1]. Intact skin constitutes a highly effective, impermeable barrier to *Candida* penetration. Disruption of the skin from burns, wounds, and ulceration permits invasion by colonizing opportunistic organisms. Similarly, indwelling intravascular devices provide an efficient conduit that bypasses the skin barrier. The major defense mechanisms operating at the mucosal level to maintain colonization and prevent invasion include normal protective bacterial flora and cell-mediated immunity. The importance of the latter mechanism is highlighted by chronic mucocutaneous candidiasis, a congenital *Candida* antigen-specific deficiency manifested by chronic, intractable, and severe mucocutaneous infection. However, candidemia and disseminated candidiasis are rare in the presence of an intact humoral and phagocytic system.

An effective phagocytic system is the critical defense mechanism that prevents *Candida* deep tissue invasion, thereby limiting candidemia and preventing dissemination. Polymorphonuclear and monocytic cells are capable of ingesting and killing blastospores and hyphal phases of *Candida*, a process that is enhanced by serum complement and specific immunoglobulins. Severe leukocyte qualitative dysfunction (e.g., chronic granulomatous disease) is associated with disseminated, often life-threatening candidal infections. Myeloperoxidase deficiency also results in increased susceptibility to invasive infection.

Several *Candida* virulence factors contribute to their ability to cause infection, including surface molecules that permit adherence of the organism to other structures (human cells, extracellular matrix, prosthetic devices), acid proteases, phospholipase, and the ability to convert from yeast to hyphal form [20, 21].

Candidal colonization is at the highest levels in patients at the extremes of age, neonates and adults older than 65 years. Numerous risk factors are associated with increased colonization. Once the colonized mucosal surface is disrupted by chemotherapy or trauma, organisms penetrate the injured areas and gain access to the bloodstream. Although the yeast phase of *Candida* is capable of penetrating intact mucosal cells, the more virulent hyphal phase is more often associated with tissue invasion. Indwelling central venous catheters appear to be a frequent route of bloodstream invasion, accounting for at least 20% of candidemias. Total parenteral nutrition (TPN) constitutes an independent risk factor. The risk of fungemia is increased with prolonged duration of catheterization, which also increases the risk of local phlebitis, occasionally progressing to suppurative thrombosis. Tunneled catheters (e.g., Hickman and Broviac) are less commonly the source of candidemia, but the intravascular portion may become colonized and infected as the result of candidemia originating from a second independent focus or portal of entry. Fungal invasion from colonized wounds occurs rarely, except in patients with extensive burns. Similarly, the respiratory tract, although frequently colonized, is not a common site for *Candida* invasion and rarely is a source of dissemination. However, it can be a marker for more widespread colonization.

Following invasion of the bloodstream, efficient phagocytic cell function rapidly clears the invading organisms, especially when the inoculum is small. More prolonged candidemia is likely in granulocytopenic patients, especially when diagnosis and treatment are delayed. This results in increased risk of hematogenous spread and metastatic seeding of multiple visceral sites, primarily the kidney, eyes, liver, spleen, skin, and central nervous system. Manifestations of metastatic infection may be apparent immediately or may be delayed several weeks or even months, long after predisposing factors (e.g., granulocytopenia) have resolved.

A third route for bloodstream invasion is persorption via the GI wall, following massive colonization with a high titer of organisms that pass directly into the bloodstream. Candidemia and disseminated candidiasis almost invariably follow serious bacterial infections, especially bacteremia. An association with ventilator-associated pneumonia due to *Pseudomonas*, with enterococcal bacteremia, and with *Clostridiodes difficile* colitis, has been established [22, 23].

Clinical Manifestations

Candida infections can present in a wide spectrum of clinical syndromes, depending on the site of infection and the degree of immunosuppression of the host.

Cutaneous Candidiasis Syndromes

Generalized cutaneous candidiasis manifests as a diffuse eruption over the trunk, thorax, and extremities. Patients have a history of generalized pruritus with increased severity in the genitocrural folds, anal region, axillae, hands, and feet. Physical examination reveals a widespread rash that begins as individual vesicles and spreads into large confluent areas.

Intertrigo affects any site where skin surfaces are in close proximity, providing a warm, moist environment. A red pruritic rash develops, beginning with vesiculopustules, enlarging to bullae, which then rupture causing maceration and fissuring. The area involved typically has a scalloped border, with a white rim consisting of necrotic epidermis that surrounds the erythematous macerated base. Satellite lesions are frequently found. These may coalesce and extend into larger lesions. *Candida* folliculitis is predominantly found in hair follicles and rarely becomes extensive. Paronychia and onychomycosis are frequently associated with immersion of the hands in water, especially in patients with diabetes mellitus. These patients usually have a history of a painful and erythematous area around and underneath the nails and nail beds.

Chronic mucocutaneous candidiasis describes a unique group of individuals with *Candida* infections of the skin, hair, nails, and mucous membranes that tend to have a protracted and persistent course. Most infections begin in infancy or the first two decades of life, whereas onset in people older than 30 years is rare. These chronic and recurrent infections frequently result in a disfiguring form called *Candida* granuloma. Most patients survive for long periods and rarely experience disseminated fungal infections. Chronic mucocutaneous candidiasis is frequently associated with multiple endocrinopathies. Examination reveals disfiguring lesions of the face, scalp, hands, and nails occasionally associated with oral thrush and vitiligo. These forms of candidiasis are associated with primary immune defects and are extremely difficult to treat, as patients usually require multiple and escalating courses of antifungal therapy, resulting in antifungal resistance.

Oropharyngeal Candidiasis (OPC)

OPC occurs in association with serious underlying conditions such as diabetes, leukemia, neoplasia, corticosteroid use, antimicrobial therapy, radiation therapy, dentures, and HIV infection. Persistent OPC in infants may be the first manifestation of childhood AIDS or chronic mucocutaneous candidiasis. Samonis et al. reported that 28% of cancer patients not receiving antifungal prophylaxis developed OPC. In a similar immunocompromised, hospitalized population, Yeo et al. observed OPC in 57% of patients [24, 25].

In the past, approximately 80–90% of patients with HIV infection developed OPC at some stage of their disease. The presence of OPC should alert the physician to the possibility of underlying HIV infection. Untreated, 60% of HIV-infected patients develop an AIDS-related infection or Kaposi's sarcoma within 2 years of the appearance of OPC. Many AIDS patients experience recurrent episodes of OPC and esophageal candidiasis as HIV progresses, and multiple courses of antifungals administered may contribute to the development of antifungal resistance. Antifungal agents are less effective and take longer to achieve a clinical response in HIV-positive patients than in cancer patients. There has been a significant increase in the incidence of non-*albicans Candida* recovered from HIV-positive patients, this is particularly true for *C. dubliniensis*, which was thought to be associated with HIV infection and was thought to be more likely to be resistant to fluconazole.

C. albicans remains the most common species responsible for OPC (80–90%). *C. albicans* adheres better *in vitro* to epithelial cells than non-*albicans Candida*.

The clinical manifestations of OPC (commonly called thrush) vary significantly, from none to a sore, painful mouth, burning tongue, and dysphagia. Frequently, patients with severe objective (examination) changes are asymptomatic. Clinical signs include a diffuse erythema with white patches (pseudomembranous) that appear as discrete lesions on the surfaces of the mucosa, throat, tongue, and gums. With some difficulty, the plaques can be wiped off, revealing a raw, erythematous, and sometimes bleeding base. OPC impairs quality of life and results in a reduction in fluid or food intake. The most serious complication of untreated OPC is extension to the esophagus. Fungemia and disseminated candidiasis are uncommon.

Chronic atrophic stomatitis or denture stomatitis is a very common form of OPC, with soreness and burning of the mouth. Characteristic signs are chronic erythema and edema of the portion of the palate that comes into contact with dentures. Denture stomatitis is found in 24–60% of denture wearers and is more frequent in women than in men. Notably, *C. glabrata* has been identified in 15–30% of all cultures, a higher prevalence than generally found in the mouth. Angular cheilitis (perlèche), also called cheilosis, is characterized by soreness, erythema, and fissuring at the corners of the mouth. Chronic hyperplastic candidiasis (*Candida* leukoplakia) produces oral white patches, or leukoplakia, that are discrete, transparent-to-whitish, raised lesions of variable sizes found on the inner surface of the cheeks and, less frequently, on the tongue. Midline glossitis (median rhomboid glossitis, acute

atrophic stomatitis) refers to symmetrical lesions of the center dorsum of the tongue characterized by loss of papillae and erythema.

Esophageal Candidiasis

Candida esophagitis occurs in predisposed individuals. *C. albicans* is the most common cause. The prevalence of *Candida* esophagitis increased during the first two decades of the AIDS epidemic and with increased numbers of transplant, cancer, and severely immunocompromised patients.

Esophageal candidiasis in an HIV-infected patient may be the first manifestation of AIDS. *Candida* esophagitis tends to occur later in the natural history of HIV infection and almost invariably at a much lower CD4 count. In cancer patients, factors predisposing to esophagitis include recent exposure to radiation, cytotoxic chemotherapy, antibiotic and corticosteroid therapy, and neutropenia. Clinical features include dysphagia, odynophagia, and retrosternal pain. Constitutional findings, including fever, occur only occasionally. Rarely, epigastric pain is the dominant symptom. Although esophagitis may occur as an extension of OPC, in more than two-thirds of published reports, the esophagus was the only site involved; more often infection involved the distal two-thirds of the esophagus. *Candida* esophagitis in AIDS patients may occur in the absence of symptoms despite extensive objective esophageal involvement. Kodsi classified *Candida* esophagitis on the basis of its endoscopic appearance [26]. Type I cases refer to a few white or beige plaques up to 2 mm in diameter. Type II plaques are larger and more numerous. In the milder grades, plaques may be hyperemic or edematous, but there is no ulceration. Type III plaques may be confluent, linear, nodular, and elevated, with hyperemia and frank ulceration, and type IV plaques additionally have increased friability of the mucosa and occasional narrowing of the lumen. Uncommon complications of esophagitis include perforation, aortic-esophageal fistula formation, and, rarely, candidemia or bacteremia.

A reliable diagnosis can only be made by histologic evidence of tissue invasion in biopsy material. Nevertheless, antifungal therapy is frequently initiated empirically with minimal criteria in a high-risk patient. The mere presence of *Candida* within an esophageal lesion as established by brushings, smear, or culture does not provide sufficient evidence to distinguish *Candida* as a commensal from *Candida* as the responsible invasive pathogen.

Radiographic studies have been replaced by endoscopy, which not only provides a rapid and highly sensitive diagnosis, but also is the only reliable method of differentiating among the various causes of esophagitis. The characteristic endoscopic appearance is described as yellow-white plaques on an erythematous background, with varying degrees of ulceration. Differential diagnosis includes radiation esophagitis, reflux esophagitis, cytomegalovirus, or herpes simplex virus infection. In the AIDS patient, it is not uncommon to identify more than one etiologic agent, such as herpes viruses or cytomegalovirus, as causes of esophagitis.

Respiratory Tract Candidiasis

Laryngeal candidiasis is seen primarily in HIV-infected patients and occasionally in those with hematologic malignancies. The patient presents with a sore throat and hoarseness, and the diagnosis is made by direct or indirect laryngoscopy.

Candida tracheobronchitis is a rare form of candidiasis seen in HIV-positive or severely immunocompromised subjects, complaining of fever, productive cough, and shortness of breath. Physical examination reveals dyspnea and scattered rhonchi. The diagnosis generally is made during bronchoscopy.

Candida pneumonia is also a rare form of candidiasis. The most common form of infection appears to be multiple lung abscesses due to the hematogenous dissemination of *Candida*. As there may be a high degree of colonization and isolation of *Candida* from the upper respiratory tract, diagnosis requires the visualization of *Candida* invasion on histopathology. Patient history usually reveals similar risk factors for disseminated candidiasis, and patients complain of shortness of breath, cough, and fever. Sputum or endotracheal secretions positive for *Candida* usually indicate upper respiratory tract colonization, have low predictive value for pneumonia, and, unfortunately, are incorrectly used as a pretext for initiation of antifungal therapy. Current guidelines strongly discourage treating these patients, except for the context of this finding representing colonization and being taken as a risk factor for disseminated disease [27].

Vulvovaginal Candidiasis

In the United States, *Candida* vaginitis is the second most common vaginal infection. During the childbearing years, 75% of women experience at least one episode of vulvovaginal candidiasis (VVC), and 40–50% of these women experience a second episode. A small subpopulation of women experiences repeated, recurrent episodes of *Candida* vaginitis. *Candida* may be isolated from the genital tract of about 10–20% of asymptomatic, healthy women of childbearing age.

Candida vaginitis can be classified as complicated or uncomplicated, depending on factors such as severity and frequency of infection and the causative *Candida* species (Table 9.2). Increased rates of asymptomatic vaginal coloni-

Table 9.2 Classification of *Candida* vaginitis

	Uncomplicated (90%)	Complicated (10%)
Severity	Mild or moderate	Severe
Frequency	Sporadic	Recurrent
Organism	*Candida albicans*	Non-*albicans* species of *Candida*
Host	Normal	Abnormal (e.g., uncontrolled diabetes mellitus)

zation with *Candida* and *Candida* vaginitis are seen in pregnancy (30–40%), with the use of oral contraceptives with a high estrogen content, and in uncontrolled diabetes mellitus. The hormonal dependence of the infection is illustrated by the fact that *Candida* is seldom isolated from premenarchal girls, and the prevalence of *Candida* vaginitis is lower after menopause, except in women taking hormone replacement therapy. Other factors include corticosteroid and antimicrobial therapy, the use of an intrauterine device, high frequency of coitus, and refined-sugar eating binges. An increased frequency of mycotic vulvovaginal infections in Type 2 diabetics receiving SGLT2 inhibitors especially non-albicans *Candida* spp. has been reported [28].

Vulvar pruritus is the most common symptom of VVC and is present in most symptomatic patients. Vaginal discharge is often minimal and occasionally absent. Although described as being typically 'cottage cheese-like' in character, the discharge may vary from watery to homogeneously thick. Vaginal soreness, irritation, vulvar burning, dyspareunia, and external dysuria are common. Malodorous discharge is characteristically absent. Typically, symptoms are exacerbated during the week before menses, while the onset of menstrual flow frequently brings some relief.

Examination reveals erythema and swelling of the labia and vulva, often with discrete pustulopapular peripheral lesions. The cervix is normal. Vaginal mucosal erythema with adherent whitish discharge is typically present.

In most symptomatic patients, VVC is readily diagnosed by microscopic examination of vaginal secretions. A wet mount of saline preparation has a sensitivity of only 40–60%. A 10% potassium hydroxide preparation (KOH) is more sensitive in diagnosing the presence of budding yeast. Patients with *Candida* vaginitis have a normal vaginal pH (4.0–4.5). A pH of more than 4.5 suggests bacterial vaginosis, trichomoniasis, or mixed infection. Routine cultures are unnecessary, but in suspicious cases with negative microscopy cases, vaginal culture should be performed. Although vaginal culture is the most sensitive method available for detecting *Candida*, a positive culture does not necessarily indicate that *Candida* is responsible for the vaginal symptoms. A small percentage of women experience recurrent vulvo-vaginal candidiasis, which can be disabling and severely affect quality of life. These women often require multiple courses of escalating antifungal therapy and not infrequently develop fluconazole resistance especially in *C. albicans* vaginal isolates [29].

Urinary Tract Candidiasis

Candiduria is rare in otherwise healthy people. Although epidemiologic studies have documented candiduria in approximately 10% of individuals sampled, many of these culture results reverted to negative when a clean-catch technique was used. The incidence of fungal urinary tract infections (UTIs), specifically candiduria, has dramatically increased recently, especially among patients with indwelling urinary catheters.

Platt et al. reported that 26.5% of all urinary tract infections related to indwelling catheters were caused by fungi. *Candida* are the organisms most frequently isolated from the urine samples of patients in surgical intensive care units (ICUs) and 10–15% of nosocomial UTIs are caused by *Candida* [30, 31].

Diabetes mellitus may predispose patients to candiduria by enhancing *Candida* colonization of the vulvovestibular area (in women), by enhancing urinary fungal growth in the presence of glycosuria, by lowering host resistance to invasion by fungi as a consequence of impaired phagocytic activity, and by promoting stasis of urine in those with neurogenic bladder.

Antibiotics also increase colonization of the GI tract by *Candida*, which are normally present in ~30% of immunocompetent adults. In patients receiving antibiotics colonization rates approach 100%. Candiduria is almost invariably preceded by bacteriuria. Indwelling urinary catheters serve as a portal of entry for microorganisms into the urinary drainage system. Other risk factors include the extremes of age, female sex, use of immunosuppressive agents, venous catheters, interruption of urine flow, radiation therapy, and genitourinary tuberculosis.

In a large multicenter study by Kauffman et al., *C. albicans* was found in 51.8% of 861 patients with funguria. The second most common pathogen (134 patients) was *C. glabrata* [32]. Other non-*albicans Candida* are also very common and far more prevalent than in other sites (i.e., oropharynx and vagina), possibly as a function of urine composition and pH selectivity for non-*albicans* species. In approximately 10% of patients, more than one species of *Candida* are found simultaneously.

Ascending infection is by far the most common route for infection of the bladder. It occurs more often in women because of a shorter urethra and frequent vulvovestibular colonization with *Candida* (10-35%). Ascending infection that originates in the bladder can infrequently lead to infec-

tion of the upper urinary tract, especially if vesicoureteral reflux or obstruction of urinary flow occurs. This may eventually result in acute pyelonephritis and, rarely, candidemia. A fungus ball consisting of yeast, hyphal elements, epithelial and inflammatory cells, and, sometimes, renal medullary tissue secondary to papillary necrosis may complicate ascending or descending infections.

Hematogenous spread is the most common route for renal infection (i.e., renal candidiasis). *Candida* have a tropism for the kidneys; one study revealed that 90% of patients with fatal disseminated candidiasis had renal involvement at autopsy. Frequently, when renal candidiasis is suspected, blood cultures are no longer positive. Patients with renal candidiasis usually have no urinary tract symptoms.

The finding of *Candida* organisms in the urine may represent contamination, colonization of the drainage device, or infection. Contamination of a urine specimen is common, especially with suboptimal urine collection from a catheterized patient or from a woman who has heavy yeast colonization of the vulvovestibular area. Given the capacity of yeast to grow in urine, a small number of yeast cells that migrate into the collected urine sample may multiply quickly. Therefore, high colony counts could be the result of yeast contamination or colonization. Colonization usually refers to the asymptomatic adherence and settlement of yeast, usually on drainage catheters or other foreign bodies in the urinary tract (i.e., stents and nephrostomy tubes), and it may result in a high concentration of the organisms on urine culture. Simply culturing the organism does not imply clinical significance, regardless of the concentration of organisms in the urine. In the asymptomatic patient, candiduria almost always represents colonization, and elimination of underlying risk factors, such as indwelling catheter, is frequently adequate to eradicate candiduria. Diagnostic tests on urine often are not helpful in differentiating colonization from infection or identifying site of infection.

Infection is caused by superficial or deep tissue invasion. Kozinn showed that colony counts of $>10^4$ colony-forming units (cfu)/ml of urine were associated with infection in patients without indwelling urinary catheters, although clinically significant renal candidiasis has been reported with colony counts of 10^3 cfu/ml of urine. Pyuria supports the diagnosis of infection in patients with a urinary catheter but can result from mechanical injury of the bladder mucosa by the catheter or from coexistent bacteriuria. In summary, absence of pyuria and low colony counts tend to rule out *Candida* infection, but the low specificity of pyuria and counts $>10^3$ cfu/ml require that results be interpreted in their clinical context. The number of yeast cells in urine has little value in localizing the anatomical level of infection. Rarely, a granular cast containing *Candida* hyphal elements is found in urine, allowing localization of the infection to the renal parenchyma. Declining renal function suggests urinary obstruction or renal invasion. For candiduria patients with sepsis, is it not only necessary to obtain blood cultures, but also, given the frequency with which obstruction and stasis coexist, essential to perform radiographic visualization of the upper tract. Any febrile patient for whom therapy for candiduria is considered necessary should be investigated for the anatomic source of candiduria. In contrast, patients without sepsis require no additional studies unless candiduria persists after the removal of catheters [33].

Candiduria is most often asymptomatic, usually in hospitalized or nursing home patients with indwelling catheters. These patients usually show none of the signs or symptoms associated with UTI. Symptomatic *Candida* cystitis is uncommon. Cystoscopy, although rarely indicated, reveals soft, pearly white, elevated patches with friable mucosa underneath and hyperemia of the bladder mucosa. Emphysematous cystitis is a rare complication of lower UTI, as is prostatic abscess and epididymal orchitis.

Upper UTIs present with fever, leukocytosis, and costovertebral angle tenderness, indistinguishable from bacterial pyelonephritis and urosepsis. Ascending infection almost invariably occurs in the presence of urinary obstruction and stasis, especially in patients with diabetes or nephrolithiasis.

A major complication of upper UTI is obstruction caused by fungus balls (bezoars), which can be visualized on ultrasonography. Renal colic may occur with the passage of fungal 'stones,' which are actually portions of these fungus balls.

Patients with hematogenous seeding of the kidneys caused by candidemia may present with high fever, hemodynamic instability, and variable renal insufficiency. Blood culture results are positive for *Candida* in half of these patients. Retinal or skin involvement may suggest dissemination, but candiduria and a decline in renal function are often the only clues to systemic candidiasis in a febrile, high-risk patient.

Abdominal Candidiasis, Including Peritonitis

Candida infection has been increasingly recognized as a cause of abdominal sepsis and is associated with a high mortality. Peritoneal contamination with *Candida* follows either spontaneous, traumatic, or surgical gastrointestinal or genitourinary perforation or incision. However, after contaminating the peritoneal cavity, *Candida* organisms do not inevitably result in peritonitis and clinical infection. Risk factors for peritonitis include recent or concomitant antimicrobial therapy, inoculum size, and acute pancreatitis. Translocation of *Candida* across the intact intestinal mucosa has been shown experimentally in animals and in a volunteer. Additional risk factors for invasive candidiasis include diabetes, malnutrition, ischemia, hyperalimentation, neoplasia, and multiple abdominal surgeries. Pancreatic transplan-

tation, especially with enteric drainage, is associated with intra-abdominal *Candida* abscess formation. *Candida* have a unique affinity for the inflamed pancreas, resulting in intrapancreatic abscesses or infecting accompanying pseudocysts. In *Candida* peritonitis, *Candida* usually remains localized to the peritoneal cavity, with the incidence of dissemination at about 25%.

The clinical significance of *Candida* isolated from the peritoneal cavity during or after surgery has been controversial. Several earlier studies concluded that a positive culture did not require antifungal therapy. Calandra et al., in a review of *Candida* isolates from the peritoneal cavity, determined that *Candida* caused intra-abdominal infection in 19 of 49 (39%) patients [34]. In 61% of patients, *Candida* isolation occurred without signs of peritonitis. Accordingly, in each patient, clinicians should consider the clinical signs of infection and other risk factors when deciding whether to initiate antifungal therapy.

Candida peritonitis as a complication of continuous ambulatory peritoneal dialysis (CAPD) is more common, but it infrequently results in positive blood cultures or hematogenous dissemination. In a series of CAPD patients followed for 5 years, fungal peritonitis, most commonly due to *Candida*, accounted for 7% of episodes of peritonitis. Seventeen cases of fungal peritonitis were reported, with eight associated deaths. Few risk factors have emerged except for recent hospitalization, previous episodes of peritonitis, and antibacterial therapy. Clinically, fungal peritonitis cannot be differentiated from bacterial peritonitis except by Gram stain and culture of dialysate.

Yeast in the bile is not uncommon, especially after biliary surgery, and has the same significance as asymptomatic bactibilia (i.e., colonization only), however *Candida* is an infrequent cause of cholecystitis and cholangitis. Other risk factors include diabetes, immunosuppression, abdominal malignancy, liver or pancreatic transplantation, and the use of biliary stents. Biliary infection is usually polymicrobial and *Candida* is a pathogen that should not be ignored when isolated.

Candida Osteomyelitis and Arthritis

Although previously rare, *Candida* osteomyelitis is now not uncommon, usually as the result of hematogenous dissemination, with seeding of long bones in children and the axial skeleton in adults. Sites of bone infection include the spine (vertebral and intravertebral disk), wrist, femur, humerus, and costochondral junctions.

Osteomyelitis may present weeks or months after the causal candidemic episode; therefore, at presentation, blood cultures are usually negative and radiologic findings nonspecific. Diagnosis usually requires a bone biopsy.

Occasionally, postoperative wound infections may spread to contiguous bone such as the sternum and vertebrae. Regardless of the source, manifestations resemble bacterial infection but run a more insidious course, with a significant delay in diagnosis.

Candida arthritis generally represents a complication of hematogenous candidiasis and rarely follows local trauma, surgery, or intra-articular injections. Patients with underlying joint disease (e.g., rheumatoid arthritis, prosthetic joints) are at increased risk. *Candida* arthritis can occur in any joint, is usually monoarticular (knee), but has been reported to effect multiple joints in up to 25% of cases. Infection resembles bacterial septic arthritis, but chronic infection often develops with secondary bone involvement because of the delay in diagnosis and suboptimal treatment.

Candidemia and Disseminated Candidiasis

Clinical presentation of candidemia varies from fever alone and absence of any organ-specific manifestations to a wide spectrum of manifestations, including fulminant sepsis. Accordingly, acute candidemia is indistinguishable from bacterial sepsis and septic shock. In general, there are no specific clinical features associated with individual *Candida* species.

Candidemia may also present with manifestations of systemic and invasive metastatic candidiasis, although when these occur, blood cultures have frequently become negative. Accordingly, candidemia is a marker, although insensitive, of deep invasive candidiasis. Only 50% of patients with disseminated candidiasis will have positive blood cultures, and an antemortem diagnosis is even lower (15–40%). Dissemination to multiple organs may occur with candidemia, especially to the kidney, eye, brain, myocardium, liver, and spleen in leukemia patients, but infection can also involve the lungs, skin, vertebral column, and endocardium.

The possibility of asymptomatic disseminated infection drives the treatment principles of candidemia. Transient candidemia can occur from any source but most often follows intravascular catheter infection, with prompt resolution of candidemia following catheter removal. Prolonged candidemia, especially when blood cultures remain persistently positive on appropriate antifungal treatment, suggests a persistent focus or source (e.g., intravascular catheter, abscess, suppurative thrombophlebitis, endocarditis, severe neutropenia) or antifungal resistance, which albeit rare, is more common with some of the non-*albicans Candida*. When candidemia is diagnosed, a general physical examination rarely reveals clinical signs of dissemination, but a thorough examination, including a dilated funduscopic examination, is mandatory. The crude mortality rate reported in patients with candidemia ranges from 40–60%, with an attributable mor-

tality of 38%, exceeding that of most bacteremias. A 50% reduction in national mortality rates for invasive candidiasis since 1989 was reported after a steady increase in mortality in the previous decades, reaching 0.62 death/100,000 population. The decrease in mortality, despite increased invasive disease, may be related to increased awareness, earlier diagnosis, and increased therapeutic options, primarily fluconazole and echinocandins.

Ocular Candidiasis

Candida organisms especially *C. albicans* gain access to the eye by one of two routes: direct inoculation during eye surgery or trauma, or as the result of hematogenous spread (endogenous) causing vitritis or chorioretinitis. Once endophthalmitis occurs, therapy, especially if delayed, is often insufficient to prevent blindness. Given the recent increased incidence of nosocomial candidemia, a parallel increase in endophthalmitis has occurred. Endophthalmitis should raise the suspicion of concomitant, widely disseminated candidiasis. Estimates of the incidence of eye involvement during candidemia have been as high as 37%, but recent studies indicate a reduced rate of less than 10%. Only half of patients diagnosed with endophthalmitis have a history of recent candidemia.

Symptoms of chorioretinitis vary, may be absent in patients too ill to complain, and include visual blurring, floaters, scotomata, and blindness. Funduscopic examination reveals white, cotton ball-like lesions situated in the chorioretinal layer that may rapidly progress to extend into the posterior vitreous. Indirect ophthalmoscopy with pupillary dilation is necessary to achieve complete visualization. For the lesions to be visible, they require the presence of leukocytes; thus, in the presence of neutropenia, ocular lesions may be absent.

Cardiac and Endovascular Candidiasis

Candida myocarditis is the result of hematogenous dissemination with formation of microabscesses within the myocardium usually detected only on autopsy. Studies in the pre-antifungal drug era reported that 62% of 50 patients with disseminated candidiasis had myocardial involvement at autopsy.

Candida may reach the pericardium from adjacent endocarditis or myocarditis, but pericardial involvement is most often the result of hematogenous seeding or direct inoculation during cardiac surgery. Pericarditis is purulent in nature, resembles bacterial infection, and may be complicated by constrictive pericarditis.

The advent of prosthetic cardiac valve replacement surgery and the increase in IV drug abuse have resulted in a dramatic increase in the incidence of *Candida* endocarditis, which previously had been rare. Fungal endocarditis is responsible for <10% of all cases of infective endocarditis.

Endocarditis following prosthetic valve surgery (PVE) remains the most common form of *Candida* endocarditis (>50%). Most episodes occur within 2 months of surgery, though endocarditis can also occur much later (>12 months). Specific risk factors for PVE include complicated surgery, antibiotics, prolonged postoperative use of catheters, and candidemia, even if transient. Non-*albicans Candida* species are increasingly responsible for prosthetic valve endocarditis, especially *C. parapsilosis*. Damaged endocardium and prosthetic material, especially suture lines, serve as foci for *Candida* adherence. Pacemaker-associated endocarditis from *Candida* has also been reported.

Clinical findings and complications in *Candida* endocarditis are similar to those seen in bacterial endocarditis, with the exceptions of increased frequency of large vegetations and large emboli to major vessels. Aortic and mitral valve involvement is the most common. The higher incidence of embolization is frequently manifested as focal and global neurologic deficits. Some studies have found a reduced incidence of cardiac failure, changing heart murmurs, and splenomegaly. Prosthetic valve endocarditis may recur several years after a putative cure with medical therapy, so long-term follow up is necessary.

Most patients with *Candida* endocarditis have positive blood cultures. Improved diagnosis is the result of greater awareness of the significance of candidemia, newer blood culture techniques, and echocardiography. Visualizing large vegetations via echocardiogram in patients with negative blood culture is strong circumstantial evidence of *Candida* endocarditis. Mycologic examination including culture and histopathology should be performed on all surgically removed emboli.

Candida endocarditis mortality remains high. Before cardiac surgery was available, mortality exceeded 90%. With combined treatment using surgery and aggressive antifungal therapy, mortality rates of ~45% are now typical.

Phlebitis due to *Candida* is common and often associated with subcutaneous catheters. Delay in treatment often results in extensive vascular thrombosis and suppuration. Prolonged candidemia, despite adequate antifungal treatment, is not uncommon. Venous thrombi, even after removal of responsible catheters, impair drug penetration and contain persistent microabscesses, with resultant prolonged candidemia. Surgical excision of thrombi is often required in addition to prolonged antifungal therapy. Complications include superior vena cava obstruction, tricuspid valve endocarditis, right-sided mural endocarditis, and pulmonary vein thrombosis.

Central Nervous Systemic (CNS) Candidiasis

CNS candidal infections usually occur as a manifestation of acute disseminated candidiasis or as a complication of a neurosurgical procedure especially involving an intracranial device. The most common presentation is that of meningitis or multiple small abscesses throughout the brain; however, large solitary brain and epidural abscesses have been described. CNS involvement is more commonly seen at the extremes of life; low birth weight neonates are particular vulnerable to metastatic CNS involvement secondary to candidemia.

Chronic Disseminated Candidiasis (CDC)

Hepatosplenic candidiasis is a chronic form of disseminated candidiasis that develops as a complication of invasive candidiasis during granulocytopenia. Many now prefer the term chronic systemic or disseminated candidiasis, because other organs (e.g., eyes, skin, and soft tissue) may also be involved [35]. In the last 2 decades, reports of HSC have increased, probably as a result of improved diagnostic imaging and increased rates of candidemia. Candidemia, although frequently secondary to intravascular catheter infection, generally follows *Candida* colonization of the gut, together with disruption of the GI mucosa, organism reach the submucosal blood vessels that drain into the portal venous system and into the liver, where focal lesions are established. Thus, many patients with chronic disseminated candidiasis have no history of documented candidemia. As patients recover from neutropenia, the lesions that were established during the neutropenic phase become more prominent, especially in the liver, spleen, and kidneys.

Clinically, most patients have a history of a hematologic malignancy, cytotoxic chemotherapy, or recent recovery from neutropenia, during which time they were febrile and received antibacterial therapy. Upon recovery from neutropenia, symptoms of antibiotic-resistant fever and abdominal pain begin and worsen as the neutrophils infiltrate foci of *Candida* in the liver and spleen. Serum alkaline phosphatase increases, paralleling the increase in leukocytes, although hepatic transaminases are not invariably elevated.

Diagnosis

In superficial candidiasis, wet mount smears use scrapings or smears from skin, nails, oral or vaginal mucosa examined under the microscope to identify hyphae, pseudohyphae, or budding yeast cells. Potassium hydroxide smear, gram stain, or methylene blue stain may help directly demonstrate fungal cells. For diagnosis of invasive candidiasis, laboratory studies are nonspecific and lack sensitivity. Clinicians are required to act definitively based on a high index of suspicion. In the past, many patients with life-threatening candidiasis died without receiving antifungal therapy. For therapy to be effective, clinicians must act early, often empirically in patients who are febrile and at risk.

In candidemia and disseminated candidiasis, blood cultures are helpful, but they are positive in only 40–60% of cases of disseminated disease. Urinalysis may be helpful and may be indicative of colonization or renal candidiasis. Nonculture, antigen-based diagnostic assays are not available in the United States. Occasionally, blood cultures obtained via central catheters may indicate contamination. Nevertheless, febrile patients with a single positive blood culture for *Candida* should *always* initially be considered to have a proven infection. Given the low sensitivity of blood cultures, as well as the lack of an adequate test for the diagnosis of invasive candidiasis, detection of hematogenous dissemination remains poor.

Cultures of nonsterile sites, although not useful in establishing a diagnosis, may demonstrate high degrees of candidal colonization. This may be useful in deciding whether to initiate antifungal therapy in patients with fever unresponsive to broad-spectrum antimicrobials. Positive blood cultures and cultures from sterile sites, on the other hand, are indicative of definite infection using established international definitions of EORTC/MSG organization [36]. Lesions of chronic disseminated candidiasis (CDC) may be detected by imaging techniques such as CT scan, ultrasonography, and magnetic resonance imaging. The characteristic "bull's-eye" lesions seen on ultrasound and/or CT are not detectable until neutrophil recovery. However, the lesions are not specific for CDC as they resolve during therapy: they may either disappear completely or calcify. Ultrasonography appears to be less sensitive but possibly more specific than CT scanning in demonstrating these lesions; however, MRI appears to be the most sensitive imaging tool. Diagnosis may be confirmed by histopathologic examination and culture of hepatic tissue obtained by either percutaneous biopsy or laparoscopy. The appearance of hyphae in a granulomatous lesion is itself not specific for *Candida* and may be caused by other fungi such as *Trichosporon*, *Fusarium*, and *Aspergillus*. Additionally, metastatic tumors may simulate the appearance of CDC. Often biopsy visible organisms do not grow in culture, leading some investigators to propose that CDC might reflect immune reconstitution only. Diagnosis of *Candida* endophthalmitis is usually made on the basis of clinical context and characteristic funduscopic picture. Aspiration of the anterior chamber is justified, but often culture-negative; vitrectomy is often helpful. PCR studies on the aspirate may prove the presence of *Candida*.

Species identification of *Candida* is critically important because of the increase in non-*albicans Candida* infections.

CHROMagar *Candida* media allows for the presumptive identification of several *Candida* species by using enzymatic reactions in specialized media that produce differing colony colors. Several biochemical assays, usually based upon fermentation reactions, can be used to identify the different *Candida* species with more accuracy. Assays that evaluate the assimilation of a number of carbon substrates and generate profiles are used in the identification of different fungal species. Once colonies are present on an agar plate or blood culture growth is recognized, significantly more rapid identification of *Candida* species taking only two hours is now possible using peptide nucleic acid fluorescence in situ hybridization (PNA Fish) allowing more rapid acid fluorescence in situ hybridization (PNA Fish) allowing more rapid administration of appropriate antifungals.

Serum levels of beta-1,3-glucan are useful as an aid to the diagnosis of invasive candidiasis but are not widely used or always timely available. Sensitivity for *Candida* infections of greater than 80% has been reported. The test often provides a positive test days before clinical signs and symptoms appear, allowing earlier initiation of therapy [37]. A negative test seems to have good negative predictive value. False-positive results may occur with antibiotics, surgical gauze, hemodialysis, mucosal disruption, or use of IV immunoglobulins. Although not commercially available, homegrown PCR tests may be useful in febrile patients, culture negative with disseminated or invasive candidiasis [38]. More recently, automated molecular diagnosis test, coupled with T2 magnetic resonance detection directly from blood, has been shown to be sensitive and specific when compared to blood cultures, with faster times of identification down to the species level, at least for the five most common *Candida* species [38, 39].

EUCAST and CLSI microbroth dilution methodologies have standardized reference antifungal susceptibility testing for *Candida* species and several companies are now offering automated methods that correlate well with the reference standards. Antifungal susceptibility testing has become the standard of care for isolates from blood and sterile sites, as antifungal resistance to azoles and echinocandins is becoming more prevalent and more clinically relevant [40].

Management

Treatment of *Candida* infections varies considerably and is based on the anatomic location of the infection, the patient's underlying disease and immune status, the patient's risk factors for infection as well as the species of *Candida* responsible for infection, and, in some cases, the susceptibility of the strain to antifungal drugs (Table 9.1). In 2016, the Infectious Diseases Society of America published updated practice guidelines for the treatment of candidiasis [27].

Although azoles have traditionally been the mainstay of therapy for superficial infections for many years, including many topical and systemic agents. Polyenes include amphotericin B, lipid-based amphotericin B formulations, and topical nystatin. The echinocandin class of antifungals has excellent fungicidal activity against *Candida* species, and has become the cornerstone of therapy for invasive disease and this class has been associated with decreased mortality [41].

Cutaneous Candidiasis

Most localized, cutaneous candidiasis infections can be treated with topical antifungal agents, such as clotrimazole, econazole, ciclopirox, miconazole, ketoconazole, and nystatin. If the infection is a paronychium, the most important aspect of the therapy is drainage of the abscess, followed by oral antifungal therapy with either fluconazole or itraconazole. In cases of extensive cutaneous infections, infections in immunocompromised patients, folliculitis, or onychomycosis, systemic antifungal therapy is recommended. For *Candida* onychomycosis, oral itraconazole appears to be the most efficacious of azoles. Two treatment regimens are available: a single daily dose of itraconazole taken for 3–6 months or a pulsed-dose regimen that requires a slightly higher dose daily for 7 days, followed by 3 weeks off therapy. The cycle is repeated every month for 3–6 months. Also effective and well tolerated is terbinafine 250 mg daily for 6 weeks.

Gastrointestinal Candidiasis

Oropharyngeal candidiasis may be treated with topical antifungal agents (e.g., nystatin, clotrimazole, miconazole, amphotericin B oral suspension) or with systemic oral azoles (e.g., fluconazole, posaconazole, itraconazole) (Table 9.3).

Candida esophagitis requires systemic therapy, usually with fluconazole for at least 14–21 days. Parenteral therapy with fluconazole may be required initially if the patient is unable to take oral medications. Daily suppressive antifungal therapy with fluconazole 100–200 mg/day is effective in preventing recurrent episodes, but it should only be used if the recurrences become frequent or are associated with malnutrition from poor oral intake and wasting syndrome. In patients with advanced AIDS and severe immunodeficiency, recurrent candidal esophagitis due to fluconazole-resistant *C. albicans* or *C. glabrata* can be treated with voriconazole, posaconazole or any of the echinocandins or AmB [27].

Table 9.3 Treatment options for oropharyngeal and esophageal candidiasis

Drug/formulation	Dose	Comments
Nystatin		
Pastilles or lozenge	200,000 U qid[a]	Unpleasant taste; may cause nausea and gastrointestinal disturbances
Suspension	500,000 U by swish and swallow qid	
Vaginal tablet	100,000 U dissolve 1 tablet tid	Vaginal tablets in combination with unsweetened mints or chewing gum better tolerated; not recommended for esophagitis
Clotrimazole troches	Dissolve in mouth 5 times each day	More palatable than nystatin but contains dextrose, which may promote dental caries; not recommended for esophagitis
Miconazole mucoadhesive buccal tablet	50 mg, apply to gum once daily	Adheres to gum above incisor tooth; convenient once daily but expensive
Fluconazole Oral suspension or tablet	100 mg/day; loading dose of 200 mg for severe disease	Superior to nystatin, clotrimazole, ketoconazole. High doses (up to 800 mg/day) can be used in difficult cases. Success has been obtained even in cases with in vitro resistance
Itraconazole		
Solution	200 mg (20 ml) by swish and swallow daily	Solution has been tested only among HIV patients, but is much better absorbed and has shown efficacy equivalent to that of fluconazole
Capsule	200 mg/d (with food) × 14–28 days	Limited bioavailability; absorption improved if taken with fatty meal
Posaconazole tablet	400 mg bid, then 400 mg daily. Esophagitis 400 mg bid Delayed-release tablet 300 mg daily	Oral solution availability but tablet preferred
Isavuconazole tablet	400mg loading dose, then 100mg daily for 14–28 days or 400 mg once weekly for 4 weeks	Noninferior to fluconazole, unclear if it has any advantages
Amphotericin B		
Suspension	1 ml (1 mg/ml) swish and swallow qid	Agent considered second-line option; reserved for severe cases and documented failures to azoles; parenteral dosing necessary for esophagitis
Lozenge	100 mg qid	
Tablet	10 mg qid	
Parenteral	0.4–0.6 mg/kg/day IV	

U units, *qid* four times daily, *tid* three times daily, *bid* two times daily, *IV* intravenously

[a] All given for 7–14 days for oropharyngeal candidiasis and up to 21 days for esophageal candidiasis orally, unless otherwise stated

Genital Tract Candidiasis

Vulvovaginal candidiasis can be managed with either topical antifungal agents or single-dose oral fluconazole in uncomplicated infections (Table 9.4). Single-dose (150 mg) oral fluconazole is the preferred method of treatment and typically preferred by women. This therapy has been shown to have clinical and microbiologic efficacy as good as that of topical antifungal agents.

A small percentage of women (5–7%) suffer from recurrent VVC infections, which often require chronic or prophylactic oral azole therapy for control. In women who suffer from recurrent attacks, the recommended regimen is fluconazole at a dose of 150 mg every third day for 3 doses, followed by weekly fluconazole at a dose of 150 mg for 6 months. This regimen prevents recurrent infections in more than 90% of women although symptomatic recurrence is common following cessation of maintenance suppressive prophylaxis [42]. Recently, a new antifungal called ibrexafungerp has been approved for the treatment of VVC and it will likely play a key role in recurrent infections and in the setting of azole resistance [43].

Urinary Tract Candidiasis (Candiduria)

Asymptomatic candiduria in urinary catheterized patients is extremely common and most commonly reflects yeast colonization of the catheter and lower urinary tract; hence, no antifungal therapy is indicated. Treatment for asymptomatic candiduria should only be considered in a high risk for dissemination subgroup including infants with very low birth weight, neutropenic subjects and patients due to undergo urologic manipulation. Symptomatic candiduria reflects deep tissue or parenchymal invasion and results in organ specific as well as constitutional symptoms (e.g., fever, frequency, dysuria (lower urinary tract) or fever, renal angle pain, nausea, vomiting, and even sepsis (pyelonephritis). While amphotericin B IV has been the mainstay of indicated therapy, accompanying drug nephrotoxicity limits its use.

Table 9.4 Azole therapy for vaginal candidiasis

Drug	Formulation	Dosage
Butoconazole	2% cream 2% vaginal suppository	5 g × 3 days (single dose) 1 suppository (5 g) once daily × 7–14 days
Clotrimazole	1% cream 10% cream 100 mg vaginal tablet 100 mg vaginal tablet 500 mg vaginal tablet	5 g × 7–14 days 5 g single application 1 tablet × 7 days 2 tablets × 3 days 1 tablet once
Econazole	150 mg vaginal tablet	1 tablet × 3 days
Fenticonazole	2% cream	5 g × 7 days
Miconazole	2% cream 100 mg vaginal suppository 200 mg vaginal suppository 1,200 mg vaginal suppository	5 g × 7 days 1 suppository × 7 days 1 suppository × 3 days 1 suppository once
Tioconazole	2% cream 6.5% cream	5 g × 3 days 5 g single dose
Terconazole	0.4% cream 0.8% cream 80 mg vaginal suppository	5 g × 7 days 5 g × 3 days 80 mg × 3 days
Fluconazole	Oral tablet	150 mg single dose
Itraconazole	100 mg tablet	200 mg × 3 days

Fluconazole 200–400 mg, daily for two weeks, achieves high urinary concentrations and has emerged as the drug of first choice with small dose adjustments required for coexistent renal insufficiency. None of the other azoles, including voriconazole, are excreted in urine. Similarly, the echinocandins and lipid formulations of amphotericin B achieve subtherapeutic urine concentrations. A useful agent for eradicating non-*albicans* candiduria, especially *C. glabrata*, is oral flucytosine in the absence of renal failure. Irrigation of the bladder with amphotericin B resolves candiduria in 80–90% of patients with disease confined to bladder especially in infections caused by non-*albicans Candida* spp. Deep tissue invasion of kidneys or bladder can be treated by all the systemically active antifungals [44].

Candidemia and Acute Disseminated Candidiasis

Candidemia requires treatment in all patients (Tables 9.5 and 9.6) and is related to the presence of an intravascular catheter in up to 80% of nonneutropenic patients. Removal of intravascular catheters shortens the duration of candidemia and has been associated with reduced mortality [41]. Although some patients have been cured by catheter removal alone, even transient episodes of candidemia can be associated with hematogenous spread and subsequent diagnosis of endophthalmitis or osteomyelitis. Thus, all episodes of candidemia mandate antifungal therapy. A dilated retinal examination is important in all candidemic patients.

Table 9.5 Dosing for drugs used for first-line therapy for candidemia and invasive candidiasis

Polyenes
– Amphotericin B deoxycholate 0.5–0.7 mg/kg IV daily
– Liposomal amphotericin B 3–5 mg/kg IV daily
– Amphotericin B lipid complex 3–5 mg/kg IV daily
Azoles
– Fluconazole 400–800 mg IV daily
– Voriconazole 6 mg/kg IV q12h × 2 doses, followed by 3 mg/kg IV q12h
Echinocandins
– Caspofungin 70 mg IV × 1 dose, followed by 50 mg IV daily
– Micafungin 100 mg IV daily
– Anidulafungin 200 mg IV × 1 dose, followed by 100 mg IV daily

IV intravenously, *PO* by mouth, *q12h* every 12 h

Table 9.6 Management of candidemia and disseminated candidiasis

Situation	Drugs of choice	Comments
First-line therapy	Caspofungin Micafungin Anidulafungin	Echinocandins are associated with decreased mortality as initial therapy when compared to other drug classes
Alternative therapy	Lipid-based amphotericin B Fluconazole Voriconazole	Lipid-based amphotericin B may be preferred in severe or refectory disease Voriconazole preferred for ocular involvement Fluconazole may be used as initial therapy in stable patients in resource-limited settings or for susceptible isolates
Step down options	Fluconazole Voriconazole	Step-down therapy usually done on day 3–5 after echinocandin initiation for stable patients with susceptible isolates

Antifungal therapy has progressed dramatically from the period when only desoxycholate amphotericin B (AmB) was available and clinicians now have a wide choice of antifungals. AmB was previously the standard drug for candidemia or invasive candidiasis. However, in spite of broad-spectrum activity against most *Candida* species except *C. lusitaniae*, its significant nephrotoxicity dramatically reduces its use. Lipid formulations of AmB have almost entirely replaced the desoxycholate formulation. However, these agents are expensive and, although less toxic, are not therapeutically superior.

Fluconazole, a triazole and antifungal, has been widely used for all forms of candidiasis for almost two decades and has an excellent safety profile. Several large studies have shown equivalent activity and success when compared with polyene agents and echinocandins in nonneutropenic hosts [41]. Voriconazole has superior in vitro activity than fluconazole and is active against fluconazole-resistant *C. krusei* and

some but not all (<50%) *C. glabrata* strains. IV followed by convenient oral voriconazole was shown to be equivalent or noninferior to AmB followed by fluconazole in the treatment of candidemia and invasive candidiasis. Nevertheless, evidence of superiority of voriconazole over fluconazole clinically has not been forthcoming. Its additional costs and less favorable toxicity profile have resulted in voriconazole being reserved for selected patients with fluconazole-resistant but voriconazole-sensitive *Candida* species. In a recent randomized controlled trial, isavuconazole failed to meet the noninferiority endpoint when compared to caspofungin; therefore, it is not recommended, at least for initial therapy of candidemia or invasive candidiasis [27, 41].

Echinocandins are noncompetitive inhibitors of synthesis of beta-1,3-glucan, a major component of fungal cell wall. The class includes caspofungin, anidulafungin, and micafungin. All 3 drugs have excellent activity against all *Candida* species and are considered therapeutically equivalent. A new long active echinocandin, rezafungin, is under clinical development and is expected to be dosed once weekly [45]. Echinocandins have become the initial therapy of choice for invasive candidiasis due to a mortality benefit described in a patient-level meta-analysis [41]. Although drug concentrations of echinocandins (MICs) *in vitro* required to inhibit *C. parapsilosis* are slightly higher than for other *Candida* species (1–2 μg/mL), clinical efficacy is comparable and resistant organisms are extremely rare. On the other hand, recent studies identified acquired resistance to all agents in this class to be increasing among *C. glabrata* isolates especially those with fluconazole resistance although this phenomenon is still uncommon. Prevalence of fluconazole resistance (7% of all *Candida* spp.) remains unchanged. As discussed, some strains of *C. auris* exhibit echinocandin resistance [4].

Antifungal therapy should be started in patients who are critically ill and have risk factors for systemic candidiasis after other causes of fever have been excluded. First-line choices are an echinocandin or fluconazole; however, if *C. krusei* or *C. glabrata* is suspected or documented, an echinocandin is recommended. Similarly, this class is advised in critically ill, unstable patients and in patients who have had exposure to an azole. Recommended alternatives are a lipid formulation [27].

In the presence of neutropenia, a slightly different order of preference is recommended. This is because febrile neutropenic patients are usually already receiving oral prophylactic azole drugs, and because of the higher risk of molds, less susceptible to azoles. Accordingly, initial antifungal recommended includes either AmB/lipid formulation AmB or an echinocandin.

Combinations of either fluconazole or amphotericin B with flucytosine at 100–150 mg/kg/day may be useful in some patients, but the precise role of this combination is unclear. The required duration of antifungal therapy is undetermined, but therapy is usually continued for about 2 weeks after the last positive blood culture [27]. With this approach, the rate of subsequent recurrent infection at a hematogenously seeded site is about 1%. For *Candida* endophthalmitis or complicated candidemia with metastatic *Candida* infection, duration of treatment should be at least 4–6 weeks.

It appears likely that the GI tract is the most common source of candidemia in neutropenic patients. In these patients, removal of intravenous catheters is still important. This applies particularly to *C. parapsilosis* fungemia, which is highly associated with intravascular catheters in cancer patients. Patients with refractory or highly invasive disease, particularly of the CNS, bone, or heart, will most likely benefit from a combination of a lipid formulation of amphotericin B and flucytosine [27].

Patients may develop candidemia while receiving antifungal therapy, including prophylactic antifungals. Such breakthrough candidemia may be the result of an infected unremoved intravascular catheter. In cancer patients, breakthrough candidemia has been associated with a higher mortality and has occurred more often during an ICU stay, during prolonged neutropenia, and with the use of corticosteroids. In this setting, immunosuppression should be reduced and factors that might alter antifungal drug delivery or clearance excluded. Intravenous catheters should be changed and the possibility of drug resistance considered, especially since non-*albicans Candida* are frequently responsible. Antifungal drug susceptibility tests should be performed and therapy should be changed to an antifungal of a different class.

Crude (30–40%) and attributable mortality rates in patients with candidemia and invasive candidiasis remains unacceptable high. *C. parapsilosis* has lowest mortality rate (20–25%) and *C. krusei*, followed by *C. glabrata* that has highest crude mortality rate. Risk factors for increased mortality include neutropenia, high APACHE scores, delay in institution of appropriate antifungal therapy, infection with *C. tropicalis*, and failure to remove IV catheters in ICU units [15, 41].

Central tunneled catheters in febrile neutropenic patients do not require mandatory removal, because alternate vascular access sites are less available, removal is more difficult, and, most importantly, such catheters are less likely to be the source of candidemia, although they may become infected secondarily to bloodstream infection. Occasionally, these valuable access sites can be salvaged using the controversial antibiotic lock method using amphotericin B, but results are unpredictable.

Infections involving chorioretinal layer are more easily treated, because they are highly vascular and systemic antifungal agents reach adequate concentrations with these structures; however, sight-threatening lesions near the mac-

ula and invasion into the vitreous usually necessitate intravitreal injection of antifungal agents, with or without vitrectomy in addition to systemic antifungal agents, such as lipid formulations of amphotericin B or voriconazole [46].

Chronic Disseminated Candidiasis

Therapy of chronic disseminated candidiasis traditionally consists of prolonged therapy with amphotericin B alone, especially in acutely ill patients, but this approach has not been uniformly successful. Amphotericin B 0.5–1.0 g, followed by a prolonged course of fluconazole 200–400 mg/day for 2–14 months, is associated with cure rates of greater than 90%. Use of oral fluconazole is also recommended 400 mg (6 mg/kg) daily for clinically stable patients. Lipid-based amphotericin B has also been used successfully. For azole-resistant *Candida* species, an echinocandin or lipid formulation of amphotericin B is recommended. If the lesions have stabilized and the patient is clinically improved, antineoplastic therapies (including those that induce neutropenia) may be restarted, while antifungal therapy is continued. The duration of antifungal therapy is determined by imaging studies of the liver and spleen. Use of corticosteroids is controversial.

Prevention

Prophylaxis of Candidiasis in Transplant Patients

Invasive candidal infections are a concern in these high-risk groups. Institutions with recipients of solid organ and bone marrow transplants usually consider prophylaxis with fluconazole for the prevention of candidiasis in selected patients only. Fluconazole is generally started 1 day before neutropenia, and although controversial, some investigators support its use for 75–100 days after bone marrow transplantation. In liver transplants, short-term (30 days) fluconazole prophylaxis is indicated in selected high-risk patients [27].

Prophylaxis of Superficial Candidiasis in HIV-Positive Patients

There is little support for primary or secondary prevention of OPC, esophageal candidiasis, or vaginal candidiasis in HIV-positive patients. Concern about potential development of resistance or colonization by resistant species or strains of *Candida* exists. Prophylaxis may be indicated in a select group of patients with recurrent episodes of symptomatic candidiasis only [27].

Empirical Anticandida Treatment

Empirical use of antifungal agents in febrile patients in ICUs is widely used without data to support its use. Given the existent difficulties in diagnosing invasive candidiasis, it appears reasonable to recommend empirical antifungal therapy in selected febrile, high-risk patients with persistent antibiotic-resistant fever [27].

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Infections Due to Non-Candidal Yeasts

10

Rhonda E. Colombo and Jose A. Vazquez

Introduction

Yeasts exist throughout nature in association with soil, plants, mammals, fish, and insects. For that reason, humans are constantly exposed to many yeast genera through varying routes. Depending on the interaction between host defense mechanisms and fungal virulence factors, the association between yeast and humans can be either transient or persistent, and can be associated with colonization, local infection, or systemic disease. Most yeast organisms are of low virulence and generally require significant alterations or reductions in host defenses prior to tissue invasion. Recently, however, because of the increased population of immunocompromised patients, the frequency of invasive infections due to yeasts as well as the number of organisms causing disease continues to grow [1–6] (Table 10.1).

Table 10.1 Yeasts other than *Candida* and *Cryptococcus* that occasionally cause human infection

- *Trichosporon*
  - *T. asahii (T. beigelii)*
  - *T. inkin*
  - *T. mucoides*
- *Saccharomyces*
  - *S. cerevisiae*
  - *S. boulardii*
- *Rhodotorula*
  - *R. mucilaginosa (rubra)*
  - *R. glutinis*
- *Malassezia*
  - *M. furfur*
  - *M. pachydermatis*
- *Saprochaete*
- *S. capitata (Blastoschizomyces capitatus,Geotrichum capitatum, Trichosporon capitatum)*
- *S. clavata (Geotrichum clavatum)*
- *Sporobolomyces*
  - *S. salmonicolor*
  - *S. holsaticus*
- *Geotrichum*
  - *G. candidum*
  - *Kodamaea*
  - *Kodamae ohmeri (Pichia ohmeri)*

Trichosporon

Trichosporon asahii was first described in 1865 by Beigel, who identified it as the causative agent of hair infections [7]. Infections due to *Trichosporon* may be classified as superficial or deep. Disseminated infections have been increasingly recognized in the compromised host over the past few decades and are frequently fatal [1–6]. One of the first reported cases of disseminated disease was described in a 39 year-old woman with lung cancer who subsequently developed a brain abscess [8].

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_10

Etiologic Agents

The genus *Trichosporon* was first reported by Behrend [9]. Gueho and colleagues have suggested that the species known as *T. asahii* may include several different *Trichosporon* species with epidemiological and pathogenic differences [10]. Kemker et al., using isoenzyme delineation and PCR DNA fingerprinting, suggested that strains that produce superficial infections are distinctly different than those strains that produce invasive infection [11]. Molecular sequencing has since led to significant evolution in fungal taxonomy, including reassignment of organisms previously characterized as *Trichosporon* to other species [12, 13]. There are currently 12 species of *Trichosporon* [14]. These include *T. asahii* (formerly, *T. beigelii*), the most frequently recovered species from invasive infections, *T. inkin*, *T. faecale*, and *T. coremiiforme*, among others [1, 2, 13–16]. *T. asteroides* generally produces superficial skin infections, while *T. ovoides* generally causes white piedra of the scalp and *T. inkin*, white piedra of the pubic hair. *Trichosporon capitatum* is now known as *Magnusiomyces capitatus*, although it has also previously been referred to as *Blastoschizomyces capitatus* and *Geotrichum capitatum* [11–17]. In addition, *Trichosporon dermatis*, *Trichosporon cutaneum*, and *Trichosporon mucoides* have been reclassified as *Cutaneotrichosporon dermatis, Cutaneotrichosporon cutaneum*, and *Cutaneotrichosporon mucoides*, respectively.

Trichosporon species are characterized by true hyphae, pseudohyphae, arthroconidia, and blastoconidia [10, 18] (Fig. 10.1). *T. asahii* grows readily on Sabouraud dextrose agar, producing smooth, shiny gray to cream-colored yeast-like colonies with cerebriform radiating furrows that become dry and membranous with age [19]. Colonies are pale blue on CHROMagar [19]. The presence of arthroconidia and the ability to hydrolyze urea supports presumptive identification of *Trichosporon* species. All *Trichosporon* species are easily identified using commercially available carbohydrate assimilation assays. MALDI-TOF mass spectrometry with an extended database may be useful for identifying *Trichosporon* species. Molecular diagnostics, particularly intergenic spacer 1 (IGS) rDNA sequencing, facilitate definitive identification of *Trichosporon* isolates to the species level [13, 19, 20].

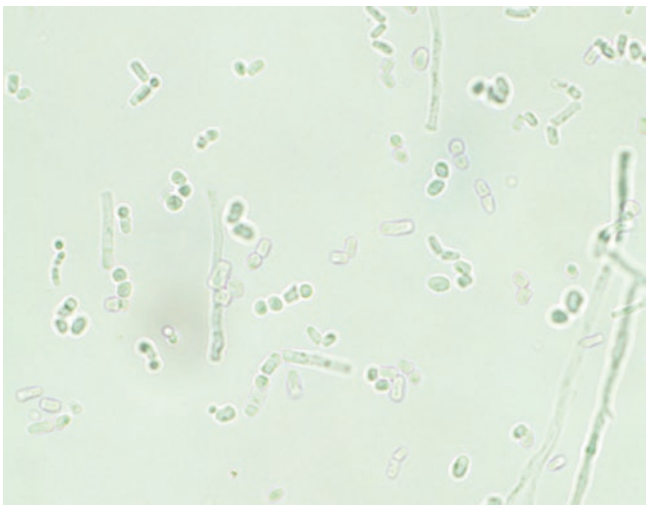


Fig. 10.1 *Trichosporon* species produce yeast-like colonies in culture and are unique in their production of hyphae, pseudohyphae, arthroconidia, and blastoconidia (budding) both in culture and in disease. Lactophenol blue preparation

Epidemiology

T. asahii is generally found in the soil, but may also be recovered from air, rivers and lakes, sewage, and bird droppings [1, 2, 12–15]. It rarely colonizes the inanimate environment, but can colonize the mucosal surfaces of the oropharynx, the lower gastrointestinal tract, and the skin of humans [11, 12, 15–19].

More than 500 cases of severe infection due to *Trichosporon* species have been reported; of these, approximately 200 fulfill criteria for a diagnosis of proven or probable invasive infection [20]. *T. asahii* was the causative pathogen in the majority of these cases [1, 2, 12–19]. The major risk factors associated with infection include hematologic malignancies (acute leukemia, chronic leukemia, multiple myeloma), solid tumors, and neutropenia [1, 2, 12–20]. In non-neoplastic, non-neutropenic cases, the major risk factors include corticosteroids, prosthetic valve replacement, solid organ transplantation, chronic active hepatitis, and occasionally intravenous drug use (Table 10.2). The most common portal of entry appears to be the either the respiratory or gastrointestinal tracts. Central venous catheters and other indwelling medical devices such as peritoneal dialysis catheters have also been implicated [12–22].

Table 10.2 Risk factors associated with *Trichosporon* infection

- Hematologic malignancy
- Solid organ transplantation (kidney, heart, liver)
- Neutropenia
- Broad-spectrum antibiotics
- Corticosteroids
- Use of intravenous lipids
- Bone marrow transplant
- Chronic active hepatitis
- Injection (IV) drug use (IVDU)
- Central venous catheters
- Continuous ambulatory peritoneal dialysis (CAPD)
- Burns

Clinical Manifestations

Trichosporonosis is classified into superficial infections (white piedra [hair shaft infection], onychomycosis, and otomycosis) and invasive infections.

Deep tissue infections may involve either a single organ or multiple organs. The most commonly infected tissue is the lungs, which account for approximately 33% of all localized deep tissue infections [1, 2, 12–19]. Other sites of infection may include the peritoneum, heart valves (natural and prosthetic), retina, liver, spleen, kidneys, gallbladder, and central nervous system (brain abscess and chronic fungal meningitis) [1, 2, 11–26].

The signs and symptoms of disseminated infection resemble those of systemic candidiasis and include fungemia with associated organ infection. Moreover, disseminated infections may present as either acute or chronic disease. Acute disseminated trichosporonosis often has a sudden onset and progresses rapidly, especially in neutropenic patients. Patients may develop skin lesions (~33%), pulmonary infiltrates (~30–60%), renal and ocular involvement [19, 20]. The metastatic cutaneous lesions generally begin as an erythematous rash with raised papules on the trunk and the extremities. The rash eventually evolves into macronodular lesion, followed by central necrosis of the nodules and occasionally formation of hemorrhagic bullae. The pulmonary infiltrates may present as lobar consolidations, bronchopneumonia, or reticulonodular patterns.

Renal involvement occurs in >75% of the disseminated infection cases. Renal disease may manifest as proteinuria, hematuria, and RBC casts, with either acute renal failure or acute glomerulonephritis [15–17, 19–22]. Urine cultures positive for *Trichosporon* suggest disseminated disease, especially in immunocompromised patients [26].

Chorioretinitis is not uncommon in disseminated infection and may be a cause of visual alterations due to retinal vein occlusion or retinal detachment [15–20, 22]. For unexplained reasons, *Trichosporon* has been found to have tropism for the choroid and retina. However, unlike candidal endophthalmitis, *Trichosporon* infects uveal tissues, including the iris, but spares the vitreous [27].

During disseminated infection, any tissue in the body may become infected. The organs most frequently include the lung, liver, spleen, gastrointestinal tract, lymph nodes, myocardium, bone marrow, pleura, brain, eye, adrenal gland, and thyroid gland [1, 2, 15–29].

In chronic disseminated infection, subtle manifestations may be present for several weeks and frequently include persistent fever of unknown etiology [1, 2, 15–19]. The infection is similar to the entity known as chronic disseminated (hepatosplenic) candidiasis. It is generally a chronic infection of the liver, spleen, and other tissues after recovery from neutropenia. Laboratory studies frequently reveal an elevated alkaline phosphatase. CT scan or MRI frequently reveals hepatic or splenic lesions compatible with abscesses. A tissue biopsy is needed to confirm the diagnosis.

Diagnosis

The diagnosis is made with histologic examination and culture of affected tissue obtained from biopsy of the skin or involved organs. Blood cultures may occasionally be useful in deep tissue infection, but are positive only late in the course of infection. *Trichosporon* grows readily in conventional blood culture and on standard fungal media, including Sabouraud dextrose agar [18, 30]. The presence of *Trichosporon* in the urine of a high-risk patient should increase the suspicion of disseminated infection.

Although there are no standardized serologic assays, the serum latex agglutination test for *C. neoformans* may be positive. A potential usefulness of this assay has been postulated based on the report of positive serum latex agglutination test for *C. neoformans* in several patients with disseminated *Trichosporon* infection [31, 32]. Although not specific for *Trichosporon*, β-D-glucan may also be detected in the serum of patients with disseminated *Trichosporon* infection. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using a qualified database containing the majority of clinically significant *Trichosporon* spp. can provide rapid, accurate identification of *Trichosporon* isolates [33–35]. Where available, panfungal PCR assays with sequencing of intergenic spacer 1 (IGS1) region or internal transcriber spacer (ITS) region may allow direct detection and species level identification of *Trichosporon* in clinical specimens [20, 30, 35].

Treatment

Disseminated trichosporonosis has a mortality rate of approximately 45–70% [2–6, 15, 16, 19–22]. In most cases, however, underlying host factors such as co-morbidities and presence of neutropenia contribute greatly to the overall mortality. First-line, optimal antifungal therapy has not been established. The initial step in the management of disseminated *Trichosporon* infection should be to decrease or reverse immunosuppression.

In vitro susceptibility studies of *Trichosporon* species are limited (Table 10.3), and clinical break points have not been established. In vitro susceptibility assays of *T. asahii* reveal wide MIC_{90} ranges for fluconazole, itraconazole, voriconazole, posaconazole, and amphotericin B, depending on the method of susceptibility testing and the isolate's country of origin [20]. In general, most strains have relatively high MICs for polyenes, flucytosine, and echinocandins, with

Table 10.3 In vitro antifungal activity against emerging yeast infections

		Minimum inhibitory concentration (MIC; μg/mL) range[a]								
Organism	Flu	Itra	Vori	Posa	Isuv	Mica	Cas	Anid	AMB	5FC
Trichosporon [13, 20, 34–36]	0.25– > 64	0.0125–32	0.008–32	0.008–32	≤0.015–2	16–64	8–32	16 – > 16	0.0125–64	16 – > 512
Saccharomyces [37]	0.5–64	0.03–4	0.015–2	0.12–1.0	≤0.007–1	NA	0.25–2	0.25–1	0.032–4	< 0.125–1
Rhodotorula [1, 38, 39]	8– > 256	0.5– > 16	0.5– > 8	0.5– > 32	0.125–4	> 64	8– > 64	>64	0.12–1.0	0.6–0.25
Malassezia [40, 41]	0.5–128	0.03–8	0.03–8	0.03–32	0.03–0.5	NA	NA	NA	0.25– > 16	> 100
Saprochaete [42, 43]	4– > 64	0.03–0.50	0.03–2	0.12–0.25	0.125– ≥ 8	NA	1– > 8	1–4	0.125–1	0.12–16
Sporobolomyces [44]	1.25– > 64	1.0–2.0	0.25–4.0	NA	NA	> 64	NA	NA	0.14–1.0	NA

Flu fluconazole, *Itra* itraconazole, *Vori* voriconazole, *Posa* posaconazole, *Isuv* isavuconazole [36], *Mica* micafungin, *Cas* caspofungin, *Anid* anidulafungin, *AMB* amphotericin B, *5FC* flucytosine [20, 35, 38], *NA* data not available

[a]Clinical Laboratory Standards Institute (CLSI) testing criteria and breakpoints have not been established for any of these fungi

Table 10.4 Suggested antifungal agents for use in treatment of emerging yeast infections

Yeast	Antifungal therapy[a]
Trichosporon	Voriconazole 200 BID
	Posaconazole 400 mg BID Fluconazole 400 mg/day
	Itraconazole 400–600 mg /day
Saccharomyces	Echinocandin
	Amphotericin B
	Azoles (e.g., voriconazole, posaconazole, ketoconazole)
Rhodotorula	Amphotericin B + 5-flucytosine
Malassezia	Liposomal amphotericin B 5 mg/kg/day
	Amphotericin B 0.7–1 mg/kg/day
	Voriconazole 200 mg BID Fluconazole 400 mg BID
Saprochaete	Amphotericin B 1–1.5 mg/kg/day
Sporobolomyces	Amphotericin B
	Voriconazole

[a]No antifungal agents have specific FDA approval for therapy of any of these infections. No randomized clinical trials have been performed. Selections are based on in vitro data, limited animal studies, and/or individual case reports

relatively low MICs for the azoles. In fact, *Trichosporon* spp. are considered to have intrinsic resistance to echinocandins, and breakthrough *Trichosporon* fungemia has been reported in patients receiving echinocandins. Fluconazole has species- and strain-dependent activity, and high fluconazole MICs have been increasingly observed likely related to antifungal pressure [13]. Among the newer triazoles, voriconazole, itraconazole, and posaconazole have demonstrated excellent in vitro activity [13, 34, 35, 45–48]. Isavuconazole also shows significant *in vitro* activity against most strains of *Trichosporon* (Table 10.4) [36, 47]; in fact, it appears to have better activity than fluconazole [25]. In vitro and animal models suggest that azoles and not polyenes are more effective in the eradication of *Trichosporon* species [1, 2, 27]. Voriconazole 3 mg/kg IV or 200 mg orally twice daily has emerged as the first-line therapy for treatment of disseminated *Trichosporon* infection. Posaconazole 400 mg orally twice daily, fluconazole 400–800 mg/day, itraconazole 400–600 mg/day or isavuconazole 372 mg/day are suggested alternatives (Table 10.4) [13, 20, 34–36, 48]. A potential option to consider in patients failing azole therapy is a combination of an azole with a polyene. Serena et al. demonstrated in vitro synergy and improved outcomes in an animal model of trichosporonosis with either the combination of amphotericin B/micafungin or fluconazole/micafungin [16, 28, 46, 47]; however, clinical data supporting combination therapy in humans is lacking. Given the high MICs of *T. asahii* to polyenes and echinocandins, as well as reported clinical treatment failures with these agents, these antifungals should not be used alone or as first-line therapy. In a patient with disseminated infection and poor response to therapy, in vitro susceptibility testing of recovered isolates may be a helpful adjunct [13, 31, 35, 36, 49]. Source control, e.g., removal of a central venous catheter in central line associated bloodstream infections and valve replacement in *Trichosporon* endocarditis, is recommended.

Saccharomyces

Saccharomyces is an ascomycetous yeast found throughout nature. *Saccharomyces* is commonly known as "brewer's yeast" or "baker's yeast." It is best known for its commercial use in beer and wine production, in health food supplements, and more recently, its use in DNA recombinant technology. Occasionally, these yeasts have been reported to cause severe infection in immunocompromised hosts [18]. Species include *S. cerevisiae*, *S. boulardii* (a subtype of *S. cerevisiae*), *S. fragilis*, and *S. carlsbergensis*. *Saccharomyces* may occasionally be part of the normal flora of the gastrointestinal and genitourinary tracts [1, 2, 50]. *S. cerevisiae* has been found to cause mucosal and disseminated infection in humans, primarily in immunocompromised hosts [37, 50–52].

Etiologic Agents

Cells are oval to spherical and exist as either haploids or diploids. When present, ascospores, one to four in number, are in either tetrahedral or linear arrangement and stain gram negative; vegetative cells stain gram positive. Colonies are smooth, moist, and either white or cream colored. On CHROMagar, colonies are dark pink. *Saccharomyces* spp. are generally non-pathogenic due to innate low virulence [18, 37, 53–55]; however, have rarely been reported as causes of invasive infection. Investigators evaluating more than 3300 yeast cultures obtained from cancer patients found only 19 isolates of *S. cerevisiae* [30, 37]. Clemons et al. (1994) showed that some strains of *S. cerevisiae*, when introduced into CD-1 mice, can proliferate and resist clearance in vivo, supporting the role of *S. cerevisiae* as a cause of clinical infection in humans [37, 55]. In the last decade, approximately 100 cases of *Saccharomyces* infections have been reported in the medical literature [30].

Epidemiology

Isolation of *Saccharomyces* species from human surfaces is rarely of any clinical significance. It has been recovered from the bloodstream, lungs, peritoneal cavity, bone, esophagus, urinary tract, and vagina [2–6, 50, 53, 56]. Genotyping studies evaluating the relatedness between clinical strains and commercial strains of *S. cerevisiae* have demonstrated that commercial products may occasionally be a contributing factor in human colonization and infection [57, 58]. Nyirjesy et al. reported that four women suffering from recurrent *S. cerevisiae* vaginitis had also experienced exposure to bread dough that contained identical strains of *S. cerevisiae* [52].

The risk factors associated with *Saccharomyces* infections are similar to the risk factors associated with candidemia and invasive candidiasis, including central venous catheters, neutropenia, antimicrobials, gastrointestinal tract surgery, and occasionally in HIV-positive individuals [51, 59–62]. The portal of entry for invasive disease is most likely the oropharynx or gastrointestinal tract [60].

Clinical manifestations are generally nonspecific and indistinguishable from those associated with candidemia and invasive candidiasis. In addition, *Saccharomyces* has been associated with bloodstream infections, endocarditis, peritonitis, disseminated disease, and vaginitis [1–6, 52, 56, 59–65]. A case of post-traumatic *Saccharomyces cerevisiae* osteomyelitis in a baker has also been reported [62].

Fungemia is the most common form of infection, occurring in approximately 70% of reported cases. As in invasive candidiasis, it is seen primarily in the immunocompromised host and tends to be associated with use of intravascular catheters, chemotherapy, and/or antimicrobials [50, 51, 60]. Translocation of *S. boulardii*, taken as a probiotic, across gastrointestinal mucosa has also been implicated in fungemia development in severely immunocompromised patients. Manifestations are similar to those of systemic candidiasis and candidemia. Overall, fever unresponsive to broad spectrum antimicrobials is the most frequent manifestation. Unlike infections due to *Candida* species, most patients survive.

In addition, it is not uncommon for other organ systems to become infected, including the respiratory tract, with several documented episodes of pneumonia and empyema. Diagnosis is generally established by histopathology, since *Saccharomyces* can colonize the respiratory tract without producing invasive disease [36, 50, 60, 65]. *Saccharomyces* has also been reported to produce peritonitis, cholecystitis, and endocarditis [50, 60]. All cases of endocarditis were associated with prosthetic valves and intravenous heroin use. Furthermore, two out of the three patients were cured with antifungal therapy alone; only one patient had their valve replaced. There have also been documented cases of urinary tract infections due to *S. cerevisiae* [52]. All patients with *S. cerevisiae* UTI had underlying urologic abnormalities or associated fungemia [60].

Mucosal infections due to *S. cerevisiae* have also been reported. Sobel et al. reported on 17 women with difficulty to manage vaginitis due to *S. cerevisiae* [48, 52]. In fact, the women with symptomatic vaginitis had manifestations indistinguishable from those caused by *C. albicans.* All patients had a history of chronic vaginitis unresponsive to conventional antifungals and all but two had systemic or local predisposing factors.

Diagnosis

Because of the fact that *Saccharomyces* species have a tendency to be non-pathogenic, the decision to attribute a causal role to *S. cerevisiae* is difficult [2–6]. Diagnostic difficulty occurs when the organism is recovered from body sites that may be colonized by *Saccharomyces,* especially in the absence of symptoms of infection. Unless the organism is found in the bloodstream, it is frequently necessary to determine whether these yeasts are causing true infection versus colonization. This is generally done via a histopathologic examination. *S. cerevisiae* readily grows from blood culture bottles and on Sabouraud dextrose media [2–6, 37, 57]. MALDI-TOF MS and ITS sequencing may be used to help identify *Saccharomyces* to the species level.

Treatment

It is often difficult to assess the role of antifungal therapy in patients with infection due to *Saccharomyces*. There are several reports that document resolution of fungemia and infection just by removing the intravascular catheter without providing antifungal therapy [59, 60]. As with most fungi, *Saccharomyces* are capable of producing biofilms, and most experts advocate removing the focus of infection, whether it is an indwelling or tunneled intravenous catheter, and the concurrent use of antifungal agents [60]. Currently, there are not any established treatment guidelines for *Saccharomyces* infections. *In vitro* susceptibility studies reveal that isolates of *S. cerevisiae*, when compared to *C. albicans* isolates, tend to have higher MICs to fluconazole and other azoles [1–5, 48] (Table 10.3). Although clinical trials have not be conducted and *in vitro* susceptibility assays are not standardized, the majority of *Saccharomyces* species appears to be susceptible to most antifungals, including amphotericin B, 5-flucytosine, echinocandins, and extended spectrum azoles [1–5, 48] (Table 10.4). Thus, empiric treatment with an echinocandin, amphotericin B, or an extended spectrum azole is reasonable, with specific choice dependent on clinical characteristics, including site of infection and patient comorbidities. In vitro susceptibility testing is recommended for all *Saccharomyces* isolates recovered from sterile sites, in case of treatment failure with the initial antifungal regimen.

Rhodotorula

Yeasts of the genus *Rhodotorula* are found worldwide from a variety of environmental sources. *Rhodotorula* is generally considered a contaminant when isolated; however, infections occasionally occur, primarily in immunocompromised hosts [2–6].

Etiologic Agents

Yeasts of the genus *Rhodotorula* are imperfect basidiomycetous yeasts belonging to the family Cryptococcaceae. There are over 50 recognized species in the genus *Rhodotorula* [2–6, 66]; however, few have been implicated as a human pathogen. *Rhodotorula mucilaginosa* (formerly *R. rubra*) is the species most frequently associated with human infections, followed by *R. glutinis* and *R. minuta.* Most *Rhodotorula* species produce red-to-orange colonies due to the presence of carotenoid pigments [18, 67]. The yeast is mucoid, encapsulated, and readily grows on many types of culture media. In tissue sections, *Rhodotorula* may be difficult to distinguish from *Cryptococcus* spp., thus culturing the tissue is critical. Molecular diagnostics may also aid identification.

Epidemiology

Rhodotorula can be isolated from a variety of sources including seawater, plants, air, food, fruit juices, and occasionally, from humans [1–6, 18, 66, 67]. It is not unusual to recover it as an airborne laboratory contaminant. *Rhodotorula* can also be recovered from shower curtains, bathtub-wall junctions, and toothbrushes. In humans, *Rhodotorula* can be been recovered from skin, nails, respiratory tract, urinary tract, gastrointestinal tract, and the bloodstream [1–6, 18, 68–71]. Although present worldwide, the highest percentage of isolates are recovered in the Asia-Pacific region [72].

R. mucilaginosa and *R. glutinis* account for approximately 0.5% of yeast isolated from the oral cavity and more than 12% of yeast isolates recovered from stool and rectal swabs [38]. The recovery of *Rhodotorula* from non-sterile human sources such as mucosal sites is of questionable clinical significance. Although still uncommon, in the last decade, invasive infections due to *Rhodotorula* spp. have been increasingly documented [38, 73]. *Rhodotorula* spp. are the third to fourth most common non-candidal yeasts isolated from clinical specimens [39, 72, 74]. Risk factors for invasive *Rhodotorula* infection include underlying immune compromise or immunosuppression (malignancy, neutropenia, hematopoietic stem cell transplantation, HIV, corticosteroids, collagen vascular disease, cirrhosis, and uncontrolled diabetes mellitus), use of broad spectrum antimicrobials, and central venous catheters [38, 42].

Clinical Manifestations

Manifestations of infection are generally non-specific, and may vary from subtle and mild, to severe, including septic shock. *Rhodotorula* have been incriminated in a wide spectrum of infections, including bloodstream infections, endocarditis, peritonitis, meningitis, and disseminated disease [2–6, 38, 39, 42, 66, 68, 71–83] (Table 10.5).

Fungemia is the most common form of infection and is generally associated with an intravascular catheter [1–6, 38, 39, 42, 66, 68, 71–83]. Fever of unknown etiology that is

Table 10.5 Clinical manifestations of *Rhodotorula* infections

- Fungemia
- Endocarditis
- Meningitis
- Peritonitis
- Disseminated disease

unresponsive to broad spectrum antimicrobials is the most frequent manifestation of *Rhodotorula* fungemia.

Meningitis has also been described in patients with acute leukemia, HIV infection, and post-operatively [79, 84]. The organisms are generally recovered from the CSF on culture, and frequently seen on an India ink stain. In addition, several cases of *R. rubra* peritonitis have been described in patients undergoing continuous ambulatory peritoneal dialysis. In these patients, environmental cultures revealed a possible common source outbreak. In all peritonitis patients, the symptoms were subtle and intermittent at first, consisting of abdominal pain, anorexia, nausea, and occasional diarrhea [2, 80, 83].

Diagnosis

In most proven infections, *Rhodotorula* is recovered from a sterile site of infection. In these cases, the decision to attribute a causal role to *Rhodotorula* is relatively simple, and the patient should be treated appropriately for an invasive fungal infection. The more difficult decision is when the organism is recovered from non-sterile body sites that may normally harbor *Rhodotorula* species, especially in the absence of specific signs or symptoms of infection [66, 71, 83]. In this setting, it is essential to establish the presence of infection and not just colonization.

Treatment

As with many of the uncommon yeast isolates, it is difficult to assess the role of antifungal therapy in patients infected with *Rhodotorula*. Optimal management of patients with indwelling catheters and infection due to *Rhodotorula* has not been well defined. There are several case reports that document the clearance of fungemia and the resolution of infection by removing the intravascular catheter without providing antifungal therapy [2–6, 48, 80–85]. On the other hand, there are also several documented case reports that have suggested that antifungal treatment alone may suffice without having to remove the central venous catheter. Since infections due to *Rhodotorula* may be severe and life threatening, it is probably best to manage these infections aggressively with catheter removal and antifungal therapy.

In vitro susceptibility studies reveal that *Rhodotorula* are susceptible to amphotericin B and flucytosine, but less susceptible to azoles and resistant to echinocandins [2–6, 48, 80–85] (Table 10.3). Even isavuconazole, the newest expanded triazole, has demonstrated high MICs against some strains of *Rhodotorula* species. Although clinical trials have not been conducted to inform treatment guidelines, amphotericin B with or without flucytosine is generally considered the preferred antifungal therapy or invasive *Rhodotorula* infections [1–6]. In view of the reduced susceptibility of *Rhodotorula* to the azoles, these agents should not be used as monotherapy unless in vitro susceptibility activity has been determined. Additionally, echinocandin monotherapy should be avoided due to intrinsic resistance. Although sometimes given, there is limited information on the efficacy of combination antifungal therapy for this organism.

Malassezia

Malassezia furfur is a yeast commonly found on human skin. It has been well documented to cause superficial skin infections such as pityriasis (tinea) versicolor and folliculitis. In addition, in the immunocompromised host it may occasionally cause invasive infection.

Etiologic Agents

The genus *Malassezia* currently consists of 18 species, grouped into four clusters (A, B1, B2, and C). Some of the more common species isolated include *M. furfur, M. pachydermatis, M. sympodialis, M. slooffiae, M. globosa, M. obtuse*, and *M. restricta,* and others [1–6, 18]. *M. furfur* is the dominant species recovered in humans as a fungal pathogen. *M. pachydermatis*, *M. sympodialis,* and *M. restricta* are the only other *Malassezia* (besides *M. furfur*) that have been implicated in invasive infection. Over 100 cases of systemic *Malassesia* infections have been published to date, with *M. furfur* accounting for nearly 70% of the cases, *M. pachydermatis* 28%, and *M. sympodialis* less than 3%. *M. restricta* was isolated from splenic lesions in a neutropenic child [86].

M. furfur is a dimorphic, lipophilic yeast that is unable to synthesize medium or long chain fatty acids and thus has a strict *in vitro* requirement for exogenous fatty acids of the C_{12} and C_{14} series [18, 86–88]. Although it exists primarily in the yeast form, it may also form filamentous structures on the skin when the organism is associated with superficial infections [87–90]. Because of its nutritional requirements, *M. furfur* is difficult to recover from clinical specimens unless its presence is suspected and special preparations are made by the microbiology laboratory. Thus, the true incidence of *M. furfur* infections is likely underestimated. The second most common species is *M. pachydermatis*, which is generally associated with otitis externa infections in dogs [87–89]. Occasionally, however, it has been implicated in human infections [87–89]. Both *Malassezia* species, when grown under favorable conditions, produce clusters of oval to round, thick-walled yeast cells, with unipolar buds that form repeatedly from the same pole of the parent cell. This

gives rise to the characteristic "collarette" at the bud site. Media such as Sabouraud dextrose agar, chocolate agar, trypticase soy agar with 5% sheep blood all require the addition of supplements such as olive oil, in order to permit the growth of most *Malassezia* species, including *M. furfur* [18]. *M. pachydermatis,* however, does not require exogenous lipids for growth and thus can be recovered on conventional fungal media. Colonies tend to be dry, and white to creamy in color. All three of the pathogenic *Malassezia* spp. are capable of forming biofilms, a characteristic thought to contribute to their virulence. Additionally, high lipolytic activity has been demonstrated in *M. furfur* strains causing fungemia, which may help explain the contribution of parenteral lipids in the pathogenesis of invasive *Malassezia* infections.

Epidemiology

Malassezia is frequently found colonizing the skin of healthy individuals. In fact, *Malassezia* spp. are a predominant component of the human mycobiome [89, 90]. The distribution of this colonization tends to correlate with oilier areas of the body, most likely because these areas satisfy the organism's requirement for exogenous fatty acids. Thus, *Malassezia* is found primarily on the scalp, shoulders, chest, and back [88–92]. The highest colonization rates (>90%) are found in teenagers.

The isolation of *M. furfur* from newborns is reported to be less than 10% in non-intensive care settings, but greater than 80% in neonatal intensive care units [87–92]. Risk factors associated with increased colonization rates in neonates include prematurity, duration of hospitalization in the ICU, use of occlusive dressings, and prolonged antimicrobial use [87–92]. Although the epidemiology of disseminated infection in adults has not been well studied, there appear to be several risk factors that are frequently associated with deep-seated infections (Table 10.6). These include central venous catheters, total parenteral nutrition, parenteral lipid preparations, and immunocompromised state [88, 92–95]. Molecular epidemiologic studies using DNA fingerprinting have concluded that within the neonatal ICU there is longitudinal persistence of both *M. furfur* and *M. pachydermatis* strains [88–92].

Table 10.6 Risk factors associated with *Malassezia* infections

- Prematurity (neonates)
- Longer duration of hospitalization
- Use of occlusive dressings
- Administration of antibiotics
- Use of central venous catheters
- Use of intravenous lipids

Clinical Manifestations

Malassezia most commonly produces superficial skin infections, such as pityriasis (tinea) versicolor or folliculitis. From time to time, *Malassezia* may produce a deep-seated or hematogenous infection [88–95]. The majority of invasive *Malassezia* infections are diagnosed in premature infants. Occasionally, they may occur in adults. The first reported case of systemic infection was described in 1981 in a premature neonate who developed fungemia and vasculitis while on lipid therapy [94]. Since then, numerous reports describing disseminated infection have been published [87–92]. The manifestations of disseminated infection vary from subclinical with mild symptomatology, such as fever, to sepsis with associated multiorgan dysfunction [87–92]. The most commonly reported manifestations of systemic infection include fever unresponsive to broad spectrum antimicrobials, bradycardia, respiratory distress, hepatosplenomegaly, and lethargy.

Diagnosis

Laboratory findings are nonspecific, and may include leukocytosis and thrombocytopenia. Chest X-ray frequently reveals bilateral pulmonary infiltrates (>50%) [1–6, 88, 92]. Occasionally, the diagnosis of disseminated infection can be made by a gram stain of the buffy coat of blood. The budding yeast cells may be observed using different stains such as Giemsa, periodic acid Schiff (PAS), Gomori's methenamine silver (GMS) or Calcofluor white. Blood cultures are usually negative unless the infection is initially suspected and the laboratory is alerted to use a lipid-enriched media. The recovery of the organisms is enhanced by using the lysis centrifugation blood culture technique [88, 96]. Palmitic acid (3%) supplementation may also improve the recovery of *Malassezia* [88, 96]. Automated systems commonly used by clinical laboratories to identify organisms in the blood, such as the BacT/Alert system, does not reliably detect *M. furfur* [89, 90]. On the other hand, *M. pachydermatis*, with its more favorable growth characteristics in routine blood cultures, is detectable by these systems. MALDI-TOF MS and molecular techniques such as ITS or 28S rRNA sequencing are useful for identifying these organisms to the species level [97–100].

Treatment

Management of *M. furfur* fungemia and disseminated infection are controversial. Most authorities recommend prompt removal of the central venous catheter and discontinuation of intravenous lipids [88, 92–94]. In cases without a deep-

seated infection, removal of the central venous catheter and discontinuation of lipids may be all that is needed to clear the infection. This treatment modality accomplishes two objectives: it eradicates the nidus of infection, and removes the nutritional requirements of the organism. If fungemia persists or there is evidence of deep-seated infection, it is prudent to initiate antifungal therapy. Fortunately, *Malassezia* species are often susceptible to azoles and polyenes [1–6, 40, 41, 101] (Table 10.3), although there may be species and genotype variations in antifungal susceptibility patterns [89, 90]. For instance, *M. pachydermatis* and *M. sympodialis* tend to be more susceptible to azoles than *M. furfur*. *In vitro* susceptibility assays of *M. furfur*, strains have demonstrated a wide range of MIC values for amphotericin B and azoles (Table 10.3) [40, 41, 101]. Most of the isolates are intrinsically resistant to flucytosine (MIC $_{90}$ > 100 μg/mL) and the echinocandins (MIC >32 μg/mL) [1–6, 40, 41, 48, 88, 101]. Although randomized clinical trials on which to base recommendations have not been conducted, in most situations, amphotericin B deoxycholate 0.7–1 mg/kg/day or liposomal amphotericin B 5 mg/kg/day is sufficient to eradicate systemic *Malassezia* infection (Table 10.4). Fluconazole 400 mg/day may also be appropriate if the patient was not on azole prophylaxis at the time of fungemia development. Extended spectrum azoles such as itraconazole and voriconazole may also be considered as alternatives. Echinocandins and flucytosine should not be used as monotherapy due to intrinsic resistance, although cases have been reported wherein flucytosine was successfully used in combination with amphotericin B. Given the observed heterogeneity in antifungal susceptibility patterns according to *Malassezia* species and strains, species-level identification may be useful and is recommended. Although challenging to apply clinically due to lack of established breakpoints, in vitro susceptibility testing provides valuable epidemiologic information and the results may aid clinical decision-making in cases that are poorly responsive to first-line therapy.

Other Emerging Yeasts

In addition to the yeasts discussed thus far, fewer reports have been published of infection due to other yeasts. These include *Saprochaete*, *Sporobolomyces*, *Geotrichum*, *Kodamae* (formerly *Pichia* or *Hansenula*), and *Exophiala*.

Saprochaete

Saprochaete capitata (formerly *Magnusiomyces capitatus, Blastoschizomyces capitatus*,,*Geotrichum capitatum*, or *Trichosporon capitatum*) infections, although less common than those due to *T. asahii*, have been well described in the literature [2–6, 102]. *S. clavata* (formerly *Geotrichum clavatum*), the other species in this genus, appears to be less pathogenic than *S. capitata*, and has primarily been described in cluster outbreaks in patients with hematologic malignancies [18, 103–105]. *S. capitata* is found in wood and poultry, but has also been recovered from sputum and normal intact skin [18]. Geographically, it appears to be the opposite of *T. asahii*, with *S. capitata* infections found primarily in Europe and *T. asahii* found in North America [2–6, 103]. In most cases of invasive *Saprochaete* infections, the major risk factors include neutropenia and underlying hematologic malignancies. Although the portal of entry is unknown, it is suspected to be either the respiratory tract, gastrointestinal tract, or central venous catheter [2–6, 103].

Infection may involve a single organ or multiple organs and may be associated with fungemia. The clinical spectrum of disseminated infection is similar to that of systemic candidiasis and includes fungemia with or without organ infection [2–6, 103], metastatic skin lesions, and hepatosplenic abscesses. Osteomyelitis, meningitis, pneumonia, and prosthetic valve endocarditis have also been described [104, 105]. By and large, the manifestations begin with fever of unknown etiology unresponsive to antibiotics. Diagnosis can be made with blood cultures, or on biopsy of the skin or affected organs. Blood cultures are reportedly positive in >80% of cases [103]. *S. capitata* easily grows in blood culture bottles and on fungal specific media [18]. Colonies are white to cream in color; isolates grown in culture produce hyphae, psuedohyphae, and conidia. Although skin lesions are commonly seen, fungal stains and cultures from biopsied skin lesions are frequently negative [18, 103]. MALDI-TOF MS with an extended library and molecular diagnostic techniques (ITS and 28S rRNA sequencing) can be used for species-level identification.

Mortality rates between 60–80% are generally described [43, 44, 103, 105]. However, underlying disease, persistent neutropenia, and concurrent infections are significant contributing factors to this overall mortality rate. Optimal therapy has not yet been established. Until recently, however, most patients have received amphotericin B [2]. As with all invasive fungal infections, the initial step is to decrease or reverse the immunocompromised state. In vitro susceptibility testing breakpoints have not been established and therapy is typically guided by species identification. In vitro studies demonstrate amphotericin B usually has activity (MIC_{90} 0.12 μg/mL), although high MICs (2.0 μg/mL) have been described in some strains. Fluconazole MICs tend to be higher (16–32 μg/mL), as do those of ketoconazole (0.04–32 μg/mL). Despite these higher MICs, it appears that high dose fluconazole has produced favorable outcomes against *S. capitata* in animal models. Extended spectrum triazoles such as itraconazole, posaconazole, voriconazole, and isavucon-

azole typically have low MICs against *S. capitata* [36]. Although isolates may have low MICs to flucytosine, resistance has been described (2, 18). Flucytosine is often avoided in these patients due to potential bone marrow suppression [2, 18] (Table 10.3). The current recommendation is to use amphotericin B at a dose of 1–1.5 mg/kg/day with or without flucytosine [1–6] or voriconazole as initial therapy. Posaconazole and isavuconazole have demonstrated good in vitro activity and may also be suitable alternatives [30, 104]. Echinocandins should not be used as monotherapy due to intrinsic resistance; however, there have been some case reports of combination therapy with voriconazole and an echinocandin for potential synergy [43].

Sporobolomyces

Sporobolomyces are yeast-like organisms that belong to the family Sporobolomycetaceae. These yeasts are found throughout the world in soil, bark, and decaying organic material. They have occasionally been associated with infections in humans. There are at least 15 known species of *Sporobolomyces*, but only three have been documented to cause disease, *S. salmonicolor, S. holsaticus*, and *S. roseus* [30]. Invasive cases of *Sporobolomyces* are very rare. To date, there have been only 14 documented cases of *Sporobolomyces* infections worldwide, including a case of endopthalmitis, a central venous catheter-associated blood stream infection, an infected cranioplasty, and two cases of disseminated infection in patients with AIDS (lymph node and bone marrow) [2, 42, 106–109].

Sporobolomyces can have a similar appearance to *Rhodotorula* in culture, growing as salmon-pink to red colonies on standard media. *Sporobolomyces* produce kidney-shaped ballistoconidia that lead to the formation of small satellite colonies. Hyphae and pseudohyphae are visible on microscopic exam. Some rapid identification systems may misidentify this yeast, and MALDI-TOF MS using standardly available databases does not reliably identify *Sporobolomyces* [110, 111]. Molecular identification using panfungal PCR with ITS sequencing can confirm the species.

In vitro susceptibility studies show that *S. salmonicolor* is susceptible to amphotericin B and the imidazoles [1, 2, 18, 44, 106] (Table 10.3). Despite the fact that these organisms are saprophytic, the case reports indicate their potential ability to produce invasive infection in humans, especially, in a compromised host. If a central venous access device is implicated in the infection, removal is advised, although there is limited data on which to base this recommendation. Treatment with either liposomal amphotericin B 3–5 mg/kg/day or voriconazole is recommended based on case reports and available susceptibility data.

Geotrichum

Geotrichum spp. are closely related to the *Saprochaete* genus. In fact, the two main *Saprochaete* species (*S. capitata* and *S. clavata*) were formerly classified as *Geotrichum capitatum* and *Geotrichum clavatum.* They have been reassigned based on their genetic sequencing. *Geotrichum candidum* is the only remaining species in the *Geotrichum* genus known to be pathogenic in humans.

Geotrichum candidum is an arthroconidia-producing yeast similar to *Trichosporon* spp.; however, *Geotrichum* does not form true blastoconidia. In addition to being ubiquitous in nature, *Geotrichum candidum* is used in the production of soft French cheese and has been found in contaminated milk [112]. Cases have been associated with ingestion of each. Less than 30 cases of invasive *Geotrichum candidum* infections have been reported worldwide. As with the other rare noncandidal yeasts, immunocompromising conditions including hematologic malignancy, HIV/AIDS, and uncontrolled diabetes mellitus are risk factors for invasive infection [113, 114]. Trauma and surgery have also rarely been associated with cases of invasive *Geotrichum candidum*. Fungemia is the most common manifestation of invasive *Geotrichum* infection in immunocompromised patients. This often presents as fever unresponsive to broad-spectrum antibiotics; skin and/or pulmonary lesions (on CT scan) accompany the fungemia in some cases. Endocarditis has been reported, and echocardiography should thus be considered in disseminated *Geotrichum candidum* [115].

Geotrichum spp. have long, thin, septated hyphae with variable branching angles. Gram stain of blood cultures may reveal yeast-to-hyphal structures [114–116]. On Sabouraud's dextrose agar, *Geotrichum* colonies are fast growing, moist, and white to cream colored. They may have dry fimbriate margins. MALDI-TOF MS and molecular diagnostic methods (e.g., panfungal PCR targeting the ITS or 28S rDNA) can provide species-level identification of *Geotrichum*.

As with the other rare yeasts, clinical breakpoints have not been established for in vitro susceptibility testing of *Geotrichum*. The lowest MICs against *Geotrichum* have been reported with voriconazole and posaconazole. Isavuconazole may also have good activity (MIC_{50} 0.25, MIC range $\leq$ 0.007–2), but data is limited [36]. Fluconazole and itraconazole have variable activity against *Geotrichum*. Low MICs have been reported for micafungin. Most strains also have low MICs for amphotericin. Currently, treatment with either voriconazole or an amphotericin B formulation with or without flucytosine is recommended for invasive *Geotrichum candidum* infection. Echinocandins are also anticipated to be effective although there is limited clinical data to date to support their use.

Kodamaea

Kodamaea ohmeri, formerly *Pichia ohmeri* (the anamorph form of *Candida guilliermondii* var. *membranefacians),* is the primary human pathogen in the genus *Kodamaea* [117]. This species of environmental yeast belongs to the family Saccharomycetaceae, and can be found on fruit, leaves, and in fermented foods.

Cases of *K. ohmeri* fungemia, endocarditis, wound infections, and peritonitis have been documented. Over 130 cases have been reported worldwide, with the majority of cases occurring in southeast Asia [39, 74, 117–120]. Infections may occur in immunocompromised or immunocompetent individuals, the latter typically in association with indwelling central venous catheters [121]. Similar to other noncandidal yeasts, prematurity, prolonged hospitalization, ICU admission, indwelling central venous catheter, burn injury, and immunity compromise are risk factors associated with invasive *K. ohmeri* infection in pediatric populations [121–123]. Hematologic malignancy, prolonged ICU course, HIV, diabetes mellitus, prolonged corticosteroids, and antibiotic use have been identified as predisposing factors in adults.

Blood cultures may reveal budding yeast forms on gram stain. *K. ohmeri* quickly grows as radial, flat, white colonies. Similar to *Candida tropicalis*, *K. ohmeri* colonies change from pink to blue-grey over time when subcultured on CHROMagar™ Candida. *K. ohmeri* produce pseudohyphae, but not true hyphae. Unlike *Trichosporon*, this yeast is urease-negative.

There is limited data on use of MALDI-TOF MS for *K. ohmeri* identification [117]. Molecular identification via ITS sequencing reliably provides species-level identification of *Kodamaea*.

Although clinical breakpoints have not been established for *K. ohmeri*, in vitro susceptibility testing is recommended for epidemiologic purposes and as a potential guide for treatment failures. Voriconazole, posaconazole, and itraconazole appear to have lower MICs than fluconazole for *K. ohmeri* [36, 117, 119, 124]. There is limited data for isavuconazole; however, low MICs (≤0.007–0.25 mg/L) have been reported [125]. Echinocandin and amphotericin B MICs also tend to be low. The optimal treatment for *K. ohmeri* has not yet been established. Amphotericin B, echinocandins, and fluconazole are all considered appropriate first-line treatment options. Voriconazole was also reported to be effective in one case [119]. Source control is recommended when feasible; removal of the catheter is recommended in central venous catheter associated infections, and valve replacement is recommended in cases of *K. ohmeri* endocarditis.

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Aspergillosis

11

Helen W. Boucher and Thomas F. Patterson

Introduction

Aspergillosis is caused by *Aspergillus*, a hyaline mold responsible not only for invasive aspergillosis but also a variety of noninvasive or semi-invasive conditions. These syndromes range from colonization to allergic responses to *Aspergillus*, including allergic bronchopulmonary aspergillosis (ABPA), to semi-invasive or invasive infections, spanning a spectrum from chronic necrotizing pneumonia to invasive pulmonary aspergillosis.

The genus *Aspergillus* was first recognized in 1729 by Micheli, in Florence. He described the resemblance between the sporulating head of an *Aspergillus* species and an aspergillum used to sprinkle holy water. In 1856, Virchow published the first complete microscopic descriptions of the organism [1].

The frequency and severity of invasive fungal infections in immunocompromised patients have increased steadily over recent decades with the growing population of patients undergoing transplantation and the persistent challenges in preventing, diagnosing, and treating these infections [2]. Invasive aspergillosis is a major cause of mortality in high-risk patients, including those with underlying hematologic malignancy, bone marrow or solid organ transplantation, and may be related to several factors, including diagnostic and therapeutic inadequacies [2–5]. Invasive aspergillosis has also been observed in individuals hospitalized with severe illnesses, including ICU admission or respiratory failure due to influenza and SARS-CoV-2 [6–8]. Other risk factors have emerged, including use of small molecule biological agents and monoclonal antibodies targeting the immune system [8, 9].

Successful therapy depends not only on an early diagnosis—which is often difficult to establish—but even more importantly, on reversal of underlying host immune defects, such as neutropenia or high dose immunosuppressive therapy [2, 10]. Non-culture-based tests and radiological approaches can be used to establish an early diagnosis of infection and may result in improved outcomes of infection [2, 11]. Even when therapy is begun promptly, efficacy of therapy is poor, particularly in patients with disseminated or central nervous system disease [2, 12, 13]. Recent developments include more widespread use of newer diagnostic approaches and improved understanding of how best to use available antifungal agents [8].

Etiologic Agents

Aspergillus fumigatus is one of the most ubiquitous of the airborne saprophytic fungi [14]. *Aspergillus fumigatus* has emerged worldwide as a frequent cause of opportunistic or nosocomial infections and may be regarded as the most important airborne pathogenic fungus [2, 14]. As *Aspergillus* species can be readily found in the environment, invasive aspergillosis is widely believed to occur as a consequence of exogenous acquisition of the

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_11

conidia (spores) of the species [15]. The most common route of transmission of *Aspergillus* infection is the airborne route. *Aspergillus* conidia are resilient and may survive for long periods in fomites (any substance that can absorb, retain, and transport infectious species, e.g., woolen clothes or bedding) [10]. *Aspergillus* infection occurs less frequently through damaged mucocutaneous surfaces (e.g., following surgery or through contaminated dressings). However, the sources of *Aspergillus* may be broader than traditionally thought, as waterborne transmission of *Aspergillus* conidia through contaminated aerosols has been suggested [16].

The most common species causing invasive aspergillosis include: *Aspergillus fumigatus* (Fig. 11.1), by far the most common, *A. flavus*, *A. terreus*, and less commonly for invasive infection, *A. niger* [13] (Table 11.1). Other less common species have emerged, including *A. terreus* (which is frequently resistant to polyenes) and other unusual less pathogenic species, as the etiologic agents of invasive infection [17–19].

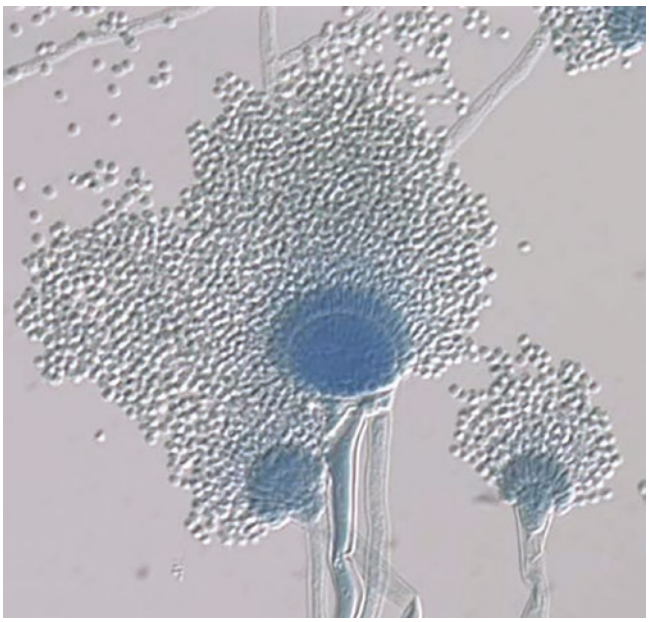


Fig. 11.1 Microscopic morphology of *Aspergillus fumigatus* showing a single role of phialides (uniseriate) bearing smooth conidia in a columnar fashion. (Courtesy of www.doctorfungus.org)

Table 11.1 Characteristics of common *Aspergillus* species complexes

Aspergillus species	Mycological characteristics	Clinical significance	Mycoses
A. flavus	Olive to lime green colonies	Second most common species, produces aflatoxin, may be less susceptible to polyenes	Sinusitis, cutaneous infection, pulmonary and disseminated disease
A. fumigatus	Smoky, blue- or gray green, small, smooth conidia (2–2.5 μm)	Most common species causing invasive infection	Invasive pulmonary aspergillosis, disseminated infection, CNS, others
A. niger	Typically black colonies, radiate conidial head, large rough conidia	Common cause of otomycosis, produces oxalate crystals which may be seen in host	Otomycosis, cutaneous, endophthalmitis, aspergilloma, invasive pulmonary or disseminated disease is uncommon
A. terreus	Beige to buff colonies, globose accessory conidia along hyphae	Increasing frequency, associated with soil, usually resistant to polyenes	Pulmonary, disseminated, cutaneous, keratitis, CNS
A. lentulus	Poorly sporulating variant of *A. fumigatus*	May be multidrug resistant, recently described variant, may be underdiagnosed	Invasive pulmonary, disseminated, other sites

Epidemiology

The incidence of invasive aspergillosis has increased substantially during the last few decades because of the use of more intensive cytotoxic anticancer chemotherapy and small molecule chemotherapeutic agents (e.g., Bruton's tyrosine kinase inhibitors [ibrutinib and others]) and the introduction of novel immunosuppressive therapies for organ transplant recipients, all of which have prolonged the period of risk for many individuals [9]. The increasing number of patients undergoing solid organ, bone marrow, and hematopoietic stem cell transplantation has also contributed to the increased incidence. The changes in epidemiology of invasive aspergillosis may also be the result of growing awareness of aspergillosis among clinicians, the introduction of non-invasive diagnostic tools and improved microbiological laboratory techniques.

Invasive fungal infections are an important cause of morbidity and mortality among patients with severely compromised immune systems. Although there have been significant advances in the management of immunosuppressed patients, invasive aspergillosis remains an important life-threatening complication, and is the leading cause of infection-related mortality in many immunocompromised patient groups [20].

Immunosuppression and breakdown of anatomical barriers, such as the skin, are the major risk factors for fungal infections, including *Aspergillus* [21, 22]. Individuals at risk for invasive aspergillosis include those with severely comprised immune systems as a result of anticancer chemotherapy, solid organ or bone marrow transplantation, acquired immunodeficiency syndrome (AIDS), or use of high-dose corticosteroids. Patients with hematological disorders, such as prolonged and severe neutropenia, those undergoing transplantations, and those treated with corticosteroids and immunosuppressive therapies such as the tumor necrosis factor-α antagonists (e.g., infliximab), JAK/STAT and IL-6 inhibitors (e.g., tocilizumab and barcitinib) are considered to be at highest risk for invasive aspergillosis [9, 22, 23]. Admission to an intensive care unit has been identified being a risk factor for invasive aspergillosis even in the absence of traditional risk factors associated with invasive aspergillosis, such as neutropenia, immunosuppressive conditions, receipt of cytotoxic chemotherapy, and transplantation [24, 25]. Non-traditional risk factors for invasive aspergillosis in the ICU have included chronic obstructive pulmonary disease (COPD) with or without corticosteroid use, other lung diseases, history of pulmonary infections, and acute respiratory distress syndrome [26].

Another risk factor for invasive aspergillosis in the ICU is the diagnosis of severe respiratory infection due to influenza (Influenza Associated Aspergillosis [IAA]) and other respiratory viruses, including SARS-CoV-2 (COVID-19 Associated Invasive Aspergillosis [CAPA]) [7, 27]. Invasive aspergillosis associated with respiratory failure requiring ICU admission has been associated with influenza A (H1N1) and influenza B, but also avian influenza H7N9 and other respiratory viruses such as respiratory syncytial virus (RSV) and now SARS-CoV-2 [27–30]. For influenza and other respiratory viruses, IPA occurs secondary to epithelial airway injury leading to *Aspergillus* colonization and invasion. In CAPA, treatments associated with COVID-19 infection including dexamethasone and other corticosteroids, tocilizumab and other IL-6 inhibitors, and baricitinib appear to be risk factors for this fungal infection [30]. The incidence of IAA varies significantly in different locations and studies. In a Belgian and Dutch study over seven years, the incidence associated with influenza requiring ICU admission was 19% and mortality was 51% compared to 28% with severe influenza alone [31]. The incidence of CAPA has varied widely in case series (0–33%) [32, 33]. This wide variation likely reflects differences in diagnostic suspicion and testing, inconsistent case definitions, underlying treatment practices, and geographic variation [30, 34]. Risk factors for CAPA appear to be older age (>65 years), immunosuppressive agents (especially corticosteroids and tocilizumab), and chronic lung disease [32, 33].

Antifungal fungal resistance has emerged as a significant issue in aspergillosis [35–37]. Some species inherently have different susceptibilities to antifungal drugs: *A. terreus* is less susceptible to polyenes and more susceptible to mold active azoles; others like *A. calidoustus* demonstrate resistance to most antifungals [38, 39]. Although resistance of *A. fumigatus* to azole antifungals has been relatively uncommon in the USA, global clonal spread of azole resistant *A. fumigatus* has been reported [40–42]. The mechanism of resistance results from a tandem repeat (TR) mutation in the promoter region associated with point mutations in the target gene [40–42]. In the Netherlands and Western Europe where this was first reported, resistance rates of >25% have been noted and are frequently associated with pan-azole resistance [41–43]. This resistance mechanism is associated with environmental exposure of *Aspergillus fumigatus* to agricultural fungicides and is frequently seen in patients without prior azole use [36]. Mortality rates are high and require treatment with liposomal amphotericin or combination antifungal therapy [44].

Pathogenesis and Immunity

Invasive aspergillosis most frequently originates via inhalation of *Aspergillus* conidia into the lungs, although other routes of exposure such as inhalation of water aerosols contaminated with *Aspergillus* conidia have been suggested [16].

In the absence of effective pulmonary host defenses, the inhaled small resting conidia enlarge and germinate, then transform into hyphae with subsequent vascular invasion and eventual disseminated infection. The incubation period for conidial germination in pulmonary tissue is variable, ranging from 2 days to months [45]. Hydrocortisone significantly increases the growth rates of *Aspergillus*; likely one of the reasons corticosteroids pose a risk factor for invasive disease [46].

Although infection in apparently normal hosts can occur, invasive aspergillosis is uncommon in immunocompetent hosts [13]. Normal pulmonary defense mechanisms usually contain the organism in a host with intact pulmonary defenses. The first line of defense against *Aspergillus* is ciliary clearance of the organism from the airways and limited access to the alveoli due to conidia size. This feature is one reason for the increased pathogenicity of *A. fumigatus* as compared with other species of *Aspergillus* [46]. Once conidia reach the alveoli, pulmonary macrophages are generally capable of ingesting and killing *Aspergillus* conidia [47]. When macrophages fail to kill the conidia (e.g., high fungal inoculum, decreased number or function of macrophages), conidia germinate and begin to form hyphae. Polymorphonuclear leukocytes are recruited via complement activation and production of neutrophil chemotactic factors and extracellularly kill both swollen conidia and hyphae [48]. Antibodies against *Aspergillus* are common due to the ubiquitous nature of the organism, although they are not protective, nor are they useful in the diagnosis of infection in high-risk patients due to the lack of consistent seroconversion following exposure or infection [49].

Corticosteroids play a major role in increasing susceptibility to *Aspergillus*, by decreasing oxidative killing of the organism by pulmonary macrophages, by increasing the linear growth rate by as much as 30–40% and cell synthesis by greater than 150%. As such, they are likely a major factor contributing COVID-19 associated pulmonary aspergillosis (CAPA) [7, 46].

Many *Aspergillus* species produce toxins, including aflatoxins, ochratoxin A, fumagillin, and gliotoxin. Gliotoxin works in several ways to help evade host defenses:

- Inhibition of phagocyte NADPH oxidase activation (key in host defense versus filamentous fungi)
- Inhibition of macrophage ingestion of *Aspergillus*
- Suppression of functional T cell responses [50, 51]

In tissues, invasive aspergillosis causes extensive destruction across tissue planes via vascular invasion with resulting infarction and necrosis of distal tissues.

Differences in the host response determine the balance between allergic responses to *Aspergillus*, colonization, and invasive infection [8]. Differences in host genetics also impact individual risk. Polymorphisms within the genes encoding pentraxin-3, toll-like receptors 2 and 4, and dectin-1 have been associated with increased risk in at-risk patient populations (e.g., HSCT or SOT recipients) [15, 52].

Clinical Manifestations

The clinical syndromes associated with aspergillosis are diverse, ranging from allergic responses to the organism, including allergic bronchopulmonary aspergillosis (ABPA), asymptomatic colonization, superficial infection, and acute or subacute, and chronic invasive disease. The clinical presentation generally corresponds to the underlying immune defects and risk factors associated with each patient group, with greater immune suppression correlating with increased risk for invasive disease. Although this chapter focuses on invasive aspergillosis, a brief description of other presentations follows. The reader is encouraged to reference other sources for more in-depth discussion of those conditions [1, 15].

Allergic Bronchopulmonary Aspergillosis

Allergic bronchopulmonary aspergillosis (ABPA) is a chronic allergic response to *Aspergillus* characterized by transient pulmonary infiltrates due to atelectasis. The incidence of ABPA is estimated to range from 1–2% in patients with persistent asthma, and in approximately 7% (with a range from 2–15%) of patients with cystic fibrosis [53]. Specific criteria are used to establish the diagnosis of ABPA, as no single finding is diagnostic for the condition, although some presentations, like central bronchiectasis in patients with asthma, highly suggest the diagnosis [53–55]. ABPA typically progresses through a series of remissions and exacerbations, but can eventually lead to pulmonary fibrosis, which is associated with a poor long-term prognosis [54]. Management of ABPA is directed at reducing acute asthmatic symptoms and avoiding end-stage fibrosis. Corticosteroid therapy is commonly used for treating exacerbations, although few randomized trials have been conducted for their use [56]. The role for antifungal therapy was evaluated with a randomized double-blind, placebo-controlled trial that showed itraconazole at 200 mg per day for 16 weeks significantly reduced daily corticosteroid use, reduced levels of IgE, and improved exercise tolerance and pulmonary function [55, 57].

Aspergilloma

A pulmonary fungus ball due to *Aspergillus* or "aspergilloma" is a solid mass of hyphae growing in a previously existing pulmonary cavity, typically in patients with chronic lung disease

such as bullous emphysema, sarcoidosis, tuberculosis, histoplasmosis, congenital cyst, bacterial lung abscess or, very rarely, in a pulmonary bleb from *Pneumocystis* pneumonia in AIDS [58, 59]. On chest radiograph, a pulmonary aspergilloma appears as a solid round mass in a cavity. In many patients the fungus ball due to *Aspergillus* remains asymptomatic, but in a significant number, hemoptysis occurs and can be fatal [60]. Surgical resection is considered the definitive therapy, but the dense pleural adhesions adjacent to the fungus ball and the poor pulmonary reserve of most patients with this condition makes surgery hazardous. Contamination of the pleural space with *Aspergillus* and the common complication of bronchopleural fistula in the postoperative period can lead to chronic *Aspergillus* empyema. Dense adhesions make pleural drainage difficult, often requiring pleural stripping, further compromising lung function [60].

Aspergillus can also be associated with fungal balls of the sinuses without tissue invasion [28]. The maxillary sinus is the most common site for a sinus aspergilloma to occur [58]. Clinical presentation is similar to that for any chronic sinusitis. Management is usually directed at surgical removal and a generous maxillary antrostomy for sinus drainage, along with confirmation that invasive disease has not occurred.

Other Superficial or Colonizing Syndromes

Other superficial or colonizing syndromes of aspergillosis include otomycosis, a condition of superficial colonization typically due to *A. niger* [61]; onychomycosis which, although rare, can become chronic and respond poorly to antifungal agents [62]; and keratitis, particularly following trauma or corneal surgery [63].

Chronic Pulmonary Aspergillosis

Denning and colleagues have described three distinct syndromes of chronic pulmonary aspergillosis in order to better characterize those patients who develop chronic pulmonary disease related to *Aspergillus* [64]. These conditions include chronic cavitary pulmonary aspergillosis, which is characterized by the formation and expansion of multiple cavities, which may contain fungus balls; chronic fibrosing aspergillosis, which as its name suggests, involves extensive fibrosis; and chronic necrotizing aspergillosis or subacute aspergillosis, in which slowly progressive infection occurs usually in a single thin-walled cavity. In all of these conditions, the diagnosis is suggested by radiological and clinical features and the role for therapy remains speculative, although it appears that long-term antifungal therapy may be beneficial in a subset of patients, perhaps even with the extended spectrum triazole antifungals [64, 65].

Invasive Pulmonary Aspergillosis

Invasive pulmonary aspergillosis is the most common form of invasive aspergillosis in immunocompromised patients. This infection typically occurs following approximately two weeks of neutropenia [66] or during the course of therapy for graft versus host disease, a common risk factor in hematopoietic stem cell transplant recipients [67]. Symptoms include fever (may be absent in the presence of high dose corticosteroid therapy), dry cough, shortness of breath, pleuritic chest pain, and hemoptysis. Pulmonary infiltrates may lag behind disease progression. In lung transplant patients and those with AIDS, *Aspergillus* tracheobronchitis can present with cough, wheezing, and shortness of breath and chest radiographs show normal lungs with or without atelectasis [68].

Disseminated Aspergillosis

A variety of signs and symptoms are seen with disseminated invasive aspergillosis, based on the organs involved. Involved organs include the kidneys, liver, spleen, and CNS (signs and symptoms of stroke or meningitis) most frequently, followed by the heart, bone, skin, and other organs [69]. Aspergillosis of the skin can occur either as a manifestation of disseminated disease or by direct extension from a local inoculation, for example, from an intravenous catheter [70].

Sinusitis

Aspergillosis of the sinuses presents clinically like rhinocerebral mucormycosis, but is more common in neutropenic patients than in those with diabetic ketoacidosis, and inflammatory signs may thus be less frequent. Fever, nasal congestion, facial pain can progress to visual changes, proptosis, and chemosis if the infection spreads to the orbit. Posterior extension to the brain can lead to cranial nerve palsies, other focal neurologic deficits as well as a depressed level of consciousness [71].

Endocarditis

Aspergillus endocarditis is the second most common form of fungal endocarditis after that caused by *Candida* species and occurs in prosthetic valve recipients and in native cardiac valves in intravenous drug users and patients with indwelling central venous catheters [72]. Clinically, these patients present with fever and embolic complications. Blood cultures are rarely positive even with extensive disease [73].

Diagnosis

The availability and utility of non-culture based diagnostics have improved the diagnosis of invasive aspergillosis. However, the clinician must rely on the combination of risk factors, a high index of suspicion, clinical judgment, and the mycologic markers of infection (Table 11.2) [74]. The diagnosis of proven invasive aspergillosis requires both tissue biopsy demonstrating invasion with hyphae and culture positive for *Aspergillus* species [75]. *Aspergillus* produce hyaline, 3–6 μm wide septate hyphae that typically branch at acute angles [43] (Fig. 11.2). In tissue, these features can often distinguish *Aspergillus* from agents of mucormycosis, but they cannot distinguish *Aspergillus* from a large number of other opportunistic molds, including *Fusarium* and *Scedosporium* (*Pseudallescheria*) [76, 77]. Thus, culture is needed to confirm the diagnosis and also provides the possibility of obtaining fungal susceptibility testing [78, 79]. Invasive procedures for tissue biopsy are infrequently undertaken in high-risk patients. Bronchoalveolar lavage (BAL) testing is less invasive and can help establish a diagnosis, especially with use of non-culture-based diagnostics [75, 80].

Plain chest radiography is of limited utility in invasive aspergillosis as it has low sensitivity and specificity in this disease [81]. In contrast, chest CT scans have proven useful in early diagnosis of invasive pulmonary aspergillosis as the "halo sign" of low attenuation surrounding a pulmonary nodule has successfully been used as a marker for early initiation of therapy in high-risk patients with neutropenia or who have undergone hematopoietic stem cell transplantation [3, 11, 82]. Of note, these radiographic findings are also consistent with other infections such as *Nocardia* species, which may increase over the first week of therapy even when the patient in improving; follow-up scans should be ordered and interpreted cautiously with full attention to the clinical progress of the patient [11].

Table 11.2 Diagnosis of invasive aspergillosis

Diagnostic method	Comment
Respiratory culture	Not frequently positive early in course of infection; positive result in high-risk patient (bone marrow transplant, neutropenia) highly correlates with infection; may indicate colonization in other populations (chronic pulmonary diseases, lung transplant)
Galactomannan	*Aspergillus* Platelia system (BioRad, Redmond, WA) with variable sensitivity—low (~40%) with single samples or prior antifungal therapy or prophylaxis; better yield with reduced threshold for positivity (≥0.5), serial samples, testing on BAL samples
1,3-β-D-glucan	Nonspecific detection of cell wall glucan. Commercially available Fungitell™ assay (Associates of Cape Cod, Falmouth, MA), false positives common
PCR	Accepted as diagnostic criteria for aspergillosis; laboratory developed test in USA; may be useful for detecting some resistance mutations; lower cycle thresholds associated with greater likelihood of diagnosis
Computed tomography	In high-risk patient, "halo" sign and/or pulmonary nodules without other documented cause may be a frequent and early sign of invasive pulmonary aspergillosis

BAL bronchoalveolar lavage

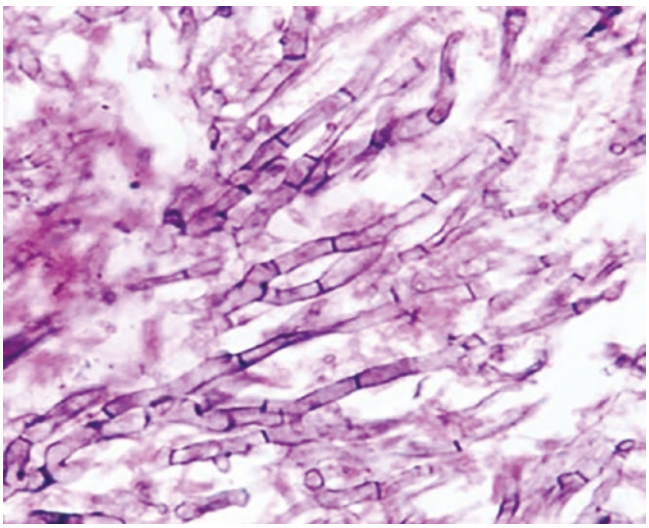


Fig. 11.2 Periodic Acid-Schiff (PAS) stained tissue section of lung showing dichotomously branched, septate hyphae of *Aspergillus fumigatus*. (Courtesy of www.doctorfungus.org)

Non-culture diagnostic tests have also been used to diagnose aspergillosis and in attempts to preempt difficult to treat proven disease. *Aspergillus* galactomannan detection (Platelia *Aspergillus*, BioRad, Redmond, WA) is used for serum and BAL fluid testing and is accepted as criteria to establish a diagnosis of invasive aspergillosis [75, 83]. Extensive studies have documented the utility of this method, with BAL frequently more likely to be positive than serum. Serial testing in asymptomatic patients receiving mold-active prophylaxis significantly reduces test sensitivity, but in symptomatic patients testing for galactomannan is still useful [84]. Less data are available to support the utility of serial measurement of galactomannan measurements to assess outcome, but serial progression of galactomannan is a poor prognostic sign [85–87].

Extensive studies of PCR methods have led to PCR as a diagnostic criterion for invasive aspergillosis [88–90]. In the USA, these are only available as laboratory-developed tests that are done in reference laboratories. Notably, these methods may also be able to detect some antifungal drug resistance mutations and can help guide therapy [91]. The utility of PCR testing is increased when combined with other non-culture-based methods and clinical assessment [80, 89].

Other non-culture-based methods for the diagnosis of invasive aspergillosis include detection of the non-specific fungal marker 1,3-β-D-glucan. This assay (Fungitell™, Associates of Cape Cod, Falmouth, MA) is commercially available and is positive in infections due to many fungi including *Aspergillus* and *Candida*, but not *Cryptococcus* or Mucorales (which contain little or no 1,3-β-D-glucan) [92, 93]. Since detection of beta-D-glucan can occur in infection due to yeast or molds, it has resulted in beta-D-glucan not being included as a diagnostic criterion for mold infection, including aspergillosis. Interpretation of results is also complicated with false positive results, including those due to hemodialysis with cellulose membranes, intravenous immunoglobulin, albumin, gauze packing of serosal surfaces, some bacterial bloodstream infections [94–96].

Treatment

The goals of treatment of patients with invasive aspergillosis are to control infection and to reverse any correctable immunosuppression. Patients at high-risk of developing invasive aspergillosis should be treated based on clinical or radiological criteria alone if microbiological or histological diagnosis would significantly delay treatment [2].

Treatment of *Aspergillus* infection is challenging due to difficulty in diagnosis, the presence of advanced disease in many by the time of diagnosis and the presence of severe, often irreversible, immunosuppression. Mortality rates are high in patients with invasive aspergillosis and the efficacy of currently available treatments is limited by spectrum of activity, extensive drug–drug interactions and serious toxicity. Treatment failure with currently available antifungal medication in patients with invasive aspergillosis has been reported to be 40% or higher in some series [3–5]. Mortality in recent clinical trials of mold-active azoles was noted to be 19–20% at 6 weeks, with 12-week mortality ranging from 30–36%. Antifungal therapies with activity against *Aspergillus* include triazoles (voriconazole, posaconazole, and isavuconazole), lipid formulations of amphotericin B, and the echinocandins (caspofungin, micafungin, and anidulafungin), all of which offer options for therapy of this disease [2] (Table 11.3). Guidelines developed by the Infectious Diseases Society of America, the American Thoracic Society,

Table 11.3 Antifungal agents for treating invasive aspergillosis

Agent	Typical dose / route of administration	Comments
Azole		
Voriconazole	6 mg/kg IV q12 h × 2 doses, then 4 mg/kg IV q12 h; 200 mg PO bid (although weight based dosing should be considered)	Recommended primary therapy for invasive aspergillosis; drug interactions common, hepatic toxicity (10–15%) may be dose limiting; visual effects common (~30%), but not usually dose limited and no long-term toxicity reported [97]
Posaconazole	Extended release tablets – 300 mg bid × 2 doses, then 300 mg daily; intravenous – 300 mg bid × 2 doses, then 300 mg daily:	Recommended for salvage therapy; for prophylaxis; limited metabolism with favorable tolerance in clinical studies [98]; similar efficacy and fewer adverse events vs. voriconazole [5]; posaconazole-induced pseudoaldosteronism (PIPH) syndrome reported [99]
Isavuconazole	200 mg PO or IV every 8 h for 6 doses, then 200 mg once daily	Similar efficacy vs. voriconazole with fewer adverse events; less cytochrome 3A4 interactions compared to other mold-active azoles; associated with shortened QTc interval
Itraconazole	200 mg tid for 3 days, then 200 mg PO bid (oral solution)	Second-line agent for invasive aspergillosis; erratic bioavailability, improved with oral solution; drug interactions, including chemotherapeutic agents; intravenous formulation no longer available [2]
Polyene		
Amphotericin B deoxycholate	1.0–1.5 mg/kg IV daily	Prior "gold standard"; associated with significant toxicity and limited efficacy; most guidelines recommend against use [2]
Liposomal amphotericin B	3–6 mg/kg IV daily	Alternative primary therapy; well tolerated; limited nephrotoxicity or infusion-related reactions; anecdotal reports of efficacy with higher doses (7.5 mg/kg/d or more) [100]
Amphotericin B lipid complex	5 mg/kg IV daily	Indicated for salvage therapy or intolerance to standard agents, generally well tolerated [101]
Echinocandin		
Caspofungin	70 mg × 1 dose, then 50 mg IV daily	Indicated for salvage therapy of aspergillosis, experimental and clinical data for use in combination therapy; well tolerated [2, 102]
Micafungin	Investigational for aspergillosis (IV)	Used in doses of 100 mg/day in salvage studies; 50 mg/day for prophylaxis; well tolerated [103]
Anidulafungin	Investigational for aspergillosis (IV)	In vitro activity; studied at doses of 100 mg/day after 200 mg loading dose in other fungi; well tolerated; studies in combination with voriconazole for invasive aspergillosis [104]

IV, intravenous; *PO* orally, *bid* twice daily, *qid* four times daily

European Conference on Infections in Leukemia (ECIL)-6, and the European Society of Chemotherapy and Infectious Diseases-European Confederation of Medical Mycology-European Respiratory Society (ESCMID-ECMM-ERS) provide summaries of existing data as well as recommendations [2, 105, 106]. Although large randomized trials of all 3 mold active azoles have been conducted, many questions remain, including the question of optimal regimens and the use of combination therapy. Thus, many recommendations stem from non-randomized and non-comparative studies, as well as expert consensus [2].

Azoles

Voriconazole is a potent, broad-spectrum, triazole that has fungicidal activity against many *Aspergillus* species, including *A. terreus*, is approved for therapy of invasive aspergillosis, and has replaced amphotericin as the recommended primary therapy for most patients with invasive aspergillosis [2, 105]. This recommendation is based on data from a randomized trial that compared voriconazole to conventional amphotericin B for the primary treatment of invasive aspergillosis, with each agent followed by other licensed antifungal therapy if needed for intolerance or progression of disease, in severely immunocompromised patients with invasive aspergillosis [3]. In this trial, voriconazole was superior to amphotericin B with successful outcomes in 52% of patients as compared to only 31% in those receiving amphotericin B. In addition, voriconazole demonstrated a survival advantage to amphotericin B with an absolute 13% difference in mortality between treatment groups.

In clinical trials, voriconazole has been adequately tolerated and offers an advantage of oral and intravenous therapy. There are a number of issues to consider, including important drug interactions, especially those with immunosuppressive agents such as cyclosporine, tacrolimus, and sirolimus and intolerance to the drug. The most common adverse event has been a transient and reversible visual disturbance, described as an altered perception of light which has been reported in approximately 30% of treated patients. This effect has not been associated with pathologic changes and rarely requires drug discontinuation [3]. Other adverse events include liver function test abnormalities in 10–15%, which can be dose limiting, and skin rash in 6% (sometimes associated with sun exposure). Long term voriconazole therapy has been associated with skin cancer and periostitis related to high fluoride levels [107–109].

Both toxicity (e.g., liver function abnormalities and CNS side effects increase with higher levels) and efficacy (i.e., poorer outcomes with lower levels) have been associated with voriconazole concentrations. As voriconazole metabolism varies between patients and is affected by so many relevant drug–drug interactions, therapeutic drug monitoring is recommended in most settings [2]. Serum concentrations between 2 and 5.5 μg/mL are associated with successful outcomes, with more toxicity associated with higher levels, particularly involving the central nervous system [110, 111].

Itraconazole is approved for use as salvage therapy of aspergillosis. Its utility has been limited due to the fact that the only reliably absorbed formulation is an oral solution, as its intravenous formulation is no longer marketed in the USA. For these reasons, itraconazole is more frequently used in less immunosuppressed patients who are able to take oral therapy and those with chronic pulmonary aspergillosis syndromes [2]. A new SUBA-itraconazole formulation of itraconazole provides increased bioavailability of the drug [112].

Isavuconazole has been studied in a large blinded, randomized, controlled trial compared to voriconazole for treatment of invasive aspergillosis [4]. In that study, isavuconazole was as effective as voriconazole with significantly fever adverse events, particularly eye findings, skin, and hepatobiliary events [4]. Notably, bioavailability of isavuconazole was very good, with consistent drug levels detected. In contrast to other azole drugs, isavuconazole is associated with a shortened QTc interval which may offer an advantage of its use in patients requiring other drugs, which may prolong the QTc interval [4]. Isavuconazole is available in an intravenous prodrug formulation, isavuconazonium, which does not require the use of a cyclodextrin. In comparison to other mold-active azoles, isavuconazole appears to have less cytochrome P450 interaction [113]. The routine measurement of isavuconazole may not be routinely required due to consistent bioavailability and may be reserved for patients with progressive disease, potential drug–drug interactions, or toxicities [2].

Posaconazole is FDA approved for invasive aspergillosis and for prophylaxis of fungal infections in neutropenic patients. It has also been studied in patients who failed to tolerate or had fungal infections refractory to standard therapy [114]. In 2005, posaconazole was approved in the EU for salvage therapy of invasive aspergillosis. Initially available only as an oral suspension, in 2013, the FDA approved delayed release tablets with higher absorption and less dependency on having a full stomach, and in 2014, an intravenous formulation was approved. These oral and intravenous formulations dramatically improved bioavailability of the drug [115]. A large randomized trial compared posaconazole to voriconazole in patients with invasive aspergillosis [5]. In this trial, outcomes with posaconazole were similar to voriconazole, with mortality in patients with proven or probable invasive aspergillosis at 6 weeks 19% and 20% in the two arms, respectively [5]. Adverse events were 10% more frequent with voriconazole as compared to posaconazole. Notably, metabolic effects, especially hypokalemia, were

more common with posaconazole (6% versus 2%). A syndrome of posaconazole-induced pseudohyperaldosteroism (PIPH) due to inhibition of enzymes in the cortisol metabolism pathway has been described [99, 116–118]. This syndrome is associated with hypertension, hypokalemia, and alkalosis [118]. PIPH appears to be associated with higher posaconazole levels (>4 μg/mL) [99]. Drug levels with the intravenous and extended release tablet formulations are significantly improved compared to earlier formulations, but low serum concentrations have been reported in some high-risk patients (e.g., patients with graft versus host disease) [119]. Measurement of posaconazole levels may be necessary to document adequate concentrations in some patients and to evaluate for possibility toxicities [2].

Polyenes

Amphotericin B deoxycholate was the previous "gold standard" therapy in patients with invasive aspergillosis, but it is associated with unacceptable toxicities and lack of efficacy [2]. Most guidelines recommend against the use of amphotericin B deoxycholate due to its intolerability [2, 105]. The lipid formulations of amphotericin B are associated with decreased toxicity and allow the administration of higher doses of drug [100, 101]. Few comparative studies of the efficacy of lipid formulations of amphotericin B in treating invasive aspergillosis have been conducted, though studies of these drugs as salvage therapy led to the approval of three lipid formulations [120]. Extensive clinical experience supports the use of lipid formulations in aspergillosis and their inclusion in guidelines for treatment of invasive aspergillosis [2, 105]. A double-blind trial of liposomal amphotericin B in patients with confirmed aspergillosis (most with hematologic malignancy and neutropenia) compared the efficacy of 10 mg/kg per day versus 3 mg/kg per day dosing for the first 14 days of treatment, followed by receipt of 3 mg/kg/day [121]. Patients treated with higher initial doses experienced more nephrotoxicity and success rates were similar. Based on these data, liposomal amphotericin B at 3 mg/kg/day is recommended as alternative primary therapy for those patients unable to tolerate voriconazole or in whom voriconazole or another mold-active azole is contraindicated because of drug interactions or other reasons. Amphotericin B lipid complex (usually at initial doses of 5 m/kg once daily) is also a reasonable alternative [2].

Echinocandins

Echinocandins are natural cyclic hexapeptide antifungal compounds that non-competitively inhibit 1,3 β-D-glucan synthase, an enzyme complex that is unique to a number of fungi that forms glucan polymers in the fungal cell wall [122]. These agents are active against *Candida* species and *Pneumocystis*. Specific modifications to the N-acyl aliphatic or aryl side chains expand the antifungal spectrum to include *Aspergillus* [122]. These agents are all poorly bioavailable and produced in intravenous formulation only.

Caspofungin is approved for treating patients refractory to or intolerant of standard therapies for invasive aspergillosis based on an open-label trial that demonstrated therapeutic efficacy in 22 of 54 (41%) patients studied [102]. Caspofungin has been very well tolerated in clinical trials; in the aspergillosis study, only approximately 5% of patients discontinued therapy. Drug interactions with these agents are minimal. In March 2005, micafungin was approved for the treatment of esophageal candidiasis and prevention of *Candida* infections. In the one prophylaxis study used to support this approval, micafungin may have reduced the number of *Aspergillus* infections as compared to standard prophylaxis with fluconazole [123]. Micafungin also demonstrated efficacy when used as salvage therapy and in prevention of invasive fungal infection in patients with hematologic malignancy at high-risk due to neutropenia or graft versus host disease [103]. Anidulafungin is another echinocandin with activity against *Aspergillus* spp. that appears to have a favorable toxicity profile similar to the other echinocandins. It was approved by the FDA in February 2006 for candidemia and other *Candida* infections (including abdominal abscess, peritonitis, and esophagitis). Notably, these agents are neither classically fungicidal nor fungistatic for *Aspergillus,* but exert their effect on the growing hyphal tips where the glucan synthase target is located [124]. For this reason, they have not frequently been used for primary therapy where outcomes have been poor and have been more frequently used as salvage therapy, or more recently, in combination regimens [104, 125].

Combination Therapies and Therapeutic Approaches

Outcomes for patients with invasive aspergillosis remain poor despite the advent of newer antifungal agents. With the poor outcomes seen in high-risk patients and with the availability of several antifungal drugs and drug classes against *Aspergillus,* interest has increased in the use of combination antifungal therapy for invasive aspergillosis [126, 127]. Marr and colleagues reported on a historical control study of caspofungin and voriconazole compared with voriconazole alone in patients who failed amphotericin formulations in 2004. In this study, the use of combination salvage therapy was associated with an improved 3-month survival rate [128]. A subsequent randomized trial evaluated voriconazole versus the combination of voriconazole with anidulafungin

for the treatment of invasive aspergillosis in patients with hematologic malignancies and/or hematopoietic cell transplant [104]. Among the 277 patients with proven or probable invasive aspergillosis, six-week mortality was 19.3% for combination therapy patients and 27.5% for those treated with voriconazole monotherapy (95% CI -19.0 to 1.5). A post-hoc analysis of patients with probable invasive aspergillosis—diagnosed with a positive galactomannan—showed a significant difference in mortality (16% with combination therapy versus 27% with voriconazole monotherapy; 95% CI -22.7 to −0.4). Most current guidelines do not recommend routine use of initial combination therapy, but these results suggest that some subgroups of patients may benefit from such an approach. Based on these data, current recommendations are to consider combination therapy in patients who fail to respond to initial therapy and in select patients as primary therapy [2].

Preventative strategies include prophylaxis and targeted preemptive therapy in high-risk patients. Two large randomized clinical trials in patients with graft versus host disease and in acute leukemia or myelodysplastic syndromes showed the benefit of posaconazole prophylaxis in those patients, with improved survival and decreased invasive mycoses, including aspergillosis [129, 130]. Other strategies include intensive use of diagnostic tools in conjunction with early antifungal therapy in order to reduce the number of invasive fungal infections [131].

Adjuvant therapies, including surgical resection or use of granulocyte transfusions and growth factors, in invasive aspergillosis can augment antifungal therapy, although their utility has not been established in randomized trials. In older studies, surgical resection of isolated pulmonary nodules prior to additional immunosuppressive therapies was shown to improve outcome of infection. With the use of newer, more effective therapies, like the mold-active azoles, resection may not be necessary or indicated [2, 132] . Recent studies also suggest that the majority of patients will have bilateral infection when the diagnosis is first made, limiting the utility of this approach. Surgical resection may be most appropriate in patients with severe hemoptysis or with lesions near the hilar vessels or pericardium.

Summary

In summary, prompt diagnosis and aggressive initial therapy remain critical in improving the outcome of this infection [133]. Radiography and measurement of galactomannan or testing with PCR may facilitate an early detection of aspergillosis in high-risk patients, for whom outcomes are especially poor [82]. Primary therapy with voriconazole is recommended in most patients [2]. Other mold-active agents such as isavuconazole and posaconazole may have fewer adverse effects than voriconazole and offer similar efficacy. In patients who are intolerant of voriconazole, have a contraindication to the drug, or have progressive infection, alternative agents include lipid formulations of amphotericin B in addition to the other mold-active azoles or echinocandins [2, 134]. Primary use of combination therapy is not recommended for routine use, but may be useful in patients with extensive infection and in those at high risk for poor outcomes [2]. Sequential therapy with oral azoles after initial intravenous therapy may be a useful option [13]. Although the optimal duration of antifungal therapy is not known, improvement in underlying host defenses is crucial to successful therapy. While substantial advances have recently been made in the management of invasive aspergillosis, newer approaches to therapy, including the potential of more targeted combination therapy and newer diagnostic tools, are needed to improve the outcome of this disease.

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Hyalohyphomycosis: Infection Due to Hyaline Molds

12

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Introduction

The term hyalohyphomycosis is used to refer to invasive mycoses that have in common the invasion of tissue of hyaline hyphae, and is a counterpart to the term phaeohyphomycosis, in which fungi appear in tissues as septate pigmented hyphae. The mycoses encompassed in this group are very heterogeneous, with only the presence of hyaline hyphae in tissue (without pigment in the wall) as a common characteristic. The term hyalohyphomycosis is clinically useful when the pathogen (genus and/or species) is not identified, and hyaline septate hyphae are observed on histopathology. When the causative agent is recovered (e.g., *Fusarium* species) a more specific term (e.g., fusariosis) should be used.

Hyalohyphomycosis includes infection caused by *Fusarium*, *Scedosporium*, *Paecilomyces*, *Acremonium*, *Scopulariopsis*, *Talaromyces*, and others. Of note, *Aspergillus* species present in tissue as hyaline hyphae. Therefore, a case of invasive aspergillosis may be considered as hyalohyphomycosis whenever the genus *Aspergillus* is not identified. Some species of *Scedosporium* and *Scopulariopsis* produce darkly pigmented hyphae and may be included in the phaeohyphomycoses.

The spectrum of infection caused by hyalohyphomycetes is broad, and includes superficial or localized infection in immunocompetent hosts, usually as a result of direct inoculation of the fungus following trauma, and disseminated or invasive disease in immunocompromised hosts, such as patients with acute leukemia, hematopoietic cell transplant (HCT) and solid organ transplant (SOT) recipients.

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Fusariosis

Fusarium species are widely found in nature, including water, soil, and air [1]. They are found in water biofilms, including municipal and hospital water systems, and are important plant pathogens [2, 3]. In humans, the most frequent infections caused by *Fusarium* species are onychomycosis and keratitis.

Etiologic Agents

While more than 20 phylogenetically distinct species complexes of *Fusarium* have been described [4, 5], medically important species are grouped into seven species complexes (Table 12.1): *Fusarium solani* species complex (FSSC), *Fusarium oxysporum* species complex (FOSC), *Fusarium fujikuroi* species complex (FFSC), *Fusarium incarnatum-equiseti* species complex (FIESC), *Fusarium chlamidosporum* species complex (FCSC), *Fusarium dimerum* species complex (FDSC), and *Fusarium sporotrichoides* species complex (FSAMSC) [6]. Approximately 70% of cases of invasive disease are caused by FSSC and FOSC [1]. However, geographic variations may be observed, with a predominance of FSSC in cases reported in Brazil [7, 8], and FFSC (especially *F. verticillioides,* and *F. proliferatum*) predominating in European countries [9].

Fusarium species grow rapidly on many media without cycloheximide. On potato dextrose agar, the colonies have velvety to cottony surfaces, and are colored in pink, yellow, red, gray, with purple shades, or white (Fig. 12.1). Direct exam of biological materials growing *Fusarium* species

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_12

Table 12.1 Fusarium species complex and their respective species within each complex

Species complex	Species
Fusarium solani	*F. falciforme*, *F.keratoplasticum*, *F. lichenicola*, *F. petroliphilum*, *F. pseudensiforme*
Fusarium oxysporum	*F. Oxysporum*, *unnamed*
Fusarium fujikuroi	*F. acutatum*, *F. anthophilum*, *F. andiyazi*, *F. fujikuroi*, *F. nygamai*,*F. proliferatum*, *F. verticillioides*
Fusarium incarnatum-equiseti	*F. incarnatum*, *F. equiseti*, *unnamed*
Fusarium chlamidosporum	*F. chlamydosporum*
Fusarium dimerum	*F. dimerum*, *F. delphinoides*, *F. penzigii*
Fusarium sporotrichoides	*F. Aermeniacum*, *F.brachygibbosum*, *F. langsethiae*, *F.sporotrichioides*

shows irregular hyaline septate branched hyphae and swollen cells (Fig. 12.2). They produce fusoid macroconidia (hyaline, with several transverse septa, banana-like clusters with foot cells at the base), microconidia (hyaline, unicellular, ovoid to cylindrical in small slimy heads or in chains) and blastoconidia (produced singly on polyblastic cells) (Fig. 12.3). Hyaline, thick-walled chlamydospores may be present, intercalary or in terminal position. Species identification is difficult and requires molecular methods [10–12]. Matrix-assisted laser desorption/ionization flight time (MALDI-TOF) has been evaluated in species identification, both in pure colonies and directly from bottles of positive blood cultures [13, 14].

In tissue, the filaments are hyaline, septate, with 3–8 μm in diameter, and dichotomize at acute and at right angles. In

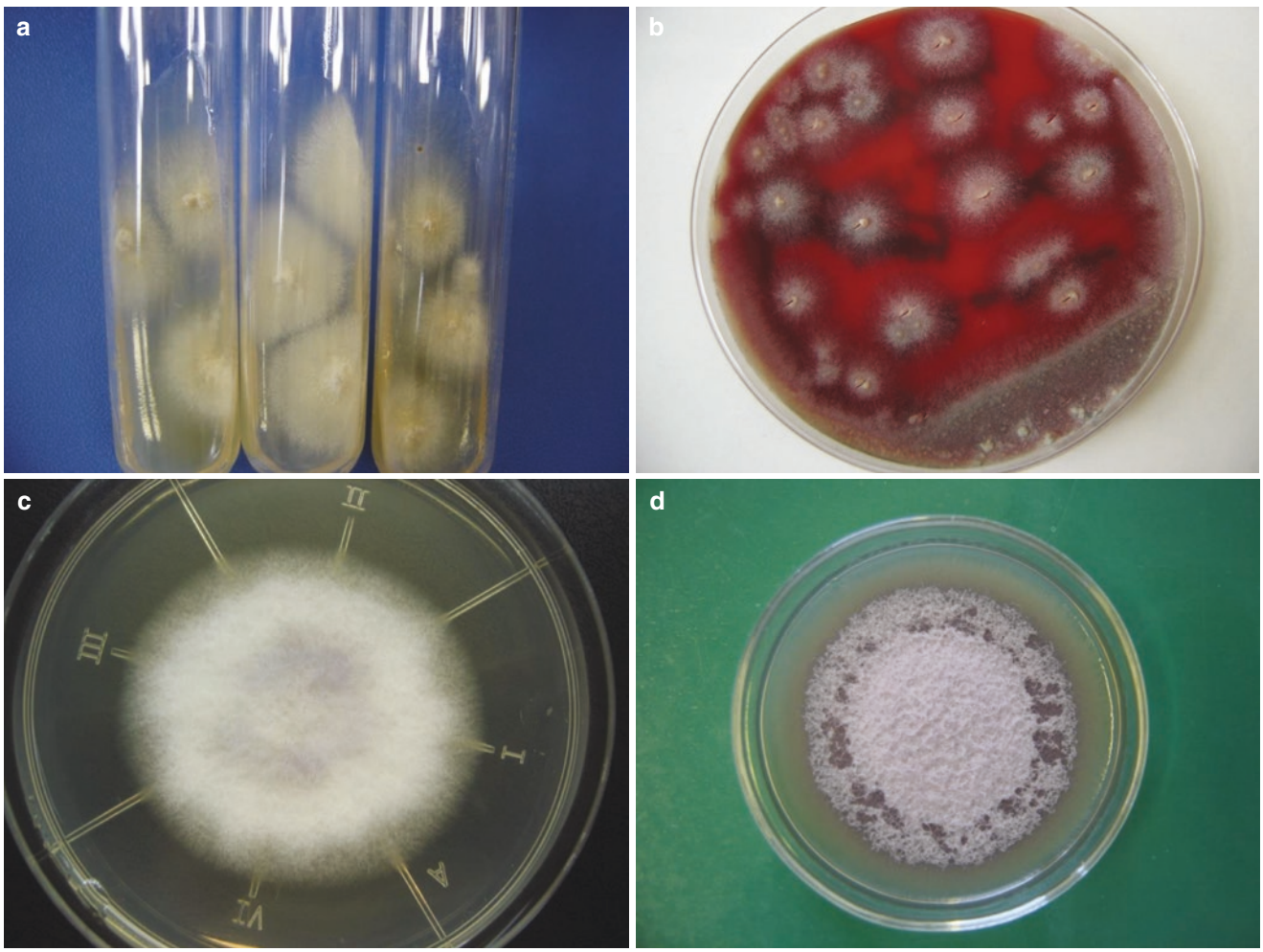


Fig. 12.1 (**a**) Colonies of *Fusarium* species grown on Sabouraud's Dextrose Agar, Brain Heart Infusion agar, and Yeast-Extract agar; (**b**) Primary culture on Blood-agar; (**c**) colonies on potato dextrose agar; (**d**) Colony on oat meal agar with shades of purple

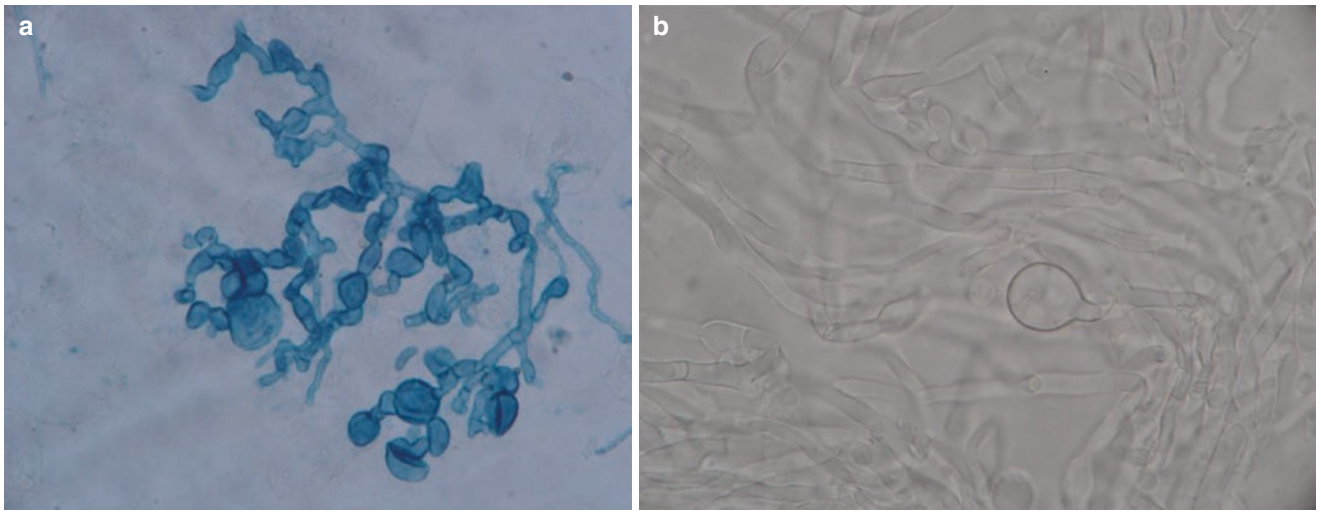


Fig. 12.2 Direct mycological exam of skin scraping (culture: *Fusarium* species) showing irregular hyaline septate branched hyphae and swollen cells (**a**) KOH 20% + Parker Ink (Permanent Black), 400×; (**b**) KOH 20%, 400×

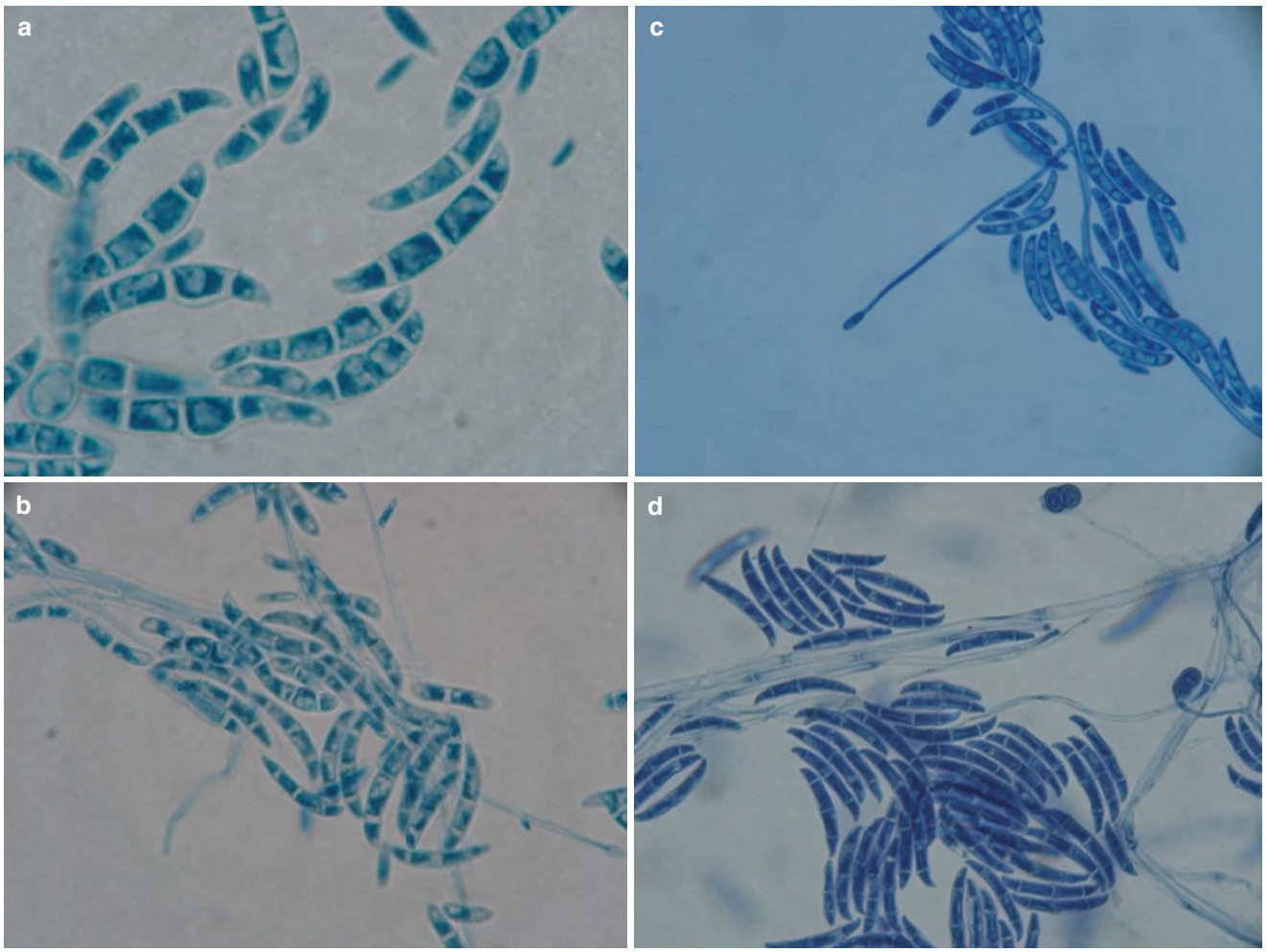


Fig. 12.3 Microscopy of *Fusarium* species. Slide cultures on Potato Dextrose Agar – Lactophenol cotton blue – 400× (**a**–**d**) fusoid septate macroconidia; (**c**) monophialide; (**d**) chlamydospores

addition, adventitious sporulation, producing yeast-like structures (aleuroconidia) may be present. In severely immunocompromised patients, these structures invade the bloodstream and cause disseminated disease, with metastatic skin lesions and frequently positive blood cultures [15]. The finding of hyphae and yeast-like structures together in tissue is highly suggestive of fusariosis in the high-risk population.

Epidemiology and Clinical Spectrum of Fusariosis in Non-immunocompromised Patients

Fusariosis can present in a variety of superficial and deep infection in non-immunocompromised patients (Table 12.2). Keratitis and onychomycosis are the most frequent clinical forms. Keratitis usually occurs after trauma (including surgery), or in contact lens users [16]. Predisposing factors include ocular surface problems, agricultural work, topical corticosteroids or antibiotics, and diabetes [17, 18]. Outbreaks of keratitis have been reported. In one outbreak, the occurrence of 164 cases of keratitis in the USA were associated with the use of a specific contact lens solution (ReNu with MoistureLock) [19]. Other outbreaks were subsequently reported in France and Singapore [20–22].

Onychomycosis is usually caused by dermatophytes, but cases of onychomycosis caused by *Fusarium* species have been increasingly reported [23–26]. In addition to onychomycosis, cases of interdigital intertrigo, tinea pedis, and hyperkeratotic plantar lesions caused by *Fusarium* species may occur [27, 28]. The reasons for the emergence of non-dermatophyte molds as causes of superficial skin infections are not clearly understood.

Fusarium species may also cause skin and soft tissue infection as a result of burns or trauma, presenting as cellulitis with necrosis, abscess formation, and ulcers. Nodules and eumycetomas, with tumefaction and draining secretions with grains, may also occur [29–32].

Other forms of localized infection caused by *Fusarium* species include peritonitis in patients receiving continuous ambulatory peritoneal dialysis [33, 34] and in patients with liver cirrhosis [35], sinusitis [36], pneumonia (including in a patient with Covid-19) [37–40], fungemia [32, 41], thrombophlebitis [42], septic arthritis [43], osteomyelitis [44], and endophthalmitis [45, 46]. Sinusitis may manifest as both allergic and chronic non-invasive disease [47]. An allergic form of pneumonia has also been described [48].

Table 12.2 Clinical spectrum of fusariosis in non-immunocompromised patients

Eye infection
Keratitis
Endophthalmitis
Skin and subcutaneous tissues
Onychomycosis
Interdigital intertrigo
Tinea pedis
Cellulitis after burn or trauma
Peritonitis
Arthritis
Sinusitis and pneumonia
Allergic
Chronic invasive

Epidemiology and Clinical Spectrum of Fusariosis in Immunocompromised Patients

Immunocompromised patients at high risk to develop fusariosis are those with severe neutropenia caused by an underlying hematologic disease (usually acute leukemia) and/or its treatment, and HCT recipients with or without neutropenia [1]. A study describing the epidemiology of invasive fusariosis in HCT recipients treated in centers in Brazil and in the USA reported an overall incidence of ~6 cases per 1000 HCT, being lowest in autologous (1.5–2/1000 HCT), intermediate in HLA matched donor allogeneic HCT (2.5–5/1000) and highest (20/1000 HCT) in mismatched related donor HCT [49]. A trimodal distribution was observed, with a first peak occurring in the pre-engraftment period, in the context of severe neutropenia, the second occurring ~day+70, in the context of acute graft versus host disease (GVHD), and the third occurring >1 year, in the context of chronic GVHD.

Another prospective epidemiologic study conducted in eight Brazilian Hematology centers, evaluated 378 allogeneic HCT, 322 autologous HCT, and 237 patients with acute myeloid leukemia (AML) or myelodysplasia (MDS) undergoing induction remission chemotherapy [50]. Transplant recipients were followed for one year, and patients with AML or MDS were followed until the end of consolidation therapy. Invasive fusariosis was the most frequent invasive fungal disease, with 23 episodes, and an incidence of 5.2% in AML/MDS, 3.8% in allogeneic HCT, and 0.6% in autologous HCT. The 6-week probability of survival of patients with invasive fusariosis was 41%. Risk factors for invasive fusariosis in this patient population were identified. Among patients with AML/MDS, active smoking was the only significant variable, whereas in allogeneic HCT, receipt of antithymocyte globulin, hyperglycemia, and AML as underlying disease were risk factors for invasive fusariosis in the pre-engraftment period, while non-myeloablative conditioning regimen, grade III-IV GVHD and previous invasive mold disease were risk factors in the post-engraftment period [51].

More recently, a prospective study evaluated the epidemiology of invasive fungal disease occurring during neutropenia in 71 allogeneic and 51 autologous HCT recipients, 46 patients with AML, and 24 patients with acute lymphoid leukemia (ALL) [52]. Three cases of invasive fusariosis were diagnosed: two in patients with AML and one in an autologous HCT recipient (incidences of 4.3% and 2.0%, respectively).

Epidemiologic studies conducted in other countries reported much lower incidences of invasive fusariosis in patients with hematologic malignancies and in HCT recipients. The TRANSNET study, conducted in centers in the USA, reported an incidence <0.3% [53] and a multicenter Italian study reported an incidence of 0.2% in HCT recipients [54]. The reasons for these differences in the incidence of invasive fusariosis are not clear.

Nosocomial outbreaks of invasive fusariosis occurring in immunocompromised patients have been reported. In a prospective study, *Fusarium* species were recovered from the hospital water system: water, water storage tanks, shower and sink drains, shower heads, and sink faucet aerators and from hospital air and other environments. In addition, showering and other water-related activities increased the dispersion of airborne fusarial conidia. The acquisition of invasive fusariosis from the hospital water was documented, as shown by the close molecular relatedness between water and patients' isolates [55]. Another study conducted in a Brazilian hospital reported an increased incidence of invasive fusariosis in hematologic patients. Genetic relatedness was observed among five isolates from patients' blood cultures and seven isolates from the hospital air [56].

In our hospital, an increase in the incidence of invasive fusariosis with a cutaneous portal of entry (onychomycosis and interdigital intertrigo) was observed in 2007.We suspected that patients with previous skin lesions in the extremities became colonized and subsequently infected by *Fusarium* in the hospital, since *Fusarium* had been recovered from the hospital water system [57]. However, while cases of invasive fusariosis were caused by FSSC, most environmental isolates were FOSC, and among concordant species, most did not exhibit genotypic similarities [58]. Furthermore, in the same period, we observed an increase in the recovery of *Fusarium* species from skin lesions of immunocompetent patients from the Dermatology outpatient unit [57]. Taken together, these data indicated a community origin of invasive fusariosis, and underscore the lack of a complete understanding of the sources and epidemiology of *Fusarium* both in the community and in the hospital.

Other immunocompromised patients, such as SOT recipients, patients with rheumatic diseases, solid tumor, or infection by HIV may develop invasive fusariosis, but the incidence is much lower compared with patients with hematologic malignancies [59–61].

Invasive fusariosis in immunocompromised patients may involve any organ, and usually occurs in the context of disseminated disease (Table 12.3). For example, in a series of 233 cases of invasive fusariosis treated in 44 centers worldwide, lung involvement was present in 114 patients. Only nine had pneumonia as the sole manifestation of invasive fusariosis [62].

Clinical Manifestations of Fusariosis in Non-immunocompromised Patients

The clinical presentation of keratitis caused by *Fusarium* species is indistinguishable from other causes of keratitis, with complaints of reduced visual acuity, redness, pain, photophobia and corneal opacity. The serrated edge finding on ophthalmic examination is frequent (up to 79%), but not pathognomonic [16].The typical clinical presentation of onychomycosis caused by *Fusarium* species is that of distal subungueal lesion in the toe nails, occurring more frequently in females [27].

Clinical Manifestations of Fusariosis in Immunocompromised Patients

Invasive fusariosis in immunocompromised patients manifests in four major clinical presentations. The most frequent is disseminated disease, with the sudden appearance of one or (more frequently) various skin lesions (detailed description below) in the context of severe neutropenia and persistent or recurrent fever [63]. Patients usually complain of generalized myalgia, and have a toxemic appearance. Other clinical presentations of invasive fusariosis include pneumonia, fever, and positive blood culture for a mold, and superficial infection in the feet with subsequent lymphangitis.

Table 12.3 Clinical spectrum of fusariosis in immunocompromised patients

Endophthalmitis
Cellulitis at sites of skin breakdown, onychomycosis or interdigital intertrigo
Fungemia
Disseminated disease
Sinusitis
Pneumonia
Arthritis

Skin Involvement

Skin lesions occurring in the context of disseminated disease are painful, erythematous, papular, or nodular, with or without central necrosis, with an echthyma gangrenosum-like appearance. Target lesions (a thin rim of erythema of 1–3 cm in diameter surrounding the above-mentioned papular or nodular lesions) may be present. The lesions may appear in any part of the body, with predominance in the arms and legs. Lesions at different stages of evolution (papules, nodules, and necrotic lesions) may be present [32].

Skin involvement in fusariosis may also represent a primary site of infection, serving as a portal of entry for disseminated disease. Onychomycosis with periungueal cellulitis and interdigital intertrigo with lymphangitis are the most frequent presentations [57].

Pneumonia

Pneumonia is a frequent manifestation of invasive fusariosis, and occurs more frequently in the context of disseminated disease. The majority of patients presents with bilateral involvement [40]. In a review of 13 cases of invasive fusariosis with lung involvement, the most frequent lesions were macro (>1 cm) nodules, centrilobular micro (<1 cm) nodules, and ground-grass infiltrates. Comparing with invasive aspergillosis, patients with fusariosis were less likely to have macronodules with halo sign (23.1% vs. 62.5% in cases of invasive aspergillosis). In four of the 13 cases, pneumonia was the sole manifestation of invasive fusariosis [64].

Fungemia

A remarkable characteristic of invasive fusariosis is the high frequency of positive blood cultures, mostly in the context of disseminated disease. In a series of 233 cases of invasive fusariosis in hematologic patients, fungemia occurred in 37% [62]. Catheter-related fungemia may occur occasionally, usually in absence of neutropenia. Antifungal treatment and catheter removal resolves the infection in the majority of cases [65].

Diagnosis

In severely immunocompromised patients, the appearance of metastatic skin lesions or a positive blood culture for a mold are highly suggestive of invasive fusariosis. The definitive diagnosis is made by direct exam, culture, and histopathology. Fungal antigens, such as galactomannan and 1,3-beta-D-glucan are frequently positive in patients with disseminated disease, but their actual contribution to the diagnosis is not clear [66, 67]. In patients with skin lesions, the fastest way to approach the diagnosis is to perform direct exam of skin biopsy. The finding of septate, acute branching hyphae is highly suggestive of invasive fusariosis, and prompts treatment initiation.

Treatment

Antifungal susceptibility tests show that *Fusarium* species exhibit high minimum inhibitory concentrations (MICs) to most antifungal agents, including voriconazole, posaconazole, isavuconazole, and amphotericin B, with FSSC exhibiting higher MICs compared with FOSC [68]. *In vitro* susceptibility tests are of little help in defining treatment, because there is no correlation between MIC and the outcome [69]. This is illustrated by the good clinical response to voriconazole in patients with invasive fusariosis, with rates similar to those achieved by lipid amphotericin B, despite the high MICs exhibited in the susceptibility test studies [8, 62].

The treatment of invasive fusariosis varies according to the immune status of the host and the site(s) of infection. The treatment of keratitis caused by *Fusarium* species is challenging because of the poor tissue penetration of antifungal agents. Topical antifungal agents with or without systemic therapy is usually indicated. Natamycin is the drug of choice for therapy, whereas voriconazole is a good option as systemic treatment [70–72]. Onychomycosis caused by *Fusarium* species is difficult to treat, with a long duration of treatment and high failure rates with both systemic and topical therapy. Treatment options include amphotericin B solution and topical and oral terbinafine [73, 74].

In immunocompromised patients, the success of treatment of invasive fusariosis depends on the recovery of the immune function. In an analysis of 84 hematologic patients with invasive fusariosis, the actuarial survival was 0% in patients with persistent neutropenia and receiving corticosteroids, 4% in those with persistent neutropenia only, 30% in patients on corticosteroids but no neutropenia, and 67% in patients without any of these two factors [75].

The optimal treatment of invasive fusariosis in immunocompromised patients is difficult to define, since there are no randomized trials. In the largest series ever published (236 patients), the 90-day probability of survival was 53% in patients receiving voriconazole, 48% in those receiving a lipid formulation of amphotericin B, and 27% in patients treated with deoxycholate amphotericin B. The response rate was similar among patients receiving combination therapy and monotherapy [62]. Based on these results, recently published guidelines recommend voriconazole, a lipid formulation of amphotericin B or a combination of both as primary therapy for invasive fusariosis (Table 12.4) [76].

Prevention

Primary prophylaxis for invasive fusariosis is indicated in high-risk patients (acute leukemia or HCT) who present with superficial skin lesions in the extremities (usually onycho-

Table 12.4 Principles of the management of invasive fusariosis in immunocompromised patients

1. General principles
(a) Antifungal susceptibility tests are of little help in defining treatment
(b) The success of treatment depends on the recovery of the immune function
2. Primary treatment (either option)
(a) Lipid amphotericin B (liposomal or lipid complex)
(b) Voriconazole
(c) Combination therapy with voriconazole and a lipid formulation of amphotericin B
3. Prophylaxis
(a) Primary prophylaxis
Voriconazole in patients who present with superficial skin lesions in the extremities (usually onychomycosis or interdigital intertrigo) with positive cultures for *fusarium* species
(b) Secondary prophylaxis
Voriconazole in patients who had invasive fusariosis and will receive any treatment with a potential to induce neutropenia or T-cell immunodeficiency

mycosis or interdigital intertrigo) with positive cultures for *Fusarium* species. The agent of choice is voriconazole [76, 77]. Secondary prophylaxis (voriconazole) is also indicated for patients who had invasive fusariosis and will receive any treatment with a potential to induce neutropenia or T-cell immunodeficiency [78].

Scedosporiosis and Lomentosporiasis

The genus *Scedosporium* contains 10 species: the *Scedosporium apiospermum* complex comprising *Scedosporium angustum*, *S. apiospermum*, *Scedosporium boydii, Scedosporium ellipsoideum,* and *Scedosporium fusarium*; and *Scedosporium aurantiacum*, *Scedosporium minutisporum, Scedosporium desertorum, Scedosporium cereisporum,* and *Scedosporium dehoogii* [79]. The species *Scedosporium prolificans* has been recently separated from the genus *Scedosporium*, and was redefined as *Lomentospora prolificans* [80]. The majority of infections in humans are caused by *S. boydii*, *S. apiospermum*, *S. arantiacum*, and *L. prolificans*.

These fungi are saprophytes found in soil, and can cause localized infections in immunocompetent patients and disseminated infections in immunocompromised patients [81]. *Scedosporium* species are distributed worldwide, but are most commonly found in temperate areas, with regional differences in the species causing infection [82, 83]. By contrast, *L. prolificans* predominates in arid climates of Australia, Spain, and southwest USA [84, 85]. *Scedosporium* species cause eumycotic mycetoma, a variety of infections in the normal host, including keratitis, endophtalmitis, otitis, sinusitis, central nervous system (CNS) infections, and pneumonia after near-drowning, and deep-seated infections in the immunocompromised host, manifesting as pneumonia, sinusitis, and infection in the CNS. In addition, patients with chronic lung diseases, such as cystic fibrosis, may be colonized by *Scedosporium* species and occasionally develop invasive disease [86]. Infections by *L.prolificans* typically occur in immunocompromised patients, including patients with hematologic malignancies, HCT recipients, and SOT recipients. In these hosts, the disease is usually disseminated. Less frequently, *L. prolificans* may cause localized infection after trauma or surgery in non-immunocompromised patients [85].

Etiologic Agents

Scedosporium species and *L. prolificans* grow rapidly on Sabouraud's dextrose agar, but the growth of *L. prolificans* is inhibited by the presence of cycloheximide. *Scedosporium* species produce spreading, hairy, grayish-brown colonies. Microscopy shows unicellular sessile or formed conidia on cylindrical or flask-shaped conidiogeneous cells (Fig. 12.4). *Lomentospora prolificans* produces greyish-white to olive-grey to black colonies (Fig. 12.5). Microscopically, *L. prolificans* differs from *Scedosporium* species by its characteristic flask-shaped and annellated conidiogenous cells, but identification needs to be confirmed by molecular methods (Fig. 12.5).

Epidemiology and Clinical Manifestations

Infection is secondary to inhalation of airborne conidia, or by direct inoculation, such as after trauma. The most frequent groups of immunocompromised patients with infection secondary to *Scedosporium* species and *L. prolificans* are SOT recipients, patients with hematologic malignancies (with the infection occurring in the context of severe neutropenia), and allogeneic HCT recipients with GVHD. In a series of 118 immunocompromised patients, 58 were SOT recipients, 21 had cancer, and eight were HCT recipients. Most SOTs were kidney or lung transplants, and the disease occurred at a median of 365 days and 82 days after kidney and lung transplant, respectively. Most patients with cancer had a hematologic malignancy. Among 90 immunocompetent patients, surgery (n = 17), trauma (n = 17), and near drowning (n = 12) were the most frequent predisposing factors [87]. In lung transplant recipients, *Scedosporium* species and *L. prolificans* are the second most frequent causes of invasive fungal disease, following aspergillosis [88].

The clinical presentation of infection caused by *Scedosporium* species and *L. prolificans* is non-specific, and reflect the invasiveness of the affected organs. The clinical spectrum of infection is largely dependent on the immune

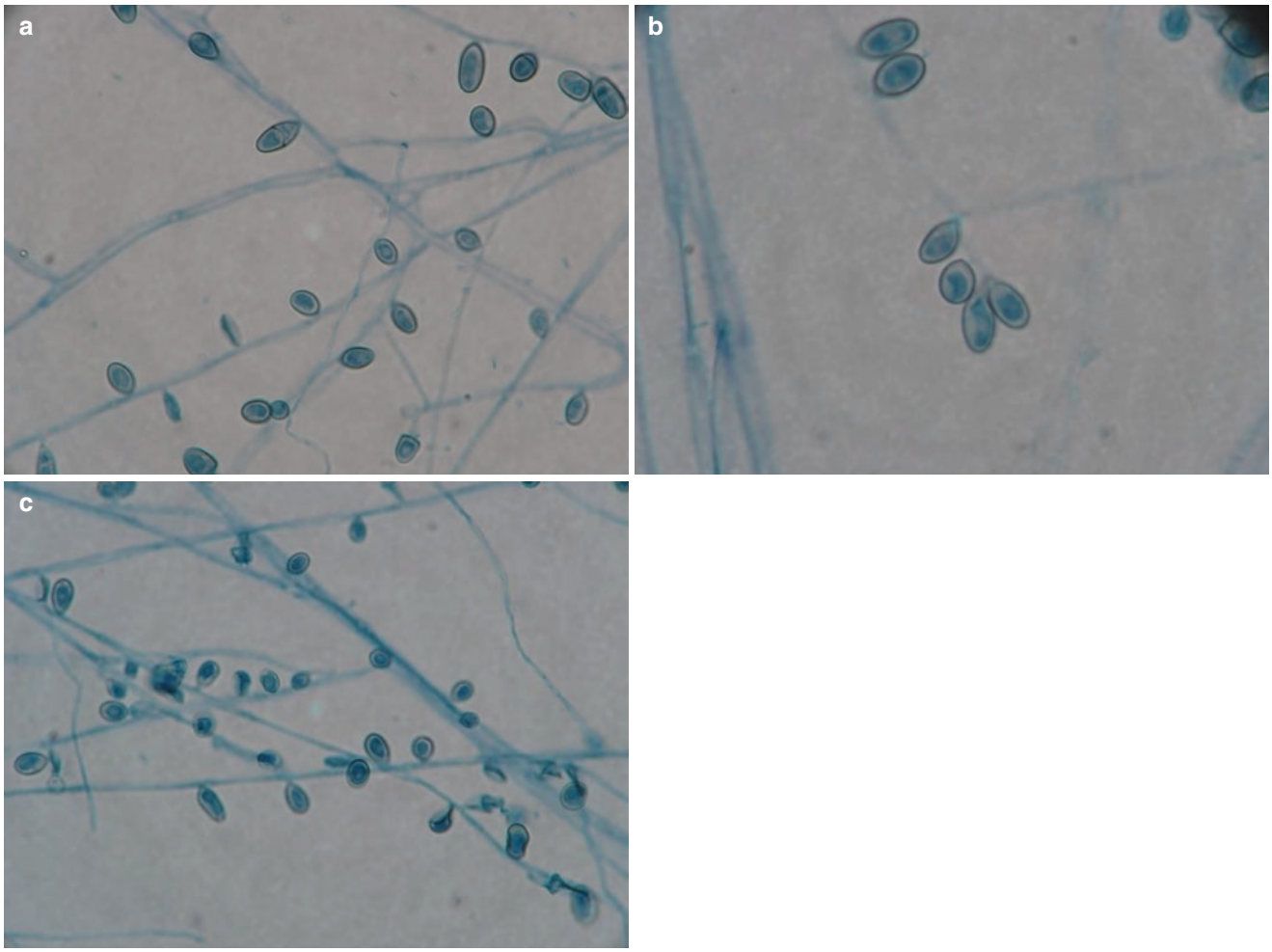


Fig. 12.4 Microscopy of *Scedosporium* species. Slide cultures on Potato Dextrose Agar – Lactophenol cotton blue – 400× (**a**–**c**) conidia one-celled

status of the host (Table 12.5). The most frequent infections by *Scedosporium* species in non-immunocompromised individuals are eumycetoma, keratitis, endophtalmitis, sinusitis, pneumonia, colonization of a lung cavity (fungus ball), and brain lesions [81]. Tsunami, earthquake, and near drowning are predisposing factors for infection by *Scedosporium* species [89–93]. In the latter, a syndrome of sinopulmonary and CNS disease has been described [94]. In immunocompromised patients, the disease is more frequently disseminated, with or without fungemia. Organ involvement includes sinuses, lungs (unilateral or bilateral pulmonary infiltrates), soft tissue, and CNS.

Most patients who develop infection by *L. prolificans* are severely immunosuppressed, and the disease is usually disseminated with fungemia and metastatic skin lesions, with or without central necrosis (Table 12.5). In a review of 162 patients with infection or colonization by *L. prolificans*, cancer was the most frequent underlying condition (74 cases, 45.7%). There were 14 SOT recipients and eight patients with AIDS [85]. Localized organ involvement may occur in the sinuses, lungs, skin and soft tissues, and CNS. The most frequent infections in immunocompetent individuals are sinusitis, endophtalmitis, and soft tissue infections [95].

In addition to invasive disease, patients with cystic fibrosis may be colonized by *Scedosporium* species or *L. prolificans*. A study evaluating 128 patients with cystic fibrosis reported that 8.6% were colonized by *Scedosporium* species [96], and other study found *L. prolificans* in 5.8% of 69 patients with cystic fibrosis [97]. The clinical significance of colonization by these fungi in patients with cystic fibrosis is not clear.

Diagnosis

Despite recent advances in non-culture-based diagnostics, classical mycology, with direct exam and culture of the causative fungal agent from clinical specimens is the backbone of the diagnosis of infection by *Scedosporium* species and *L. prolificans*. For species identification, molecular techniques

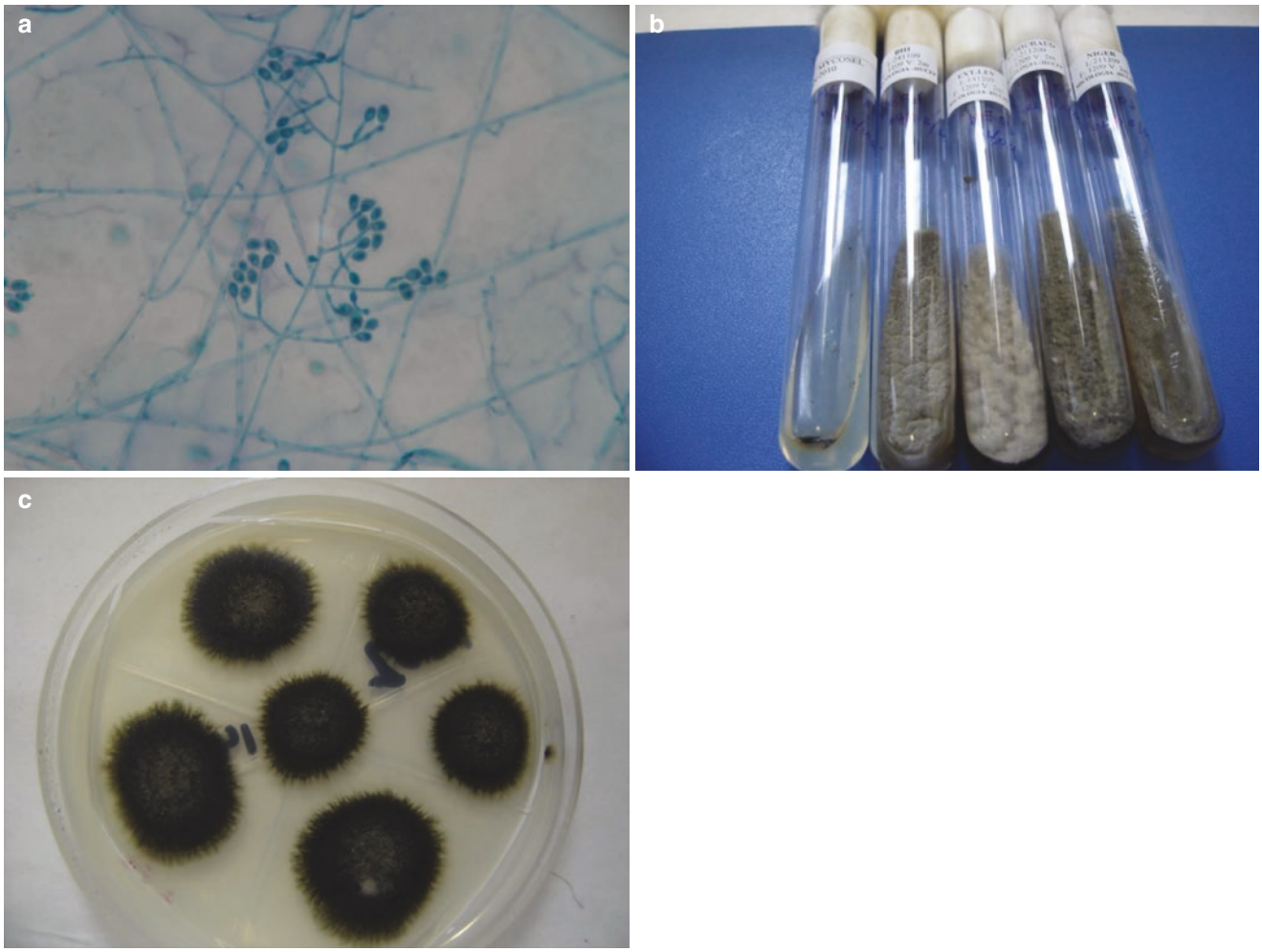


Fig. 12.5 *Scedosporium prolificans* (**a**) slide culture on Potato Dextrose Agar – Lactophenol-cotton blue – 400× Conidiogenic cells and conidia (**b**) Primary culture on several routine media; (**c**) black colonies subcultured on Potato Dextrose Agar

Table 12.5 Clinical spectrum of infection by *Scedosporium* species and *Lomentospora prolificans*

Scedosporium species	
Non-immunocompromised	Immunocompromised
Eumycetoma	Sinusitis
Eye infection	Pneumonia
Keratitis	Soft tissue infection
Endophthalmitis	Central nervous system infection
Sinusitis	Fungemia
Pneumonia	Disseminated infection
Central nervous system infection	
Lomentospora prolificans	
Non-immunocompromised	Immunocompromised
Sinusitis	Sinusitis
Endophthalmitis	Pneumonia
Soft tissue infection	Soft tissue infection
	Disseminated infection

have been increasingly used. In addition, proteomic approaches, such as MALDI-TOF have shown excellent performance, likely replacing phenotypic methods as routine species identification. Serology has no role in establishing the diagnosis [98].

The histopathology shows invasion of tissue by hyaline, septate, acute-branching hyphae, best seen with Gomori methenamine silver stain. Sometimes hyphae have branches bridging two parallel hyphae to form an H-shaped pattern, which is considered by some experts to be highly suggestive of *Scedosporium* species and *L. prolificans* [99]. Not infrequently, the hyphae of *L. prolificans* are melanized [100].

Treatment

Patients with localized infection usually respond to surgical removal of lesions. Voriconazole is the drug of choice for the treatment of most infections caused by *Scedosporium* species, while amphotericin B has a very limited role [76]. As with other fungal infections occurring in severely immunosuppressed patients, the success of treatment is largely

dependent on immune reconstitution, with 100% death rate in persistently neutropenic patients [101]. *L. prolificans* is intrinsically resistant to almost all currently available antifungal agents. The best results appear to be a combination therapy of voriconazole with terbinafine [76], but the outcome is usually poor, especially in persistently immunosuppressed patients.

Paecilomyces and *Purpureocillium* Species

Paecilomyces species are filamentous fungi widely encountered globally, and can cause infection in animals and humans, especially immunocompromised patients. Historically, *Paecilomyces variotii* and *Paecilomyces lilacinus* have been members of the genus. More recently, the two species have been reclassified. *P. variotii* represents a species complex with *P. variotii sensu stricto*, and four other species: *Paecilomyces formosus*, *Paecilomyces divaricatus*, *Paecilomyces brunneolus*, *and Paecilomyces dactylethromorphus*, and *P. lilacinus* has been shifted to *Purpureocillium lilacinum* [102]. While *P. variotii* belongs to the order Eurotiales, phylogenetic analyses showed that *P. lilacinum* belongs to the order Hypocreales [103].

Etiologic Agents

Paecilomyces variotii and *P. lilacinum,* the two main agents of infection, grow rapidly on various media, including blood, chocolate, and Sabouraud dextrose agar without cycloheximide. *P. variotii* grows well at temperatures as high as 50 °C, while *P. lilacinum* does not grow or presents restricted growth at 37 °C. The colonies of *P. variotii* are powdery to floccose, yellow-brown or sand colored. *P. lilacinum* produce pink or vinaceous to lilac colonies, with a basal felt. On microscopy, *Paecilomyces* species produce unicellular conidia that can be ovoid or fusoid, formed in divergent chains. Phialides have a swollen base and a long tapered neck (Fig. 12.6). *Paecilomyces variotii* sensu stricto produce olive brown conidia and chlamydospores. By contrast, the conidia of *P. lilacinus* are lilac, and chlamidospores are absent.

Epidemiology and Clinical Manifestations

The clinical spectrum of infection by *P. variotii* and *P. lilacinus* varies according to host immunity. Non-immunocompromised patients may develop localized infection in various organs, such as nails, skin and subcutaneous tissues, eyes, bones and joints, sinuses, lungs, and peritoneum [104–111]. Some of these infections have been

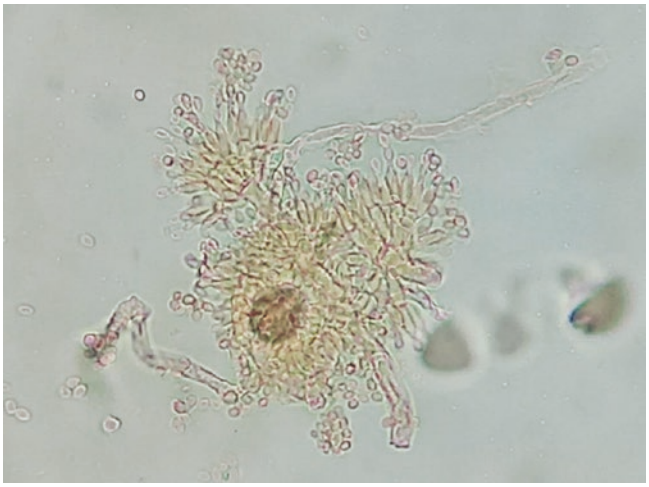


Fig. 12.6 *Paecilomyces* species Colony microscopy showing phialides and conidia in chains. Lactophenol 400×

associated with contaminated prosthetic implants. Immunocompromised patients may have localized or disseminated infection.

In a review of 59 cases of invasive infection caused by *P. variotii*, predisposing factors included indwelling devices including peritoneal catheters (33.9%) and prosthetic heart valves (10.2%), cancer (32.2%), surgery (18.6%), and diabetes mellitus (16.9%). Peritonitis (20 cases) and pneumonia (16 cases) were the most frequent infections. The overall mortality was 28.8%, and death was attributed to infection in ten cases [112].

Most cases of infection by *P. lilacinus* are ocular, or cutaneous and sub-cutaneous, as shown in a review of 119 reported cases between 1964 and 2004. In this review, keratitis and endophthalmitis were the most common clinical manifestations, and were frequently associated with intraocular lens implantation, trauma, or wearing contact lens. Skin and soft tissue infections were the second most frequent clinical presentation, occurring both in immunocompetent and, more frequently, in immunocompromised patients such as SOT recipients, and patients with cancer, diabetes, and AIDS. A third group of non-cutaneous, non-ocular infections comprised infection in various organs, such as lungs, sinuses, bone, as well as catheter-related fungemia [113]. Disseminated disease with multiple skin lesions has been also reported [114].

Diagnosis

The diagnosis of infection by *P. variotii* and *P. lilacinus* relies on the identification of septate hyaline hyphae in tissue and the identification of the agent in culture. Molecular and mass spectrometry (MALDI-TOF) can be used for species identification [102, 115].

Treatment

Antifungal susceptibility tests have shown that amphotericin B, terbinafine, and the echinocandins have *in vitro* activity against *P. variotii*, while the azoles have different activity, with lower MICs with itraconazole and posaconazole, and higher MICs with voriconazole. Regarding *P. lilacinus*, amphotericin B, itraconazole, and the echinocandins have high MICs, while voriconazole, posaconazole, and terbinafine have lower MICs [116].

The drug of choice for the treatment of invasive infection by *P. variotii* is a lipid formulation of amphotericin B. Posaconazole and itraconazole are alternatives. Surgical debridement of necrotic tissue and removal of catheter may be of help [76]. For infection by *P. lilacinus*, voriconazole is the drug of choice [76].

Acremonium and *Sarocladium* Species

Acremonium species are commonly found in soil, decaying vegetation, and in decaying food. *Acremonium* species can cause onychomycosis and keratitis in non-immunocompromised, and invasive disease in immunocompromised patients. Two species that cause infection in humans, *Acremonium kiliense* and *Acremonium strictum*, have been moved to the genus *Sarocladium* [117], and other species have been transferred to the genus Gliomastix [118].

Etiologic Agents

Phylogenetic analyses of *Acremonium* species have been undertaken, and two species that cause infection in humans, *Acremonium kiliense* and *Acremonium strictum*, have been moved to the genus *Sarocladium* [117], while various other species have been transferred to the genus Gliomastix [118].

Acremonium species grow fast on Sabouraud dextrose agar and form velvety white, salmon, or yellowish-green colonies with raised centers. The hyphae are narrow, with unbranched needle-shaped phialides and conidia variable in shape (subglobose, obovate, or ellipsoidal) (Fig. 12.7). *Sarocladium* species form elongated phialides which rise singly on vegetative hyphae or on conidiophores. These conidiophores are sparsely or repeatedly branched, and besides, abundant adelophialides with elongated conidia are also formed.

Epidemiology and Clinical Manifestations

In a review of 309 cases of human infections by *Acremonium* and *Sarocladium* species, fungemia was the most frequent clinical presentation (106 cases), followed by ocular infections (104 cases) and sinusitis or pneumonia (18 cases). Other infections included skin and soft tissue infection, mycetoma, arthritis, osteomyelitis, peritonitis, and CNS infections. Most patients (125 of 309) had underlying condi-

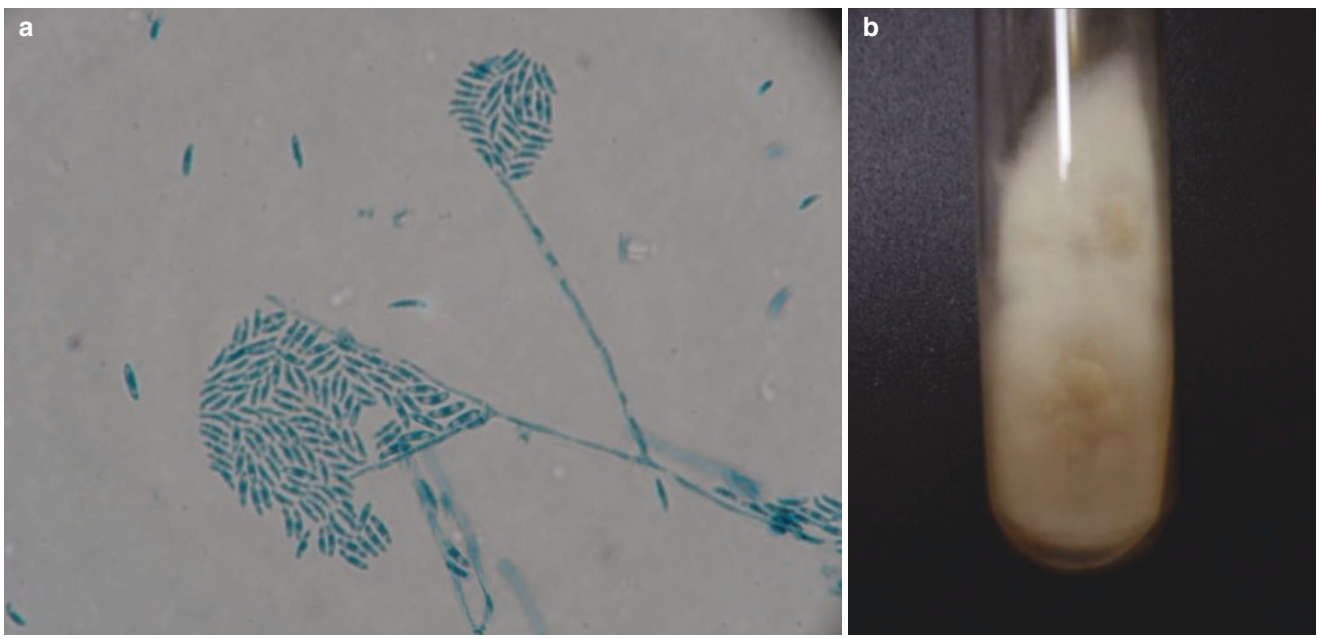


Fig. 12.7 *Acremonium* species. (**a**) slide culture on Potato Dextrose Agar – Lactophenol cotton blue – 400× - conidia grouped in slimy heads at the phialide tip (**b**): culture on Sabouraud's Dextrose Agar

tions, and hematologic malignancies (44 patients) and solid tumors (24 patients) were the most frequent. In total, 125 patients were immunosuppressed [117].

Among the 106 cases of fungemia, 83 were caused by *S. kiliense*, and occurred in the context of an outbreak in Chile (67 cases) and Colombia (16 cases), associated with the intravenous administration of contaminated antiemetic drug used in cancer patients. In the investigation of the outbreak, whole genome sequencing showed relatedness between patients' isolates from Chile and Colombia, and medication vials [119]. Keratitis caused by *Acremonium* and *Sarocladium* species usually occur in the context of trauma or contact lens wearing [120]. Most patients with infection in the respiratory tract caused by *Acremonium* and *Sarocladium* species were immunosuppressed (leukemia, AIDS, SOT, and others), and the clinical presentation was non-specific [117]. As with fusariosis, patients with disseminated disease may have positive blood cultures and metastatic skin lesions which appear as nodules with or without central necrosis [121, 122].

Diagnosis and Treatment

The diagnosis of infection by *Acremonium* and *Sarocladium* species relies on culture of biologic materials, and for invasive infection, culture and histopathology. In vitro susceptibility test of *Acremonium* and *Sarocladium* species show high MICs for most antifungal agents, except terbinafine [123]. Data on the treatment of invasive infection are scarce. Voriconazole, amphotericin B, and posaconazole have been recommended as agents of choice [124].

Other Agents of Hyalohyphomycosis

Scopulariopsis species are saprophytes found in soil, plants, food, and air. The taxonomy of these fungi has been reviewed, and according to morphologic and molecular analyses, six related genera have been defined: *Scopulariopsis*, *Microascus*, *Acaulium*, *Pithoascus*, *Yunnannia*, and *Pseudoscopulariopsis* [125]. The most frequent agent of infection in humans is *S. brevicaulis*. In non-immunocompromised patients, onychomycosis and keratitis are the most frequent clinical forms [126, 127]. In a review of 73 published cases, deep cutaneous infection (18 cases), disseminated infection (13 cases), pulmonary infection (12 cases), and sinusitis (10 cases) were the most frequent clinical presentations. An underlying hematologic malignancy was present in 31 cases [128]. Patients with disseminated infection may present with fungemia and metastatic skin lesions [129]. Antifungal susceptibility tests usually show high MICs to most antifungal agents. Primary treatment for invasive infection includes monotherapy with a lipid formulation of amphotericin B, voriconazole or isavuconazole, or a combination therapy with lipid amphotericin B and one of these two azoles [76].

Trichoderma species are recovered from soil. The most frequent agent of infection in humans is *T. longibrachiatum* [130, 131]. Invasive infection occurs in immunosuppressed patients such as SOT recipients and patients with hematologic malignancies, and is usually disseminated, with frequent lung involvement [132]. Peritonitis in patients undergoing peritoneal dialysis has also been reported [133]. Based on in vitro susceptibility tests, first-line treatment with voriconazole with or without an echinocandin is suggested [134].

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Phaeohyphomycosis: Infection Due to Dark (Dematiaceous) Molds

13

Sanjay G. Revankar

Introduction

Dematiaceous, or darkly pigmented fungi are a large, heterogeneous group of organisms that have been associated with a wide variety of clinical syndromes. These are uncommon causes of human disease, but can be responsible for life-threatening infections in both immunocompromised and immunocompetent individuals. These fungi have become increasingly recognized as important pathogens and the spectrum of diseases they are associated with has also broadened.

The clinical syndromes caused by the dark-walled fungi are typically distinguished based on characteristic histologic findings into chromoblastomycosis, mycetoma and phaeohyphomycosis. Chromoblastomycosis and mycetoma are caused by a small group of fungi that are associated with characteristic structures in tissue and are usually seen in tropical areas [1]. These are discussed in Chap. 25 (Fungal Infections of Implantation). Phaeohyphomycosis is a term introduced by Ajello et al. in 1974, which literally means "infection caused by dark walled fungi" [2]. It is a catch-all term generally reserved for the remainder of clinical syndromes caused by dematiaceous fungi that range from superficial infections and allergic disease to brain abscess and disseminated disease [3, 4]. These fungi are alternately called phaeoid, dematiaceous, dark, or black molds. While typically phaeohyphomycosis is a term limited to infections caused by the dark molds, there are dark yeasts that rarely cause infection, and these are also included under this grouping by many experts.

Etiologic Agents

Over 150 species and 75 genera of dematiaceous fungi have been implicated in human disease [4]. The common characteristic among these fungi is the presence of melanin in their cell walls, which imparts the dark color to their conidia or spores and hyphae. Their colonies are typically brown to black in color as well. As the number of patients immunocompromised from diseases and medical therapy increases, additional species are being reported as causes of human disease, expanding an already long list of potential pathogens. Common genera associated with specific clinical syndromes are listed in Table 13.1. Some important taxonomic name changes are *Curvularia spicifera* replacing *Bipolaris spicifera*, *Rhinocladiella mackenziei* replacing *Ramichloridium mackenziei*, and *Verruconis gallopava* replacing *Ochroconis gallopava* [5–8].

Guidelines are available regarding the handling of potentially infectious fungi in the laboratory setting. Cultures of certain well-known pathogenic fungi, such as *Coccidioides* species and *Histoplasma* species, are suggested to be worked with in a Biosafety Level (BSL) 3 facility, which requires a separate negative pressure room. Recently, certain agents of phaeohyphomycosis, in particular *Cladophialophora (C.) bantiana,* have been included in the list of fungi that should be kept under BSL-2 containment, though some guidelines suggest BSL-3 [9, 10]. This is due to their propensity, albeit rarely, for causing life-threatening infection in normal individuals. It should be noted that no laboratory-associated infections have been reported to date [9].

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_13

Table 13.1 Clinical spectrum and treatment of phaeohyphomycosis

Clinical syndrome	Commonly associated fungi	Therapy
Onychomycosis	*Onychocola*, *Alternaria*	Itraconazole or terbinafine
Subcutaneous nodules	*Exophiala*, *Alternaria*, *Phialophora*	Surgery ± voriconazole
Keratitis	*Curvularia*, *Exserohilum*, *Lasiodiplodia*	Topical natamycin ± voriconazole
Allergic fungal sinusitis/allergic bronchopulmonary mycosis (disease)	*Curvularia*	Corticosteroids ± voriconazole
Pneumonia	*Verruconis*, *Exophiala*, *Chaetomium*	Voriconazole, (amphotericin B if severe)
Brain abscess	*Cladophialophora* (*C. bantiana*), *Rhinocladiella* (*R. mackenzei*), *Verruconis*	See text
Disseminated disease	*Lomentospora* (*L. prolificans*), *Curvularia*, *Exophiala*	See text

Epidemiology

These fungi are typically soil organisms and generally distributed worldwide [11]. Surveys of outdoor air for fungal spores routinely observe dematiaceous fungi [12]. This suggests that most, if not all, individuals are exposed to them, though they remain uncommon causes of disease. However, there are species that do appear to be geographically restricted, such as *Rhinocladiella mackenzei*, which has almost exclusively been seen in patients from the Middle East [7, 13]. Exposure is thought to be from inhalation or minor trauma, which may not even be noticed by the patient. Anecdotal reports suggest that smoking (including marijuana use) may be a risk factor in patients who are immunodeficient [14]. In 2012, a large nosocomial outbreak of infections primarily due to *Exserohilum rostratum* occurred in the United States, traced to contaminated corticosteroid injections [15, 16]. Clinical manifestations included osteomyelitis, septic arthritis, and meningitis, with multiple fatalities [16]. Interestingly, some of the clinical syndromes had never been reported previously due to *Exserohilum* spp., suggesting that natural routes of infection are limited for this species. Dematiaceous fungi may also be found to be contaminants in cultures, making the determination of clinical significance problematic. At one institution, only 10% of positive cultures were associated with clinical disease [17]. A high degree of clinical suspicion as well as correlation with appropriate clinical findings and histopathology is required when interpreting culture results.

Pathogenesis and Immunology

Little is known regarding the pathogenic mechanisms by which these fungi cause disease. One of the likely virulence factors is the presence of melanin in the cell wall, which is common to all dematiaceous fungi. It may confer a protective advantage by scavenging free radicals that are produced by phagocytic cells in their oxidative burst that normally kill most organisms [18]. In addition, melanin may bind to hydrolytic enzymes, thereby preventing their action on the plasma membrane [18]. In the yeasts *C. neoformans* and *W. dermatitidis*, disruption of melanin production leads to markedly reduced virulence in animal models [19, 20]. Melanin has also been associated with decreased susceptibility of fungi to certain antifungals, possibly by binding these drugs [21, 22]. It is interesting to note that almost all allergic disease and eosinophilia is caused by *Curvularia* spp., though the virulence factors responsible for eliciting allergic reactions are unclear at present [23].

CARD-9 is a protein involved in signal transduction from pathogen recognizing receptors such as Dectin-1,2,3 that recognize beta-glucans, alpha mannans, and glycolipids present in fungal cell walls [24]. This includes phosphorylation of receptors that cascade to activation of CARD-9 that leads to release of proinflammatory cytokines and chemokines [24]. In animal models, CARD-9 deficiency has been associated with decreased Th17 cells when under fungal stimuli as well as decreased cytokine production [24]. More recently, in a report encompassing 14 countries, invasive phaeohyphomycosis associated with CARD-9 deficiency was reported in 10 out of 58 cases, with 22 mutations identified either as nonsense, missense, small deletions, small insertions, or single nucleotide substitution. Melanized fungi seen in these cases included *Phialophora verrucosa, Corynespora cassiicola, Exophiala* spp., and *Aureobasidium pullulans* [24]. The description of familial invasive fungal disease should prompt the investigation of a genetic mutation, including in CARD-9 [25, 26].

Clinical Manifestations

Superficial Infections

Superficial infections are the most common form of disease associated with phaeohyphomycosis. These may be divided into tinea nigra, onychomycosis, subcutaneous lesions, and

keratitis and are generally associated with minor trauma or other environmental exposure. Although many pathogens have been reported, relatively few are responsible for the majority of infections.

Tinea nigra is primarily seen in tropical areas, and involves only the stratum corneum of the skin. Patients are generally asymptomatic, presenting with brownish-black macular lesions, almost exclusively on the palms and soles. *Hortaea werneckii* is the most commonly isolated species, though *Stenella araguata* has also been cultured from lesions [27]. Tinea nigra may be confused with a variety of other diseases, including dysplastic nevi, melanoma, syphilis, or Addison's disease. Diagnosis is made by scrapings of lesions and culture. As it is a very superficial infection, simple scraping or abrasion can be curative, though topical treatments such as keratolytics or imidazole creams are also highly effective [27].

Dematiaceous fungi are rare causes of onychomycosis, and the term fungal melanonychia has been used to describe this entity, which is seen predominantly in tropical regions [28]. Clinical features may include a history of trauma, involvement of only one or two toenails, and lack of response to standard systemic therapy [29]. Twenty-one species have been implicated as causes, including *Alternaria, Curvularia* and *Scytalidium* have been reported, with the latter being highly resistant to therapy [28].

There are numerous case reports of subcutaneous infection due to a wide variety of species [30, 31]. Minor trauma is the usual inciting factor, though it may be unrecognized by the patient. Lesions typically occur on exposed areas of the body and often appear cystic or papular. Immunocompromised patients are at increased risk of subsequent dissemination. Occasionally, these infections may involve joints or bone.

Fungal keratitis is an important ophthalmologic problem, particularly in tropical areas of the world. In one large series, 40% of all infectious keratitis was caused by fungi, almost exclusively molds [32]. The most common fungi are *Fusarium* and *Aspergillus*, followed by dematiaceous fungi (up to 8–17% of cases) [33]. Approximately half the cases are associated with trauma; prior eye surgery, diabetes, and contact lens use have also been noted as important risk factors [33]. In a study from the USA, of the 43 cases of *Curvularia* keratitis, almost all were associated with trauma [34]. Plants were the most common source, though several cases involving metal injuries were seen as well.

Allergic Disease

Relatively few species have been associated with allergic disease. *Alternaria alternata* is thought to be involved in some cases of asthma [35]. Whether dematiaceous fungi may be responsible for symptoms of allergic rhinitis is unclear, as it is difficult to quantitate exposure and to distinguish them from other causes [36].

Curvularia is responsible for most cases of allergic fungal sinusitis (AFS) and allergic bronchopulmonary mycosis (ABPM). Patients with AFS usually present with chronic sinus symptoms that are not responsive to antibiotics. Previously, *Aspergillus* was thought to be the most common fungus responsible for allergic sinusitis, but it is now appreciated that disease due to dematiaceous fungi actually comprises the majority of cases [37]. Criteria have been suggested for this disease, and include (1) nasal polyps, (2) presence of allergic mucin, containing Charcot-Leyden crystals and eosinophils, (3) hyphal elements in the mucosa without evidence of tissue invasion, (4) positive skin test to fungal allergens, and (5) on computed tomography (CT) scans, characteristic areas of central hyper-attenuation within the sinus cavity [38]. Diagnosis generally depends on demonstration of allergic mucin, with or without actual culture of the organism.

Allergic bronchopulmonary mycosis (ABPM) (or disease [ABPD]) is similar in presentation to allergic bronchopulmonary aspergillosis (ABPA), which is typically seen in patients with asthma or cystic fibrosis [39]. Criteria for the diagnosis of ABPA in patients with asthma include: (1) asthma, (2) positive skin test for fungal allergens, (3) elevated IgE levels, (4) *Aspergillus*-specific IgE, and (5) proximal bronchiectasis [40]. Similar criteria for ABPM are not established, but finding allergic mucin (Charcot-Leyden crystals and eosinophils) without tissue invasion, as in AFS, makes this diagnosis highly likely [41].

Pneumonia

Non-allergic pulmonary disease is usually seen in immunocompromised patients, and may be due to a wide variety of species, in contrast to allergic disease [23, 41–44]. Clinical manifestations include pneumonia, asymptomatic solitary pulmonary nodules and endobronchial lesions which may cause hemoptysis.

Brain Abscess

This is a rare but frequently fatal manifestation of phaeohyphomycosis [45]. Interestingly, over half of reported cases have occurred in patients with no risk factors or known immunodeficiency. Lesions are usually solitary. Symptoms may include headache, neurologic deficits, and seizures, though the classic triad seen in bacterial brain abscess (fever, headache, and focal neurologic deficit) is not usually present. The most commonly isolated organism is *C. bantiana*, particularly in immunocompetent patients. The pathogenesis

may be hematogenous spread from an initial, presumably subclinical, pulmonary focus. However, other risk factors such as chronic sinusitis or smoking have been implicated in case reports [14, 46]. It remains unclear why these fungi preferentially cause CNS disease.

Disseminated Infection

This is the most uncommon manifestation of infection seen with dematiaceous fungi. Most patients are immunocompromised, though occasional patients without known immunodeficiency or risk factors have developed disseminated disease as well [47]. In contrast to most invasive mold infections, blood cultures are often positive. The most commonly isolated fungus, *L. prolificans*, may also be associated with septic shock. Peripheral eosinophilia, seen in 11% of cases, is more commonly associated with *Curvularia*. Disseminated infection due to *Exophiala* spp. has also been seen in apparently immunocompetent patients, especially of Asian origin [24, 48, 49].

Diagnosis

In contrast to other common mycoses that cause human disease, there are no specific serologic or antigen tests available to detect these fungi in blood or tissue. However, the non-specific serum 1,3-β-D-glucan test may be positive in certain cases of invasive disease [50], and certain species have been demonstrated to contain the *FKS* gene responsible for its production [51]. The diagnosis of phaeohyphomycosis currently rests on pathologic examination of clinical specimens and careful gross and microscopic examination of cultures (Fig. 13.1). Hospital laboratories can generally identify the most common genera associated with human disease (Fig. 13.2), though referral to a reference laboratory is often needed to identify unusual species. As many of these are rarely seen in practice, a high degree of clinical suspicion is required when interpreting culture results. Increasingly, molecular techniques such as internal transcribed sequence (ITS) sequencing are being used to definitively identify isolates to the species level and are becoming the standard to distinguish between closely related strains and establish novel species [4].

In tissue, these fungi will stain strongly with the Fontana-Masson stain, which is specific for melanin (Fig. 13.3) [3]. This can be helpful in distinguishing these fungi from other species, particularly *Aspergillus*. In addition, hyphae typically appear more fragmented in tissue than seen with *Aspergillus*, with irregular septate hyphae and beaded, yeast-like forms [3].

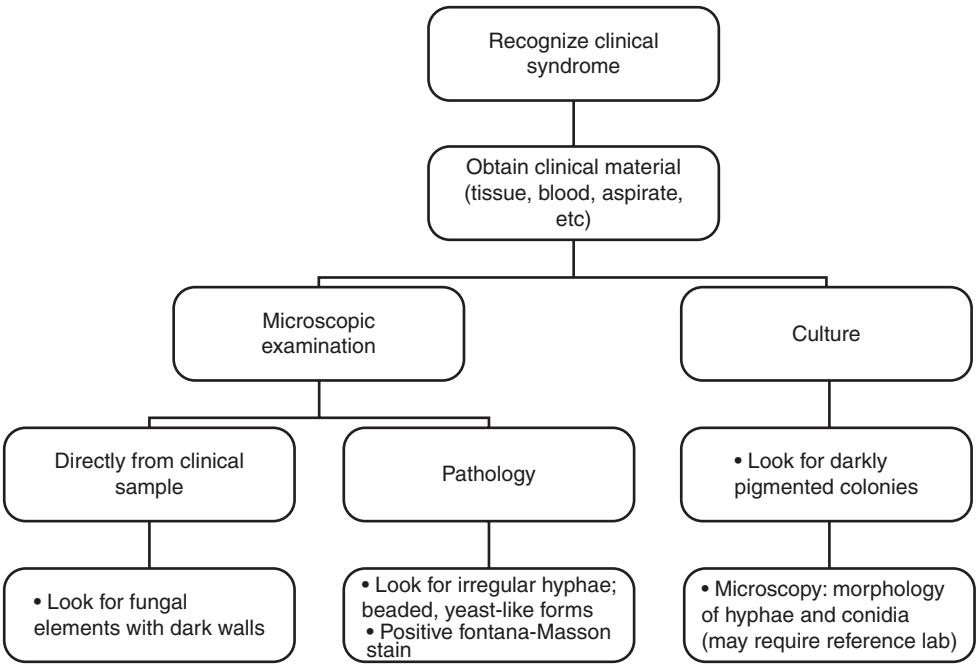


Fig. 13.1 Diagnostic approach for phaeohyphomycosis

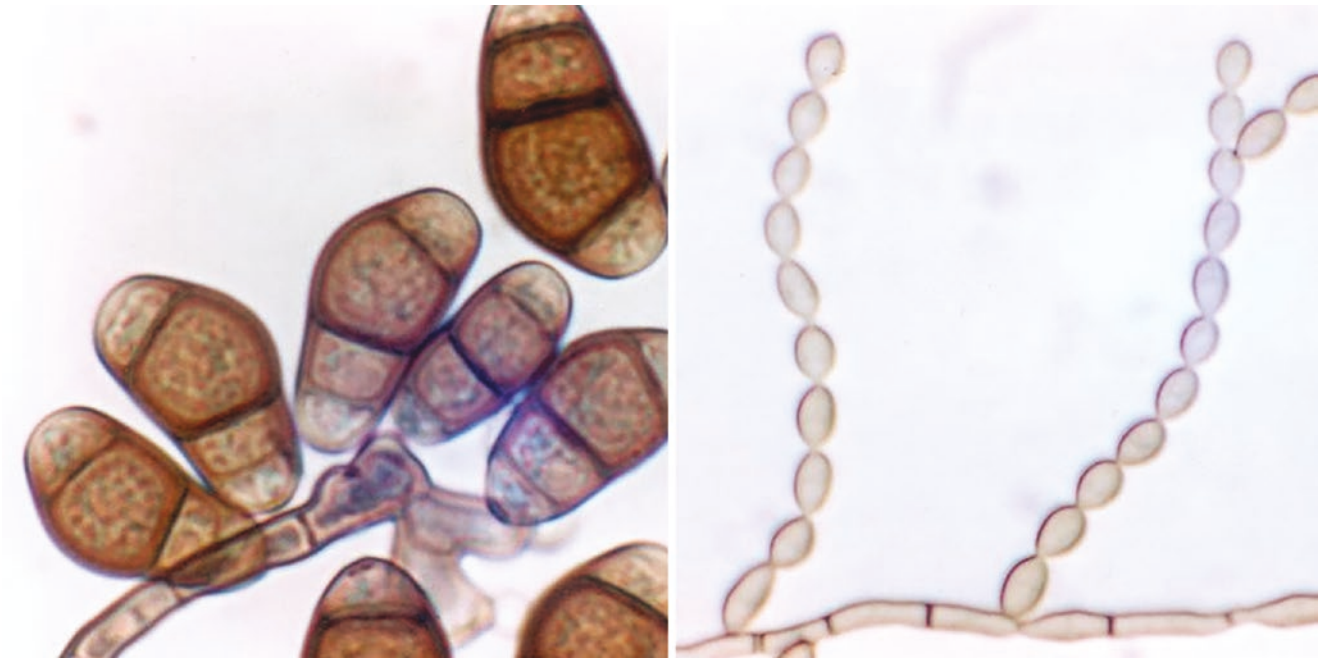


Fig. 13.2 Commonly seen fungi causing phaeohyphomycosis. Left panel, *Curvularia lunata*; right panel, *Cladophialophora bantiana*

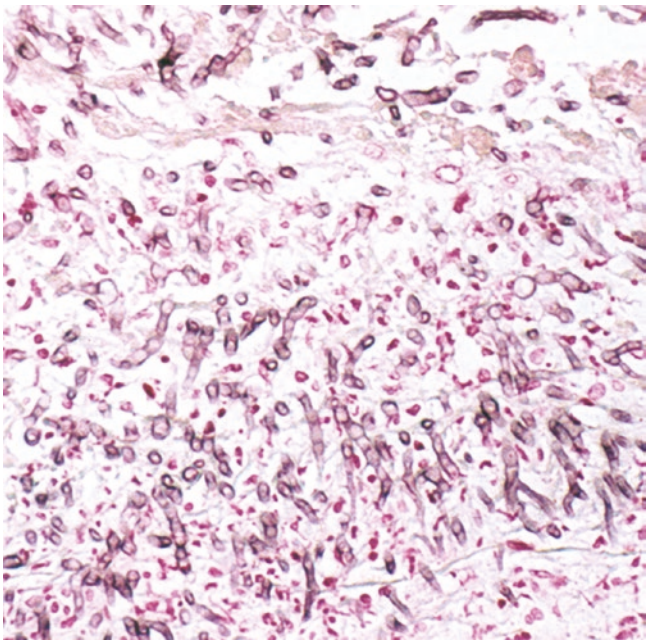


Fig. 13.3 Fontana-Masson stain of *Curvularia* infection in the lung, demonstrating irregular hyphae and beaded yeast-like forms

Treatment

Therapy is not standardized for any of these clinical syndromes, and randomized trials are unlikely given the sporadic nature of cases, though guidelines have been published based mostly on expert opinion [52]. Itraconazole, voriconazole, and posaconazole demonstrate the most consistent in vitro activity against this group of fungi [4]. Initial clinical experience accumulated with itraconazole, though voriconazole and posaconazole have become preferred agents due to good efficacy and better tolerability [4, 53, 54]. Isavuconazole is a more recently approved triazole with good *in vitro* activity, though limited clinical data [55–57]. Amphotericin B may be used for severe infections in unstable patients; high doses of lipid formulations may have a role in the treatment of refractory cases or in patients intolerant of standard amphotericin B. However, some species of dematiaceous fungi are resistant to this agent [4]. Once the infection is under control, longer term therapy with a broad spectrum oral azole is often reasonable until complete response is achieved, which may require several weeks to months [54].

Other agents have limited roles in treating these fungi. Ketoconazole is not well tolerated, and fluconazole has poor activity against these fungi in general. Terbinafine and flucytosine have occasionally been used for subcutaneous infections in patients refractory to other therapy [58]. Echinocandins do not appear to be very useful as single agents. Combination therapy is a potentially useful therapeutic strategy for refractory infections, particularly brain abscess and disseminated disease, though it has not been well studied [52, 54, 59]. Suggested therapies for specific infections are summarized in Table 13.1.

Superficial Infections

Itraconazole and terbinafine are the most commonly used systemic agents for onychomycosis, and may be combined with topical therapy for refractory cases [60]. There is no published experience with voriconazole.

Subcutaneous lesions will often respond to surgical excision alone [61]. Oral systemic therapy with a broad spectrum azole antifungal agent in conjunction with surgery is frequently employed and has been used successfully, particularly in immunocompromised patients [62, 63].

For keratitis, topical 5% natamycin is used almost exclusively, with only a few severe cases requiring adjunctive therapy, usually with an azole [32, 64]. Itraconazole has the best in vitro activity. The majority of isolates are resistant to flucytosine. Surgery, including penetrating keratoplasty, is often needed. Enucleation is occasionally required due to poor clinical response. Many patients do not recover complete visual acuity despite aggressive therapy.

Allergic Disease

Corticosteroids are the mainstay of treatment for allergic disease caused by these fungi, especially in asthma, though other modalities may have a role in specific clinical situations. For example, therapy for AFS consists of systemic corticosteroids and surgery to remove the mucin, which is often tenacious. Antifungal therapy, usually in the form of itraconazole, may play a role in reducing the requirement for corticosteroids, but this is not routinely recommended [65]. Other azoles have only rarely been used for this disease.

Allergic bronchopulmonary mycosis (ABPM) can be treated with systemic corticosteroids as in ABPA; prednisone at a dose of 0.5 mg/kg/day for two weeks, followed by a slow taper over 2–3 months or longer [39]. Itraconazole has been used as a steroid-sparing agent in APBA, but its efficacy is not clear and routine use of itraconazole is not generally recommended [39].

Pneumonia

Therapy consists of systemic antifungal agents, usually amphotericin B or itraconazole initially, followed by itraconazole for a more prolonged period [23]. Mortality rates are high in immunocompromised patients [66]. Voriconazole is becoming more commonly used [67].

Brain Abscess

Therapy published in the literature has varied greatly depending on the case report, and there is no standard treatment. A retrospective analysis of 101 reported cases suggested that the combination of amphotericin B (high-dose lipid formulation), flucytosine and itraconazole may be associated with improved survival, though it was not frequently used [45]. Voriconazole may also prove useful. High doses of azoles have been suggested as an option, though there are no studies confirming this approach. Based on animal models and anecdotal reports, some form of combination therapy may be optimal, though specific regimens have not been established [4, 52]. Complete excision of brain abscesses may lead to better outcomes than aspiration or partial excision [45, 52]. Overall mortality is greater than 70%.

Disseminated Infection

A review of 72 cases found the mortality rate to be greater than 70%, despite aggressive antifungal therapy [47]. There were no antifungal regimens associated with improved survival in disseminated infection [47, 54]. High dose lipid amphotericin B may be reasonable for initial therapy, given its fungicidal activity for many fungi. Addition of a broad spectrum azole or echinocandin could be considered in those failing therapy. Infection with *S. prolificans* has been associated with a nearly 100% mortality in the absence of recovery from neutropenia, as it is generally resistant to all available antifungal agents. In vitro and clinical studies have suggested that the combination of itraconazole or voriconazole with terbinafine may be synergistic against this species, though the clinical relevance of this finding is unclear [59, 68, 69].

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Mucormycosis

14

Charalampos Antachopoulos, Ruta Petraitiene, Emmanuel Roilides, and Thomas J. Walsh

Introduction

The class Mucormycetes (formerly Zygomycetes) includes a variety of filamentous fungi that may cause life-threatening human diseases and, over the past two decades, have emerged as increasingly important causes of morbidity and mortality among immunocompromised patients [1, 2]. The first case of mucormycosis (formerly zygomycosis) in humans was reported in 1885 by Platauf as Mycosis Mucorina. In many of the cases reported thereafter, the infection was identified as "mucormycosis" or *Mucor* infection based solely on histological findings of wide, rarely septate hyphae, without culture confirmation. The use of the term "mucormycosis" was further promoted by the original classification of most of the pathogenic species of Zygomycetes as members of the genus *Mucor* [3]. Consequently the term "zygomycosis," instead of "mucormycosis," was widely used during the past three decades for infections caused by any of the species within the class of Zygomycetes.

Many experts preferred the use of "mucormycosis" and emphasized that the opportunistic disease due to those fungi in the order Mucorales differed substantially from those caused by members of the order Entomophthorales, which was also included within the class Zygomycetes [4]. Diseases caused by members of the order Mucorales, such as *Rhizopus arrhizus (*formerly *R. oryzae)*, are typically opportunistic infections of the lungs, sinuses, and brain with angioinvasion leading to ischemia, infarction, and necrosis. Diseases caused by members of order Entomophthorales, such as *Conidiobolus* spp. and *Basidiobolus* spp., are typically subcutaneous infections in immunocompetent hosts [3, 5, 6]. With advances in molecular taxonomy, these clinical and pathophysiological distinctions correlated with the observation that the class of Zygomycetes is not monophyletic. A phylogenetic analysis found that the Zygomycetes comprise two clades with a paraphyletic relationship, the Mucoromycota and the Zoopagomycota. The order Mucorales belongs to the subphylum Mucoromycotina of the Mucoromycota clade. The order Entomophthorales belongs to the subphylum Entomophthoromycotina of the Zoopagomycota clade [7, 8]. As the orders Mucorales and Entomophthorales are preserved, the preferred names for the respective diseases should now be mucormycosis and entomophthoramycosis. The focus of this chapter is mucormycosis. Please see Chap. 25 (Fungal Infections of Implantation) for discussion of entomophthoramycosis.

Etiologic Agents

The majority of cases of mucormycosis in humans are caused by members of the order Mucorales. Molecular phylogenetic studies over the last decade have resulted in several changes in species names as well as family assignment [7–9]. Current nomenclature and taxonomic classification of medically important species of Mucorales is presented in Table 14.1. Organisms of the genus *Rhizopus* are the most common clinical isolates, with *R. arrhizus* being the most frequently recovered species. *R. arrhizus* has been further classified as *R. arrhizus* var. *arrhizus* and *R. arrhizus* var. *delemar* [10]. Some experts describe *R. delemar* as belonging to its own clade. Next in order of frequency are *Mucor* spp. and *Lichtheimia* (formerly *Absidia*) spp., while *Cunninghamella*, *Apophysomyces*, *Saksenaea*, *Rhizomucor*, and other genera each represent a significantly smaller percentage of clinical

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_14

Table 14.1 Recent taxonomic classification of clinically relevant species of Mucorales [8]

Family	Genus	Species causing human disease
Cunninghamellaceae	*Cunninghamella*	*C. bertholletiae, C. blakesleeana, C. echinulata, C. elegans*
Lichtheimiaceae	*Lichtheimia*	*L. corymbifera, L. ornata, L. ramosa*
	Rhizomucor	*R. miehei, R. pusillus*
Mucoraceae	*Cokeromyces*	*C. recurvatus*
	Mucor	*M. amphibiorum, M. ardhlaengiktus, M. circinelloides* complex, *M. griseocyanus, M. indicus, M. irregularis, M. janssenii, M. lusitanicus, M. plumbeus, M. racemosus, M. ramosissimus, M. variicolumellatus, M. velutinosus*
Rhizopodaceae	*Rhizopus*	*R. arrhizus (R. oryzae), R. delemar, R. homothallicus, R. microsporus, R. schipperae*
Saksenaeaceae	*Apophysomyces*	*A. elegans, A. mexicanus, A. ossiformis, A. trapeziformis, A. variabilis*
	Saksenaea	*S. erythrospora, S. loutrophoriformis, S. trapezispora, S. vasiformis*
Syncephalastraceae	*Syncephalastrum*	*S. racemosum*

isolates [1, 3, 11, 12]. The species within each genus are delineated in Table 14.1.

The Mucorales are characterized in culture by broad, nonseptate, or sparsely septate hyphae and by the presence of sporangiophores supporting sporangia, which contain sporangiospores. *Cunninghamella bertholettiae* is characterized by sporangiola rather than sporangiospores. During sexual reproduction in culture, zygospores may be produced. The Mucorales are characterized in tissue by the formation of wide, ribbon-like, hyaline, aseptate (coenocytic) or sparsely septate hyphae with wide-angle (approximately 90°) nondichotomous branching. The substantial differences among these and other structures allow organisms to be diagnosed by genus and species in the mycology laboratory [3, 13], although molecular methods have evolved as useful tools for species identification [2].

Epidemiology

The Mucorales are ubiquitous in soil and can be isolated from decaying organic matter, including hay, decaying vegetation, and a variety of food items. Human infection is usually acquired through inhalation of sporangiospores from environmental sources. Acquisition via the cutaneous or percutaneous route is also common, either through traumatic disruption of skin barriers or with the use of catheters and injections. Less commonly, infection through the gastrointestinal route may occur [1, 3, 5, 14]; however, gastrointestinal mucormycosis is relatively common (54% of total cases reported) in premature neonates [14].

Mucormycosis is approximately 10- to 50-fold less common than invasive *Candida* or *Aspergillus* infections, with a prevalence of 1–5 cases per 10,000 autopsies and an estimated incidence of 1.7 cases per million per year in a study conducted in 1992–1993 in the San Francisco Bay area [15]. The prevalence of mucormycosis-related hospitalizations in US hospitals between 2005 and 2014 was estimated at 0.12 per 10,000 discharges [16]. In France, the prevalence of mucormycosis increased from 0.7 cases per million in 1997 to 1.2 per million in 2006 [11]. In a university hospital of Belgium, the annual incidence of mucormycosis also increased from 0.019 cases/10,000 patient-days in 2000 to 0.148 cases/10,000 patient-days in 2009 [17]. The observed increase in incidence in these and other studies over the last years may be attributed to better diagnostic methods and increased awareness, but also to the increase of population at risk for mucormycosis. A clear male predisposition has been observed for this infection, as demonstrated by an approximate 2:1 male-to-female ratio among reported cases [1].

Unlike other opportunistic filamentous fungi that target mainly immunocompromised patients, the Mucorales cause disease in a wider and more heterogeneous population. The most common underlying condition for development of mucormycosis is diabetes, both type I and type II. A significant proportion of these patients will present with ketoacidosis, while in others, mucormycosis may even present as the diabetes-defining illness. Other significant underlying conditions include the presence of hematological malignancy, solid organ or hematopoietic stem cell transplantation, deferoxamine therapy, and injection drug use [1, 2, 5]. During the past three decades, the percentage of pediatric and adult patients with hematological malignancy, solid organ transplantation or hematopoietic stem cell transplantation (HSCT), and injection drug use among all cases of mucormycosis has significantly increased [1, 18, 19]. In the aforementioned groups of hematological patients and transplant recipients, factors associated with this infection have been reported to include prolonged neutropenia, corticosteroid use, and graft versus host disease (GvHD) [2, 5, 19]. Less commonly, the Mucorales may cause invasive disease in the presence of renal failure, diarrhea, and malnutrition, in low birth weight infants and in HIV patients. Occasionally mucormycosis has developed in patients with persistent metabolic acidosis secondary to causes other than diabetes [1, 5].

A significant proportion of mucormycosis cases have as well been observed in persons with no primary underlying disease at the time of infection. In many of these cases, there was a history of penetrating trauma, surgery, or burn prior to the development of infection [1, 2, 5]. Penetrating injuries

that are sustained following tornadoes and tsunamis have been complicated by severe myocutaneous mucormycosis. The epidemiology, pathogenesis, and management of trauma-related mucormycosis is discussed in detail elsewhere [20].

Mucormycosis also has been recently associated with Coronavirus Disease (COVID-19) [21]. Among the potential risk factors for development of COVID-19-Associated Mucormycosis (CAM), dexamethasone and other immunosuppressive modalities may have an important role in otherwise non-immunocompromised hosts. Diabetes mellitus, which is an important risk factor for moderate to severe COVID-19, also is a covariate in increasing the risk for development of CAM.

Pathogenesis and Immunology

The epidemiologic profile of mucormycosis cases, such as those with diabetes mellitus, hematological malignancies, and transplant recipients, may in part be explained by our current understanding of the pathogenesis of these infections. As with other filamentous fungi, an effective immune response following inoculation of sporangiospores requires the presence of adequate phagocytic activity of the host effector cells, including tissue macrophages and neutrophils. Pulmonary alveolar macrophages ingest the sporangiospores to inhibit germination, while the neutrophils are involved in hyphal damage [22]. Consequently, the host immune response against the Mucorales may be compromised if phagocytic cells are insufficient in number as in the case of chemotherapy-induced neutropenia, or dysfunctional, as in the case of corticosteroid treatment or diabetes mellitus [22, 23].

Experimental evidence also suggests an important role of iron in the pathogenesis of infections caused by the Mucorales, whose growth is promoted in the presence of increased iron uptake. The *FTR1* gene, encoding a high-affinity iron permease, mediates iron uptake and transport in these organisms. Knockdown of *FTR1* reduces virulence and iron uptake by *R. arrhizus* in infected mice [24]. Deferoxamine, an iron chelator, has siderophore activity for these fungi, allowing significant increase in iron uptake. Furthermore, the availability of serum iron is increased in the presence of acidic pH, suggesting an additional mechanism for the development of mucormycosis in patients with diabetic ketoacidosis [22].

An almost universal feature in infections caused by the Mucorales is the presence of extensive angioinvasion associated with thrombosis and ischemic necrosis [3, 23]. This is likely an important mechanism by which these organisms survive antifungal therapy, since adequate blood supply is necessary for the delivery of antifungal agents. In vitro studies also have demonstrated the ability of *R. arrhizus* sporangiospores or hyphae to adhere to subendothelial matrix proteins and human endothelial cells [23]. Sporangiospores of *R. arrhizus* are able to damage endothelial cells in vitro, following adherence to and phagocytosis by these cells. *R. arrhizus* viability is not required for endothelial cell damage, suggesting that in the setting of established infection, even fungicidal therapy may not prevent subsequent tissue injury [23]. The glucose-regulated protein 78 (GRP78) has been identified as the endothelial cell receptor for *R. arrhizus* germlings but not for *Aspergillus fumigatus* or *Candida albicans*. The expression of GRP78 has been shown to increase in the presence of elevated glucose or iron concentrations, allowing invasion and damage of endothelial cells by *R. arrhizus* in a receptor-dependent manner [25]. The fungal ligands involved in the binding of Mucorales to GRP78 are the spore coat protein homolog (CotH) cell surface proteins, in particular CotH3. *R. arrhizus* mutants with reduced CotH expression exhibited impaired invasion and damaging of endothelial cells [26]. More recent studies also demonstrate that *Rhizopus delemar* invades endothelial cells by binding of its CotH proteins to the host receptor GRP78 and that antibody to these proteins prevents murine disseminated mucormycosis [27]. Further contributing to the pathogenesis of mucormycosis is a ricin-like toxin, known as mucoricin [28].

Clinical Manifestations

The clinical manifestations of human infection caused by the Mucorales can be classified as sinus disease, localized or extended to the orbit and/or brain, pulmonary, cutaneous, gastrointestinal, disseminated, and miscellaneous infection [1, 29, 30]. Some of these manifestations may occur with increased frequency in patients with certain underlying conditions (Table 14.2) [1, 5]. However, this is not always the case, and mucormycosis in these patient groups may still present with any of the above patterns.

Table 14.2 Predominant site of Mucorales infection according to the patient's underlying condition (Roden 2005; Roilides 2009)

Underlying condition	Predominant site of infection
Diabetes	Paranasal Sinuses
Hematological Malignancy	Pulmonary and Sinuses
Solid organ transplantation	Pulmonary
Bone marrow transplantation	Pulmonary and Sinuses
Deferoxamine therapy	Pulmonary, Sinuses, Disseminated[a]
Injection drug use	Cerebral
Prematurity	Gastrointestinal
COVID-19[b]	Paranasal Sinuses
Trauma and burns	Cutaneous

[a] No clear predominance among the three sites
[b] Diabetes mellitus, corticosteroid therapy, and other immunosuppressive modalities are important covariates for development of COVID-19 associated mucormycosis

Paranasal Sinus Infection

Paranasal sinus disease may be confined to the sinuses or infiltrate the orbit (sino-orbital) and/or the brain parenchyma (rhinocerebral). This form represents approximately two-thirds of all cases of mucormycosis in diabetic patients [1, 30]. The infection originates in the paranasal sinuses following inhalation of sporangiospores. Initial symptoms may suggest sinusitis and include sinus pain, discharge, soft tissue swelling, and perinasal cellulitis/paresthesia. Fever is variable and may be absent in up to half of cases [29, 31]. The tissues involved become red, violaceous, and finally black, as vascular thrombosis leads to tissue necrosis. A blood-tinged nasal discharge may be present. In sinus disease, nasal endoscopy may show black necrotic crusts on the nasal septum and turbinates; in the early phases, the mucosa may still look pink and viable. We refer to these necrotic ulcers along the nasal mucosa or turbinates as "sentinel eschars,", as they may represent an early phase of infection or may be more amenable to biopsy than a deep maxillary sinus infection.

Extension of the infection to the mouth may produce painful necrotic ulcerations in the hard palate. Extension into the periorbital area and ultimately the orbit may be manifested by periorbital edema, lacrimation, chemosis, and proptosis. Subsequent ocular or optic nerve involvement is suggested by pain, diplopia, blurring, or loss of vision. Alteration of mental status and cranial nerve palsies may signify invasion of the central nervous system. Occasionally thrombosis of the cavernous sinus or the internal carotid artery may follow, with resultant neurological deficits, while dissemination of the infection also may occur [5, 31, 32].

Pulmonary Infection

Pulmonary disease is most commonly observed in patients with hematological malignancies, solid organ, or HSCT recipients and those receiving deferoxamine treatment [1, 30]. Not infrequently, it may occur with concomitant sinus disease (sinopulmonary infection) [33]. Lung involvement may be manifested as infiltrates, consolidation and solitary nodular or cavitary lesions (Fig. 14.1) [34, 35]. Fungal invasion of the pulmonary vessels may result in thrombosis and subsequent infarcts in the lung parenchyma (Fig. 14.2). Angioinvasion may also lead to intraparenchymal bleeding or even hemoptysis, which can be fatal if major vessels, such as the pulmonary artery, are involved. Extension of the infection to the chest wall, pericardium, myocardium, mediastinum, and diaphragm has been described [5, 35]. A predilection for the upper lobes has been reported; however, any part of the lung may be involved, and bilateral disease is not uncommon [35]. Recent studies of the presenting signs and symptoms are nonspecific and include fever, cough, chest pain, dyspnea, hemoptysis, tachypnea, crackles, decreased breath sounds, and wheezing [34, 35]. Endobronchial findings include stenosis or airway obstruction, erythematous mucosa, fungating or polypoid mass and, less often, granulation tissue or mucosal ulceration [35].

Cutaneous Infection

Cutaneous mucormycosis is often observed in individuals with no underlying condition as a result of infection of a preexisting lesion, such as skin trauma or burn [30, 36].

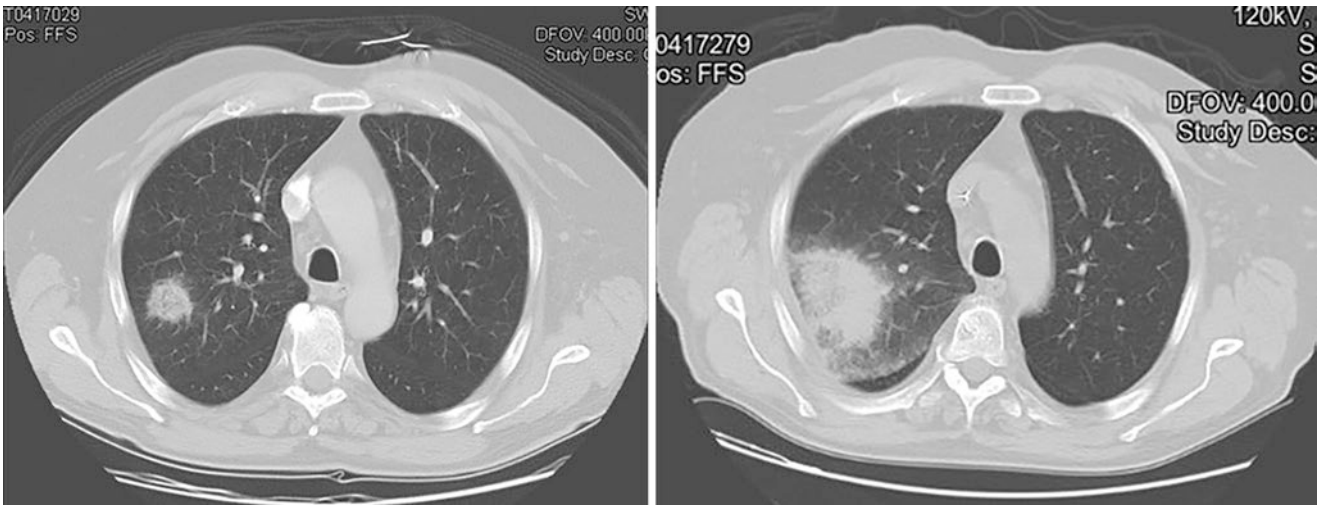


Fig. 14.1 Thoracic CT scan of profoundly neutropenic patient with pulmonary mucormycosis demonstrates rapid evolution of pulmonary nodule to involve the pleural surface and to manifest a halo sign at the interface with radiologically normal lung. The two scans are separated by 5 days

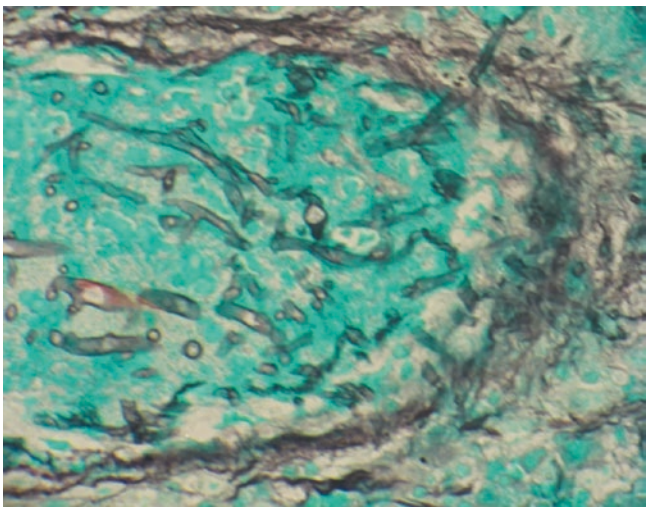


Fig. 14.2 Histopathology of pulmonary mucormycosis in this figure is characterized by broad non-septate ribbon-like hyphae with non-dichotomous branching invading a pulmonary blood vessel. The specimen was obtained from the lung lesion seen on CT scan in Fig. 14.1

Alternatively, it may occur in the context of disseminated disease or extensive local infection in immunocompromised hosts [1, 3, 37]. In the case of primary cutaneous inoculation, the lesion appears acutely inflamed with redness, swelling, induration, and frequent progression to necrosis. Extensive local invasion may occur involving the adjacent subcutaneous fat, muscle, bone tissues, and facial layers (Fig. 14.3). When cutaneous disease is the result of disseminated infection, it usually presents as nodular subcutaneous lesions that may ulcerate [3, 5, 37].

Gastrointestinal Infection

Gastrointestinal disease is rare, occurring mainly in malnourished patients and premature neonates, where it can present as necrotizing enterocolitis [1, 14, 38]. After ingestion of the sporangiospores, fungal invasion of the mucosa, submucosa, and vascular structures of the gastrointestinal tract may occur, often resulting in necrotic ulcers, rupture of the intestinal wall and peritonitis. Symptoms are nonspecific, including fever, abdominal pain, distention, vomiting, and gastrointestinal hemorrhage [3, 30].

Disseminated Infection

Disseminated infection refers to involvement of at least two non-contiguous sites and is commonly observed in patients receiving deferoxamine therapy [1]. Dissemination occurs through the hematogenous route and may originate from any of the above sites of primary infection; although, it seems to be more frequently associated with lung disease. The most common site of dissemination is the brain, but other organs may also be involved [30, 39].

Other Infection

Isolated cerebral mucormycosis is usually observed in injection drug users [1, 30]. Endocarditis is a potential complication of cardiac surgery. Isolated peritonitis is often associated with peritoneal dialysis. Renal infection and external otitis also have been reported [5].

Diagnosis

As infections caused by the Mucorales in humans may be rapidly fatal, timely diagnosis is crucial to avoid treatment delay. While confirmation of the diagnosis and species identification of the causative organism should be pursued, treatment should be initiated as soon as the diagnosis is suspected, due to the severity of these infections.

Currently, the diagnosis of mucormycosis relies on a constellation of the following: high index of suspicion, assessment of presenting signs and symptoms, imaging studies, cultures of clinical specimens, and histopathology (Fig. 14.4).

Clinical Assessment

The high index of suspicion should be based on the knowledge of the underlying conditions that predispose to mucormycosis and the usual presentation of the infection in each of these conditions (Table 14.2). Nevertheless, less common manifestations of the disease should not be excluded. A common scenario is the development of mucormycosis in oncological patients or transplant recipients that are receiving antifungal therapy for prophylaxis or treatment of other opportunistic fungal infections, such as invasive aspergillosis. If antifungal agents being administered to the patient are not active against the Mucorales (including, fluconazole, voriconazole, and the echinocandins), then clinical deterioration or appearance of new signs and symptoms in these patients should alert the clinician to the possibility of mucormycosis [33].

Most of the signs and symptoms that are associated with the clinical manifestations of mucormycosis are nonspecific. However their diagnostic significance may increase if they are interpreted in relation to the patient's underlying condition. For example, the development of sinusitis in a leukemic or diabetic patient should raise the suspicion of mucormycosis. Other findings have probably greater specificity for this infection, such as the presence of blood-tinged nasal dis-



Fig. 14.3 Development of mucormycosis in the skin and subcutaneous tissues of the right lower extremity in a patient with cutaneous T-cell lymphoma. The top left panel depicts the lesions of cutaneous T-cell lymphoma, which were possibly infected by direct inoculation. The top right panel reveals the extensive necrosis and destruction of soft tissue caused by the rapidly invading hyphae. The entire region was anesthetic to any tactile or pressure stimuli. The bottom panel demonstrates the soft tissues following extensive surgical debridement to resect infected skin, fascia, and muscle

charge or necrotic eschars in the hard palate. In addition, the presence of hemoptysis in a susceptible host is consistent with angioinvasion and should raise the possibility of mucormycosis [35]. Finally, even after the diagnosis has been established, careful periodic clinical assessment should be performed in order to detect progression of the disease. For example, in a patient with pulmonary mucormycosis, palpation of the skin for subcutaneous nodules and neurological evaluation for changes in mental status and focal neurological signs should be repeatedly performed in order to detect dissemination to the skin and brain, respectively.

Diagnostic Imaging

Imaging studies are helpful in assessing the burden of the disease, involvement of adjacent tissues, and response to treatment. They are also helpful in guiding more invasive procedures to obtain biopsy specimens for histopathology and culture [40]. Although imaging findings may be suggestive of mucormycosis in the appropriate clinical setting, they are not sufficiently specific to establish the diagnosis. In sinus disease, computerized tomography (CT) detects subtle mucosal thickening or bony erosions of the sinuses, but it is less sensitive than magnetic resonance imaging (MRI) for the detection of extension of the infection to the soft tissues of the orbit [29, 41]. In the case of pulmonary disease, high-resolution CT is more sensitive than chest radiograph for early diagnosis of the infection and can more accurately determine the extent of pulmonary involvement. Radiographic features consistent with pulmonary mucormycosis include nodular infiltrates, pleural effusions, cavity, consolidation, and reverse halo sign (Fig. 14.1). The air crescent and halo signs, which are recognized radiologic features of invasive

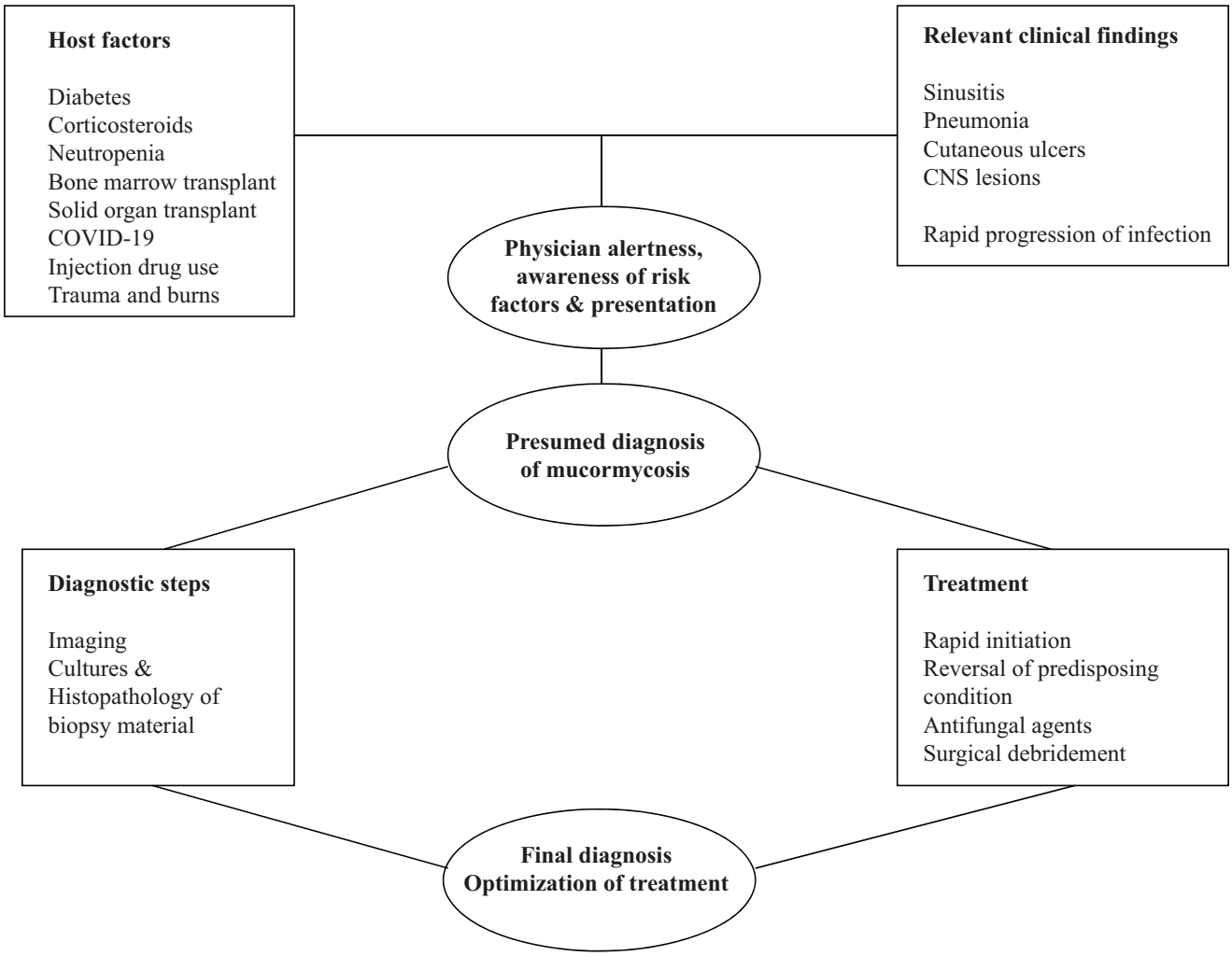


Fig. 14.4 Approach to diagnosis and treatment of mucormycosis. The initial diagnosis of mucormycosis requires a recognition of known risk factors in a patient who presents with clinical manifestations of sinusitis, pneumonia, cutaneous lesions, or CNS infection. A diagnosis is facilitated by accurate imaging and established with culture and biopsy of the infected site. Effective treatment is predicated upon the triad of rapid initiation of antifungal therapy, reversal of immunosuppression, and surgical intervention, as indicated

aspergillosis, have been reported as well for mucormycosis, while the reverse halo sign in neutropenic patients may have more specificity for pulmonary mucormycosis [34, 35, 42, 43]. In patients with pulmonary mucormycosis, the presence of an air crescent sign seems to be associated with increased risk for massive hemoptysis [35]. Another suggestive finding could be expansion of a mass or consolidation across tissue planes, in particular toward the great vessels in the mediastinum [44]. In the case of cutaneous disease, MRI is superior to CT scan for assessment of extension of the infection to the adjacent soft or bone tissues.

Culture

Recovery of Mucorales from cultures of clinical specimens allows not only establishment of diagnosis but also identification of the causative organism to the species level. Although the Mucorales may contaminate laboratory material, their isolation from clinical specimens of susceptible hosts should not be disregarded as contamination. Despite the ability of these organisms to invade tissues, they are rarely isolated from cultures of blood, even when dissemination has occurred, for example, from urine, cerebrospinal fluid, feces, sputum, paranasal sinuses secretions, bronchoalveolar lavage, or swabs from infected areas [29, 35, 45–48]. Tissue specimens submitted for fungal culture should be processed by mincing (cutting a tissue into small pieces) instead of homogenization (grinding the whole tissue into suspension) before inoculating onto culture plates. The recovery of Mucorales from biopsy material may be compromised if processing of the specimens involves tissue grinding, a procedure that may kill the non-septate hyphae of these fungi [49]. Therefore major clinical microbiology reference texts

emphasize the importance of mincing tissue instead of homogenizing when mucormycosis is suspected [50–52]. Culture recovery may be significantly enhanced and the fungal growth optimized, if clinical specimens are inoculated onto appropriate media saturated with carbohydrates, such as Sabouraud glucose agar, and incubated at 25°C or 37°C [53]. The recovery rate is, however, enhanced if thin slices of minimally manipulated tissue are placed onto the culture medium. Consequently, for proper handling of the specimens, the laboratory should be notified of the possibility of mucormycosis. In any case, negative cultures do not diagnostically exclude mucormycosis.

Colonies typically appear within 24–48 h unless residual antifungal agents, such as amphotericin B, are present, which can suppress growth. Most mucoraceous species fill a culture dish within 3–5 days and demonstrate a grayish-white, aerial mycelium with a wooly texture. The colonies readily separate from the agar surface. Identification of the species of the Mucorales in most laboratories are based upon colonial and microscopic morphology [53].

Histopathology

Given the above limitations of cultures or imaging studies, diagnosis of mucormycosis is almost always based on histopathologic examination of appropriately collected samples (Fig. 14.2). The latter should be pursued in the presence of strong suspicion for mucormycosis if the cultures or imaging studies are negative or nonspecific. Depending on the presentation of the disease, the specimens may be collected by fiberoptic bronchoscopy, radiographically guided transthoracic needle aspiration, open lung biopsy, nasal endoscopy, paranasal sinus biopsy or debridement, and biopsies of skin or other infected tissues [5, 35, 40, 54].

Because the hyphae of Mucorales in tissue specimens may stain poorly with hematoxylin and eosin (H&E), a second more fungus-specific tissue stain should also be used, such as Grocott-Gomori methenamine silver (GMS) or periodic acid Schiff (PAS). As already mentioned, the hallmark of mucormycosis is the demonstration of broad (6–20 μm in diameter), ribbon-like, aseptate (coenocytic), or sparsely septate hyphae with wide-angle branching in biopsy specimens (Fig. 14.2). For Mucorales infections, the hyphae are seen to invade the adjacent blood vessels. Mycotic emboli may thrombose small vessels in which they are lodged. Neutrophilic, granulomatous, or nonspecific inflammatory variations, extensive tissue necrosis, or hemorrhage may be observed [3, 55]. Fine needle aspiration biopsy may help to determine the diagnosis when focal pulmonary nodules or masses are apparent [56, 57]. Although histopathology is sensitive and reliable for diagnosing mucormycosis, obtaining biopsy material from hematological patients may not always be feasible due to concomitant thrombocytopenia. Finally, a new approach to rapid intraoperative diagnosis and staging of mucormycosis utilizes fluorescent microscopy and cell wall stain of resected fresh tissue in lieu of frozen sections with special stains [58].

Non-culture Diagnostic Methods

Although there are no standardized molecular or antigen detection methods available to date for primary diagnosis of mucormycosis, there are important advances being achieved. Among these advances are nucleic acid amplification methods, including PCR, for laboratory detection of circulating mucoralean DNA in blood and bronchoalveolar lavage fluid [59]. Reference laboratory support for detection of mucormycosis is available through Viracor (https://www.viracor-eurofins.com/test-menu/3200-mucorales-real-time-pcr/). Expansion of molecular diagnostic platforms Matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF MS) is being increasingly used for identification of molds to the species level once an organism is recovered by culture [60]. Detection of microbial cell-free DNA in whole blood using metagenomic next generation sequencing is yet another promising tool for the detection of invasive fungal infections, including mucormycosis. One such commercially available metagenomic system is Karius (https://www.kariusdx.com/).

Treatment

There are four cornerstones of successful management of mucormycosis: (1) rapid initiation of therapy; (2) reversal of the patient's underlying predisposing condition; (3) appropriate choice and dosage of antifungal agents; and (4) surgical debridement of infected tissues (Fig. 14.4) [5]. If not treated or diagnosed with delay, infections caused by the Mucorales in humans are typically fatal [1, 18, 19]. Even if a timely diagnosis is made, treatment is challenging due to a number of reasons such as the underlying condition of the patient, the rapid progression of the disease, and the high degree of angioinvasion and thrombosis that compromises the delivery of antifungal agents active against the causative organisms. The value of a comprehensive diagnostic and interdisciplinary management approach, incorporating molecular diagnostics, repeated surgical debridement until clean biopsies are obtained and optimized antifungal therapy based on in vitro susceptibility results and therapeutic drug monitoring (TDM) was recently demonstrated in a series of 13 hematological patients with mucormycosis [61].

As previously mentioned, in the presence of certain conditions such as diabetes, immunosuppression, and others (Table 14.2), treatment for mucormycosis should be initiated empirically as soon as a strong suspicion for this infection is raised without awaiting formal confirmation of the diagnosis, which may take time. Meanwhile, of course, all the required actions to establish the diagnosis should be undertaken, as already outlined (Fig. 14.4).

Reversal of the underlying condition can be fairly quickly achieved in certain circumstances, such as diabetic ketoacidosis, which should be promptly corrected, or deferoxamine therapy, which should be discontinued. However, timely reversal of the disease- or treatment-related immunosuppression in patients with hematological malignancies or transplant recipients is challenging. In these patients, temporary discontinuation of corticosteroid treatment or myelotoxic chemotherapy should be strongly considered until the infection is brought under control. However, even with these measures, spontaneous restoration of phagocytic activity or recovery from neutropenia is likely to occur after several days, during which time the infection may progress. In vitro and in vivo studies as well as case reports have suggested that the administration of cytokines, such as granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-gamma, may accelerate immune recovery [62]. In particular, interferon-gamma and GM-CSF have been shown to enhance neutrophil activity against *R. oryzae* and other species of Mucorales [63]. An alternative approach has been the administration of granulocyte transfusions in neutropenic patients with invasive fungal infections [64]. These immunomodulatory interventions may be considered on an individual patient basis as adjunctive therapy for mucormycosis in immunocompromised hosts. Nonetheless, as a caveat, there is a lack of adequately powered clinical trials to evaluate their clinical efficacy and potential complications [5, 62].

Amphotericin B

Liposomal amphotericin B is currently the drug of choice for first-line treatment of mucormycosis [59]. This polyene agent exerts good in vitro and in vivo activity against the Mucorales. Although currently no validated antifungal susceptibility breakpoints are available for Mucorales, apparent in vitro resistance, with elevated minimal inhibitory concentrations (MICs) of amphotericin B, may be observed among clinical isolates and is relatively common among *Cunninghamella* species [65–68]. The efficacy of amphotericin B in the treatment of mucormycosis was demonstrated in a review of 929 cases, where survival was 61% for patients treated with amphotericin B deoxycholate versus 3% for those who received no treatment [1]. The lipid formulations of amphotericin B (mainly amphotericin B lipid complex and the liposomal formulation) also have been used in the treatment of mucormycosis. These formulations are associated with significantly less toxicity than amphotericin B deoxycholate and demonstrate at least equivalent clinical efficacy [1, 53, 69].

When treatment with amphotericin B is initiated for documented mucormycosis, full doses should be given from the onset, foregoing the past practice of dose escalation [59]. The optimal dosage of amphotericin B formulations for the treatment of mucormycosis has not been systematically evaluated in clinical studies. A study of the safety, tolerance, and plasma pharmacokinetics of liposomal amphotericin B in patients with invasive fungal infections found no demonstrable dose-limiting nephrotoxicity or infusion-related toxicity over a dose range of 7.5–15 mg/kg/day [70]. Plasma concentrations of liposomal amphotericin B achieved an upper limit at 10 mg/kg/day and were not increased by further dosage increases. Nevertheless, the efficacy of higher dosages of liposomal amphotericin B compared to the Food and Drug Administration(FDA)-approved dosage of 3–5 mg/kg/day for aspergillosis has not been investigated through clinical trials in mucormycosis. In the absence of such studies, an increased dosage of liposomal amphotericin B to 5.0–10 mg/kg/day should be considered for treatment of mucormycosis and adjusted based upon renal function, stage of disease, and degree of immunosuppression.

A prospective pilot study of high-dose (10 mg/kg/day) liposomal amphotericin B monotherapy (combined with surgery in 71% of cases) in 33 patients with proven or probable mucormycosis demonstrated an overall response rate of 45% at week 12. Serum creatinine doubled in 16 (40%) of patients and normalized by week 12 in 10/16 (63%) of them [71]. Currently recommended dosage of liposomal amphotericin B for first-line treatment of mucormycosis is 5–10 mg/kg/day, with 10 mg/kg/day in case of brain involvement [59].

Amphotericin B lipid complex (ABLC) also has been used for successful treatment of mucormycosis, albeit with considerably fewer reported cases. Amphotericin B lipid complex (ABLC) was studied in an open-label, single-patient, emergency-use salvage study of patients who were refractory to or intolerant of conventional antifungal therapy. Among the 291 mycologically confirmed cases evaluable for therapeutic response, there was a complete or partial response to ABLC in 17 (71%) of 24 cases of mucormycosis [72]. ABLC for mucormycosis has usually been dosed at 5 mg/kg/day.

Several studies have also found that ABLC also exerts immunomodulatory activity on phagocytic effector cells. For example, ABLC interacted synergistically with human neutrophils in inducing augmented hyphal damage to *Rhizopus arrhizus*, *Rhizopus microsporus*, and *Lictheimia corymbifera* [73].

Triazoles

Isavuconazole is a new extended spectrum triazole with in vitro and in vivo activity against the Mucorales that was recently approved for primary treatment of mucormycosis. Reduced susceptibility may be observed in vitro for some isolates, in particular for *Mucor* spp. [74–76]. The clinical efficacy of isavuconazole was demonstrated in a single-arm, open-label trial (VITAL study), including 37 patients with mucormycosis, of whom 21 received isavuconazole as primary and 16 as salvage treatment. The overall response rate (complete and partial response) at day 42 and at end-of-treatment was 54% and 31%, respectively. The day 42 crude all-cause mortality of 21 patients who received primary therapy with isavuconazole was similar to that of 33 matched controls from the FungiScope Registry who received primary amphotericin B-based treatment (33% vs. 39%, $p = 0.77$) [77]. A number of case reports and small series, including pediatric patients, also suggest the clinical efficacy of isavuconazole in mucormycosis [78–80]. Currently, isavuconazole is recommended as salvage therapy for patients with mucormycosis not responding or intolerant to liposomal amphotericin B or as primary therapy in those with pre-existing renal impairment [59]. Isavuconazole is available in intravenous and oral (capsules) formulations. The recommended adult dosage for both formulations is 200 mg (372 mg of isavuconazonium sulfate) three times daily for the first 2 days, followed by 200 mg once daily. Routine TDM for isavuconazole is currently not recommended, although some experts recommended TDM if the causative organism exhibits high (≥2 μg/mL) MIC for isavuconazole [81].

Posaconazole is active in in vitro and in vivo against many of the Mucorales [66, 68, 82–84]. However, in a comparative in vitro susceptibility study of 217 clinical isolates of Mucorales, the percentage of isolates (particularly of *Rhizopus* and *Mucor* spp.) exhibiting reduced susceptibility to posaconazole was higher compared to amphotericin B [65]. There are several reports of salvage therapy with posaconazole of patients with mucormycosis refractory to amphotericin B [85–87]. The intravenous formulation of posaconazole contains sulfobutyl ether cyclodextrin as a carrier, which may be accumulated in patients with creatinine clearance <50 mL/min. Oral formulations of posaconazole constitute an option for long-term, outpatient treatment of mucormycosis; these include suspension and, more recently, delayed-release tablets. The later exhibit improved bioavailability compared to the suspension, resulting in more predictable drug exposure [88]. The recommended adult dosage for intravenous and tablet formulations of posaconazole is 300 mg twice daily for the first day followed by 300 mg once daily. The recommended dosage for the suspension is 200 mg 4 times daily (or 400 mg twice daily). In the 2019 guidelines of the European Confederation of Medical Mycology (ECMM) for the management of mucormycosis, posaconazole could be used as salvage therapy in patients not responding or intolerant to liposomal amphotericin B. It could also be considered as first-line therapy for patients with pre-existing renal compromise. The intravenous formulation or delayed-release tablets should be preferred over the oral suspension [59]. Due to significant inter-patient variability in drug exposure, TDM is suggested for patients receiving oral suspension, with target trough levels >1 μg/mL [81].

Of the triazole agents, fluconazole and voriconazole have little or no activity against the Mucorales [65, 83]. Itraconazole is active in vitro against some of these organisms, but has demonstrated poor efficacy in animal models [82, 84].

Combination Antifungal Therapy

As mucormycosis is difficult to treat, combination antifungal therapy has attracted scientific interest over the last years. Although echinocandins have no intrinsic in vitro activity against the Mucorales, their combination with lipid formulations of amphotericin B improved outcome in murine models of mucormycosis compared to amphotericin B alone [89]. In a retrospective study, a small number ($n = 6$) of patients treated with polyene-caspofungin combination for mucormycosis had improved response and survival rate compared to those treated with polyene monotherapy [90]. However, in another retrospective study of 101 cases of mucormycosis in high-risk patients, the increased use of amphotericin B-echinocandin combination therapy was not associated with improved survival [91].

The combination of amphotericin B with posaconazole also has been studied in animal models of invasive mucormycosis yielding conflicting results [89]. In a retrospective study of 106 patients with hematological malignancies and mucormycosis, survival was compared among those (47 or 44%) who received monotherapy (liposomal amphotericin B or posaconazole) as initial treatment and those (59 or 56%) who received combination therapy (of whom 27% received liposomal amphotericin B with posaconazole, 46% liposomal amphotericin B with echinocandins, and 27% triple combination with these agents). No statistically significant difference in survival was found between monotherapy and combination therapy [92].

Based on available data, no definitive recommendations can be made regarding the use of antifungal combination therapy for mucormycosis [59].

Surgery

Appropriate and early surgical debridement is a critical intervention for the successful management of mucormycosis for a number of reasons: the infection progresses rapidly, vascular thrombosis compromises the delivery of antifungal agents to the site of infection, and there is massive tissue necrosis. Several retrospective studies have demonstrated that the survival of patients treated with antifungal therapy combined with surgical debridement was significantly higher than that of patients treated with antifungal therapy alone [1, 5, 35]. Surgical treatment should aim at removing all necrotic tissues and should be considered for any of the clinical presentations of mucormycosis (sinus disease, pulmonary or cutaneous). It should be performed early in the course of treatment and repeated if necessary. It may include excision of the infected sinuses, debridement of retro-orbital space, or even enucleation in the case of sinus/sino-orbital disease, and wedge resection, lobectomy or pneumonectomy, in the case of pulmonary disease [29, 31, 35, 37, 93]. If the patient survives the infection, plastic surgery is likely to be needed in order to correct disfigurement resulting from debridement.

Hyperbaric Oxygen

Besides the above important aspects of management of mucormycosis, hyperbaric oxygen is a therapeutic modality that has been occasionally used as adjunctive treatment. Hyperbaric oxygen has a theoretical potential for being beneficial in the treatment of mucormycosis since it is known to inhibit fungal growth at high pressures, correct tissue hypoxia and lactic acidosis, promote healing, and enhance phagocytosis [94, 95]. In a number of case reports and small case series of mucormycosis, administration of hyperbaric oxygen was associated with a favorable outcome [95]. Currently, however, the absence of randomized controlled clinical trials on the efficacy of hyperbaric oxygen in this setting does not allow firm recommendations regarding its use as adjunctive treatment of mucormycosis.

Prognosis

The prognosis of mucormycosis largely depends on the patient's underlying condition, the clinical presentation of the infection, the time of initiation of therapy, and the type of treatment provided. Mortality may range from approximately 10% for localized sinus disease to 100% for disseminated infection; survival is higher for patients treated with antifungal therapy plus surgery compared to those receiving antifungal therapy alone [1, 30, 96].

Underlying conditions are critical prognostic factors in management of mucormycosis. Relapsed or refractory hematological malignancies carry a poor prognosis. Persistent neutropenia and pharmacological immunosuppression, such as corticosteroids, also carry a poor prognosis unless reversed. Control of diabetes mellitus also is important for improved prognosis.

Prevention

Prevention may be feasible for a proportion of cases through adequate control of diabetes and judicious use of deferoxamine and corticosteroids. For severely immunocompromised hosts, measures to reduce the risk of exposure to airborne sporangiospores should be undertaken, including HEPA-filtration of air supply, positive room air pressures, exclusion of plants from the wards, and wearing of masks when leaving the room. Oral posaconazole formulations have been approved by the Food and Drug Administration and European Medicines Agency for primary prophylaxis of invasive fungal infections in patients with acute myelogenous leukemia or myelodysplastic syndromes [88]. Due to the relatively low incidence of mucormycosis, the cost-effectiveness of prophylactic treatment solely for this infection would probably be questionable; the development of a pre-emptive therapy approach, however, based on validated early indicators of the disease and risk assumption, should be a target for research in the near future. In the meantime, physicians caring for susceptible patients should maintain a high level of suspicion and be alert to the early signs and symptoms of mucormycosis, in order to achieve early diagnosis and timely initiation of treatment.

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Pneumocystosis

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Introduction

Pneumocystis infection does not produce discernible disease in a normal healthy host, yet infection of the immunocompromised host results in a pneumonitis that is universally fatal, if untreated. The organism was first identified in the early 1900s, but was not recognized as a human pathogen until after World War II, when outbreaks of *Pneumocystis* pneumonia (PCP) occurred in orphanages in Europe. These young infants likely suffered from immunosuppression secondary to severe malnutrition and developed what was termed "interstitial plasma cell pneumonitis." Two subsequent events firmly established *Pneumocystis* as a major opportunistic pathogen; the development of successful cancer chemotherapy in the late 1950s and 1960s, and the start of the AIDS epidemic in the early 1980s. The diagnoses of this "rare" pneumonia, PCP, in a cluster of apparently healthy young men over a short period of time led to the discovery that a new syndrome (i.e., AIDS) and infection (i.e., HIV) had emerged [1, 2].

Presently the population of patients at risk of developing PCP is growing steadily as novel and potent immunosuppressive therapies enter clinical use for the treatment of malignancies, organ failure, autoimmunity, and inflammatory diseases. For example, as survival rates improve in solid organ transplant recipients, so does the recognition that these patients are clearly at risk of developing PCP, if not on specific prophylaxis [3, 4]. Most recently, the addition of anti-tumor necrosis factor and other immunomodulatory therapies to the management of patients with Crohn's disease, rheumatoid arthritis, and other inflammatory conditions has resulted in the occurrence of PCP in populations that previously had not been considered to be at risk [5, 6]. The importance of PCP as an opportunistic pneumonia is likely to increase as the use of immunosuppressive biologic response modifiers increases.

Etiologic Agent

All species and strains of *Pneumocystis* are extracellular organisms found exclusively in the lungs of mammals. Until recently, the taxonomic placement of these organisms had not been clearly established, largely due to the inability to observe its complete life cycle in a culture system. However, comparative genomics studies revealed that *Pneumocystis* is most closely related to the ascomycetous fungi [7, 8], despite possessing morphologic features and drug susceptibility profiles that are similar to those of protozoa. *Pneumocystis* organisms display strict host–species restriction, and each mammalian species is infected by a genetically and antigenically unique strain or species of *Pneumocystis* [9–11]. Animal studies demonstrating that *Pneumocystis* organisms are not transmissible from one mammalian species to another confirmed the biological consequence of these differences [12, 13]. This tightly restricted host range is the one biologic characteristic of this pathogen that might achieve the level of uniqueness sufficient to define distinct species of *Pneumocystis*.

Although the specific mechanisms regulating the host–species specificity have not been identified, the clear biological distinction of the *Pneumocystis* organisms from different hosts has led to the division of these fungi into multiple unique species. The nomenclature *P. jirovecii* is now used to refer to *Pneumocystis* infecting humans [14], while *Pneumocystis* capable of infecting rats and mice are referred to as *P. carinii* and *P. murina*, respectively [15]. The proposal for a change in nomenclature was initially questioned because speciation was based on molecular phylogeny [16]. However, the finding of host-restricted transmission and replication supports the change, and the new nomenclature has

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_15

been largely adopted in the literature. *Pneumocystis* that has not had a new name submitted for consideration can still be clearly defined using "special form" nomenclature (e.g., *P. carinii* f. sp. mustela for ferret *P. carinii*). Clinicians should be aware that these variations appear in the medical and research literature.

The lack of an *in vitro* culture system to propagate any of the *Pneumocystis* species has significantly hampered the study of these respiratory fungal pathogens. Complete genome sequencing of *P. jirovecii*, *P. carinii*, and *P. murina* indicates that *Pneumocystis* species have lost many genes that are deemed essential for autonomous survival [17–19]. These include enzymes involved in amino acid synthesis and other critical metabolic pathways. Therefore, *Pneumocystis* is most likely an obligate parasite that requires the lung environment to complete its life cycle. Unfortunately, researchers have not been able to replicate the critical aspects of the lung that promote replication in an in vitro culture system. Another consequence of the failure to successfully culture *Pneumocystis* is that a clearly defined life cycle has not been determined. However, different life forms of *Pneumocystis* are evident in the alveolar spaces of infected mammals. Thick-walled cysts, or asci (Fig. 15.1), are 5–8 μm in diameter and may contain up to eight distinctive intracystic sporozoites or ascospores. Trophozoites, or trophic forms, are 2–5 μm diameter cells with a typical cell membrane, but lacking the thick cell wall characteristic of the cyst. They are thought to be derived from excysted sporozoites, and are often observed closely attached to the alveolar epithelium. While it has been proposed that trophic forms replicate asexually through binary fission, at least some of the trophic forms are thought to mature into the cyst form to complete the life cycle. Recent work challenges the capability of trophic forms to independently replicate, and suggests that progression through a cyst stage is obligatory for replication [20]. The terminology "sporozoite" and "trophozoite" are based on the morphological similarities to protozoa, while ascus and ascospore are terms derived from fungal nomenclature.

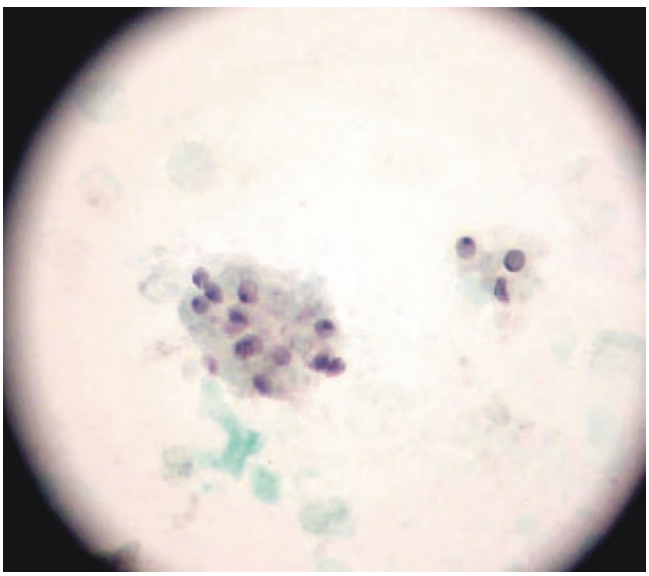


Fig. 15.1 Silver stained bronchoalveolar lavage specimen showing characteristic clusters of *Pneumocystis* cysts

Epidemiology

Symptomatic PCP occurs only in patients who are significantly immunosuppressed, typically with abnormalities in $CD4^+$ T lymphocytes or B cells. However, serologic studies have demonstrated that a high proportion of the population has evidence of current or prior infection, and that seroconversion typically occurs during childhood. By 20 months of age, > 85% of the infants in a prospective longitudinal study had seroconverted [21], indicating that they were exposed to *P. jirovecii*. The high prevalence of *P. jirovecii* primary infection during childhood has also been demonstrated by molecular techniques [22, 23]. Aside from these data, *Pneumocystis* was not known to actually infect the immunologically intact host. However, animal studies have proved that *Pneumocystis* produces a typical pattern of infection, transmission, and resolution in the normal immunocompetent host [24]. Together, these data suggest that *Pneumocystis* is able to evade innate immunity in both immunocompetent and immunosuppressed hosts, which likely contributes to the widespread distribution of this fungi throughout the population [25]. Recent studies suggest that the trophic form of *Pneumocystis* may suppress immune function, possibly contributing to immune evasion [26].

As detailed above, an important biological feature of *Pneumocystis* is that the species from any given mammalian host is transmissible only to members of the same host species. Cross-species transmission has never been convincingly demonstrated. Because early seroconversion to *P. jirovecii* was followed by disease later in life, PCP was initially postulated to result from reactivation of a latent infection in immunosuppressed patients. However, no evidence for latency has ever been demonstrated, and studies of mouse and rat models of PCP have determined that latency does not develop after infection [27]. Thus, it seems most likely that human cases of PCP develop as a result of a new exposure to *P. jirovecii* rather than reactivation of a latent infection. Person-to-person airborne transmission is likely, based on the cumulative experience in animal models, but is difficult to directly prove. However, genetic typing of *P. jirovecii* isolates from individual patients has shown that cases of PCP caused by genetically identical organisms can occur in clusters [3, 4]. In addition, *P. jirovecii* DNA has been detected in

air samples taken from the hospital rooms of infected patients [28, 29].

Without prophylaxis, PCP develops in approximately 70% of adults and 40% of infants and children with AIDS, and 10% of patients with organ transplants. It is often the sentinel event identifying infants with severe congenital immunodeficiencies such as severe combined immunodeficiency syndrome (SCID). PCP also is a frequent occurrence in patients being treated for malignancies, occurring with an overall frequency of 10–15%. The actual incidence for any given malignancy depends on the treatment regimen, and is positively correlated with the number of chemotherapeutic agents and intensity of treatment.

Pathogenesis and Immunology

Control of infection is dependent on normally functioning $CD4^+$ T lymphocytes. Studies in patients with AIDS show an increase in the occurrence of PCP as $CD4^+$ T lymphocyte numbers drop. Specific antibody-mediated depletion of $CD4^+$ T cells in rodent models has confirmed the requirement of these cells for effective host defense [30]. For adults and children over 6 years of age, a $CD4^+$ T cell count of 200 cells/μL or lower is a marker of very high risk for development of PCP. Based on the occurrence of PCP in certain patients and mouse strains with various immunologic defects which result in impaired B cell function or defective antibody production, a possible role for $CD4^+$ T lymphocytes could be to provide help for the production of specific antibody. Passively administered antibody has been shown to aid in the clearance of *Pneumocystis* in mouse models [31, 32], and active immunization of mice with a protective subunit vaccine has been shown to protect against PCP even upon subsequent $CD4^+$ T cell depletion and infection [33]. Thus, antibody could be involved in the clearance of organisms through interaction with complement, phagocytes, and/or T lymphocytes.

The mechanism by which *Pneumocystis* damages the lung is not yet fully defined, but animal models have advanced our understanding of PCP-related immunopathogenesis [34]. Infection of SCID mice with *Pneumocystis* produces very little alteration in lung histology or function until very late in the course of the disease when heavy lung burdens are achieved. However, if *Pneumocystis*-infected SCID mice are immunologically reconstituted with normal splenocytes, there is a rapid onset of pulmonary inflammation characterized by an intense alveolar infiltrate, pulmonary function deficits, surfactant dysfunction, and significant hypoxia. These findings are also typical in humans with PCP, and demonstrate that immunopathogenesis is a key contributor to PCP-associated respiratory impairment. T cell subset analyses have shown that $CD4^+$ T lymphocytes produce an inflammatory response that clears the organisms, but also causes lung injury. In contrast, $CD8^+$ T lymphocytes are usually ineffective for the eradication of *Pneumocystis,* but do produce immunopathology, especially in the absence of $CD4^+$ T lymphocytes [35]. However, specific subsets of $CD8^+$ T cells may offer host defense against PCP in the absence of $CD4^+$ T cells [36].

Immune reconstitution inflammatory syndrome (IRIS), also called immune restitution disease or immune reconstitution syndrome, is a manifestation of pulmonary infection in AIDS patients with *Pneumocystis*, *Mycobacterium tuberculosis*, and other pulmonary pathogens. These patients experience a rapid recovery of their immune system due to the initiation of effective antiretroviral therapy [37]. In general, the severity of IRIS is directly related to the degree and rapidity of T cell recovery. Mouse models of PCP suggest that $CD8^+$ T lymphocytes help limit the level of immunopathogenesis generated by the $CD4^+$ T lymphocyte response, but as mentioned above, they can also drive lung injury. Variability in the nature of the T cell response may be responsible for disparities in the presentation and outcome of PCP in different patient populations.

The inflammatory processes taking place during PCP do not appear to result in major long-term damage to the lung in those who recover. A long-term follow up of 23 children with cancer and PCP showed a return to normal lung function by six months in all survivors [38]. Similar studies in adults are complicated by the fact that adult patients, especially those with AIDS, might have multiple pulmonary insults. While some studies, primarily of adult AIDS patients, suggest long-term pulmonary damage following PCP, other studies of renal transplant recipients have shown pulmonary function returned to nearly normal after recovery from PCP [39].

Clinical Manifestations

Pneumocystis Pneumonia

There are at least three distinct clinical presentations of PCP. In patients with profound immunodeficiency, such as young infants with congenital immunodeficiency, severe malnutrition, or AIDS patients with low $CD4^+$ T lymphocyte counts, the onset of hypoxia and symptoms is subtle with cough, dyspnea on exertion, or tachypnea, often without fever. Infants may show progression to nasal flaring, intercostal, suprasternal, and infrasternal retractions. As the disease progresses, patients develop hypoxia, with cyanosis in severe cases. In the sporadic form of PCP, occurring in children and adults with underlying immunodeficiency, the onset of hypoxia and symptoms is usually more abrupt with fever, tachypnea, dyspnea, and cough, progressing to severe respiratory compromise. This latter type accounts for the majority

of cases, although the severity of clinical expression may vary. Rales are usually not detected on physical examination. The third pattern of disease is that associated with rapid restoration of immune function referred to as IRIS. It has been best described in newly diagnosed AIDS patients who are severely immunocompromised and present with PCP as their initial manifestation of AIDS [37]. These patients appear to respond well to therapy for PCP, but 3–6 weeks after beginning treatment, they experience an unexpected recurrence of pulmonary symptoms and CXR abnormalities that coincide with return of immune function. IRIS may also occur in bone marrow transplant patients who engraft while infected with *Pneumocystis*.

Extrapulmonary Infections

Extrapulmonary infection with *Pneumocystis* is rare. The incidence is not well defined, but is estimated to be 1000-fold less likely than PCP itself [40]. The most commonly reported sites of infection include the ear and eye. Why these two sites seem to predominate is unclear, but may reflect the fact that infection at these sites may quickly produce readily apparent signs and symptoms. Other sites of involvement are the thyroid gland, liver, kidney, bone marrow, lymph nodes, spleen, muscle, and GI tract. How the organism arrives at these sites is unknown. Response to treatment is usually good when extrapulmonary infections occur in the absence of pulmonary infection.

Diagnosis

Pulmonary symptoms in at-risk patients should always raise the suspicion of PCP. The classic chest radiograph reveals bilateral diffuse alveolar disease with a granular pattern (see Fig. 7.8, Chap. 7). The earliest densities are perihilar, and progression proceeds peripherally, typically sparing the apical areas until the last. Less common chest radiograph appearances in PCP include cystic lesions, pneumothorax, or isolated focal infiltrates. In patients receiving aerosolized pentamidine for prophylaxis, there may be a predisposition for upper lobe infiltrates. The arterial oxygen tension (PaO_2) is invariably decreased.

A clinical pearl is that an elevated lactate dehydrogenase (LDH) may be a hint that one is dealing with PCP. This is due to the fact that LDH is useful as a marker of alveolar and inflammatory cell damage. Because *Pneumocystis* is a diffuse alveolar infection, it tends to result in higher and more often elevated levels of LDH than some other more focal opportunistic pulmonary infections. For example, a recent analysis of LDH and pulmonary opportunistic infections in AIDS patients showed that about 90% of those with definite PCP had elevated serum LDH [41]. Thus, while not specific for PCP, very high LDH levels should raise one's suspicion for PCP and normal levels make the diagnosis of PCP much less likely.

PCP can only be definitively diagnosed by demonstrating *Pneumocystis* in the lungs of a patient with compatible pulmonary signs and symptoms. Appropriate specimens for analysis include bronchoalveolar lavage, tracheal aspirate, transbronchial lung biopsy, bronchial brushings, percutaneous transthoracic needle aspiration, and open lung biopsy. Induced sputum samples are gaining popularity, but are helpful only if positive; the absence of *Pneumocystis* in an induced sputum sample does not exclude infection. The open lung biopsy is the most reliable method, although bronchoalveolar lavage is generally more practical. Estimates of the diagnostic yield of the various specimens are as follows: induced sputum 20–40%, tracheal aspirate 50–60%, bronchoalveolar lavage 75–95%, transbronchial biopsy 75–95%, and open lung biopsy 90–100%. Once obtained, the specimens are typically stained with one of four commonly used stains: Gomori methenamine silver (GMS) and toluidine blue stains only stain cyst forms; polychrome stains, such as Giemsa, stain both trophozoites and sporozoites; and the fluorescein-labeled monoclonal antibody also stains both trophozoites and cysts. *Pneumocystis* can also be visualized by Papanicolaou and Gram-Weigert stains. Polymerase chain reaction analysis of respiratory specimens offers a rapid diagnostic method to identify *Pneumocystis* in sputum or BAL samples. Collected specimens should also be inoculated onto culture media to test for bacteria, fungi, and viruses.

Treatment

The clear drug of choice for the treatment of PCP is trimethoprim–sulfamethoxazole (TMP–SMX) (Table 15.1). Generally, TMP–SMX is administered intravenously, but it may be given orally if disease is mild and no malabsorption or diarrhea is present. The duration of treatment is generally three weeks for patients with AIDS and two weeks for other patients. Adverse reactions occur frequently with TMP–SMX, more so in adults than children. These include rash, fever, and neutropenia in patients with AIDS. These side effects are less common in non-AIDS patients. For patients who cannot tolerate or fail to respond to TMP–SMX after 5–7 days, pentamidine isethionate may be used. Adverse reactions are frequent with pentamidine and include renal and hepatic dysfunction, hyperglycemia or hypoglycemia, rash, and thrombocytopenia. Atovaquone is an alternative treatment that has been used primarily in adults with mild to moderate disease. For adults and adolescents, atovaquone is given twice a day with food. Less information is available for

Table 15.1 Recommended treatment for *Pneumocystis* pneumonia[a]

Drug	Adults	Children
Treatment of first choice		
Trimethoprim–sulfamethoxazole (TMP–SMX)	TMP 15–20 mg/kg/d with SMX 75–100 mg/kg/d IV divided into 3 or 4 doses; PO for mild disease	TMP 15–20 mg/kg/d with SMX 75–100 mg/kg/d IV divided into 4 doses; PO for mild disease
Alternate treatment regimens		
Pentamidine	4 mg/kg/d IV as single dose	4 mg/kg/d as single dose
Atovaquone	750 mg PO bid	3–24 mo of age: 45 mg/kg/d PO divided into 2 doses; 1–3 mo and over 24 mo: 30 mg/kg/d in 2 divided doses (max. daily dose 1500 mg)
Dapsone plus trimethoprim	Dapsone 100 mg, PO once daily; TMP 15 mg/kg/d PO in 3 divided doses	Dapsone 2 mg/kg/d (100 mg max.) PO once daily; TMP 15 mg/kg/d PO in 3 divided doses
Primaquine plus clindamycin	Primaquine 15–30 mg, PO once daily; clindamycin 600 mg IV every 8 h	Primaquine 0.3 mg/kg (max 30 mg) PO once daily; clindamycin 40 mg/kg/d IV in 4 divided doses (no pediatric data)
Trimetrexate plus leucovorin	Trimetrexate	
	<50 kg: 1.5 mg/kg/d IV once daily	45 mg/m^2 IV once daily
	50–80 kg: 1.2 mg/kg/d IV once daily	
	>80 kg: 1.0 mg/kg/d IV once daily	
	Leucovorin (continue 3 days beyond trimetrexate)	
	<50 kg: 0.8 mg/kg/d IV or PO every 6 h	20 mg/m^2 IV or PO every 6 h
	>50 kg: 0.5 mg/kg/d IV or PO every 6 h	

IV, intravenous; PO, orally; mg/kg, milligrams/kilogram; mg/kg/d, milligrams/kilogram/day; mo, months of age; bid, twice daily

[a] Duration of therapy is typically 3 weeks in patients with AIDS and 2 weeks in other immunosuppressed patients

the treatment of younger children with this agent. Other effective therapies include trimetrexate glucuronate or combinations of trimethoprim plus dapsone or clindamycin plus primaquine. Recent studies raise the possibility that combined treatment with Caspofungin and TMP–SMX may lead to improved outcomes for PCP patients [42].

Administration of corticosteroids in addition to antibiotic drugs increases the chances for survival in moderate and severe cases of PCP [43]. The recommended regimen of corticosteroids for adolescents older than 13 years of age and for adults is oral prednisone, 80 mg/day divided in 2 doses on days 1–5, 40 mg/day once daily on days 6–10, and 20 mg/day once daily on days 11–21. While specific studies of adjunctive corticosteroid therapy in young children are not available, a reasonable regimen for children is oral prednisone, 2 mg/kg/day for the first 7–10 days, followed by a tapering regimen for the next 10–14 days.

Prevention

PCP is effectively prevented by the use of antimicrobial prophylaxis, thus all patients at high risk for PCP should be placed on chemoprophylaxis. As noted above, CD4$^+$ T-cells are the key cells in determining susceptibility to PCP. However, defining the risk for PCP is not always clear. In AIDS patients, there is a clear cut correlation between cell number and function so that firm cutoffs can be given. In adults with AIDS, prophylaxis is indicated at CD4$^+$ T-cell counts of below 200 cells/μL. Because of rapid changes in CD4$^+$ T-cell counts in young infants, prophylaxis is recommended for all HIV-infected children during their first year of life. Thereafter, prophylaxis is started at CD4$^+$ T-cell counts drop below 750 cells/μL for infants 12–23 months of age, 500 cells/μL for children from 2 to 6 years of age, and 200 cells/μL for those 6 years and older. Prophylaxis is also recommended for all ages if CD4$^+$ T-cell percentages drop below 15%. In other disease states, where patients are placed at risk of PCP from immunosuppressive drug, treatment, both lymphocyte number and function will be affected. Thus, while a patient may have a lymphocyte count above the threshold for susceptibility to develop PCP, suppressed function of remaining lymphocytes may place them at risk for PCP. Because of the demonstrated increased risk of PCP with increasing intensity of chemotherapy in patients with cancer, it would seem prudent, in our opinion, to consider prophylaxis for patients receiving prolonged (more than 6–8 weeks) therapy with two immunosuppressive agents and to give prophylaxis to all patients receiving three or more immunosuppressive agents.

TMP–SMX is the drug of choice for *Pneumocystis* prophylaxis and may be given for three days each week, or, alternatively, each day (Table 15.2). The original study testing less than daily administration of TMP–SMX used a schedule of three consecutive days on TMP–SMX and four days off with the idea of reducing potential bone marrow suppression from the TMP–SMX. Subsequent studies have used alternate day schedules such as dosing on Monday, Wednesday, and Friday. The double strength tablet is preferred for adults receiving three days a week dosing. Alternatives for prophylaxis, all of which are inferior to TMP–SMX, include dapsone, atovaquone, and aerosolized pentamidine. Additional experience with pentamidine demonstrates that monthly IV pentamidine is an acceptable prophylaxis for PCP [44, 45]. Recent reviews of patient records have determined that patients receiving the anti-inflammatory drug sulfasalazine were protected from *Pneumocystis* infec-

Table 15.2 Recommended antibiotic prophylaxis for *Pneumocystis* pneumonia

Drug	Adults	Children
Trimethoprim–sulfamethoxazole (TMP–SMX)	1 single or double strength tablet daily or 3 days/week	TMP 5 mg/kg/d with SMX 25 mg/k/d given once daily or divided into 2 doses
Dapsone	100 mg daily or twice weekly	2 mg/kg/d as single dose (max. 100 mg/dose)
Atovaquone	1500 mg once daily	30 mg/kg/d as single dose for children aged 1–3 months and older than 24 mo; 45 mg/kg/d as single dose for children 4–23 mo
Aerosolized Pentamidine	300 mg monthly given by Respigard II nebulizer	For children ≥5 years—same as for adults
Pentamidine	4 mg/kg IV monthly	4 mg/kg IV monthly

IV, intravenous; PO, orally; mg/kg/d, milligrams/kilogram/day; mo, months of age

tion [46]. Prospective studies will be needed to understand the significance of this observation.

Prophylaxis must be continued as long as the patient remains immunocompromised. Studies in adult AIDS patients who reconstitute adequate immune response during antiretroviral therapy show that prophylaxis may be withdrawn without risk of developing PCP. Small studies in children have provided similar results. Patients who maintain their $CD4^+$ T-cell count at or above the at-risk threshold for age, e.g., 200 cells/μL for older children and adults, for at least three months are candidates for discontinuation of both primary and secondary prophylaxis. Guidelines for the management of PCP in adults and children have been published [47, 48].

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Cryptococcosis

16

Eileen K. Maziarz and John R. Perfect

Introduction

Cryptococcosis is an infectious disease with a wide range of clinical presentations caused by pathogenic encapsulated yeasts in the genus *Cryptococcus*. In the current *Cryptococcus* complex, there are two species of these fungi that commonly cause disease in humans: *Cryptococcus neoformans,* which causes cryptococcosis in both immunocompetent and immunocompromised hosts, and *Cryptococcus gattii*, which is primarily a pathogen in apparently immunocompetent patients, but can also cause disease in those immunocompromised. *C. neoformans* was first identified as a human pathogen in 1894 by two German physicians, Otto Busse and Abraham Buschke, when they described a circular yeast-like microorganism in a lesion on the tibia of a woman; the yeast was initially named *Saccharomyces hominis* [1]. However, the name *Cryptococcus neoformans* has been consistently adopted in both the mycology and medical literature since 1950 [2]. In the mid-1970s, when Kwon-Chung discovered two mating types of *C. neoformans* that produced fertile basidiospores, the yeasts were subsequently separated into two varieties, var. *neoformans* (serotypes A and D) and var. *gattii* (serotypes B and C). These two varieties were separated into two species, *C. neoformans* and *C. gattii*, based on their genetic background and phylogenetic diversity, as proposed by Kwon-Chung in 2002 [3] and has become standard clinical nomenclature since. The incidence of cryptococcosis began to rise in the late 1970s. Early case reports of cryptococcal infections were primarily associated with expanding immunocompromised populations related to treatment of cancer and autoimmune diseases, organ transplantation, and receipt of corticosteroids [4]. A major surge in new cases of cryptococcosis occurred during the first two decades of the HIV epidemic, when cryptococcal infection was an important opportunistic infection in all parts of the world. Furthermore, around 2000, *C. gattii* strains (previously geographically restricted to tropical and subtropical regions) caused a localized outbreak of cryptococcosis in apparently immunocompetent individuals on Vancouver Island [5]. This outbreak increased recognition that these fungi can exploit new geographical environments and cause disease in both immunocompromised and apparently immunocompetent hosts. Despite the development of effective ART, which has decreased overall rates of HIV-associated cryptococcosis worldwide, the burden of cryptococcal infection remains very high in countries with high numbers of immunosuppressed individuals, particularly when access to healthcare is limited. Despite widespread ART expansion, the global burden of HIV-associated cryptococcal disease remains unacceptably high, with an estimated 270,000 new cases per year and over 180,000 annual deaths due to cryptococcosis worldwide [6].

Etiologic Agents

Cryptococcus is a genus of heterobasidiomycetous fungi containing more than 30 species. Molecular taxonomic classifications have placed many of these species in other genera. However, the primary pathogenic cryptococcal species have been divided into several sibling species or clades based on molecular taxonomic classification (Table 16.1). *Cryptococcus* has been divided into either the *C. neoformans* species complex or the *C. gattii* species complex [7]. The *C. neoformans* species (variety *grubii*) group includes several distinct genotypes (all serotype A strains by capsular serotyping): VNI, VNII, VNBI and VNBII. VNIII is a diploid hybrid (i.e., AD serotype) of *C. neoformans* VNI (serotype A) and VNIV (serotype D). VNIV is a serotype D by capsular serotyping and has been designated a sibling species called *C. deneoformans*. The *C. gattii* species complex has been divided into several sibling species that exhibit B or C

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_16

Table 16.1 Classification of *Cryptococcus neoformans* and *Cryptococcus gattii*

Serotype	Species and varieties	Molecular types
A	*C. neoformans*	VN I, VN II, VNBI, VNBII
B	*C. gattii*	VG I, VG II, VG III, VG IV, VGV
C	*C. gattii*	VG I, VG II, VG III, VG IV
D	*C. deneoformans*	VN IV
AD	*C. neoformans*	VN III

capsular serotyping: *C. gattii* (VGI genotype); *C. deutero-gattii* (VGII genotype). *C. bacillisporus* (VGIII genotype), *C. tetra gattii* (VGIV genotype), the hybrid species *C. deca-gattii* (VGII c/VGIV) [8] and the newly-discovered VGV lineage [9]. With further sequencing of strains and as whole genome sequencing becomes more common, it is expected these groups will be further divided into certain sequence clusters and more common strains may be broken down further to geographical relationships.

The life cycle of *C. neoformans* and *C. gattii* involve asexual (yeast) and sexual (basidiospores/hyphae) forms. The asexual form is the encapsulated yeast that reproduces by narrow-based budding and is found most commonly in clinical specimens; whereas the sexual stage, which exists in one of two mating types, "alpha" or "a," is observed only under certain conditions, resulting in meiosis to form basidiospores. The vast majority of clinical infections and environmental isolates are caused by "alpha" mating-type locus strains. Since the sexual stage of *C. neoformans* and *C. gattii* has been described, their teleomorphs were named *Filobasidiella neoformans* and *Filobasidiella bacillospora*, respectively.

C. neoformans and *C. gattii* usually appear as white-to-cream, opaque, and mucoid colonies that grow to several millimeters in diameter on agar plates within 48–72 h. With some strains, a few colonies occasionally develop sectors with different pigmentation or different morphologies (e.g., wrinkled, smooth, mucoid). Both cryptococcal species will grow readily on most fungal culture media without cycloheximide at 30–37 °C in aerobic conditions. However, *C. neoformans* is generally more thermotolerant than *C. gattii*, and, within this species, serotype A strains are generally more tolerant than serotype D strains (*C. deneoformans*). In addition to their ability to grow at 37 °C, the yeasts produce a thick, shedding polysaccharide capsule, melanin pigments, and the enzymes urease and phospholipase, which allow *Cryptococcus* to be readily identified from other yeasts. These characteristics are also considered to be some of the yeast's virulence factors.

Epidemiology

Cryptococcosis was once considered an uncommon infectious disease, associated with certain malignancies such as lymphomas and chronic leukemias, organ transplantation, and certain immunosuppressive treatments. However, as the AIDS epidemic expanded beginning in the early 1980s, the incidence of cryptococcosis increased significantly and between 6 and 10% of persons with AIDS developed cryptococcosis [10] and 80% of cryptococcosis cases worldwide were HIV-associated. *C. neoformans* remains a major opportunistic infection in persons living with HIV/AIDS, with the majority of cases occurring in those with $CD4^+$ cell counts below 100 cells/μL. Following widespread implementation of ART, the incidence of cryptococcosis among patients with HIV/AIDS has fallen in most high-income countries, while the incidence of cryptococcal infection in persons not infected with HIV has remained stable over time. On the contrary, in low- and middle-income countries, even where ART is available, the prevalence of morbidity and mortality associated with cryptococcosis remain unacceptably high, accounting for up to 180,000 deaths per year [6]. Besides HIV infection, other risk factors for acquiring cryptococcal infections include many conditions and therapeutics that result in a net (or, in some cases, specific) state of immune compromise (Table 16.2). Although both *C. neoformans* and *C. gattii* can cause cryptococcosis in apparently normal hosts, the percentage of *C. gattii* infections causing disease in such patients is higher than for *C. neoformans*.

Table 16.2 Predisposing factors of cryptococcosis

HIV infection
Malignancy[a] (e.g., Hodgkin's disease, other lymphomas, and chronic lymphocytic leukemia)
Lymphoproliferative disorders[a]
Idiopathic $CD4^+$ T cell lymphopenia
Anti GM-CSF autoantibodies
Rheumatologic or immunologic disease[a]
Sarcoidosis
Systemic lupus erythematosus
Rheumatoid arthritis
Hyper-IgM syndrome or hyper-IgE syndrome
Monoclonal antibodies (etanercept, infliximab, alemtuzumab)
Bruton's tyrosine kinase inhibitors (i.e., ibrutinib)
Corticosteroid and/or immunosuppressive therapies
Diabetes mellitus
Solid organ transplantation[a]
Chronic pulmonary diseases
Renal failure and/or peritoneal dialysis
Intravenous drug use
Chronic liver diseases[b]

a Immunosuppressive therapies add to the risk
[b] Poor prognosis

C. neoformans is found throughout the world in association with excreta from certain birds such as pigeons and in a variety of tree hollows. *C. gattii* is commonly associated with several species of eucalyptus and other deciduous trees. While the link between a direct environmental source of infection and cryptococcosis cases is not precise, there is evidence to suggest an increased risk of cryptococcosis and asymptomatic cryptococcal antigenemia following intense bird exposures. There has been a strong link between the *C.*

Table 16.3 Distribution of *C. neoformans* and *C. gattii*

Cryptococcus species	Primary areas of distribution
C. neoformans	Worldwide; pigeon guano, tree hollows
C. gattii	Tropical and subtropical regions: southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa; eucalyptus trees, firs, and oak trees
C. deneoformans (serotype D)	Europe: Denmark, Germany, Italy, France, Switzerland; less common in the environment than serotype A

gattii outbreak in humans on Vancouver Island and common environmental yeast exposures.

Approximately 95% of cryptococcal infections are caused by serotype A strains (*C. neoformans*), with the remaining infections caused by serotype D (*C. deneoformans*) or serotype B and C strains (*C. gattii*). Whereas *C. neoformans* is found worldwide, *C. gattii* is found primarily in tropical and subtropical regions such as southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa and, more recently identified in temperate climates, including Vancouver Island and the Pacific Northwest region of the United States. *Cryptococcus deneoformans* is predominantly found in European countries (Table 16.3). In areas where it is endemic, such as Australia and New Zealand, *C. gattii* may account for up to 15% of all cases of cryptococcosis, but *C. neoformans* remains the predominant serotype [11]. To date, only *C. gattii* strains have been reported to cause a localized, defined outbreak of disease [5].

Pathogenesis and Immunology

Cryptococcosis occurs primarily by inhalation of the infectious propagules, either dehydrated (poorly encapsulated) yeasts or basidiospores, into pulmonary alveoli. Direct inoculation into tissue due to trauma can be a portal of entry in occasional cases and, potentially, yeasts may enter through the gastrointestinal tract. After the yeasts are inhaled into the lungs of a susceptible host, they encounter alveolar macrophages, and other inflammatory cells are recruited through release of cytokines and chemokines such as IL-12, IL-18, monocyte chemotactic protein (MCP)-1, and macrophage inflammatory protein (MIP)-1α. Cryptococcal infection primarily involves granulomatous inflammation, which is typically the result of a successful helper T cell (Th1) response with cytokines, including tumor necrosis factor, interferon-γ, and IL-2 [12]. In many circumstances, the yeasts are either killed or remain dormant (yet viable) in hilar lymph nodes or within pulmonary foci in an asymptomatic individual for years, with the potential to disseminate outside these complexes if local immunity is suppressed, similar to that which is observed in cases of reactivation tuberculosis or histoplasmosis. In a patient with compromised cellular immunity or high inoculum exposure, however, the yeasts may immediately proliferate at the site of infection and disseminate to other body sites causing progressive clinical symptoms.

Recent advances in the molecular biology of *Cryptococcus* have confirmed several virulence factors at the genetic level. The three classical virulence factors of *C. neoformans* include: capsule formation, melanin pigment production, and the ability to grow well at 37 °C [12]. The prominent antiphagocytic polysaccharide capsule, comprising glucuronoxylomannan (GXM), which can shed extracellularly, is unique to *Cryptococcus* and considered an essential virulence factor that has multiple effects on host immunity. In addition, *C. neoformans* possesses an enzyme that catalyzes the conversion of diphenolic compounds to form melanin, which may have a biological role to protect the yeasts from host oxidative stresses and may partially explain the yeast's neurotropism in a site enriched for certain diphenolic substrates. Finally, its ability to grow at 37 ° C is a basic part of the virulence composite for most of the human pathogenic fungi, including *Cryptococcus*, as molecular studies have linked high temperature growth with certain signaling pathways and enzymes that this yeast has acquired or adapted over time in order to enhance its pathogenicity. Other virulence factors include phospholipase and urease production and multiple enzymes associated with protection against oxidative stresses. Many of these classic phenotypes have been linked to specific gene or genetic pathways. However, even these classic virulence factors cannot explain significant strain differences in virulence despite possessing all the known virulence phenotypes. There is still much to know about how this yeast produces disease.

Clinical Manifestations

C. neoformans and *C. gattii* have a predilection for establishing clinical disease in the lungs and central nervous system (CNS). Other organs that may be involved in cryptococcosis include skin, prostate, eyes, bone, and blood. In fact, this yeast may cause disease in any organ of the human body and widely disseminated cryptococcal infection can affect function of multiple organs at the same time in severely immunosuppressed patients (Table 16.4).

Pulmonary Infection

The respiratory tract serves as the most important portal of entry for this yeast and there are many clinical manifestations of pulmonary cryptococcosis, ranging from asymptomatic transient or chronic colonization of the airways or simply a pulmonary nodule on radiograph, to life-threatening fungal pneumonia with acute respiratory distress syndrome (ARDS) [2, 13]. Although cryptococci can be isolated from endo-

Table 16.4 Clinical manifestations of cryptococcosis

Organs	Common clinical manifestations
Central nervous system	Acute/subacute/chronic meningoencephalitis
	Cryptococcomas (abscesses)
	Spinal cord granuloma
	Chronic cognitive impairment (sequelae of hydrocephalus)
Lung	Asymptomatic airway colonization
	Pulmonary nodule(s)
	Hilar or mediastinal lymphadenopathy
	Lobar/interstitial infiltrates
	Miliary infiltrates
	Lung cavities
	Endobronchial lesions
	Pleural effusion/empyema
	Pneumothorax
	Acute/subacute pneumonia
	Acute respiratory distress syndrome
Skin	Papules with central ulceration (molluscum contagiosum-like)
	Subcutaneous abscesses
	Nodules/papules
	Cellulitis
	Draining sinuses
	Ulcers
Eye	Papilledema
	Endophthalmitis
	Optic nerve atrophy
	Chorioretinitis
	Keratitis
	Paresis of extraocular muscles
Genitourinary tract	Prostatitis
	Cryptococcuria
	Renal abscess
	Genital lesions
Bone and joints	Osteolytic lesion(s)
	Arthritis (acute/chronic)
Cardiovascular system	Cryptococcemia
	Endocarditis (native/prosthetic)
	Mycotic aneurysm
	Myocarditis
	Pericarditis
Other organs	Myositis
	Peritonitis
	Hepatitis
	Nodular/ulcerative GI mucosal lesions
	Pancreatic mass
	Breast abscess
	Adrenal mass and adrenal insufficiency
	Thyroiditis or thyroid mass
	Sinusitis
	Salivary gland enlargement

Adapted from Casadevall, A, Perfect, JR. *Cryptococcus neoformans*. Washington: ASM Press; 1998: 409 [2]

bronchial specimens of humans without disease (colonization), clinicians should always be alert for subclinical disease or the potential for invasive disease whenever *Cryptococcus* is isolated from any clinical specimen. In normal hosts, asymptomatic pulmonary cryptococcosis can occur in up to one-third of patients with pulmonary infection and patients may present to care with only an abnormal chest radiograph. The most common radiologic findings of cryptococcosis include well-defined single or multiple noncalcified nodules (Fig. 16.1) and pulmonary infiltrates (Fig. 16.2); other less frequent radiographic findings include pleural effusions, hilar lymphadenopathy, and lung cavitation. Patients with symptomatic pulmonary cryptococcosis may present with acute onset of fever, productive cough, respiratory distress, chest pain, and weight loss [13]. Severe presentations can occur in apparently immunocompetent individuals. In immunocompromised hosts, especially those with underlying HIV, cryptococcal pneumonia is usually symptomatic and can progress rapidly to ARDS, even in the absence of CNS

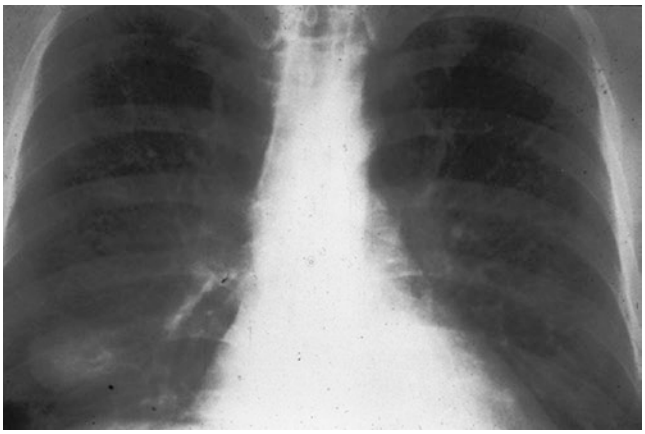


Fig. 16.1 Chest radiograph of pulmonary cryptococcosis presents as a single nodule in the lung at right lower lung field. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)



Fig. 16.2 Chest radiograph of pulmonary cryptococcosis presents as left lobar infiltrates. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)

involvement. Most immunocompromised patients with cryptococcal infection, however, present with CNS rather than pulmonary symptoms. Imaging findings of pulmonary cryptococcosis in immunocompromised patients can be similar to what is seen in immunocompetent patients, but alveolar and interstitial infiltrates are more frequently observed and imaging can occasionally mimic *Pneumocystis* pneumonia. In general, immunocompromised patients with cryptococcal pneumonia are more likely than immunocompetent hosts to present with symptomatic and accelerated presentations. In pulmonary cryptococcosis, if the infection is confined to the lung, serum cryptococcal polysaccharide antigen (CrAg) is generally negative. However, while a positive serum CrAg may indicate the dissemination of the yeast from the lung, it does not confirm CNS involvement. In immunocompromised individuals with pulmonary cryptococcosis, a lumbar puncture to rule out CNS disease should be considered, regardless of the patient's symptoms or serum CrAg results. The only setting in which a screening lumbar puncture may not be required is in a patient with *Cryptococcus* isolated from a respiratory specimen in an asymptomatic, immunocompetent patient in whom the disease appears limited to the lungs.

Central Nervous System (CNS) Infection

Clinical manifestations of CNS cryptococcosis include headache, fever, cranial neuropathy, alteration of consciousness, lethargy, memory loss, or signs of meningeal irritation [2]. These findings are usually present for several weeks and therefore produce a clinical syndrome of subacute meningitis or meningoencephalitis. However, on some occasions, patients can present more acutely with only 1–2 weeks of symptoms or even lack typical features of CNS infection, including headache. In HIV-associated CNS cryptococcosis, the fungal burden in the CNS is usually high. As a result, these patients may have a shorter duration of symptoms, higher CSF CrAg titers, elevated intracranial pressure, and slower CSF sterilization after starting effective antifungal treatments.

Different cryptococcal species may produce differences in clinical manifestations. For instance, *C. gattii* species may have a predilection to cause disease in brain parenchyma rather than the meninges. In certain areas of the world, *C. gattii* tends to produce cerebral cryptococcomas (Fig. 16.3) and/or hydrocephalus with or without large pulmonary mass lesions more frequently than *C. neoformans*. Meningoencephalitis may occur in these patients with substantial brain parenchymal involvement, who may have high intracranial pressures, cranial neuropathies, and respond poorly to antifungal therapy.

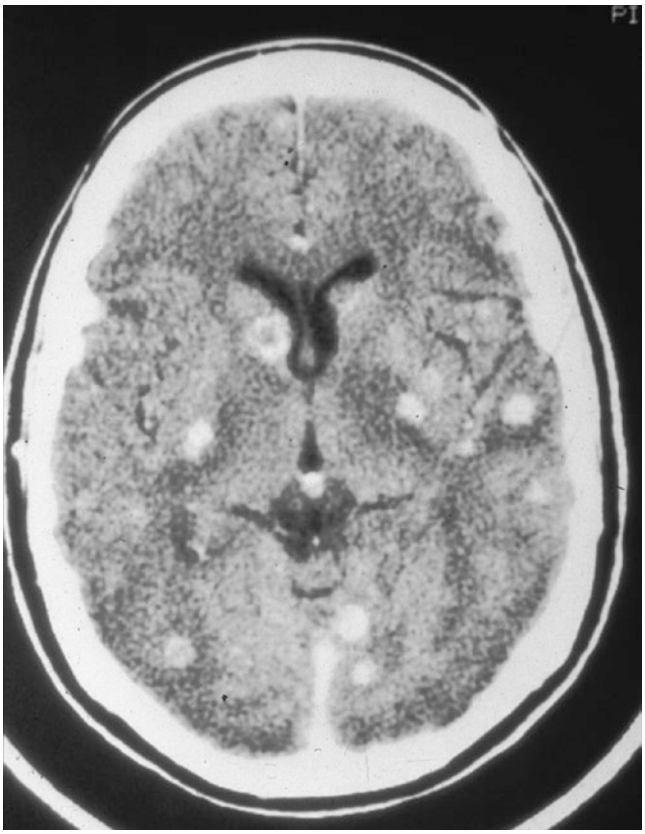


Fig. 16.3 CT scan of the brain showing multiple cryptococcomas in an apparently normal host. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)

Skin Infection

Cutaneous infections are the third most common clinical manifestations of cryptococcosis, occurring in up to 15% of patients with disseminated disease. A variety of cutaneous findings have been described and, in general, *Cryptococcus* can produce any type of skin lesion. A common cutaneous manifestation is a papule or maculopapular rash with central ulceration that may be described as "molluscum contagiosum-like." These lesions are indistinguishable from those due to other fungal infections, including *Histoplasma capsulatum*, *Coccidioides immitis*, and *Talaromyces marneffei*. Other cutaneous manifestations include acneiform lesions, purpura, vesicles, nodules, abscesses, ulcers (Fig. 16.4), granulomas, pustules, plaques, draining sinus, and cellulitis. Because there are many skin manifestations in cryptococcosis that mimic other infectious as well as malignant conditions, a skin biopsy with culture and histopathology are essential for definitive diagnosis. While cutaneous *Cryptococcus* most commonly indicates disseminated infection, primary cutaneous cryptococcosis can occur, usually associated with direct inoculation of the yeasts following a



Fig. 16.4 Skin ulceration and cellulitis as cutaneous cryptococcosis

traumatic skin injury. Solid organ transplant (SOT) recipients on tacrolimus seem to be more likely to develop skin, soft tissue, and osteoarticular infections due to *Cryptococcus* [14]. Tacrolimus has anti-cryptococcal activity at high temperatures, but loses this activity as environmental temperatures decrease; this might in part explain the increased frequency of cutaneous cryptococcosis in these patients. Despite this series of patients, however, the most common site of disseminated infection in SOT recipients still remains the CNS, including patients receiving tacrolimus.

Prostate Infection

Prostatic cryptococcosis is usually asymptomatic, and the prostate gland is considered to be a sanctuary site for this yeast. The prostate may serve as an important reservoir for relapse of cryptococcosis in patients with a high fungal burden [15]. Latent *C. neoformans* infection of the prostate has even been recognized to disseminate to the bloodstream following prostate surgery [16]. Cultures of urine or seminal fluid may remain positive for *Cryptococcus* after initial antifungal treatment of cryptococcal meningitis in patients with AIDS [17], strongly supporting the need for prolonged antifungal treatment to also clear the prostate in these severely immunocompromised patients.

Eye Infection

In the early reports of cryptococcal meningitis before the AIDS epidemic, ocular signs and symptoms were noted in approximately 45% of cases [18]. The most common manifestations were ocular palsies and papilledema. In the HIV era, several other manifestations of ocular cryptococcosis have been identified, including the presence of extensive retinal lesions with or without vitritis, which can lead to irreversible blindness. Furthermore, catastrophic loss of vision without evidence for endophthalmitis has also been reported [19]. Visual loss may be due to one of two pathogenic processes. The first caused by infiltration of the optic nerve with the yeasts, producing rapid visual loss with few effective treatments. The second is due to increased intracranial pressure and compression of the ophthalmic artery. In this setting, patients have slower visual loss and treatment with serial lumbar punctures or ventricular shunts can prevent or slow down visual loss.

Infection at Other Body Sites

In addition to the lung, CNS, skin, prostate, and eye, *C. neoformans* can cause disease in many other organs (Table 16.4). Cryptococcemia can occur in severely immunosuppressed patients, but rarely causes endocarditis. Bone involvement of cryptococcosis typically presents as one or more circumscribed osteolytic lesions in any bone of the body, occasionally associated with "cold" soft tissue abscesses, and has been associated with sarcoidosis. Bone marrow infiltration can be observed in severely immunocompromised hosts. Cryptococcal peritonitis [20] and cryptococcuria are also reported in several case series. Any organ of the human body can be a site of cryptococcal infections.

Diagnosis

There are several methods used for the diagnosis of cryptococcosis. These techniques include direct examination of the fungus in body fluids, histopathology of infected tissues, culture of body fluids or tissues, serology and molecular methods, including multiplex polymerase chain reaction (PCR) platforms for a series of CNS pathogens, including *Cryptococcus neoformans* and *gattii*.

Direct Examination

The most rapid method for diagnosis of cryptococcal meningitis is direct microscopic examination for encapsulated yeasts by an India ink preparation of CSF. *Cryptococcus* can be visualized as a globular, encapsulated yeast cell with or without budding, ranging in size from 5 to 20 μm in diameter. It is easily distinguished in a colloidal medium of India ink when mixed with CSF (Fig. 16.5). Approximately 1–5 mL of specimen is recommended for use in the India ink preparation. India ink examination can detect encapsulated yeasts in a CSF specimen with a threshold between 10^3 and 10^4 colony forming units of yeasts per milliliter of fluid. The sensitivity of India ink preparation technique is 30–50% in

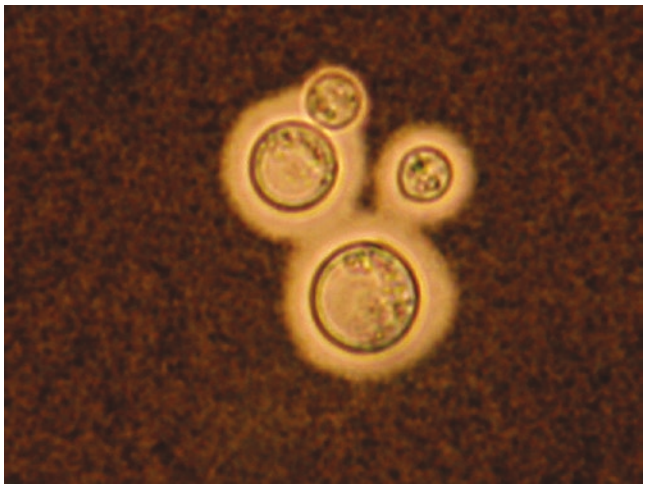


Fig. 16.5 India ink preparation showing budding encapsulated yeasts of *C. neoformans*

non-AIDS-related cryptococcal meningitis and up to 80% in AIDS-related disease. Some false positive results can be found from intact lymphocytes, myelin globules, fat droplets, and other tissue cells. Also, dead yeast cells can remain in the CSF and be visualized by India ink preparation for varying periods of time during and after appropriate antifungal treatment. This is a limitation of direct microscopy of CSF during the management of cryptococcal meningitis. In most settings, the less precise India ink microscopy has been largely replaced by lateral flow assays (LFA) for detection of cryptococcal antigen, as these assays are inexpensive, do not require technical expertise, and demonstrate superior performance.

Cytology and Histopathology

Cryptococcus can be identified by histological staining of tissues from lung, skin, bone marrow, brain, or other organs [21]. Histopathological staining of centrifuged CSF sediment is more sensitive for rapid diagnosis of cryptococcal meningitis than the India ink method [22]. Peritoneal fluid from chronic ambulatory peritoneal dialysis, seminal fluid, bronchial wash or bronchoalveolar lavage fluid can also be used for cytology preparations in the diagnosis of cryptococcal infections, whereas India ink preparations from these body fluids are difficult to interpret [23, 24]. Fine needle aspiration for cytology of peripheral lymph nodes, adrenal glands, or vitreous aspiration, percutaneous transthoracic biopsy under real-time ultrasound guidance, or video-assisted thoracoscopic lung biopsy on pulmonary nodules, masses, or infiltrative lesions can be used for obtaining tissues for cytology/histopathology [25].

A variety of positive staining methods has been described to demonstrate the yeast cells in tissue or fluids; ranging from the nonspecific Papanicolaou or hematoxylin and eosin stains, to the more specific fungal stains such as Calcofluor, which binds fungal chitin, or Gomori methenamine silver (GMS), which stains the fungal cell wall [2, 23] (Fig. 16.6). Several stains can identify the polysaccharide capsular material surrounding the yeasts. These stains can be especially useful in presumptively identifying *Cryptococcus* when cultures do not grow or are not obtained. They include Mayer's mucicarmine, periodic acid-Schiff (PAS), and alcian blue stains. The Fontana-Masson stain appears to identify melanin in the yeast cell wall. The fungus is observed as a yeast that reproduces by formation of narrow-based budding with a prominent capsule. Gram stain is not optimal for identification of this yeast, but may show *C. neoformans* as a poorly stained gram-positive budding yeast (Fig. 16.7). The recognition of *C. neoformans* in gram-stained smears of purulent exudates may be hampered by the presence of the large gelatinous capsule that apparently prevents definitive staining of the yeast-like cells.

Serology

Diagnosis of cryptococcosis has improved significantly over the last several decades with the development of serological tests for cryptococcal polysaccharide antigen (CrAg) and/or antibody. Use of serum cryptococcal antibodies for diagnosis of cryptococcosis has not been adopted. In contrast, detection of cryptococcal CrAg in serum or body fluids by a latex agglutination (LA) technique has been robust in its performance and, until recently, was considered the gold standard diagnostic test for serological diagnosis of cryptococcosis. This test uses latex particles coated with polyclonal cryptococcal capsular antibodies or anti-glucuronoxylomannan monoclonal antibodies and has overall sensitivities and specificities of 93–100% and 93–98%, respectively [26, 27]. The false-positive rate of cryptococcal capsular polysaccharide antigen testing is 0–0.4% [28]. The majority of false positive results can be explained by technical error (improper boiling/treatment), presence of rheumatoid factor or interference proteins, and infections with *Trichosporon beigelii* [29] or some bacterial species [30]. However, most of the false-positive results of LA testing for cryptococcal polysaccharide antigen have initial reciprocal titers of 8 or less [26]. Therefore, results of such low titers must be carefully interpreted within the clinical context. False-negative results of LA test for cryptococcal polysaccharide antigen in cryptococcal meningitis are unusual, but can be seen due to a prozone effect, and, therefore, high-risk negative specimens should be diluted and retested [31]. Low fungal burden, as in chronic low-grade cryptococcal meningitis or in the very early stages of cryptococcal infection, and improper storage of patient sera can also cause false-negative results in LA cryptococcal polysaccharide antigen tests [32].

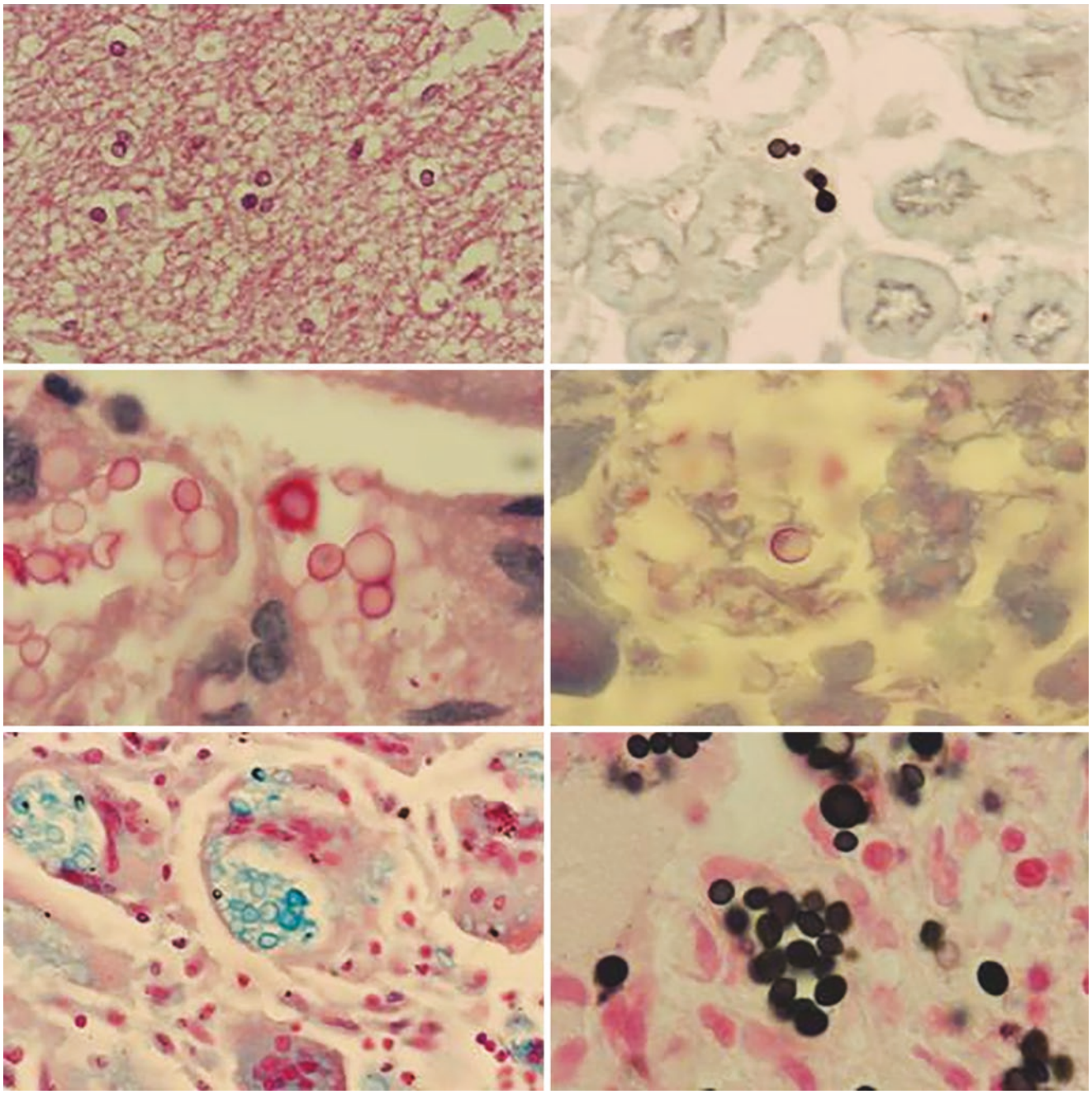


Fig. 16.6 Mouse tissues stained with various stains used to identify cryptococcal infection. Upper left panel is of brain stained with H&E showing meningoencephalitis with encapsulated yeast cells of *C. neoformans*. The upper right panel is of kidney stained with GMS. The middle left panel demonstrates lung stained with Mayer's mucicarmine. Note orange-red staining of polysaccharide capsular material of *C. neoformans*. The middle right panel is liver tissue stained with PAS. Lung stained with Alcian blue stain is seen in the bottom left panel. Lung stained by Fontana-Masson method is seen in the bottom right. Melanin pigment in the cell wall of *C. neoformans* stains dark with this stain. (Courtesy of Dr. W. A. Schell)

Enzyme immunoassays (EIA) for detection and quantification of cryptococcal polysaccharide antigen of all four serotypes of *C. neoformans* in sera and CSF have been developed to detect the major component of the polysaccharide capsule, glucuronoxylomannan (GXM), with sensitivities and specificities of 85.2–99% and 97%, respectively [26, 33]. This methodology is automated and overcomes some of the practical limitations of LA testing. Previous studies have compared EIA and LA assays and revealed no significant difference between these testing methods. EIA for cryptococcal polysaccharide antigen does not give discrepant results with rheumatoid factor or serum macroglobulins and

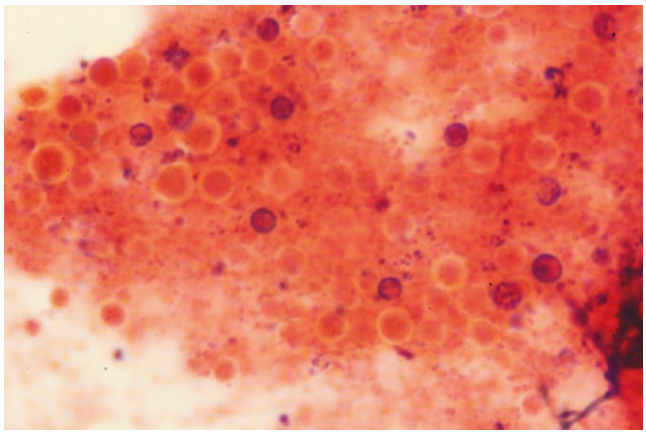


Fig. 16.7 Gram stain of sputum of a patient with pulmonary cryptococcosis. *C. neoformans* appears as poorly stained gram-positive budding yeasts. (Courtesy of Dr. W. A. Schell, Duke University Medical Center)

is not affected by prozone reactions. Both LA and EIA testing have been rigorously studied and are recommended for use in both serum and CSF samples.

A semi-quantitative lateral flow assay (LFA) for detection of cryptococcal antigen offers many advantages over the other serological techniques, including rapid turn-around (approximately 15 min), minimal requirements for specialized laboratory infrastructure, stability at room temperature, and low cost [34]. The LFA has been evaluated against both EIA and culture, with sensitivities of 96–100% for serum and plasma and 70–94% for urine samples [34–37]. This assay has good performance across a broad range of clinical settings, including resource-limited settings and among cohorts with low burden of HIV infection and high rates of *C. gattii* infection, for which some EIA and LA tests are known to be insensitive [34–39]. The outstanding performance of LFA combined with its established cost-effectiveness and practical advantages support its use as a point-of-care testing [34, 35] and has become the primary method for CrAg detection today.

While a positive serum CrAg is suggestive of dissemination of cryptococcal infection outside the lung, the precise value of CrAg for diagnosis of non-disseminated pulmonary cryptococcosis remains less certain. Generally speaking, detectable CrAg in serum should make clinicians consider active extrapulmonary infection. In a high-risk patient with clinical symptoms suggestive of meningitis, identification of CrAg in serum is rapid, specific, noninvasive, and virtually diagnostic of meningoencephalitis or disseminated cryptococcosis even when the India ink examination or culture is negative [40, 41]. Likely because of its sensitivity, the detection of CrAg in serum may precede clinically obvious disseminated cryptococcal disease ("isolated cryptococcal polysaccharidemia") in severely immunosuppressed patients [42–44]. The management of these cases, in which there is a positive serum CrAg and other nonspecific clinical findings in HIV-infected patients with negative fluid or tissue cultures has created new paradigms of preemptive therapy. Persons of high risk with isolated positive CrAg benefit from antifungal therapy to prevent or delay the development of symptomatic disease [42]. Such preemptive approaches have been evaluated and found to be cost-effective for high-risk patients (see *Prevention*, below).

Baseline cryptococcal polysaccharide antigen titers in serum and CSF correlate with fungal burden and carry prognostic significance in patients with cryptococcal meningitis [45, 46]. A study in HIV-related acute cryptococcal meningitis indicated that a baseline titer of CSF cryptococcal polysaccharide antigen of 1:1024 or greater was a predictor of death during systemic antifungal treatment [46]. While baseline CrAG values are important in both prognosis and diagnosis and a rise in CSF cryptococcal polysaccharide antigen titer during suppressive therapy has been associated with relapse [47], monitoring serial CrAg titers to make therapeutic decisions in the acute setting is not recommended, as the kinetics of polysaccharide elimination from humans are difficult to predict and change in CrAg titer over time is not correlated with clinical response to antifungal therapy.

Culture and Identification

Cryptococcus can be easily grown from clinical samples such as CSF, sputum, and skin biopsy on routine fungal and bacterial culture media. Colonies can usually be observed on solid agar plates after 48–72 h incubation at 30–35 °C in aerobic conditions. Antibacterial agents, preferably chloramphenicol, can be added to the media when bacterial contamination is considered. The yeast, however, does not grow in the presence of cycloheximide at the concentration used in selective fungal isolation media (25 μg/mL). Despite relatively rapid growth for most strains, cultures should be held for 3–4 weeks before discarding, particularly for patients already receiving antifungal treatment. Conversely, cultures may be negative despite positive microscopic examinations (India ink) due to nonviable yeast cells, which may persist for a prolonged period of time at the site of infection. Positive blood cultures are frequently reported in AIDS patients and may actually be the first positive test for cryptococcal infection in a febrile high-risk patient.

C. neoformans colonies will appear on routine fungal media as opaque, white, creamy colonies that may turn orange-tan or brown after prolonged incubation. The mucoid appearance of the colony is related to the capsule size around the yeasts. *Cryptococcus* does not routinely produce hyphae or pseudohyphae, or ferment sugars, but is able to assimilate inositol and hydrolyze urea. *C. neoformans* and *C. gattii* have the ability to use galactose, maltose, galactitol, and sucrose. There are special media such as

canavanine-glycine-bromthymol blue (CGB) agar that can be used to differentiate *C. gattii* strains from *C. neoformans* strains.

Molecular Identification Methods

A number of molecular techniques has been developed for identification of cryptococcal species from biological specimens, the most clinically important of which is a multiplex PCR assay for detection of a number of CNS pathogens, including *C. neoformans/gattii* (BioFire FilmArray®). This rapid-test has comparable diagnostic performance to CSF CrAg testing when fungal CSF burden is high, but may be falsely-negative when CSF fungal burden is low [48]. A number of studies have replicated the high degree of correlation with FilmArray® testing and CSF culture results [49, 50], suggesting there may be a role for this testing in predicting culture sterility/positivity in patients with established cryptococcal meningitis. Other molecular assays include random amplified polymorphic DNA (RAPD), PCR restriction fragment length polymorpism (RFLP) analysis, multilocus sequence typing (MLST), and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) [51–56]. These highly sensitive and specific methods have been evaluated with a variety of biologic samples [57] and can rapidly identify to the species and subspecies/genotypic level, including identification of recognized and novel strains within geographical niches [58]. While the expense and specialized techniques required of these methods preclude widespread use in clinical practice, their use in larger scale investigations will continue to enhance our understanding of the epidemiology, pathogenesis, and nuances of antifungal management, as well as identify micro-evolution of different strains [59].

Treatment

The Infectious Diseases Society of America Clinical Practice Guidelines for the Management of Cryptococcal Disease (summarized in Tables 16.5 and 16.6), updated in 2010, continue to provide a suitable framework for therapeutic decision-making [60]. The guidelines provide detailed rec-

Table 16.5 Treatment recommendations for cryptococcal meningoencephalitis[a]

Human immunodeficiency virus-infected individuals[b]	
Induction therapy:	
Primary regimen:	
Liposomal AmB (3–4 mg/kg/day) or AmB lipid complex (ABLC; 5 mg/kg/day) pls 5-FC(100 mg/kg/day) for patients predisposed to renal dysfunction	2 weeks
AmBd (0.7–1 mg/kg/day) plus flucytosine (5-FC) (100 mg/kg/day)	2 weeks
Alternative regimens**c**:	
AmBd (0.7–1 mg/kg/day) or liposomal AmB[d] (3–4 mg/kg/day) or ABLC (5 mg/kg/day) if flucytosine-intolerant	4–6 weeks
AmBd (0.7–1 mg/kg/day) plus fluconazole (800 mg/day)	2 weeks
Fluconazole (≥800 mg/day, preferably 1200 mg/day) plus 5-FC (100 mg/kg/day)	6 weeks
Fluconazole (800–2000 mg/day, preferably 1200 mg/day)	10–12 weeks
Itraconazole (200 mg BID)	10–12 weeks
Consolidation therapy: Fluconazole (400–800 mg/day)	8 weeks
Maintenance or suppressive therapy: Fluconazole (200 mg/day)	≥1 year[e]
Alternative regimens[c]:	
Itraconazole (200 mg BID)	≥1 year[e]
AmBd (1 mg/kg IV per week)	≥1 year[e]
Organ transplant recipients[f]	
Induction therapy:	
Primary regimen:	
Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day) plus 5-FC (100 mg/kg/day)	2 weeks
Alternative regimen (if flucytosine-intolerant):	
Liposomal AmB[d] (up to 6 mg/kg/day) or ABLC (5 mg/kg/day)	4–6 weeks
AmBd (0.7 mg/kg/day)[f]	4–6 weeks
Consolidation therapy: Fluconazole (400–800 mg/day)	8 weeks
Maintenance or suppressive therapy: Fluconazole (200–400 mg/day)	6–12 months
Non-HIV infected and non-transplant patients	
Induction therapy:	
Primary regimens:	
Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day) plus 5-FC (100 mg/kg/day)	4–6 weeks[g,h]
AmBd (0.7–1 mg/kg/day) plus 5-FC (100 mg/kg/day)	4–6 weeks[g,h]
AmBd (0.7–1 mg/kg/day)	6 weeks
Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day)	6 weeks
Consolidation therapy: Fluconazole (400–800 mg/day)	8 weeks

Table 16.5 (continued)

Non-HIV infected and non-transplant patients	
Maintenance therapy: Fluconazole (200 mg/day)	6–12 months

[a] Adapted from the 2010 IDSA Clinical Practice Guideline for the Management of Cryptococcal Disease with personal suggestions [60]
[b] Initiate ART 2–10 weeks after beginning antifungal regimen
[c] Can be considered as alternative regimen in circumstances in which primary regimen is not available, but are not encouraged as equivalent substitutes
[d] Liposomal Amphotericin can be safely administered in doses as high as 6 mg/kg per day
[e] After 1 year of therapy, if successful response to ART (CD4 count ≥100 and viral load low or undetectable for >3 months), can consider discontinuation of antifungal therapy. Consider reinstitution if CD4 count falls below 100
[f] Caution due to concomitant calcineurin inhibitor use
[g] Consider stepwise de-escalation of immunosuppressive regimen if allograft function permits. Shorter duration (i.e., two weeks) of induction therapy can be considered for certain low-risk patients
[h] If CSF culture remains positive at 2 weeks of therapy or initial presentation with neurologic complications, longer therapy preferred

Table 16.6 Treatment recommendations for nonmeningeal cryptococcosis

Immunosuppressed[a] and immunocompetent[b], mild-to-moderate pulmonary disease	
Fluconazole (400 mg/day)	6–12 months[c]
Alternatives (immunocompetent): itraconazole 200 mg BID	
Voriconazole 200 mg BID, posaconazole 300 mg daily	12 months
Immunosuppressed or immunocompetent, severe pulmonary disease	
Treat as CNS disease	
Nonmeningeal, nonpulmonary cryptococcosis	
Patients with cryptococcemia	
Treat as CNS disease	12 months
No CNS disease, no fungemia, isolated focus of infection	
Fluconazole 400 mg/d	6–12 months

[a] CSF sampling should be performed to rule out CNS involvement
[b] CSF sampling can be considered, but not required in the absence of neurological symptoms or high serum cryptococcal antigen
[c] If successful response to ART (CD4 count ≥100 and viral load low or undetectable for >3 months) and stable serum cryptococcal antigen, can consider discontinuation of antifungal therapy after 12 months of therapy

ommendations for specific "at-risk" populations and address different management strategies based on host, site of infection, and potential complications of cryptococcal infection. While subtle nuances exist based on host and site of infection, general principles for management of cryptococcal infection can provide the cornerstone of a treatment plan in most cases.

Basic Management Principles/Role of Combination Therapy

Amphotericin B remains the mainstay of treatment for disseminated cryptococcosis and severe cryptococcal infection. While Amphotericn B deoxycholate (AmBd) at 0.7–1 mg/kg/day is supported by randomized controlled trials and remains an important component of induction therapy in resource-limited settings, liposomal amphotericin B (AmBisome) at 3–4 mg/kg/day has become the preferred treatment with similar outcomes to that of AmBd, but with less nephrotoxicity, and is specifically recommended for primary induction in organ transplant patients as well as patients at risk for renal dysfunction [60–62]. Higher doses of AmBd have been shown to be more rapidly fungicidal [63, 64]. Flucytosine (5-FC) is primarily used in combination therapy with AmBd or liposomal amphotericin B for first-line therapy in cryptococcal meningitis or severe pulmonary cryptococcosis at a dosage of 100 mg/kg/day in divided doses in patients with normal renal function [65, 66]. 5-FC should be dose-adjusted for renal dysfunction, with therapeutic monitoring performed 3–5 days after initiation of therapy, to maintain 2-h post-dose levels under 100 μg/mL (goal 30–80 μg/mL), to reduce its primary side effect of bone marrow suppression. Early studies in HIV-associated cryptococcal meningitis demonstrated increased rates of CSF sterilization and fewer relapses with the combination of AmBd and 5-FC followed by azole maintenance [65]. This initial combination regimen has since been compared against multiple alternatives, with the superiority of its fungicidal activity consistently confirmed [67]. Similar results have been observed among the most severe cases of cryptococcal infection [64, 68]. Early mycological failure (as defined by persistently positive CSF cultures at day 14) has for many years been associated with late treatment failure and poor outcome [69], and lack of 5-FC has been independently associated with both early [70] and late [68] mycological failure. The combination of AmBd and 5-FC represents the most potent fungicidal regimen with more rapid sterilization of CSF cultures at two weeks as demonstrated across multiple studies [64, 65, 67], which has been shown to translate into a direct survival benefit compared with AmBd monotherapy and other alternatives, with improved survival at ten weeks lasting up to six months [64, 71].

Alternative Combination Regimens

Though combination induction with AmBd and 5-FC for two weeks remains the recommended standard of care for severe

cryptococcosis, including cryptococcal meningitis, limited resources to safely administer this combination and limited availability of 5-FC in certain settings presents significant challenges for managing patients in areas where the disease burden and mortality rates are highest. A large follow-up study evaluated alternative induction regimens, including shorter courses and all-oral induction regimens for cryptococcal meningitis in less-resourced settings and confirmed the value and mortality benefit of the combination of amphotericin B plus flucytosine [71]. In this study, the optimal regimen for success was one week instead of two weeks of combination therapy with AmBd (1 mg/kg/day) plus 5-FC, followed by one week of fluconazole 1200 mg/day. The benefit of shorter induction in this study may have been related to management of toxicities. As such, in settings where resources are not limited, the standard combination induction length remains at two weeks. Alternative combination therapies have been investigated; the most studied has been AmBd (0.7 mg/kg/day) plus fluconazole (800 mg/day), which has demonstrated improved rates of a composite endpoint of CSF culture negativity, neurological improvement and survival compared with AmBd alone or in combination with lower doses of fluconazole [72]. Fluconazole (at doses of 800–1200 mg/day) in combination with AmBd (standard dosing) has been shown to demonstrate similar rates of fungal clearance from CSF as standard AmBd plus 5-FC in a randomized study performed in HIV-infected patients in South Africa [73] and offers a potential viable option [71] for effective initial therapy in settings where access to 5-FC is limited. Whether the survival benefit observed with AmBd plus 5-FC will continue to be observed with this regimen remains uncertain. Additional alternative regimens for primary therapy are available in the guidelines but their use is not encouraged based on limited data on the success of these regimens [74]. Despite slower rates of CSF fungal clearance compared to AmBd-containing regimens, an all-oral combination regimen of fluconazole (1200 mg/day) plus 5-FC (100 mg/kg/day) regimen has been shown to be an effective alternative [71] and better than fluconazole alone. Use of fluconazole alone for induction therapy in the absence of a polyene is not recommended given the fungistatic nature of this drug, poor success, higher relapse rates, and increased resistance in relapse cases when used as monotherapy for induction [60, 75]. However, in areas without access to AmBd and flucytosine, high doses (1200 mg per day) of fluconazole should be commenced.

Host Considerations

Cryptococcal Meningitis in HIV Patients

A 3-stage regimen of induction/consolidation/maintenance is employed in the treatment of cryptococcal meningitis in all patients, irrespective of host risk factors [60, 65]. In HIV-infected patients, initial induction treatment usually begins with combination therapy with AmBd or AmBisome plus 5-FC for at least two weeks as above. Patients with a favorable response to induction are then transitioned to consolidation treatment with fluconazole 400–800 mg/day (higher dose preferred in patients with higher baseline fungal burden) for eight weeks. Following consolidation, a long-term suppressive/maintenance phase is commenced with oral fluconazole, 200–400 mg given once daily. This approach has been demonstrated to effectively reduce relapse rates from ~40% to less than 5% in the pre-ART era [76]. Maintenance antifungal therapy is discontinued after one year in patients who respond to ART with rise in $CD4^+$ cell counts to greater than 100 cells/μL and decline in viral load (HIV RNA) to undetectable levels for at least three months [60, 77, 78].

Itraconazole can be used as an alternative consolidation treatment for cryptococcosis, but first-line therapy is with fluconazole. Despite its poor CSF penetration and inconsistent oral bioavailability, itraconazole has been successfully used in the treatment of cryptococcal meningitis; however, it has been shown to be inferior to fluconazole during suppression phase [79] and requires therapeutic drug monitoring due to its poor bioavailability. Newer triazoles including posaconazole, voriconazole, and isavuconazole are not specifically incorporated into practice guidelines, but are active against cryptococcal isolates in vitro and have been shown to demonstrate moderate efficacy in patients with refractory disease [80–82].

In patients with HIV-associated cryptococcal infection, ART has a major impact on long-term prognosis. However, given concerns regarding IRIS, optimal timing for ART initiation in the setting of opportunistic infections has been studied. Early retrospective studies suggested an increased risk of IRIS among HIV-infected patients initiated on ART early after the diagnosis of an opportunistic infection (OI) [83, 84]. Further studies have demonstrated conflicting results regarding outcomes of cryptococcal infection based on timing of ART initiation [85–89]. However, the COAT study provides the best evidence for current recommendations regarding timing of ART initiation in patients with cryptococcal meningitis [90]. ART-naïve patients were randomized to receive immediate (within 48 h) or deferred (greater than four weeks) ART following a minimum of seven days of antifungal therapy with AmBd and high-dose fluconazole. This trial was stopped early after interim analyses showed poorer early survival among patients receiving immediate ART (55% vs. 70%), particularly among patients with altered mentation and low CSF white blood cell count. Although a trend toward increased rates of and earlier IRIS was observed in the immediate ART group, this was not statistically significant.

The above data support present recommendations to delay initiation of ART in patients with cryptococcal meningitis for a minimum of four weeks after starting antifungal therapy (potentially longer if primary regimen does not include AmBd) and after demonstration of a sustained clinical response to antifungal therapy [60, 91]. Interruption of ART and/or corticosteroid treatment may be used to control symptoms if severe cryptococcal IRIS occurs.

Organ Transplant Recipients

Organ transplant recipients with CNS cryptococcal infection are managed similarly to HIV-infected patients, with a clear preferential use of lipid formulations of amphotericin B to limit nephrotoxicity [60]. The principles of induction, consolidation, and maintenance therapy remain the same. Repeat CSF sampling at two weeks is recommended in this population and a longer course of induction therapy should be pursued if CSF cultures remain positive at two weeks, as this scenario is associated with increased 6-month mortality [92]. Unlike HIV-infected patients, relapse rates among organ transplant recipients are quite low (~1.3%), such that a shorter course of maintenance therapy with fluconazole (6–12 months) can be considered following standard consolidation [60, 92]. Drug interactions between fluconazole and immunosuppressive agents should be anticipated due to fluconazole-induced CYP3A4 inhibition, and preemptive adjustment (reduction) in calcineurin inhibitors should be made. Management of immunosuppression in the setting of cryptococcal infection requires recognition of the increased risk of IRIS associated with abrupt withdrawal or reduction of immunosuppression in organ transplant recipients with increased rates of allograft loss reported in some patients [93, 94]. Therefore, stepwise reduction in immunosuppression regimens is recommended, though the approach should be individualized for each patient.

Non HIV-Infected, Nontransplant Patients

Screening for HIV, CD4 lymphopenia, and anti-GM CSF autoantibodies [95] is recommended among patients who present with cryptococcosis without apparent risk factors [60]. Very little prospective data is available on management of cryptococcal infection among this heterogeneous group of "apparently immunocompetent" patients lacking classical risk factors for cryptococcosis. What is known is based on early studies that included a heterogeneous mix of patients with multiple underlying diseases and were performed prior to acceptance of the standard algorithm of induction, consolidation, and maintenance therapy and higher dose polyene therapy [66]. Recommendations for longer induction therapy (four weeks or more) in this population are based on the recognition of poorer outcomes and higher mortality rates in this group of patients both in early [66, 96] as well as contemporary [97] studies. However, therapeutic decisions should be individualized; patients who respond well to initial therapy and have low risk for relapse can often be managed with standard 2-week induction. An additional two weeks of therapy should be considered if 5-FC is not included in the induction regimen [60]. Recommendations for consolidation and maintenance parallel those for HIV-infected and transplant patients and reflect early reports of relapse rates approaching 30% within the first year prior to introduction of consolidation and maintenance antifungal therapy [60, 66]. Criteria for stopping treatment in these patients include resolution of symptoms, generally following at least one year of suppressive therapy. Patients may have prolonged positive CrAg tests and/or slightly abnormal CSF findings for months during successful therapy and if there are concerns about cure, follow-up CSF culture should be considered.

Nonmeningeal Cryptococcosis

Just as host factors influence management approaches for cryptococcal infection, site of infection also matters. Airway colonization in a non-immunosuppressed individual poses a low risk for invasive pulmonary infection (and dissemination) and treatment can be deferred. Some experts would still favor treatment with fluconazole in this scenario, given the relative benign nature of this therapy. However, among immunocompromised patients with isolated pulmonary cryptococcosis, treatment is recommended to prevent dissemination [60]. It should be emphasized that a thorough evaluation to rule out systemic disease/dissemination is warranted in this group of patients to provide optimal treatment. This includes blood and CSF cultures as well as serum and CSF CrAg. If the results of the above evaluation are negative, symptoms are mild, and there is no evidence of diffuse pulmonary infiltrates or ARDS, oral fluconazole (400 mg/day) is recommended for 6–12 months. However, in any patient in whom cryptococcemia is identified, symptoms are severe, ARDS is present or CSF examination reveals asymptomatic CNS involvement, treatment for cryptococcal meningitis is recommended [60]. Cerebral cryptococcomas often can be managed with prolonged antifungal therapy without need for surgical removal unless mass effect or other evidence of obstruction is identified. At least six weeks of induction therapy with AmBd or AmBisome plus 5-FC, followed by 6–18 months of maintenance therapy with fluconazole (400–800 mg/day) is recommended for management. Surgery should be considered for large lesions (>3 cm) or the presence of obstructive hydrocephalus [60]. Localized infection of extra-pulmonary, non-meningeal sites can occasionally occur with direct inoculation, but more commonly represents disseminated infection. Suspicion for the latter must be

maintained when *Cryptococcus* is identified from a sterile body site, as management strategies will differ if disseminated disease is present. Consultation with ophthalmology is indicated in cases of cryptococcal eye disease [60].

Immune Reconstitution Inflammatory Syndrome (IRIS)

Restoration of pathogen-specific immunity as a result of ART or following reduction of immunosuppression in SOT recipients can result in a destructive inflammatory response known as the *immune reconstitution inflammatory syndrome* (IRIS). IRIS is best characterized in association with *C. neoformans* infection of the CNS, particularly among HIV-infected patients and is associated with significant morbidity and mortality [83, 84, 86, 87, 98–106]. Proposed criteria for IRIS include onset of symptoms within 12 months of ART initiation (with concomitant CD4+ recovery) [107]. In addition, IRIS is estimated to occur in 5–11% of SOT recipients with cryptococcal infection and has been associated with increased risk of allograft failure [93, 108–112]. Finally, a similar entity of post-infectious inflammatory response syndrome (PIIRS) with features of spinal arachnoiditis following treatment of cryptococcal meningitis has been characterized among non-HIV, non-transplant patients and has been shown to respond to corticosteroids [113, 114].

Clinical features of cryptococcal IRIS are similar to cryptococcal infection, most commonly presenting as CNS disease, although lymphadenitis, pneumonitis, multifocal disease, soft-tissue involvement, and mediastinitis have all been reported. Meningeal disease is the most frequent and most serious presentation [107], most often manifest as aseptic meningitis with associated intracranial hypertension and CSF pleocytosis [98, 100, 101, 103, 104, 106]. Clinically, this entity has been categorized as either "unmasking" IRIS when a previously unrecognized infection develops after early ART initiation or "paradoxical" IRIS when clinical worsening of a pre-existing infection, previously controlled with treatment, occurs following ART. A hallmark histopathologic finding is suppurative or necrotic granulomatous inflammation with yeast seen in tissues despite negative tissue cultures [93, 110, 114, 115]. The presence of a positive CSF culture in cases of suspected cryptococcal IRIS should raise suspicion for direct antifungal failure or drug resistance, particularly in settings where fluconazole therapy is widely used as the standard of care [86].

Cryptococcal IRIS represents unchecked reversal of a Th2 (anti-inflammatory) to Th1 (pro-inflammatory) immune response in the setting of immune recovery in the host [116]. Prospective cohort studies of ART-naïve individuals indicate that an ineffectual host immune response to initial infection or less optimal antifungal therapy are associated with a greater likelihood of future IRIS [103]. A three-phase theory of cryptococcal immune reconstitution has been postulated, marked by: (1) failure of antigen clearance due to inappropriate Th2 response; (2) lack of effector response despite inflammatory signaling; and, ultimately, (3) vigorous pro-inflammatory responses (both Th1 and Th17) to residual antigen, which is recognized clinically as IRIS [98].

There are no reliable diagnostic tests for IRIS, and establishing the diagnosis remains a considerable challenge for clinicians [99, 117]. The differential diagnosis includes progressive disease due to persistent immune deficiency, failure of antimicrobial therapy (due to resistance or nonadherence), co-infection with other OIs, and drug toxicity. A high index of suspicion is necessary for recognizing atypical presentations or manifestations at distant sites. Nevertheless, when assessing the patient with clinical deterioration, distinguishing between disease progression related to ongoing immune deficiency with fungal persistence and clinical deterioration due to restoration of host immune capacity has important management implications. CSF analyses and biomarkers may be useful in distinguishing between relapse and IRIS. Prospective studies have demonstrated that CSF opening pressure [87] and WBC count [98, 103] at the time of an IRIS event are significantly higher than baseline values for individual patients, and higher CSF opening pressures may help to distinguish IRIS from relapsed infection [100].

Treatment options for cryptococcal IRIS are based largely on expert opinion [60]. Implicit in management is ensuring the efficacy of antifungal therapy, particularly in settings where access to AmBd may be limited and fluconazole resistance may account for recurrent meningitis episodes [75, 118]. In the absence of disease relapse or direct antifungal resistance, modification of antimicrobial therapy is not indicated [60]. Once the diagnosis of IRIS is suspected, consideration of disease severity is warranted. A significant proportion of minor cases will improve without specific treatment [84, 86, 106]. Corticosteroids have been shown to reduce the need for hospitalization and to improve short-term quality of life and functional status without increased risk of complications in paradoxical TB-associated IRIS [119]; however, the role of corticosteroids in cryptococcal IRIS is not as well-established and should be reserved for life-threatening cases, particularly in light of their association with increased mortality in one study [120]. Other anti-inflammatory agents have been used in cryptococcal IRIS, but the number of patients treated with any of these agents is too small to draw substantive conclusions [84, 121, 122]. Other management strategies, including therapeutic lumbar drainage in the setting of intracranial hypertension [60, 119, 123] and, at times, surgical drainage of suppurative lymph nodes [114, 115] or lung masses are important adjunctive therapies that may be considered in severe disease.

Although no controlled studies have been performed, continuation of ART in the setting of IRIS is recommended and has been performed safely without adverse effects in several studies [85, 86, 101, 117, 124]. Similarly, careful withdrawal or reduction of immunosuppressive agents is standard practice in managing infectious complications in SOT recipients [109]. Given the putative risk of IRIS with abrupt withdrawal or discontinuation of immunosuppressive agents in these patients, gradual de-escalation during the initiation of antifungal therapy is advised to reduce the risk of future IRIS [93, 109, 110].

Persistent and Relapsed Infection

Persistent and relapsed infection must be distinguished from IRIS, as management strategies will differ significantly. Persistent disease is defined as persistently positive CSF cultures after one month of antifungal therapy, whereas relapse requires new clinical signs and symptoms and repeat positive cultures (at same or distant sites) after initial improvement and fungal sterilization [60]. Surrogate markers, including biochemical parameters, India ink staining, and CrAg titers, are insufficient to define relapse or alter antifungal therapy. General recommendations for management in these cases include resumption of induction therapy, often for a longer duration and at increased dosages, if tolerable, and pursuance of antifungal susceptibility testing [60].

Antifungal Susceptibility Testing

While routine in vitro susceptibility testing of cryptococcal isolates at the time of initial therapy is not recommended, there is a role for such testing in cases of suspected relapse or persistent infection [60]. It is generally recognized that primary antifungal resistance to most agents is rare, although reduced susceptibility to flucytosine has been observed in untreated and treated patients [125] and echinocandins have no reliable activity against this yeast. Reduced susceptibility to fluconazole has been described in cases of culture-positive relapsed cryptococcal meningitis associated with prior fluconazole therapy [75, 126, 127]. No standard minimum inhibitory concentration (MIC) breakpoint for *Cryptococcus* has been yet defined; however, a fluconazole MIC ≥16 μg/mL raises concern for potential drug resistance.

Management of Elevated CSF Pressure

Along with the optimization of antifungal therapy, management of increased intracranial pressure (ICP) is critically important. Elevated ICP is correlated with overall fungal burden, and is thought to be due to CSF outflow obstruction by clumped yeast forms [128]. An ICP of 250 mmH_2O or greater is considered elevated and is associated with increased morbidity and mortality [120]. Persistently elevated ICP after two weeks of treatment is associated with poorer clinical responses among patients with HIV-associated cryptococcal meningitis [60]. Intracranial imaging should be performed prior to lumbar puncture if impaired mentation or focal neurologic deficits are present. A baseline measurement of CSF pressure should be obtained in all patients with suspected cryptococcal meningitis. Ongoing aggressive attempts to manage increased ICP should occur if there are signs/symptoms to suggest increased ICP (headache, mental status changes, and new focal neurological findings). Treatment options for managing acutely elevated ICP include repeated lumbar punctures (daily until pressure and symptoms are stable for >2 days), lumbar drain insertion, ventriculostomy, or ventriculoperitoneal shunt (Table 16.7) [120]. Routine adjunctive corticosteroid use in cryptococcal meningitis has been associated with reduced antifungal activity and increased morbidity and is not recommended in the absence of severe forms of IRIS [129]; other medical treatments such as mannitol and acetazolamide have been used in rare cases, but are similarly not recommended for routine use in management of increased ICP pressure in cryptococcal meningitis [130]. Some patients may develop symptoms of obstructive hydrocephalus, necessitating placement of a permanent ventriculoperitoneal (VP) shunt during the first 1–2 years of treatment, and occasionally at the time of initial presentation. Sterilization of CSF is not required prior to placement of a VP shunt, which can be inserted once a patient is receiving appropriate antifungal therapy [131].

Table 16.7 Management of elevated intracranial pressure in HIV-infected patients with cryptococcosis[a]

Initial lumbar puncture
Normal opening pressure
Initiate medical therapy, with follow-up lumbar puncture at 2 weeks
Opening pressure ≥250 mmH_2O with signs or symptoms
Lumbar drainage sufficient to achieve closing pressure <200 mmH_2O or 50% of initial opening pressure[b]
Follow-up for elevated pressure
Repeated drainage daily until opening pressure <200 mmH_2O and symptoms/signs are stable
If elevated pressure persists, consider
Lumbar drain or ventricle peritoneal shunt

[a] Based on the IDSA Practice Guideline for the Management of Cryptococcal Diseases [60]

[b] Recommendations are not evidence-based and provided as a guide only

Prevention

Prevention of cryptococcal disease is best achieved by use of ART in HIV-infected patients. Routine fluconazole prophylaxis has been shown to be effective for preventing cryptococcosis in AIDS patients with persistently low $CD4^+$ cell counts (below 100 cells/μL) [132, 133], but due to concerns regarding antifungal resistance, this approach is not currently recommended unless there is a high incidence disease and poor access to diagnostics. ART still remains the best strategy for prevention of cryptococcal disease in this population. Serum CrAg screening at the time of HIV diagnosis and ART initiation, especially in patients with very low CD4 counts (less than 100 cells/μL), is recommended [134, 135] to identify asymptomatic patients in whom preemptive therapy with fluconazole should be commenced to treat to mitigate progression of early infection into overt invasive cryptococcal disease. Patients who screen positive with serum CrAg should undergo careful clinical assessment to evaluate for signs and symptoms of cryptococcal meningitis. Lumbar puncture should be performed if any symptoms are identified (even subtle) or if serum CrAg titer is ≥1:160 as this titer is associated with higher rates of subclinical meningitis and, in the setting of CNS symptoms, increased mortality [136, 137]. Preemptive fluconazole therapy followed by ART initiation is indicated for asymptomatic CrAg-positive patients with low CrAg titer or negative lumbar puncture and should be continued until the CD4 count is greater than 100 cells/μL for at least three months. This approach has demonstrated clinical benefit, including reduction when paired with additional support [138] and has been shown to be cost-effective across a number of settings [139–141]. One meta-analysis suggests additional benefit in patients with even higher CD4 counts (between 101 and 200 cells/μL); however, the optimal preemptive treatment strategy in this group of patients remains uncertain [142]. Routine screening for cryptococcal infection and/or prophylaxis are not recommended in asymptomatic SOT recipients, even when immunosuppression is augmented in patients with previously (appropriately) treated infection [143]. Although cryptococcal GXM-tetanus toxoid conjugate vaccine and specific monoclonal antibodies to cryptococci have been developed, clinical trials have not been initiated to determine their usefulness in human subjects [144, 145].

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Blastomycosis

17

Donna C. Sullivan and Rathel L. Nolan III

Introduction

Blastomycosis is a systemic mycosis, primarily involving the lungs, historically associated with the thermally dimorphic fungus *Blastomyces dermatitidis*. First described by Gilchrist as a cutaneous disease [1], later analysis showed that the lung was the primary route of infection [2] and that skin disease or other organ involvement occurred secondary to hematogenous dissemination. Pulmonary blastomycosis may be asymptomatic or manifest as acute or chronic pneumonia. Hematogenous spread of the organism frequently results in extrapulmonary disease. Blastomycosis has been reported in North America, Africa, India, and parts of Europe, but the majority of cases are from the endemic region around the Mississippi and Ohio Rivers of the USA, and in areas of southern Canada near the Great Lakes [3, 4].

Etiologic Agents

The thermally dimorphic fungi *Blastomyces, Histoplasma, Emmonsia, Paracoccidioides, Emmonsiellopsis,* and *Emergomyces* are the members of the family Ajellomycetaceae, order Onygenales [5, 6]. For over 100 years, the genus *Blastomyces* included only one species, *B. dermatitidis*. Recently, sequencing techniques have been used to construct taxonomic relationships among Ajellomycetaceae family members, resulting in the addition of species to the genus *Blastomyces* [6].

B. gilchristii is a North American strain previously identified as a cryptic species and is not phenotypically distinguishable from *B. dermatitidis* [5]. *B. helicus* was previously identified as *Emmonsia helica* [5, 7]; and is a North American strain, albeit with a more western distribution than *B. dermatitidis* or *B. gilchristii*. Clinically, *B. helicus* more frequently infects immunocompromised patients. Outside North America, *B. percursus* and *B. emzantsi* have been associated with extrapulmonary blastomycosis in South Africa and Israel. *B. parvus*, previously classified as *Emmonsia parva,* does not cause blastomycosis in humans but is associated with adiaspiromycosis, occasionally in humans, but more typically in small mammals [8, 9].

Phenotypic Characteristics

B. dermatitidis and *B. gilchristii* are the imperfect (asexual) stage of the corresponding *Ajellomyces* species, which exhibit thermal dimorphism growing as a mold (mycelial) form at 25–30 °C and as a yeast form at 37 °C (Fig. 17.1). The distinction between the two species is based on sequence analysis and genetic differences [10, 11]. Phenotypically, they are indistinguishable and the descriptions below will be derived from the *B. dermatitidis* literature. The mycelia produce terminal conidia which, when disturbed in the environment, easily become airborne. Human and animal infections typically occur following the inhalation of conidia, which convert to large budding yeast cells inside the lungs associated with the temperature shift to 37 °C [3].

Primary isolation of *B. dermatitidis* from clinical specimens is most reliable when grown as the mycelial form at 30 °C. Mycelial colonies, which are white to brown in color, grow on agar in 1–3 weeks. Positive identification of *B. dermatitidis* requires conversion to the yeast form at 37 °C or nucleic acid amplification methods, which allow early identification of mycelial phase growth. Yeast-like colonies are wrinkled and cream to tan in color. Asexual reproduction is by budding of single 8–15 μm, broad-based, thick walled, multinucleated daughter cells (Fig. 17.1). The same morphologic characteristics are observed in tissue samples from infected individuals. In the appropriate clinical situation, this allows a presumptive diagnosis of blastomycosis [12].

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_17

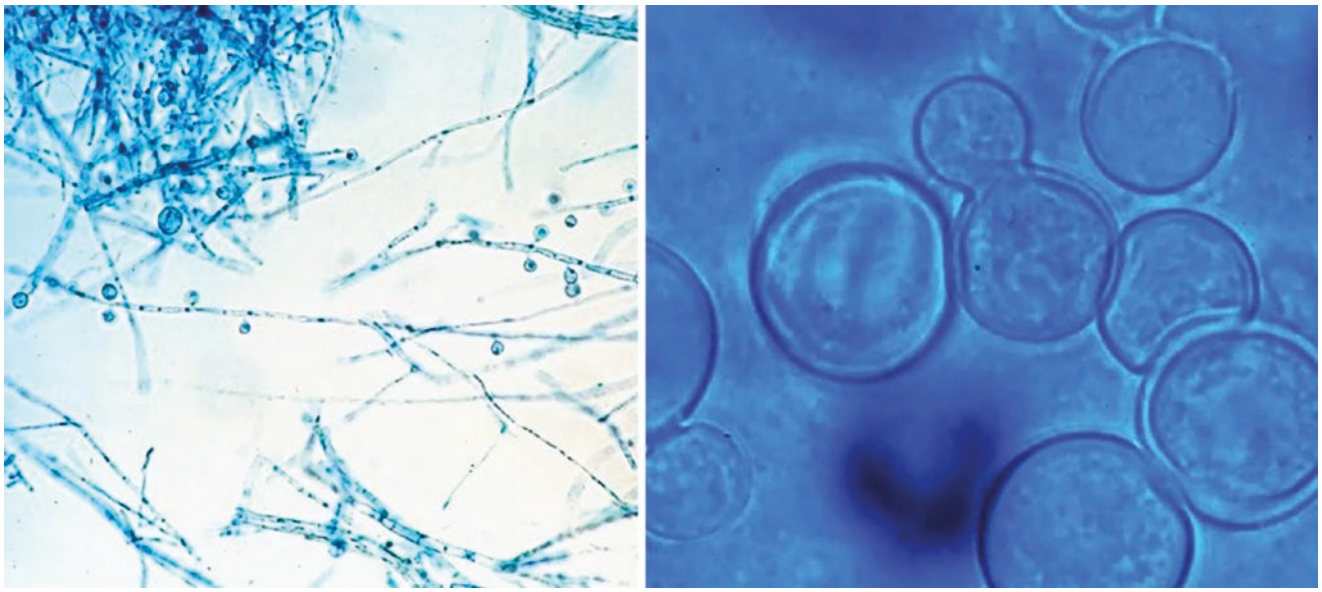


Fig. 17.1 The mycelial phase of *B. dermatitidis* (left) produces no unique characteristics which allow organism identification. As a yeast (right), *B. dermatitidis* has characteristic thick walled, multinucleated cells with single broad-based daughter cells

Several newly recognized *Blastomyces* species associated with atypical blastomycosis are phenotypically distinct from *B. dermatitidis/gilchristii*. *Emmonsia helica* was transferred to the genus *Blastomyces* as *B. helicus.* The mold phase of *B. helicus* does not produce conidia but exhibit spiral hyphae in laboratory settings [8]. The yeast phase produces broad-based buds which are 4–5 μm and sometimes appear as chains [13]. *B. percursus* produces conidiophores in clusters, unlike the terminal conidia of *B. dermatitidis*, but the yeast phase is similar [5, 6]. *B. parvus,* previously *E. parva*, resembles *B. dermatitidis* in its mold phase but produces adiaspores at 35 °C.

Genotypic Characteristics

A. dermatitidis, the telomorphic form of *B. dermatitdis*, has been shown to have heterothallic mating type cultures [12, 14]. Mating is controlled by a highly conserved domain that determine plus (MAT1) and minus (MAT2) strains. The complete sexual cycle has been defined by the DNA structure of the *MAT* locus (alpha or HMG domain), mating assays, and genetic recombination [15]. The *B. dermatitidis MAT* locus is similar to those of other dimorphic fungi in which the mating type gene is linked to *APN2, SLA2*, and *COX13* genes [15].

A variety of nucleic acid techniques have been employed to examine the genetic diversity and geographic distribution of *B. dermatitidis* isolates. In an analysis of 106 clinical and environmental isolates of *B. dermatitidis* from Wisconsin, Georgia, and Africa, polymerase chain reaction (PCR) based assays targeting the promoter region of the *BAD-1* virulence gene identified two major genotypic groups A and B, representing 47.2% and 48.1%, and three minor groups (C, D, and E) [16]. Recently, the technique of microsatellite analysis has been applied to a number of fungal species as well as *B. dermatitidis* [10, 17–20]. Microsatellite analysis offers several advantages over both PCR-RFLP and random amplified polymorphic DNA (RAPD) methods. Meece and coworkers used a panel of 27 microsatellite markers to distinguished 112 geographically diverse isolates into two genetically distinct *Blastomyces* species, *B. dermatitidis,* and *B. gilchristii* [10]. The α and HMG mating types were almost evenly divided between the two species.

The identification of species by sequencing a genetic marker has become common practice among mycologists [21–23]. Genealogical concordance phylogenetic species recognition (GCPSR) techniques have been used to identify populations, delineate cryptic species, and document genetic recombination and gene flow in a number of fungal pathogens [17, 19, 24–27]. GCPSR employing seven nuclear genes to evaluate 78 human, canine, and environmental *B. dermatitidis* isolates from diverse geographic regions also revealed two distinct monophyletic clades within *B. dermatitidis*, phylogenetic species 1 (PS1, clade 1) and phylogenetic species 2 (PS2, clade 2), which represent a genetically divergent novel cryptic species, *B. gilchristii* [11]. Although only a few isolates were subjected to both multi-locus satellite analysis and GCPSR, GCPSR defined PS1 (*B. dermatitidis)* and PS2 (*B. gilchristii*) correspond to the highly diverse Group 2 and monomorphic Group 1 as described by Meece and colleagues [10].

Table 17.1 Recent taxonomic additions to the genus *Blastomyces*

Taxon name	Geographic distribution	Yeast morphology	Mold morphology	Syndromes
Blastomyces dermatitidis	Mississippi/Ohio River Valleys	Broad-based budding yeast, 8–15 μm	Hyphae with right angle branching, terminal conidia	Pulmonary, systemic, and extrapulmonary blastomycosis
Blastomyces gilchristii	Canada, some northern US states	Broad-based budding yeast, 8–15 μm	Hyphae with right angle branching, terminal conidia	Pulmonary, systemic, and extrapulmonary blastomycosis
Blastomyces helicus	Alberta, Saskatchewan, Montana, Colorado, Idaho, Utah, Nebraska, New Mexico, Texas, California	Broad-based budding yeast in multiple sites prior to separation, 4–5 μm	Spiral hyphae with no conidiation	Fatal pulmonary and systemic disease in immunocompromised
Blastomyces percursus	Israel, South Africa	Similar to *B. dermatitidis*	Short conidiophores appearing in clusters resembling florets	Few reported cases with cutaneous lesions as well as pulmonary blastomycosis
Blastomyces parva	North and South America, Eastern Europe, Australia, Asia	No yeast forms, produce adiaspores	Similar to *B. dermatitidis*	Adiaspiromycosis, a granulomatous pulmonary infection
Blastomyces silverae	United States, Canada	Giant cells and yeast-like cells produced at 37 °C, conversion time prolonged	Spirally twisted hyphae with conidiophore	Reported in animals, closely related to *B. parva*

The phylogenetic relationship among the *Ajellomycetaceae* (Onygenales) have been examined using sequence analysis of DNA-directed RNA polymerase II (*rPB2*), translation elongation factor 3-α (*TEF3*), β tubulin (*TUB2*), 28S large subunit rDNA (LSU), and the internal transcribed spacer regions (ITS) in combination with morphological and physiological characteristics [6]. This study established five species in the genus *Blastomyces*: *B. dermatitidis/B. gilchristii, B. helicus, B. parvus, B. percursus,* and *B. silverae*, Table 17.1, although Jiang and colleagues could not distinguish *B. dermatitidis* and *B. gilchristii. Emmonsia parva* and *E. helica* are now synonyms of *B. parvus* and *B. helicus,* respectively [6].

Epidemiology

Blastomyces dermatitidis/gilchristii species in North America are endemic in areas bordering the Ohio and Mississippi River valleys as well as areas bordering the Great Lakes and St. Lawrence River [3, 4, 28]. Environmental isolations of *B. dermatitidis* associated with disease outbreaks indicate that the organism grows as microfoci in warm, moist soil in wooded areas that are rich in organic material [28–31]. Analysis of sporadic cases in humans and dogs, point source outbreaks, and infrequent environmental isolations has provided the major basis for the definition of endemic regions in North America [3, 4]. While most cases have been reported in Mississippi, Arkansas, Kentucky, Tennessee, and Wisconsin, up to 14% of blastomycosis cases identified from Medicare claims data occurred outside the endemic region [32, 33]. Within these known endemic regions there exist hyperendemic areas with exceptionally high attack rates [3, 4].

Recently, 25 microsatellite loci from 169 strains of *B. dermatitidis/gilchristii* were examined from collections held at the University of Alberta and the University of Texas Health Sciences Center [34]. The collection included isolates from throughout the known range of endemicity in North America. Four populations of *B. dermatitidis* and four populations of *B. gilchristii* were identified by genetic analysis. While *B. dermatitidis* was isolated from the entire endemic region, *B. gilchristii* isolates were found only in Canada and a few northern USA. Similar DNA analysis of *B. helicus* indicate its geographic range, but is not as well-described. Ten human and five veterinary isolates of *B. helicus* were identified from mountainous regions of western of Canada and the USA [13].

Other mammals, especially dogs, may become infected [35]. Early studies of endemic cases suggested middle aged men with outdoor occupations were at greatest risk for blastomycosis. A common source outbreak was reported among college students relocating prairie dog burrows in Colorado was reported [36]. Schwartz and Kauffman [37] recently suggested that this outbreak was likely due to *B. helicus* rather than *B. dermatitidis*. Subsequent reviews of reported outbreaks indicate no predilection for sex, age, race, occupation or seasonal exposure [3, 4]. Vocational or avocational exposure to soil appears to be a common factor associated with both endemic and epidemic disease.

Pathogenesis and Immunology

Most of the literature describing the life cycle of what is now considered to be *B. dermatitidis* and the cryptic species *B. gilchristii* was published before molecular genotyping. However, the two are phenotypically indistinguishable and

there are no known clinical differences [37]. The following discussion will refer to both as *B. dermatitidis* as recommended [37]. Blastomycosis is initiated by the inhalation of the conidia of *B. dermatitidis*. Following inhalation, the infectious conidia are non-specifically phagocytosed and killed by polymorphonuclear leukocytes (PMN), monocytes, and alveolar macrophages. Epithelial lung cells initiate NF-κB signaling which in turn elicits IL-17A- and GM-CSF producing lymphoid cells such as Th17 and γδ cells [38–40]. This phagocytic response represents natural or innate immunity and may in part explain asymptomatic cases observed in outbreaks.

Conidia which escape the initial phagocytic response rapidly convert to a yeast form that is more resistant to phagocytosis and killing. In in vitro studies, *B. dermatitidis* yeast evades macrophage defenses [41] and suppresses nitric oxide production by inhibition of inducible nitric oxide synthase [42]. Several virulence factors have been associated with the pathogenicity of *B. dermatitidis*. The thick cell wall of the yeast has been proposed to have antiphagocytic properties. Higher concentrations of lipids and phospholipids in cell walls in some strains have been associated with increased virulence.

Conversion of *B. dermatitidis* to the yeast form induces the expression of a yeast phase specific gene designated BAD-1 (formerly WI-1). BAD-1 (WI-1) is a 120 kDa glycoprotein adhesion and immune modulator with a number of essential properties, including CR3 and $CD14^+$ binding and an epidermal growth factor (EGF)-like domain [43–46]. The protein contains a repetitive domain that mimics thrombospondin type-1 (TSP-1) and suppresses T lymphocyte activation and effector function by binding heparin sulfate [47]. In addition, BAD-1 appears to inhibit tumor necrosis factor-α (TNF), resulting in immune suppression [48]. Transcriptome analysis of mouse models of pulmonary infection demonstrated significant upregulation of *BAD1*, genes involved in cysteine metabolism and exogenous zinc uptake (PRA1) [49]. BAD-1 is a major virulence factor and its deletion results in attenuated pathogenicity [50, 51].

Cellular immunity in humans, as determined by antigen induced lymphocyte proliferation, has been documented using whole yeast phase organisms, an alkali-soluble, water-soluble yeast extract, and BAD-1 [3, 4]. As with other endemic fungi, *B. dermatitidis* seems to require type 1-dependent cell mediated immunity (CMI) [52]. Recent vaccine studies in animal models have shown that CMI is mediated by both vaccine-induced $CD4^+$ [52] and $CD8^+$ T cells [53] which produce type 1 cytokines such as gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α). In addition, the $CD28^+$ T cell receptor has been shown to be required for the induction of protective T cell responses to *B. dermatitidis* infection [54]. $CD4^+$ cells require IL-12 for the development of CMI while $CD8^+$ cells were less dependent on IL-12 for this process [55].

Development of a vaccine to prevent blastomycosis is a major goal but is hampered by an incomplete understanding of the immune response. Bronchoalveolar macrophage (BAM) activity is limited for fungal pathogens such as *B. dermatitidis* [56]. Binding of the yeast cells to surfactant protein D results in interference with BAM TNF-α production, blunting the host defense [57]. This may be compensated by the stimulation of BAM TNF- α by the abundant 1,3-β-glycan on the *B. dermatitidis* yeast cell. Intradermal administration of an attenuated *B. dermatitidis* lacking BAD-1 protects mice against lethal pulmonary challenge but intranasal vaccine delivery fails to do so [52]. Mucosal vaccination leads to poor T cell activation by induction of matrix metalloproteinase 2, which impairs the chemokine response [50].

The development of clinical disease months to years after exposure to *B. dermatitidis* has been reported and patients can have recurrent clinical disease [37, 58, 59]. These observations suggest either reactivation or reinfection. Microsatellite analysis of isolates collected over time from two patients found evidence for both [59].

Clinical Manifestations

The clinical manifestations of blastomycosis are varied and include asymptomatic infection, acute or chronic pneumonia and extrapulmonary disease. A recent meta-analysis of blastomycosis mortality data from 1970 to 2014 identified 20 studies and a total of 2820 cases. Three case series reports identified an additional 67 cases [60]. Of these, studies in which mortality was attributed to blastomycosis were analyzed. Carignan and colleagues [60] reported an overall mortality rate of 6.6% (95% confidence interval [CI], 4.9–8.2). Immunocompromised patients had a mortality rate of 37% (95% CI, 23–51) while patients who developed acute respiratory distress syndrome (ARDS) had a 75% mortality rate (95% CI, 53–96).

A number of factors are known to influence clinical presentation of disease. Patient characteristics and comorbidities as well as genetic variations of the infecting organism affect disease outcome. The Hmong living in Wisconsin are disproportionally high rates of blastomycosis than their neighbors of European ancestry [61]. Homozygosity mapping of the genomes of Hmong blastomycosis patients detected 25 variants of the *IL6* locus as linked to increased risk of blastomycosis. Loss of IL6 production blunts the IL17 production by CD4+ T cells [62]. Univariate analysis of 16 clinical disease and patient demographic characteristics showed significant associations with *B. dermatitidis/gilchristii* [10, 11, 63]. The monomorphic group 1, corresponding

Table 17.2 Organ involvement in blastomycosis

Organ system involved	No. involved/total patients (%)
Pulmonary	369/534 (69)
Cutaneous	306/534 (57)
Osseous	116/534 (22)
Genitourinary	92/534 (17)
Central nervous system	29/534 (5)

Modified from [3]. Data obtained from clinical and autopsy findings compiled from seven studies

to *B. gilchristii* isolates identified by microsatellite analysis were more likely to be associated with pulmonary-only infections while the more genetically diverse group 2 isolates, *B. dermatitidis,* were more likely to disseminate [11, 63]. Thus, it would appear that *B. dermatitidis* isolates were more frequently seen in older patients who were smokers and had a comorbid condition [63].

Extrapulmonary disease results from the hematogenous spread of the fungus from a primary pulmonary infection. Although extrapulmonary *B. dermatitidis* infection has been reported to involve almost every organ of the human body, the skin, bones, and genital urinary system are most common (Table 17.2). It is important to note that blastomycosis mimics many other disease processes whether acute or chronic [3]. For example, acute pulmonary blastomycosis is often mistaken for bacterial community acquired pneumonia or influenza. Chronic pulmonary blastomycosis commonly mimics a malignancy or tuberculosis. Skin lesions are often misdiagnosed as pyoderma gangrenosum or keratoacanthoma. Blastomycosis of the larynx is frequently misdiagnosed as carcinoma. One case report of laryngeal blastomycosis was identified in a patient with no other systemic disease as determined by computed tomography of the head, chest, and abdomen as well as culture of the maxillary sinus [64]. A high index of suspicion and a careful histologic evaluation of secretions or biopsy material is needed.

Pulmonary Blastomycosis

Acute Infection

Initial infection occurs after inhalation of conidia into the lungs. Unless associated with an outbreak or group exposure, acute infection is frequently unrecognized. Clinical studies involving point source outbreaks of infection indicate that symptomatic acute pulmonary disease occurs in only 50% of individuals, usually after an incubation period of 30–45 days [29, 30]. Signs and symptoms of acute pulmonary blastomycosis are similar to those of influenza or bacterial pneumonia. Fever, chills, pleuritic chest pain, arthralgias, and myalgias usually occur abruptly. At onset cough is nonproductive but frequently becomes purulent as disease progresses. Chest radiographs commonly reveal alveolar

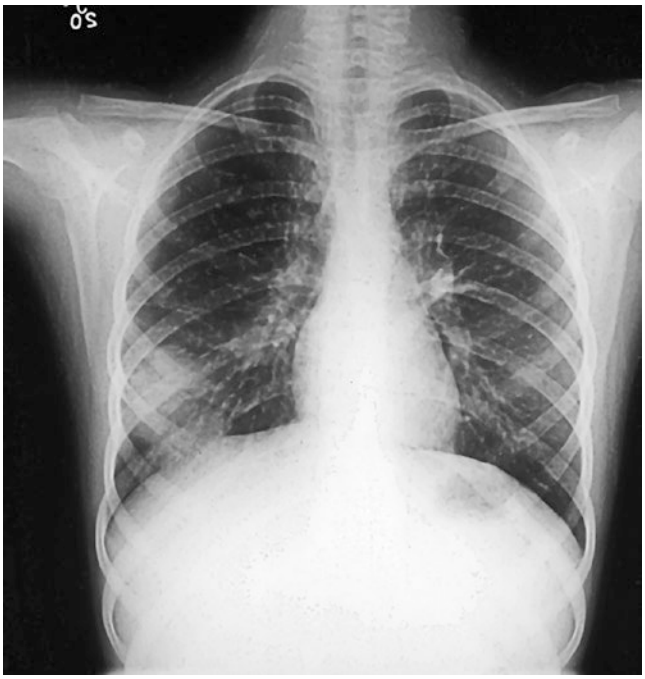


Fig. 17.2 Acute pulmonary blastomycosis. Chest radiograph reveals peripheral alveolar infiltrate which appear to be pleural based. Although this patient clinically improved with antibiotics sputum culture grew *B. dermatitidis*

infiltrates with consolidation (Fig. 17.2) [3, 4]. Pleural effusions are uncommon and, if present, are typically small in volume. Hilar adenopathy is uncommon and is a useful sign in distinguishing acute blastomycosis from acute histoplasmosis. Spontaneous cures of symptomatic acute infection have been documented, but the exact frequency of these cures has not been clearly established [3, 4]. Although not an opportunistic infection, immunosuppression is a risk factor for serious pulmonary complications such as acute respiratory distress syndrome (ARDS) [3, 4].

Chronic Infection

The majority of patients diagnosed with pulmonary blastomycosis have a chronic pneumonia which is clinically similar to tuberculosis, other fungal infections, and cancer. Symptoms include fever, weight loss, chronic productive cough, and hemoptysis. The most frequent radiologic findings are alveolar infiltrates (Fig. 17.3) with or without cavitation, mass lesions that mimic bronchogenic carcinoma (Fig. 17.4), and fibronodular infiltrates [3, 4]. Although small pleural effusions have been reported, large pleural effusions (Fig. 17.5) are distinctly uncommon and, when present, have been associated with poor outcome.

Acute Respiratory Distress Syndrome

Patients may occasionally present with ARDS associated with miliary disease or diffuse pneumonitis (Fig. 17.6).

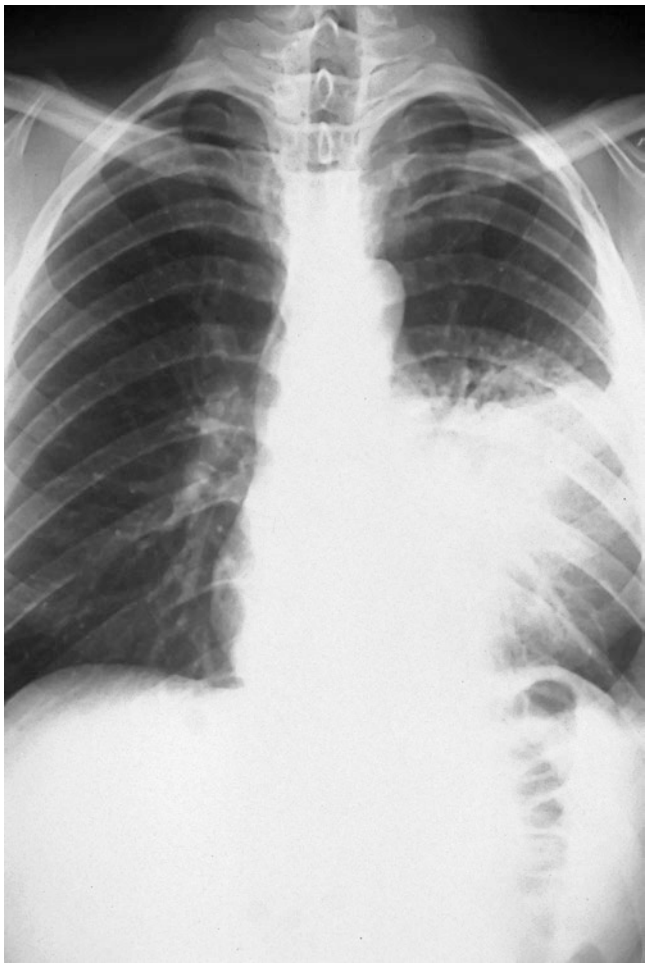


Fig. 17.3 Progressive pulmonary disease showing extensive left midlung alveolar infiltrate. This patient failed multiple courses of oral and intravenous antibiotics over a 2 month period prior to diagnosis of blastomycosis

Mortality exceeds 50% in these patients and most deaths occur within the first few days of therapy [60, 65]. Diffuse pulmonary infiltrates and respiratory failure are more likely to occur in immunocompromised patients, especially those with late stage AIDS [3, 4].

Extrapulmonary Blastomycosis

Extrapulmonary disease has been reported in as many as two-thirds of patients with chronic blastomycosis. This high frequency probably reflects selection bias as these figures were reported in earlier autopsy-based studies before effective therapy was available [3, 4]. More recent studies have documented extrapulmonary disease in only 25–40% of patients with blastomycosis [65]. Extrapulmonary disease is almost always seen in conjunction with active pulmonary disease. Putman and colleagues recently reviewed cases of intrathoracic blastomycosis and identified 25 cases of mediastinal disease [66].

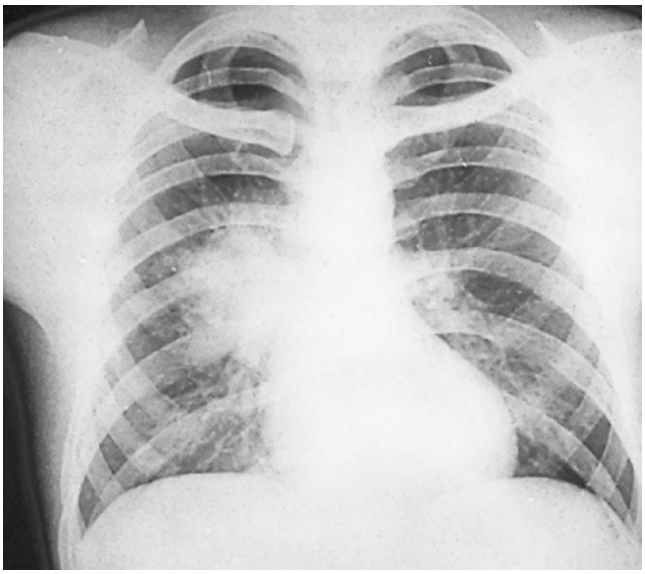


Fig. 17.4 Chest radiograph with right hilar infiltrate that mimics bronchogenic carcinoma. Bronchoscopy with biopsy and pulmonary cytology should be performed in these patients presenting with this radiographic finding to rule out concomitant disease

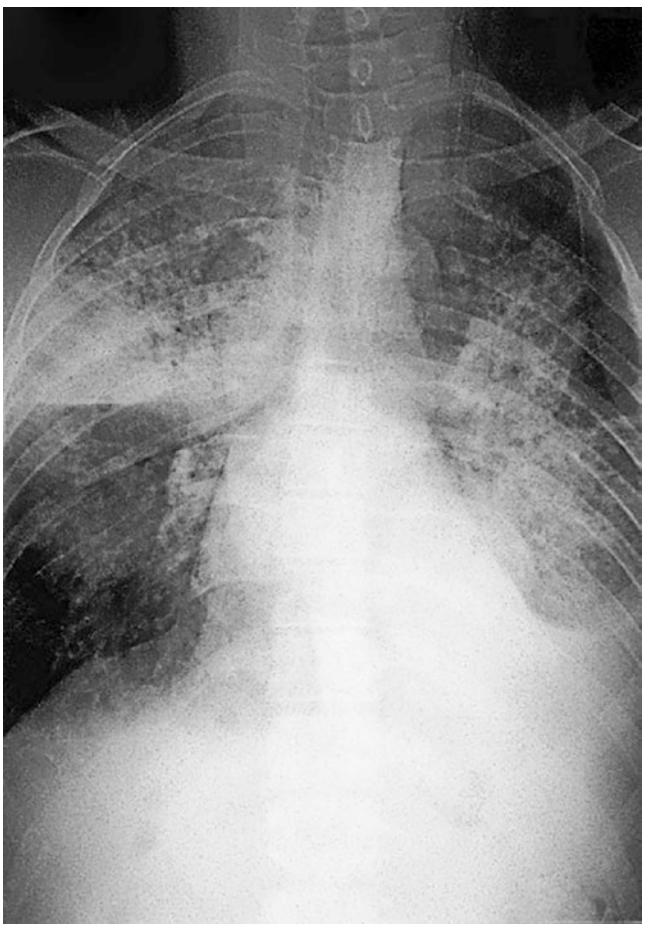


Fig. 17.5 Patient with life-threatening pulmonary disease whose chest radiograph reveals bilateral alveolar infiltrates and large left sided pleural effusion

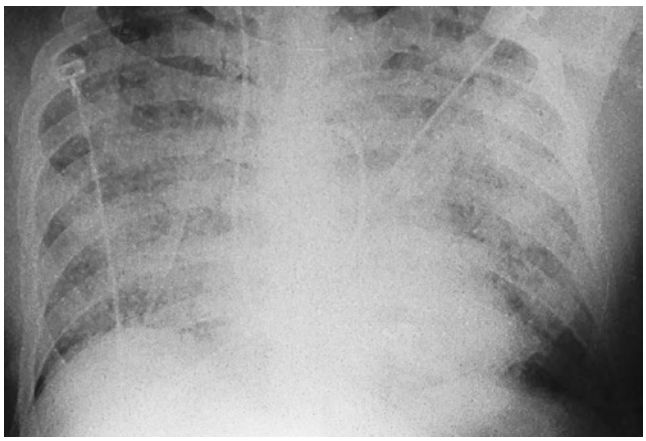


Fig. 17.6 Diffuse pulmonary infiltrates in a patient with ARDS. Patients presenting with this syndrome have a mortality rate greater than 50%

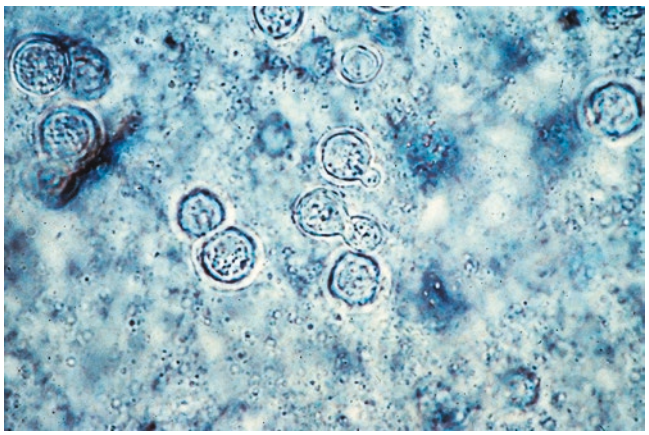


Fig. 17.8 Diagnosis of blastomycosis. This figure shows the characteristic yeast forms in a wet preparation of a skin scraping. Scraping of the edges of the verrucous and ulcerative lesions yield the best diagnostic results

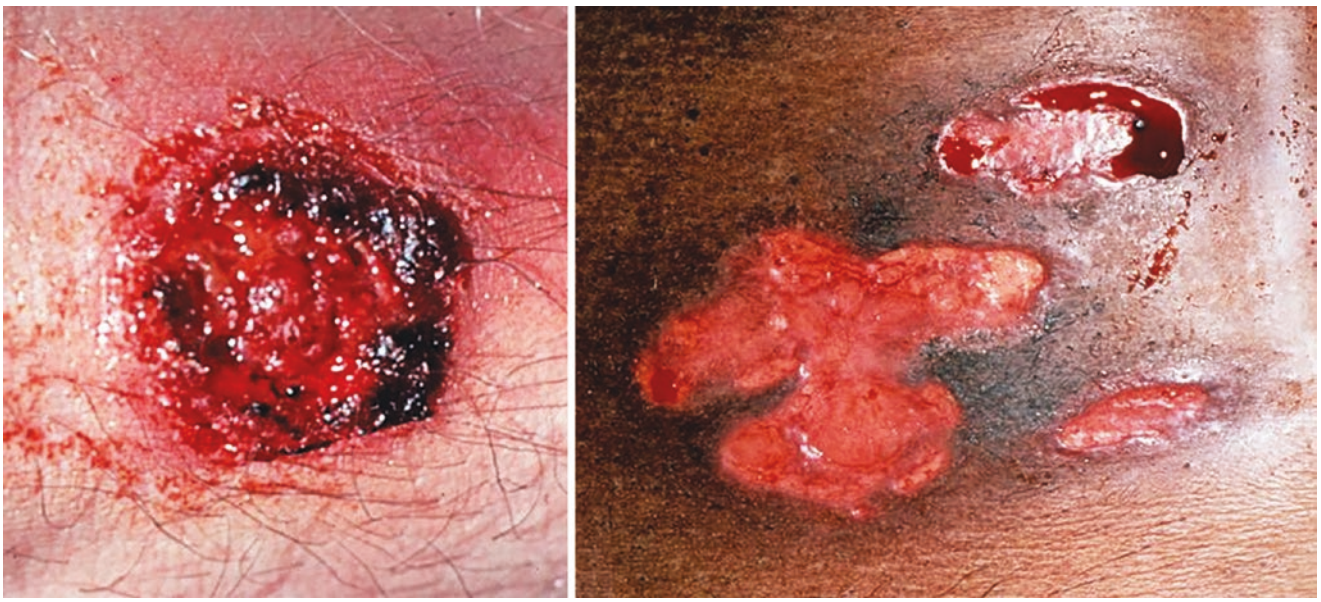


Fig. 17.7 Cutaneous blastomycosis typically produces verrucous (left) or ulcerative (right) lesions

Skin Disease

Skin disease is the most common extrapulmonary manifestation of blastomycosis. Two types of skin lesions occur, verrucous and ulcerative (Fig. 17.7). The verrucous lesion is most common, typically with well-demarcated borders from gray to violaceous in color. These lesions may mimic squamous cell carcinoma. Microabscesses develop at the periphery of these lesions. Specimens taken from the margins usually reveal the diagnostic yeast form on wet preparation (Fig. 17.8). The second type of lesion is ulcerative. These ulcers are friable and bleed easily and usually have well-demarcated, heaped up borders. The ulcers of blastomycosis develop from subcutaneous pustular lesions which spontaneously rupture and drain.

Regional lymphadenopathy is usually not present in cases of pulmonary dissemination. Inoculation blastomycosis following a dog bite or autopsy accidents often have lymphadenopathy/adenitis as a prominent feature [3, 4]. Lesions may also appear on the mucosa of the nose, mouth, and larynx. Laryngeal blastomycosis mimics well-differentiated squamous cell carcinoma both clinically and histopathologically. Subcutaneous nodules or cold abscesses may be seen in patients with multi-organ involvement.

Osseous

Osteomyelitis occurs with as many as one-fourth of *B. dermatitidis* infections [3, 4]. Although any bone may be affected the vertebrae, pelvis, sacrum, skull, ribs or long bones are most frequently involved. Granuloma formation, suppuration, or necrosis is observed in biopsy specimens. A well-circumscribed osteolytic lesion may be observed on radiographs. Such lesions are radiographically indistinguishable from other fungal, bacterial or neoplastic disease. Patients with *B. dermatitidis* osteomyelitis usually present with contiguous soft tissue abscesses or chronic draining sinuses. Although most bone lesions resolve with prolonged antifungal therapy, some may require surgical debridement for cure.

Genitourinary

In men, 10–30% of blastomycosis involves the genitourinary tract, primarily the prostate and epididymis [3, 4]. Prostatic involvement is frequently associated with symptoms of obstruction, and includes an enlarged, tender prostate, and pyuria. Urine cultures obtained following prostate massage are frequently positive. Female genitourinary blastomycosis is rare but may include endometrial infection and tubo-ovarian abscess.

Central Nervous System

CNS involvement occurs in less than 5% of cases in immunocompetent patients. Persons with AIDS have rates of CNS involvement as high as 40% [67] and other studies confirm immunosuppression as a risk factor for dissemination [68]. CNS blastomycosis may present as an abscess (epidural, cranial, or spinal) or as meningitis (Fig. 17.9) [68]. Magnetic resonance imaging (MRI), alone or in conjunction with computed tomography scans, reveals CNS abnormalities and are used to identify CNS involvement [68]. Surgical intervention may be necessary for both diagnosis and to prevent neurologic deterioration [69].

Atypical Blastomycosis

New species within the genus *Blastomyces* have been shown to be associated with atypical presentations of blastomycosis. *B. helicus* is most frequently associated with disease in immunocompromised patients: HIV/AIDS, hematological malignancy, solid organ transplant and immunosuppressed patients [13]. *B. parvus* presents as adiaspiromycosis, a granulomatous pulmonary infection clinically distinct from classic blastomycosis discussed above.



Fig. 17.9 CNS blastomycosis in an AIDS patient. Diagnosis may require aspiration of the abscesses if no active pulmonary or cutaneous disease is present

B. percursus has been reported only in a few cases in Africa and the Middle East [5].

Diagnosis

Definitive diagnosis of blastomycosis requires the growth of the organism from sputum, pus, or biopsy material. The clinical laboratory should be alerted to culture specimens from suspected cases on fungal media such as Sabourand dextrose agar. Mycelial phase cultures grown at 30° C are the preferred method for isolation of *B. dermatitidis* from clinical specimens. These usually become positive within 1–3 weeks of incubation (Fig. 17.1). Sputum cultures in pulmonary blastomycosis have a high positive yield (75% per single sample, 86% per patient), but specimens obtained by bronchoscopy yield an even higher positive rate (92% of patients).

A presumptive diagnosis is often made by visualization of the characteristic large broad-based budding yeast in sputum, pus, or histopathologic specimens (Fig. 17.8). Because colonization with *B. dermatitidis* does not occur, observation of yeast forms in clinical specimens should prompt empiric therapy in the appropriate clinical presentation. Although direct examination of wet preparations have been reported to

have relatively low diagnostic yield [3, 4], the simplicity of the procedure, low cost, and potential for rapid diagnosis warrant its use. Cytology has been shown to have a higher diagnostic yield [4].

Serologic diagnosis of blastomycosis is of limited usefulness. Complement fixation antibodies have been used for epidemiologic purposes, but are severely limited in their specificity due to cross-reactivity to antigens of other fungi, particularly *H. capsulatum* and *C. immitis*. Immunodiffusion tests for precipitating antibodies to *B. dermatitidis* are more specific than complement fixation but lack sensitivity in early disease [4]. Commercial radioimmunoassays, enzyme immunoassays, and enzyme linked immunosorbent assays have been developed which offer the promise of higher sensitivity but with specificities similar to complement fixation [4]. Immunoassays employing the BAD-1 yeast phase specific protein discussed above are not currently commercially available.

A second generation assay for *Blastomyces* that detects antigen in urine and serum has been developed by MiraVista Diagnostics (Indianapolis, IN; www.miravistalabs.com) [44]. Antigenemia is detected in 70–80% of patients with disseminated disease. Antigen detection in the urine was higher than serum, approaching 100%. Specificity is, however, reduced by the presence of cross-reactive antigens present in specimens obtained from patients with other fungal infections. This occurs in 96% of patients with histoplasmosis [70, 71]. Antigen levels are reported to decline with successful treatment and increase in treatment failure or relapse.

Nucleic acid detection techniques, both target and signal amplification methods, have been developed [47], including the chemiluminescent DNA probe (GEN-PROBE® AccuProbe®) for culture identification. This assay has been shown to cross react with *B. helicus* [13]. These facilitate early identification of *B. dermatitidis* in mycelial cultures without the requirement of conversion to the yeast form. PCR amplification of the rRNA gene along with specific probe hybridization has been used to identify yeast phase organisms in tissue specimens. A rapid, real-time assay employing TaqMan probes to detect the *B. dermatitidis* BAD-1 promoter has been reported but is not commercially available [72]. These molecular techniques offer great promise for the rapid diagnosis of blastomycosis.

Treatment

Virtually all patients require therapy. Prior to the availability of azoles, amphotericin B was the mainstay of treatment. However, a series of clinical trials performed by the NIAID Mycoses Study Group have shown itraconazole and fluconazole to be effective, relatively nontoxic agents when compared to amphotericin B for treatment of patients with mild to moderate non-CNS disease [37, 73]. Other than its use in diagnosis, the role of surgery is limited. Along with specific antifungal therapy, surgery may be helpful for the drainage of large abscesses, resection of cerebral blastomycomas, and debridement of devitalized bone.

Selection of an appropriate therapeutic regimen is based on three major considerations: the clinical form and severity of the disease, the immune status of the patient, and the toxicity of the antifungal agent. Specific recommendations of dose and duration of therapy in defined clinical settings are listed in Table 17.3 [37, 73]. For more detail concerning drug interactions, toxicities, adverse reactions and pharmacokinetics about individual antifungal agents, the reader is referred to the chapter discussing individual agents in this text (see Chap. 8).

All patients with progressive pulmonary infection or extrapulmonary disease and all immunocompromised

Table 17.3 Treatment guidelines for *Blastomyces dermatitidis* infections

Type of disease	Primary therapy	Alternate therapy
Pulmonary		
Severe	Amphotericin B 0.7–1.0 mg/kg/day; [a]total dose—1.5–2.5 g	Switch to itraconazole 200–400 mg/day once patient stabilized
Mild to moderate	Itraconazole 200–400 mg/day	Ketoconazole 400–800 mg/day, or fluconazole 400–800 mg/day
Disseminated		
CNS	Amphotericin B, 0.7–1.0 mg/kg/day; total dose—at least 2 g	If intolerant to full course of amphotericin B, fluconazole 800 mg/day
Non-CNS disease		
Serious	Amphotericin B 0.5–0.7 mg/kg/day; total dose—1.5–2.5 g	Switch to itraconazole 200–400 mg/day once patient stabilized
Mild to moderate	Itraconazole 200–400 mg/day	Ketoconazole 400–800 mg/day, or fluconazole 400–800 mg/day
Immunocompromised	Amphotericin B 0.3–0.6 mg/kg/day; [a]total dose—1.5–2.5 g	Selected patients with non-CNS disease may be switched to itraconazole, 200–400 mg/day, once clinically improved. Suppressive therapy with itraconazole should be considered in patients whose immunocompromised state continues. For patients with CNS disease or intolerant to itraconazole, consider fluconazole, 800 mg/day

Modified from [66]

[a] A lipid formulation of amphotericin B (3.0–5.0 mg/kg/day) may be substituted for conventional amphotericin B

patients should be treated [37, 73]. In immunologically normal patients with mild to moderate pulmonary or extrapulmonary disease that does not involve the CNS, the azole antifungal agents, itraconazole or fluconazole, administered for 6 months have proven to be effective, less toxic alternatives to amphotericin B. Although no randomized, blinded studies have been performed to compare different azoles, and only a few comparative trials for blastomycosis therapy have been reported, itraconazole appears to be the best tolerated and most effective azole. Itraconazole is considered the drug of choice for patients with non-life-threatening, non-CNS blastomycosis [37, 73].

Amphotericin B (including lipid-based formulations) is currently reserved for the initial treatment of patients with life-threatening disease, immunocompromised patients, and those with CNS disease. In selected patients initially presenting with life-threatening disease, itraconazole has been successfully substituted following an induction course of amphotericin B. Patients with CNS disease and severely immunocompromised patients should be treated with a full course of amphotericin B. Most experts recommend a total dose of 1.5–2.5 g. Other suggested modifications of therapy for special circumstances are reviewed in Table 17.3

Fluconazole has been used in only a limited number of patients, but appears efficacious at doses of 400–800 mg/day. Two factors may eventually lead to more extensive use of fluconazole: it has fewer side effects and adverse drug interactions and it has excellent penetration into the CNS, suggesting a role for this drug in the treatment of CNS blastomycosis. Voriconazole also has excellent CNS penetration and has activity against *B. dermatitidis* in vitro [74]. Successful outcomes have been reported in a small number of patients treated with voriconazole for CNS blastomycosis [68, 74]. Posaconazole has been effective in the treatment of two patients with refractory pulmonary blastomycosis [75].

Amphotericin B is the drug of choice for blastomycosis occurring during pregnancy, as azoles are contraindicated [37, 73]. The clinical spectrum of blastomycosis in pediatric patients is similar to that seen in adults. Recent reports indicate blastomycosis in children is more difficult to diagnosis and less likely to respond to oral therapy. Children with life-threatening disease should be treated with amphotericin B. Itraconazole has been used successfully at a dosage of 5–7 mg/kg/day in a small cohort of pediatric patients [37, 73]. CNS disease occurs in approximately 40% of patients with AIDS or other diseases or therapies associated with immunosuppression. Likewise, disseminated disease and life-threatening pulmonary disease also appear more common in the clinical setting of immunosuppression. Hence, the recommendation that amphotericin B is the drug of choice for treatment of immunocompromised patients. Frequent relapses have been reported in patients whose immunosuppression persists and chronic suppressive therapy with an oral azole is recommended by some experts [37, 73].

Patients should be followed for years for evidence of relapse, especially in the CNS. Relapse rates of less than 5% are reported in patients treated with amphotericin B and itraconazole. Owing to the problems with bioavailability of oral itraconazole, serum blood levels may be clinically useful in guiding treatment of patients whose disease progresses on either formulation [37, 73].

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Coccidioidomycosis

18

Arash Heidari, Rasha Kuran, and Royce Johnson

Introduction

Coccidioidomycosis was first described in 1892, in Buenos Aires by Posadas and Wernicke [1, 2]. They believed that the individual in their case report suffered from a malignant disease with probable infectious triggers. Organisms seen microscopically were thought to be parasites. The first extensive study of coccidioidomycosis was published by Rixford and Gilchrist in San Francisco in 1896, who recognized it as an infectious disease, albeit believed parasitic in etiology [3]. In 1900, Williams Ophuls noted that the culture of the organism always produced colonies of a mold, what we now know to be the mycelial (saprobic) growth of *Coccidioides.* The life cycle was roughly outlined in a preliminary report and the fungus was given the name of C*occidioides immitis* [4].

During the years 1925–1936, the early pathologic, epidemiologic, and mycologic studies were completed. Montenegro reported the first recovery of *C. immitis* from blood [5]. Coccidioidal infection in farm animals was described by Beck [6]. Meningitis was described first pathologically and subsequently clinically in the early part of the twentieth century [7, 8]. Two important observations were also made during this period; that the lung is the portal of entry and that *C. immitis* can be isolated from soil [9, 10].

Coccidioidomycosis was considered to be a rare and fatal infection until an accidental laboratory exposure of a medical student at Stanford University resulted in only a transient pulmonary infection. This led to a reassessment of the natural history of coccidioidal infection. The work in Kern County by Dr. Myrnie Gifford on a local respiratory illness in the San Joaquin Valley of California, known as Valley Fever, eventually elucidated the primary infection as being predominantly pulmonary [11].

During the latter part of the 1930s and 1940s, the natural history of the primary illness, the utility of the skin test, and serology were developed by Charles E. Smith and coworkers. William Winn and Hans Einstein made further contributions to disease description and therapy with amphotericin B deoxycholate, both intravenously and intrathecally for meningitis. By the 1950s, the clinical spectrum of coccidioidal infection was well described, with the publication of an excellent monograph by Fiese [12]. The Infectious Diseases Society of America published a clinical practice guideline for the treatment of coccidioidomycosis in 2016 [13].

Etiologic Agents

- Kingdom: Fungi
  - Phylum: Ascomycota
    Class: Euascomycetes
    - Order: Onygenales
      - Family: Onygenaceae
        Genus: *Coccidioides*

Coccidioides was originally described as one species, *C. immitis,* until the mid-1990s when two genetically distinct populations of *Coccidioides* were described, *C. immitis* and *Coccidioides posadasii*, correlating to separate endemic regions. Currently the *C. immitis* is maintained as the name of those isolates that are predominately found in California. The new species, *C. posadasii*, is predominately found in Texas, Mexico, Central America, and South America. Both species are found in Arizona [14]. *Coccidioides* of both species, however, show few phenotypic differences and are mycologically and clinically indistinguishable. *Coccidioides* is a thermally dimorphic fungus that exists either as myce-

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_18

lium or spherule. The fungus is found as far north as south-central Washington State and as far south as Argentina, the place of its original description. It grows in soils with relatively high salinity, and in climates with mild winters with few freezes and hot dry summers [15]. Under these ideal conditions, the mycelia grow in isolated pockets by apical extension. They produce specialized aerial hyphae that segment and form arthroconidia. The connecting links between arthroconidia are quite fragile and separate easily with minimal mechanical force or air turbulence. The arthroconidia become airborne in a form capable of deposition in the lungs if inhaled, and can travel substantial distances, perhaps as far as 75 miles or more. If they find an appropriate soil niche, can reestablish the saprophytic phase. However, if they are inhaled by an appropriate host, they undergo transformation from arthroconidia into spherules. Spherules reproduce by endosporulation, a process whereby the growing spherule is subdivided into numerous sub-compartments, each of which becomes viable daughter cells or endospores. The spherule eventually ruptures, releasing endospores, each of which may continue to propagate in tissue or revert to mycelial growth in soil or on growth media (Fig. 18.1).

Epidemiology

The disease was first described in Argentina, but other foci of infection in South America and Central America also exist. *Coccidioides* species are found solely in the Western Hemisphere, in the "lower Sonoran life zone" [17]. The majority of the soils which support the organism are found in North America, particularly in the southwestern USA and northwestern Mexico. The areas of greatest endemicity are in the southern San Joaquin Valley and South-central Arizona. The disease extends to the northern Central Valley in California and as far as south-central Washington State in North and Utah in the Great Plains [18]. The total number of infections per year is not known but it is agreed that there are more than 150,000 infections per year in the USA [19].

Sixty percent of infections are asymptomatic and 40% have an influenza-like or pneumonic illness. Of the latter, only a quarter are diagnosed. Approximately 1% of total infections disseminate. There is seasonality to the infection as reported from California and Arizona. In California, the highest incidence occurs in the fall.

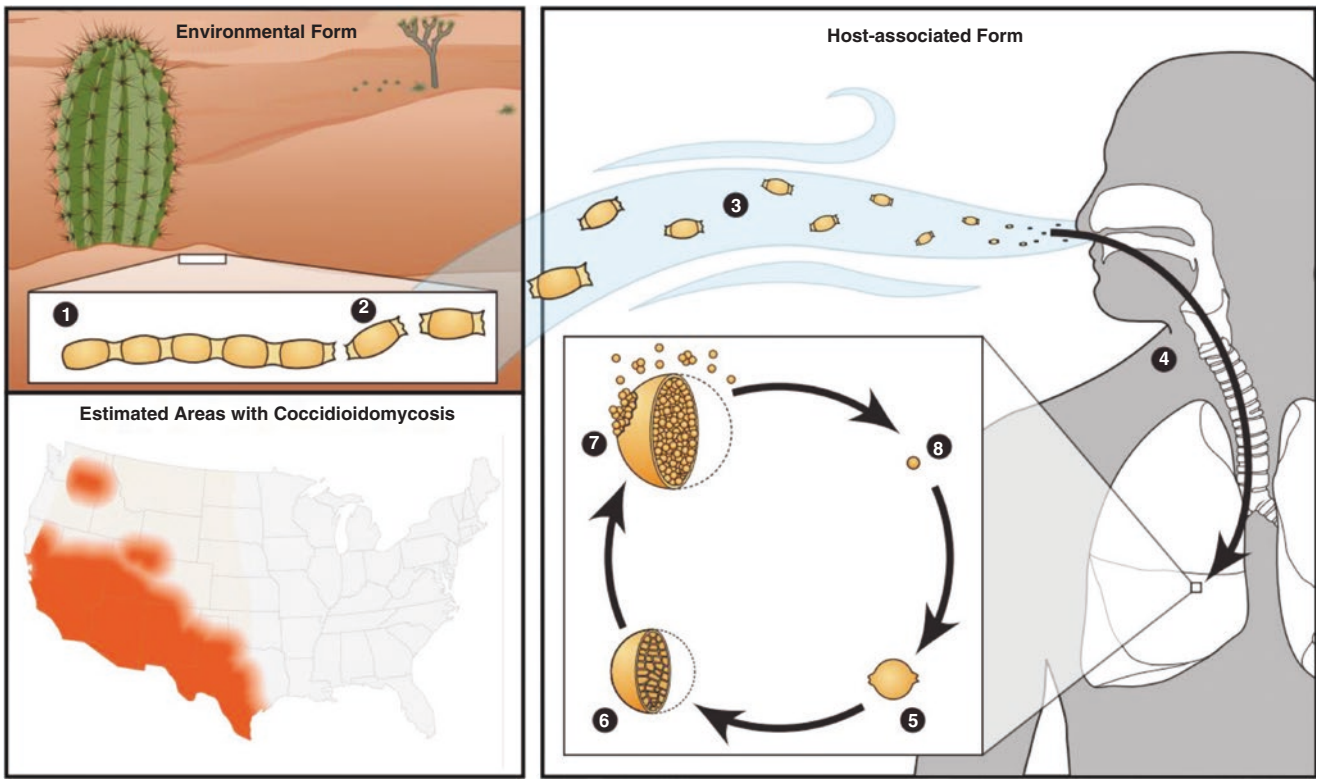


Fig. 18.1 Biology and Life cycle of Coccidioidomycosis depicting saprophytic (soil) and parasitic(host) phases with estimated areas in the USA. CDC [16]

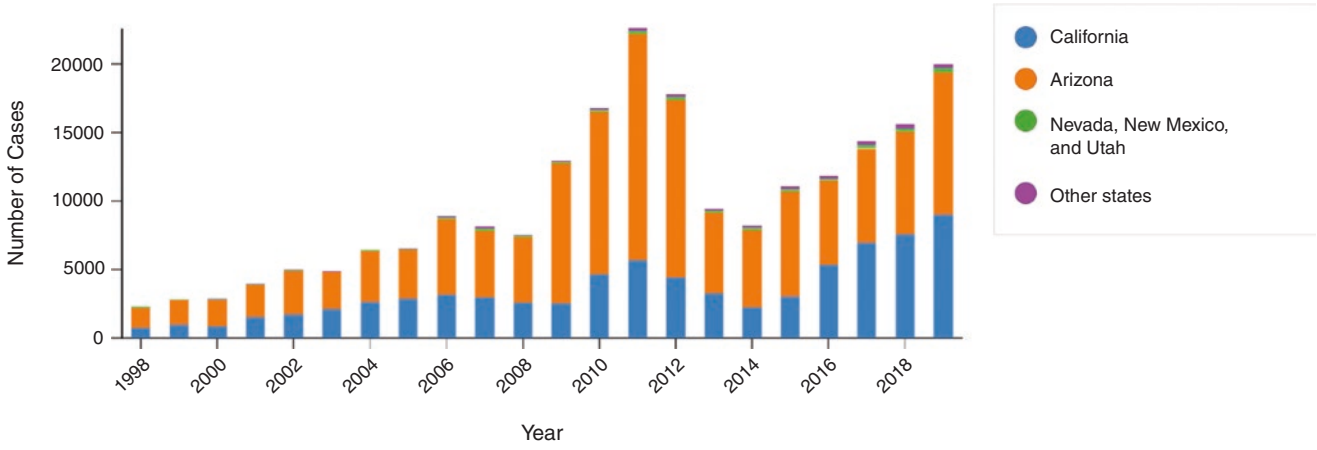


Fig. 18.2 Number of reported Valley fever cases. CDC, National Notifiable Diseases Surveillance System (NNDSS)

The incidence of coccidioidomycosis is generally increasing. Sporadic increases in reported cases follow certain phenomena such as earthquakes [20], dust storms [21], and with certain activities that involve soil disturbance [22]. More than 95% of cases are reported out of California and Arizona (Fig. 18.2).

Some of this increase may be due to increased populations, increased soil disturbance (construction), changes in case definitions (lab-only reporting), and increased diagnosis. In all probability, all of these do not account for more than a minority of the increased numbers. The annual incidence of infections in those living in the most endemic areas (Southern Arizona and Southern Central Valley, California) is probably 1–3%. Coccidioidomycosis is increasingly found in individuals who live outside of the endemic zone due to increased leisure and work-related travel.

Several studies described the increase in the absolute incidence of disease during the epidemics described in the 1990s and in the first 2 years of the new millennium [23, 24]. Despite the significant escalation in incidence, mortality associated with coccidioidomycosis has remained similar [25, 26].

Pathogenesis and Immunology

Coccidioides require animal nutrients for growth; hence, its natural distribution is largely restricted to areas of previous human or animal habitation, particularly small rodent burrows [27]. It grows in soil (and on laboratory media) as a mycelium. After a variable period, mycelia septate and produce spores known as arthroconidia. The thin septations are fragile and the arthroconidia become airborne with minimal soil disturbance (wind and digging). The arthroconidia may travel up to 75 miles and occasionally a much greater distance [28]. Infection is almost always due to the inhalation of arthroconidia.

Most of these inhalations either result in no infection or asymptomatic infections manifested as skin test conversion (60% of infections) [29, 30]. If not controlled, arthroconidium under the effect of temperature and other factors transform into spherules which subsequently internally divide into endospores. With spherule lysis, the endospores are released and become spherules, which results in exponential propagation of the pathogen. During this process, an inflammatory response ensues, and a local pulmonary lesion develops. In some infections, the *Coccidioides* species gain access to the vascular space, leaving the lungs and disseminating to other parts of the body.

The immunopathology of this host–parasite interaction has been studied for several decades but is yet incompletely understood. Both the innate and adaptive immune systems are involved. The response is sequential, multifaceted, and very complex. Neutrophils, macrophages, and dendritic cells are all involved. T-cell responses based on T-helper (Th) cells, Th1 and Th2 ratio, and Th17 and regulatory T cells ratio appear to be important. Antibodies are not demonstrably protective.

Additionally, in vitro, observations have shown that innate cellular responses, mediated by mononuclear cells or natural killer cells, may slow fungal proliferation after infection [31]. It is conceivable that interleukin (IL)-12, IL-23, and interferon-gamma may play an important role in protective immunity in coccidioidomycosis as recently demonstrated in paracoccidioidomycosis and histoplasmosis [32, 33]. Progressive and disseminated forms of coccidioidomycosis

were recently described in cases with interferon-gamma receptor 1 deficiency or mutation in the beta 1 subunit of the IL-12 receptor [34, 35].

Clinical Manifestations

Pulmonary Infection (Mild to Moderate)

Coccidioidomycosis most commonly presents as primary pulmonary disease. The first symptoms of primary infection usually appear 7–21 days after exposure, although infection is asymptomatic 60% of the time. In those patients presenting with symptomatic disease, the majority present with an influenza-like syndrome. Of the 40% of total infections with symptoms, only 1 out of 4 is diagnosed, the majority of these present with pneumonic or pleural disease.

Symptoms prevalent in primary coccidioidomycosis include fever (76%), cough (73%), chest pain (44%), fatigue (39%), shortness of breath (32%), chills (28%), erythema nodosum (26%), myalgias (23%), sputum production (22%), headache (21%), and night sweats (21%) [31]. Skin manifestations develop as part of the primary illness, most often as a transient non-pruritic fine papular rash. Erythema nodosum is fairly common in primary coccidioidal infection, with a strong predilection for women (Fig. 18.3). Less commonly, erythema multiforme and erythema sweet bullosum are seen [36, 37]. Migratory arthralgias are common; the triad of fever, erythema nodosum, and arthralgias has been referred to as "desert rheumatism." Radiographic findings in primary coccidioidomycosis typically include infiltrate only (70%), infiltrate with hilar adenopathy (10%), or infiltrate with effusion (10%) (Figs. 18.4 and 18.5). Approximately 10% of

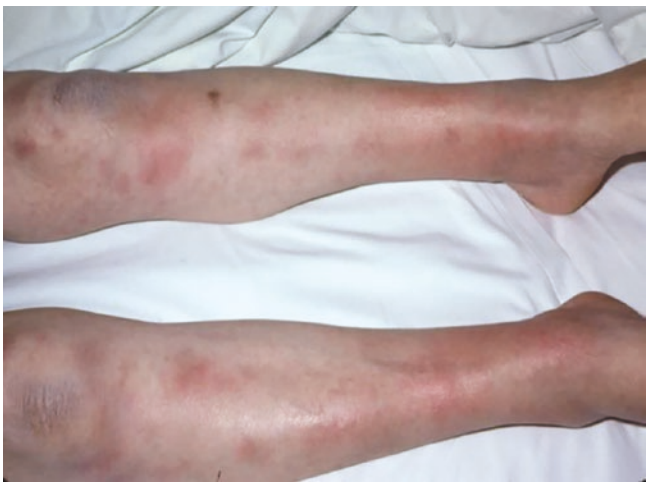


Fig. 18.3 Erythema nodosum affecting the lower extremities

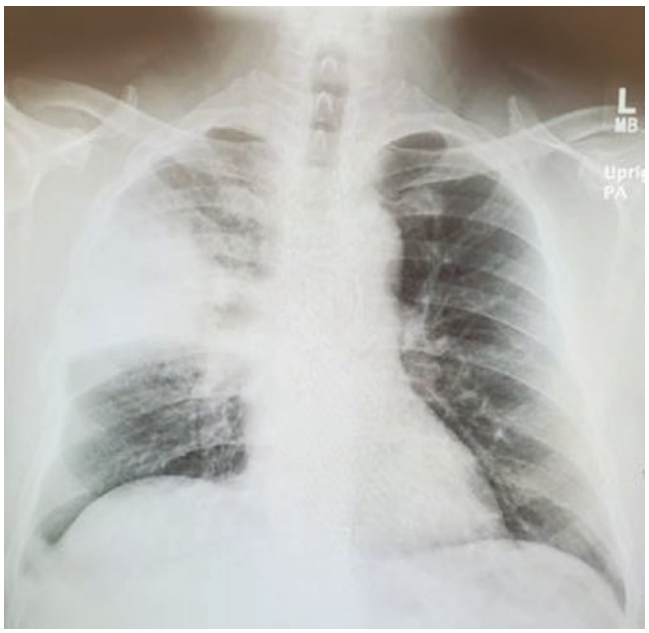


Fig. 18.4 Right upper lobe dense consolidation with associated right hilar adenopathy

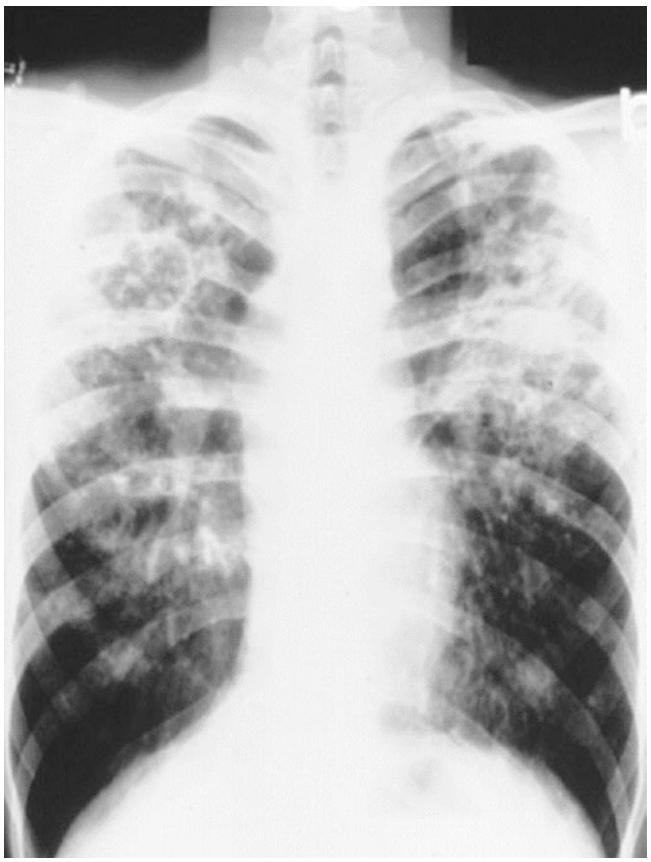


Fig. 18.5 Fibrocavitary changes involving both upper lobes. Right upper lobe cavity with air-fluid level

individuals will have a negative chest radiograph at diagnosis. Most coccidioidal respiratory infections resolve within several weeks to months without complications.

Pulmonary Infection (Severe and Complicated)

There are a number of pulmonary complications of primary coccidioidomycosis. The most common is severe and persistent pneumonia. This is defined as radiographic and clinical findings of pneumonic disease for greater than 6 weeks. Progressive primary coccidioidomycosis is a syndrome described in which the patient has resolution of their pulmonary parenchymal disease with the persistence of hilar and mediastinal lymphadenitis. Rare cases of progressive fibrocavitary coccidioidomycosis, which often resembles pulmonary tuberculosis, are described (Fig. 18.5). Solitary thin-walled pulmonary cavities are a frequent complication (Fig. 18.6). Residual nodules are often confused with a neoplasm, particularly when individuals with unrecognized primary coccidioidomycosis present with a residual nodule on routine chest radiograph long after the time of infection (Fig. 18.7) [38]. Modest amounts of pleural fibrosis, a residual of the primary infection, may also be seen.



Fig. 18.6 Right middle lobe giant cavity with air-fluid level and associated infiltrate

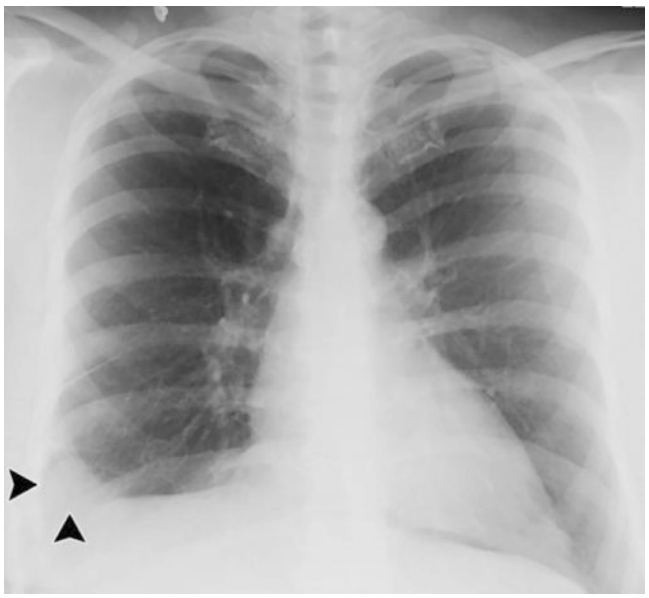


Fig. 18.7 Right lower lobe nodular coccidioidomycosis

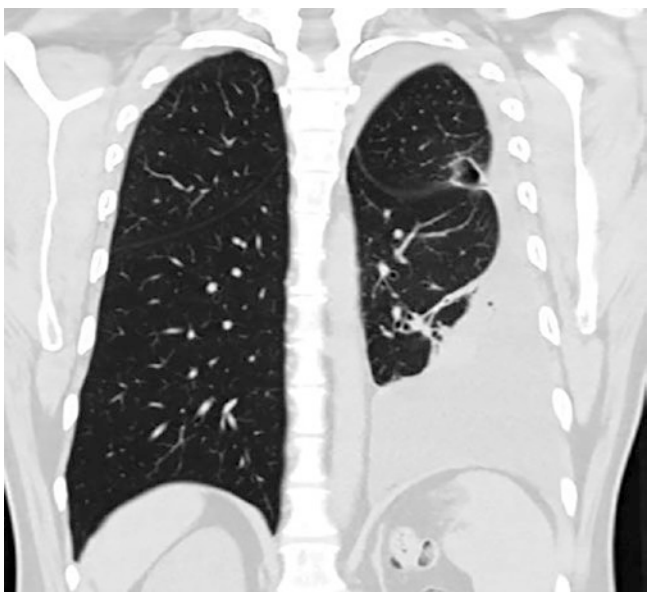


Fig. 18.8 Left upper lobe cavity with associated empyema

Lung cavities are present in about 8% of adults but are less common in children. Rarely, pulmonary coccidioidomycosis may present as a bronchial mass found on bronchoscopy. Chronic fibrocavitary pneumonia can occur, commonly in association with diabetes or preexisting pulmonary fibrosis [39].

Unrecognized or recognized pleural-based cavities may rupture into the pleural space, resulting in a hydropneumothorax often with bronchopleural fistula. This is defined as coccidioidal empyema (Fig. 18.8). The culture is commonly positive. The pathogenesis and pleural fluid are distinct in empyema and should not be confused with simple pleural

effusions which may occur as part of the primary disease process (Fig. 18.9).

Miliary disease with coccidioidomycosis is seen with significant frequency in the endemic area, where miliary coccidioidomycosis maybe ten times as frequent as miliary tuberculosis (Fig. 18.10). Overwhelming miliary and/or alveolar coccidioidomycosis can result in respiratory failure. A severe pulmonary case is defined when PaO_2 is ≤70 mmHg or the A-a O_2 gradient is ≥35 or the PaO_2-FiO_2 ratio is ≤330. These patients are hospitalized and need aggressive and prompt treatment including steroids similar to the *Pneumocystis* infection [40]. HIV-infected patients often have a fulminant presentation, particularly when CD4+ T lymphocyte counts are less than 100 cells/μL. Probably, the most common cause of death in the endemic area from coccidioidomycosis is respiratory failure.



Fig. 18.9 Primary left pleural coccidioidomycosis with effusion

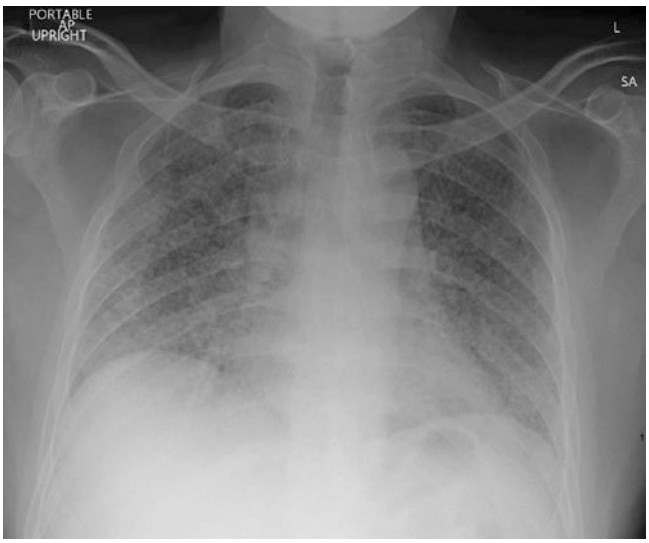


Fig. 18.10 Miliary coccidioidomycosis

Disseminated Disease

The rate of dissemination of coccidioidomycosis is highly dependent on the infected host. The majority of disseminated disease occurs in individuals with antecedent symptomatic pulmonary infection. However, in a minority of patients, disseminated disease presents without obvious primary pulmonary infection. Risk factors for dissemination include the extremes of age, male sex, African American or Filipino ancestry, tobacco smoking, and low socioeconomic status [41, 42]. Persons with immunodeficiency, including those seen with advanced HIV infection, high-dose corticosteroid therapy, lymphoma, solid organ, or bone marrow transplantation, are at greater risk of dissemination [43–48]. Pregnancy also predisposes individuals to disseminated disease [49, 50]. There are two basic mechanisms: lymphatic and hematogenous. The former is exemplified by pericarditis and supraclavicular lymphadenitis, the latter by meningitis. Clearly, the hematogenous disease is more common. There is reason to believe that at least some "uncomplicated pulmonary infection" is in fact asymptomatically disseminated [13]. The majority of dissemination is to the skin, subcutaneous tissue, bone, and joints. These sites taken together represent more than 50% of disseminated disease. Virtually every part of the human body has been described with coccidioidal infection. The sites discussed will be limited to the more commonly encountered:

Osteomyelitis

Infection has been described in almost all bones. The axial skeleton is of particular importance. The lumbar spine is the most common. The pelvic bones such as the tibia and femur are commonly involved. It is not unusual to see osteomyelitis and joint involvement in the same patient. Single bone or joint infections are most common but multiple sites may be involved, particularly in African American males. A total body technetium pyrophosphate bone scan should be routinely obtained on all cases of osteomyelitis and all other patients where any dissemination is identified. CT and more commonly MRI with gadolinium are used to evaluate the extent and the severity of disease [51]. Surgical intervention is commonly needed to debulk extensive disease or to preserve or restore structure or function.

Synovitis

Synovitis may occur as a separate entity but not uncommonly in association with osteomyelitis. In this latter circumstance, the osteomyelitis takes precedence. The knee is



Fig. 18.11 Coccidioidal synovitis of the knee with osteomyelitis of the femur and tibia

most commonly affected (Fig. 18.11); other sites are the wrist and ankle. Treatment is largely medical but for severe or refractory cases surgical interventions are required [52–54].

Lymphadenitis

This most commonly occurs as lymphatic spread from pneumonic disease to hilar lymph nodes and can be found on chest X-ray or chest CT. Subsequent spread to mediastinal lymph nodes may occur but seldom presents clinically. Subsequent spread to subclavicular, supraclavicular, and suprasternal lymph nodes may occur. Hematogenous spread to lymph nodes occasionally occurs. In the setting of known coccidioidal disease one may make a clinical diagnosis. More commonly and especially if a presenting problem, a lymph node biopsy is required. A surgical biopsy is much preferred over needle biopsy for sensitivity and specificity of diagnosis.

Soft Tissue Infections

This is a heterogeneous group of infections. Perhaps the most severe is an intramuscular infection often associated with osteomyelitis. Prevertebral abscesses are associated with vertebral osteomyelitis/discitis. These may require drainage by an interventional radiologist or surgery. Psoas and gluteal abscesses are also noted. The latter is often associated with sacroiliitis.

Another relatively common presentation is subcutaneous abscess. This may be small or quite large (up to 10 cm). Commonly, these are culture positive. One is tempted to drain these; however, even if called "abscess," they do not behave similarly to a bacterial abscess. If opened, they will drain for weeks and create an unpleasant management problem. Better are large volume aspirations, which might need to be repeated over time. Medical management is usually with azoles.

Cutaneous Disease

Cutaneous disease is one of the most common and usually the most benign form of dissemination. Depending on the circumstance, the diagnosis can be simple, as in a new skin lesion in someone with recently diagnosed coccidioidomycosis. More difficult are patients presenting primarily with an unidentified skin lesion. The visual appearance is quite variable. Small cutaneous abscesses or verrucous lesions are common and ulcerated lesions may occur (Fig. 18.12). Diagnosis is usually by biopsy. In some circumstances, clinical suspicion and serology may suffice.

Peritonitis

When it occurs in isolation, coccidioidal peritonitis can be a subtle and difficult diagnosis. Presentation is varied but most commonly ascites of uncertain origin [55]. Paracentesis yields fluid consistent with an inflammatory/malignant process. Laparoscopic evaluation shows peritoneal "studding" that is grossly identical to carcinomatosis and tuberculosis peritonitis. Serum cocci serology is almost always positive. Peritoneal fluid titers add nothing, and culture of the fluid is low yield. Histopathology is the most common positive diagnostic.

Meningitis

The most severe disseminated manifestation of coccidioidomycosis associated with devastating complications is meningitis. Both morbidity and mortality are higher than any other disease manifestation and account for 30–50% of disseminated infections.

This is the single most common dissemination site in Caucasian and Latino males. Untreated, meningitis is fatal within a few months, although there are rare reports of survival for 2 or more years [56].

Meningitis usually develops within 6 months of the initial infection [57] either with or without notable primary infection. The most common presentation is headache. Persistent and progressive confusion, focal neurological deficits, and gait disturbances (especially tandem gait) are other presenta-



Fig. 18.12 Cutaneous coccidioidomycosis of nostril and posterior chest wall

tions seen separately or in parallel. A high index of suspicion and early lumbar puncture either with or without antecedent neuroimaging are paramount. The diagnosis can be suspected based on MRI with gadolinium in 50% of cases [58]. CT is of far lower sensitivity. The lumbar puncture is the diagnostic of choice. The lumbar puncture, if possible, should be completed in the traditional lateral recumbent position. An opening pressure of 180–200 mmH_2O is normal. An opening pressure ≥250 mmH_2O requires cerebrospinal fluid (CSF) removal to obtain pressure of <50% of the opening pressure or 200 mmH_2O, whichever is greater. CSF should be analyzed for cells, differential (cytospin), glucose, protein, coccidioidal titers, and fungal culture [59]. A diagnosis is made if there is CSF pleocytosis, usually lymphocytic with decreased glucose, elevated protein, and a positive culture (best but low frequency). A positive CSF complement fixation (CF) titer at a reliable laboratory also is helpful. Eosinophils are not common, but when present, they are highly suggestive of the diagnosis of coccidioidal meningitis [60]. The presence of CSF antigen is diagnostic, but this test is not commonly available [61, 62].

Complications of coccidioidal meningitis are manifold. Included are hydrocephalus, vasculitic infarctions, focal neurological deficit of cranial nerves, arachnoiditis, syrinx, paraplegia, bowel, bladder, and erectile dysfunction [63–66].

Diagnosis

It is essential to obtain a detailed travel history for exposure to an endemic area. Exposure does not need to be over a prolonged period of time; infection has occurred after only passing through an endemic area. The diagnosis of coccidioidomycosis is dependent on a compatible clinical illness with positive laboratory confirmation by culture, histopathology, or serology.

Culture

Suitable material for culture is sputum, tissue aspirates, or biopsy specimens. *Coccidioides* species grow well on most culture media after 5–7 days of incubation in aerobic conditions at 25, 30, or 35 °C. Typically, these fungi produce a white mold, although more pigmented strains have been observed (Fig. 18.13). Laboratory cultures are highly infectious when mature arthroconidia have formed. Typically, it takes about 10–20 days for *Coccidioides* to mature and produce arthroconidia (Fig. 18.14). Because of their size, the arthroconidia are easily dispersed in the air and inhaled; therefore, *Coccidioides* is extremely hazardous when cultured in the laboratory. At a minimum, biosafety level 2 practices and facilities are recommended for handling and processing of clinical specimens. When working with known *Coccidioides*, biosafety level 3 is required [67]. Accidental percutaneous inoculation of the spherule form may result in local granuloma formation. Clinical specimens, prior to culture, however, are not infectious to personnel. The much larger size spherules are considerably less effective as airborne pathogens. *Coccidioides* will grow in most media including 5% sheep blood agar, chocolate agar, Sabouraud Dextrose agar, Mycosel agar, and Brain Heart Infusion agar with or without blood. Growth on 5% sheep blood agar and chocolate agar incubated at 35 °C can be seen in as little as 24 h. Growth on Sabouraud Dextrose agar and Mycosel agar incubated at 25 °C (room temperature) can be seen after 3–4 days. Specimens from known or suspected cases should not be cultured on unsealed plated media. The use of a tubed media is suggested (Fig. 18.13). Presumptive identification may be made based on colony morphology, growth rate, and the production of segmented hyphae. Care must be taken when attempting to identify *Coccidioides* as other mycoses may have similar macroscopic and microscopic morphologies, especially if arthroconidia are not abundant (Fig. 18.14). Laboratories that are not experienced with working with



Fig. 18.13 Growth of *Coccidioides* in tubed fungal medium



Fig. 18.14 Arthroconidia of *Coccidioides immitis*

Coccidioides should refer these suspected isolates to qualified reference laboratories.

Coccidioides species are dimorphic fungi that have the ability to grow vegetatively at 25 °C as molds, and at 37 °C in tissue or in special medium (Converse liquid medium) in 10% CO_2 incubator as spherules. Confirmation traditionally was performed by animal inoculation with identification of endosporulating spherules on histopathology. Exoantigen tests and the production of spherules in Converse liquid medium could also be used. These methods have now been supplanted by molecular testing; a DNA probe is available commercially.

Histopathology

Diagnosis may also be confirmed histopathologically with the demonstration of spherules (20–80 μm) with endosporulation, usually in the setting of granulomatous inflammation.

Serologic Testing

The most common method of diagnosis is serologic testing in individuals who have typical clinical features. Correctly performed serologic tests are both sensitive and specific for the disease. A negative serologic test, however, does not exclude the presence of infection, especially if recently acquired, and should be repeated over the course of 2 months. Many serologic tests for *Coccidioides* are available. The most commonly used currently are enzyme immunoassay (EIA), immunodiffusion (ID), and complement fixation (CF) antibody tests. The EIA allows the detection of IgM antibodies for the determination of recent infection. Although this test has many false positives, it is probably the most sensitive test for early infection. The ID IgM test has somewhat less sensitivity but a better specificity than the EIA test. The EIA IgG test appears to have a significant number of false negatives which limit its utility in the diagnosis of severe and advanced disease. The ID IgG test has a high degree of specificity and fewer false negatives in individuals with significant or disseminated disease. CF tests are both sensitive and specific in the diagnosis of coccidioidal infections. The quantitative CF test is expressed as a titer and has the additional advantage of being not only diagnostic but prognostic. There is an inverse relationship of the IgG antibody titer to prognosis. Individuals with decreased amounts of IgG antibodies tend to have modest primary infections. Individuals with high amounts of IgG antibodies are more likely to have extensive primary infection or disseminated disease. It must

be understood, however, that this holds true for a population of patients. In a given individual, the extent and severity of disease cannot be accurately predicted solely on the measurement of IgG antibodies.

The majority of patients with disseminated disease will eventually have a titer ≥1:16. At present, EIA results should be confirmed with the more established ID or CF tests [68, 69]. ID and CF IgG tests will have a false-negative rate of approximately 1% in disseminated disease. Most individuals with disseminated disease who have falsely negative *Coccidioides* serology are HIV infected. The CF titer in individuals with meningitis is higher than in individuals with primary disease but lower than those with other forms of disseminated disease [70].

It is worth mentioning that cross-reactivities of serology between blastomycosis, histoplasmosis and coccidioidomycosis have been observed. The antigen used for serology testing should be monitored frequently for specificity and reliability.



Fig. 18.15 Coccidioidal meningitis with severe arachnoiditis of spine

Skin Testing

Skin testing detects the delayed-type hypersensitivity reaction to *Coccidioides* [71]. Skin test positivity with Spherusol indicates past or present infection and commonly remains positive for life in most people. Skin test conversion (negative to ≥5 mm bidirectional induration) indicates infection in the intervening time. False-negative skin test reactions can occur in immunocompromised individuals and suggest genetic or acquired failure of cellular immune response. Skin testing, therefore, is limited as a screening procedure for recent infection but may be useful in epidemiologic studies.

Other Laboratory Findings

Antigen detection and PCR are newer useful diagnostics but not widely available [72, 73]. Nonspecific laboratory tests such as the complete blood count and chemistry tests occasionally offer clues to coccidioidal infection. In an endemic area, an individual presenting with what appears to be community-acquired pneumonia who has an absolute eosinophilia (greater than 350 cells/μL) is more likely to have primary coccidioidal infection. It has also been noted that individuals who present with coccidioidomycosis and elevated alkaline phosphatase may also have liver involvement.

Radiologic Imaging

Radiographic imaging may be of great help in defining the extent and severity of both pleural pulmonary and disseminated disease. Chest radiograph is mandatory in the evaluation of primary disease. Computed tomography of the chest may be helpful in selected cases, especially with cavitary disease. Bone scan is the most frequently ordered test for osteomyelitis and plain radiograph is frequently utilized. Contrast-enhanced magnetic resonance imaging (MRI) of bone and joints will help define problematic cases. MRI of the brain and spinal cord with contrast may reveal meningeal enhancement, hydrocephalus, or vasculitic infarction (Fig. 18.15). Even though approximately 50% of patients with meningitis will have normal neuroimaging [74].

Treatment

General Approach

The treatment of coccidioidomycosis is both complex and, at times, controversial. A treatment guideline published by the Infectious Disease Society of America (IDSA) in 2016 gives a consensus framework [13]. It is clear that many individuals infected with *Coccidioides* species recover without specific therapy. This is especially true when one notes that 60% of infections are asymptomatic and, by definition, go undiagnosed and untreated. Of symptomatic individuals, large numbers also go undiagnosed and most recover uneventfully. There is some controversy as to whether those individuals who are diagnosed with symptomatic primary disease need to be treated. Some experts believe that most of these persons will recover without treatment and therefore treatment ought

not to be offered. Other authorities note that a small but significant percentage of individuals with primary disease will have either pulmonary or disseminated complications and it is difficult to predict with certainty who these individuals might be; thus, the majority should be treated. Unfortunately, there is no evidence-based study of primary disease that has examined whether improvement in the primary symptom complex, rate of pulmonary complications, or frequency of dissemination is affected by treating or not treating. What is generally agreed upon is that individuals with significant risk factors for dissemination or poor outcome should receive treatment for primary disease. Thus, advanced age; male sex; vulnerable race; presence of associated comorbid diseases such as diabetes, liver disease, and underlying lung disease; and elevated CF titers should favor treatment of primary disease. HIV infection and other conditions associated with immunodeficiency, such as lymphoma, cancer chemotherapy, and organ transplantation, mandate early and aggressive therapy of primary disease. Pregnancy represents a special circumstance. The development of primary disease during the middle trimester through the early postpartum period puts an otherwise low-risk group of individuals at much higher risk. Coccidioidomycosis has been the leading cause of maternal mortality in Kern County, California for more than 50 years [75]. Despite the risk, many women will have favorable outcomes without drug treatment, and abortions or early delivery in subjects with active infection is rare [76].

Primary Coccidioidomycosis

Most individuals with uncomplicated acute coccidioidal pneumonia, if treated, are initiated on oral azole therapy (Tables 18.1 and 18.2). Fluconazole 400 mg daily has been prescribed most often. Some institutions are initiating higher doses as primary therapy (600–800 mg). This has especially been true with the recent availability of generic fluconazole. Alternatively, itraconazole 200 mg twice daily is also commonly prescribed [77]. These two drugs have become the mainstay in the treatment of primary disease. It should be noted that none of the azoles are approved for coccidioidomycosis by the Food and Drug Administration (FDA). Duration of recommended treatment ranges from 3 to 6 months and signal to discontinue treatment is based on the resolution of symptoms, improved chest X-ray and improvement of the CF titer. Longer courses may be prescribed in diabetics, persons of African American or Filipino descent, sero-persistent (lack of serological response to therapy), and immunocompromised patients.

In individuals presenting with severe, diffuse pulmonary coccidioidomycosis or miliary disease with respiratory failure, azoles are not the initial drugs of choice. In this circumstance, liposomal amphotericin B is preferred. In addition, it is also recommended to initiate glucocorticoids on a schedule identical to pneumocystis when hypoxemia, as described, is present [40, 78].

Table 18.1 Clinical manifestations of coccidioidomycosis and treatment

Clinical presentation	Antifungal therapy	Duration of therapy
Pulmonary without significant respiratory distress	Azoles	3 months–1 year
Pulmonary with respiratory failure	Liposomal AmB (first)	Daily for 14 days, if not improved, three times a week for extra 4–6 weeks
	Azoles to follow	1 year or occasionally longer
Cutaneous	Azoles	≥3 years
Subcutaneous lesions and soft tissue abscesses	Azoles	≥3 years
Synovitis (without adjacent osteomyelitis)	Azoles	≥3 years
Axial skeleton long bones, other critical bones	Liposomal AmB (first)	Daily for 14 days loading then three times a week for 12 weeks or occasionally longer
	Azoles	≥3 years
Osteomyelitis (non-critical bones)	Azoles	≥3 years
Peritonitis	Azoles	≥3 years
	Liposomal AmB (rarely needed)	
Lymphadenopathy	Azoles	≥3 years
	Liposomal AmB (rarely needed)	
Other sites	Azoles	≥3 years
	Liposomal AmB (rarely needed)	
Meningitis	Azoles	Lifelong therapy
	Intrathecal AmB (for treatment failure)	
Intracranial hypertension/ hydrocephalus	Repeated lumbar puncture/ ventricular shunting	

AmB Amphotericin B

Table 18.2 Antifungal drugs used in the treatment of coccidioidomycosis

Class	Drug	CNS penetration	Dose	Half-life	Toxicity
Polyene	Intrathecal AmBd	100%	Initial dose: 0.1 mg, 3×/week then titrate (CNS)	Terminal half-life 127–152 h after multiple doses	++++
	L-AmB	Nearly undetectable	Intravenous: 5 mg/kg/day		++++
Azole[a]	Fluconazole	50–100%	Intravenous/tab: 800 mg daily (non-CNS), 800–1200 mg/day (CNS)	~30 h	+++
	Itraconazole	<1%	Cap/solution: 200 mg two times per day	~30 h	+++
	SUBA-itraconazole	ND	Cap: 130 mg two times per day	~30 h	+++
	Voriconazole	22–100%	Tab: 4 mg/kg two times per day	~6 h	++++
	Posaconazole	<1%	DR-Tab: 400 mg/day suspension (only if unable to take tab): 400 mg two times per day	~25 h	+++
	Isavuconazonium	ND	Cap: 372 mg/day	80–120 h	+

AmBd amphotericin B deoxycholate, *Cap* capsule, *CNS* central nervous system, *CSF* cerebrospinal fluid, *DR* delayed release, *L-AmB* liposomal amphotericin B, *ND* no data, *Tab* tablet

[a] All the aforementioned azoles have been used successfully in coccidioidal meningitis, and the presence or lack of CSF penetration does not appear to be substantiative

It appears that there is a more rapid response to amphotericin than to the azole drugs. Several weeks of therapy with amphotericin B are often required for improvement, after which oral azole therapy is employed.

Pulmonary Nodule

Antifungal therapy or resection is unnecessary for stable pulmonary nodules. If enlargement of the nodule occurs, reevaluation with sputum cultures and measurement of serum coccidioidal antibodies should be done to determine if the infection is active and warrants treatment.

Pulmonary Cavity

Asymptomatic, cavitary disease caused by *Coccidioides* often does not require intervention. Symptomatic solitary cavity coccidioidomycosis may benefit from azole therapy. A course of varying duration, until symptoms are resolved, is appropriate. Resolution of fever, cough, and hemoptysis, improvement in appetite, and decrease in CF titers, if any, may be used to guide therapy. Approximately, one-half of cavities smaller than 3 cm will resolve in 6–12 months. If the cavity persists but the symptoms abate, a trial of withdrawal of azole therapy can be undertaken. If symptoms recrudesce, reinstitution of therapy for a longer period of time is suggested. Indications for resection of the cavity include recurrent bacterial superinfections and recurrent or life-threatening hemoptysis. Rupture of the cavity into the pleural space, with the development of empyema, often requires surgical as well as medical therapy.

Chronic Progressive Fibrocavitary Pneumonia

Fibrocavitary pneumonia of coccidioidomycosis in the pre-azole era often resulted in death from respiratory failure and pulmonary hypertension. Since the advent of azoles, death is less common. Fluconazole or itraconazole is the most common therapy. Amphotericin has little role in the management of this subacute illness.

Coccidioidomycosis in Pregnancy

Because of demonstrated concerns regarding teratogenicity with high-dose azole compounds, amphotericin is the drug of choice in pregnancy for those requiring therapy. Pregnant females with distant infection or mild disease of limited extent and with low CF titers are usually followed very closely without initiation of any therapy. Those with a greater extent of disease or with high CF titers in the first trimester are immediately placed on amphotericin. In the second or third trimester, low-dose azoles can be used as an alternative [79]. This should be done in concert with an obstetrician who deals with high-risk pregnancies.

Disseminated Disease (Extrapulmonary, Nonmeningeal)

Therapy of disseminated disease requires more expertise and judgment than does uncomplicated pulmonary disease (Tables 18.1 and 18.2). It has been noted that minimal cutaneous disseminated disease may remit without specific antifungal therapy. At this time, however, no expert recom-

mends that treatment of disseminated disease not include antifungal therapy. Disseminated disease of the skin, soft tissue, joints, and bones, which is limited and not life or limb-threatening is usually treated with azole therapy. Some experts prefer itraconazole for disseminated disease, particularly bony dissemination, because of a trend toward superior resolution at 1 year with itraconazole 200 mg every 12 h when compared with fluconazole 400 mg daily [77]. However, both drugs are used, albeit fluconazole is now commonly used at doses greater than 400 mg per day. Duration of therapy substantially longer than a year is frequently recommended. Some experts are recommending therapy for 3 years for significant disseminated disease such as synovitis, lymphadenitis, peritonitis, miliary, and soft tissue infections. This should be adjunct with clinical and radiographical stability and a CF titer of <1:2 for at least 6 months before discontinuation.

Severe multifocal osseous disease that affects the axial skeleton or a major long bone may be treated with lipid preparation of amphotericin B for 12 weeks and then transitioned to azole for a total of 3 years. The lesser disease may be treated with an azole primarily. If the disease is amenable to surgical debridement, this may be a valuable adjunct. After individuals undergo treatment with amphotericin, secondary therapy with azoles is undertaken for protracted periods. Doses higher than 400 mg of fluconazole or itraconazole are frequently administered.

Disseminated Disease (Central Nervous System)

Azoles are the primary medical therapy. The duration of treatment is currently construed to be lifelong. The reason for this is the lack of available proof of cure and the significant morbidity and mortality of relapse. Fluconazole is the preferred drug, given at doses of 800–1200 mg daily as a single dose [80, 81]. Itraconazole is not as commonly used as fluconazole but has had reported success. In patients failing high-dose fluconazole therapy, voriconazole has been used and has significant theoretical appeal as rescue therapy at a dose of 4 mg/kg every 12 h [82, 83]. Despite the efficacy due to the correlation between prolonged use of voriconazole and skin cancers and liver toxicity associated experts are moving away from this azole [84]. Isavuconazonium, the latest azole that has become available has been utilized in all forms of infection including coccidioidal meningitis, and shows promising efficacy [85]. This newer azole has favorable tolerability and was effective in cases with failure to other azoles. Posaconazole has been also used in refractory cases [86]. Intrathecal amphotericin B was the primary therapy of central nervous system (CNS) coccidioidomycosis until supplanted by azole therapy. This therapy can and has been given by direct cisternal injection, via ventricular or cisternal reservoir, or via intrathecal lumbar injection or reservoir [63]. It is now used primarily in those failing other initial or secondary therapies.

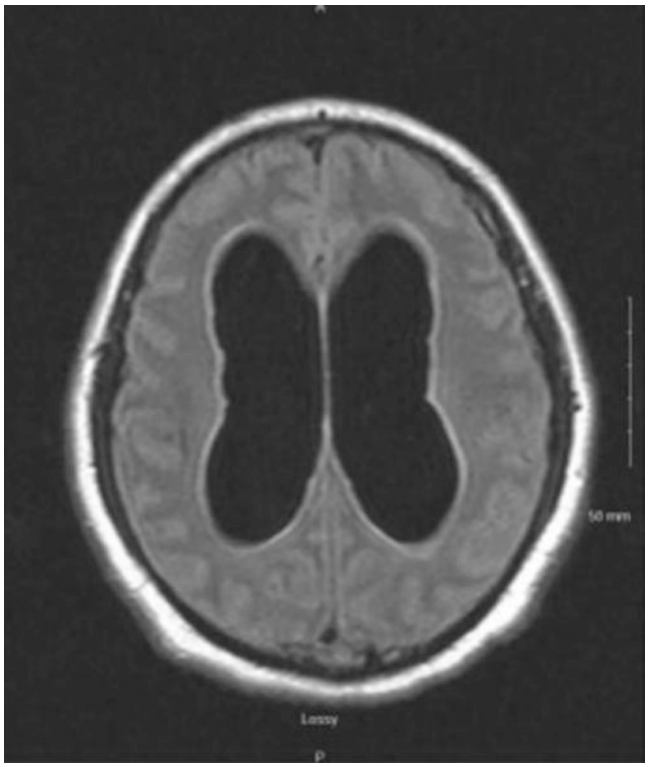


Fig. 18.16 Hydrocephalus in coccidioidal meningitis

Coccidioidal meningitis is often complicated by increased intracranial pressure (ICP) and hydrocephalus (Fig. 18.16). Severe increased ICP is defined as ≥250 mmH_2O. This complication is a known distinct problem that requires a separate management [13]. In persistent or severe cases, placement of a ventriculoperitoneal shunt is required [59].

Monitoring Therapy

Patients with primary coccidioidomycosis should be monitored at 1–3-month intervals, both with laboratory and radiologic studies. If there is suspicion for dissemination by history or on examination, biopsy and culture of suspected sites of infection should be performed. Lumbar puncture should be performed in patients who develop headaches after the initial primary infection or who develop other neurologic signs at any time. Bone scan is indicated to evaluate bony or joint involvement.

Prevention

Developing a vaccine has been a goal for many years. A formalin-killed, whole-cell spherule vaccine was used in a human field trial but was not found to be protective [87]. New research on a subcellular vaccine has been initiated but has as yet come to fruition.

Acknowledgments The authors wish to thank D. Mejia and J. Mahinan-Picar for their help in the preparation of this chapter.

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Emergomycosis

19

Ilan S. Schwartz

Introduction

Emergomycosis is an invasive fungal disease caused by dimorphic fungi from the recently described Onyenalean genus, *Emergomyces*. The disease has been reported from four continents (Europe, Asia, Africa, and North America), although by far the most cases have been reported from South Africa. Nearly all affected patients have been profoundly immunocompromised, typically with a deficiency of cell-mediated immunity such as advanced HIV infection or immunosuppression for organ transplantation. The disease is typically disseminated at diagnosis, involving skin, lungs, and often bloodstream, although other sites may also be affected. In patients with HIV, an unmasking immune reconstitution inflammatory syndrome can occur in the form of new or worsening skin lesions after initiation of antiretroviral therapy. The histopathological picture is identical to histoplasmosis. The diagnosis can be secured by isolating the fungus in culture of biopsied tissue, blood, or other clinical specimens using standard fungal media, or sequencing of broad-range fungal targets (like internal transcribed spacer) from infected tissue. Treatment of severe disease involves induction with amphotericin B followed by itraconazole or newer azole for at least 1 year pending immune reconstitution.

Etiological Agent

Emergomycosis is caused by recently described thermally dimorphic fungi in the genus *Emergomyces* [1]. The type species, *Emergomyces pasteurianus*, was first isolated in 1994 from a patient with a disseminated mycosis in the setting of advanced HIV [2]. The isolate had genetic and—in the mold phase, at least—morphological similarities to *Emmonsia*, and was described as *Emmonsia pasteurianus* [2, 3]. However, unlike *Ea. crescens* and *Ea. parvus*, which cause a granulomatous lung disease in mammals called adiaspiromycosis, named for the hallmark, large, non-replicating "adiaspores" found in lung [4], the tissue phase of *Ea. pasteurianus* was characterized by small, narrow-budding, yeast-like cells similar to *Histoplasma capsulatum*. *Ea. pasteurianus* remained a curiosity until 2013, when Kenyon et al. reported the occurrence of 13 cases of a disseminated AIDS-related mycosis in South Africa caused by a closely related dimorphic fungus [5]. That discovery, and the sporadic description of related cases from around the world [6–10], prompted a taxonomic re-examination of the Onygenales, a taxonomic order that includes a number of clinically important dimorphic fungi, including *Emmonsia, Blastomyces*, *Histoplasma*, and *Paracoccidioides* [11, 12]. This ultimately led to the description of a new Onygenalean genus, *Emergomyces*, to accommodate these previously unknown/unappreciated, closely related, thermally dimorphic, opportunistic fungal pathogens [12]. These now include *Es. pasteurianus, Es. africanus, Es. canadensis, Es. europaeus*, and *Es. orientalis*.

Epidemiology

Emergomycosis is an opportunistic infection of immunocompromised hosts that appear to have a near-global distribution (Fig. 19.1). Sporadic cases of emergomycosis have been reported from North America [13], Europe [2, 6, 14–16], Asia [7–9, 17–20], and Africa [5, 21–24], but the vast majority of cases have been reported from South Africa, where HIV-associated emergomycosis is now recognized to be the most common dimorphic fungal infection, eclipsing histoplasmosis, blastomycosis, and sporotrichosis [25]. Between 2008 and 2015, 52 cases were identified by laboratory-based passive surveillance. All but one were in

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_19

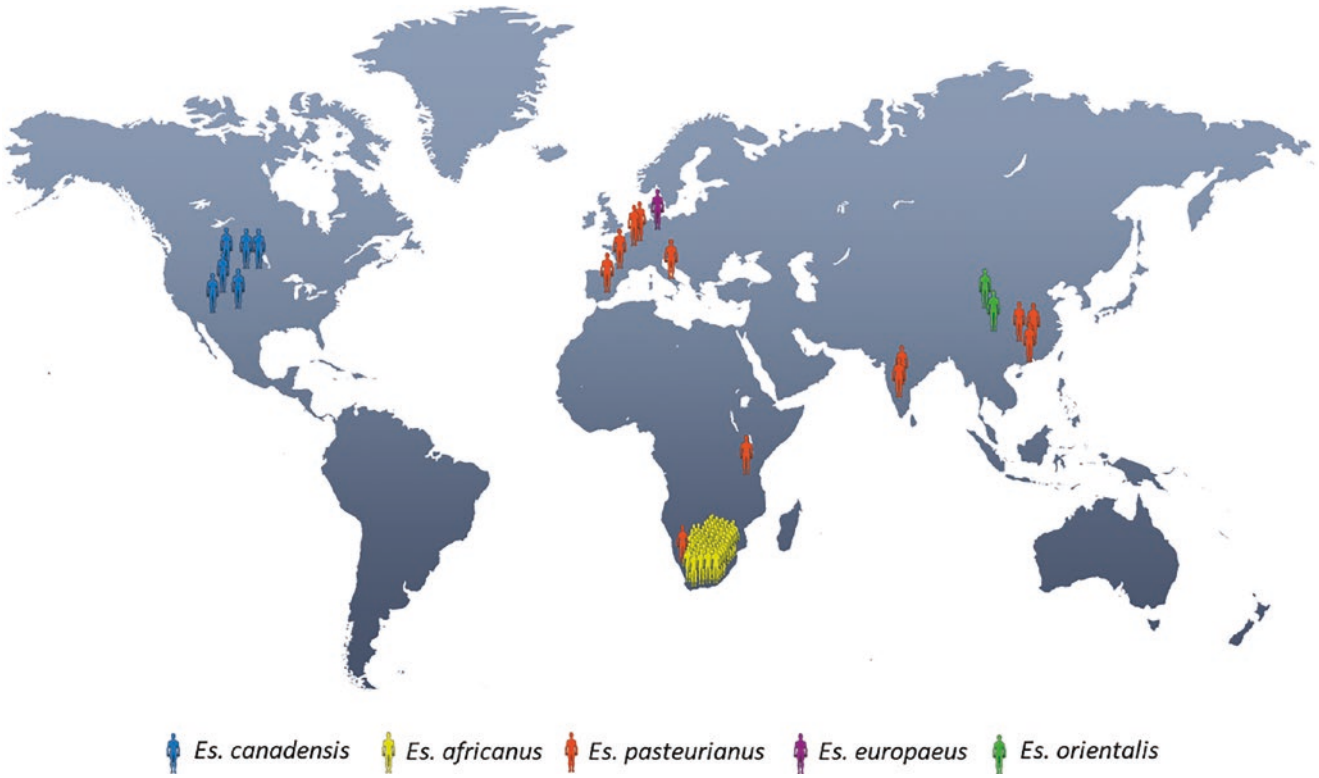


Fig. 19.1 Geographic distribution of reported cases of emergomycosis as of October 2021. The number of cases depicted in South Africa is to illustrate that it is much more common than elsewhere

patients with advanced HIV disease, among whom the median CD4 was 16 cells/mm^3, and the other was an organ transplant recipient [22]. There have since been many more cases reported [21, 26, 27], although it likely remains underdiagnosed.

There are at least five phylogenetic species of *Emergomyces*. *Es. pasteurianus*, the type species, has been reported from Europe (Italy [3], Spain [14], France [15], and The Netherlands [16]), Asia (Mainland China [8, 9], Hong Kong [17], and India [18]), and Africa (South Africa [12] and Uganda [23]). *Es. africanus* has been reported only from Africa, in South Africa and the Kingdom of Lesotho. *Es. canadensis* has been reported diagnosed in immunocompromised patients in western provinces and states, including Alberta [28] and Saskatchewan [13] in Canada, and Arizona, Utah, Colorado, and New Mexico, in the US [13]. *Es. europaeus* was reported from a single case in Germany [6] and *Es. orientalis* from two cases in China [7, 20]. Recently, a proposal was made to add two non-pathogenic taxa to the genus [29], but this has not been accepted by the community.

Little is known about the ecology of *Emergomyces* species. *Es. africanus* was detected by PCR in South Africa in soils from a range of habitats [30] and in air samples [31]. It has not been successfully isolated from environmental samples. Natural infections in animals have not been reported, and a study of 1402 animals from 26 species of terrestrial mammals in South Africa failed to identify *Emergomyces* DNA [32].

Pathogenesis and Immunology

The primary route of inoculation is thought to be inhalation, followed by extrapulmonary dissemination. *Emergomyces* yeasts are engulfed by macrophages, and spread hematogenously through the bloodstream to secondary sites of disease. It is unconfirmed whether cases of disease represent primary disease or reactivation of latent infections.

Virulence factors of *Emergomyces* have only just begun to be elucidated. *Es. africanus* produces urease, a virulence factor that is important in pathogenicity in *Cryptococcus neoformans* [33]. However, it is unclear whether this enables virulence in vivo.

Based on the high frequency of advanced HIV among patients with emergomycosis, cell-mediated immunity is thought to be critical for host defense. Approximately half of all cases of emergomycosis in South Africa were diagnosed

after clinical worsening—typically the appearance of widespread skin lesions—upon initiation of antiretroviral therapy (ART), suggesting an unmasking immune reconstitution inflammatory syndrome (IRIS) [22]. Paradoxical IRIS—worsening of previously controlled disease with immune reconstitution—has been reported, but infrequently [34].

Clinical Manifestations

Patients with emergomycosis typically have widespread, disseminated disease at the time of diagnosis.

Cutaneous lesions are the most common clinical manifestation at diagnosis, occurring in 95% of South African patients [22]. These can take the form of papules, plaques, or ulcers (Fig. 19.2).

Respiratory disease occurs in the majority of patients; in a case series of 52 patients with emergomycosis in South Africa, 86% had abnormal chest imaging [22]. Abnormalities can manifest as diffuse infiltrates, consolidation, or mediastinal lymphadenopathy (Fig. 19.3). Isolated lung disease is uncommon, but presumably is underdiagnosed. In areas where tuberculosis is highly endemic, the non-specific radiographic findings, along with constitutional symptoms like weight loss, often leads to empiric TB therapy [22].

Less commonly, disease can also involve bone marrow, lymph nodes [22], liver [35], cervix [13]. Osteoarticular disease is uncommon. Central nervous system disease appears uncommon, with no confirmed cases reported to date.



Fig. 19.2 Examples of skin lesions of emergomycosis in persons with advanced HIV disease, including: (**a**) diffuse papules, some with umbilication; and (**b**) coalescing plaques

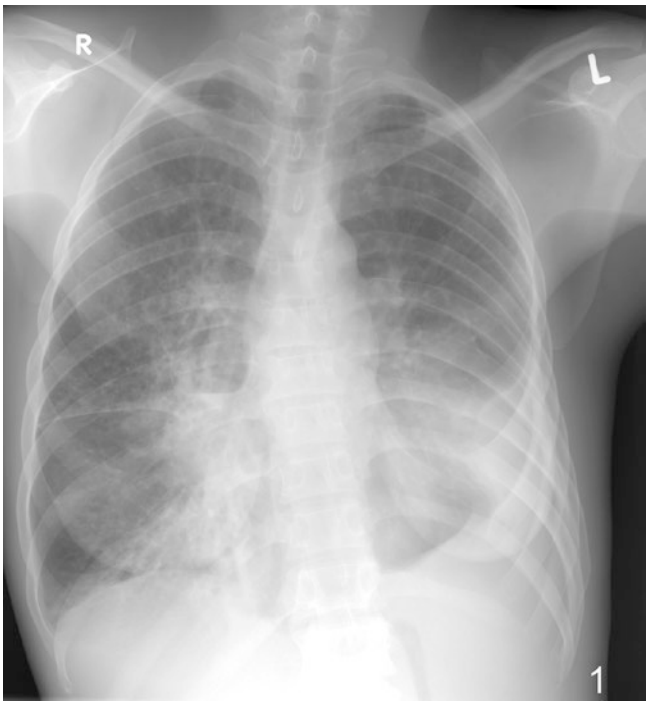


Fig. 19.3 Chest X-ray of a patient with emergomycosis and advanced HIV disease. Diffuse, nodular infiltrates are seen in this example, but this can also manifest as consolidation or lymphadenopathy

Diagnosis

In tissue, the histopathological appearance of *Emergomyces* spp. is of small, round yeast-like cells measuring 2–5 μm in diameter, with narrow based budding [21, 36] (Fig. 19.4). *Emergomyces* cannot be distinguished from *H. capsulatum* on the basis of histopathology [21]. In the absence of diagnostic cultures, the diagnosis can be made with sequencing of the internal transcribed spacer (ITS) or D1/D2 region of rRNA from fresh or formalin-fixed paraffin-embedded tissue [23].

The diagnosis of emergomycosis is most commonly made on the basis of isolation of the fungus in clinical specimens or tissue, or sequencing of infected tissue. *Emergomyces* spp. are not known to colonize humans or animals without causing disease, and so any isolation should be considered diagnostic of invasive disease. The specimens with highest yield for fungal culture are tissue (e.g., skin biopsy), blood, and bone marrow—especially with use of lysis-centrifugation (aka fungal or mycobacterial) blood culture bottles [21, 22].

Emergomyces spp. can be cultured on standard fungal media, such as Sabauraud's dextrose agar, incubated at 24–30 °C. Growth usually happens after 5–14 days [5, 11], but late growth can occur and plates should be kept for at least 4 weeks. In the mold phase, *Emergomyces* appear as yellowish-white colonies, initially glabrous and then becoming powdery, before becoming slightly raised and furrowed [11, 12] (Fig. 19.5). Microscopically, the mold phase is characterized by short, slender conidiophores that emerge at right angles from hyphae, with small, round conidia appearing in florets of 2–4 at the end of primary or secondary conidiophores [11] (Fig. 19.5). *Es. canadensis* can lead to low positive results (<400,000 relative light units) on a commercial DNA probe for *Blastomyces dermatitidis* (Accuprobe, Hologic Inc., San Diego, CA).

There are no recommended serological tests or biomarkers that have been demonstrated to be helpful for diagnosing emergomycosis. At least some species of *Emergomyces* will cross-react with testing for *H. capsulatum* antigen [13, 27], although the sensitivity of the test is insufficient to recommend it to rule out the diagnosis of emergomycosis [37].

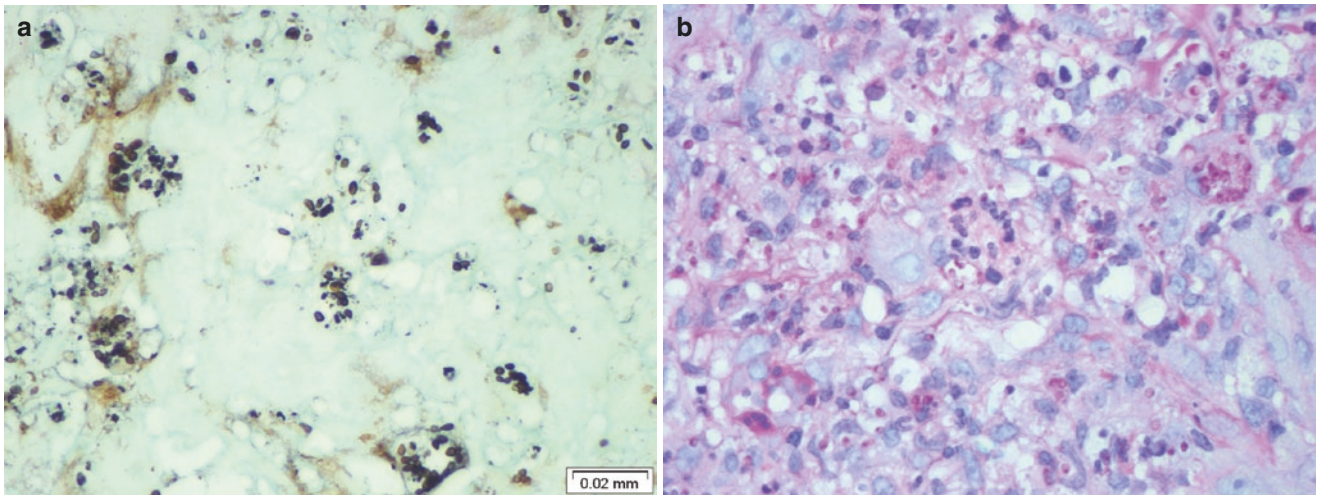


Fig. 19.4 Histopathology of skin lesions of South African patients with emergomycosis and advanced HIV. (**a**) Grocott's methenamine silver (40×), and (**b**) Periodic acid Schiff (40×) stains showing round to oval yeast-like cells of 2–5 μm in diameter with narrow based budding. The histopathological appearance is indistinguishable from histoplasmosis

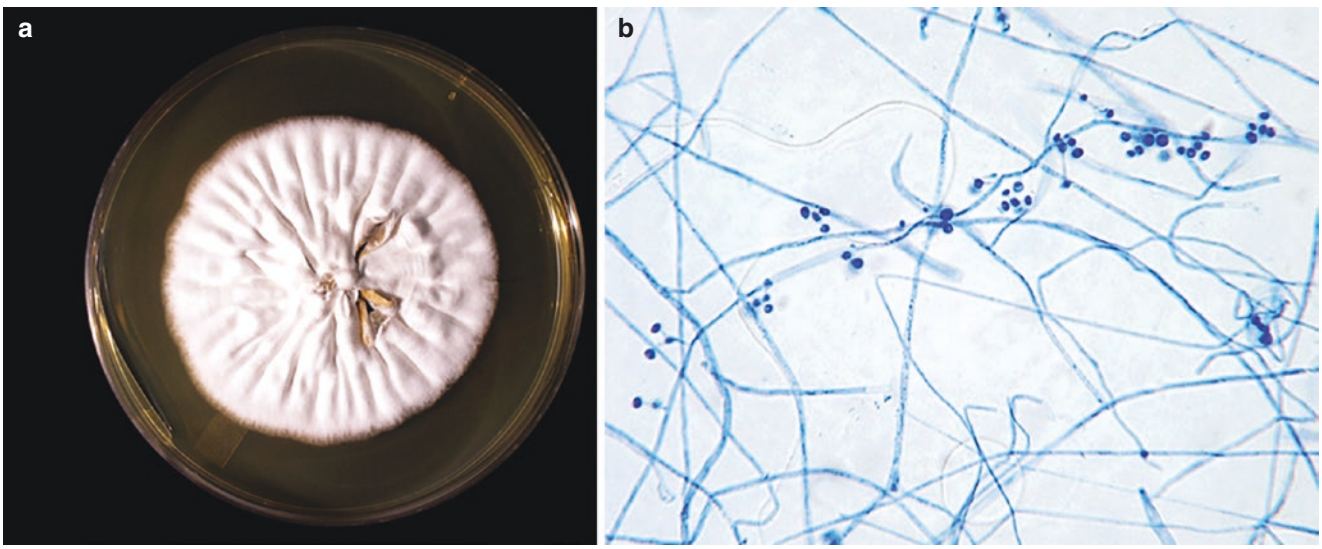


Fig. 19.5 Mold phase of *Emergomyces pasteurianus*. (**a**) Macroscopic appearance at 4 weeks on Sabouraud's dextrose incubated at 30 °C. (**b**) Microscopic appearance, showing small, round conidia in florets at the end of secondary conidiaphores that abut hyphae at right angles. (Courtesy of Yuri Amatnieks)

Treatment

Emergomycosis is treatable, but outcomes are dependent on the host immune status, stage of disease at diagnosis, and therapy. Approximately half of cases of HIV-associated emergomycosis in South Africa were fatal [22], likely reflecting the profound degree of immunodeficiency and advanced stage of infection. There are no randomized clinical trials to guide management of emergomycosis, and treatment recommendations are largely extrapolated from firmer evidence from other endemic mycoses [37]. In immunocompromised patients with disseminated disease, induction antifungal therapy with amphotericin B is recommended for 10–14 days, preferably with a lipid formulation at 3–5 mg per kg per day; amphotericin B deoxycholate at 1 mg per kg per day is a suitable alternative where lipid formulations are unavailable [37]. Thereafter, long term therapy with a triazole is required [37]. Itraconazole is preferred due because it is supported by

the most clinical experience, although in vitro susceptibility testing also supports use of newer azoles [25, 38]. Fluconazole is not recommended because of high mean inhibitory concentrations in vitro [25]. Treatment should continue for at least a year pending immune reconstitution (ideally CD4 above 200 cells/mm^3). Where immune recovery does not occur, longer term suppressive therapy can be considered.

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Histoplasmosis

20

Wassim Abdallah and Chadi Hage

Introduction

Histoplasma capsulatum is a dimorphic fungus primarily found in the Americas, Africa, and Asia. Among the endemic mycoses, histoplasmosis is the leading cause of hospitalization and death in the USA [1]. Darling first described the organism in 1906, believing it to be *a protozoan infection,* until pathologist Henrique da Rocha-Lima who recognized it as a fungus in 1912 [2]. First thought to cause a progressive and fatal disseminated disease, histoplasmosis was subsequently found to be very common and usually asymptomatic or clinically self-limited infection.

Etiologic Agent

Histoplasma is a dimorphic fungus, defined by its ability to grow as a mold in the environment and as a yeast at 37 °C. Clinical specimens viewed via potassium hydroxide (KOH) preparation, calcofluor white, Giemsa, or hematoxylin and eosin (H&E) stains may demonstrate the 2–4 μm budding yeast cells (Fig. 20.1). *Histoplasma capsulatum* var. *capsulatum* causes the vast majority of clinical disease, while its closely related variant *Histoplasma capsulatum* var. *duboisii* is the etiologic agent of African histoplasmosis. There is also the var. *farciminosum* known to be a horse pathogen. More recently, using DNA sequencing, eight clades of *Histoplasma capsulatum* were identified (mainly distributed based on geographic predominance/location of discovery of clade), and when these clades were used to link them to the three original groups, scientists found them to be intermixed, making the original nomenclature obsolete. New information on cryptic speciation may lead to further refinements of *H. capsulatum* classifications [2].

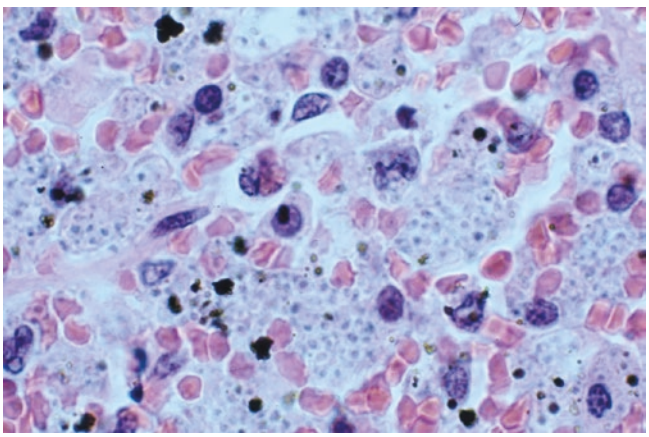


Fig. 20.1 Hematoxylin and eosin stain of yeast in macrophages

Epidemiology

Histoplasmosis is the most prevalent endemic fungal infection in the USA. It is estimated that 50 million people have latent infection, and that 500,000 new infections occur annually [2]. Histoplasmosis is most commonly reported to occur in and around the Mississippi and Ohio River valleys of the USA, in South and Central America [3, 4]. Increased availability of diagnostic modalities and immigration have caused a "re-mapping" of the *Histoplasma* distribution, rendering areas such as South East Asia, Africa, and some parts of Europe more endemic than initially thought. Also, increasing average temperatures are making *Histoplasma* more common in more Northern States like Michigan and Minnesota [5–7]. This is thought to be due to factors such as climate change, humidity, and soil acidity [8]. Large amounts of bird and bat excreta enrich the soil in which the fungi are found,

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_20

facilitating growth and accelerating sporulation. When disturbed, microfoci or niches harboring many infective particles may lead to high infectivity rates or large outbreaks. In fact, large inoculum from construction causes most progressive disseminated histoplasmosis (PDH) infections [9, 10]. In most cases, however, the exposure is small, and infection is usually unrecognized. In addition, a new effort to screen for *Histoplasma* prevalence using wildlife tissue collection and culture in Argentinean Humid Pampas showed that 32.4% of wild mammals were positive for *H. capsulatum* [11]. This shows that current efforts to look for viruses in bats (and other hosts) to predict outbreaks could also be used with other infections, such as *Histoplasma*, to better elucidate epidemiology.

Pathogenesis, Virulence, and Immunology

Infection occurs when aerosolized microconidia (spores) are inhaled (Fig. 20.2). Infection usually is asymptomatic in healthy individuals following low level exposure. Infection usually is self-limited except following heavy exposure and/or in patients with underlying diseases that impair immunity. Pulmonary infection may be progressive in patients with underlying obstructive lung disease. For unknown reasons, pulmonary infection may rarely elicit exuberant mediastinal fibrosis.

Phases of initial infection:

1. Microconidia are inhaled from environment (no human-to-human transmission outside of organ transplants). Microconidia initially face physical/mucosal barriers (pharyngeal mucous and mucociliary clearance) before reaching their target (i.e., alveoli).
2. If microconidia escape physical barriers and initial antibody responses, and reach alveoli, they are encountered by surfactant proteins that opsonize the fungus. Surfactant proteins were also shown to have fungicidal effects [2].
3. Phagocytic cells including macrophages, neutrophils, and dendritic cells are rapidly recruited to the alveolar spaces that contain conidia [12]. Entry into macrophages is beneficial to the pathogen as it allows it to avoid activation of other fungicidal pathways. Within a few days the conidia transform to yeast, which multiply within the non-activated macrophages, and disseminate via the bloodstream to extrapulmonary organs. Neutrophils and dendritic cells inhibit proliferation of the organism, and dendritic cells present antigen to T lymphocytes as the initial step in development of specific cell-mediated immunity.
4. Consequently, tumor necrosis factor alpha and interferon-gamma are induced, which activate macrophages to inhibit the growth of the organism, leading to the formation of caseating granuloma, spontaneous recovery, and immunity against reinfection in most individuals [13].

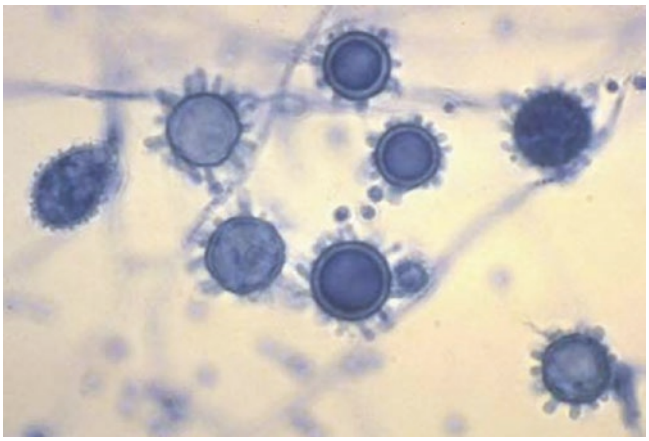


Fig. 20.2 Lactophenol cotton blue stain of the mold grown at 25 °C. Note microconidia and tuberculate macroconidia

Traditionally, humoral immunity is not felt to be important, but an experimental histoplasmosis study in 2012 found increasing levels of circulating immune complexes after *Histoplasma* infection [14], suggesting a role for humoral immune response.

A recent study found that hypoxia-inducible factor 1alpha (HIF-1alpha), a principal regulator of innate immunity to pathogens, is necessary for macrophage-mediated immunity to *H. capsulatum* in mice. It has shown that *Histoplasma* exploits an autophagic process (autophagic-related LC3-II) to allow intra-macrophage growth of *H. capsulatum*, and that an increase in HIF-1alpha would prevent this autophagic process, therefore inhibiting this intracellular growth and providing immunity against *Histoplasma* [15].

Reactivation of latent infection has been proposed as the mechanism for progressive disseminated histoplasmosis (PDH) in immunocompromised patients. However, the rarity of PDH in immunosuppressed patients (0.1–1%, even in those who reside in endemic areas) argues against reactivation as a common mechanism of pathogenesis. Histoplasmosis occurs in about 0.5% of patients undergoing solid organ transplantation in endemic areas [16]. Among 469 patients treated with TNF inhibitors for inflammatory bowel disease at a single institution, 3 (0.6%) developed histoplasmosis [17].

More likely, PDH in immunosuppressed individuals occurs because low-grade histoplasmosis was present at the time immunosuppression was initiated, or more likely de novo infection was acquired exogenously. In endemic areas, repeated exposure to *Histoplasma* spores probably occurs, permitting reinfection in immunosuppressed individuals whose immunity to *H. capsulatum* has waned.

Clinical Manifestations

Asymptomatic Infection

Infection is asymptomatic in 99% of otherwise healthy individuals, who experience low inoculum exposure, as indicated by skin test positivity rates above 50–80% in endemic areas [18]. Prior asymptomatic infection may also be identified by radiographic findings of calcified pulmonary nodules or mediastinal lymphadenopathy. It can also be identified by splenic calcifications, or by positive serologic tests performed during screening for organ or bone marrow transplantation or epidemiologic investigation. In endemic areas about 5% of healthy subjects have positive complement fixation tests for anti-*Histoplasma* antibodies [19].

Acute Pulmonary Histoplasmosis

Healthy individuals who experience a heavy exposure usually present with acute diffuse pulmonary disease 1–3 weeks following exposure [2]. Fever, dyspnea, dry cough, and weight loss are common, and physical examination may demonstrate hepatomegaly and/or splenomegaly as evidence of extrapulmonary dissemination. In many cases following heavy exposure, the illness is sufficiently severe to require hospitalization, with some individuals experiencing respiratory failure. Chest radiographs usually show diffuse infiltrates, which may be described as reticulonodular or miliary with prominent mediastinal and hilar lymphadenopathy (Fig. 20.3). Other forms of acute histoplasmosis present as reactive pericarditis or with rheumatologic syndromes such as arthritis, erythema nodosum [2].

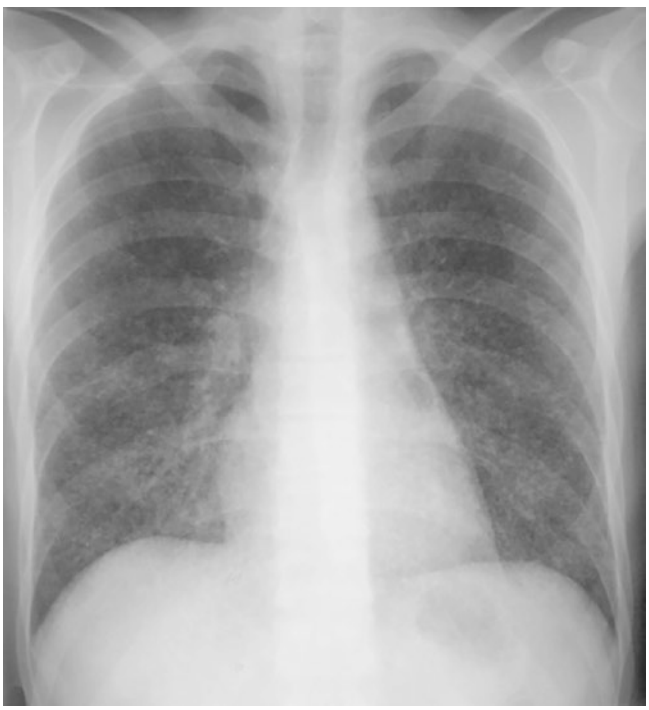


Fig. 20.3 Chest radiograph showing diffuse infiltrate seen in acute pulmonary or disseminated histoplasmosis



Fig. 20.4 Chest radiograph showing hilar lymphadenopathy seen in subacute pulmonary histoplasmosis

Subacute Pulmonary Histoplasmosis

In symptomatic cases the most common syndrome is a subacute pulmonary infection manifested by respiratory complaints and fever lasting for several weeks, then resolving spontaneously over 1 or 2 months. The chest radiograph or CT scan usually shows focal infiltrates with enlarged mediastinal or hilar lymphadenopathy (Fig. 20.4). Symptoms caused by a mediastinal adenopathy may prevail, and in some cases persist for months to years (granulomatous mediastinitis). In some cases, respiratory symptoms may be mild or absent, and findings of pericarditis or arthritis/arthralgias may be prominent. Pericarditis and this rheumatologic syndrome represent inflammatory reactions to the acute infection, rather than infection of the pericardium or joints [10].

Chronic Pulmonary Histoplasmosis

Patients with underlying obstructive pulmonary disease develop chronic pulmonary disease following infection with



Fig. 20.5 Chest radiograph showing upper lobe infiltrates with cavitation seen in chronic pulmonary histoplasmosis

H. capsulatum. The underlying lung disease prevents spontaneous resolution of the infection. Chest radiographs reveal upper lobe infiltrates with cavitation, often misdiagnosed as tuberculosis (Fig. 20.5). The course is chronic and gradually progressive, highlighted by systemic complaints of fever and sweats, associated with shortness of breath, chest pain, cough, sputum production, and occasional hemoptysis. Patients often experience repeated bacterial respiratory tract infections, and occasionally superinfection with mycobacteria or *Aspergillus*. Bronchopleural fistulas are a serious complication of chronic disease [2].

Progressive Disseminated Histoplasmosis (PDH)

Hematogenous dissemination is common during acute pulmonary histoplasmosis, but is non-progressive [20]. With the development of specific cell-mediated immunity, the infection resolves in the lung and extrapulmonary tissues. In contrast, disease is progressive in individuals with defective cell-mediated immunity (even though a large inoculum infection can result in severe disease in ~29% of individuals without immunological disorders [2]). In many cases the cause for immune deficiency is easily identified, and often includes the extremes of age, solid organ transplantation [21], treatment with immunosuppressive medications, steroid use [22], acquired immunodeficiency syndrome (AIDS) [23], idiopathic $CD4^+$ leucopenia [24], deficiency in the interferon-γ/interleukin-12 pathway [25], or malignancy [26]. In other cases, the cause for immunodeficiency remains unknown, awaiting a more complete understanding of immunity in histoplasmosis and development of better tests for diagnosis of immunodeficiency.

Clinical findings in PDH include fever and progressive weight loss, often associated with hepatomegaly or splenomegaly, and laboratory abnormalities including anemia, leukopenia, thrombocytopenia, liver enzyme elevation, and ferritin elevation [20]. An elevation in serum LDH has been associated with disseminated histoplasmosis, particularly levels greater than 600 IU/L. Pulmonary involvement is often the prominent feature in PDH, with manifestation as severe as respiratory failure requiring mechanical ventilation and vasopressor support for shock. The classical radiographic finding in PDH is diffuse reticulonodular infiltrates on chest X-ray and CT scan. Other less frequent sites of involvement include the central nervous system, gastrointestinal tract, skin, and adrenal glands. PDH also may present as culture-negative endocarditis, accompanied by other sites of dissemination or as an isolated manifestation of histoplasmosis [27].

Fibrosing Mediastinitis and Mediastinal Granuloma

Fibrosing mediastinitis is a rare complication of pulmonary histoplasmosis [10]. The mechanism for this manifestation appears to be an excessive fibrotic response to antigens released into the mediastinal tissues rather than progressive infection. It is unclear why the immune response from subclinical histoplasmosis can lead to either mediastinal granuloma formation (with inflammation and caseating necrosis) or this fibrotic process. Genetic influences, inoculum size, and host immunity are all likely factors. The clinical findings of fibrosing mediastinitis are caused by obstruction of mediastinal structures and may include involvement of the superior vena cava, airways, or pulmonary vessels. Infiltrative inflammatory mediastinal masses that do not respect fat or fascial planes are characteristic computed tomography findings of fibrosing mediastinitis. Fibrosing mediastinitis most commonly involves the right hemithorax, although bilateral involvement may occur. In most patients, obstruction does not progress, but mild to moderate symptoms persist indefinitely. In less than one quarter of patients the illness is progressive, highlighted by repeated bouts of pneumonia, hemoptysis, even respiratory failure, or pulmonary hyperten-

sion. No proven medical therapy exists for fibrosing mediastinitis due to histoplasmosis, including antifungal or corticosteroid therapy. Since the pathogenesis involves fibrosis rather than inflammation or infection, antifungal or anti-inflammatory therapy is not effective. Because fibrosis infiltrates adjacent mediastinal structures, surgical therapy has been of little benefit and is associated with a high risk for surgical morbidity and mortality. Surgical therapy is a rarely indicated and should be considered only after careful consideration of the risks and benefits by experts in the management of patients with fibrosing mediastinitis. Various procedures to relieve compression of vascular structures, and airway and esophageal compression are often employed with mixed results.

Distinguishing fibrosing mediastinitis from mediastinal granuloma is key. Mediastinal granuloma represents persistent inflammation in mediastinal or hilar lymph nodes. Enlarged, inflamed notes may cause chest pain with or without impingement upon soft mediastinal structures, such as the esophagus or superior vena cava. Fistulae may develop between the lymph nodes, airways, or esophagus. Improvement may occur spontaneously or following antifungal therapy or anti-inflammatory medications. The enlarged lymph nodes are usually encased in a discrete capsule, which can be dissected free from the adjacent tissues with a low risk for surgical morbidity or mortality. Thus, surgery may be appropriate in select patients with persistent symptoms despite antifungal therapy, weighing the risk of surgery with the severity of the clinical findings. However, studies establishing the effectiveness of surgery for mediastinal granuloma are scant, and surgical therapy is rarely necessary in such cases.

Often mediastinal lymphadenopathy is asymptomatic, identified on chest radiograms or CT scans performed for other reasons. In such cases concern often arises if to whether the mass represents malignancy. Differentiation of mediastinal lymphadenopathy caused by histoplasmosis or other granulomatous infection from that caused by a malignancy is best deferred to pulmonary disease consultants [18]. The presence of calcification strongly suggests that lymphadenopathy is caused by granulomatous infection but cannot rule out concomitant neoplasm. Conversely, the absence of calcification does not exclude granulomatous infection, and is quite typical of histoplasmosis during the first year or two after infection. PET scan has been suggested as a method to distinguish malignancy from nonmalignant causes for mediastinal or pulmonary masses. In fact, recent studies on PDG PET scans done to evaluate lung nodules found that mediastinal lymph nodes of patients with fungal nodules had increased intake compared to the nodules themselves, a finding opposite ("flip-flop fungus sign") to what is seen in lung cancers (nodules have higher intake than the mediastinal lymph nodes). This would help avoid invasive procedures when evaluating lung nodules [28]. For most patients lacking risk factors for malignancy, follow-up CT scan at 3–6-month intervals for 1 year is appropriate, lack of progressive enlargement supporting the diagnosis of histoplasmosis. *Histoplasma* serology may be useful in distinguishing between the two entities [29]. In others, especially those with risk factors for malignancy, biopsy may be necessary for definitive diagnosis. Choices include surgical excision or mediastinoscopy, although many recommend avoiding the latter due to risk of excessive bleeding.

Uncommon Presentations of Disseminated Disease

Histoplasmosis can sometimes present solely as adrenal insufficiency [30]. Some cases of infective endocarditis (usually caused by the mold form of the fungus), head and neck histoplasmosis have also been reported [27, 31, 32]. Also, hemophagocytic lymphohistiocytosis is an uncommon but frequently fatal complication of disseminated histoplasmosis [33].

African Histoplasmosis

In addition to infection with *Histoplasma capsulatum* var. *capsulatum*, *Histoplasma capsulatum* var. *duboisii* causes disease in Africa. Bony abscesses commonly involving the axillary skeleton, and skin lesions are much more common with African histoplasmosis. Pulmonary disease is rare, although infection with this variant also likely occurs via inhalation of spores. Disseminated African histoplasmosis resembling PDH as described above has been reported, with fever, multi-organ involvement, and a progressive course. The yeasts of *H. capsulatum* var. *duboisii* are 10–15 μm in diameter (much larger than var. *capsulatum*) and can be seen within giant cells. This can be confused with *Blastomyces dermatitis* or *Coccidioides immitis* on histopathological examination. DNA and antigen tests with the African variant should react similarly to *H. capsulatum* var. *capsulatum*. Likewise, therapy is similar with the exception that isolated cutaneous disease, or "cold abscesses" may heal spontaneously or with excisional surgery. Disseminated African histoplasmosis, especially with HIV co-infection, has a poor prognosis.

Diagnosis

Culture

The only test specific for histoplasmosis is culture, but the sensitivity is low, and delays up to 1 month may be required to isolate the organism [15]. Identification of *H. capsulatum* can be determined by conversion from the mold to the yeast, exoantigen detection, or through use of the commercially available DNA probe. Despite these limitations, culture should be performed unless the patient is already improving at the time diagnosis is suspected. In patients with pulmonary disease, bronchoscopy may be required, as most patients are unable to produce sputum. In those with suspected PDH, fungal blood cultures should be obtained, but cultures of tissues requiring invasive procedures may be deferred if tests for antigen are positive. In such cases failure to improve within 2 weeks after initiation of therapy would raise question about the diagnosis and support additional testing.

Antigen Detection

Antigen detection is the most useful method for rapid diagnosis of the more severe histoplasmosis syndromes, including acute diffuse pulmonary histoplasmosis and PDH [34–36]. Antigen may be detected in any body fluid, but urine and serum testing are recommended in all cases. A study comparing different urine antigen assays found a high degree of agreement between different platforms in diagnosing Histoplasmosis [37].

In some patients with pulmonary histoplasmosis, antigen may be detected in the bronchoalveolar lavage fluid but not in urine or serum [38]. Antigen may also be detected in the cerebral spinal fluid in patients with central nervous system histoplasmosis [39, 40], in pleural fluid, synovial fluid, peritoneal fluid, or pericardial fluid in patients with infection localized to those tissues. Serial measurement of antigen levels during treatment is recommended to monitor response as they decline with effective therapy [41], persist with ineffective therapy, and rebound in patients who relapse during or following treatment [42].

Histopathology

Demonstration of yeast-like structures in body fluids or tissues may provide a rapid diagnosis in some cases [18] (Fig. 20.6). Limitations of histopathology include the need for performance of an invasive procedure and low sensitivity. Histopathology may be falsely negative or falsely positive when performed by pathologists inexperienced with recognition of fungal pathogens. If histopathology is inconsistent with clinical findings or other laboratory tests, the specimens should be reviewed by a pathologist experienced with recognition of fungal pathogens.

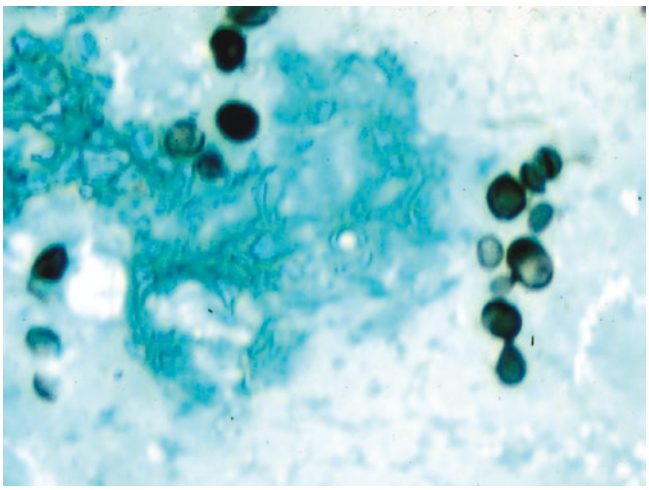


Fig. 20.6 Gomori methenamine silver (GMS) stain of yeast showing narrow neck budding

Serologic Tests

Serologic tests for antibodies are most useful in patients with subacute pulmonary histoplasmosis, chronic pulmonary histoplasmosis, granulomatous mediastinitis, pericarditis, or rheumatologic syndromes [42]. Immunodiffusion and complement fixation tests should be performed in all cases of suspected histoplasmosis. Serologic tests may be falsely negative during the first 2 months following acute exposure, limiting their usefulness in acute pulmonary histoplasmosis [35, 36]. Also, these tests may be falsely negative in immunosuppressed patients, limiting their role in PDH [16] but a study from 2019 evaluating the role of Western Blot in identifying antibodies for *Histoplasma* in patients with AIDS in Brazil found that 90% had positive antibodies against M and/or H antigens [43]. However, positive serologic tests persist for several years following recovery from histoplasmosis, and thus may provide misleading information in patients with other diseases. A newer enzyme immunoassay (EIA)-based serologic test of IgG and IgM provides a high diagnostic yield in patients with pulmonary histoplasmosis [44].

Molecular Testing

Although several publications suggest that PCR is useful for diagnosis of histoplasmosis and assays are commercially available [45], their role is uncertain. The highest sensitivity has been achieved by testing tissues or respiratory secretions [45], but whether PCR is positive in specimens in which organisms are not visible by fungal stain is uncertain [46].

A new loop-mediated isothermal amplification (LAMP) assay and nested PCR, both targeting the internal transcribed spacer (ITS) multicopy region of *Histoplasma capsulatum* had both 83% sensitivity and 92% specificity, with rapid results compared to culture, with the potential advantage of being more specific than antigen tests [47].

A metagenomic next generation sequencing has also been developed in the last few years and has helped identify H capsulatum in multiple case reports [48, 49].

Cell-Mediated Immunity Testing

A proof-of-concept study was done in 2019 to evaluate an interferon-gamma release assay (IGRA) to detect asymptomatic *Histoplasma* infections. This study was able to distinguish between individuals with past infections and uninfected individuals with high sensitivity (70–92%) and specificity (85–95%). This holds potential for a great screening (i.e., epidemiology) tool, similar to other commercially available IGRAs (like the one used to screen for tuberculosis) [50].

Conclusions

Accurate diagnosis of histoplasmosis requires skilled use of all the aforementioned laboratory methods [44]. While culture remains the gold standard for diagnosis, none of the other tests are 100% specific or sensitive. If the diagnosis is uncertain, additional testing should be performed, including repetition of the antigen test or serology, and in some cases, invasive procedures to obtain tissues for histopathology and culture. Expert advice should be considered in difficult cases.

Treatment

Practice guidelines, published in 2007 [51], are outdated, but have been updated in a recent review [52]. Treatment is indicated in most patients with acute diffuse pulmonary histoplasmosis and all patients with chronic pulmonary histoplasmosis or PDH (Table 20.1). Adjunctive treatment with methyl prednisolone or prednisone may hasten recovery in otherwise healthy individuals with respiratory distress caused by acute pulmonary histoplasmosis and can be administered safely if patients also receive effective antifungal therapy.

Table 20.1 Primary and alternate therapy for treatment for histoplasmosis

Presentation	Primary therapy	Alternate therapy
Acute pulmonary		
Mild	Itraconazole 200 mg daily or bid for 6–12 weeks	Posaconazole 400 mg bid, or voriconazole 200 mg bid, or fluconazole 800 mg daily
Moderately severe or severe	Liposomal amphotericin B 3–5 mg/kg/d for 1–2 weeks followed by itraconazole 200 mg bid for 12 weeks;[a] methyl prednisolone or prednisone 0.5–1 mg/kg/d for 1–2 weeks[b]	Amphotericin B lipid complex 3–5 mg/kg/d, or amphotericin B deoxycholate 0.7–1.0 mg/kg/d
Chronic pulmonary	Itraconazole 200 mg bid for at least 12 months	Posaconazole 400 mg bid, or voriconazole 200 mg bid, or fluconazole 800 mg daily
Disseminated	Liposomal amphotericin B 3–5 mg/kg/d for 1–2 weeks followed by itraconazole 200 mg bid at least 12 months[a]	Amphotericin B lipid complex 3–5 mg/kg/d, or amphotericin B deoxycholate 0.7–1.0 mg/kg/d

bid twice daily, *tid* three times daily, *mg/kg/d* milligram/kilogram/day

[a]Itraconazole may be given 200 mg tid × 3 days as a loading dose to achieve steady-state levels more quickly. The capsule formulation should be administered with food while the solution should be administered on an empty stomach. Measurement of drug concentrations is recommended since itraconazole drug exposure is highly variable. The intravenous formulation of itraconazole would be an alternative in a hospitalized patient with moderately severe or severe disease who is unable to be treated with any amphotericin formulation

[b]Adjunctive treatment with methyl prednisolone or prednisone may hasten recovery in otherwise healthy individuals with respiratory distress caused by acute pulmonary histoplasmosis

Treatment also should be considered in patients with subacute pulmonary histoplasmosis or granulomatous mediastinitis who are not improving within a month or two of symptoms onset. However, the effectiveness of therapy for these manifestations remains uncertain. Medical treatment for fibrosing mediastinitis is ineffective and is not indicated except in cases where granulomatous mediastinitis cannot be reasonably excluded. Treatment is not indicated in patients with calcified or non-calcified pulmonary nodules or as prophylaxis before immunosuppression in patients without evidence of active histoplasmosis within the last 2 or 3 years.

Liposomal amphotericin B is the treatment of choice for patients with severe manifestations of histoplasmosis requiring hospitalization [53]. In some cases, however, because of intolerance or cost, other lipid formulations may be used. In children, deoxycholate amphotericin B is well tolerated and preferred over the lipid formulations because of cost.

Itraconazole is recommended for mild cases not requiring hospitalization and for continued therapy following response to liposomal amphotericin B. Treatment should be continued for 6 to 12 weeks in patients with acute pulmonary histoplasmosis and 1 year or longer in patients with chronic pulmonary histoplasmosis or PDH. In patients with AIDS who achieve a good immunologic response to antiretroviral therapy, itraconazole may be stopped after 1 year if the $CD4^+$ T lymphocyte count is above 200 cells/μL and the *Histoplasma* antigen concentration in urine and serum are below 2 ng/mL [54, 55]. Similarly, lifelong maintenance therapy appears to be unnecessary in most patients with histoplasmosis complicating treatment with tumor necrosis factor inhibitors [17] or following solid organ transplantation [16]. However, in those with persistent immune deficiency, or who relapse after stopping an appropriate course of therapy, lifelong maintenance therapy may be required. Itraconazole blood levels should be monitored to assure adequate drug exposure, and the dosage should be increased, or the capsule formulation replaced with the solution if random concentrations are below 1 μg/mL as determined by high pressure liquid chromatography, or 3 μg/mL by bioassay.

If the antigen test is positive, treatment should be continued until antigen levels become undetectable or below 2 ng/mL. Furthermore, antigen levels should be monitored during the first year following discontinuation of therapy, and at the time of recurrence of symptoms suggesting relapse of histoplasmosis.

The best alternative oral therapy in patients unable to take itraconazole is posaconazole, which is highly active in vitro [56], in animal models [56], and in patients [57]. However, therapeutic concentrations of posaconazole of at least 0.5 μg/mL may be difficult to achieve using current formulations. Fluconazole is less active in histoplasmosis, and relapse associated with development of resistance has been observed in patients with AIDS [58]. Voriconazole is more active in vitro than fluconazole, but less active than itraconazole or posaconazole. Although minimum inhibitory concentrations (MICs) are lower to voriconazole than fluconazole, higher drug exposure with fluconazole offsets the lower MICs. Furthermore, prior exposure to fluconazole or voriconazole may induce resistance to voriconazole [59]. Voriconazole has not been studied in animal models or patients with histoplasmosis and offers no clear advantage over fluconazole. Furthermore, voriconazole causes several important toxicities, including photosensitivity and skin cancer, fluorosis manifesting as joint pain, and mental status changes, which must be considered before choosing it as chronic therapy in patients with mycoses that could be treated with other antifungal agents. Although CSF levels are higher with fluconazole than with itraconazole or posaconazole, the role of the former in treating *Histoplasma* meningitis is uncertain [40, 51, 52]. Measurement of voriconazole or posaconazole blood levels is recommended because of the wide variation in drug levels. Voriconazole exhibits a short half-life (~6 h), and concentrations decline in at least twofold from the peak to the trough time after administration. Accordingly, trough concentrations of voriconazole of at least 0.5 μg/mL are recommended. Posaconazole exhibits a long half-life, similar to that of itraconazole (~24 h), supporting a similar target random concentration of 2 μg/mL. The echinocandins are not active in histoplasmosis and should not be used [60, 61].DisclaimerDr. Abdallah is an Internal Medicine Resident Physician at Indiana University School of Medicine. Dr. Hage is an Associate Professor of Clinical Medicine at Indiana University School of Medicine and faculty in the thoracic transplantation program at Indiana University Health.

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Paracoccidioidomycosis

21

Angela Restrepo, Beatriz L. Gomez, Juan G. McEwen, Angela Tobon, and Gil Benard

Introduction

Paracoccidioidomycosis (PCM) was first described in 1908 by Lutz in Brazil, who with Splendore characterized both the mycosis and its etiologic agent through exacting clinical observations and detailed comparisons of fungal isolates from Latin American patients as well as from foreign cases [1, 2]. Initially, the fungus was erroneously considered to represent a strain of *Coccidioides immitis*, and it was not until 1930 that de Almeida properly differentiated the etiologic agent, *Paracoccidioides brasiliensis*. Originally, this pathology was designated as the "Brazilian disease" but later on when this ailment began to be diagnosed in other Latin American countries, it was renamed South American Blastomycosis, thus stressing its peculiar geographic boundaries [3–5]. The use of the term PCM was sanctioned later on by Latin American scientists during the First Pan American Symposium held in Colombia in 1972.

At present, five phylogenetically diverse *P. brasiliensis* species (*P. brasiliensis, P. americana, P. restrepiensis, P. venezuelensis,* and *P. lutzii*) have been recognized as etiologic agents of this mycosis [6]. *P. lutzii* diverged from the other species approximately 30 million years ago (MYA), whereas *P. venezuelensis* and *P. restrepiensis* appear closely related, with a recent divergence of less than 0.2 MYA [6–10].

These fungal pathogens are thermally dimorphic and as such exhibit two morphotypes, a mold at temperatures under 28 °C and a yeast in cultures at 35–37 °C as well as in tissues. The yeast reproduces by multiple budding, creating a shape that resembles a pilot's wheel [11, 12]. The genus *Paracoccidioides* is only known in its asexual (anamorph) stage, but molecular techniques have revealed the presence and expression of the mating type locus, thus allowing its classification within the phylum Ascomycota, order Onygenales, family Onygenacea, close to the phylogenetic branch encompassing the dimorphic fungi *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Emmonsia parva,* all of which present a teleomorphic or sexual stage in the genus *Ajellomyces* [13–16].

PCM is an endemic systemic mycosis that exhibits two main clinical presentations, the subclinical or asymptomatic infection, and the clinically manifested disease. The latter is usually chronic with involvement of the primary target, the lungs, as well as of different body organs including the mucosa, skin, adrenal glands, and lymph nodes, among others. The mycosis afflicts men more frequently than women and is more common in adults [17–19]. Latency is known to exist and is frequently prolonged [20, 21].

Angela Restrepo has died before the publication of this book.

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_21

The Etiologic Agent

The *Paracoccidioides* spp. complex is characterized by thermally dimorphic fungi that at temperatures between 4 and 25 °C grow as white molds, composed microscopically of thin septated hyphae, occasional chlamydospores, and rarely microconidia. The latter are infectious and capable of converting into yeast cells under the influence of temperature [22–24]. At 35–37 °C colonies are soft, wrinkled, and microscopically composed of oval to round yeast cells of varying sizes (4–40 μm) that reproduce by budding. The key distinguishing feature is that of a multiple budding yeast cell with a larger mother cell surrounded by multiple daughter cells (blastoconidia), a structure resembling the pilot wheel of a ship. Additionally, a thick refractive cell wall and prominent intracytoplasmic vacuoles can be observed (Fig. 21.1) [11, 18, 23, 25]. The yeast morphologic characteristics are also observed in clinical specimens (Fig. 21.2) [11, 17, 18, 23]. Since the original release in 2009 the BROAD Institute has updated the database for *P. brasiliensis* genomes, with information now available at fungidb.org and https://www.ncbi.nlm.nih.gov/genome/browse/#!/eukaryotes/334/ [26].

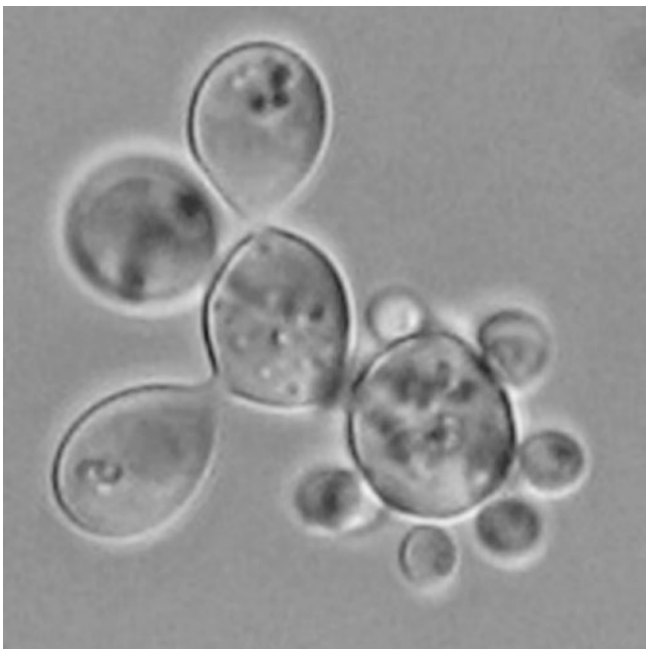


Fig. 21.1 *Paracoccidioides* spp. yeast cell surrounded by multiple budding daughter blastoconidia ("pilot's wheel")

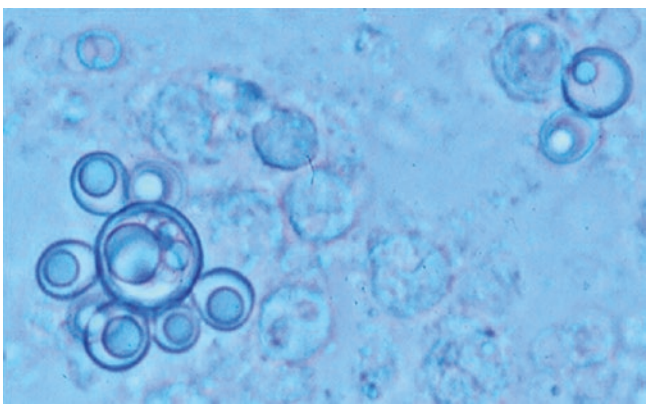


Fig. 21.2 *Paracoccidioides* spp. in KOH preparation of a sputum sample. Note abundant round to oval yeast cells, one cell has multiple buds, while other cells appear empty

Ecological Aspects

The exact locations of the fungal niches of these organisms remain unknown [11, 17, 27]. Experimental animal studies have shown that after inhalation of the infectious particles (conidia, short mycelial fragments) produced by the fungus' saprophytic form, these fungal elements promptly transform into the yeast/tissue form [28, 29]. Of note, this infectious process is coupled with a thermally regulated transition (dimorphism) from a suspected soil-dwelling filamentous form bearing conidia to a yeast-like pathogenic structure in tissue [30]. Molecular epidemiology studies have shown that *P. brasiliensis* predominates in Brazil, Argentina, Paraguay, Peru, and Venezuela; *P. americana* prevails in Brazil and Venezuela, while *P. restrepiensis* is mainly found in Colombia [10, 15]. *P. lutzii* occurs preferentially in the central and northern regions of Brazil [8, 15].

Epidemiological Aspects

As a disease, PCM has a peculiar geographic distribution limited to Latin America. Approximately 80% of the confirmed cases have been reported from Brazil, followed by patients from Colombia, Venezuela, and Ecuador [12, 31–33]. The infection has been reported as far north as Mexico and as far south as Argentina [34, 35]. However, PCM has not been observed in certain countries of the South American continent such as Chile, Surinam, Guyana, Nicaragua, Belize, and most of the Caribbean islands [18, 32, 36]. Brazil has the largest number of cases, all distributed in three clearly delineated regions [37]. PCM is extremely rare in the Caribbean Islands, where only single cases have been described from Trinidad, Grenada, and Guadalupe. No cases have been reported from Chile, Surinam, or Guyana [37–39].

Approximately 100 PCM cases have been reported from countries outside of the recognized endemic areas, mostly in Europe, as this continent receives a large number of immigrants from Latin America [11, 21]. In 2010–2012, eight cases were reported outside the endemic area [32, 40]. These patients had all lived in the Latin American endemic regions,

up to 14 years prior to the appearance of clinical manifestations in their new residences in non-endemic countries [32, 40]. This prolonged dormant period confirms Borelli's postulates [38], indicating that although the primary infection occurred in an endemic area, it would manifest as a disease far away from the recognized endemicity areas.

In endemic countries, patients' residence appeared associated with juvenile PCM cases, as such cases had all resided in close connection to coffee plantations, areas that were characterized by a humid atmosphere (high rainfall indexes, presence of humid forests, and waterways) plus stable, mild temperatures (17–24 °C) [41]. Despite the above data, the exact habitat of *Paracoccidioides* spp. remains largely unknown, as this agent has been isolated only from very few, diverse environments, including soils and certain substances such as animal forage, bat guano, and penguin feces; and using molecular detection in aerosols [42–44]. Barroso et al. analyzed the records of 91 acute/subacute patients in whom infection was estimated to have occurred 1–2 years previously and through multiple regression analyses determined that weather variability was a significant factor. Construction of a good fitting model provided significant estimates of the effect of absolute air humidity, soil water storage, and Southern Oscillation Index [45]. These environmental factors explained 49% of the incidence variance of the cases and suggested that such variables influenced the conditions favoring infection [45–47].

The armadillos *Dasypus novemcinctus* and occasionally also *Cabassous centralis* have been shown to harbor *Paracoccidioides* spp. in their internal organs; however, isolation of the fungus in those armadillos' burrows has failed [48, 49]. PCM has also been confirmed, or suspected to exist, via molecular or immunological methods in other feral and domesticated animals [50]. Evidence indicates that *Paracoccidioides* spp. occur preferentially in humid and shady disturbed forests in a strong association with armadillos.

Researchers in the area of clinical PCM have observed an estimated reduction in the usual number of cases that are being diagnosed in public health centers. This finding is thought to be attributable to changing agricultural practices whereby there has been a gradual substitution of coffee plantations to sugar cane farming and cattle pastures [19, 44]. The area occupied by sugar cane crops has significantly increased, especially in some areas of the State of Sao Paulo, in Brazil's Southern Region. As sugar cane plantations employ practices such as burning and widespread use of pesticides, herbicides, and fungicides, prior habitats of *Paracoccidioides* spp. may be subjected to significantly elevated soil temperatures with concomitant extinction of the accompanying saprophytic soil microorganisms [46].

Argentina has two endemic PCM areas, one in the northeast (NEAT) region, close to the borders of Paraguay and Brazil that comprise the area with the highest incidence of the chronic adult forms; however, between 2010 and 2012, several acute/subacute juvenile cases (ages 10–16 years) were also diagnosed. A common finding was that the histories traced their living quarters to the NEAT region, where important climatic and anthropogenic changes had taken place, including deforestation and the El Niño phenomenon, all which occurred during the period indicated above. Additionally, the significant ecological alterations brought about by the construction of the largest hydroelectric dam in the whole of South America also exerted their influence on emergence of this mycosis in children in the area [34].

Pathogenesis and Immune Response

Infection gives rise to an intense host response with alveolitis and presence of neutrophils. These cells are later replaced by migrating mononuclear cells which, in turn, convert into epitheloid cells, thus initiating the formation of granulomas and attracting various subtypes of T lymphocytes of which the relative proportions depend on the host immune status [11, 51–53]. In mice with pulmonary infection with *P. brasiliensis*, fungal load was controlled by CD8+ T cells, whereas antibody production and delayed type sensitivity reactions were regulated by CD4+ T cells [54]. Lymphocytes from PCM patients are poorly activated, express low levels of IL-15R alpha, and produce only basal levels of cytotoxic granules [54–57]. These findings may account for an observed defective in vitro cytotoxic activity [51, 52, 55, 56].

The characteristics of the immune responses in patients with overt disease have been the subject of numerous studies [58]. Earlier studies have shown that the immune reactivity to *P. brasiliensis* antigens is characterized by depressed Th1 cellular immune responses that revert to normal or almost normal ranges with treatment and patient improvement [55, 59]. Patients with the acute-type disease not only have depressed Th1, but have also polarized Th-2 responses, characterized by the increased release of the cytokines IL-4, IL-5, and IL-10, high levels of circulating anti-*P. brasiliensis* antibodies of the IgG4 and IgE subclasses, and marked eosinophilia [52, 53]. This pattern is reflected by abundant fungal multiplication and extra-pulmonary dissemination with progression of disease [54–56]. This Th-2 polarization is regularly reverted with treatment. A Th-1 cellular immune response to *P. brasiliensis* is detected in those individuals who were once exposed to the fungus but did not develop the disease [54]. More recently, a role for T regulatory cells in cell-mediated immune suppression has been found in patients with active disease, which was also reverted with treatment [52]. Beyond the Th-1/Th2 paradigm, the participation of the

newly described Th-17, Th-21 and Th-9 subsets in the patients' immune response has recently been documented revealing Th-17 and Th-21 responses in the case of the adult-type disease while Th-9 response was detected mostly in the juvenile-type disease [55].

The factors that determine the different outcomes of the infection are not known. In most instances the subclinical infection is resolved, possibly leaving viable yeast cells that eventually may enter into an ill-defined latent state for decades, although histopathological evaluation of asymptomatic persons accidentally diagnosed with silent PCM lesions (paracoccidioidomas) showed within these lesions small amounts of active, multiple budding yeast cells amidst non-sporulating cells, suggestive of a subclinical disease [20, 60]. In the rare instances when the primary infection is not checked by the host's immune responses, *P. brasiliensis* disseminates through the lymphohematogenous route resulting in the acute- or juvenile-type disease [11, 53, 61, 62]. Overall, the prevalence of the disease is low even in endemic areas, while that of infected-only individuals is high, reaching up to 60% of the population in certain highly endemic settings according to skin testing studies [11, 63–65]. A tentative schematic view of the natural history of the mycosis is provided in Fig. 21.3.

In PCM, neutrophils (PMN) have been implicated not only in phagocytosis and pathogen destruction but also in granuloma formation. In cases presenting only loose granulomas, a greater quantity of fungi is to be found in comparison to those presenting well-organized granulomas. In either case neutrophils are present in tissues and participate in granuloma formation, largely contributing to the inflammatory response [66]. The major biological significance of the granuloma is the limitation of the infection to a local area, preventing dissemination [11, 67].

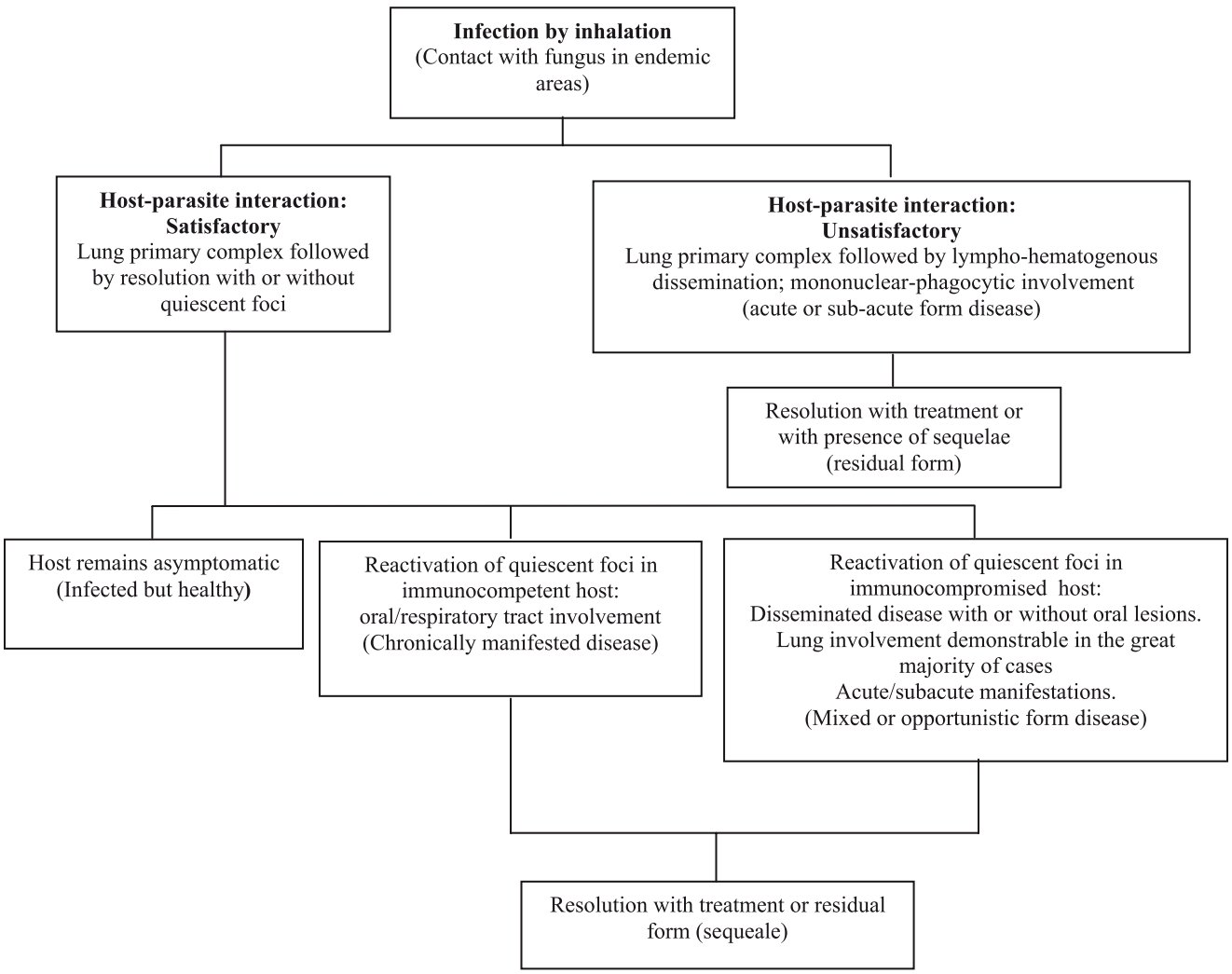


Fig. 21.3 Presumed natural history of paracoccidioidomycosis.

Clinical Manifestations

PCM is a disorder characterized by protean manifestations that in most patients tend to run a chronic progressive course involving various organs and systems with mortality rates that average 0.9/1,000,000 inhabitants in Brazil [68]. Most patients exhibit constitutional symptoms such as general malaise, asthenia, adynamia, weight loss, and fever, as well as symptoms related to the infected organs. The primary infection occurs in the lungs, but often neither the patient nor the clinical examiner finds abnormalities at this site. On the basis of the clinical presentation and the host immune response to PCM, the disease is categorized as (1) subclinical infection, or (2) symptomatic infection, which is subdivided in two forms, the acute/subacute juvenile and the chronic adult type. A third residual form characterized by fibrotic sequelae is also recognized [68–70]. On the basis of gallium image studies and autopsies, it is presently accepted that the various manifestations of PCM entail multiple organ involvement, thus negating the former division of the disease into an unifocal or a multifocal process [68, 70]. With the increase in the number of immunocompromised individuals, particularly those with AIDS, the mycosis is being recognized more frequently [71, 72]. In such patients, the corresponding clinical presentation does not allow to categorize the process as chronic or acute but rather as a mixed (opportunistic) form [71, 72]. Table 21.1 summarizes the key factors leading to consider a diagnosis of PCM.

Table 21.1 Key factors leading to consider the diagnosis of paracoccidioidomycosis

- History of residence in an endemic country (even if many years previously to initiation of symptoms/lesions)
- History of working in agriculture or related occupations. Mining also to be considered
- Being an adult male with a chronic, progressive illness
- Complaints related to external manifestations (mucous membrane, skin and/or lymph node enlargement/draining)
- General malaise, weight loss, fever
- Signs and symptoms of adrenal gland dysfunction
- No major pulmonary signs/symptoms contradicted by marked X-rays or imaging abnormalities
- In children, young adults, and immunocompromised individuals (mainly AIDS), hypertrophy of lymph node structures and/or involvement of liver/spleen
- Multiple skin lesions or bone abnormalities in the abovementioned groups

Remember: Paracoccidioidomycosis often exhibits protean manifestations, thus hindering proper diagnosis

Subclinical Infection

The subclinical infection has no special characteristics and is detected mostly by a reactive paracoccidioidin skin testing and sometimes by abnormal chest radiographs [65]. Additionally, more sensitive serological or molecular techniques may also identify healthy infected people [52, 55, 63, 64, 73, 74]. However, *Paracoccidioides* spp. may remain latent in the infected host, giving rise to symptomatic PCM years after the initial contact, as demonstrated by the cases diagnosed outside the endemic areas, in persons with prior endemic exposure [20, 73].

Symptomatic Infection

Clinically manifested disease varies with patient's age and the person's immune response. The role of inoculum size or frequency of re-exposure awaits further investigation.

Acute or Subacute Disease (Juvenile Type)

The juvenile-type disease is a serious disorder that afflicts children, individuals of age 30 years or younger, and immunocompromised individuals of either sex. Older non-immunocompromised patients with acute-subacute disease have occasionally been reported. It represents less than 10–15% of all cases. Infection characteristically involves the reticuloendothelial system with lymphadenopathy, hepatomegaly, and/or splenomegaly. Skin lesions, often multiple, are observed in 31% of the cases. Constitutional abnormalities such as fever, marked weight loss, and general malaise are hallmarks and become associated with anemia, hypoalbuminemia, eosinophilia, and hypergammaglobulinemia [36, 75–77]. Abdominal and digestive tract manifestations such as presence of abdominal masses, lymph node enlargement, diarrhea, vomiting, abdominal distention or pain, and ascites are also more common than in the chronic adult disease. Although respiratory symptoms are infrequent (5–11%), the fungus can be found in respiratory secretions, and computed tomography studies not infrequently reveal abnormalities such as enlarged hilar lymph nodes or, more rarely, miliary infiltrates [78]. The juvenile-type disease may evolve rapidly, in weeks, and consequently, prompt diagnosis accompanied by institution of antifungal therapy and supportive measures are required. Mortality rates are higher than in the adult disease, reaching up to 10% in some series [68, 75, 79], but a trend for lower rates has been reported more

recently. In children, mortality has decreased from 9.5% to 2.6% in recent years, thus approaching that of adults [79].

Chronic Disease, Adult Type

The chronic, adult-type disease predominates in all case series (80–90%) [32, 60]. It occurs preferentially in male patients (13 men:1 female), ages 30 years or more, with agriculture-related occupations. The disease course is characterized by protracted pulmonary and extra-pulmonary organ damage, especially of the mucous membranes and the skin (90.8%) with the lesions tending to be ulcerative, granulomatous, infiltrated, and painful with hemorrhagic points, "mulberry stomatitis" (Fig. 21.4) [68, 80, 81]. Sialorrhea, dysphagia, and dysphonia are common, as well as lost tooth due to gingivitis. Regional lymph nodes are hypertrophied and may spontaneously drain forming fistulae. The adrenals glands may also become involved, with decrease in functional reserve in 48% of the cases. Central nervous system (CNS) involvement occurs in 25% of the cases, including pseudotumoral, meningeal, or spinal forms [82–84]. At least 80% of these patients also present pulmonary abnormalities [80, 85].

High-resolution CT studies have revealed lung abnormalities in 98% of the patients with architectural distortion, reticulate and septal thickening and centrilobular and paraseptal emphysema [86–89]. Chest radiographs reveal mixed infiltrates, mostly interstitial but at times also alveolar; these are predominantly bilateral and located in the central and lower fields respecting the apices in a one third of the patients (in contrast with tuberculosis) [86–89]. Sequelae represented by pulmonary fibrosis were observed in most patients, with 30% of them developing significant respiratory limitation [70, 80, 86, 88, 89]. Not infrequently, image studies show more important lesions in comparison with the patient's symptoms or even without findings at auscultation [90–92]. Fibrosis may also occur in other organs and systems and may be associated with functional impairments (Fig. 21.5). In absence of treatment, adult mortality may be as high as 30–45% based on multiple series [36, 90, 93–95].



Fig. 21.4 Cutaneous and mucosal lesions in a patient with paracoccidioidomycosis. Note lip's edema, ulceration, and scarring

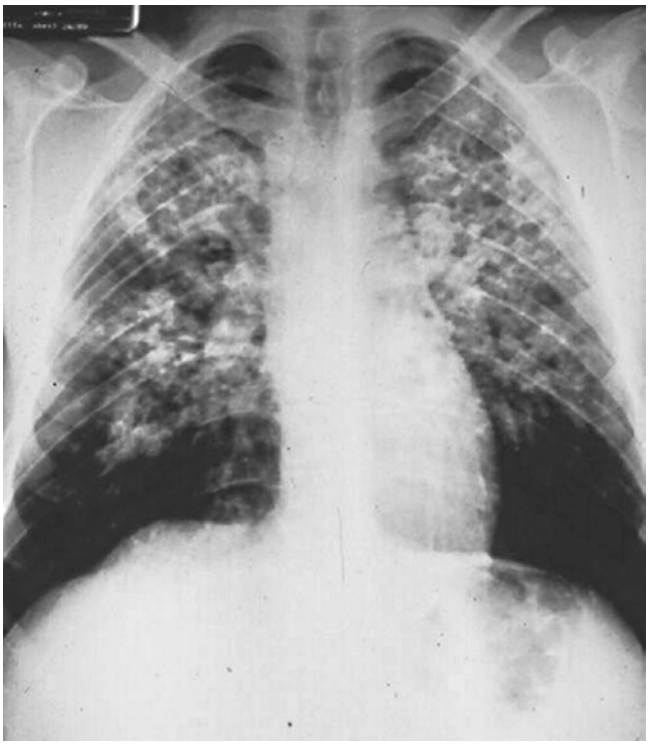


Fig. 21.5 Pulmonary paracoccidioidomycosis. Note abundant fibronodular infiltrates in central fields and basal bullae

Disease in Immunocompromised Patients

Despite the fact that PCM is uncommon in immunocompromised patients, HIV-PCM coinfection has been reported in endemic areas. In the last 30 years, 136 PCM cases in HIV-infected patients have been identified in the literature, 80% with CD4 counts lower than 200 cells/mm^3, presenting the mixed clinical form with fever, widespread lymphadenopathy, splenomegaly, skin lesions, and pulmonary involvement (70%), likely as a result of inefficient immune control of the initial subclinical pulmonary foci, followed by lymphohematic dissemination [60]. Coinfected AIDS patients may represent 5% of all PCM cases in highly endemic areas [96, 97]. In a 1986–2004 cohort study, a trend for a decrease in these numbers followed the advance of the antiretroviral therapy. Fungemia and other opportunistic infections constitute a risk factor for mortality [95]. Prophylaxis with trimethoprim/sulfamethoxazole did not appear to prevent PCM infection [71, 96, 97].

Among the neoplastic hemopathies, PCM is more often associated with lymphoma. Also, PCM has been confused with carcinoma of the respiratory tract, with its presentation in the same anatomic site of the fungal infection in 58% of the cases [71, 98–100].

PCM in organ transplant recipients is mostly associated with the late phase of kidney transplantation and the involvement of skin, mucosae, and lungs with infiltrates accompanied or not by lymph node enlargement [97]. In patients receiving immunotherapy the development of PCM chronic form has been reported after 1 year of biological therapy [71].

PCM and Tuberculosis (TB)

PCM can be associated with TB in 15–20% of the cases, as the latter disorder also produces similar respiratory abnormalities, but with minor (0.4%) mucocutaneous involvement in TB, as well as no frequent involvement of the lung apices by PCM, sites more frequently attacked in TB [12, 80, 81].

Diagnosis

The classical PCM diagnosis combines clinical evaluation and laboratory studies, including routine direct examination, histopathology, culture-based techniques, as well as immunological and molecular assays.

Direct Examination and Histopathology

In clinical specimens from oral, pharyngeal and cutaneous lesions, sputum, bronchoalveolar lavage fluid, lymph nodes, adrenal glands, or biopsy materials from other tissues, *Paracoccidioides* spp. can be identified in up to 85% of the cases by means of fresh or wet potassium hydroxide (KOH) preparations, as well as on calcofluor and immunofluorescence preparations. *P. brasiliensis* appears as an oval to round translucent-walled yeast cell, often having multiple peripheral buds, typically with the "pilot wheel" shape, considered a pathognomonic finding in the diagnosis of PCM (Fig. 21.1). Histopathologic preparations stained with hematoxylin and eosin (H&E), Gomori methenamine-silver stain (GMS), or periodic acid-Schiff (PAS) are also useful as they reveal the multiple budding yeast elements. Frequently, with H&E stains, infected tissues reveal a mixed inflammatory reaction characterized by the presence of granulomas centered on the multiple budding yeasts with the granuloma being further characterized by the presence of neutrophils, mononuclear cells, and epithelioid cells, as well as by multinucleated giant cells, with the latter constituting the hallmark of granulomatous inflammation (Fig. 21.6). It is important to differentiate *Paracoccidioides* spp. from *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and even *Histoplasma capsulatum* [11, 12, 19, 59, 101].

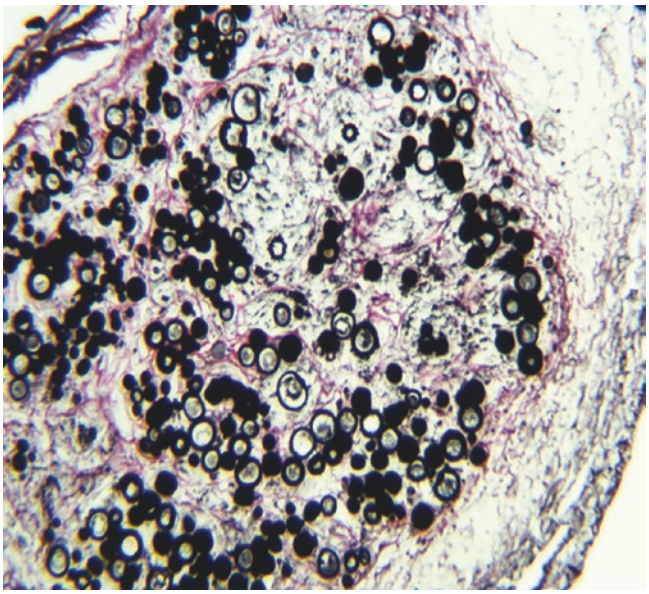


Fig. 21.6 Tissue biopsy with abundant *Paracoccidioides* spp. yeasts enclosed within a granuloma. GMS

Culture

Paracoccidioides spp. can be recovered from clinical specimens such as sputum or tissue in 86–100% of infections. However, *Paracoccidioides* spp. will take an average of 2–3 weeks to start growing and culture requires a battery of selective and nonselective culture media such as Sabouraud dextrose, brain-heart infusion (BHI) plus glucose, or Mycosel™ agar. The addition of antibacterial drugs (chloramphenicol or gentamicin) and mold inhibitors (cycloheximide) to the above media has resulted in improved recovery rates of around 80%, thus providing a useful differential tool in the identification of the fungus [11, 12, 19, 101, 102]. Modified Sabouraud's (Mycosel) agar and yeast extract agars incubated at room temperature (19–24 °C) are the best media for primary isolation but the growth is slow and may take 20–30 days. Microscopically, the mold shows thin septated hyphae and chlamydospores (15–30 μm). Under conditions of nutritional deprivation, some isolates produce conidia, which vary in structure from arthroconidia to microconidia, and measure less than 5 μm. Conidia respond to temperature changes, germinating into hyphae at 20–24 °C or converting into yeasts at 36 °C on appropriate media. The mycelial form is not distinctive and dimorphism must be demonstrated for identification [11, 12, 19, 101, 102]. At

37 °C the *Paracoccidioides* spp. yeast form grows in 8–10 days as a cerebriform, cream-colored colony. Microscopically, oval to spherical yeast cells (2–40 μm) are observed. As mentioned above, the large mother yeast cell bearing multiple blastoconidia (pilot's wheel) is characteristic of this fungus [11, 12, 101]. There is no commercial DNA probe test for identification of *Paracoccidioides* spp.

Immunodiagnostic Tests

Immune-based methods for antibody and antigen detection are useful not only for diagnosis but also for monitoring the patient's treatment course [101–108]. Antibody detection has been based upon antigen preparations using either mycelial or yeast cell lysates and includes a variety of techniques such as immunodiffusion (ID), counterimmunoelectrophoresis (CIE), enzyme-linked immunosorbent assay (ELISA), immunoblotting, dot blot, and latex agglutination assay. These are available at various reference laboratories. While a series of immunoreactive antigens (27 kDa, 43 kDa, 70 kDa, 87 kDa) are present in these lysates, the predominant antigen is the 43 kDa glycoprotein, gp43 [101, 104, 105, 107, 109, 110].

Because of its simplicity, the gel immunodiffusion (ID) test is regularly used in endemic countries. This test demonstrates circulating antibodies in 65–95% of the cases and is highly specific. Commercial mycelial-form culture-filtrate antigen can be obtained for in-house use in ID from IMMY (*Paracoccidioides* ID Antigen; IMMY), but its sensitivity has not been widely studied [101]. Complement fixation (CF) test is performed with *P. brasiliensis* yeast-form culture-filtrate antigen but this reagent is not commercially available. CF is less specific than the ID test, and cross-reactions can occur in cases with histoplasmosis. However, CF titers of ≥1:8 are considered presumptive evidence of PCM with falling CF titers being often predictive of successful treatment [101, 107].

Several laboratories have developed their own "in-house" immunodiagnostic tests based on methods such as ELISA, inhibition ELISA, competition ELISA, Western blot, or dot blot [101, 104, 105, 107]. Improvements include the detection of antibodies against chemically characterized and/or recombinant *P. brasiliensis* antigens, notably gp43, pb27, and the 87-kDa heat-shock protein [111–117]. A combination of two recombinant products has resulted in increased sensitivity (92%) and specificity (88%) [118, 119]. Antigen detection tests have some important advantages over antibody detection in the diagnosis of PCM, particularly in immunocompromised patients and in those previously exposed to *Paracoccidioides* spp. who may have pre-existing antibody titers [104, 111–113, 115, 118, 120]. The gp43 glycoprotein and the 87-kDa heat-shock protein have been described as useful targets for serum antigen detection [118]; the 43-kDa glycoprotein has also been detected in urine, cerebrospinal fluid, and BAL samples [115]. However, these methodologies are not yet available as routine diagnostic tests. Authors have described that serum antigen levels diminished or even disappeared during successful treatment [104, 111, 119, 121].

Nucleic Acid Detection

The number of clinical tests available for the molecular detection of *Paracoccidioides* spp. in clinical samples is very limited. Several molecular diagnostic methods, such as PCR, nested PCR, PCR-enzyme immunoassay, real-time (RT) PCR, and loop-mediated isothermal amplification, have been successfully used to detect specific *Paracoccidioides* spp. DNA sequences; however no commercially available systems exist for the detection of *Paracoccidioides* spp. DNA in human clinical samples. The number of clinical tests available for the molecular detection of *Paracoccidioides* spp. in clinical samples is very limited [122, 123].

As mentioned before, a new challenge and extra care has to be taken regarding *Paracoccidioides* spp. and its recently described cryptic species, which are not always considered in experimental design. A few tests are suitable for investigating clinical samples aiming to detect *Paracoccidioides* species' DNA during infection, and none of them are designed to discriminate among the cryptic species described over the last decade [123]. A Real Time-Polymerase Chain Reaction (RT-PCR) amplifying the recombinant protein PB27 gene was designed and primers and probe sequences were deposited in the Brazilian Coordination of Technological Innovation and Transfer (CTIT), under patent reference number BR1020160078830 [124]. Preliminary results using 78 clinical samples from confirmed cases showed a sensitivity of 94% and specificity of 100%. More studies are needed to further validate this test [125].

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is also a new tool that was first applied for *Paracoccidioides* spp. identification by Almeida Jr et al. [126], aiming to standardize an assay that will differentiate the spectra obtained from proteins expressed by *P. brasiliensis* complex and *P. lutzii*. No misidentifications were found among the 22 strains tested. This technology is not currently available for identification of *P. brasiliensis* directly from clinical samples [126].

Treatment

Antimicrobial agents from three different classes are currently used to treat PCM patients. They include the sulfonamides, the polyene amphotericin B and its lipid formulations, and certain azoles (Table 21.2) [127, 128]. Generally, pro-

Table 21.2 Antifungals recommended for the treatment of paracoccidioidomycosis

Antifungals	Dose (daily)	Route	Duration
Itraconazole (tablets or solution)	Adults: 200–400 mg Child (>6 years old): 5–10 mg/K	PO	6–12 months
Trimethoprim/sulfamethoxazole tablet 80 mg/400 mg	2–3 tablets bid	PO	18–24 months
Amphotericin B	Deoxycholate formulation 0.7–1 mg/kg/day 1–2 g (cumulative dose)[a] Lipid formulation: 3–5 mg/K[a]	IV	2–4 weeks (until clinical improvement)

PO oral, *IV* intravenous
[a] To be followed by an oral medication

longed treatment is required to avoid fungal persistence in the host due to the patients' low immune responses. Intravenous medication should be used in severe disease, and should be followed by oral treatment when the patient shows clinical improvement [11, 101, 127, 129]. Azoles, such as itraconazole and voriconazole, have emerged as therapeutic options for the control of the disease's moderate forms over shorter periods of time [129, 130].

Additional measures such as adequate nutrition and interruption of both alcohol intake and smoking are a must in order to accelerate recovery and diminish fibrous sequelae. In severe cases, particularly in those with the acute-type disease, the antifungal agents may not have sufficient time to act leading to fatalities [131]. Use of corticosteroids has been advocated in the presence of important tissue inflammation that can result in severe clinical complications (e.g., laryngeal narrowing, compressive syndromes) [132].

Sulfonamides are also effective and low in cost. In Brazil, the combination sulfamethoxazole-trimethoprim has been preferentially used for treating PCM patients (Table 21.2). In less severe forms of the disease, this medication should be administered for a period of 2 years, although high dropout rates have been reported (Table 21.2) [129, 133]. Tolerability is good, with myelotoxicity (leukopenia) being the main side effect. Patients should be monitored for leukopenia, as folinic acid administration can be used to reverse this effect, without modification of the therapeutic regimen, in many patients. Other side effects include rash, gastritis, and interstitial nephritis, all reversible with the interruption of treatment. The above combination has the advantage of permitting parenteral administration when necessary. Development of resistance to trimethoprim-sulfamethoxazole is clinically suspected occasionally, but has rarely been documented in vitro [134]. Brazilian physicians often employ trimethoprim-sulfamethoxazole (80 mg of trimethoprim and 400 mg of sulfamethoxazole per tablet) given at a dose of two or three tablets and administered orally at 12-h intervals for adults or 8–10 mg/Kg/day (based on the trimethoprim) component in pediatric cases. This treatment can also be used in association with amphotericin B. In severe forms of the disease, the treatment regimen involves intravenous (IV) administration of sulfamethoxazole 800 mg/trimethoprim 160 mg, every 8 h until clinical improvement of the patient, followed by administration of oral antifungal drugs [134].

Another treatment option for severe PCM forms is the administration of amphotericin B deoxycholate (AmBd) at a dose of 1 mg/kg/day (maximum 50 mg/day), dissolved in dextrose 5%, administered daily (Table 21.2). Patients may need to be hospitalized for side effects caused by the drugs [134, 135]. The patient should remain in treatment and under follow-up management until achieving apparent resolution, with assessment based on clinical, radiological, and serological parameters. To avoid AmBd nephrotoxicity, a liposomal AmB formulation (AmBisome) may be used in some cases [127]. Three AmB lipid formulations have been developed and are now available in most countries: AmB colloidal dispersion (ABCD), AmB lipid complex (ABLC), and liposomal AmB (Ambisome). These are highly effective, but due to their high cost their use is often restricted to specific situations such as severe illness, renal involvement, patients with nephrotoxicity risks, severe gastrointestinal disease associated with malabsorption syndromes, extensive gastrointestinal symptoms leading to deficient food intake, and CNS involvement [136].

Most of the systemically absorbed azole antifungals are effective for PCM therapy. Currently, ketoconazole has been replaced by itraconazole, which is considered the best option for the treatment of PCM [134]. A comparative study showed that although both itraconazole and trimethoprim-sulfamethoxazole resulted in restoration of patients' health, itraconazole promoted a more rapid clinical and serological cure [137]. Itraconazole is regularly administered at a dose of 200–400 mg/day for at least 6 months of therapy: duration is based on cure parameters. In severe adult cases, higher doses (400 mg/day) can be used. Itraconazole has been shown to be effective in 95% of the patients with minimal adverse effects; relapses occur in 5% of the cases. Despite this high response rate, itraconazole has not reduced the fibrous pulmonary sequelae [80]. With the administration of capsules of itraconazole, medications that reduce gastric acidity such as proton pump inhibitors and histamine-2 blockers should be avoided. Itraconazole oral solution exhibits superior bioavailability, near 80%, and this absorption is

not affected by gastric acidity or food intake [134]. Although used in a small number of patients, voriconazole and isavuconazole have been shown to have inhibitory action in vitro and in vivo [130, 138]. Fluconazole has been less used than itraconazole in the treatment of PCM due to its higher in vitro MICs, although initial in vivo studies showed good results [139], and, for its better passage through the blood-brain barrier, it has occasionally been used with good results in neuro-PCM cases. Echinocandins have poor antifungal activity against *Paracoccidioides* spp.

Cure (if ever attained considering the long-lasting latency periods of *Paracoccidioides* spp. [20]) should be evaluated by using clinical, mycological, radiological, and serological parameters such as (1) absence or regression of signs and symptoms, (2) disappearance of the fungus especially from respiratory samples, (3) presence of scarring and fibrosis on radiologic studies, and (4) negative antibodies titers or stabilization of titers at low levels [134].

Prevention

Preventive measures are difficult to place into effect as the source of infection is unknown. PCM is not transmissible from person to person. Nonetheless, precaution against aerosols is recommended when felling trees in known endemic areas or hunting armadillos in the forest [11, 140].

The use of antifungals in combination with immunomodulatory compounds, as well as biological therapies or prophylactic vaccination employing a *Paracoccidioides* spp. recombinant antigen, may successfully attenuate pulmonary inflammation and fibrosis, and is a promising therapeutic strategy. It will be necessary to move these experimental studies to clinical trials [141].

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Sporotrichosis

22

Carol A. Kauffman and Marisa H. Miceli

Introduction

Sporotrichosis is a subacute to chronic fungal infection of skin and subcutaneous tissues that occurs throughout the world [1]. Most cases of sporotrichosis arise from direct inoculation of the organism from soil, vegetation, or wood into the subcutaneous tissues. Subsequent spread along the lymphatics draining the primary lesion is common, but hematogenous spread is rare. The organism is occasionally inhaled from the environment and causes pneumonia. Zoonotic transmission has been highlighted in the last two decades by a large outbreak of cat-associated sporotrichosis that began in Rio de Janeiro, Brazil and has subsequently spread to other areas [2].

Etiologic Agent

Sporothrix schenckii, named after Dr. Schenck who described the first case of sporotrichosis in Baltimore in 1898, is the cause of sporotrichosis. However, it is now clear that *S. schenckii* is not a single species, but rather a complex of several phylogenetically different organisms, each with different geographic sites and each with varying levels of pathogenicity for humans [2–6]. *S. schenckii* sensu stricto remains the most common etiologic agent of sporotrichosis; it has a worldwide distribution and predominates in almost all countries except for those in Latin America. Most clinical laboratories do not use the term "sensu stricto" and do not differentiate among the species within this complex; they merely refer to the isolate as *S. schenckii*. That simplified terminology will be used in this chapter, as well.

Currently, it appears that there are at least 5 other species in addition to *S. schenckii* sensu stricto in the *S. schenckii* complex [7]. The most important is *S. brasiliensis*, which occurs in Brazil and other countries in Latin America and is responsible for a large zoonotic outbreak among cats and humans in Rio de Janeiro [2]. *S. brasiliensis* shows important differences in epidemiology, virulence, and antifungal susceptibility from other species in the complex. *S. globosa* is found worldwide, but most human infections are reported from Asia [8]. *S. mexicana* is present in Mexico and other Latin American countries. *S. luriei* and *S. pallida* rarely cause infection in humans [1].

S. schenckii is a dimorphic fungus that exists as a mold in the environment and as a yeast in tissues [9]. The dimorphism is temperature dependent. In the environment and in the laboratory, at 25–27 °C, *S. schenckii* is a mold with thin, septate, branching hyphae that have conidia that can be either dark or hyaline and that tend to arrange themselves along the hyphae in "bouquet-like" configurations (Fig. 22.1). In the laboratory, on Sabouraud's dextrose agar, growth of a white to cream-colored mold occurs within 1–2 weeks. The colony becomes brown or black and assumes a wrinkled appearance over the ensuing weeks (Fig. 22.2).

In tissues and in vitro at 37 °C, *S. schenckii* assumes a yeast-like form. The yeasts are 4–6 μm in diameter and show budding that can be single or multiple; they are classically described as being cigar-shaped, although round and oval forms are also seen (Fig. 22.3). In the laboratory, growth of the yeast phase is accomplished by incubation at 35–37 °C using enriched media, such as brain heart infusion (BHI)

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_22

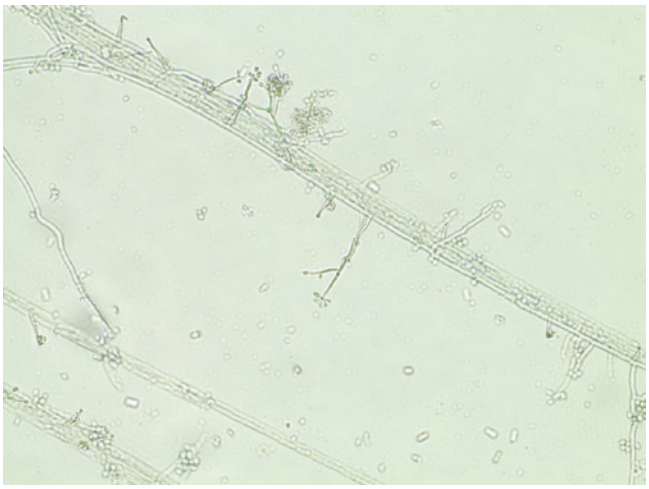


Fig. 22.1 Microscopic view of the mold form of *Sporothrix schenckii* grown at 25 °C on Sabouraud dextrose agar. Note the thin septate hyphae with conidiophores that bear oval conidia that appear "bouquet-like". (Courtesy of Dr. D. R. Hospenthal)

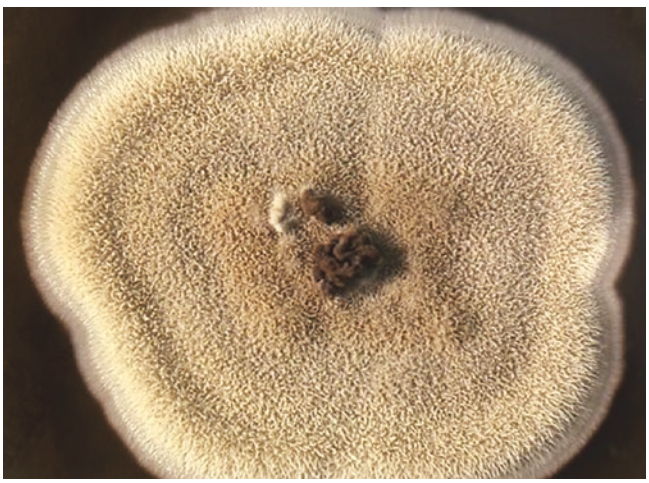


Fig. 22.2 Colony of *Sporothrix schenckii* grown at 25 °C on malt extract agar. Initially cream-colored, the colony darkens over time

agar. The colony morphology of *S. schenckii* in the yeast phase is usually off-white and wrinkled. Some strains of *S. schenckii* do not grow well at 37 °C but do grow at 35 °C.

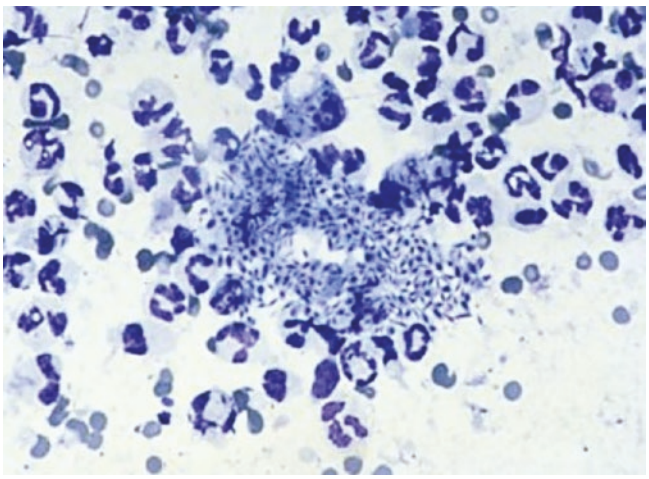


Fig. 22.3 Smear of an ulcerated lesion caused by *Sporothrix schenckii* showing a large number of oval and cigar-shaped yeasts, 4–6 μm in diameter. (From Reed et al. Zoonotic transmission of sporotrichosis: case report and review. Clin Infect Dis 1993;16:384–387. Reprinted with permission from Oxford University Press. Courtesy of Dr. K. Reed)

Epidemiology

Sporothrix species are found throughout the world [1]. In the environment, these organisms are found in sphagnum moss, decaying wood, vegetation, hay, and soil. For infection to occur, one must be exposed to an environmental source, and the organism must be inoculated through the skin. This can occur with motor vehicle accidents, hay baling, landscaping, tree farming, and in developing countries, often just the activities of daily living [10–13].

The typical person who develops sporotrichosis is a healthy person whose occupation or hobby takes him or her into the out-of-doors. Classically, landscapers and gardeners develop sporotrichosis because they are exposed to contaminated materials and their activities frequently lead to nicks and cuts on their extremities, allowing easy access for the organism.

Zoonotic transmission can occur from infected animals or from soil transferred from the nails of burrowing animals,

such as armadillos [14, 15]. Cats develop ulcerated skin lesions, often on the face, and many die of the infection. These ulcers are teeming with organisms and are highly infectious [1]. Sporotrichosis also has occurred in laboratory workers who, in the course of handling infected animals or culture material, have inoculated themselves or splashed material into their eye [16].

Outbreaks of sporotrichosis are not uncommon and have been traced back to a variety of point sources: contaminated timbers in a mine, sphagnum moss packed around Christmas trees, bushes, or seedlings, and hay used for Halloween parties [10, 17–19]. However, the outbreak in Rio de Janeiro associated with transmission of *S. brasiliensis* from infected cats has proved to be massive and has continued since at least 1998 [20]. This outbreak has affected women and children disproportionately, perhaps because they have the most exposure to cats. The majority of those infected live in the poorer areas of the city. Over 4000 cases in humans and similar numbers in cats have been reported from one medical center in Rio de Janeiro alone [2]. Spread has been documented into other areas of Brazil and to other countries in Latin America [2, 21]. *S. brasiliensis* is the cause, and the organisms appear to have originated from one common source [22].

Cats usually have ulcerated lesions, often on the face, that contain a huge number of organisms. Cats that appear well or have only mild respiratory symptoms can be colonized and spread the organism to other cats and to humans [20, 23]. Dogs have been noted to develop sporotrichosis after contact with ill cats, but do not appear to be a source for transmission to humans [24].

Pathogenesis and Immunology

Infection with *S. schenckii* is almost always initiated when the mold that is present in the environment is inoculated into the skin, usually through minor trauma. Inhalation of the conidia of *S. schenckii* is the presumed method of transmission in the uncommon syndrome of primary pulmonary sporotrichosis. Components of the cell wall of *Sporothrix* species, especially a 70 kDa glycoprotein (gp70), mediate adhesion to extracellular matrix and endothelial cell surface proteins, initiating invasion [25, 26]. Studies comparing *S. brasiliensis* with *S. schenckii* have noted that *S. brasiliensis* has a thicker cell wall with a greater amount of chitin and rhamnose and less gp70 glycoprotein; these changes might explain the greater virulence of *S. brasiliensis* [27]. Melanin is likely an important virulence factor as it is in many fungi [28].

The innate immune system plays a crucial role in the initial response to *Sporothrix* species [9]. Neutrophils, monocytes, and macrophages that can ingest and kill the yeast cells of *S. schenckii* are the first line of defense; these cells are triggered primarily by toll-like receptors and other pattern recognition receptors that recognize cell wall components of *Sporothrix* species [29–31]. Antibody appears to play a minimal, if any, role in immunity to *Sporothrix* species. In contrast, cell-mediated immunity appears to be crucial in containing infection [32, 33]. The importance of cell-mediated immunity is supported primarily by clinical reports of disseminated sporotrichosis occurring in patients who have advanced HIV or hematological cancers, have received a solid organ transplant, or have been treated with tumor necrosis factor antagonists 34–41].

Clinical Manifestations

The usual manifestation of sporotrichosis is localized lymphocutaneous infection. Most patients who present with typical lymphocutaneous sporotrichosis are healthy hosts. Extensive disseminated cutaneous lesions and spread to other structures, including joints, meninges, lungs, and other organs almost always occur in those who have certain underlying illnesses. Alcoholism and diabetes mellitus are two risk factors for more severe sporotrichosis and for non-cutaneous forms of sporotrichosis [33, 42–44]. Chronic obstructive pulmonary disease is almost always present as an underlying condition in patients who have pulmonary sporotrichosis [45], and disseminated sporotrichosis is rare unless cell-mediated immunity is suppressed [33] (Table 22.1).

Table 22.1 Clinical manifestations of sporotrichosis

Clinical syndrome	Known risk factors	Initiation of infection
Lymphocutaneous	None	Local inoculation
Fixed cutaneous	None	Local inoculation
Osteoarticular	Alcoholism, diabetes	Local inoculation or hematogenous spread
Pulmonary	COPD, alcoholism	Inhalation
Meningitis	HIV, other immunosuppression	Hematogenous spread
Other focal disease (eye, breast, larynx, pericardium, epididymis, rectum, spleen, liver)	None known	Hematogenous spread or local inoculation
Disseminated	HIV, TNF antagonist therapy, other immunosuppression	Hematogenous spread

COPD chronic obstructive pulmonary disease, *TNF* tumor necrosis factor



Fig. 22.4 Typical skin lesions in lymphatic distribution seen in a patient who was a horticulturist and had inoculation of *Sporothrix schenckii* in the subcutaneous tissue of the wrist. (From Watanakunakorn C. Answer to Photo Quiz. Clin Infect Dis 1996, 22:765. Reprinted with permission from Oxford University Press)



Fig. 22.5 Fixed cutaneous skin lesion of sporotrichosis. In this form of the disease, lymphatic spread does not occur, and the lesion may remain for months to years until treated. (From Kauffman CA. Sporotrichosis. Clin Infect Dis 1999;29:231–237. Reprinted with permission from Oxford University Press)

Lymphocutaneous Sporotrichosis

The first manifestation of infection generally occurs several days to weeks after cutaneous inoculation of the fungus when a papule appears at the site of inoculation. This primary lesion becomes nodular and usually will eventually ulcerate. Drainage from the lesion is minimal, is not grossly purulent, and has no odor. Pain is generally mild, and most patients have no systemic symptoms. Over the next few weeks, new nodules that often ulcerate appear proximal to the initial lesion along the lymphatic distribution (Fig. 22.4).

The most important diseases in the differential diagnosis are infection with *M. marinum* or another non-tuberculous *Mycobacterium* (NTM), leishmaniasis, and nocardiosis, especially that caused by *Nocardia brasiliensis* [46, 47]. Rarely, other bacterial, fungal, and even viral infections can be associated with a similar lymphocutaneous syndrome [47].

Fixed cutaneous sporotrichosis is uncommon in North America, but common in South America (Fig. 22.5). Patients with this form of sporotrichosis manifest only a single lesion, often on the face, that can be verrucous or ulcerative [12]. The lesion may regress and flare periodically and can be present for years until it is treated. Pain and drainage are not prominent symptoms.

Pulmonary Sporotrichosis

Primary pulmonary sporotrichosis is a subacute to chronic illness that usually occurs in patients who have chronic obstructive pulmonary disease; alcoholism is a risk factor in a smaller proportion of patients [45, 48–50]. The symptoms mimic those of reactivation tuberculosis. Patients have fever, night sweats, weight loss, and fatigue; dyspnea, cough, purulent sputum, and hemoptysis also occur frequently. Chest radiography shows unilateral or bilateral fibronodular or cavitary disease; the upper lobes are preferentially involved (Fig. 22.6). This form of sporotrichosis must be differentiated from tuberculosis, NTM infections, chronic cavitary histoplasmosis, blastomycosis, and sarcoidosis. Patients with primary pulmonary sporotrichosis may or may not have disease elsewhere, especially involving skin and osteoarticular structures.

Pulmonary sporotrichosis that occurs as a manifestation of disseminated infection differs from primary pulmonary sporotrichosis in several regards. This form of pulmonary involvement occurs almost entirely in immunocompromised patients [33]. The mode of infection of the lungs is hematogenous from cutaneous or visceral lesions. Cavitary pulmonary lesions are uncommon, and reticulonodular or diffuse infiltrates favoring the lower lobes are the usual radiographic finding [44].

Osteoarticular Sporotrichosis

Osteoarticular sporotrichosis is an uncommon manifestation of infection with *S. schenckii* that can occur after local inoculation, but can also arise from hematogenous spread [51–53]. It is found most often in middle-aged men and appears to occur more frequently in alcoholics. Overlying cutaneous lesions may or may not be present, and one or more joints may be involved. Most commonly, the knees, elbows, wrists,

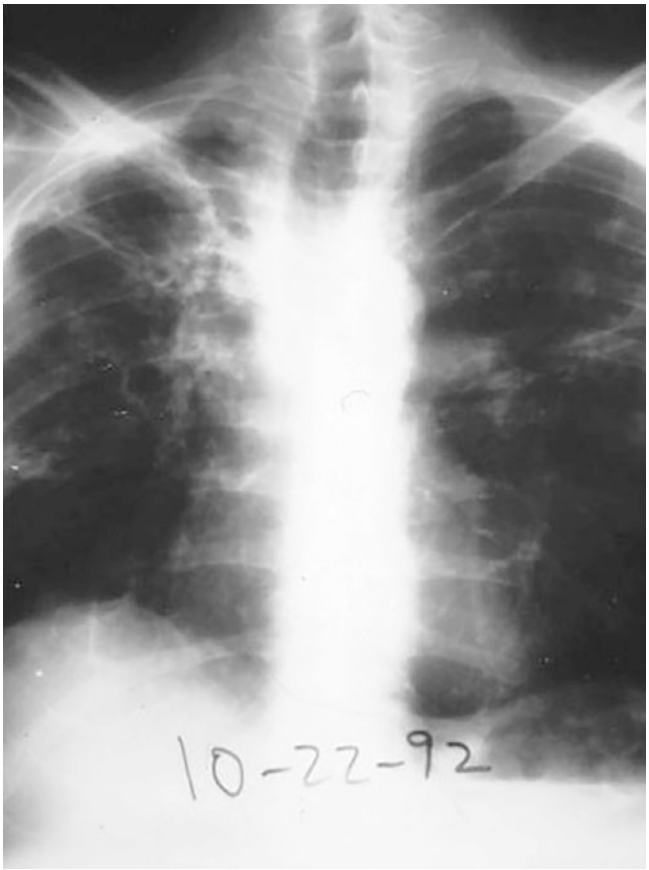


Fig. 22.6 Chest radiograph of a patient with pulmonary sporotrichosis. The patient was an alcoholic who also had diabetes mellitus and chronic obstructive pulmonary disease

and ankles are infected. Bone involvement usually occurs contiguous to an infected joint (Fig. 22.7). Bursitis and tenosynovitis, the latter presenting as nerve entrapment, also have been described [54].

Central Nervous System Sporotrichosis

Central nervous system sporotrichosis can present as isolated chronic meningitis, but more commonly is just one manifestation of widespread disseminated infection [55]. Chronic meningitis caused by *Sporothrix* species mimics tuberculous meningitis or meningitis caused by *Histoplasma capsulatum* and has been reported primarily in non-immunocompromised hosts [56–58]. This form of meningitis presents with weeks to months of gradually increasing headaches, confusion, and gait disturbance.

Meningitis as a manifestation of disseminated infection almost always occurs in patients who have cellular immune defects, primarily those with advanced HIV infection [59, 60]. Acute onset of fever, headache, and mental status changes are prominent symptoms.



Fig. 22.7 Radiograph of the elbow of a patient who had osteoarticular sporotrichosis manifested by infection of both elbows and one knee. There is destruction of the joint and adjacent osteomyelitis of the radius, ulna, and humerus

Disseminated Sporotrichosis

Disseminated sporotrichosis is very uncommon. Almost all patients have had cellular immune deficiencies, and most cases have occurred in persons with AIDS [59, 61–63]. Fungemia has been noted in a few patients [63]. *Sporothrix* species have been reported very rarely to cause infection of eye, larynx, breast, pericardium, spleen, liver, bone marrow, lymph nodes, rectum, and epididymis. There is a suggestion that these atypical presentations might be more commonly seen in patients infected with *S. brasiliensis* [64].

Diagnosis

Culture yielding a *Sporothrix* species is the gold standard for establishing the diagnosis. Biopsy or material aspirated from a cutaneous lesion should be sent to the laboratory for culture. Sputum, synovial fluid, blood, and cerebrospinal fluid can also yield the organism in patients who have visceral involvement. Material obtained for culture should be inoculated onto Sabouraud's dextrose agar and incubated at room temperature to allow growth of the mold phase. Growth usually occurs within 1–3 weeks. The characteristic arrangement of conidia on the hyphae makes the diagnosis likely, but conversion to the yeast phase at 35–37 °C, which may take several additional weeks, allows definitive identification of the organism as belonging to the genus *Sporothrix*.

Of increasing scientific and epidemiological importance is the ability to differentiate the various species within the *S. schenckii* complex. This requires either specific tests that show phenotypic differences in growth characteristics and assimilation of several sugars [3, 4] or molecular methods that involve calmodulin sequencing, restriction fragment length polymorphisms, or real-time PCR techniques [2, 65, 66]. None of these techniques is available in clinical laboratories, and for most patients, identification of the infecting organisms as *S. schenckii* complex is sufficient. It is likely that mass spectrometry (MALDI-TOF MS) soon will provide clinical laboratories with identification to the species level within the complex [67].

The histopathology of sporotrichosis reveals a mixed granulomatous and pyogenic inflammatory process [68]. The organism is an oval to cigar-shaped yeast, 3–5 μm in diameter, and can exhibit multiple buds. However, it is difficult to visualize the organisms within tissues, even with the use of methenamine silver or periodic acid Schiff stains primarily because few organisms are needed to cause lymphocutaneous disease in humans. In contrast, cytopathological examination of material from lesions in cats with sporotrichosis is a sensitive method for diagnosis because the lesions usually are teeming with large numbers of organisms [65]. In some cases, an asteroid body, in which the basophilic yeast is surrounded by eosinophilic material radiating outward like spokes on a wheel, can be seen [69]. This is also known as the Splendore–Hoeppli phenomenon and is not specific for sporotrichosis, but can be seen in various parasitic, fungal, and bacterial infections. This reaction is thought to be due to deposition of antigen–antibody complexes around the organism in tissues.

Studies have been ongoing for several years to develop a serological test to aid in the diagnosis of sporotrichosis [66]. A recently developed enzyme immunoassay has the potential to be sensitive and specific enough to become a useful diagnostic test [70, 71], but it is not widely available. An assay available from Mayo Laboratories is similar to a latex agglutination assay that was shown decades ago to be useful for the diagnosis of sporotrichal meningitis; in some individual cases this assay been shown useful for diagnosing this rare form of sporotrichosis [57].

In early studies, a nested polymerase chain reaction (PCR) assay showed high sensitivity and specificity in a small number of tissue samples from humans with sporotrichosis [72]. Modifications of this technique have resulted in enhanced sensitivity, and further modifications have allowed detection and differentiation of DNA from different *Sporothrix* species in tissue samples [73, 74]. However, PCR assays are available only through reference laboratories at the present time.

Treatment

In general, most patients who have sporotrichosis are treated with an oral azole antifungal agent. Those patients who have disseminated infection, meningitis, or severe pulmonary involvement are treated initially with an intravenous amphotericin B formulation. Guidelines for the management of the various forms of sporotrichosis have been published by the Infectious Diseases Society of America and the European Confederation of Medical Mycology [75, 76]. The suggestions that follow are modified from these guidelines (Table 22.2).

As molecular methods have defined new species in the *S. schenckii* complex, in vitro studies have shown different patterns of susceptibility for these different species [22, 77–80]. *S. brasiliensis* appears to be slightly more susceptible and *S. mexicana* more resistant, but in vitro susceptibility studies do not necessarily translate into efficacy in vivo and very few strains of the uncommon species have been tested. Overall, the most active drugs in vitro are itraconazole, terbinafine, and posaconazole. Fluconazole and voriconazole exhibit high MICs for *Sporothrix* species.

Table 22.2 Treatment of sporotrichosis

Clinical syndrome	Primary therapy	Alternate therapy	Duration
Lymphocutaneous and cutaneous	Itraconazole 100–200 mg/d	SSKI, titrated dose Terbinafine 500 mg bid Hyperthermia	Until lesions resolve; usually 3–6 months
Pulmonary	Itraconazole 200 mg bid	Lipid AmB, 3–5 mg/kg/d for severe disease	AmB: several weeks Itraconazole: at least 1 year
Osteoarticular	Itraconazole 200 mg bid	Lipid AmB, 3–5 mg/kg/d	AmB: several weeks Itraconazole: at least 1 year
Meningitis	Lipid AmB, 5 mg/kg/d, then transition to itraconazole, 200 mg bid	AmB deoxycholate, 0.7–1 mg/kg/d, then transition to itraconazole, 200 mg bid	AmB: 4–6 weeks Itraconazole: at least 1 year or life-long suppression
Disseminated	Lipid AmB, 3–5 mg/kg/d Then transition to itraconazole, 200 mg bid	AmB deoxycholate, 0.7–1 mg/kg/d, then transition to Itraconazole 200 mg bid	AmB: several weeks Itraconazole: at least 1 year or life-long suppression

AmB amphotericin B, *bid* twice daily, *SSKI* saturated solution potassium iodide

Lymphocutaneous Sporotrichosis

Itraconazole is the drug of choice for the treatment of this form of sporotrichosis [75, 76, 81–83]. The dosage recommended is 200 mg daily, and response rates as high as 90% are commonly noted. Barros et al. reported a 90% response rate among 610 patients treated with a dosage of only 100 mg daily [81]. The oral suspension achieves higher serum levels, but is less well tolerated and is more expensive than the capsule formulation. If itraconazole capsules are used, the patient cannot take acid-inhibiting drugs, such as antacids, proton pump inhibitors, or H2 blockers and should take the capsules with food to ensure adequate absorption. A new formulation of "super bioavailable" itraconazole (SUBA-itraconazole) with rapid and reliable absorption in adults is now available; however, data to support its use for the treatment of sporotrichosis are not available. Treatment should continue until the lesions have resolved; this usually takes 3–6 months.

Saturated solution of potassium iodide (SSKI) has been used successfully to treat lymphocutaneous sporotrichosis for decades. It still is not clear how SSKI inhibits *S. schenckii* [84]. The initial dose is 5–10 drops three times daily in juice or milk, increasing weekly to a maximum of 40–50 drops three times daily, as tolerated. Side effects are very common and include nausea, rash, metallic taste, fever, salivary gland swelling, and thyroid dysfunction [85, 86].

Several other less effective options exist if the patient is unable to tolerate itraconazole or SSKI. Terbinafine is active in vitro against most *Sporothrix* species [22, 77, 80]. One study noted that high doses of terbinafine (500 mg twice daily) should be used, but another used only 250 mg twice daily with similar results [87, 88]. However, experience is limited, and this agent should be used only in patients who fail standard therapy with itraconazole.

Higher dosages of fluconazole (400–800 mg daily) have been used with only modest success in a few patients, and this agent is not recommended for treatment of sporotrichosis [89]. Posaconazole has in vitro activity similar to that of itraconazole [22, 80], and it was efficacious in a murine model of systemic sporotrichosis [90]. Posaconazole has been used successfully in a cat with sporotrichosis and in a small number of patients [39, 91–93]. Voriconazole exhibits only modest activity against *Sporothrix* species and should not be used to treat sporotrichosis [79, 80]. There are no data to support the use of isavuconazole for the treatment of sporotrichosis.

Local hyperthermia can be used to treat cutaneous sporotrichosis; it is less effective for lymphocutaneous sporotrichosis [94, 95]. A variety of different warming devices are available, but each must be used faithfully for months to effect improvement in cutaneous lesions. Local hyperthermia can be useful in pregnant women who should not be treated with azole agents.

Pulmonary Sporotrichosis

Pulmonary sporotrichosis can be quite recalcitrant to therapy. If the patient is seriously ill, amphotericin B, preferably as a lipid formulation, 3–5 mg/kg daily, should be used initially [44, 75]. When the patient is stable, therapy can be changed to oral itraconazole, 200 mg twice daily. The duration of therapy should be at least 1 year and perhaps longer for some patients. Surgical resection should be considered, especially for those patients who have a single cavitary lesion. Outcomes have improved in the last decade, but the response rates are still only about 50%.

Osteoarticular Sporotrichosis

This form of sporotrichosis, which is almost always chronic and not life threatening, can be treated with an oral antifungal agent [75]. Itraconazole is the agent of choice, and the dosage is 200 mg twice daily. Therapy should continue for at least 1 year and perhaps longer in some patients. Amphotericin B, preferably a lipid formulation at 3–5 mg/kg/day is the only remaining option. Intra-articular, amphotericin B is not recommended [96]. Even if cure occurs, joint function rarely is recovered.

Central Nervous System Sporotrichosis

A lipid formulation of amphotericin B, 5 mg/kg daily, for 4–6 weeks is the preferred treatment [55, 75]. Itraconazole is used as step-down therapy after the patient has responded to amphotericin B. The total length of therapy is at least 1 year, and long-term suppressive therapy is needed in patients who remain immunocompromised.

Disseminated Sporotrichosis

For those rare individuals who appear to have disseminated disease confined to the skin, oral itraconazole has been used as sole therapy [34]. However, for most patients with disseminated sporotrichosis, initial therapy should be with a lipid formulation of amphotericin B, 3–5 mg/kg daily. Outcomes in AIDS patients were dismal in cases reported prior to effective anti-retroviral therapy. However, current response rates are reported to be as high as 81% [34]. For

sporotrichosis involving other organ systems, therapy is individualized based on the organ involved and the immune status of the host.

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Talaromycosis

23

Thuy Le, Vu Quoc Dat, and H. Rogier van Doorn

Introduction

Talaromycosis (formerly penicilliosis) is an invasive fungal infection caused by the dimorphic fungus *Talaromyces marneffei* (formerly *Penicillium marneffei*) endemic in Southeast Asia, southern China, and northeastern India. Talaromycosis is one of the seven endemic mycoses of medical importance. Talaromycosis primarily affects individuals with a compromised immune system, but occasionally affects apparently healthy individuals [1, 2]. Talaromycosis is characterized by subacute infection of the reticuloendothelial system that can be localized to the lungs or to the gastrointestinal tract, or disseminated to multiple organ systems, including the liver, spleen, lymph nodes, skin, blood stream, bone marrow, bone, joints, and central nervous system [2–4].

*T. marneffei*was discovered in the liver of a captive bamboo rat (*Rhizomys sinensis*) used for experimental Tsutsugamushi infection in Vietnam in 1956 and was named after Dr. Hubert Marneffe, Director of Institute Pasteur in Vietnam at the time [5]. Human infection was first described by Gabriel Segretain in 1959 as a laboratory accident involving self-inoculation resulting in a cutaneous infection with lymphangitis [6]. The first natural infection was described in 1973 in an American man with Hodgkin lymphoma who developed a splenic mass 3 years after travel to Southeast Asia [7]. The HIV pandemic has led to a rapid rise in incidence, with talaromycosis accounting for 4% to 16% of HIV admissions in the endemic area [4, 8, 9]. Incidence is rising due to increasing international travels and migration [10, 11] and due to increasing use of immunosuppressive therapy for autoimmune diseases, cancers, and organ transplantation [12]. The infection has substantial morbidity and mortality, with case fatality ranging from 10% to 50% despite antifungal therapy [4, 8, 9].

Etiologic Agent

T. marneffei was formerly classified under the *Penicillium* subgenus *Biverticillium* based on morphological characteristics. The subgenus *Biverticillium* was later found to form a monophyletic group with *Talaromyces* that is distinct from *Penicillium* and was taxonomically unified with the *Talaromyces* genus in 2011 [13]. Hence, *Penicillium marneffei* was changed to *Talaromyces marneffei*, and the disease penicilliosis is now called talaromycosis.

T. marneffei is the only known species in the genera of *Talaromyces* that exhibits temperature-dependent dimorphism and is pathogenic in humans. It demonstrates the ability to switch from its environmental mold form at temperatures 25 °C to 35 °C to a parasitic yeast form at body temperature (above 35 °C). This dimorphic switching process is completely reversible in the laboratory [14]. When cultured in artificial medium in the laboratory, the mold form produces yellow green colonies and a bright red pigment that diffuses into the medium, the latter is very specific for *T. marneffei*. In human clinical tissue the yeast form is round or oval with a diameter of 2–3 μm (Fig. 23.1). However, in laboratory media, the yeast form has a transitional sausage shape and does not produce a red diffusible pigment. Unique among pathogenic yeasts which predominantly divide by budding, *T. marneffei* divides by binary fission. The identifi-

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_23

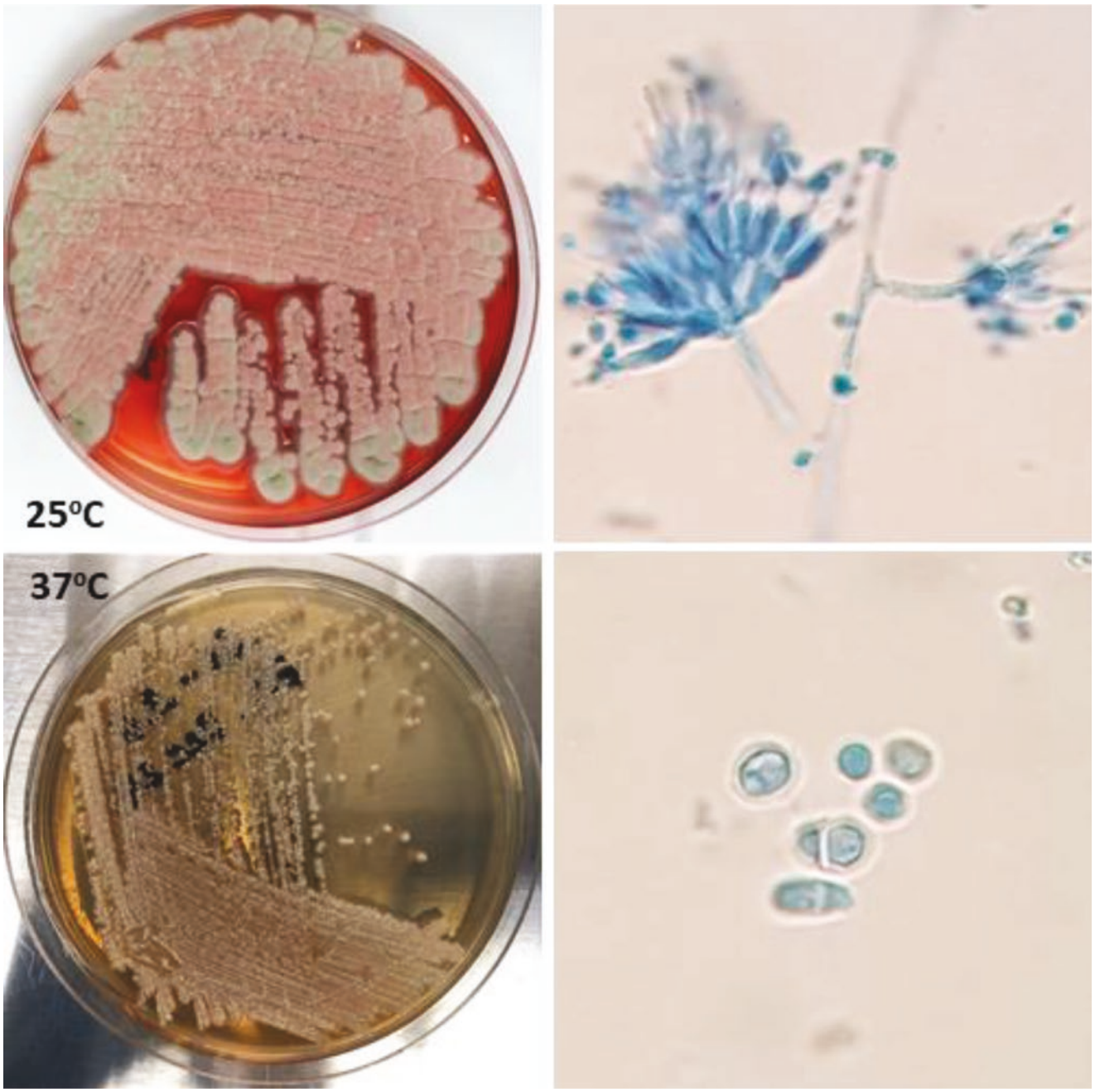


Fig. 23.1 Morphology of *Talaromyces marneffei* colonies and cells grown at 25 °C and at 37 °C on Sabouraud agar medium

cation of a midline septum in a dividing yeast cell in clinical specimens or cultures of clinical specimens, the demonstration of thermal dimorphism in culture, and the presence of a diffusible red pigmentation in the mold form are pathognomonic for *T. marneffei*.

The draft genome of *T. marneffei* is comprised of at least 7 chromosomes [15]. The complete genomes of *T. marneffei* in two patients in Vietnam consisted of 8–9 contigs with a total length of 28.2 Mb and a GC contents of 46.8%. An intrachromosomal rearrangement was observed between the two isolates. The number of protein coding genes was approximately 10,000 [16].

Epidemiology

Talaromycosis is endemic in the tropical and subtropical zones of Southeast Asia (particularly in northern Thailand, Vietnam, Myanmar), East Asia (southern China, Hong Kong, Taiwan), and South Asia (northeastern states of India) [17]

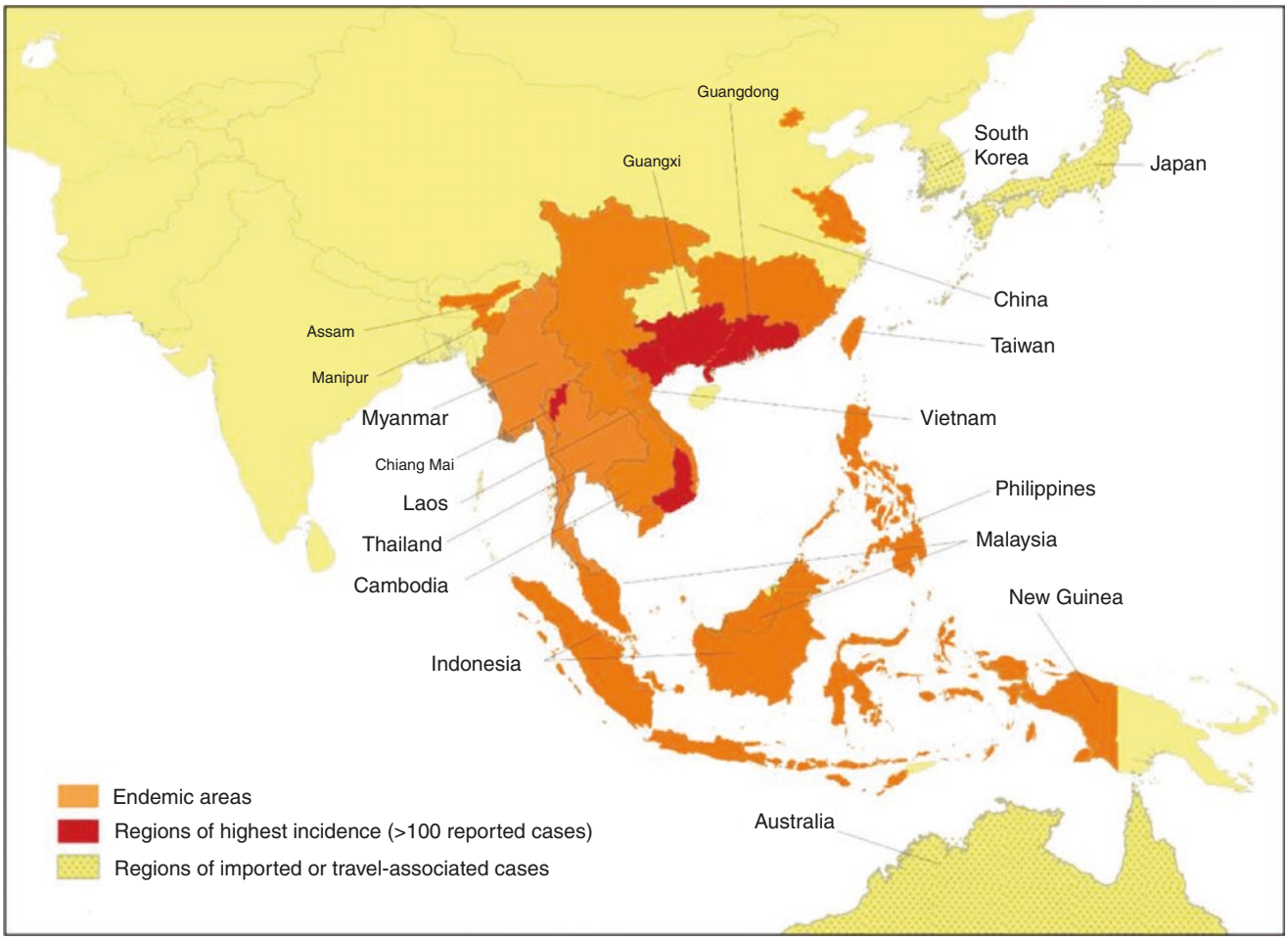


Fig. 23.2 Geographical distribution of talaromycosis

(Fig. 23.2). Talaromycosis has been reported worldwide in returning travelers or immigrants from the endemic region [10, 11]. At least 288,000 talaromycosis cases (95% confidence interval [CI]: 146,000–613,800) and 87,900 deaths (95% CI: 37,200–204,300) have been reported in the literature in 33 countries to the end of 2018 [18]. The vast majority of cases (99.7%) occur in the endemic region, with 0.5% occurring in children as young as 3 month old [18, 19]. HIV is the major risk factor for talaromycosis, accounting for 90% of global talaromycosis burden [18]. The prevalence of talaromycosis in patients with advanced HIV disease (CD4 counts <100 cells/mm^3) was the highest in southern China (15.0%), followed by Vietnam (6.4%) and Thailand (3.9%) [20]. Talaromycosis is increasingly recognized in individuals who have a primary immunodeficiency condition (e.g., idiopathic CD4 lymphopenia, anti-interferon-gamma autoantibody-associated immunodeficiency in Southeast Asian descent, conditions due to mutations in CYBB, CD40L, or gain-of-function mutation in STAT1/STAT3 pathways) or a secondary immunodeficiency conditions (e.g., autoimmune diseases in patients on corticosteroids and/or other immunosuppressive therapy, solid and hematological malignancies, solid organ transplantation, hematopoietic stem cell transplantation, and therapy with novel target therapies such as monoclonal antibodies against CD20 and kinase inhibitors) [12].

Humans and all four species of soil-burrowing bamboo rats living in the highlands of Southeast Asia (*Rhizomys sinensis, R. pruinosus, R. sumatrensis, and Cannomys badius*) are the only known natural reservoirs of *T. marneffei* [2]. The geographic distribution of cases follows the natural distribution of bamboo rats; however, case control studies have suggested that human talaromycosis is a result of exposure to soil-related agricultural cultivation of farms and livestock, rather than from exposure or consumption of bamboo rats [21, 22]. Cases increase 30% to 70% during the rainy months [4, 23, 24] and are associated with humidity rather than precipitation [25, 26]. Collectively these studies suggest that humidity facilitates an expansion of disease reservoir in the environmental resulting in increased infections. One case of presumed laboratory-acquired infection was reported outside of the endemic region in an HIV-

infected man from Africa who participated in a mycology course at the Institute of Pasteur in Paris where *T. marneffei* colonies were shown [27]. However, laboratory-acquired infection has not been reported from the endemic countries where *T. marneffei* is routinely cultured and handled in standard microbiology laboratories. A case of donor-acquired transmission has been reported in a lung transplant recipient from Belgium. The donor had traveled to Myanmar 3 months prior and visited several caves and pagodas in rural areas during a 20-day trip [28].

Pathogenesis and Immunology

The lung is the dominant portal of entry of *T. marneffei.* Studies in wild bamboo rats have found the highest fungal burden in the lungs (83.3%), followed by the liver (33.3%) and spleen (33.3%) [29]. A murine inhalation model delivering nebulized *T. marneffei* conidia in a chamber to mice demonstrated that the lungs were the primary organ of infection, with invasive pulmonary disease occurring in 65% of exposed mice [30]. The dimorphic switching from mold to yeast form is important for virulence and is associated with alteration of cell wall composition and expression of yeast-growth specific proteins used to evade the immune system [31, 32]. At the host–pathogen interphase, *T. marneffei* conidia adhere to bronchial epithelial cells by attaching to extracellular matrix laminin and are phagocytosed by pulmonary alveolar macrophages [33]. *T. marneffei* evades macrophage killing by producing superoxide dismutase and catalase-peroxidase to prevent digestion by lysosomes [34]. *T. marneffei* further evades the host defense by downregulating IL-6, a proinflammatory cytokine produced by bronchial epithelial cells [35], and by producing a large quantity of galactomannan protein Mp1p, a virulent factor that effectively captures arachidonic acid and disrupts the host proinflammatory cascade [36]. The establishment and proliferation of infection inside macrophages enables *T. marneffei* to disseminate through the reticuloendothelial system causing multi-organ infection.

Defects in cellular immunity and $CD4^+$lymphopenia are the major predisposing factors for talaromycosis, as demonstrated by the high burden of disease among individuals with advanced HIV infection and other T cell immunosuppression [12]. Development of talaromycosis among individuals with primary immune deficiencies provides insights into the immune mechanisms essential for disease control. The adult-onset immunodeficiency condition due to anti-interferon-γ autoantibodies inhibits signal transducer and activator of transcription 1 (STAT1) phosphorylation and IL-12 production, leading to a severely compromised Th1 response [37]. Isolated STAT1 deficiency, both inherited and sporadic, has been described, as well as hyper-IgE (Job's) syndrome caused by STAT3 mutations resulting in Th-17 deficiency [38]. Hyper-IgM syndrome caused by CD40 ligand deficiency results in downregulation of activated T cells and downstream decreased signaling through NF-κB and reduction in IL-12 production [38]. These immune deficiencies reveal the central roles of IL-12 and INF-γ interaction for macrophage activation and Th17 effector cells in host control of *T. marneffei*.

Clinical Manifestations

Upper Respiratory Tract Infections

Upper respiratory tract infection is a unique feature of talaromycosis among the endemic mycoses [39] and has been described in immunocompromised as well as apparently immunocompetent individuals [40–43]. Pharyngeal and laryngeal lesions have been identified in patients presenting with odynophagia, dysphagia, hoarseness, soft tissue masses, papules, and mucosal ulceration [43–46]. Tracheal and endobronchial lesions are often accompanied by cervical lymphadenopathy, pulmonary infiltrates, or post-obstructive pneumonia [47, 48]. These lesions can cause structural obstruction or collapse of the large airways [47, 49]. Tracheobronchial infections can lead to long-term sequelae, requiring tracheostomy and reconstructive surgery [42]. Biopsy for histopathology and cultures for both fungi and mycobacteria should be performed to differentiate talaromycosis from tuberculosis, head and neck cancer, lymphoma, or Kaposi's sarcoma.

Lower Respiratory Tract Infections

Lower respiratory tract infections are diverse, encompassing alveolar consolidation, solitary or multiple nodules, mediastinal lymphadenopathy, pulmonary mycetoma, cavitary disease, and pleural effusion [40, 50, 51]. Radiological characteristics are similarly diverse, including thick and thin-walled cavities, patchy consolidation, ground glass changes, reticular nodular changes, and hilar and mediastinal lymphadenopathy [52–54]. Cavitary disease had been thought to predominate in non-HIV-infected patients; however, a study found that talaromycosis was the most common cause of cavitary lung lesions (23.5%) in 81 patients with HIV/AIDS, followed by cryptococcosis (13.6%) and tuber-

culosis (13.6%) [55]. The diagnosis of pulmonary diseases in patients with advanced HIV disease is challenging due to the frequency and clinical mimicry of other infections, including *Pneumocystis jiroveci* pneumonia, tuberculosis, cryptococcosis, and histoplasmosis, posing diagnostic and therapeutic dilemmas.

Pleural effusion is a common manifestation of talaromycosis in non-HIV-infected people. In a non-HIV-infected cohort of talaromycosis from China, 42/61 (69%) had pleural effusions; all cases were initially misdiagnosed as tuberculous [56]. Compared to tuberculous pleural effusions, talaromycosis effusions have a lower protein content and lymphocyte count and a higher neutrophil count. The diagnosis is often made by pleural biopsy and culture. Thoracoscopy demonstrates fibrous pleural adhesions and multiple pleural nodules [56]. Talaromycosis may be unique among endemic mycoses and other opportunistic mycoses in causing pleural effusions and should be considered in the differential diagnosis in a susceptible host [40].



Fig. 23.3 Skin lesions of talaromycosis in a patient with HIV infection

Localized Infections Outside of the Lungs

Localized infections outside of the lungs are more commonly seen in non-HIV-infected individuals and include abdominal abscess [57], genital chancroid-like lesions [58], chronic cutaneous ulcers in patients with systemic lupus erythematosus [59], pericarditis with pericardial effusion [60], and osteoarticular lesions of ribs, long bones, skull, lumbar vertebrae, scapular, and temporomandibular region [61, 62].

Disseminated Infection

Most patients with advanced HIV disease and profound immunodeficiency develop disseminated infection involving multiple organ systems. Infection has a subacute onset; the median duration of illness is 4 weeks (ranging between one and 24 weeks) prior to diagnosis [63]. Infection is characterized by constitutional symptoms of fever, weight loss, and malaise, cough, abdominal pain, or diarrhea. Common signs include anemia, hepatosplenomegaly, and lymphadenopathy. Skin lesions are the most specific sign but are a late physical manifestation of talaromycosis and occur in 40% to 70% of patients with disseminated infection [4, 24, 64]. Skin lesions have characteristic appearance of rolled-border papules with central necrosis appearing first on the face and spreading to the truck and extremities (Fig. 23.3). Meningoencephalitis is a rare manifestation of disseminated infection, occurring in less than 1% of individuals. Most patients have elevated intracranial pressure and abnormal brain computed tomography findings including intracranial lesions or hydrocephalus [3, 65].

Talaromycosis Immune Reconstitution Inflammatory Syndrome (IRIS)

IRIS is characterized by an excessive inflammatory response to an occult infection (unmasking IRIS) or by a paradoxical worsening of a treated infection upon initiation of HIV therapy (paradoxical IRIS). The fast decline of HIV viral load and the opposing rise of CD4+ T cells lead to a shift of the immune response from the Th2 anti-inflammatory response to the Th1 proinflammatory response. Patients with very high HIV viral loads and very low CD4+ T cells are at increased risk of IRIS with intracellular pathogens including viral pathogens, fungal pathogens, and mycobacteria (particularly tuberculosis) [66]. Talaromycosis IRIS is shown to occur within 1–5 months of antiretroviral therapy initiation and in the setting of rapid CD4+ T cell recovery [67]. Unmasking talaromycosis IRIS tends to present similarly to disseminated talaromycosis [68, 69]. Talaromycosis paradoxical IRIS, however, usually present with some inflammatory features including synovitis of small joints and occasionally erythema nodosum (Fig. 23.4) [67].



Fig. 23.4 Bilateral synovitis of the proximal interphalangeal joints in the index and middle fingers and a large erythema nodosum lesion on the left arm of two HIV-infected patients with talaromycosis paradoxical IRIS

Diagnosis

Microscopy and Culture

Microscopically *T. marneffei* can be seen as oval or round intracellular and extracellular yeasts in smears or biopsies of cutaneious lesions, aspirates of lymph nodes or bone marrow using Wright, Giemsa, or Gomori–Grocott methenamine (GMS) stains. A presumptive diagnosis can be made rapidly based on smears of skin lesions or lymph node aspiration showing round to oval intracellular and extracellular yeast approximately 2–3 μm in diameter with central septation (Fig. 23.1). This allows empirical antifungal therapy to be initiated while awaiting culture confirmation. However, skin lesions are absent in up to 60% of patients with talaromycosis, and skin lesions are late manifestation of infection. Occasionally infection can be diagnosed from a peripheral blood smear of patients with disseminated infection and high fungal burden in the blood [2].

A definitive diagnosis is based on culture isolation demonstrating thermal dimorphism. Unlike other endemic dimorphic fungi, *T. marneffei* grows more readily in standard BACTEC culture, yeast peptone dextrose (YPD), or Sabouraud dextrose agar (SDA) media. The classical culture characteristics of thermal dimorphism and the production of a red pigment in the mold form must be demonstrated. Culture yield is the highest in bone marrow (100%), followed by skin lesions (90%) and blood (70%) [4, 70]. Cultures take 4–28 days for identification, leading to diagnostic and treatment delays, particularly in patients presenting without fever or skin lesions. Diagnostic delay is the most critical barrier to progress, as 7% of patients die before culture diagnosis is made [63], and the mortality doubles from 24% to 50% when the diagnosis is delayed [71].

Molecular Diagnosis

Polymerase chain reaction (PCR) technology has been developed for detection of *T. marneffei* based on specific oligonucleotide primers designed from the internally transcribed spacer regions and the 5.8S rRNA and the 18S rRNA genes of *T. marneffei* [72]. A PCR-hybridization assay based on the 18S rRNA gene was shown to be specific when tested against 7 other fungi with sensitivities of 0.5 pg/μL and 0.1 pg/μL of DNA by PCR and Southern hybridization, respectively [73]. However, hybridization technique is labor-intensive and requires high level of competency in the laboratory. A nested PCR assay based on 18S rRNA had a sensitivity of 24/35 (68.6%) and specificity of 100% when tested in sera of 35 *T. marneffei*-infected patients, and 15 non-talaromycosis patients and 300 healthy people [74]. Conventional PCR methods are still relatively labor-intensive and prone to post PCR contamination. Several real-time PCR assays have been developed to detect *T. marneffei* in peripheral blood samples of patients and showed specificities approaching 100% and

sensitivities approaching blood culture at 60–86% [75–78]. These assays have the potential to make a diagnosis within a few hours of patient presentation. However, none has been standardized and prospectively validated to determine their usefulness in clinical management of talaromycosis.

Immunodiagnostics

Various methods have been developed to detect *T. marneffei* antigen and host antibody production, including immunoblot, indirect fluorescent antibody test (IFAT), latex agglutination, and enzyme-linked immunosorbent assay (ELISA). However, these prior assays have used polyclonal antibodies which have been shown to have low sensitivity and specificity [79, 80].

The *Aspergillus* galactomannan has high homology to that of *Talaromyces*, and the commercial galactomannan assay has been investigated to detect *T. marneffei* infection. The sensitivity of the serum galactomannan for detection of talaromycosis ranges between 80.6% and 95.8%, but specificity is only 90.9% [78, 81]. This test has the potential for use as a screening assay in areas of high endemicity for talaromycosis, but has a substantial cross reactivity issue with other endemic fungi.

Two promising monoclonal-antibody-based antigen detection immunoassays have been developed for detection of *T. marneffei* in blood and urine of patients. The first assay, developed by a research group from Thailand, used pooled monoclonal antibodies (MAb) 4D1 against a 30–150 kDa protein in an inhibition ELISA that showed a sensitivity of 97% and a specificity of 93% [82]. This group further developed a MAb 4D1 immunochromatographic test from urine samples and showed a sensitivity of 88% and a specificity of 100% [83]. The test utilizes a simple nitrocellulose membrane strip that has the potential as a rapid point-of-care diagnostic tool, but has not been prospectively validated.

The second assay, developed by researchers from Hong Kong, used MAbs and polyclonal antibodies (PAb) generated against an abundantly secreted cell wall galactomannan protein Mp1p that is specific for *T. marneffei* [84, 85]. This test has recently been validated in a large patient cohort in Vietnam showing a sensitivity of 86.3% (in 372 culture-proven talaromycosis cases) and a specificity of 98.1% (in 517 controls) [86]. In a recent prospective validation study in hospitalized patients with advanced HIV disease, the Mp1p ELISA has a sensitivity of 97.5% (compared to all cultures of 85.2%) and a specificity of 96.5% and can detect infection up to 16 weeks before cultures turn positive [87]. A commercial Mp1p ELISA has been approved for clinical use in China since October 2019, and a lateral flow antigen (LFA) platform is being developed in collaboration with IMMY for point-of-care diagnosis. These antigen detection assays have improved sensitivity compared to cultures, can speed up diagnosis, and are being studied as a screening tool for preclinical talaromycosis, enabling early antifungal treatment thereby reducing disease associated morbidity and mortality.

Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF)

MALDI-TOF method has recently been used for identification of *Talaromyces* to the species level from cultured yeast cells based on an in-house database generated from the researchers' institution's *T. marneffei* clinical strain collection [88, 89] or from the comprehensive National Institutes of Health MDL Mold Library [90]. Once these databases are incorporated into commercially available libraries, the MALDI-TOF represents a rapid and promising tool for downstream fungal identification, potentially eliminating the need to subculture and demonstrate thermal dimorphism.

Treatment

Talaromycosis is fatal if untreated. All 12 patients in a case series of disseminated infection who did not receive antifungal therapy died or left the hospital to die at home [91]. Mortality ranges from 10% to 50% on antifungal therapy, with higher mortality associated with delays in diagnosis and treatment [4, 63, 71]. Antifungal therapy for talaromycosis is guided by observational studies [4, 52, 92–94] and a single randomized controlled trial [63], all conducted in patients with advanced HIV disease.

Treating Acute Disease

Antifungal therapy is divided into induction, consolidation, and maintenance phases (Table 23.1). The Itraconazole versus Amphotericin B for Penicilliosis (IVAP) trial randomizing 440 patients with HIV-associated microbiology-confirmed talaromycosis in 5 hospitals across Vietnam demonstrated that induction therapy with amphotericin B reduced 6-month mortality from 21% to 11% (absolute risk difference: 9.7% point, 95% confidence interval: 2.8 to 16.6, $P = 0.006$), reduced incidence of relapse and IRIS, and had a fourfold faster rate of fungal clearance from blood [63]. The difference in mortality was not dependent on disease severity (based on baseline blood fungal load, CD4 count, antiretroviral therapy status, intravenous drug use, and requirement for oxygen support at presentation). This provides the evidence for the updated 2019 international recommendations of amphotericin B as the first choice of induction therapy

Table 23.1 Treatment regimen for talaromycosis

	Preferred therapy	Alternative therapy
Induction therapy	Liposomal amphotericin B 3–5 mg/kg/day IV for up to 2 weeks	Deoxycholate amphotericin B 0.7 mg/kg/day IV for up to 2 weeks OR Voriconazole 6 mg/kg IV every 12 h for 1 day (loading dose) and then voriconazole 4 mg/kg IV every 12 h for up to 2 weeks
Consolidation therapy	Itraconazole 200 mg PO every 12 h for 10 weeks	Voriconazole 200 mg PO every 12 h, OR itraconazole 200 mg PO every 12 h for 10 weeks
Maintenance therapy	Itraconazole 200 mg PO daily	Voriconazole 200 mg PO daily
Discontinuing maintenance therapy	CD4 count >100 cells/mm^3 for ≥6 months in response to antiretroviral therapy OR virologic suppression for ≥6 months on antiretroviral therapy	

Modified from [96]. *PO* per oral (by mouth). *IV* intravenous

regardless of disease severity [95]. Where available, liposomal amphotericin B (3 to 5 mg/kg/day) is preferred to amphotericin B deoxycholate (0.7 mg/kg/day) formulation to reduce infusion related reactions and renal and bone marrow toxicities. For patients unable to tolerate any form of amphotericin B, induction therapy with intravenous voriconazole (6 mg/kg every 12 h on day 1, then 4 mg/kg every 12 h) or with oral voriconazole (600 mg every 12 h on day 1, then 400 mg every 12 h) is recommended based on safety and efficacy in two non-comparative studies [92, 94]. After up to 2 weeks of induction therapy, consolidation therapy is initiated with oral itraconazole or voriconazole (200 mg every 12 h) for 10 weeks, followed by maintenance therapy with either oral itraconazole or voriconazole (200 mg daily) until the CD4 count rises above 100 cells/μL for at least 6 months [95]. The optimal regimen and duration of antifungal therapy for non-HIV-infected patients remains to be defined and are likely to be individualized based on the underlying immunodeficiency state, antifungal and immunosuppressive drug–drug interactions, and the expected duration of immune dysfunction.

Emerging Treatment Strategies

The IVAP trial showed that the vast majority (96%) of patients in the amphotericin B arm completely cleared fungemia by day 8 [63]. This suggests that the duration of amphotericin B induction therapy can be significantly shortened to reduce drug related toxicities while preserving antifungal potency. The newer azole drugs posaconazole and isavuconazole have low in vitro minimum inhibitory concentrations (MIC) against *T. marneffei* (MICs 0.001 μg/mL to 0.002 μg/mL) and are likely to provide effective induction or consolidation therapy, but remain to be tested in humans. The echinocandin drugs caspofungin, micafungin, and anidulafungin have intermediate to high MICs against *T. marneffei* (2 μg/mL to 8 μg/mL) and are less likely to be effective [96, 97]. Flucytosine has low MICs (0.015 μg/mL to 0.9 μg/mL) against *T. marneffei* [91], and it remains to be determined whether the combination of amphotericin B plus flucytosine would be more effective than amphotericin B alone for induction therapy of talaromycosis, as it is for cryptococcal meningitis.

Preventing Recurrence

Effective HIV therapy and reversal or reducing immunosuppression are the most effective strategies to prevent recurrence. A study prior to the era of antiretroviral therapy showed that more than 50% of patients not treated with antiretroviral therapy relapsed within 6 months after discontinuation of antifungal drug. A double-blind, placebo-controlled trial conducted in Thailand demonstrated that secondary prophylaxis with itraconazole 200 mg daily reduced talaromycosis relapse rate from 57% to 0% [98]. Secondary prophylaxis with itraconazole or voriconazole should be used until the CD4 count rises above 100 cells/μL for at least 6 months on antiretroviral therapy [95] or until the underlying immunosuppression is improved.

Preventing Disease

Primary prophylaxis with itraconazole 200 mg daily has been shown to reduce incidence of invasive fungal infections (predominantly cryptococcosis and talaromycosis) in HIV-infected patients with a CD4 count of less than 200 cells/μL in a double-blind, placebo-controlled trial [99]. However, primary prophylaxis has not been adopted in national programs due to the lack of a mortality benefit and concerns of toxicity, drug–drug interaction, drug resistance development, and costs. A diagnostic-driven strategy of antigen screening of high-risk patients, such as those with advanced HIV disease (CD4 count <100 cells/μL), and providing pre-emptive antifungal therapy is likely to be more cost-effective at preventing development of disease and is a subject of active research.

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Dermatophytosis (Tinea) and Other Superficial Fungal Infections

24

Gloria M. González and Alexandro Bonifaz

Introduction

Superficial dermatophyte infections have been identified with a variety of terms through the ages, although the term "tinea" has persisted as the most common. Dermatophytes are taxonomically classified into nine current genera, with four being the most frequent, *Trichophyton*, *Microsporum*, *Nannizzia* and *Epidermophyton* [1]. Topical preparations have been the historic method of treatment, with the first effective oral medication being griseofulvin, developed in 1958. Current medical treatment for dermatophyte infection is based on griseofulvin, allylamines, imidazoles, and triazoles, with other drugs becoming available as research in therapeutic efficacy of new antifungals proceeds.

Dermatophytes require keratin for growth. Because of this they usually affect hair, nails, and superficial skin. The clinical manifestations are named according to the affected region: tinea capitis (scalp), tinea corporis (body), tinea cruris (groin), tinea pedis (feet), tinea manuum (hands), tinea barbae (affecting the beard in men), tinea faciei (face), and tinea unguium (nails) [2]. "Ringworm" is the popular term used to identify tinea infections because lesions develop as a dermatosis characterized by a circular or oval clear area surrounded by a red, scaly, elevated border ("ring"). Tinea unguium is the term used for onychomycosis caused by dermatophytes, in contrast to nail infections caused by *Candida* or non-dermatophyte molds.

In memory of Dr. Oliverio Welsh.

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Superficial skin infections are also caused by other genera of fungi, including *Malassezia*. *Malassezia* is lipid dependent or lipophilic basidiomycetous yeast that inhabits the skin of humans and other animals. This genus was first recognized as a pathogen in 1846. Culture was not successful until 1927 when it was found that these organisms require lipids for growth. Initially two species were described under the genus *Pityrosporum*, and by 1970 three species were recognized, *P. ovale*, *P. orbiculare*, and *P. pachydermatis* [3]. The existence of yeast and mycelial forms made identification difficult. Genetic research in the 1990s confirmed at least seven species of *Malassezia*. Since then more have been identified. At present, the genus currently comprises 17 species. *Malassezia* is known to cause pityriasis versicolor and is associated with seborrheic dermatitis. These superficial conditions will be included in this chapter together with dermatophyte infections.

Etiologic Agents

Four fungal genera cause most tinea: *Microsporum*, *Trichophyton, Nannizzia*, and *Epidermophyton* [1]. Human infection can spread from one person to another (anthropophilic), from an animal (zoophilic), or less commonly, from soil (geophilic). The major causative species differ geographically and may change in prevalence over time due to population movements. Anthropophilic dermatophytes are the most frequent causes of onychomycosis and other superficial dermatophytoses with the most common agents being *T. rubrum, T. interdigitale,* and *T. mentagrophytes*. *T. tonsurans* is currently the most frequent cause of tinea capitis in North America (Northern Mexico, the USA and Canada). *M. canis* is a zoophilic organism frequently picked up by humans from contact with animals such as dogs and cats [4].

Malassezia yeast species cause the superficial skin fungal infections pityriasis versicolor, seborrheic dermatitis, folliculitis, atopic dermatitis, and other skin or systemic disorders.

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_24

Among the most frequent species are *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. slooffiae*, *M. restricta*, and *M. obtusa* [5]. *M. pachydermatis* is typically associated with animal infection [3]. Up to six new species have been described in recent literature: *M. dermatis*, *M. equi*, *M. japonica*, *M. yamatoensis*, *M. arunalokei,* and *M. nana* [5]. The most common *Malassezia* species contributing to pityriasis versicolor lesions seborrheic dermatitis and folliculitis are *M. globosa* (50–60%), *M. sympodialis* (3–59%), *M. furfur*, and *M. slooffiae* (1–10%) [6]. *M. furfur* has been identified from deep-seated infections, such as blood, urine, and vagina, habitually associated with immune compromised patients, or from septicemia in neonates that received lipid supplementation via catheters [7] (see Chap. 10).

Epidemiology

Dermatophytosis is a common fungal infection. The risk of acquiring the disease is 10–20% in an individual's lifetime [8]. Overall rates of dermatophyte infection were measured in the USA using the National Ambulatory Medical Care Survey (NAMCS) from 1990 to 1994 [9]. This survey determined that an estimated 21.6 million physician office visits were for cutaneous fungal infections during this period, breaking down the data obtained into types of infection: tinea corporis, 27.2%; tinea cruris, 16.9%; tinea pedis, 16.7%; tinea unguium, 15.6%; tinea of hair and beard, 6.9%; and tinea manuum, 1.0%.

Tinea Pedis/Manuum

Tinea pedis is estimated to affect 10% of the world population [9]. Infections are more frequent in tropical climates and may also be associated with use of occlusive footwear. Men are more often affected than women for both tinea pedis and tinea manuum, with most infections occurring in the space between the fourth and fifth toes [10]. Children less frequently develop tinea pedis. Patients with atopic dermatitis or immunosuppressive disorders may develop tinea pedis. Predisposing factors for tinea manuum include manual work that results in repeated trauma to the hands, hyperhidrosis, and frequent use of soap.

Tinea Corporis/Cruris

Tinea corporis and tinea cruris are common and have a worldwide distribution [11]. Little data on prevalence in North America has been published, but tinea corporis was found to be the most common dermatophytosis for which patients sought treatment during the NAMCS (27.2% of all dermatophytoses with an estimated 2.3 million physician visits) [9]. A subset of tinea corporis affecting only the non-bearded regions of the face, tinea faciei, makes up 3–4% of tinea corporis cases and is more frequently seen in warm, humid climates [13].

Tinea Capitis

The genus *Trichophyton*, particularly *T. tonsurans*, is the predominant cause of tinea capitis in North America. In Western Europe, *M. canis* and *T. violaceum* are the most common pathogens of tinea capitis; *T. tonsurans* and *M. canis* are dominant in the Caribbean and South America. *M. canis*, *T. mentagrophytes*, and *T. violaceum* dominate in the Middle East [4].

Tinea capitis is prevalent in children over the age of 6 months and before puberty. African Americans develop tinea capitis more frequently than the general US population. *Trichophyton* species affect men and women equally, although *M. audouinii* and *M. canis* are more frequent in men. The spread of infection may increase in conditions of overcrowding, poor hygiene, and poverty [4].

Onychomycosis

Onychomycosis has an estimated prevalence of 6.5% to 12.8% in North America, accounting for up to 50% of all nail disease [14, 15]. It is more common in men and in people over the age of 60 years. Other predisposing factors include nail trauma, diabetes, peripheral artery disease, and immunodeficiency. Tinea pedis may be present in patients with toenail onychomycosis [15].

Pityriasis Versicolor

Pityriasis versicolor has a worldwide distribution. Prevalence in summer months of tropical climates has been reported at 30–40%, compared to 1–4% in temperate climates. This evidence points to the importance of endogenous host factors in the development of the disease [3, 16]. Pityriasis versicolor is less frequent in prepubescent children and more frequent in adults when sebaceous gland activity is greatest [6, 16]. Equal prevalence between the sexes has been noted [6].

Seborrheic Dermatitis and Folliculitis

Seborrheic dermatitis is a chronic, recurrent disorder affecting between 1 and 5% of immunocompetent adults [3, 16, 17]. It is typically referred to as dandruff. Its mild form

affects a large proportion of North American population, but reported numbers are likely underestimated as people tend not to seek medical advice for dandruff. Men are more frequently affected than women. The disease is more severe in winter and improves with summer sun exposure [18]. Seborrheic dermatitis chiefly affects adolescents, young adults, and adults over age 50 [19]. Incidence may increase in immunocompromised persons such as those infected with HIV, where estimates of incidence of seborrheic dermatitis are as high as 83% [3]. Data on frequency or prevalence of *Malassezia* folliculitis does not exist, but this disorder is increasingly reported in young adults. It can be observed after treatment with antibiotics for acne and with the use of corticosteroids [20].

Pathogenesis

Dermatophytes colonize keratinized tissue of the stratum corneum. Invasion by anthropophilic species usually results in less inflammation than those of zoophilic or geophilic species [21]. The epidermis functions as a barrier to microorganisms and commensal flora may help reduce infection [3]. Entry to the stratum corneum may result from trauma or some other breach of the skin. Excessive sweating and occlusive clothing or footwear aid in providing a warm, moist environment that favors tinea infection, which can be transferred from one area of the body to another. It can also be transmitted between individuals by direct or indirect contact with scales containing fungal arthroconidia from infected individuals, as occurs in contact sports including wrestling and rugby [11].

The establishment of dermatophytosis is subject to the abilities of the microorganism to overcome the barriers of the host, such as the acidic nature of the skin and nails and a combination of molecules derived from glands and epidermal cells: fatty acids and antimicrobial peptides, phagocytosis, sweat, and skin desquamation. Some proteins present in the epidermis, and some skin lipids in the scalp and hair are fungicidal to certain, but not all, dermatophyte species [3]. Adhesins are in the fungal cell wall and allow the attachment to host tissue. Arthroconidia must geminate and the hyphae must penetrate the body surface. Then, dermatophytes seek nutrients for growth and produce hydrolytic enzymes such as lipases, phosphatases, DNAses, and keratinolytic proteases [22].

Dermatophyte glycopeptides prompt development of delayed hypersensitivity. Patients with inflammatory infection are more likely to demonstrate this type of reaction than patients with non-inflammatory chronic disease [23]. Dermatophytes and other microorganisms can activate the alternate complement pathway of immune response, causing production of molecules which prompt the chemotaxis of neutrophils into the skin. Immunoglobulins are secreted onto the skin surface via sweat, and commensal organisms, including *Malassezia* species, have been found to be coated with these immunoglobulins [3].

Malassezia organisms are a normal part of human commensal skin microbiota. They are found particularly in sebaceous skin such as the chest, back, and head. They are usually found in the yeast form rather than the mycelial form. In pityriasis versicolor, *Malassezia* cells massively acquire a distinctive hyphal morphology that probably denotes the profuse availability of nutrients [24]. *Malassezia* species vary in the antigens presented and can alter their expressed antigens throughout their growth cycle. The ability of *Malassezia* to elicit activities of the human immune system is not well defined [3]. *Malassezia* in atopic dermatitis and seborrheic dermatitis induce skin disease through allergic and irritant pathways. In atopic dermatitis, a sensitization stage against *Malassezia* antigens seems to be almost universal. Current data in seborrheic dermatitis point toward an irritant and/or toxic effect of the yeast metabolic products. At a histologic level, *Malassezia* folliculitis is marked by the presence of an inflammatory infiltrate consisting of lymphocytes, histiocytes, and neutrophils with focal rupture of the follicular epithelium with budding cells. The inflammatory response may be a result of the ability of the *Malassezia* yeasts to hydrolyze triglycerides into free fatty acids [25].

People and animals, though uninfected, may still be asymptomatic carriers. Fomites also play a significant role in transmission. Autoinoculation can occur; for example, tinea pedis spreading to tinea cruris, tinea capitis to tinea corporis, or onychomycosis to tinea pedis [26]. High levels of perspiration may predispose to infection, as fungal arthroconidia persist to a greater extent on the scalp with higher levels of oils [11].

Clinical Manifestations

Clinical presentations and differential diagnoses for the various superficial infections are summarized in Table 24.1.

Tinea Pedis/Manuum

Tinea pedis, also known as "athlete's foot," has three common presentations: interdigital, moccasin, and vesiculobullous [10].

Interdigital is the most common and typically infects the toe webs, particularly between the fourth and fifth toes (Fig. 24.1). Interdigital infection may show fissuring, scaling, maceration, and erosion. Hyperhidrosis, pruritis, and foul odor may also be present; The latter may well be related to the bacterial association. Dermatophytosis simplex is an

Table 24.1 Clinical presentations and differential diagnoses of common dermatophyte and superficial fungal infections

Condition	Presentation	Differential diagnosis
Tinea pedis	*Interdigital:* Scaling, fissuring, maceration, erosions, hyperhidrosis, pruritis, odor *Moccasin*: Fine silvery scales with underlying pink or red skin on soles, heels, sides of feet *Vesiculobullous:* Inflammatory vesicular or bullous lesions, particularly at instep	Candidiasis, erythrasma, bacterial infection, psoriasis, contact dermatitis, dyshidrotic eczema, Reiter's syndrome
Tinea manuum	Dry, scaly, hyperkeratotic skin particularly of the palmar area, minimal erythema	Contact dermatitis, atopic dermatitis, pompholyx, psoriasis lamellar
Tinea corporis	Annular erythematous plaques with raised leading edges and scaling, over glabrous skin of trunk; may be central clearing	Impetigo, nummular dermatitis, secondary or tertiary syphilis, psoriasis, lichen planus, seborrheic dermatitis, pityriasis rosea, pityriasis rubra pilaris, candida intertrigo, atopic dermatitis, cutaneous lupus, pityriasis versicolor
Tinea cruris	Annular erythematous plaques with raised leading edges and scaling, over pubic area, perineal and perianal skin, typically not affecting the scrotum or labia majora	Psoriasis, seborrheic dermatitis, candidiasis, erythrasma, lichen simplex chronicus, Darier's disease, pemphigus vegetans
Tinea capitis	*Non-inflammatory:* Erythematous papules around hair shaft spreading out with fine scaling in noticeable patches and partial or complete alopecia *Black dot:* Noticeable black dots where hair breakage at scalp level occurs, scaling with little inflammation (particularly with *T. tonsurans* or *T. violaceum*) *Inflammatory:* Kerion with pustules, loose hair, discharge of pus *Favic*: Large yellow crusts on the scalp	Seborrheic dermatitis, psoriasis, atopic dermatitis, tinea amiantacea, alopecia areata, trichotillomania, lupus erythematosus, lichen planopilaris, traction folliculitis, bacterial pyoderma
Onychomycosis	*Distal lateral subungual (DLSO):* Infection at the distal end of nail plate; discoloration and thickening of nail plate, onycholysis, subungual debris *Superficial white (SWO):* White spots or patches on the surface of the nail plate *Proximal subungual (PSO):* Infection of the proximal nail fold, and extending distally, typically whitish in color *Endonyx:* Milky white discoloration of the nail plate without hyperkeratosis, onycholysis; may show lamellar splitting of the nail plate (typically caused by *T. soudanense* or *T. violaceum*)	Psoriasis, chronic onycholysis, chronic paronychia, trachyonychia, hemorrhage, onychogryphosis, lichen planus, alopecia areata, subungual malignant melanoma, subungual squamous cell carcinoma
Pityriasis versicolor	Well-defined, hyperpigmented or hypopigmented lesions of areas with high concentrations of sebaceous glands such as scalp, chest, back, upper arms, and face; showing fine scaling in most cases (caused by *Malassezia* species)	Vitiligo, chloasma, tinea corporis, pityriasis rotunda, erythrasma
Seborrheic dermatitis	Red, flaky, greasy-looking patches of skin on scalp, nasolabial folds, eyebrows, and ears: "Dandruff"; may also affect groin, axillae, anterior chest; pruritis, irritation may be associated with *Malassezia* infection.	Psoriasis, contact dermatitis, atopic dermatitis, tinea capitis, rosacea, lupus erythematosus
Folliculitis	Pustules measuring 2–5 mm, without comedo, and papulo-follicular lesions, located especially on the trunk, shoulders, and on the face. It mostly affects young adults and occurs after the use of antibiotics and corticosteroids (caused by *Malassezia* species)	Acne vulgaris, bacterial folliculitis, eosinophilic folliculitis

uncomplicated form of interdigital tinea pedis. In contrast, dermatophytosis complex is associated with concomitant bacterial infection that can be facilitated by breakdown of the skin in preliminary infection. This form of tinea is characterized by inflammation, maceration, and odor [10]. This clinical form should be ruled out of interdigital erythrasma, which usually fluoresces coral-red in Wood's light.

Moccasin tinea pedis presents as fine silvery scales with underlying pink to red skin on the soles, heels, and sides of feet (Fig. 24.2) [10]. More severe cases may show cracked, inflamed skin, erythema, and odor. In the most chronic cases, they present with extensive areas of plantar hyperkeratosis. In some patients, two or more clinical varieties may coexist. It is important to distinguish it from palmar-plantar hyper-



Fig. 24.1 Interdigital tinea pedis and vesiculobullous tinea



Fig. 24.2 Moccasin tinea pedis, with close-up of the fine scaling

keratosis, this type of infection is commonly produced by *T. rubrum* and secondly by *T. interdigitale*.

Vesiculobullous tinea is the least common form of tinea pedis and appears as acute inflammatory vesicular or bullous lesions, typically at the instep; however, inflammation may spread over the sole [10]. This form is considered an acute phase. When the vesicles break, they leave areas of scale and meliceric crusts; it is a very itchy variety and it may be related to the presence of dermatophytid-reaction in hands [27]. This dermatophytosis is associated with *T. mentagrophytes* and *T. interdigitale* infection.

Tinea manuum is an uncommon clinical presentation that affects the palms of the hands as a chronic dermatosis with minimal erythema and dry, scaly, hyperkeratotic skin [10]. When extended to the dorsum of the hand, it is presented with its classic active border. Infections are frequently caused by *T. rubrum*. Tinea manuum can be associated with tinea pedis or onychomycosis and a two-feet-one hand syndrome [28]. The latter consists of tinea manuum with excoriation of infected tinea pedis and/or toenail onychomycosis. It is important to mention the so-called two feet-one hand syndrome, affecting mainly the feet in the form of tinea in moccasin and only one hands almost always in a hyperkeratotic form [29].

Tinea Corporis/Cruris

Tinea corporis is a superficial dermatophyte infection of the glabrous skin, excluding the scalp, beard, face, hands, feet, and groin (Fig. 24.3), which is more common in men and children [11, 12]. Tinea faciei is a subset of tinea corporis that affects only the face, excluding the beard region [13].

Tinea cruris, also known as "jock itch," is a dermatophyte infection of the genitalia, pubic area, perineal skin, and perianal skin (Fig. 24.4). The scrotum and labia majora are typically not affected. This form of infection is more common in men [11].

Tinea cruris and tinea corporis present as annular erythematous plaques with raised leading edges and scaling. Central clearing of the lesion may be noticed, but nodules may remain present throughout the lesion [11]. Infection is typically associated with pruritus and an erythematous pap-



Fig. 24.3 Tinea corporis



Fig. 24.4 Tinea cruris

ule or series of vesicles can also be present. This condition is very itchy; it is exacerbated by humidity and maceration; constant scratching causes lichenification and secondary bacterial infection. With the use of topical corticosteroids, the process is exacerbated and spreads, easily causing the addition of candidiasis or other bacterial infections.

Tinea incognito can occur anywhere in the body, it is due to the use of topical and systemic steroids, immunomodulators such as tacrolimus and pimecrolimus. Clinically, the lesions are less scaly, with pustules, more extensive and erythematous and can simulate other diseases such as atopic dermatitis, rosacea, seborrheic dermatitis, lupus erythematous, or contact dermatitis among others [30]. Another atypical form of dermatophytosis is Majocchi's granuloma, where the fungi are located in a deeper plane, forming nodules, generally present on the legs and trunk and regularly associated with diabetes and the use of steroids.

Significant inflammation may result from infection with zoophilic organisms such as *T. verrucosum*, which produces



Fig. 24.5 Tinea faciei

large pustular lesions, a kerion, or formation of frank bullae causing tinea corporis bullosa. This condition must be distinguished from inguinal candidiasis and inverted psoriasis. The main etiological agents are: *T. rubrum, T. interdigitale,* and *E. floccosum* [11].

Tinea faciei has a broad range of presentations. Infection may begin as flat, scaly macules that develop a raised border that advances outward in all directions, with or without development of papules, vesicles, and crusts (Fig. 24.5) [13]. Due to a lack of a correct diagnosis and the use of topical steroids, lesions may not be annular. The central area may become hypo- or hyperpigmented. Lesions may occur singly or in multiple patches and can extend to other sites; that is to say that in the face it is more frequent its manifestation as tinea incognito [13, 30].

Tinea imbricata or Tokelau is a chronic tinea of glabrous skin caused by *T. concentricum*, an anthropophilic dermatophyte. It presents as distinctive scaly, concentric, overlapping plaques that typically begin on the face and spread, involving large areas of the body [31]. This infection is endemic in Polynesia, and in Central and South America; particularly in rural areas.

Tinea Capitis

Infection of the scalp involves hyphal proliferation in the stratum corneum that extends into the hair follicle orifice and hair shaft. This clinical form is caused by exposure of the scalp to the inoculum from an infected individual, animal, or contaminated soil [32].

Non-inflammatory or epidemic tinea capitis may begin as a small erythematous papule around the hair shaft which spreads outwards, developing fine scaling in noticeable patches (Fig. 24.6a) [32]. Partial or complete alopecia may result because brittle hair breaks off a few millimeters from the scalp. Affected hair may appear gray due to a coating of

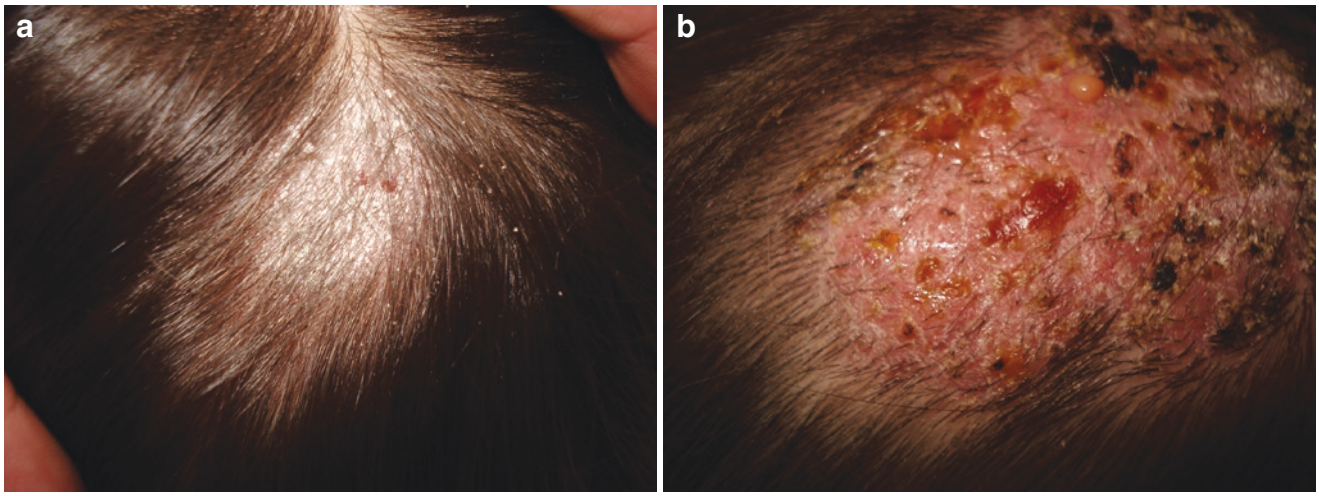


Fig. 24.6 Tinea capitis non-inflammatory and tinea capitis inflammatory (Celsi kerion)

fungi. Non-inflammatory infection is associated with *M. audouinii* and *M. ferrugineum*; however, *T. tonsurans* and *M. canis* may sometimes cause non-inflammatory infection.

Black dot tinea capitis is most frequently associated with *T. tonsurans* or *T. violaceum* infection and results from hair breakage at the level of the scalp, showing diseased hair in the follicle as a "black dot." Scaling is typically present with little inflammation, though inflammatory kerion, which is characterized by an oozing mass with pustules, loose hair, and discharge of pus, is possible [32]. Signs of systemic illness may be present, including fever and lymphadenopathy.

Inflammatory tinea capitis is associated with zoophilic or geophilic species such as *M. canis* or *N. gypsea*, but may also occur with *T. verrucosum*, *T. schoenleinii*, *T. tonsurans*, and *M. audouinii* [32].

Favic infection is rare in North America and it is usually caused by *T. schoenleinii*, leading to large yellow crusts [32, 33].

Tinea capitis in adults is observed more often in older women, but remains a relatively rare entity and may related to contact with pets or sick children. This can present in the form of non-inflammatory tinea (black dots) or as seborrheic dermatitis.

Onychomycosis

The most common presentation of onychomycosis is distal lateral subungual onychomycosis (DLSO), which presents as a nail with discoloration and varying degrees of hyperkeratosis, onycholysis (separation of nail from nail bed), subungual debris, and pachyonychia or thickening [34, 35] (Fig. 24.7). DLSO begins at the distal edge of the nail (hyponychium) and travels proximally through the stratum corneum of the nail bed, involving the nail plate (Fig. 24.8). The most severe



Fig. 24.7 Onycholysis. Bottom right-psoriasis

grades of DLSO may progress to total dystrophic onychomycosis (TDO) where the nail plate becomes friable and crumbles away to a varying degree, leaving an exposed thickened nail bed and subungual debris. Within the spectrum of DLSO presentations, infections may spread relatively evenly across the nail plate. Alternatively, infection may penetrate only the lateral edge or edges of the nail (lateral infection) or may penetrate longitudinally in a "spike" formation. Infection may also develop as a dermatophytoma, where debris and fungi clump densely to form a thick, hyperkeratotic mass [36]. These presentations may not respond well to therapy.

Sometimes, infection may present as superficial white onychomycosis (SWO), proximal subungual onychomycosis (PSO), or endonyx onychomycosis [34, 35]. SWO involves infection of the superficial nail plate, showing patches of white discoloration on the nail surface. Multiple nails may be affected, and varying degrees of nail plate area may be covered (Fig. 24.9). The rare presentation PSO results from

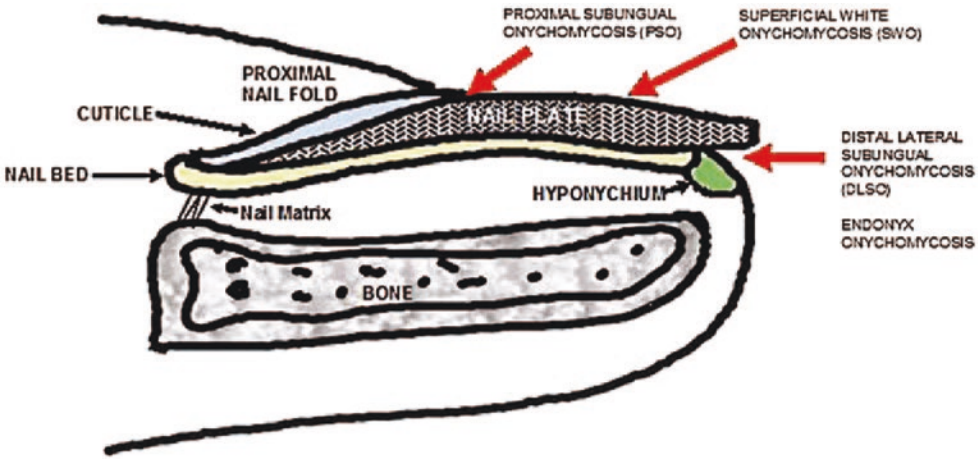


Fig. 24.8 Routes of infection causing the typical presentations of onychomycosis



Fig. 24.9 Typical presentation of superficial white onychomycosis (SWO) on the third toenail with distal lateral subungual onychomycosis (DSLO) presented in the great toenail



Fig. 24.10 Proximal subungual onychomycosis

invasion of the proximal nail fold and extending distally along the underside of the nail plate as a white patch of infection (Fig. 24.10). PSO is more common in immunodeficient individuals, such as HIV-positive patients and is a clinical marker of importance. It may serve as a hallmark for immunodeficiency [37]. Endonyx presents as a diffuse milky white discoloration of the nail in the absence of hyperkeratosis and onycholysis, with the nail plate surface and thickness remaining normal [34]. The nail plate may show lamellar splitting with invasion of the superficial and deep layers of the nail without excessive thickening or discoloration [35]. Endonyx infections are usually caused by *T. soudanense* or *T. violaceum* [34, 35]. In general, the main agent is *T. rubrum*. It should be noted that many cases of onychomycosis caused by non-dermatophyte mold fungi (*Aspergillus spp, Scopulariopsis brevicaulis,* and *Fusarium* spp) give the same clinical forms, and their differentiation is necessary because the treatment may be different [38].

Pityriasis Versicolor

Pityriasis versicolor presents as well-defined lesions with fine scales caused by desquamation. These are either hyperpigmented (pink, tan, dark brown, or black) or hypopigmented (white, or lighter than normal skin). Hypopigmentation may not always exhibit scaling (Fig. 24.11a) [39]. It is a superficial fungal infection that is largely asymptomatic, with the exception of occasional mild pruritus [6]. There is a large variation in lesion size from macules to entire trunk coverage [40]. Lesions are predominant in areas with a high number of sebaceous glands such as the scalp, chest, and back, as well as the upper arms and face [6, 39]. Facial lesions are more common in children [3]. Hypopigmentation may occur independently or following the hyperpigmented stage. Exceptionally, pityriasis versicolor can present in other clinical forms; papular-like and hypertrophic, they are usually chronic and associated with the use of steroids [39]. In general, it seems that the most common *Malassezia* species cultured from lesions of pityriasis versicolor are *M. globosa* and *M. sympodialis*. Other species such as *M. slooffiae* and *M. furfur* are relatively less common.

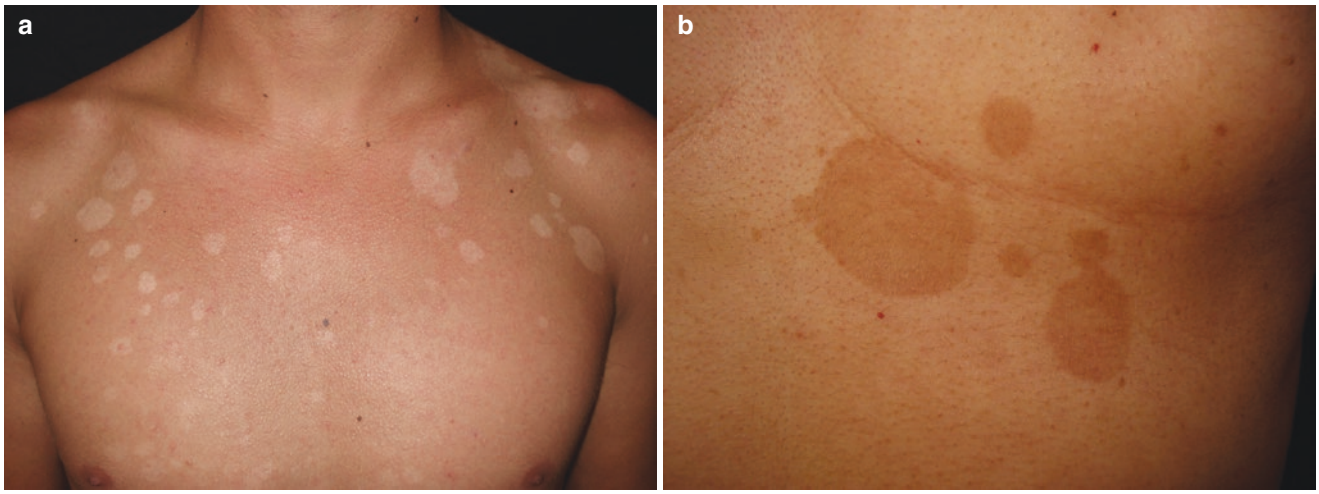


Fig. 24.11 Pityriasis versicolor hypopigmented and pityriasis versicolor hyperpigmented

Seborrheic Dermatitis and Folliculitis

Seborrheic dermatitis is the most common disease associated with *Malassezia* yeast, occurring in 1–3% of the general population. It presents as red, flaking, greasy-looking patches of skin on the scalp and hair-bearing areas of the face such as the nasolabial folds, eyebrows, and ears (Fig. 24.12) [18]. Seborrheic dermatitis is a more severe form of dandruff involving body sites of abundant sebaceous gland activity. It may occur on the groin, axillae, anterior chest, or inside/behind the ears [18, 41]. Dandruff can appear as loosely adherent white or gray flakes, while severe seborrheic dermatitis may be thick, oily, yellow-brown crusts. Pruritus, irritation, and a tight, dry feeling may be associated with the afflicted area [42]. Some cases present with little erythema, while others present as a sore scalp with occasional pustules [17]. The species that have been shown to be most closely associated with this condition is *M. globosa* and *M. restricta* [43]. However, some authors have also reported *M. furfur, M sympodialis, M. obtuse, M. slooffiae* [44].

Malassezia folliculitis is a superficial infection of the pilosebaceous follicle. It is typically located on the trunk (anterior and posterior), shoulders, and on the face, and mostly affects young adults. It usually occurs after the use of antibiotics and corticosteroids. Its most common clinical form is pustules arranged around each follicle, measuring 2–5 mm, without comedo, which distinguishes it from acne. Some patients report itching; there is another form with papulo-follicular lesions that can give rise to nodules [20, 45].

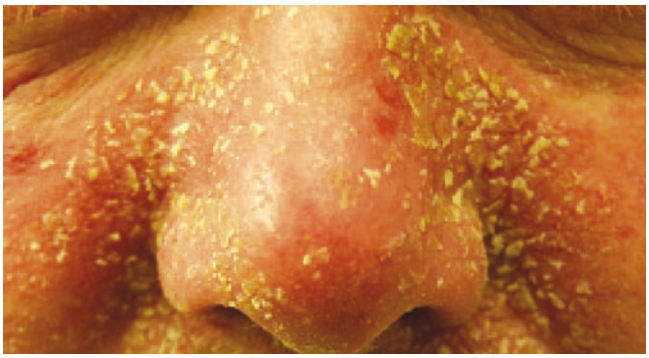


Fig. 24.12 Seborrheic dermatitis—severe presentation

Diagnosis

A simple and non-invasive technique is dermoscopy. It is very useful for tinea capitis, observing most frequently perifollicular scaling, diffuse scaling, short broken hairs, black dots, corkscrew hairs, and comma hairs. Fluorescence in Wood's light (greenish yellow) is also very useful, being positive for cases caused by *Microsporum* and *Nannizzia* species.

Dermoscopy in onychomycosis allows us to differentiate between traumatic onycholysis and melanonychia. The most common pattern of dermoscopy in onychomycosis includes ruin appearance; homogeneous leukonychia; blackish, yellowish, or greenish discoloration and punctate leukonychia with longitudinal striations.

Definitive diagnosis of tineas requires confirmation of dermatophyte organisms by microscopic examination and fungal culture methods. For skin infections, scrapings or swabs can be taken from the leading edge of a lesion. Nail clippings and subungual debris can similarly be obtained. Direct examination with 15% or 20% potassium hydroxide (KOH) is easy, economical, and fast and its initial results allow the physician to establish treatment. KOH is added to the samples to dissociate hyphae from keratinocytes. In dermatophytosis, direct examination can demonstrate the existence of hyphae or arthroconidia [46].

In pityriasis versicolor, dermoscopy is more useful for hyperchromic cases. Fine adherent scales and pigmented lesions and network composed of pigmented stripes are observed. *Malassezia* folliculitis dermoscopy shows follicular centric papules and pustules with erythema [47]. Direct mycological examination can reveal the typical "spaghetti and meatball" (mixture of yeasts and short hyphae) appearance. Microscopic examination may show mycotic nodules. When clinical samples are of good quality and the observer is experienced, KOH has a high sensitivity. Unfortunately, the test has a low specificity because it fails to identify the genus and species [48]. KOH examination of hairs may help differentiate types of tinea capitis infection. Ectothrix infection can be distinguished from endothrix infection because in the former arthroconidia appear as chains on the surface of the hair shaft or as a mosaic sheath around the hair. Inspection under Wood's light (filtered ultraviolet light with a peak of 365 nm) may aid in diagnosis [32]. Ectothrix infections with *M. audouinii*, *M. canis,* and *M. ferrugineum* show bright green fluorescence under the Wood's light. *T. schoenleinii* shows dull green fluorescence. *T. tonsurans*, however, does not fluoresce, and the utility of the Wood's lamp for diagnosis is currently limited in countries where this is the major infecting agent.

Cultures are essential to establish an accurate identification of the causative microorganism. Species identification is established by the macroscopic and microscopic characteristics of the culture, but it has a low sensitivity and a high specificity. In addition, a 1–4 week turnaround time and substantial mycological expertise are needed when performing identification. Typical dermatophyte strains can be frequently recognized from primary cultures, but subcultures on specific media are usually required. Potato dextrose agar is used most frequently for enhancing sporulation and producing pigments. Biochemical and physiological tests such as urease activity, in vitro hair perforation test, and nutritional requirements tests are currently not used [49].

Molecular diagnostic methods are becoming generally available with higher sensitivity, specificity, and minimum time to report results than the traditional methods. The most extensively used is conventional PCR; a simple and economical molecular technique for fast recognition of etiological agents in clinical cases. The species-specific primers, pan dermatophyte primers, or panfungal primers that typically target ITS, 28S region of ribosomal DNA, or the sequences coding for topoisomerase II or chitin synthase I have been utilized for the identification of microorganisms implicated in dermatophytosis. Some of the most used procedures are real-time PCR, multiplex PCR, PCR-ELISA (enzyme-linked immunosorbent assay), nested-PCR, and PCR-RFLP (restriction fragment length polymorphism) [50].

In a study of 107 clinical samples by Gordon et al. [51], the real-time PCR was able to identify and discern between cultures of dermatophytes and non-dermatophyte microorganisms, emphasizing the potential of the test to detect several microorganisms causing dermatophytoses.

The RT-PCR assay utilized by Bergmans et al. [52] differentiated 11 dermatophyte species from nail, skin scale and hair samples with a short period of time (4 h after overnight lysis). In 120 clinical samples analyzed, real-time PCR was positive in 74 samples (45 = *T. ruburm,* 23 = *T. interdigitale*, and 6 = other dermatophyte species). In 45 of 57 culture-positive and/or microscopy-positive samples, PCR identification was also possible (27 = *T. ruburm*, 12 = *T. interdigitale*, and 6 = other dermatophyte species).

Diagnostic methods in medical mycology have been changing and new techniques are an improvement over former conventional procedures. We now have optional molecular methods for rapid identification of pathogenic fungi, establishing treatment and determining epidemiological trends with more certainty.

Treatment

Treatment for superficial fungal infections varies widely. Antifungal medications that are used typically belong to the azole or allylamine drug classes (Table 24.2). Topical antifungals are effective in circumscribed areas of the skin surface. Some topical antifungals exhibit anti-inflammatory and antibacterial effects as well as antifungal activity and are indicated for infections with inflammation and potential bacterial secondary infection. Available oral antifungals include griseofulvin, terbinafine, itraconazole, fluconazole, albaconazole, and ketoconazole. These agents are indicated in severe or widespread infection, as well as for immunocompromised patients where a prompt, thorough resolution of infection is necessary. They can also be used as an alternative to daily topical therapy.

Safety and drug interactions are typically only a concern with oral therapy, as serum absorption tends to be minimal with topical drug use. With topical agents, most adverse effects are limited to skin reactions at the application site, which usually are mild and transient. Oral antifungals are occasionally associated with severe hepatic toxicity, rare

Table 24.2 Treatment options available for dermatophytoses and other superficial fungal infections[a]

	Terbinafine	Itraconazole	Fluconazole	Ketoconazole	Griseofulvin	Topicals	
Tinea pedis/ manuum[a]	[b]Cream: apply twice daily × 1–4 weeks [b]1% Solution: apply twice daily x 1 wk. Oral: 250 mg/day x 2 wks	Oral: 200 mg bid × 1 week	Oral: 150 mg once weekly × 2–6 weeks	[b]2% Cream: apply once daily × 6 weeks [b]Oral: 200–400 mg/ day × >4 weeks *(seldomly used)*	[b]Microsize: 1 g/day Ultramicrosize: 660 or 750 mg/ day × 4–8 weeks	[b]Ciclopirox 0.77% cream or gel: twice daily × 4 weeks Antifungal powder for prevention	[b]Clotrimazole, [b]Miconazole, [b]Butenafine, [b]Econazole, [b]Sertaconazole, [b]Luliconazole, [b]Naftifine
Tinea corporis/ cruris	[b]Cream: apply twice daily × 1–4 weeks [b]1% solution: apply twice daily × 1 week Oral: 250 mg/ day × 2–4 weeks	Oral: 200 mg/ day × 1 week	Oral: 150–300 mg once weekly × 2–4 weeks	[b]2% cream: apply once daily × 2 weeks [b]Oral: 200–400 mg/ day × 4 weeks *(seldomly used)*	[b]Microsize: 500 mg/ day Ultramicrosize: 330–375 mg/ day × 2–4 weeks	[b]Ciclopirox 0.77% cream or gel: Twice daily × 4 weeks	Clotrimazole miconazole, Butenafine, Econazole, Luliconazole, Sertaconazole, Naftifine
Tinea capitis	See Table 24.3 for pediatric dosing	See Table 24.3 for pediatric dosing	See Table 24.3 for pediatric dosing	Only effective against *Trichophyton*. 2% shampoo used as adjunct therapy *(seldomly used)*	See Table 24.3 for pediatric dosing	Selenium sulfide shampoo 1% as adjunct therapy	Corticosteroid adjunct therapy for severe inflammatory varieties
Onychomycosis	[b]Oral: 250 mg/day *Toenail: 12 weeks* *Fingernail:* 6 weeks	[b]Oral: [b]Continuous therapy: 200 mg/day × 12 weeks Pulse therapy: 200 mg bid for 1 week, followed by 3 itraconazole-free weeks *toenails: 3 pulses* [b]*Fingernails only: 2 pulses*	Oral: 150 mg once weekly *Toenail:* 9–15 months *Fingernail*: 4–9 months	Oral: 200–400 mg/day × 6 *not recommended due to hepatotoxicity risk*	[b]Microsize 1 g/day Ultramicrosize 660 or 750 mg/ day × 4–12 months	[b]Ciclopirox 8% lacquer: Once daily/48 weeks [b]Efinaconazole 10% laquer: Once daily/48 weeks [b]Tavaborole 5% sol. Once daily/48 weeks	Amorolfine 5% lacquer—*Not approved in North America (approved in Australia and UK)*
Pityriasis versicolor	[b]1% solution: apply twice daily × 1 week Oral: not effective	Oral: 200 mg/ day × 5–7 days	2% shampoo: 5 days Oral: 300 mg once weekly × 2 weeks	[b]2% cream: apply once daily × 2 weeks Oral: 200 mg/ day × 2 weeks, 10 days or 5 days; 400 mg per week × 2 weeks; 400 mg per day × 3 days; 3 doses of 400 mg given every 12 h	Not effective	[b]Ciclopirox 0.77% cream [b]Selenium disulfide	[b]Clotrimazole, [b]Miconazole, [b]Butenafine, [b]Econazole
Seborrheic dermatitis	1% solution: once daily × 4 weeks Oral: 250 mg/ day × 4 weeks	Oral: 200 mg/ day × 1 week	2% shampoo: twice a week × 4 weeks	[b]2% cream: apply twice daily × 4 weeks [b]Shampoo: twice a week × 4 weeks	Not effective	[b]Ciclopirox 0.77% cream, shampoo, or gel [b]Selenium sulfide [b]Coal tar [b]Hydrocortisone	[b]Zinc pyrithione, Metronidazole, Bifonazole, Miconazole

bid twice daily

[a]There are no approved treatments specifically for tinea manuum; treatments shown are for tinea pedis, which are effective in the treatment of tinea manuum

[b]FDA-approved indications

serious skin events such as Stevens-Johnson syndrome, and possible drug–drug interactions due to metabolism through the cytochrome P-450 system. For this reason, clinicians need to evaluate the patient's medical history and be aware of potential drug interactions prior to prescribing medication.

Relapse has been noted with most dermatophyte infections. Patients must be encouraged to complete a full treatment cycle, as infection can be present without visible symptoms. Assessment must include microscopic examination and culture to confirm elimination of the pathogen. Infection transmission from symptom-free carriers such as family members and pets should be controlled.

Tinea Pedis/Manuum

Griseofulvin and topical terbinafine, butenafine, miconazole, econazole, ketoconazole, clotrimazole, sertaconazole, luliconazole, and ciclopirox are FDA-approved treatments (Table 24.2) [8, 21]. Studies have shown that oral terbinafine and itraconazole may be the most effective, as they have produced a higher cure rate than has been shown with topical allylamines [21, 53]. Topical formulations may be used for milder, limited presentations. For widespread or more severe infections, oral formulations may be required. Relapses are more common with topical agents [53].

Broad spectrum topical agents may be useful, and agents with antibacterial activity may be preferred when superimposed bacterial infection is suspected (e.g., miconazole nitrate 1%, ciclopirox olamine 1%, naftifine hydrochloride 1%, sulconazole nitrate 1%). Once daily formulations may be preferred to twice daily usage, to aid patient compliance (e.g., naftifine 1% cream, bifonazole 1%, ketoconazole cream 2%). Chronic infection may warrant the use of oral antifungals, particularly if previous topical regimens have failed. Oral itraconazole, terbinafine, and fluconazole have been used successfully used in tinea pedis, though none of these agents is currently approved by the FDA for use in tinea pedis. These oral agents are preferred over ketoconazole, due to the potential for severe hepatic side effects with ketoconazole. Oral griseofulvin is less effective than newer antifungals [10]. There are no approved treatments specifically for tinea manuum; treatments for tinea pedis are effectively used to treat tinea manuum.

Tinea pedis may frequently recur. Proper foot hygiene may help prevent reinfection. Patients should avoid walking barefoot in communal areas such as bathrooms, showers, or swimming areas and ensure that feet are dried thoroughly after bathing, showering, or swimming. Additionally, patients should avoid occlusive footwear, alternate shoes every 2–3 days, and change socks often [54].

Tinea Corporis/Cruris

Griseofulvin and topical terbinafine, butenafine, econazole, miconazole, ketoconazole, clotrimazole, and ciclopirox are FDA-approved treatments (Table 24.2) [26]. Topical formulations may be used for infections in smaller areas (e.g., sulconazole, oxiconazole, miconazole, clotrimazole, econazole, ketoconazole, sertaconazole, luliconazole) [11]. Oral therapy may be required when large areas are involved, or when infection is chronic or recurrent. Topical corticosteroid should be avoided as it may lead to suppression of signs of infection [11]. Oral itraconazole, terbinafine, and fluconazole have been used successfully for tinea corporis/cruris, although none of these agents is currently approved by the FDA for use in these indications. These oral agents are preferred over ketoconazole, due to the potential for severe hepatic side effects. As tinea cruris can be associated with *Candida* infections in the inguinal region; the use of systemic triazoles (itraconazole or fluconazole) is preferred. Griseofulvin is not recommended as it does not adequately bind the keratin in the stratum corneum, reducing efficacy [11].

Tinea faciei is typically cleared with topical treatment. Topical ciclopirox and terbinafine may provide good anti-inflammatory effects as well as antifungal activity [13]. Miconazole, ketoconazole, and sertaconazole or similar azoles may also be effective. Azoles should be used for 3–4 weeks, or at least 1 week after resolution of lesions. Resistant lesions, cases of extensive disease, or more severe cases may require oral therapy [13].

Tinea imbricata is best treated with oral terbinafine or griseofulvin, though a high rate of recurrence has been noted. Itraconazole and fluconazole have not been effective. Adjunctive therapy with keratolytic creams such as Whitfield's ointment (benzoic and salicylic acids) may increase treatment efficacy [31].

Tinea Capitis

Oral therapy is required to adequately treat tinea capitis. Topical antifungals such as shampoos (selenium sulfide, povidone iodine, zinc pyrithione) may be used as adjunct therapy with or without oral antifungals to prevent reinfection or to treat asymptomatic carriers [32, 54]. As most infections occur in children, dosing regimens are modified from typical adult regimens provided for other indications and are usually given on a weight-based schedule (Table 24.3). Furthermore, infections with *Microsporum* may require higher dosing than infections with *Trichophyton*, or longer regimens of therapy [32, 54]. Griseofulvin is the only FDA-

Table 24.3 Pediatric tinea capitis dosing regimens

Regimen		Duration[a]	Weight (kg)				
			10–20	21–30	31–40	41–50	50+
Terbinafine (continuous)	5 mg/kg/day[b]	2–4 weeks	62.5 mg/day	125 mg/day	125 mg/day	250 mg/day	250 mg/day
Itraconazole (continuous)	5 mg/kg/day	2–4	100 mg every other day	100 mg/day	100 mg once daily alternating with twice daily	200 mg/day	200 mg/day
Itraconazole (pulse)[c]	Capsules: 5 mg/kg/day	1– 3 pulses	100 mg every other day	100 mg/day	100 mg once daily alternating with twice daily	200 mg/day	200 mg bid[d]
	Oral suspension: 3 mg/kg/day	1– 3 pulses					
Fluconazole (continuous)	Oral suspension: 6 mg/kg/day	20 days					
Fluconazole (pulse)[e]	Oral suspension: 6 mg/kg/day	8–12 weeks					
Griseofulvin (continuous)	Microsize: 20–25 mg/kg/day	6–12 weeks					
	Ultramicrosize: 10–15 mg/kg/day	6–12 weeks					
	Oral suspension: 15–25 mg/kg/day[f]	6–12 weeks					

bid twice daily

[a]Durations of treatment are for *Trichophyton tonsurans* infection. Longer durations are often required for *Microsporum canis* infections

[b]Drugs are given by once daily dosing unless otherwise specified

[c]Itraconazole pulses are given for 1 week, with 3 weeks "off" before starting the next pulse

[d]Itraconazole adult dose 200 mg bid (approved for pulse use in fingernail onychomycosis). No standard has been established in clinical trials for tinea capitis for children >50 kg; use varies from once daily as with continuous regimen to twice daily 200 mg dosing

[e]Fluconazole pulses are 1 day on, 6 days off, before beginning next pulse

[f]Dosing based on Grifulvin V suspension 125 mg/5 mL

approved oral treatment; however, terbinafine, itraconazole, and fluconazole have frequently been used for the successful resolution of tinea capitis. Itraconazole, terbinafine, and fluconazole have shorter treatment durations than griseofulvin [32]. Liquid formulations are available for griseofulvin, itraconazole, and fluconazole. These allow pediatric dosing, though dosing regimens may vary from that suggested for tablet/capsule formulations. In general, meta-analyzes indicate that griseofulvin is better for *Microsporum* and terbinafine for *Trichophyton* [55].

Infected children do not need to be kept out of school once treatment is initiated, particularly children in higher grades [54, 56, 57]. Infection transmission from symptom-free carriers such as family members and pets may need to be controlled. Objects that may act as fomites, such as hats, combs, pillows, blankets and scissors, should be disinfected with bleach [54].

An "id" reaction has been observed with tinea capitis patients following initiation of drug therapy and can be confused with allergic drug reaction [32]. An "id" reaction may present as symmetrical, skin colored or erythematous papules and plaques on the face, neck, and upper body, and sometimes be generalized. The reaction may also be present prior to initiating treatment.

Onychomycosis

Onychomycosis is difficult to cure and has a high rate of recurrence [8, 14, 58]. Typically, oral therapy is required to adequately treat this form of infection (Table 24.2). Following successful treatment, the infected nail must grow out, gradually becoming replaced by normal healthy nail. This process may take 9–18 months, depending on the nail growth rate. Fingernails may show better response rates than toenails, as they grow faster. Where the nail has been injured or shows other abnormal growth patterns, nail outgrowth may be slow, and the nail may never regain a normal appearance. Furthermore, relapse is frequent. Patient expectations should be discussed, so that the patient understands that successful treatment is unlikely to occur quickly and that long-term follow-up may be necessary to detect relapses.

Topical therapy may be effective in mild to moderate cases. Ciclopirox 8% lacquer, efinaconazole 10%, and tavaborole are the three topical therapies approved for onychomycosis by the FDA. Amorolfine 5% nail lacquer has not been approved for use in North America but is approved in Australia and UK [59–61]. Routine nail debridement may be needed to provide effective drug delivery to the infected area and to reduce the burden of fungal material needing treat-

ment [58]. Efinaconazole 10% solution was recently evaluated in a double blind multicenter study to define its value as topical treatment. After 52 weeks, 17.8% achieved a complete cure with efinaconazole. Typically, efinaconazole is applied daily for 48 weeks [62]. Tavaborole 5% (topical solution) is an oxaborol derivative that inhibits the fungal leucyl-tRNA synthetase, an essential component in fungal protein synthesis, leading to fungal cell death, with a broad spectrum of activity against dermatophytes and *Candida*. This agent is also applied daily for 48 weeks. The effectiveness of these 4 topical antifungal agents for onychomycosis is variable. They are typically recommended for cases where oral therapy cannot be given or are used concomitantly with oral therapy [63].

The oral agents, terbinafine and itraconazole, are frequently used for onychomycosis. Ketoconazole is currently not used due to potential hepatic side effects. Griseofulvin is also not recommended because the required regimens are significantly longer than those of itraconazole or terbinafine, and even more important, its efficacy is poor [64]. Fluconazole has shown high efficacy, low relapse rates, and usefulness with yeast coinfection; however, there have been few studies of this treatment method [26, 58, 61].

Approved oral therapy regimens for onychomycosis are: terbinafine 250 mg/day for 12 weeks (toenails) or 6 weeks (fingernails only); itraconazole 200 mg/day for 12 weeks (toenails with or without fingernail involvement); and itraconazole 200 mg twice daily as pulse therapy (one pulse: 1 week of itraconazole followed by 3 weeks without itraconazole) using 2 pulses (fingernails only). Though only a continuous regimen of itraconazole is FDA-approved for toenail onychomycosis, the current standard of care of toenail onychomycosis used by US dermatologists is a pulse itraconazole regimen (1 pulse: 1 week of itraconazole followed by 3 weeks without itraconazole; a total of 3 pulses is given).

Both terbinafine and itraconazole are readily taken up in the nail from the nail bed and matrix and may remain in the nail for a significant period after dosing is completed. Itraconazole tends to be fungistatic, while terbinafine is fungicidal [64]. Mycological cure rates (KOH negative and culture negative) for terbinafine use are estimated at 76% in a meta-analysis of clinical trial data [65]. By comparison, itraconazole mycological cure rates are 59% (continuous therapy) and 63% (pulse therapy). Clinical response rates (infection cleared or showing marked improvement) were: terbinafine, 66%, itraconazole continuous therapy, 70%, and itraconazole pulse therapy, 70% [65].

Itraconazole may be associated with more drug interactions than terbinafine due to its metabolism through the CYP 3A4 pathway, limiting its use in some patients. Itraconazole is also prohibited in patients showing ventricular dysfunction such as current or past congestive heart failure [65]. A current country-specific product monograph should be consulted for complete listing of known drug interactions, warnings, and monitoring requirements prior to prescribing. Rare cases of hepatic injury have been reported, and monitoring of hepatic enzymes is recommended for subjects with pre-existing hepatic abnormality or a history of liver toxicity with use of other medications. Capsules must be taken with a meal or a cola beverage to ensure adequate absorption [64].

Terbinafine may interfere with the metabolism of CYP 2D6 substrates, and some other drug interactions have been noted. A current country-specific product monograph should be consulted for complete listing of known drug interactions, warnings, and monitoring requirements prior to prescribing. Rare cases of hepatic injury have been reported with terbinafine. Terbinafine is not recommended for patients with existing liver disease, and all patients should be screened for hepatic enzyme abnormalities (alanine transaminase, ALT, and aspartate transaminase, AST) prior to initiating terbinafine [64]. Terbinafine may be taken in the fasted or fed state without affecting absorption.

Both pulsed itraconazole and continuous terbinafine have been used for the management of onychomycosis due to non-dermatophyte molds (*Aspergillus, Fusarium,* and *Scopulariopsis*). Although the data are limited, a retrospective review of the mycological and complete cure rates of terbinafine (69.8% and 17%) and itraconazole (67.5% and 22%) showed no significant difference in efficacy [66].

Photodynamic therapy has been successfully used in isolated cases in which oral therapy was contraindicated [67]. Laser has recently been evaluated by several authors. Kozarev and Vizintin used VSP Nd:YAG 1064 nm laser with fluences in the range of 35 to 40 J/cm^2, a 4 mm spot size, a pulse duration of 35 ms, and a pulse rate of 1 Hz for a total of three passes to treat fungal onychomycosis. They used laser energy to completely cover the nail plate in 72 patients with a confirmed diagnosis of onychomycosis caused by *Trichophyton rubrum* (37 patients), *Trichophyton mentagrophytes* (22 patients), *Candida* species (10 patients), and *Aspergillus niger* (3 patients). At 3 month follow-up 95.8% of patients were cleared of fungal disease. At 6 and 12 months they found 100% clearance of fungal infection with no other treatment [68]. In contrast, Carney et al. performed a 4-part in vitro, in vivo study. The first part evaluated 3 different nail pathogens in suspension at 7 heat and time exposures. The second and third parts irradiated pure fungal colonies. A fungicidal effect for *T. rubrum* was found in vitro at 50 °C for 15 min. No growth inhibition was seen for the *T. rubrum* colony treated with direct laser irradiation with fluences of 5, 15, 20, and 50 J/cm^2 with a pulse rate of 7–10 Hz. Temperature of the media during laser treatment was 40 °C. The in vivo assessment was a 24-week pilot study of 10 patients using a submillisecond (Nd:YAG) 1064-nm laser as the only treatment for onychomycosis. Fluences of 16 J/cm^2, a pulse duration of 0.3 ms with a total of 5 passes per session were

applied. Outcome showed no improvement in the Onychomycosis Severity Index score [69].

These results need to be further researched and validated in a well-designed multicenter, randomized, comparative study with independent evaluators using the same laser administration parameters to define the true usefulness of this therapeutic procedure.

In a systematic review and meta-analysis the treatment of onychomycosis with laser was evaluated; the authors observed that the general mycological cure rate with different types of laser therapy was 63%, while the cure rate with 1064 nm Nd:YAG laser was 63%. The authors concluded that laser therapy in onychomycosis is effective and safe; that the most effective laser modality is CO2, and the most common adverse events were pain and bleeding. Currently, this technique is being combined with the administration of topical and systemic antifungal drugs, but there are still no conclusive data [70].

Routine nail debridement may be used in conjunction with oral or topical therapy, particularly where the nail is thickened, or when disease presents as a dermatophytoma, spike or lateral infection. Caution must be taken not to damage the underlying skin during debridement, particularly in subjects who are vulnerable to severe lower limb complications, such as individuals with diabetes or lower limb peripheral vascular disease.

As with tinea pedis, proper foot and nail hygiene may help prevent reinfection. Patients should avoid walking barefoot in public areas such as restrooms, showers, or swimming pools and make sure that feet have been dried thoroughly after bathing, showering, or swimming [71]. Nails should be kept short and clean. Shoes should fit properly and socks should be made from absorbent material such as cotton.

Pityriasis Versicolor

A variety of topical agents may be used to treat pityriasis versicolor (Table 24.2). Topical azoles (ketoconazole, fluconazole, bifonazole, clotrimazole, miconazole) have been effective in treating *Malassezia*, both in cream formulation or shampoos. Terbinafine solution, cream, gel, or spray, but not the oral formulation, has also been effective [6]. Topical ciclopirox provides both antifungal and anti-inflammatory activity against *Malassezia*.

Systemic therapies may be warranted in severe cases, or cases with widespread body involvement. Patients may also prefer a short-duration oral therapy to frequent application of a topical agent. Oral therapy with ketoconazole, itraconazole, and fluconazole has been effective for pityriasis versicolor, and the regimens reported in the literature provide similar, high efficacy rates [6]. Oral ketoconazole is no longer recommended due to side effects. Oral terbinafine and griseofulvin are not effective for pityriasis versicolor [6].

Relapse of pityriasis versicolor is commonly due to endogenous host factors. Recurrence rates have been reported as high as 60–90% in 2 years post-treatment [71]. Both ketoconazole (single 400 mg dose or 200 mg daily for 3 days once monthly) and itraconazole (single 400 mg dose once monthly for 6 months or with the administration of 100 mg / day for 10 days) have been used for prophylaxis of pityriasis versicolor [72].

Individual treatments for hyper- and hypopigmented variations of pityriasis versicolor do not exist. Although fungal organisms may be eradicated after 2 weeks of therapy, it may take significantly longer before normal skin pigmentation is restored, particularly with hypopigmented lesions [6].

Seborrheic Dermatitis

There is no definitive cure for seborrheic dermatitis; it is a recurrent disease requiring prophylactic treatment [18]. Topical corticosteroid lotions have typically been used but are being replaced by antifungals in the form of shampoos, gels, and creams (Table 24.2).

Topical ketoconazole (cream, shampoo, gel, emulsion) is the most prescribed azole for SD [73]. Bifonazole, miconazole, and fluconazole may also be effective. Low-potency corticosteroids may be useful in providing an anti-inflammatory effect, though many newer antifungal agents such as ciclopirox may also provide anti-inflammatory activity comparable to corticosteroids [73]. Ciclopirox (cream, gel, or shampoo) provides effective antifungal treatment and also has antibacterial and anti-inflammatory activities [74, 75]. Zinc pyrithione shampoos are safe and effective in controlling dandruff and SD of the scalp and exhibit strong keratolytic and antifungal activity against *Malassezia* [18, 76]. Some patients benefit as well from non-antifungal, keratolytic agents (selenium sulfide, sulfur, salicylic acid) or antiproliferative (coal tar) shampoos [17, 42]. Tar shampoos often cause sensitivity of the skin to sunlight and are not as favorable cosmetically [42]. Topical 1% terbinafine solution has been effectively used for scalp seborrheic dermatitis [73].

Oral therapy should be reserved for severe inflammatory seborrheic dermatitis, widespread seborrheic dermatitis, or seborrheic dermatitis that has been refractory to topical treatment [73]. Oral ketoconazole and oral itraconazole have been used effectively for seborrheic dermatitis. Oral itraconazole is safer than ketoconazole and is effective in severe cases which have not responded to other antifungals [18].

Similar to pityriasis versicolor, *Malassezia* folliculitis responds better to oral treatments. The most effective is ketoconazole, but this agent is no longer recommended due to

side effects. Good response has been reported with itraconazole and fluconazole. This can also be managed with topical imidazoles and keratolytics [72].

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Fungal Infections of Implantation (Chromoblastomycosis, Mycetoma, Lobomycosis, and Entomophthoromycosis)

25

Flavio Queiroz-Telles
and Daniel Wagner de C. L. Santos

Introduction

Fungi may infect mammalian hosts by several manners, including inhalation and traumatic implantation through the skin. The respiratory route may result in allergic manifestations, pulmonary diseases, and/or systemic mycoses, based on the fungal pathogen and the host immune response. Among the fungal pathogens, there is a group of organisms that gain access though different kinds of transcutaneous wounds, leading to implantation mycoses. This group of fungal disease is also called the "subcutaneous mycoses," but this term is inadequate as in most of these infections, the tissue involvement may go beyond the cutaneous and subcutaneous structures. The implantation mycoses encompass a group of unrelated fungal diseases whose etiologic agents are transported from their saprobiotic niche to the cutaneous tegument through inoculation traumas. The list of implantation mycoses includes sporotrichosis, eumycetoma, chromoblastomycosis (chromomycosis), phaeohyphomycosis, lobomycosis (lacaziosis or Jorge Lobo's disease), and entomophthoromycosis [1–7]. These diseases are a frequent health problem in tropical and subtropical areas of the world [8]. Sporotrichosis, the most globally widespread and prevalent implantation mycoses, is covered in a separate chapter in this book (Chap. 22).

Chromoblastomycosis, eumycetoma, lobomycosis, and entomophthoromycosis are geographically restricted to tropical and subtropical zones. These endemic implantation mycoses are neglected diseases, affecting the low-income populations in developing regions of Africa, Asia, and Latin America. Although they rarely disseminate, their morbidity is significant due to sequelae and incapacity seen in their most severe clinical forms. If not discovered at in earlier stages, the implantation mycoses are recalcitrant and very difficult to manage (Table 25.1) [2, 10]. These infections are characterized by initial lesions starting at the site of fungal implantation, which with time evolve according to the etiologic agent and the host immune defenses to produce subacute to chronic clinical manifestations. Although the term "subcutaneous mycoses" have been used for decades, it is not strictly correct because in some of these infections spreads beyond the cutaneous and subcutaneous tissues, to involve the lymphatic vessels, fascia, muscles, cartilage, and bones [1, 2].

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6_25

Table 25.1 Epidemiology, clinical manifestations, diagnosis, and treatment of the endemic implantation mycoses[a]

Disease	Epidemiology and geographical distribution	Clinical presentation	Diagnosis	Treatment
Chromoblastomycosis (also called chromomycosis, verrucous dermatitis, figueira[b])	Global tropical distribution, usually in men aged 30–50 affecting farmers, gardeners, and lumberjacks: a risk factor is non-use of protective gloves, footwear, or garments when working with plants and soil products Occupational risk for those working with palm trees, black tea, rubber plantations, etc. Genetic susceptibility?	Slow clinical progression usually limited to the skin and subcutaneous tissue in which initial erythematous papular lesions may gradually evolve to display polymorphic clinical aspects, usually nodular and verrucous cutaneous lesions It may affect feet and legs most frequently; may cause chronic lymphedema and transform into squamous cell carcinoma	**Mycology** The observation of muriform cells (sclerotic bodies) is mandatory. Examination of scrapings, exudate or aspirates or vinyl adhesive tape preparations on wet mount examination Culture with molecular identification is necessary for species identification **Histopathology** Pseudoepitheliomatous hyperplasia, granulomatous reaction, and epidermic abscess associated with muriform cells are usual	Surgery effective in early stages; itraconazole (200–400 mg/d), terbinafine (250–500 mg/d), terbinafine (500 mg/d) plus itraconazole (50–100 mg/d); combination therapy (itraconazole with terbinafine or 5-flucytosine) for severe cases, posaconazole (400 mg po bds) in patients with disease refractory to itraconazole or who are intolerant of itraconazole; cryotherapy
Eumycetoma (also called mycetoma, Madura foot, maduromycosis)	Men aged 20–40 who work as herders, farmers, or other field laborers [9]; increasingly in travelers to tropical endemic areas	Local chronic, progressive, multifistulous, suppurative, tumoral lesions discharging grains. Infection involves cutaneous and subcutaneous tissues, fascia, and eventually muscle and bone	Observation of grain color and texture; deep surgical biopsies containing grains that can be cultured or fixed for histopathology; immunodiffusion, ELISA, PCR with DNA sequencing; MRI or CT to determine bone involvement	For *S. apiospermum* and melanized fungi causing "black grain" eumycetoma, surgery and antifungal therapy with itraconazole (400 mg/po), often given for 7–12 mo; posaconazole (400 mg po bid) in patients with disease refractory to or who are intolerant of itraconazole for fusarium spp. infections, voriconazole or posaconazole is indicated. Fosravuconazole for M. mycetomatis infection (experimental)
Entomophthoromycosis (also called subcutaneous zygomycosis, subcutaneous phycomycosis, basidiobolomycosis, conidiobolomycosis)	Infections usually caused by *Entomophthorales* species; usually in immunocompetent persons Basidiobolomycosis usually in children and conidiobolomycosis mostly in adults	**Basidiobolomycosis** Usually chronic and progressive course; hard nodules that spread, often over thighs and buttocks, eventually ulcerating overlying skin Other affected sites include maxillary sinus, palate, gastrointestinal tract, retroperitoneal space, and the lungs **Conidiobolomycosis** Begins with swelling of inferior nasal cones and extends to facial and subcutaneous tissues and paranasal sinuses Subcutaneous nodules may attach to underlying tissues, causing facial disfigurement	**Histology** Wide sparse septate, thin-walled hyphae with right-angle branching. Splendore–Hoeppli phenomenon present with basidiobolomycosis, (sometimes with conidiobolomycosis)	Most used therapy is itraconazole, 100–200 mg per day; potassium iodide, terbinafine may be a second option. Amphotericin B for severe and disseminated disease

Table 25.1 (continued)

Disease	Epidemiology and geographical distribution	Clinical presentation	Diagnosis	Treatment
Lobomycosis (also called lacaziosis, Lobo's disease, Jorge Lobo's disease, paracoccidioidomycosis loboi)	Adult men living or working in the Amazon Rainforest. Farmers, miners, hunters, rubber workers, military personnel contact with sick dolphins are in risk	Lesions are indolent, evolving over many years; polymorphic cutaneous lesions, mostly plaque and nodules with keloid-like features; pinna of the ear most commonly affected; initial lesion followed by traumatic or autoinoculation; nodule distribution follows lymphatic system	Microscopy of tissue smears from lesions, examination of vinyl adhesive tape preparation; cannot be cultured. **Serologic tests** High sensitivity but lack specificity; antigenic cross-reactivity with *Paracoccidioides* spp.	Wide surgical excision, electrodessication in early stage of disease, cryosurgery; clofazimine (300 mg/d until clinical improvement, then 100 mg/d for ≥2 y). Amphotericin B, 5-fluorocytosine, and azoles usually ineffective, except for localized lesions that may respond to long courses of posaconazole

bds twice daily, *CNS* central nervous system, *CT* computed tomography, *d* day, *ELISA* enzyme-linked immunosorbent assay, *mo* month, *MRI* magnetic resonance imaging, *PCR* polymerase chain reaction, *po* orally, *SSKI* saturated solution of potassium iodide; *tds*, 3 times daily, *wk* weeks, *y* years, *TNF* tumor necrosis factor

[a]Adapted from [8]

[b]Means "fig tree," the popular name of this disease in Brazil

Chromoblastomycosis

Chromoblastomycosis or chromomycosis is one of the most prevalent implantation mycoses in tropical and subtropical zones as well as the most frequent human mycoses caused by melanized (dark pigmented) fungi [7, 11–15]. This disease was described by Max Rudolph, a German Doctor working in Brazil, in 1914 [16, 17]. Chromoblastomycosis (CBM) lesions are clinically polymorphic and if not recognized at earlier stages, they may become recalcitrant to therapy and extremely difficult to eradicate [10]. Except for the initial lesions, which should be surgically removed, moderate to severe clinical forms constitute a true therapeutic challenge for patients and clinicians. This disease presents with the following characteristics: primary lesion beginning at the site of implantation of the etiologic agent; chronic involvement of cutaneous and/or subcutaneous tissues, associated with a granulomatous, purulent, and fibrotic tissular reaction and a non-protective humoral and cellular immune responses [7, 18, 19].

Etiology

Melanized, dematiaceous, pheoid, or simply "black fungi" fungi are ubiquitous saprobes inhabiting soil, plant fragments, and water. Over 150 species from 70 genera have been implicated in human and animal disease [20, 21]. These agents contain melanin in their cell walls, which serves a key role in virulence in black fungi as a group. The list of human infections related to the melanized fungi includes phaeohyphomycosis (PHM), allergic and invasive sinusitis, fungemia, mycetoma, and chromoblastomycosis (CBM). The exact number of melanized (dematiaceous) fungi which may cause CBM is uncertain, especially as molecular tools continue to add to fungal taxonomy [15, 21–25]. It is believed that several species of the Herpotrichiellaceae family cause the disease. The main species, according to the taxonomic proposal, can be grouped into six genera: *Fonsecaea, Cladophialophora*, *Phialophora, Rhinocladiella*, *Exophiala,* and *Cyphellophora*. The most prevalent etiologic agents are members of the genus *Fonsecaea*, including *F. pedrosoi*, *F. monophora,* and *F. nubica*, occurring in the humid and hot climate, whereas *Cladophialophora carrionii* is restricted to the semi-arid areas where cactaceae is an endemic vegetation. Sporadic cases of this disease are caused by *Phialophora verrucosa, Rhinocladiella* spp. (*R. aquaspersa*, *R. tropicalis*, *R. similis*) and *Exophiala* spp (*E. dermatitidis*, *E. jeanselmei*, *E. spinifera*). Rare cases caused by *F. pugnacious*, *Cyphellophora ludoviensis* have also been described in the literature. Application of molecular tools has allowed better understanding of biodiversity and distribution of etiologic agents of CBM. As an example, while *F. monophora* is found to have a worldwide distribution, *F. pedrosoi* is typically restricted to hot and humid tropical areas, and *F. nubica* resides especially in Asia, Madagascar and occasionally in Latin America. Conversely, the currently recognized biodiversity of CBM agents in endemic areas has unknown clinical and epidemiological implications [14, 21–26].

Similar to the endemic fungi pathogens, the agents of CBM are dimorphic and present their mycelial form in natural habitats or in vitro culture media, but in tissues present as muriform cells (the hallmark of this disease). Muriform cells, also known as "sclerotic or fumagoid cells," "Medlar bodies" or "copper pennies," are a biological adaptation leading the agents to survive in the hostility of the host tissue environment [27, 28]. Muriform cells are characterized by thick melanized cell walls, isodiametric expansion, and

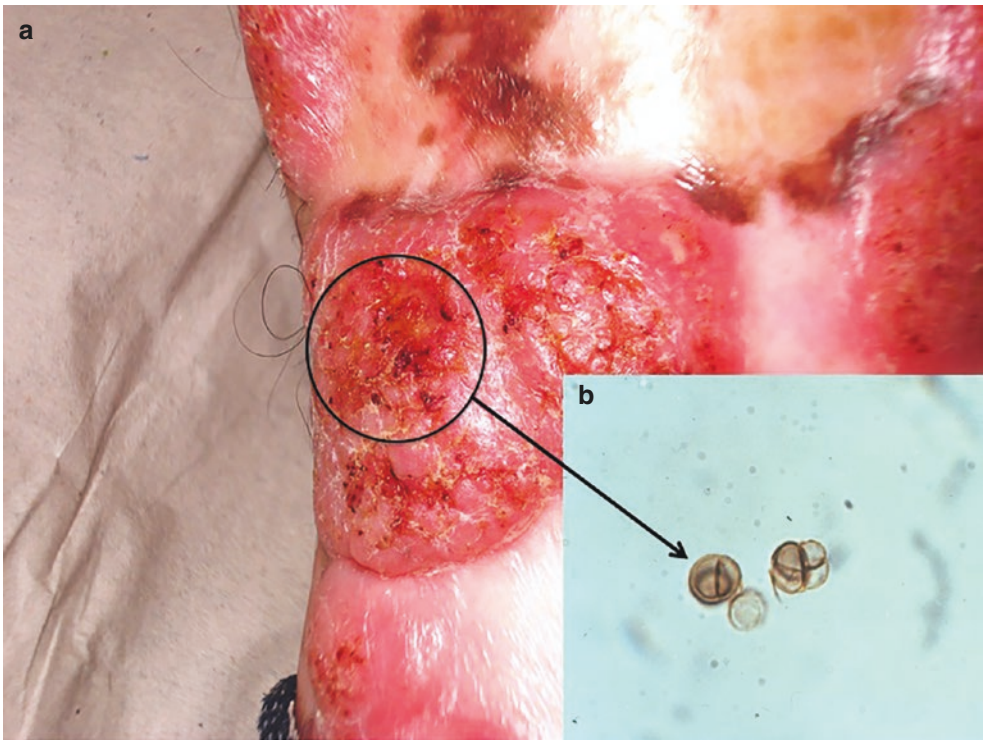


Fig. 25.1 A nodular plaque lesion of chromoblastomycosis containing several "black dots" (circle) was scraped (**a**). Muriform cells are easily observed in KOH wet mount (**b***). (*Adapted from Queiroz-Telles F and Santos DWCL. Chromoblastomycosis in the Clinical Practice. Curr Fungal Infec Rep **6**, pages 312–319 (2012) Reprinted with permission from SpringerNature)

unordered septum formation. They are polyhedric in shape, measuring 5–12 μm in diameter and divide by binary fission, forming two distinct planes of septation (Fig. 25.1). These are considered to be protective structures which also enhance resistance to antifungal drugs. Presence of muriform cells differentiates CBM from PHM [11, 19, 28].

In culture media, the agents of CBM grow as dark pigmented, velvety filamentous molds. Presumed species identification can be made by conventional mycological methods, like macro- and micromorphology and physiology characteristics, but nowadays, the definitive agent identification should be based on the molecular sequence of specific genes. Although the fungal species causing CBM do not differ in clinical aspects, they may present differences in their in vitro susceptibility to antifungal drugs. *Cladophialophora carrionii* and *Phialophora verrucosa* are considered to be more susceptible to antifungals than *Fonsecaea pedrosoi*. Even differences in susceptibility have been reported between the species of *Fonsecaea genus*, viz. *F. pedrosoi*, *F. monophora,* and *F. nubica* [14, 24, 25, 29].

Ecology and Epidemiology

Melanized fungi thrive in tropical and subtropical environments worldwide. It is believed that during their saprobic cycle, fungal agents of CBM live in soil and in different parts of plants such as thorns, leaves, spiny seeds, wood cortex, etc. [30, 31]. Consequently, CBM lesions afflict mainly adult males involved in rural labor who do not have appropriate footwear or clothing and who are more likely to be infected by trauma. CBM is diagnosed in people who live in remote and rural areas and affects some of the world's poorest and most marginalized communities, predominantly in Africa, Asia, and America. It has been widely described on all continents over the last eight decades and thrives in areas where access to adequate sanitation, clean water, and healthcare is limited [11]. In 54% to 85% of cases CBM involves the lower extremities [15, 30]. It is considered as an occupational disease in the endemic areas of the world, but scattered cases are also reported in temperate to cold regions. Chromoblastomycosis has no compulsory notification to the

health authorities, thus its real burden remains unknown. Conversely, data from published case reports and surveys shows that incidence rates may range from 1:6800 (Madagascar) to 1:8,625,000 (USA) [12, 19]. Most of the reported cases occur in Latin America, the Caribbean, Asia, Africa, and Australia. Madagascar, Brazil, Mexico, Dominican Republic, Venezuela, Cuba, South Africa, Republic of the Congo and Democratic Republic of the Congo, India, Japan, and Southern China contribute with the majority of cases. The disease is less frequent in the Northern Hemisphere, including European countries and in the USA, where it has been reported in Massachusetts (Boston), Texas, Missouri, Michigan, Georgia, Louisiana (New Orleans), and Pennsylvania (Philadelphia) [2, 4, 5, 11, 19].

Recently, Santos et al. described the most important and comprehensive review of CBM cases published between 1914 and 2020, showing a total of 7740 cases of CBM on all continents except Antarctica. For the first time, a world map was built with the prevalence of the disease in each country. The highest prevalences of CBM were reported in Costa Rica, Dominican Republic, Venezuela, French Guiana, Gabon, Madagascar, Botswana, Reunion and Comoro Islands, New Zealand, and Solomon Island [11].

Although children may present with the disease, the vast majority of patients are adult males. The main risk factors associated with CBM infection are adult age, male sex, rural work or outdoor activities, lack of protective shoes, gloves or garments, and poor nutrition and hygienic habits [2, 9]. In a series of 100 patients reported from the Brazilian South Region, the majority of patients were male (4:1) of 50–59 years of age (36). In another study of 325 cases in the Brazilian Amazon region, the main age group affected by the diseases ranged from 41 to 70 years old, 86.1% of the patients were agricultural workers, and 93.2% of were male [2]. In both reports CBM lesions predominated on the lower limbs (feet and legs) and *F. pedrosoi* was the main etiologic agent [13, 14, 32]. Conversely, a series of cases in children and adolescent patients ranging from 2 to 19 years old have been reported in an endemic Venezuelan semi-arid zone. Infection was related to *C. carrionii*, probably transmitted by cactaceae thorn implantation [9].

Immunopathogenesis

Human infection by CBM agents start when fungal elements are transferred from their environmental saprobe life across the cutaneous barrier through transcutaneous trauma. If the parasite survives and adapts to the new hostile environment represented by the host's cutaneous/subcutaneous tissues, the infection may progress to disease [18, 19]. Because labor-related trauma is very frequent among rural workers living in tropical regions, unsuccessful infections may be the rule since melanized fungi are ubiquitous in nature, but CBM is not an everyday disease (even in the endemic areas). So other factors must play a role in the pathogenesis of CBM, including fungus virulence, host genetic susceptibility, and continuing exposure to the agents during life. It has not been proved if hormonal factors are able to protect females like in paracoccidioidomycosis [18, 31, 33–35].

The knowledge of immunology of CBM is poorly understood because, to date, there are no reproducible animal models of this disease. Mechanisms of immunity may include humoral- and cell-mediated responses. Like other chronic fungal infections, CBM patients produce specific antibodies, but not protective antibodies against the progression of the disease. It has been demonstrated that the cell-mediated immunity plays a key role in the clinical presentations of this disease. The mixed tissue response in CBM shows a granulomatous reaction associated with micro-abscesses, suggesting ineffective phagocytosis of muriform cells and chronic infection [19, 36, 37].

Clinical Manifestations

Following implantation, and after an uncertain period of time, the initial lesion may be produced at the site of infection. It may start as a solitary macular lesion and latter; it may progress to a papular shape lesion with a pink smooth surface that gradually increases over a few weeks and then develop a scaly surface. The initial skin lesion may progress and evolve with diverse clinical polymorphism, eliciting differential diagnoses including many infectious and non-infectious diseases (Table 25.2). According to the modified Carrion's classification the CBM lesions are characterized as nodular, tumoral (cauliflower-like), verrucous, cicatricial, or plaque (Fig. 25.2) [15, 26, 28]. In advanced and more severe cases, more than one type of lesion can be observed in the same patient. The lesions may also be graded according to severity, which may help clinicians plan a patient's therapy (Table 25.2) [19, 38]. Initially the mild form lesions are asymptomatic, but with time itching becomes the predominant symptom of the disease, which in the moderate forms is

Table 25.2 Lesion types, severity rating, and differential diagnosis of chromoblastomycosis

Type of lesions	Severity of disease	Differential diagnosis
Nodular type Moderately elevated, fairly soft, dull to pink violaceous growth. Surface smooth, verrucous or scaly. With time lesions may gradually become tumorous	**Mild form** A solitary plaque or nodule measuring less than 5 cm in diameter	**Infectious diseases** **Fungi** Paracoccidioidomycosis Blastomycosis Fixed sporotrichosis Coccidioidomycosis Phaeohyphomycosis Lobomycosis Eumycetoma Granulomatous candidiasis Granulomatous trichophytosis **Bacteria** Cutaneous verrucous tuberculosis Leprosy Actinomycetoma, nocardiosis Botryomycosis Tertiary syphilis, yaws Ecthyma Mycobacteriosis (*M. marinum, M. fortuitum*) **Protozoa** Cutaneous leishmaniasis Rhinosporidiosis **Viral** Verrucae, papilloma
Tumorous type Tumor like masses, prominent, papillomatous, sometimes lobulated; "cauliflower-like." Surface partly or entirely covered with epidermal debris and crusts. More exuberant on lower extremities		
Verrucous type Hyperkeratosis is the outstanding feature Warty dry lesions. Frequently encountered along the border of the foot	**Moderate form** Solitary or multiple lesions: Nodular, verrucous, or plaque types, existing alone or in combination, covering one or two adjacent cutaneous regions, measuring less than 15 cm in diameter	
Cicatricial type Non-elevated lesions that enlarge by peripheral extension with atrophic scarring, while healing takes place at the center. Usually with annular, arciform, or serpiginous outline. Tends to cover extensive areas of the body		
Plaque type Slightly elevated, with variously sized and shaped areas of infiltration. Reddish to violaceous in color presenting a scaly surface, sometimes shows marked lines of cleavage. Generally found on the higher portions of the limbs	**Severe form** Any type of lesion alone or in combination, covering extensive cutaneous regions whether adjacent or non-adjacent	**Helmintic** Filariosis **Noninfectious diseases** Scamous cell carcinoma Bowen disease Psoriasis Sarcoidosis Lupus erythematosus Podoconiosis Mossy foot

intense and may be accompanied by local pain. As severity increases, edema and bacterial secondary infections may lead to limitation or incapacity to continue working. Because the CBM lesions are very pruritic, it is believed that disease dissemination usually occurs by autoinoculation and contiguous lymphatic spread [11, 12]. However, lymphatic dissemination has been reported in a few cases. In very advanced cases, chronic lymphedema, ankylosis, and malignant transformation may occur [7, 39, 40].

A recent study involving 191 CBM cases in the North of Brazil, *Fonsecaea* species was isolated from 136 patients. After ITS isolate sequencies, no correlation was demonstrated between distinct *Fonsecaea spp* haplotypes and clinical forms or severity of the disease [14].

Diagnosis

As CBM lesions have a diverse morphology leading to an equally diverse differential diagnosis, this disease must always be confirmed by mycological exams (direct examination and culture) and/or histopathology (Figs. 25.1 and 25.3) [19].

Muriform cells may easily be observed on direct exams of crusts, skin scrapings, aspirates, debris or tissue fragments taken from the lesions after KOH (10–40%) digestion. The sensitivity of the direct examination ranges from 90 to 100%. Fungal elements are easily found at the lesional surface, resembling "black dots" which may be easily observed on the clinical examination or under dermoscopy. They consist of small hematic crusts with cellular debris and fungal structures, resulting from their transepithelial elimination (Fig. 25.1). Occasionally, near the cutaneous surface, these muriform structures may germinate and undergo dimorphic transformation into filamentous fungal forms [41, 42].

In histologic sections, CBM lesions are characterized by pseudoepitheliomatous epidermal hyperplasia, hyperkeratosis, irregular acanthosis, alternating with areas of atrophy and collections of inflammatory cells forming epidermic abscesses. Granulomatous reaction with different grades of



Fig. 25.2 Clinical classification of types of chromoblastomycosis. Lesions. Initial lesion (**a***), nodular (**b***), tumoral (**c**), plaque (**d***), verrucous (**e**), and cicatricial (**f***). (*From Queiroz-Telles F, et al. Subcutaneous mycoses. Infect Dis Clin N Am 2003;17(1):59–85. Reprinted with permission from Elsevier Limited)

fibrosis can be found at the dermal level. Muriform cells may be observed among these structures or inside Langerhans giant cells (Fig. 25.3) [43].

In culture, all the CBM agents are slow-growing dark fungi. Initially the colonies are deep green, developing a velvety dark aspect on their surface with time. Traditionally, their correct identification has been made through microscopic examination of the asexual reproductive structures like conidiophores, conidiogenous cells, and conidia (Fig. 25.3) [19].

However, advances in molecular taxonomy have shown that sequencing of specific genes is necessary for correct identification at the species level [24, 25].

Serological tests have not been standardized for CBM and are not used in the routine laboratory [44].

Therapy

Over the century that has passed since Max Rudolph first reported the disease, several therapeutic regimens have been proposed, including physical therapeutic methods and chemotherapy with antifungals. As comparative trials on this disease are lacking, evidence that helps to select optimal therapy is based on a few open clinical studies and many expert opinions. Though no "gold standard" therapy for CBM has been identified, several treatment options are available. Except for its initial localized lesions, which may be treated with surgical excision, most of the CBM clinical forms require long-term continuous systemic antifungal therapy.

Based on non-comparative trials, patients are mostly treated with itraconazole 200–400 mg per day, terbinafine 500 mg per day or with a combination of both drugs in refractory patients. The combination of itraconazole (200–400 mg daily) with 150–200 mg/kg/day of 5-flucytosine may also be successful in recalcitrant cases [45–52]. Among the new triazole drug, posaconazole has achieved favorable results in severe refractory cases of CBM, while voriconazole and isavuconazole have not been effectively tested [53, 54].

There are several reports indicating that physical methods, including photodynamic, laser, and thermo therapy (topical cold or heat), may shorten the needed duration of systemic antifungal treatment [55–60]. It is important to emphasize that physical methods must be used in combination to antifungal drugs. A few refractory cases of CBM were treated with adjuvant immunomodulation therapy, like topical imiquimod or intramuscular glucan, with partial response [19].

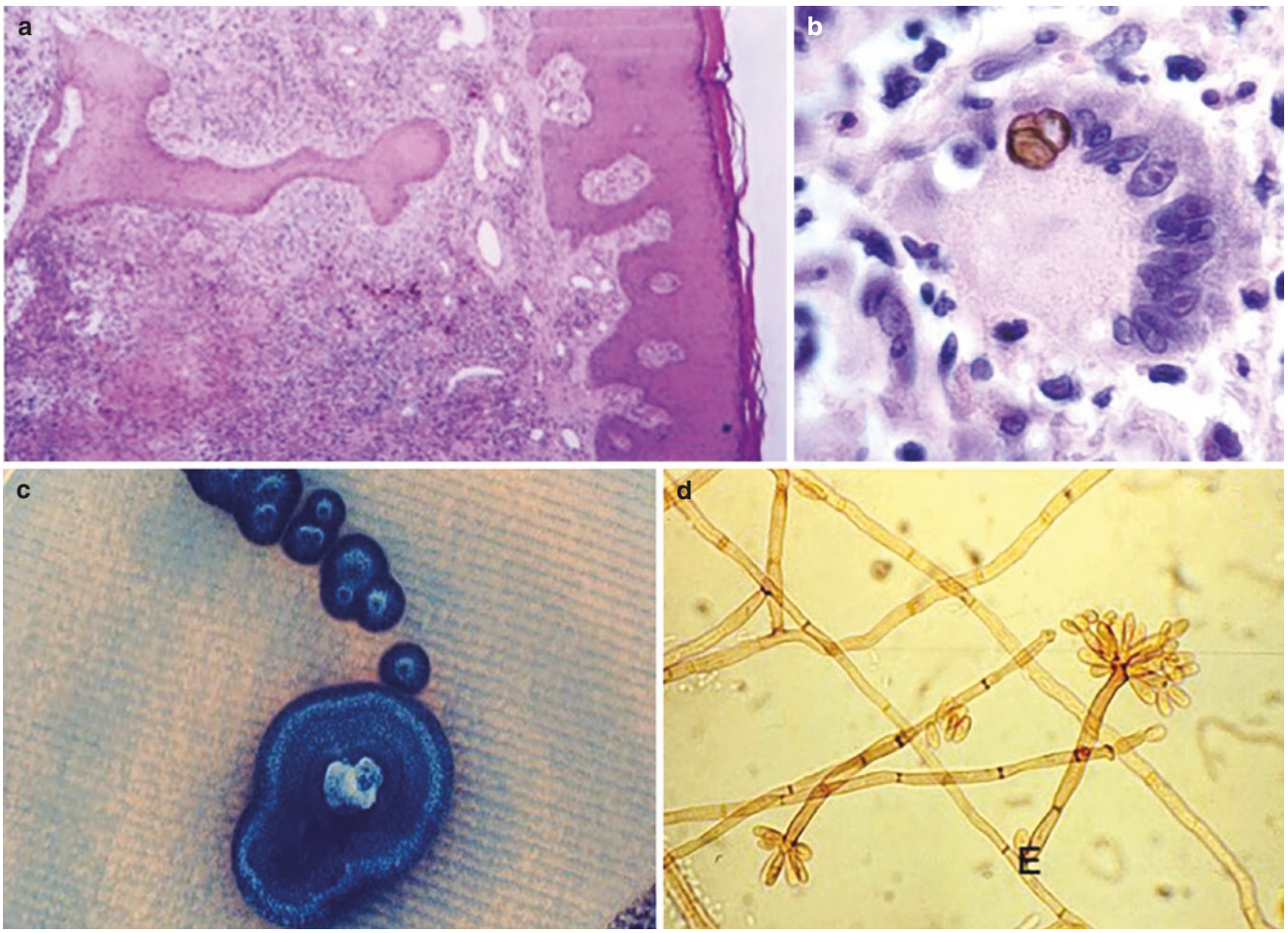


Fig. 25.3 Skin biopsy of patient with chromoblastomycosis with hyperkeratosis, acanthosis, and pseudoepitheliomatous epidermal hyperplasia. The derma depicts a mixed inflammatory reaction containing granulomata and abscesses. HE × 100 (**a**). Granuloma detail showing a Langham's giant cell with a muriform cell. PAS × 1000 (**b**). Melanized colony on Sabouraud dextrose agar (**c**) and microscopic morphology of *Fonsecaea pedrosoi* conidiogenesis. Slide culture × 600 (**d**)

Mycetoma

Mycetoma is a clinical syndrome usually composed of swelling, fistulous lesions which drain small grains. This disease can be caused by fungi (eumycetoma) or by microaerobic Actinomycetales members (actinomycetoma). Both diseases are endemic, been acquired by the inoculation route, mainly affecting rural workers around the world. Several bacterial etiologic agents may cause actinomycetoma but *Nocardia* spp. and *Actinomadura madurae* are the most prevalent agent in Latin America, especially in Mexico, Argentina, and Brazil. Eumycetoma is caused by several hyaline or melanized fungi. It is a chronic, progressive and granulomatous disease that although causing minimal mortality may have significative impacts in terms of mobility because of its devasting and incapacitant sequels, especially among the low incoming population leaving in the endemic area.

The term mycetoma derives from the Greek terms *mykes* (fungus) and *oma* (tumor). The disease was first mentioned in *Atharva Veda,* an ancient Indian religious book, as *pada valmikan,* which means foot anthill. The oldest known case of mycetoma most likely dates from the Byzantine period, as evidence was found in a human skeleton based on the bone morphological characteristics [61]. However, the disease was first described by John Gill in a dispensary report of the Madras Medical Service of the British Army in India in 1842, where it was named "Madura foot," as many cases originated from the city of Madurai [62]. The first description of mycetoma in the medical literature was given by Godfrey in 1846, who called it "*morbus tuberculosis pedis*" [63]. The fungal nature of the disease was established by Vandyke Carter, who also established several features, including its natural history, the body parts that are most commonly affected, the grain colors, the presence of bone

destruction, and the higher incidence among males [64]. It was also named mycetoma in 1860, which is the term by which it is currently known. In 1913, Pinoy subdivided the disease as a function of its bacterial or fungal etiology into two categories, actinomycetoma and eumycetoma, respectively [65].

Mycetoma is known under various names, such as fungus disease of India, Godfrey and Eyre's disease, endemic degeneration of the bones of the feet, *morbus pedis entophyticus-affection singulière* and perforating ulcer of the foot.

Like CBM, mycetoma has been officially recognized as a neglected tropical disease by the WHO and this will promote substantial improvements in care for these stigmatized patients [66–69].

Ecology and Epidemiology

The true incidence of this globally distributed implantation mycosis is unknown due to its slow and chronic natural history and late presentation. A recent meta-analysis has estimated that 8763 mycetoma cases have been reported worldwide. Approximately 60% of the cases of mycetoma are caused by filamentous bacteria, while the remainder of the cases are caused by fungi [70]. In fact, the prevalence of eumycetoma is high in the so-called mycetoma belt, which extends between latitudes 15° South and 30° North. The areas with high prevalence of mycetoma are fairly arid and have a short rainy season, lasting 4–6 months, annual precipitation of 50–1000 mm, relative humidity of 60–80%, and a constant temperature of 30–37 °C during the night and day. The rainy season is followed by a dry one that lasts 6–8 months, with 12–18% relative humidity, a daytime temperature of 45–60 °C, and a nighttime temperature of 15–18 °C. Such extreme temperature variation might be a requisite for the survival of the etiologic organisms in their natural niches. Within the mycetoma belt, the largest number of cases occur in Sudan, where the disease is particularly endemic and highly disabling. According to some studies, 300– 400 new cases occur in Sudan every year [69, 71, 72].

Mycetoma is also highly endemic in Mexico, with an average of 70 new cases per year, most of them caused by bacteria. The ecology of the various etiological agents of mycetoma might be currently changing in the Americas, as in Brazil the ratio of actinomycetoma to eumycetoma is 1:1. In Argentina, most cases are classified as eumycetoma. Ecological factors clearly determine the geographical distributions of the etiological agents. However, the considerable displacement of large populations and the ease with which people currently move from one place to another also contribute to the changes in the pattern of disease [2, 4, 73]. A few cases of mycetoma have been reported in the USA (77).

Mycetoma more frequently affects individuals who encounter frequent and direct contact with the soil, especially those in rural areas, such as farmers and herdsmen who are frequently exposed to soil and plants harboring the etiologic agents. That fact notwithstanding, the disease is not exclusive to those individuals, as cases among urban workers, homemakers, travelers, members of humanitarian organizations, and archeologists have also been reported [67–69, 73, 74].

The disease is known to mostly affect men, most likely as a function of their greater involvement in rural activities. The male to female ratio is around 3:1 to 5:1 and most affected women are also rural workers. These facts notwithstanding, a recent study suggested that the progesterone levels exhibited by women might inhibit the growth of some etiological agents of mycetoma, such as *Madurella mycetomatis*.

Mycetoma occurs more frequently among individuals aged 20– 40 years old, whereas in endemic areas, it might also affect children and older adults [2, 4, 67, 73].

Etiology

More than 30 different species of fungi have been associated with eumycetoma, some of which (melanized fungi) are able to cause a broad range of diseases, including phaeohyphomycosis and chromoblastomycosis [2, 61, 73, 75].

The etiological agents of mycetoma depend on factors such as temperature, precipitation, type of soil, vegetation, and the demographic characteristics of the susceptible population. These agents are classified according to the color of the grains, which might be black, yellow, or white (Fig. 25.4). The grains are also known as sclerotia and consist of aggregates of fungal hyphae embedded in a hard concrete-like matter (Fig. 25.4) [61, 74].

The most common etiological agents of eumycetoma are summarized in Table 25.3. More than 90% of cases of eumycetoma reported worldwide are caused by just four agents: *Madurella mycetomatis, Madurella grisea, Leptosphaeria senegalensis,* and *Scedosporium apiospermum (Pseudallescheria boydii).* Less frequently, *Biatriospora mackinnonii, (P. mackinnonii)* and *Pseudochaetosphaeronema larense* (*Chaetosphaeronema larense*) are involved. They are all melanized (black) fungi, causing "black grain mycetoma." *Scedosporium apiospermum,* which is the probable asexual state of *Pseudallescheria boydii*, *Aspergillus*, *Fusarium,* and *Acremonium* species, are non-pigmented fungi that may cause "white our yellowish grain mycetoma"[7, 61].

The prevalence of eumycetoma etiologic agents is related to the regional ecological characteristics, including type of soil, vegetation, pluviometry, temperature, and pollution [8].

M. mycetomatis and *L. senegalensis* are the most common pathogens in Africa. *Acremonium* spp. and *M. grisea* occur

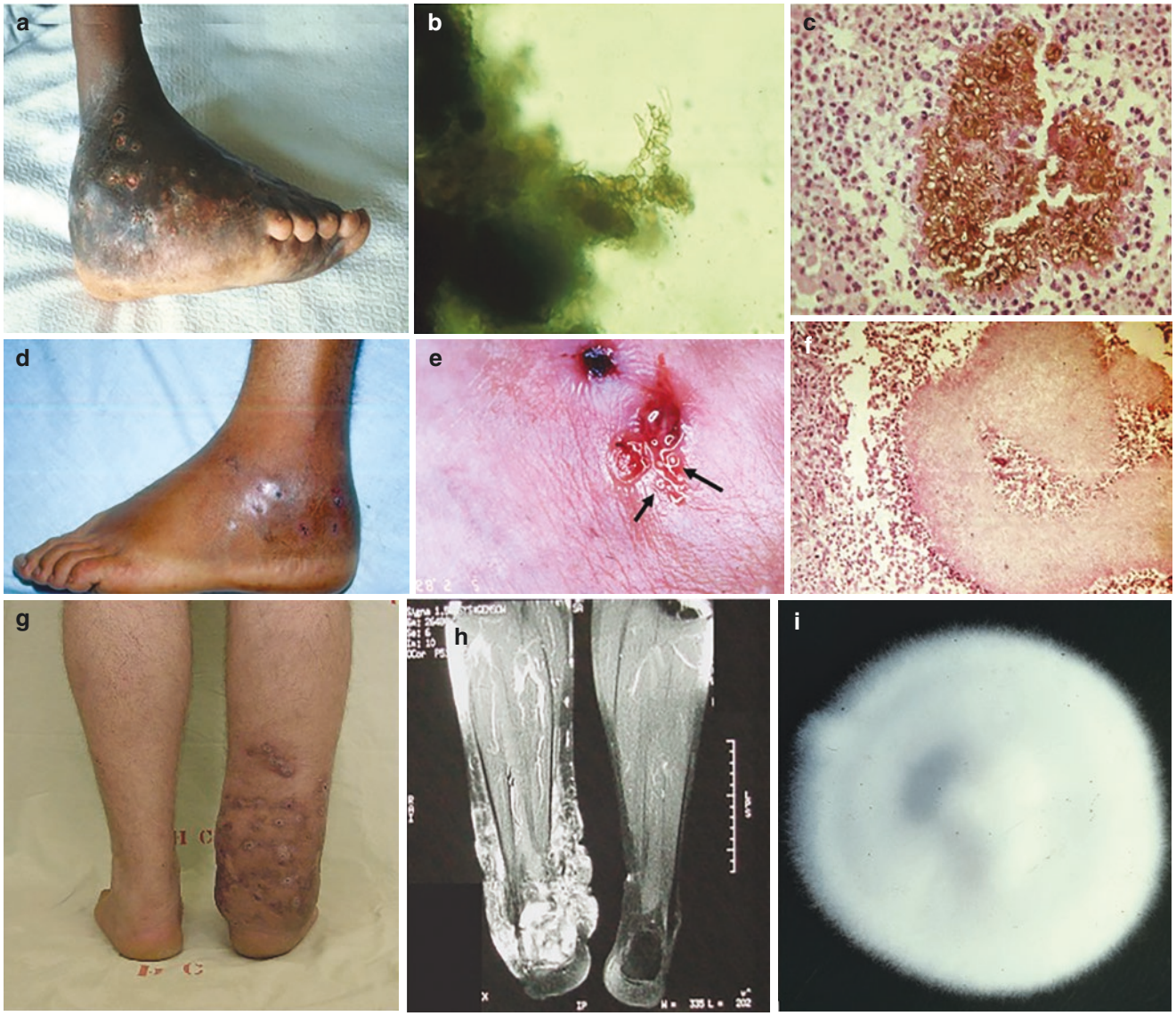


Fig. 25.4 Advanced mycetoma foot caused by *Exophiala jeanselmei.* (**a**) Wet mount of *E. jeanselmei* grain, showing shot pigmented septate hypha (**b**) and micromorphological aspects of dark melanized grain produced by *E. jeanselmei*, made of dak cement-like material and vesicular cells (**c**). Eumycema of the left foot (**d**). Macrophotography of the fistulae ostium, showing white to yellowish grains (arrow) resulting *S. apiospermum* in culture (×20) (**e**). Histopathologic section of a *S. apiospermum* grain (**f**). Multifistulous lesion affecting the left lower limb caused by a *Scedosporium apiospermum* mycetoma (**g**). Magnetic resonance showing extensive inflammatory aspect of the lower leg and calcaneus (**h**). White cottony culture of *S. apiospermum* (**i**). (*From Queiroz-Telles F, et al. Subcutaneous mycoses. Infect Dis Clin N Am 2003;17(1):59–85. Reprinted with permission from Elsevier Limited)

frequently in South America, but not in African dry areas. Although *M. grisea* is the most frequent of these organisms in Argentina, the prevalence of *S. apiospermum* and some species of *Acremonium* are also considerable. In India, the most frequent agent is *M. grisea,* followed by *M. mycetomatis, Acremonium* spp., and *Medicopsis* (*Pyrenochaeta*) *romeroi. M. mycetomatis* exhibits the largest global distribution and is predominant in Eastern Africa, especially Sudan. It is also the main etiological agent of eumycetoma in Yemen, Morocco, Tunisia, Saudi Arabia, and Senegal. In Western Africa, *L. senegalensis* stands out. Hyaline fungi are less frequently mentioned in the reported series; however, *S. apiospermum* was the most common agent found in North America and Iran, and white grains are most frequently found in the UK. Conversely, no eumycetoma with white grains has yet been reported in Chad or Djibouti [1, 2, 67–69, 73, 76–78].

Table 25.3 Main features of eumycotic and actinomycotic grains

	Fresh examination	Histology (HE)
Eumycetoma[a]		
Scedosporium apiospermum	<2 mm, yellowish or white, soft, oval to lobed, "fig seed like"	Compact, no cement, interwoven hyaline hyphae <5 μm and swollen cells <20 μm, eosinophilic border
Acremonium kiliense	<1.5 mm, white, soft, irregular shape	Compact, no cement, hyaline hyphae <4 μm, swollen cells <12 μm
Aspergillus nidulans fusarium moniliforme	<2 mm, white, soft, oval to lobed	Compact, no cement, interwoven hyaline hyphae <5 μm, eosinophilic border
Neotestudina rosatii	White to brownish, soft, <1 mm, fragmented angulated mass	Cement and swollen cells at periphery embedded in cement and some central vesicles
Madurella mycetomatis	<2 mm, black, firm to brittle (coal consistence), oval to lobed	**Compact type**, with brown-staining cement **Vesicular type**, with hyaline center and brown-staining cement and prominent <15 μm at edge
Madurella grisea	<1 mm, black, soft to firm, oval to lobed	Little brownish cement and polygonal cells at the periphery and central hyaline hyphae
Exophiala jeanselmei	<0.5 mm, black, soft, irregular to vermicular	No cement, hollow center, with melanin pigmented vesicular cells <10 μm associated with short hyphae <4 μm
Leptosphaeria senegalensis	1 mm, black, soft, irregular shape	Cement in outer zone, dark periphery with hyaline vesicular center
Pyrenochaeta romeroi	<2 mm, black, firm to brittle, oval to lobed	Brownish cement at periphery, no vesicles
Actinomycetoma[b]		
Nocardia brasiliensis	<0.5 mm, white, soft, irregular	Small, basophilic stained fringe in layers, homogenous loose clumps of bacterial filaments and rare clubs. Positive gram and Kinyoun stains
Actinomadura madurae	5 mm, yellowish to pink, oval to lobed	Anamorphous empty center with a dense basophilic or pink border associated with loose fringe and clubs. Gram positive
Actinomadura pelletieri	<1 mm, red, hard, oval to lobed	Homogenous dark staining with light periphery and no clubs. Easily fractured. Gram positive
Streptomyces somaliensis	<2 mm, yellow, hard, round to oval	Amorphous center amorphous center with basophilic layers associated with pink patches and dark bacterial filaments at the edge and no clubs. Gram positive

[a]Other eumycetoma agents: *Acremonium falciforme, Acremonium recifei, Aspergillus flavus, Leptosphaeria tompkinsii, Pyrenochaeta mackinnonii, Curvularia geniculata, C. lunata, Fusarium solani, F. oxysporum, Pseudochaetosphaeronema larense, Exserohilum rostrata*, etc.
[b]Other actinomycetoma agents: *Nocardia asteroids, N. caviae, N. farcinica, N. dassonvillei*, etc.

Pathogenesis

Although the pathogenic mechanisms of mycetoma is not completely understood, the factors involved in the onset of the disease are probable the inoculum size and host immune response, in addition to the possible participation of individual genetic susceptibility and hormones [79]. The pathological process usually begins when the infecting agent is traumatically introduced into the host tissue through the skin, which can be caused by thorny leaves, acacia or cactus thorns, wood splinters, sharp stones, agricultural implements, knives, fish scales, cow stomps, and/or other contaminated objects [69, 70, 80].

Although trauma is the mechanism currently accepted to account for the inoculation of the infecting agent, in some cases, the skin injury might go unnoticed. The incubation period is variable and has not yet been well established; it might last from weeks to years as a function of the etiological agent involved and the host immune response. Following its introduction into the host, the fungus might exhibit local dissemination from the inoculation site to the muscles or bones, especially spongy bones, such as those in the ankles, wrists, and vertebrae, for which the fungi exhibit particular affinity. These normally weakly pathogenic organisms grow and survive through the production of grains, which are structures composed of masses of fungal mycelium and a matrix component [4, 74, 78]. The matrix material has been shown to be host derived. The hyphae often have thickened cell walls and matrix material toward the periphery, potentially conferring protection against the host immune system. Grains are observed in histopathology within abscesses containing polymorphonuclear cells. Complement-dependent chemotaxis, activated by polymorphonuclear leukocytes, has been shown to be induced by fungal antigens (*M. mycetomatis, S. apiospermum*) in vitro. Cells of the innate immune system attempt to phagocytize and inactivate these organisms but fail to accomplish this goal, which is likely attributed to failure of the neutrophil response in the individuals who develop the disease [61, 81].

The role of the immune system in the pathogenesis of mycetoma has not yet been elucidated. Several studies showed that although many residents in endemic areas

develop anti-*Madurella mycetomatis* antibodies, only a low percentage of them develop the disease. For that reason, the role of innate immunity in the host resistance to fungi causing mycetoma has been quite frequently investigated in vitro and in animal models but rather rarely in human beings. The host local response, characterized by chemotaxis of neutrophils and small vessel congestion, is nonspecific. Later on, macrophages and monocytes with microbicide properties that are activated by cytokines, interferon-γ, and tumor necrosis factor-α arrive at the site of infection. Three types of immune responses have been described in response to the etiological agents of mycetoma [81]. The **type I response** is observed as neutrophils degranulate and adhere to the grain surface, leading to gradual disintegration of the grain. The **type II response** is characterized by the disappearance of neutrophils and the arrival of macrophages to clear grains and neutrophil debris. **The type III response** is marked by the formation of epithelioid granulomas to contain the infecting organism. Those host responses do not appear to be able to control infection but likely account for the partial spontaneous healing that is observed in the disease [81]. Although most of the mycetoma patients are apparently immunocompetent, there are some reports in immunocompromised patients including diabetes, leukemia, solid organ transplant, and idiopathic CD4+ T cell lymphocytopenia [82, 83].

The disease might have multifactorial etiology as a function of the heterogeneity exhibited by the factors determinant of human and animal susceptibility. Only a rather small number of residents in endemic areas develop the disease, although the full population is exposed to the same risk factors, such as walking barefoot and being scratched by thorns. The fungi likely have a saprobiotic life in the soil, and repeated inoculations of small amounts of fungi might induce sensitization and increased susceptibility to infection.

Clinical Manifestations

In most cases, the feet are involved (80%), followed by the legs and hands (7–6%, respectively). Usually the disease begins as a single, small, painless, and slow-growing subcutaneous nodule, which is usually round and firm but might also be soft, lobulated or, more rarely, cystic. The nodule slowly increases in size, while secondary nodules are formed, which become fixed to the underlying tissue and ultimately develop sterile and deep-seated draining sinus tracts beneath the lesions. These tracts open to the surface and drain purulent, serous, or serosanguinous material with grains. The grains measure several millimeters in diameter, and their color and consistency vary as a function of the etiological agent involved. The grains might be found in drained pus and in the tissue around the sinus tracts [67, 73, 76, 77]. The grains are visible to the naked eye or under a microscope. The morphological characteristics and color of the grains—which might be black, brownish, white, yellow, red, or a mixture of colors—contribute to their identification. The draining sinus tracts might take weeks, months, or even years to form. The diagnosis of disease is based on the clinical triad of **tumefaction**, **draining sinuses,** and **grains** (Fig. 25.4).

The disease can affect the skin, subcutaneous tissue, and eventually the adjacent lymphatic vessels and underlying fascia, muscles, and bones. The skin looks soft and shiny, is usually fixed to the underlying tissue, and can exhibit hyper- or hypopigmentation with local hyperhidrosis [5, 8, 68]. The affected individuals might also exhibit ulceration with honey-like crusting and scars. Swelling is usually firm and painless, while the overlying skin is not erythematous. Muscles, tendons, and nerves do not usually suffer direct infection, but extensive local damage may cause muscle wasting, bone destruction, and deformities in the limbs secondary to bone invasion and resulting osteomyelitis. As a late and rare occurrence, lesions might affect nerves, tendons, and even organs such as the lungs and peritoneum, as observed in cases of mycetoma affecting the trunk per contiguous dissemination. There are also reports of infection of the vertebral bodies, also due to contiguous dissemination, resulting in spinal cord compression. Although patients do not usually complain of pain, but rather report itching or burning, pain is the reason patients seek medical care in 20% of cases. It has been suggested that mycetoma may produce anesthetic substances within the lesions. In later stages, pain is usually due to nerve damage caused by intense fibrotic reaction, endarteritis obliterans, or neural hypoperfusion. Pain may also be caused by bone invasion, rupture of the abscesses formed by the sinus tracts, or secondary bacterial infection (Fig. 25.4) [67–69, 73, 76, 77].

On occasion, the disease exhibits alternative clinical patterns of progression, such as minimycetoma or the sporotrichoid pattern. Minimycetoma is characterized by a lack of tumefaction and a small number of sinus tracts, and although it has been found in children and young adults, it is a much more common finding in cases of actinomycetoma. Regional lymph nodes may be primarily involved or secondarily involved because of bacterial infection. As a consequence, chronic lymphedema may occur due to lymphatic obstruction and fibrosis. Contiguous lymphatic spread occurs quite rarely, being found in only 1–3% of cases, and it might appear after surgical treatment. No cases of hematogenous spread have been documented. Both the disease and its effects are usually localized, and thus, patients do not exhibit signs or symptoms of systemic infection, except in cases with secondary bacterial infection. Untreated, mycetoma continues its progression, while bacterial superinfection might lead to local abscess formation, cellulitis, and bacterial osteomyelitis [67, 71, 77].

From the topographic point of view, the fungi tend to affect the body areas that enter in direct contact with the soil or plants, mainly the feet (70–80% of cases), followed by the legs and the hands. In Mexico, the back is affected in 20% of the cases, especially in rural workers who carry logs and hay on their backs. Other possible sites include the knees, arms, neck, thighs, and perineum, while there are a few reports of infection of the eye lids, paranasal sinuses, orbits, external auditory meatus, old surgical wounds, skull, chest, abdomen, and genital organs like testicles, scrotum, and vulva, which may or may not be associated with immunosuppression [73, 76, 77].

The differential diagnosis includes foreign body granulomas and benign or malignant soft tissue neoplasms, such as squamous cell carcinoma, lipoma, fibroma, fibrolipoma, sarcoma, melanoma, and cystic lesions. Mycetoma may be confused with folliculitis and other mycoses. Mycetoma with an ulcerative pattern of proliferation might resemble epithelioma or melanoma. Lesions without draining sinus tracts may mimic phaeohyphomycosis, hyalohyphomycosis, sporotrichosis, chromoblastomycosis, and Entomophthoromycosis. Additionally, leishmaniasis, filariasis, cutaneous and osseous tuberculosis, mycobacteriosis, like infections caused by *Mycobacterium marinum*, botryomycosis, chronic bacterial osteomyelitis, and some non-infectious diseases like podoconiosis and foreign body granuloma should be taken into consideration [1, 2].

Diagnosis

Several approaches might be used for the diagnosis of mycetoma, including morphological, immunological, molecular, and imaging methods. The most relevant diagnostic element is the observation and/or the cultivation of the actinomycotic or eumycotic grains. These macro or microscopic structures represent small colonies of the etiologic agents of these diseases (Fig. 25.4). The differentiation of actinomycotic grains from the eumycotic grains is crucial for diagnosis and therapy. Morphological studies seek to identify the genus and species of the etiological agent and include direct examination, culture, and histopathology. The assessment of spontaneously drained grains might not be useful for diagnostic purposes, as they might contain dead organisms frequently associated with contaminating bacteria. In addition, these fungi are ubiquitous in nature and specimen contamination can occur. For that reason, fine-needle aspiration of the draining sinus tract content is recommended for sample collection. This procedure is easy to perform and affords the material needed for culture, direct mycological examination, histopathology, and molecular techniques to identify the etiological agent [67, 74].

For the purpose of direct mycological examination, two grains should be crushed using two glass slides, and the content should be examined using 10% potassium hydroxide, Gram stain, modified Kenyon stain and lactophenol blue, which allow scientists to distinguish between thin or thick filaments and to identify other organisms such as filamentous bacteria.

Culture of the grains should be performed on Sabouraud agar or brain heart infusion media. Ideally, 20 to 30 grains should be collected, washed several times with sterile saline solution with antibiotics or 70% alcohol, crushed with a sterile glass rod and then seeded in the appropriate culture medium. Additionally, media containing cycloheximide or antibiotics (gentamycin or chloramphenicol) might be used, bearing in mind that some organisms, *P. boydii* in particular, are inhibited by them. The organisms that cause mycetoma grow slowly; thus, they should be grown for a minimum of 6–8 weeks and ultimately for 12 weeks [67, 75, 76]. Once the etiological agent is isolated, it should be subjected to identification by microscopic morphology and gene sequencing (if available).

Histopathology reveals a chronic granulomatous reaction containing abscesses surrounded by inflammatory reaction, pseudoepitheliomatous hyperplasia, abundant granulation, and fibrotic tissue. The grains are observed at the center of the abscesses as masses of hyphae with filaments larger than 1 μm embedded in the intercellular cement. Special staining techniques such as Grocott's, periodic acid-Schiff (PAS), and hematoxylin and eosin (H&E) may help to distinguish the grain's etiology. Fontana-Masson stain specifically detects melanin and may be used in the assessment of black grains. When H&E is used, eumycetoma grains usually present a pale pink periphery. The grains are surrounded by a basophilic band, while their centers are usually intensely basophilic and disorganized, containing hyphae (Fig. 24.5).

Immunological methods, such as immunodiffusion, counter electrophoresis, immunoenzymatically assays, immunohistochemistry as well as molecular methods may be useful, but they are not standardized and commercially unavailable. The employment of ribosomic DNA sequencing may be helpful for the identification of some eumycetoma etiologic agents [84, 85].

Imaging methods are useful to assess the extent of disease. Simple radiographs might disclose early lesions, which appear as soft tissue granulomas. The findings associated with the progression of disease include variable periosteal reaction, bone deformation, osteoporosis, and multiple bone cavities. Ultrasound is useful in the case of mycetoma exhibiting cavities with thin walls without acoustic resonance, as well as to assess the extension of lesions. The so-called dot-in-circle sign is considered a characteristic radiological finding in mycetoma, and it may be identified by ultrasound or magnetic resonance imaging (MRI) (Fig. 25.4) [86].

Ultrasound is especially useful in the diagnosis of lesions lacking draining sinus tracts. The sensitivity of computed

tomography for the detection of early bone changes is higher than that of MRI. The latter exhibits particular sensitivity for the detection of small lesions with low-intensity signal on T1- and T2-weighted images, which correspond to products of the grains' metabolism. The detection of grains on MRI depends on various factors, including the size of the grains, the quality of the images, and the device parameters [86, 87].

Treatment

The prognosis of patients with mycetoma is related to early diagnosis and bone and joints involvement. Amputation of the affected limb or multiple mutilating excisions were the only therapeutic approaches available in the past and no cases of spontaneous cure have been reported in the medical literature. For those patients with cutaneous and subcutaneous infection, antibacterians or antifungal drugs can be employed for actinomycetoma and eumycetoma, respectively. For long duration infections with the involvement of deeper anatomic structures, usually, a combination of drug therapy with surgical procedures is indicated. Eumycetoma is more resistant to pharmacological treatment than actinomycetoma and exhibits high recurrence rates (20–90%). In addition to the risk of recurrence, the pharmacological treatment of eumycetoma is associated with several side effects, high dropout rates, disfigurement, and disability. Factors influencing the effectiveness of pharmacological treatment include the etiological agents' susceptibility to the drug used, the drug concentration achieved at the lesion site, the localization and size of the mycetoma and patient adherence to treatment, tolerance to the prolonged therapeutic regimen and host's immune condition [71, 88–90].

The most used antifungal armamentary used for eumycetoma consists of triazoles compounds (itraconazole, voriconazole, and posaconazole) and less frequently, amphotericin B. Although ketoconazole, ate the daily dose of 200 mg, have been favorable used in the past, this drug is not used nowadays, due to its significative side effects [8]. Itraconazole, 200 to 400 mg per day, is the most surely drug for eumycetoma, including infections caused by *M. mycetomatis* and *S. apiospermum* [68].

For patients who are intolerant of refractory to itraconazole, voriconazole is recommended, especially for patients with *S. apiospermum* infections. A loading dose of 400 mg twice a day of voriconazole should be given at the first day, followed by 200 my every 12 h. Posaconazole in extended-release formulation is also suitable option to itraconazole. Therapy consists of 600 mg in two doses at the first day, followed by 300 mg daily. If feasible, patients under triazole therapy should be monitored for serum drug level, especially itraconazole. Although isavuconazole showed good in vitro activity against *M mycetomatis*, this compound was not evaluated in patients to date. Fosravuconazole, a new triazole derivative has been evaluated in comparative clinical trial with itraconazole, in patients with *M. mycetomatis* infections, in a single center in Sudan [91]. The echinocandins, terbinafine, and fluconazole are not recommended therapies.

Lobomycosis

Lobomycosis, also known as lacaziosis or Jorge Lobo's disease, is a chronic granulomatous infection that affects skin and subcutaneous tissues, caused by *Lacazia loboi*, previously denominated *Paracoccidioides loboi* and *Loboa loboi*. Lobomycosis is endemic in several tropical countries in Latin America and it was first described in Brazil by Jorge Lobo in 1931 [92, 93] To date, *L. loboi* has never been isolated in artificial culture media and its natural source is not precise in nature, but it is believed that *L. loboi* may be a dimorphic fungus living saprobiotically in organic niches and in the aquatic environmental of endemic Latin America endemic areas; specially in the Amazon Rainforest. *Lacazia loboi* depicts some antigenic and phylogenetic similarities to *Paracoccidioides spp*, the etiologic agent of paracoccidioidomycosis a prevalent systemic endemic mycosis in Latin America. It may affect humans and dolphins and usually lobomycosis is recalcitrant to therapy with systemic antifungals [94–96].

Etiology and Epidemiology

Lacazia loboi is a consensus for the fungus denomination, after several taxonomic denominations in the past. Molecular analyses with yeast forms revealed that the fungus is a member of the Onygenales, like *Paracoccidioides* spp. [93, 97]. In vivo, *L. loboi* forms globular yeast-like cells, with refringent, thick cell walls, measuring 5–6 × 12–14 μm, which reproduce through simple budding. Several yeast cells may be linked assuming a catenular aspect (Fig. 25.5) [98].

Lobomycosis is a disease which occurs mainly in South America's Amazon area, affecting mainly men who work in the Amazon forest areas (Table 25.1). There are reports of this mycosis in dolphins (*Tursiops truncates* and *Sotalia guianensis*) off the US coast, France, south of Brazil, and the estuary of rivers in Suriname [94, 99–102]. Although this sporadic cases of dolphin to human infections have been reported, this mode of transmission is rare with no epidemiological impact, but it may represent the expanding of the ecological endemic niche of lobomycosis [103]. Occasionally lobomycotic infections were also reported after stingray

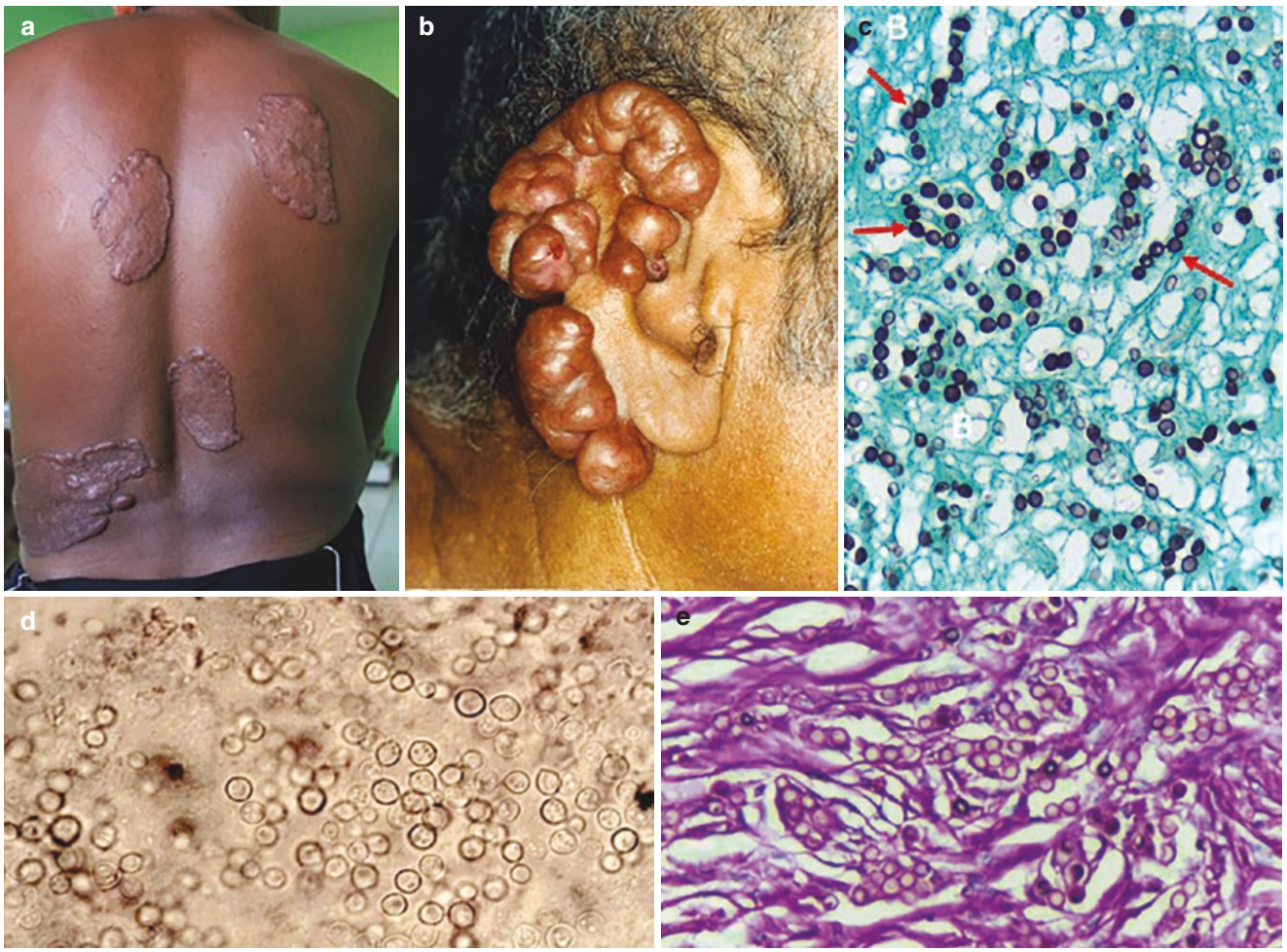


Fig. 25.5 Clinical aspects of lobomycosis (**a*** and **b****). Histologic section of skin biopsy of a patient with lobomycosis depicting several *L. loboi* yeast forms, some of them in chains (arrows). GMS × 200 (**c*****). Fresh direct examination of scrapings showing yeast budding cells of *L. loboi* × 400 (**d***). Cutaneous lesion biopsy, showing catenular yeast-like budding cells of *L. loboi* (**e**)***. (* Courtesy of Marcos Florian MD, University of Sao Paulo, Brazil. ** Courtesy of Sinesio Talliari MD, University of Amazon, Brazil, *** Courtesy of Arival de Brito MD, University of Para, Brazil)

stings and insect and snake bites. One case of this this mycosis was reported in an American patient after traumatic implantation after been exposed to high water pression in a trip to the Angel's Falls in Venezuela. A few cases of lobomycosis have been also reported in travelers visiting the endemic areas [104].

The implantation mycoses affects mainly males, between 29 and 40 years of age apparently immunocompetent. It is considered mostly an occupational disease and prevalent in forest workers, fishermen, farmers, and hunters. Although tropical areas of Central and South America are endemic for lobomycosis, most of the cases are reported from Brazil. In an analysis of 550 worldwide publications up to 2019, 330 (58.5%) were from this country [98, 105]. Lobomycosis is historically endemic among the Kaiabi Indians, living in the Brazilian Amazon region. To date, 63 (11.5%) of the global casuistic were reported in this indigenous group [105].

Clinical Manifestations

Patients with lobomycosis may present with a wide spectrum of cutaneous and subcutaneous lesions, 1 or 2 years after traumatic infection. The initial lesion may start as an indolent papule that may evolve to nodules, plaques, dyschromic macules, gummas, verruciform plaques, ulcers, scarring lesions, or keloid-like nodules. The ulcerated lesions may develop secondary bacterial superinfection, forming pustules with purulent exudate. Some infiltrative lobomycotic lesions may be similar to bur scars or even tuberculoid leprous. The pinna is affected frequently as well as the lower and upper limbs. Although mucosal involvement has not been described, 10–25% of the patients may present regional lymph adenomegaly, due to lymphatic spreading of the infection (Fig. 25.5) [106, 107].

Usually, lobomycosis lesions do not interfere with patient's general health status [2, 5, 93, 108]. Similar to CBM, the chronicity of the skin lesions associated with multiple bacterial co-infections may lead to neoplastic transformation in some patients [109].

Diagnosis

Diagnosis is made through direct visualization of the fungus, using direct microscopy and/or histopathologic stains. In the clinical material, *L. loboi* depicts round, thick-walled yeast cells with birefringent thick cell wall. Yeast cells measuring 6–7 μm in diameter are round to oval, being observed as solitary cells or in chains of 2–10 yeast cells (Fig. 25.5). Material for diagnosis can be obtained through biopsy for direct observation and histopathological examination. Histopathological examination typically reveals a granulomatous infiltrate filled with histiocytes and with a great number of yeast cells in clusters inside the cytoplasm of multinuclear histiocytes. The granuloma may be restricted to the dermis, surrounded by delicate fibrous septa [1, 2, 110]. Because this is a non-cultivated organism, to date there is no available immunodiagnosis for this disease. Differential diagnosis of lobomycosis usually is made with chronic cutaneous lesions with nodular our verrucous clinical aspect, including leprosy, leishmaniosis, and implantation mycosis like sporotrichosis, chromoblastomycosis and cutaneous manifestations of systemic mycosis like coccidioidomycosis, histoplasmosis and paracoccidioidomycosis, all occurring in Latin America.

Treatment

Excision of lesions, particularly single lesions, may result in cure. The use of cryotherapy has also produced good results. Lacaziosis is usually recalcitrant to antifungals and drugs used successfully in deep mycosis have not produced satisfactory results in lacaziosis [1, 2]. Clofazimine combined with itraconazole has been reported to produce clinical and histopathological remission of the disease in one treated patient after 1 year of therapy [111]. In another case report, posaconazole achieved reduction of the size of lesions in one patient treated with this drug [112].

Entomophthoromycosis

This rare mycosis, also known by several other names (Table 25.1), caused by fungi belonging to the phylum Glomeromycota (formerly Zygomycota), order Entomophthorales, was first described as a human disease by Paltauf in 1885 [113]. The Glomeromycota include two orders of fungi which cause human infection, the Mucorales and Entomophthorales. The order Mucorales contains the fungi which cause human mucormycosis (see Chap. 14), including the genera *Rhizopus* and *Mucor* [114, 115]. The order Entomophthorales includes three species which cause human infection, *Conidiobolus coronatus*, *C. incongruous,* and *Basidiobolus ranarum*. Infection secondary to these fungi is called Entomophthoromycosis. Infections caused by *C. coronatus* and *C. incongruous* are also called conidiobolomycosis, while basidiobolomycosis is an alternative name for infection secondary to *B. ranarum* [115].

Epidemiology

Basidiobolus spp. have been isolated from decaying plants, insects, the digestive tract of amphibians, reptiles, horses, dogs, and chimpanzees. It has a worldwide distribution and has been described in all continents, although prevalence is higher in tropical and subtropical climates. Africa has the highest number of reported cases, being identified in Uganda, Sudan, Nigeria, among others. Disease can affect any age, but most often presents in children and most commonly affects males [116]. *Conidiobolus* spp. are soil saprobes which are found in tropical areas and may infect horses. These fungi have been isolated in the UK, USA, India, and Africa [117].

Clinical Manifestations

Entomophthoromycosis is much less common than mucormycosis and is restricted to the subcutaneous and submucosal tissues. In basidiobolomycosis, infection most commonly affects the trunk and limbs. This disease starts as an isolated erytematous nodular lesion that with time me be raised and may transform in cellulitis which quickly develops a central area of necrosis with well-defined edges. These nodular lesions become confluent and, in some patients, there may be multiple lesions [118]. The lesions can spread and reaching muscle, bone or lymph nodes, and more rarely, involve adjacent organs. Disease is slowly progressive. Conidiobolomycosis affects the nasal mucosa or paranasal sinuses and spreads to the skin of the nose, cheek, upper lip, and pharynx (Fig. 25.6). These lesions are nodular, indolent, associated with diffuse edema, and may affect the entire upper face, with subsequent disfigurement. Basidiobolomycosis has also been reported as a rare cause of gastrointestinal disease with clinical manifestations similar to those of inflammatory bowel disease. In adults, gastrointestinal *B. ranarum* infection may affect normal or immuno-

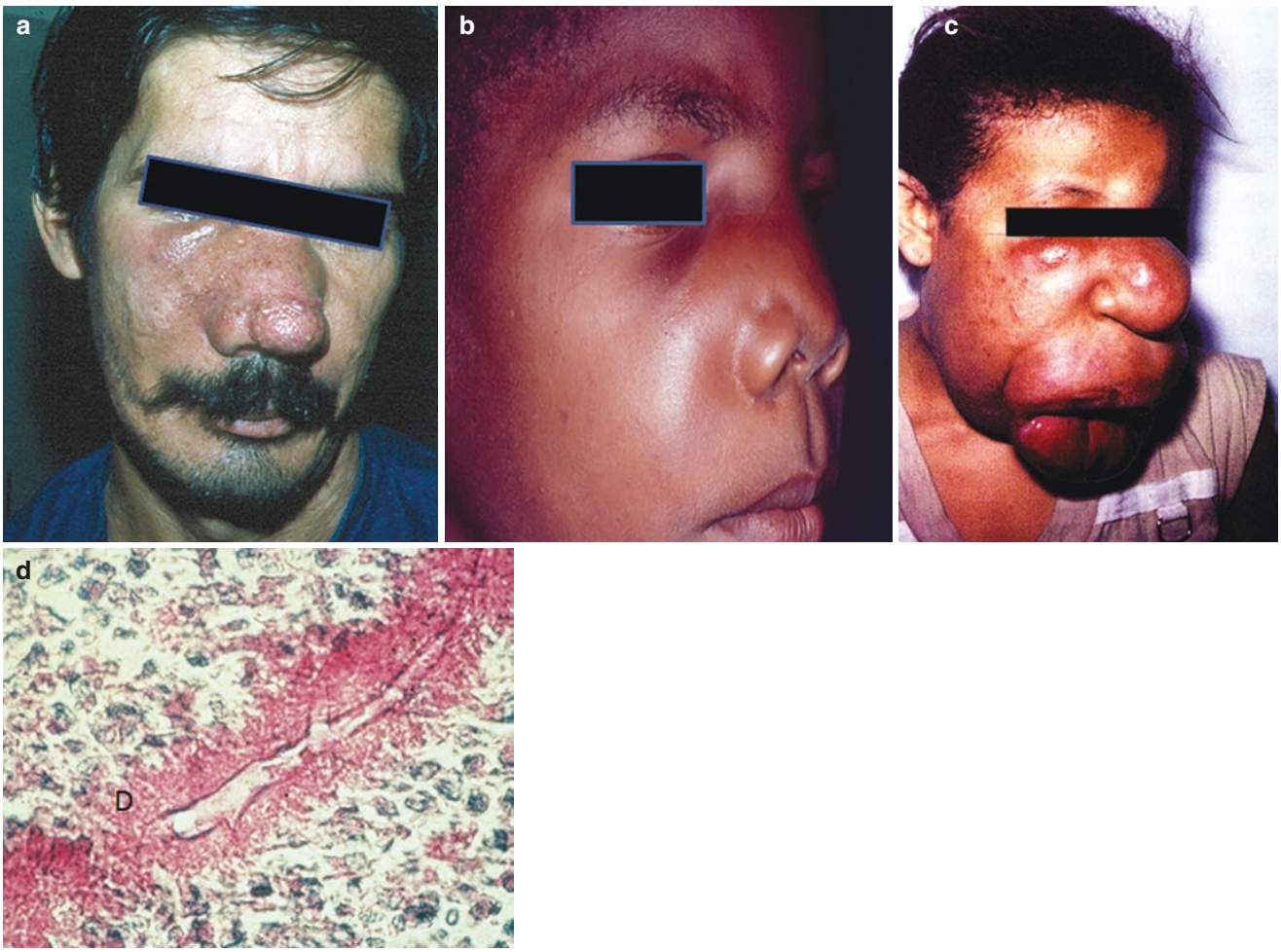


Fig. 25.6 Clinical aspects of patients with entomophthoromycosis (**a*****b*** and **c****). Histologic section showing poorly septate hyphae of *Entomophthorales*. It depicts a thin nonparallel cell wall surrounded by eosinophilic material (Splendore–Hoeppli reaction) (**d**). (*Courtesy of Angela Restrepo PhD. Medellin, Colombia **Courtesy of Jackson Mauricio M, D., Sao Luis, Brazil)

compromised hosts. Several cases of gastrointestinal basidiobolomycosis have also been reported in apparently immunocompetent children [119].

Diagnosis

Diagnosis of Entomophthoromycosis is based on clinical presentation and histopathological examination of deep lesional biopsy. Culture can be used to diagnose the fungal pathogen.

Histopathological examination shows eosinophil rich inflammation and fibrosis in the subcutaneous tissue. Aseptate hyphae 5–15 μm in diameter is usually surrounded by eosinophilic material (the Splendore–Hoeppli phenomenon) (Fig. 25.6).

The etiologic agent may be isolated by inoculating small pieces of biopsy tissue on Sabouraud glucose agar with chloramphenicol and gentamicin, with or without cycloheximide and potato dextrose agar. The colonies grow rapidly with a shade of cream to gray-green color and produce a waxy, glabrous, and cerebriform surface [118].

Treatment

The treatment of choice forEntomophthoromycosis caused by *B. ranarum* is potassium iodide at a dose of 30 mg/kg in single daily dose or divided into three doses daily for 6–12 months. Itraconazole has also been reported to produce good results at a dose of 100–200 mg/day. Itraconazole may also be combined with potassium iodide. Plastic reconstructive surgery may be indicated after treatment, as healing may be associated with fibrosis and deformity of the affected area [120–122].

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Chromoblastomycosis

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Instructive Cases

Instructive Case 1

A 57-year-old patient with acute myelogenous leukemia (AML) and 3-week history of neutropenia developed fever that was unresponsive to broad spectrum antimicrobial therapy (piperacillin/tazobactam and levofloxacin) and voriconazole prophylaxis. High-resolution computed tomography of the lungs revealed several pleural-based nodular lesions. The patient was empirically started on liposomal amphotericin B 7.5 mg/kg every 24 h for possible mucormycosis. After 5 days of therapy the patient's serum potassium declined to 2.8 mEq/L, despite supplemental KCl, while the serum creatinine and BUN remained stable.

Questions

1. The decrease in serum potassium is most likely due to what amphotericin B toxicity?
   A. Afferent arteriole constriction in the kidney
   B. Distal tubular damage in the kidney
   C. Suppression of erythropoietin synthesis
   D. Damage of the pancreatic islet cells
2. Which of the following approaches could be used if the patient's serum creatinine doubles and he begins to develop azotemia?
   A. Change liposomal amphotericin B dosing to every other day.
   B. Decrease the daily dosage of liposomal amphotericin B.
   C. Change liposomal amphotericin B to isavuconazole.
   D. Begin administering 500 ml of normal saline before and after infusion.

Instructive Case 2

While riding his dirt bike on a vacant lot in southeastern Michigan, a 22-year-old man abraded his right leg on the dirt while rounding a corner. He was taken to the emergency room where the large abrasion was cleaned and debrided. Because there was extensive loss of skin, a skin graft was placed several weeks later. About 2 weeks after the graft was placed, he noted that a few "bumps" had developed in the grafted area. These "bumps" became larger, broke open, and began to discharge what he described as a thin fluid with some pink discoloration. Similar lesions then developed in the thigh proximal to the original injury and graft. Several different antimicrobial agents were prescribed (including those with *Staphylococcus aureus* coverage), but the lesions did not respond, and in fact, new lesions appeared.

When seen by an infectious diseases consultant, the graft was beginning to breakdown and multiple nodules of various sizes were noted in the original abraded area that had been grafted and proximal to the graft into the upper thigh (Fig. A.1). Some of these were ulcerated and weeping serosanguinous fluid, and others were crusted. The nodules were not tender to palpation. The patient felt well and specifically denied having chills or fever. Biopsies were taken of several nodules for culture and histopathological examination. The tissue sections showed granulomas, but no organisms were seen. Within one week, the cultures incubated at 25 °C showed growth of an off-white mold that on microscopic examination showed tiny conidia arranged "bouquet-like" on thin hyphae. The diagnosis of sporotrichosis was made and the patient was begun on an experimental protocol using fluconazole, 400 mg daily. The lesions began to resolve by 2 months (Fig. A.2), and were finally all resolved by



Fig. A.1 Poor healing and nodular disease about lower extremity scar prior to antifungal therapy (patient later proven to have sporotrichosis)

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6

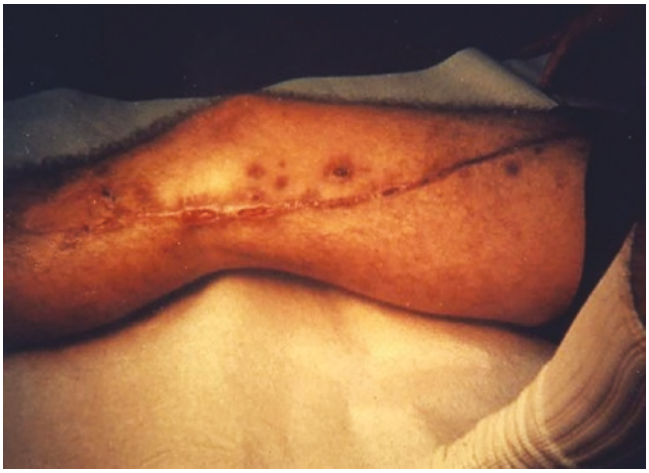


Fig. A.2 Improvement of sporotrichosis after 2 months of fluconazole therapy

5 months. Therapy was continued for a total of 6 months. No recurrences were noted. The patient was admonished to change hobbies from dirt bike riding to something less dangerous, but he chose to ignore that bit of medical advice.

Instructive Case 3

A 55-year-old Caucasian woman was in her usual state of good health until one week prior to admission when she developed a nonproductive cough, fever measured at 102 °F and a mild headache. Three days prior to admission she developed dyspnea. She did not have chest pain or a rash. Her past medical history included coronary artery disease with stent placement. Social history included previous tobacco use and recent travel to Palm Springs, California, three weeks prior to admission. On physical examination our patient was in mild distress with slight intercostal retraction. Laboratory studies included hemoglobin 12.1 g/dL, white blood count $11.8 \times 10^3/\mu L$, absolute neutrophil count $9.6 \times 10^3/\mu L$, absolute lymphocyte count $0.8 \times 10^3/\mu L$, absolute monocyte count $0.9 \times 10^3/\mu L$, and absolute eosinophil count $0.5 \times 10^3/\mu L$. Chest radiograph revealed diffuse bilateral infiltrates (Fig. A.3).

Evaluation led to a diagnosis of community-acquired pneumonia, the patient was admitted and treated with ceftriaxone and azithromycin without resolution of symptoms. All cultures were negative. Pulmonary consult on day three was followed by a bronchoscopy on day four. Bronchoalveolar lavage was negative and prednisone therapy was initiated. By day eight, the patient had improved clinically, antibiotics were stopped, and she was discharged on prednisone.

The patient was seen as an outpatient on day 14 with continued cough, but without fever, on prednisone taper.



Fig. A.3 Chest X-ray

On day 21 patient's dyspnea had worsened and the patient presented to the emergency department and was readmitted. Two days later she was transferred to the ICU due to hypoxia. On day 25 the patient developed severe respiratory failure requiring mechanical ventilation. Coccidioidomycosis serology was positive on day 26 and the patient expired on day 28.

Instructive Case 4

A 27-year-old male agricultural worker was seen in consultation to evaluate an illness of two months duration characterized by the presence of rapidly enlarging lymph nodes in both cervical chains, accompanied by pain and high fever, especially at nights. One of the nodes had drained spontaneously producing yellow-tinged purulent material. The patient also experienced productive cough without dyspnea. On examination he was pale, looked frail, and had enlarged cervical, axillary, and inguinal lymph nodes (Fig. A.4). His spleen and liver were normal by physical examination. Lung auscultation did not reveal altered breath sounds and chest radiographs were normal.

HIV serology was negative, hemoglobin was 8.8 g/dL, white blood cell count was 25,700 cells/μL. A direct KOH examination of the discharge from the ruptured nodule showed abundant yeast cells, some with the characteristic multiple budding of *Paracoccidioides brasiliensis;* this fungus was also isolated in culture. Serology with paracoccidioidin proved reactive with one band of precipitate and a CF titer of 1:1024.



Fig. A.4 Paracoccidioidomycosis. Hypertrophied cervical lymph node about to rupture. Scarring lesions of a similar process can be seen above

Instructive Case 5

A 70-year-old man with hypereosinophilic syndrome, diabetes mellitus, and long-term prednisone (25 mg/day × 25 years) use was admitted with fatigue, weakness, and shortness of breath. Physical examination was remarkable for a temperature of 99 °F, an area of erythema over the left thigh that was tender to palpation and an effusion of his right knee. On admission he had a hemoglobin of 5.9 g/dL, WBC 8600/mm^3, a serum creatinine 2.7 mg/dL, and a glucose 382 mg/dL. Aspiration of the right knee revealed a WBC of 40,500/mm^3 with 96% neutrophils; gram stain revealed gram-negative bacilli and budding yeasts. The patient was initially started on imipenem, vancomycin, tobramycin, and fluconazole 400 mg/day.

Within 72 h the blood cultures drawn on admission were found to be positive for yeast, later identified as *Trichosporon asahii (beigelii).* In addition, joint fluid cultures were also positive for *Trichosporon asahii* and *Pseudomonas aeruginosa*. Biopsy and culture of the left thigh area of erythema also grew *P. aeruginosa and T. asahii.*

After 5 days of fluconazole the blood cultures were still positive and caspofungin (Cancidas) 50 mg IV daily was added to the fluconazole. He also underwent arthroscopic irrigation of the right knee. Ten days after admission, the patient had negative blood cultures and had responded well to antimicrobial therapy. He was subsequently transferred to a rehabilitation unit in good condition.

In vitro susceptibilities of *T. asahii* revealed: MIC of fluconazole 8 μg/mg; itraconazole 0.5 μg/mL; and caspofungin 2 μg/mL.

Instructive Case 6

A 42-year-old black man presented to the University Hospital emergency department with a one-week history of fever, chills, cough productive of white sputum, night sweats, and malaise. He denied hemoptysis. Risk factors for HIV infection included a blood transfusion in 1985 and heterosexual promiscuity. He denied intravenous drug abuse or homosexual activity. Over the last eight months he had lost 80 pounds. Past medical history was significant for a prior appendectomy, a perirectal abscess which was surgically drained, and a chronic perirectal fistula for five years. There was also a history of venereal disease including primary syphilis associated with a chancre and a positive RPR of 1:32. No therapy was documented and he was lost to follow-up.

On admission, the patient was afebrile but cachectic. He was in no acute respiratory distress. On physical examination he was noted to have dry crusting lesions on the tip of his nose and both cheeks of the face (Fig. A.5). Similar lesions were noted over his shins and a small draining abscess was noted over the medial aspect of his left foot. Oral hairy leukoplakia was present on the tongue and he was noted to have prominent generalized lymphadenopathy. No rales, rhonchi, or wheezes were heard on auscultation of chest. A fistula-in-ano was also noted. Chest radiograph revealed bilateral reticulonodular infiltrates, which were more prominent in the upper lobes. The patient was lymphopenic with an absolute lymphocyte count of 410 cells/μL. Serum RPR was positive at 1:32. Skin scrapings of the lesions were performed and wet preparations of the specimens revealed characteristic thick-walled multinucleated yeast forms compatible with *Blastomyces dermatitidis* (Fig. A.6). Subsequently, the patient underwent bronchoscopy with BAL and cytology preparations of the washings also revealed yeast consistent with *B. dermatitidis*. Cultures of skin scrapings and BAL washings grew this organism. An ELISA and Western Blot confirmed HIV infection and total CD4$^+$ T lymphocyte count was 8 cells/μL. A CT scan of the head was performed because of complaints of headache. This study revealed multiple enhancing brain lesions (Fig. A.7). Owing to concerns of a second opportunistic pathogen, i.e., *Toxoplasma gondii*, the brain lesions were aspirated. Wet preparations again revealed characteristic yeast forms of *B. dermatitidis*.

The patient was begun on active antiretroviral therapy and amphotericin B deoxycholate therapy at an initial dose of 1 mg/kg/day. Intravenous penicillin, 18 million units per day



Fig. A.5 Multiple cutaneous lesions in a patient with end-stage AIDS. Similar lesions were noted on both checks of the face and both shins

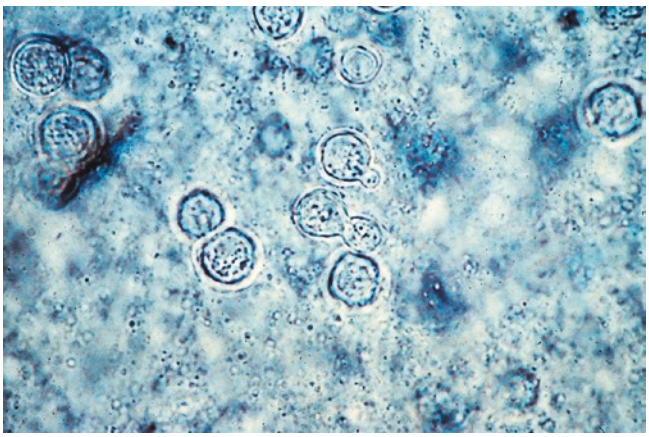


Fig. A.6 Wet preparation of skin scrapings. This figure shows the characteristic yeast forms in a wet preparation of skin scrapings. Scraping of the edges of the verrucous and ulcerative lesions yield the best diagnostic results

in divided doses, was administered for 21 days because of concerns of neurosyphilis. During the remainder of the hospitalization, the patient was carefully monitored for evidence of renal insufficiency and 500 mL of normal saline was infused prior to each amphotericin B infusion. During this hospitalization, he received a total of 1600 mg of amphotericin B. Serial CT scans documented progressive improvement of brain abscesses and oral fluconazole was substituted after the full course of amphotericin B and the patient was discharged to home. He completed eight more weeks of fluconazole therapy at a dose of 800 mg/day. Although the brain abscesses had resolved on CT scan, a maintenance suppressive dose of fluconazole of 400 mg a day was initiated. HAART was continued and a clinical response was documented by a falling HIV viral load and rising CD4 lymphocyte count.



Fig. A.7 CNS blastomycosis in an AIDS patient. Diagnosis may require aspiration of the abscesses if no active pulmonary or cutaneous disease is present

Instructive Case 7

A 45-year-old construction worker presented with a three-week history of fever, chills, myalgias, headache, and dyspnea. Examination was unremarkable and laboratory tests revealed a white blood cell count of 3300 cells/μL and a platelet count of 94,000 cells/μL. AST was 87 units/L, alkaline phosphatase 337 units/L. Angiotensin-converting enzyme was elevated at 213 units/L. Chest and abdomen CT

showed small nodules in the lungs, enlarged mediastinal lymph nodes, small pleural effusions, and splenomegaly. Transbronchial biopsies of the subcarinal lymph node and right lower lobe, and bone marrow biopsy showed noncaseating granuloma. Histopathology was negative for fungi and cultures were negative after one week of incubation. Prednisone 60 mg daily was prescribed for presumed sarcoidosis, which resulted in resolution of fever and improvement of dyspnea. The prednisone dosage was tapered over 4 weeks to 10 mg daily, which was maintained.

Two months later the patient complained of recurrent fever, 10 pound weight loss, and worsening dyspnea. Chest CT showed more extensive diffuse interstitial infiltrates and increasing splenomegaly. Hemoglobin was 9.3 g/dL, white blood cell count 2500 cells/μL, and a platelet count of 89,000 cells/μL. Alkaline phosphatase was 987 units/L. Cultures from the lung tissue, lymph nodes, and bone marrow performed during the earlier admission were negative after 4 weeks of incubation. Bronchoscopy was performed and cytology revealed small yeast like-structures resembling *Histoplasma capsulatum*, which was later confirmed by culture. Treatment was started with amphotericin B, and the patient noted progressive improvement.

Instructive Case 8

A 52-year-old man who underwent renal transplantation two years earlier presented with a slightly painful mass above his right knee. This mass began as a small lesion four weeks prior and had been slowly enlarging since first noted by the patient. He denied fever, chills, night sweats, or trauma to the area. He was taking tacrolimus and prednisone as his immunosuppressive regimen. The patient was afebrile with stable vital signs, but his physical examination was significant for a 1.5 cm firm nodule above his right knee, which was slightly tender, but without erythema or drainage. Routine laboratory studies were unremarkable.

Surgery was consulted for excision of the nodule, which was performed without complications. Pathology showed chronic inflammation and pigmented fungal elements suggestive of phaeohyphomycosis; margins were clear of infection (Fig. A.8). Culture of the specimen grew *Exophiala jeanselmei*. He was given itraconazole for three months, and no further lesions appeared.

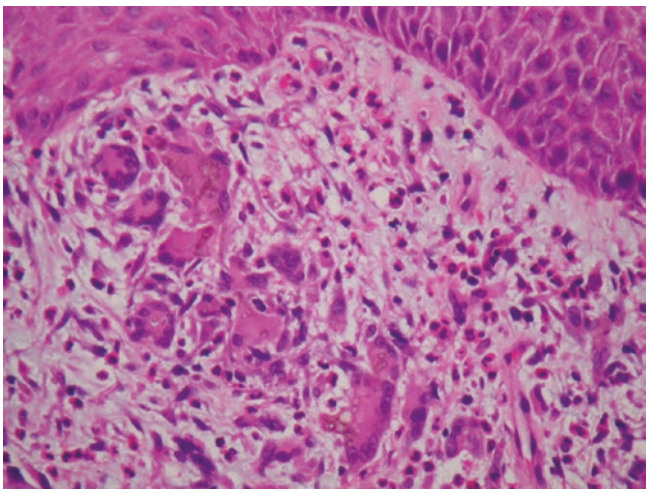


Fig. A.8 Histopathological examination of subcutaneous nodule showing granulomatous changes and dark-walled fungal elements. Phaeohyphomycosis

Instructive Case 9

A 28-year-old Caucasian man presented with a one-day history of dyspnea and anxiety. His past medical history included major depressive disorder. His past social history included marijuana and tobacco use. Physical exam revealed mild respiratory distress and anxiousness, with decreased breath sounds over the right hemithorax. Laboratory studies included hemoglobin 13.9 g/dL, white blood count 14.6 × 10^3/μL, absolute neutrophil count 12.5 × 10^3/μL, absolute lymphocyte count 2.3 × 10^3/μL, absolute eosinophil count 0.146 × 10^3/μL, and albumin 2.7 g/dL. Chest radiograph revealed complete opacity of his right hemithorax (Fig. A.9).

A chest tube was inserted and later video-assisted thoracic surgery (VATS) with right upper lobe wedge resection of a cavitary lesion was performed. Coccidioidomycosis serology was positive and fluconazole at 1000 mg daily was initiated. The patient's recovery was uneventful.

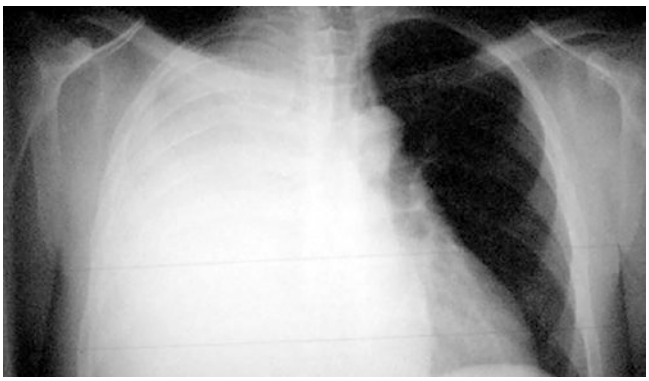


Fig. A.9 Chest radiograph

Instructive Case 10

A 68-year-old man with a long standing history of hairy cell leukemia was admitted to receive treatment with the experimental immunotoxin BL-22. He had been pancytopenic for several months and had been receiving antifungal prophylaxis with itraconazole. Upon admission, antifungal prophylaxis was changed to fluconazole. On Day 2 the patient developed fever. At that time his absolute neutrophil count was 76 cells/μL. Blood cultures recovered a highly susceptible *E. coli* and the patient defervesced promptly on ceftazidime. On Day 7 a new fever developed and meropenem, tobramycin, and caspofungin were started. A chest CT was obtained which showed a 3 cm right upper lobe (RUL) nodule (Fig. A.10). A bron-

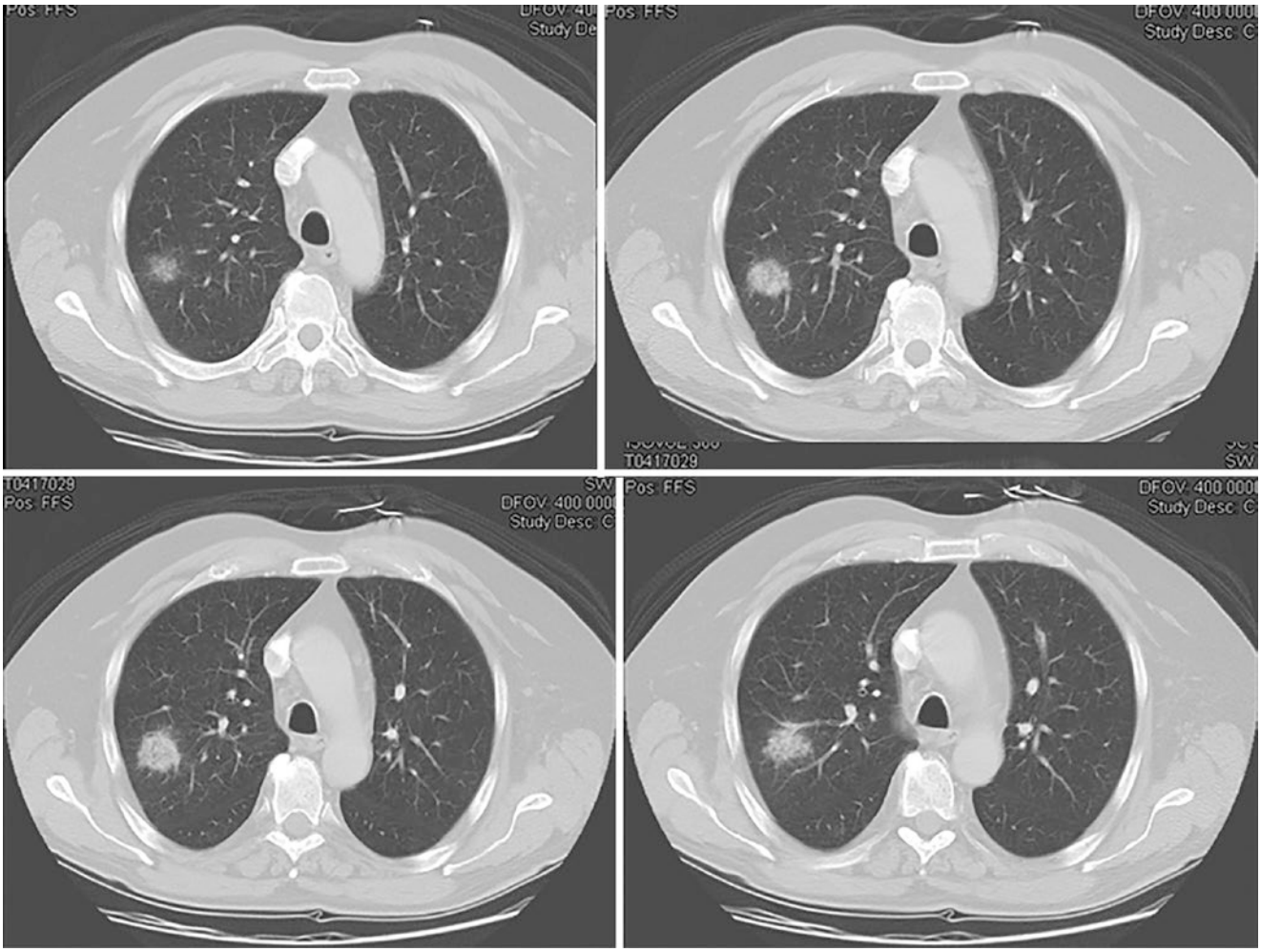


Fig. A.10 CT scan of chest revealing small pulmonary nodule in the right upper lung

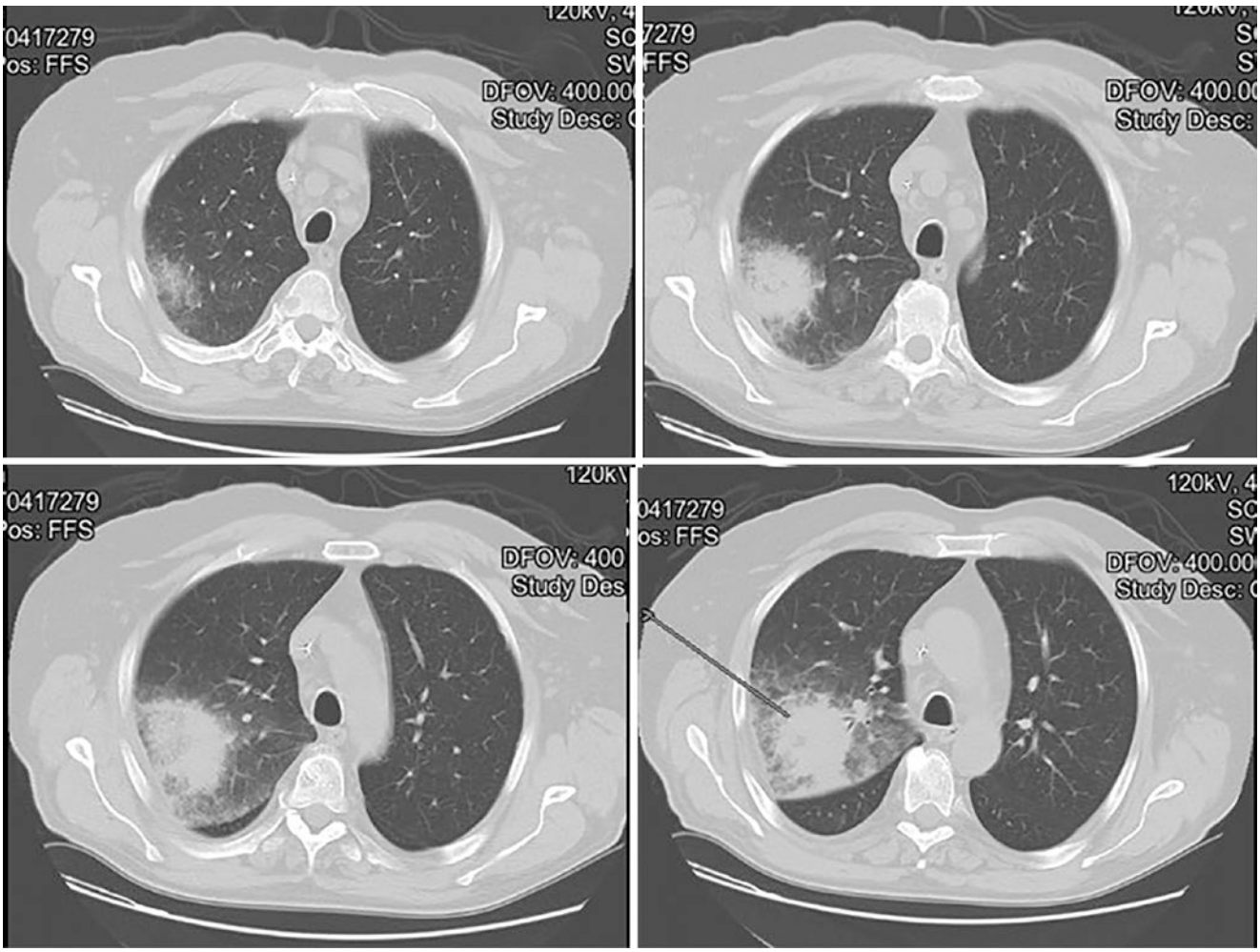


Fig. A.11 CT scan of chest revealing progression of pulmonary nodule in right upper lung field to involve the right pleural surface

choalveolar lavage (BAL) was performed, and voriconazole and levofloxacin were added. Bacterial, fungal, and viral cultures, as well as Gram, calcofluor white, acid-fast, modified-acid-fast, and Gomori methenamine silver (GMS) stains were negative. PCR for *Pneumocystis*, *Chlamydophila, Mycoplasma*, and *Legionella* was also negative. The patient continued to have fever up to 40 °C without new symptoms or hypotension. On Day 11 he complained of chest pain and dry cough. A repeat CT showed marked enlargement of the nodule with development of a halo sign and abutting of the fissure (Fig. A.11). A fine needle aspirate of the mass showed broad, ribbon-like, nonseptate hyphae (Fig. A.12). Voriconazole was discontinued and liposomal amphotericin B 7.5 mg/kg/day was started. The patient developed hemoptysis and a repeat CT showed further progression of the mass, but with apparent localization in the RUL (Fig. A.13). An emergent right upper lobectomy was performed on Day 18 (Fig. A.14). An angioinvasive mold was readily seen on the GMS stain (Fig. A.15), and was later identified as *Rhizomucor pusillus*. On the day of the surgery, granulocyte transfusions were initiated. Hemoptysis and fever resolved 2 days after the surgery. Despite local control of the fungal disease, the patient never recovered his white blood cell counts and over the next

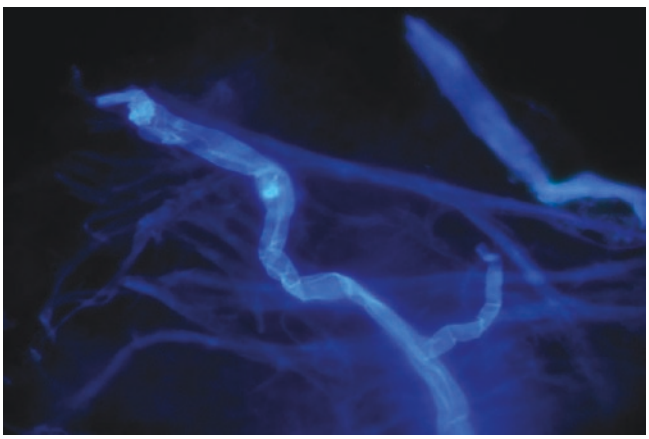


Fig. A.12 Calcofluor staining of fine needle aspiration demonstrates broad nonseptate hyphae consistent with mucormycosis

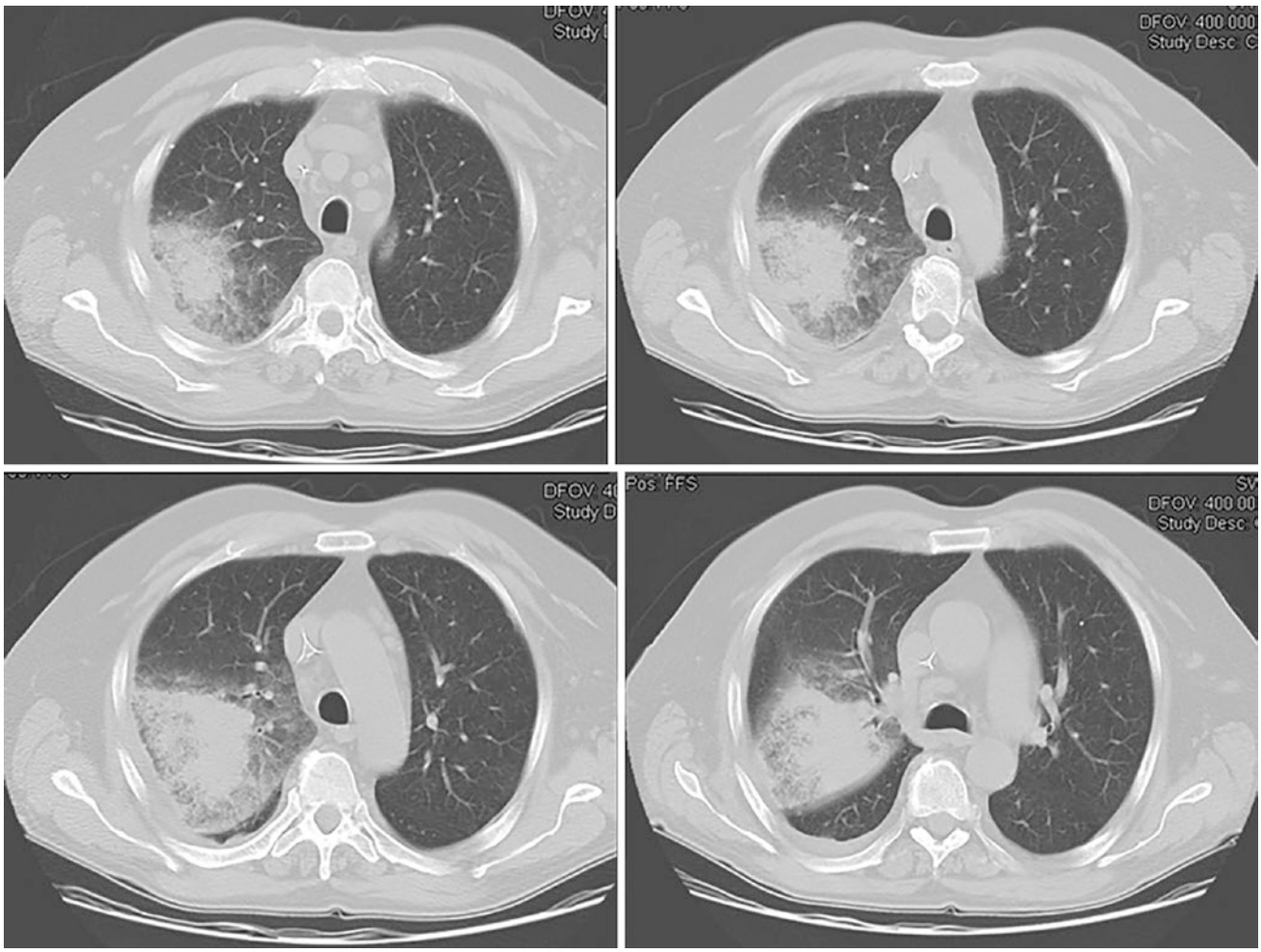


Fig. A.13 CT scan of chest with severe advancement of locally progressive disease in the right upper lung fields

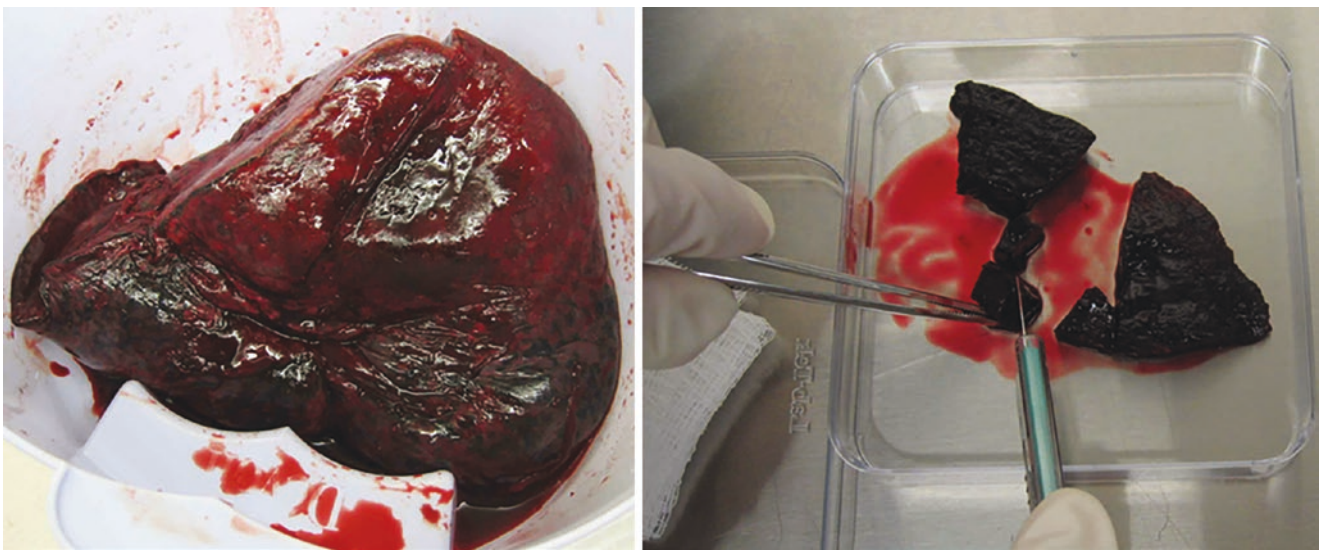


Fig. A.14 Gross pathology of right upper lobectomy

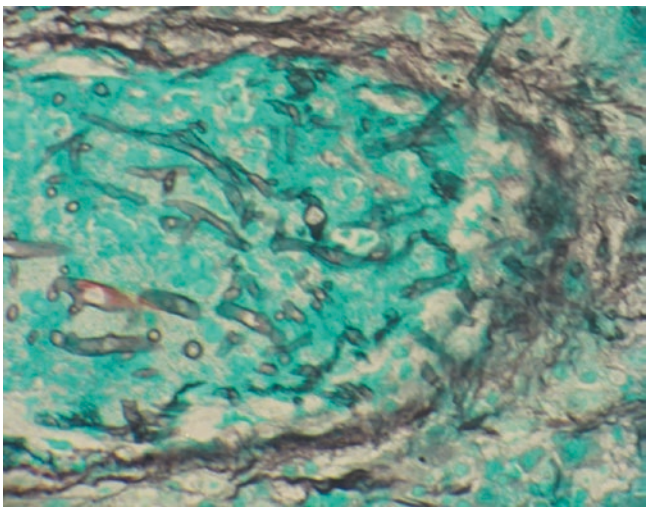


Fig. A.15 Mucormycosis with hyphae invading blood vessel, infiltrating vascular wall, and causing subsequent thrombosis. GMS

six weeks developed several complications in the ICU, including herpes simplex pneumonia. At his own request, care was withdrawn.

Instructive Case 11

A 48-year-old male rural worker was referred for consultation to evaluate the presence of painful, ulcerated lesions in the external region of his right foot (Fig. A.16). This process had gone on for 18 months and multiple local and systemic treatments had been given without success. The patient looked well and had no other symptoms. Physical examination also revealed the presence of an oral mucosal ulceration and of hypertrophied cervical lymph nodes. Lung auscultation found fine rales and the chest radiograph showed interstitial infiltrates in the central fields with fibrous zones and basal bullae (Fig. A.17).

Direct KOH examination of ulcer exudate revealed multiple budding yeasts consistent with *Paracoccidioides brasiliensis*; cultures later grew the fungus. Patient's serum gave a band of precipitate and a CF titer of 1:32 with paracoccidioidin.



Fig. A.16 Paracoccidioidomycosis. Multiple ulcerated lesions in the right foot with crusting, exudation, and hemorrhagic dots. The borders are granulomatous and show some scarring



Fig. A.17 Paracoccidioidomycosis. Bilateral interstitial infiltrates in central fields, basal bullae, and fibrous areas. The apices appear free of disease

Instructive Case 12

A 40-year-old woman with acute myelogenous leukemia presented 115 days after matched allogeneic donor hematopoietic stem cell transplantation, complicated by a suspected *Aspergillus* pneumonia, with complaints of nausea, stomach

cramping, and rash on the hands spreading up her arms. By laboratory examination, she is noted to have an ALT of 85 μ/L, AST 75 μ/L, and total bilirubin of 2.1 mg/dL. Her current medications include tacrolimus 5 mg twice daily (recent level 8 ng/mL), voriconazole 200 mg twice daily, levofloxacin 500 mg daily, valacyclovir 500 mg twice daily, metoprolol 25 mg twice daily, and benzonatate (Tessalon Perles) pearls. She is admitted to the hospital for suspected graft versus host disease exacerbation. The primary service wishes to continue voriconazole therapy as this patient has a history of poorly tolerating lipid amphotericin B formulations, but they are concerned about the possibility of drug-induced hepatitis caused by voriconazole.

Question

1. Which of the following approaches should be recommended to the primary team to manage the suspected drug-induced hepatotoxicity?
   A. Discontinue voriconazole and switch to isavuconazole.
   B. Discontinue voriconazole and switch to lipid amphotericin B with pre-medications.
   C. Continue voriconazole and lower dose by 50%.
   D. Check voriconazole trough concentration and lower dose if >6 mg/L.

Instructive Case 13

A 45-year-old Hispanic man presented with chief complaint of back pain. He was in his usual state of health until he developed fever, rigors, nonproductive cough, nausea, and vomiting. He was treated at another institution with antibiotics without improvement. Respiratory symptoms gradually abated but he continued to have night sweats, fever, weight loss, and increasing spine pain. Abscesses on his right arm and mid back were noted. Incision and drainage was attempted with "no pus" recovered.

Seven months later the patient was referred to surgery for evaluation of a possible "tumor." Pathology from surgery revealed coccidioidomycosis. Medical treatment with fluconazole 400 mg daily was initiated (later increased to 1000 mg). He was referred to specialty coccidioidomycosis clinic for evaluation and patient was admitted for further evaluation and treatment.

Past medical history included chronic low back pain for five years resulting in disability. Physical examination revealed temperature of 97 °F, pulse 86/min, blood pressure 109/68 mmHg, and respirations 18/min. He appeared chronically ill, in mild to moderate pain, with pale conjunctiva, temporal wasting, draining wounds of his right posterior upper arm and right mid back, decreased motor strength of his lower extremities, and decreased sensation to pin prick testing at T-10 level. Laboratory testing revealed hemoglobin 8.1 g/dL, white blood count 3.3 × 10^3/μL, potassium 2.8 mEq/L, creatinine 0.8 mg/dL, albumin 2.4 g/dL, and coccidioidomycosis serology (CF) 1:64. Chest radiograph showed right upper lobe nodular infiltrate with cavity (Fig. A.18), and bone scan showed evidence of osteomyelitis in distal thoracic spine. MRI of the thoracic spine revealed osteomyelitis at T-11/12 with epidural paraspinous phlegmon (Fig. A.19) and MRI of the lumbar spine, osteomyelitis at L-4/5 with paraspinous psoas phlegmon extending into the perinephric space (Fig. A.20).



Fig. A.18 Chest radiograph



Fig. A.19 MRI of thoracic and lumbar spine

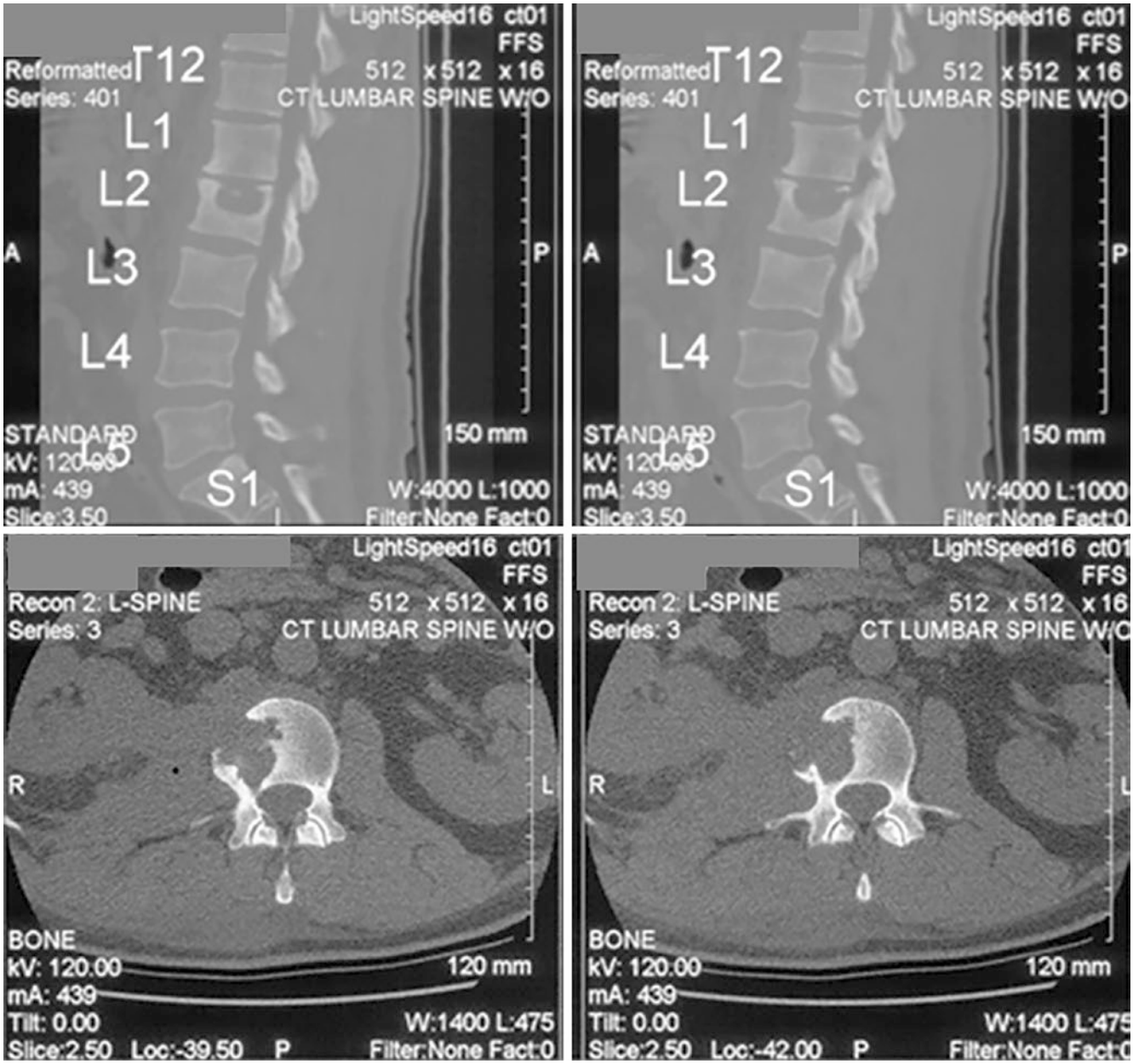


Fig. A.20 MRI of lumbar spine

Instructive Case 14

A 22-year-old woman with a failing 4- year-old renal allograft received several doses of OKT3 and high doses of corticosteroids in an attempt to reverse the acute rejection of the transplanted kidney. Three months after this increased immunosuppressive trial and still receiving her normal immunosuppressive regimen of tacrolimus, mycophenolate, and prednisone, she presented with several week course of headaches, nausea, and vomiting. Her temperature was 37.2 °C and although mental status was normal, she had bilateral clonus and papilledema on physical exam. Her laboratory results showed a normal complete blood count and a serum creatinine of 4 mg/dL. An MRI of her brain demonstrated basilar inflammation and lumbar puncture (LP) revealed a white blood cell count of 100 cells/μL with 80% lymphocytes. CSF glucose was 43 mg/dL and protein 79 mg/dL. India ink was positive for encapsulated yeasts, CSF cryptococcal polysaccharide antigen test was ≥1:256, and culture grew *Cryptococcus neoformans*. Her opening pressure was 400 mmH_2O. She was started on 5 mg/kg/day of AmBisome for 20 days and flucytosine at 25 mg/kg/day for 14 days, and

then placed on 200 mg/day of fluconazole. Patient's symptoms did not worsen and she was reevaluated at 2 weeks with a repeat LP. That LP found an opening pressure of 140 mmH_2O and India ink and culture were negative. CSF antigen was 1:256 and CSF white cell count was 28 cells/μL.

Instructive Case 15

A 47-year-old black man presented to the University Hospital emergency department complaining of pleuritic chest pain for two weeks prior to admission. He subsequently developed severe dyspnea on exertion, fever and chills, and productive cough with hemoptysis. Over the 24 h preceding admission, pleuritic chest pain, which was initially only on the left side, became bilateral and he presented to the Emergency Department for evaluation. Past medical history was pertinent for hyperthyroidism and cigarette smoking. He denied any risk factors for HIV infection.

In the emergency department, he was in moderate respiratory distress but was afebrile. Chest radiograph revealed diffuse bilateral miliary infiltrates with a mass like lesion in the lung field. White blood count was 14,400 cells/μL with a left shift. Arterial blood gases on room air revealed a pH of 7.41, pO_2 of 33 mmHg, and pCO_2 of 46 mmHg.

The patient was admitted to the hospital and placed in respiratory isolation. Differential diagnosis included severe community-acquired pneumonia, atypical pneumonia, miliary tuberculosis, and fungal disease. He was placed on supplemental oxygen, intravenous azithromycin and ceftriaxone, and a four-drug antituberculous treatment regimen. Multiple sputum samples were only remarkable for many polymorphonuclear leukocytes. DFA and urinary antigen were negative for *Legionella*. Likewise, fungal and acid-fast stains of sputum samples were negative. Testing for HIV was also negative.

On hospital day 3 the patient became febrile, chest radiograph revealed worsening bilateral pulmonary infiltrates, and intravenous trimethoprim/sulfamethoxazole was added to the existing antibacterial regimen as empiric therapy for possible *Pneumocystis* pneumonia. On the fifth hospital day he complained of increasing dyspnea and was noted as having a respiratory rate of 60 breaths per minute. Arterial blood gases on 100% oxygen by face mask revealed a pH of 7.45, pO_2 of 102 mmHg, and pCO_2 of 42 mmHg. The patient was transferred to intensive care unit where he was intubated and placed on mechanical ventilation. Chest radiographs revealed bilateral pulmonary infiltrates (Fig. A.21) with acute lung injury. Cytology samples obtained via the endotracheal tube at the time of intubation revealed numerous broad-based budding yeast forms compatible with *Blastomyces* species (Fig. A.22).

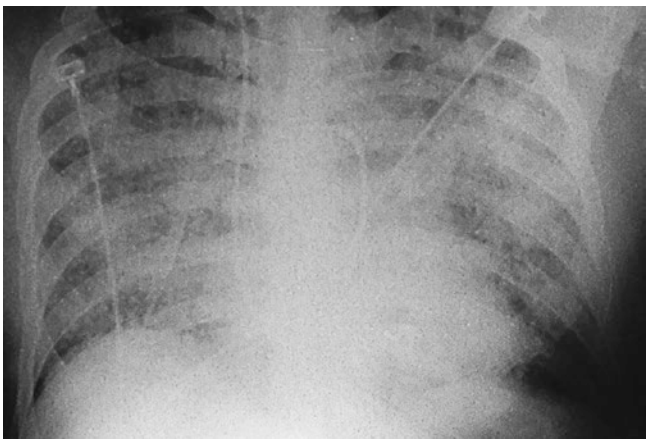


Fig. A.21 Diffuse pulmonary infiltrates in a patient with ARDS. Patients presenting with this syndrome have a mortality rate greater than 50%

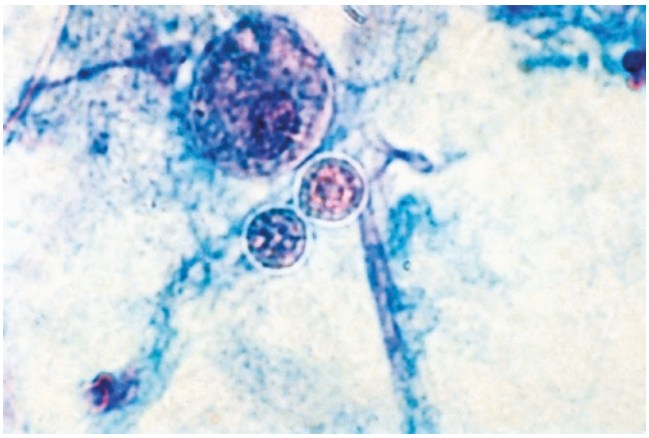


Fig. A.22 Cytology preparation of endotracheal tube specimen revealing large, thick-walled yeasts consistent with *Blastomyces dermatitidis*

The patient received intravenous amphotericin B immediately at a dose of 0.7 mg/kg/day, but was rapidly increased to 1 mg/kg/day. During the remainder of his hospitalization, the patient became increasingly difficult to oxygenate, developed hypotension requiring pressers, progressed to multi-organ failure, and died with pulseless electrical activity.

Instructive Case 16

An 18-year-old woman presented with chest discomfort. Dilated veins were noted over the chest and upper abdomen. Chest x-ray showed right hilar enlargement, which was calcified on CT. The right pulmonary artery was narrowed and the superior vena cava was occluded. Ventilation-perfusion lung scan showed reduced blood flow to the right lung. Pulmonary function tests showed normal air flow and lung

capacity. Tuberculin skin test was negative. Histoplasma immunodiffusion tests showed an M band and the Histoplasma complement fixation test showed titers of 1: 32 to the yeast and 1:16 to the mycelial antigen. Mediastinal biopsy showed chronic inflammatory cells, granuloma, and fibrosis. Culture of the mediastinal biopsy tissue was negative for fungus.

Question

Is surgery indicated to correct the obstruction of the pulmonary artery or of the superior vena cava, and should the patient receive a course of antifungal therapy?

Instructive Case 17

A 41-year-old male rural worker and heavy smoker was seen in consultation with three months of dry cough, severe progressive dyspnea, weight loss, asthenia, adynamia, and anorexia. He looked emaciated and experienced difficulties in breathing even at rest. Respiratory rate was noted to be 36 breaths per minute with accessory muscles utilization. Upon auscultation rales, rhonchi and hypoventilation were noticed. The chest X-ray (Fig. A.23) revealed the presence of a diffuse reticulonodular infiltrates predominating in both central fields with fibrous lines. Follow-up CT of chest documented widespread fibrosis (Fig. A.24). Arterial blood gas analysis revealed pH 7.44, pO_2 37 mmHg, pCO_2 23 mmHg, O_2 saturation 81%, and bicarbonate 16 mEq/L.

A bronchoalveolar lavage fluid sample was examined for acid-fast bacilli with negative results, but multiple budding cells corresponding to *Paracoccidioides brasiliensis* were seen on direct examination and recovered in culture later on. Serologic tests with paracoccidioidin were reactive with one band of precipitate and a titer of 1:1024 in the complement fixation (CF) test.



Fig. A.23 Paracoccidioidomycosis. Lung fibrosis involving specially the central field with apices appearing free. Bilateral basal bullae and pleural adhesions are seen in both lower fields



Fig. A.24 Paracoccidioidomycosis. High-resolution CT showing widespread fibrosis with honeybee aspect, bullae formation, and pleural thickening in both lower lung fields

Instructive Case 18

A 24-year-old Caucasian man presented with a chief compliant of headache of 3 weeks duration. He was in his usual state of health until 11 weeks prior to admission when he developed a headache. Nine weeks prior to admission the patient was seen at another facility with headache and confusion. Lumbar puncture without opening pressure was performed at that time and revealed meningitis. He was treated with vancomycin, ceftriaxone, fluconazole, and acyclovir. The patient was discharged home after one day with oral acyclovir. His headache continued to wax and wane, with decreasing ability to perform activities of daily living. His primary care physician referred the patient to the emergency room. Past social history included occasional marijuana use and lost job due to headaches. Physical examination revealed the patient to be alert, but listless and in mild distress. His neck was supple, but his tandem gait testing abnormal. Imaging studies including CT scan, MRI, and chest radiograph were normal. Lumbar puncture found an opening pressure of 390 mm of water, with 750 white cells/μL; 52% were neutrophils, 19% were eosinophils, and 20% were lymphocytes. The protein was 130 mg/dL and glucose was 20 mg/dL. Medical treatment was started with fluconazole 1000 mg daily and dexamethasone 20 mg daily (tapered by 5 mg every other day). A plan was made to repeat lumbar puncture daily if opening pressure was greater than 250 mm H_2O to reduce

this reading by 50% or to less 200 mm H_2O, whichever is greater. If this treatment fails the plan would be to pursue lumboperitoneal shunt placement.

Instructive Case 19

A 68-year-old woman was admitted to the hospital for a right frontal craniotomy to de-bulk a pituitary adenoma. Her past medical history was significant for poorly controlled diabetes mellitus, hypertension, and sleep apnea. Her home medications included oral prednisone. Postoperatively she was lethargic and developed diabetes insipidus due to the surgery. One week after admission, she remained obtunded and developed a temperature of 39 °C and was noted to have a brownish drainage from her craniotomy wound adjacent to a pulse-oximetry sensor. Laboratory testing was significant for a leukocytosis of 23,100 cells/μL. Underneath the sensor, a small superficial ulceration approximately 1 × 2 cm was discovered. Within 6 days, the ulceration developed into a black eschar that measured 6.5 × 7.5 cm (Fig. A.25). A biopsy of the lesion revealed necrotic tissue with arterial occlusion. Fungal stains revealed broad-based, nonseptate hyphae with acute or wide-angle branching. The culture was positive for *Mucor* species. Liposomal amphotericin B at 5 mg/kg/day was initiated and the patient underwent extensive debridement of scalp tissue, underlying bone, dura, and subdural tissue, all of which were necrotic and culture positive for *Mucor*. The patient remained comatose and expired about one week postoperatively. On autopsy, histopathology of the affected areas revealed *Mucor* infection extending from the scalp through the craniotomy incision into the pituitary tissue (Figs. A.26 and A.27).

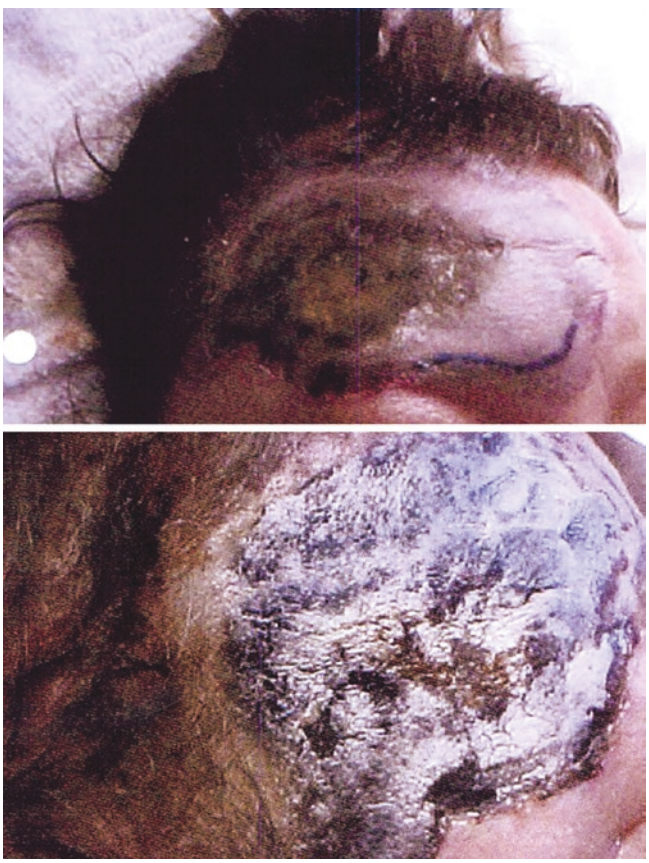


Fig. A.25 Cutaneous lesion over the right frontoparietal scalp

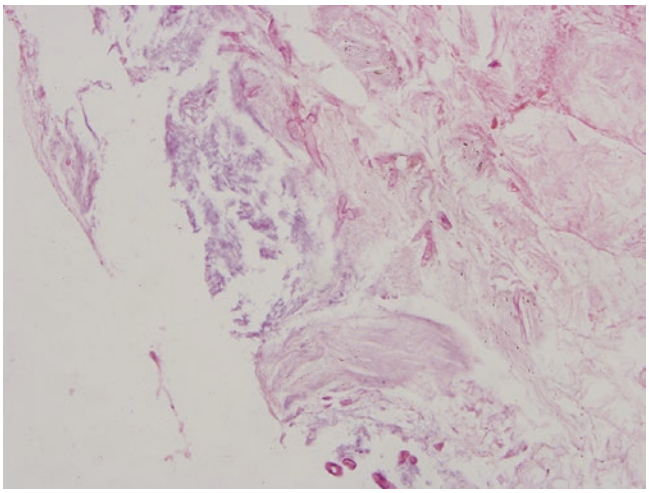


Fig. A.26 Histopathologic examination with H&E stain of the skin demonstrating the broad, nonseptate hyphae with acute or wide-angle branching

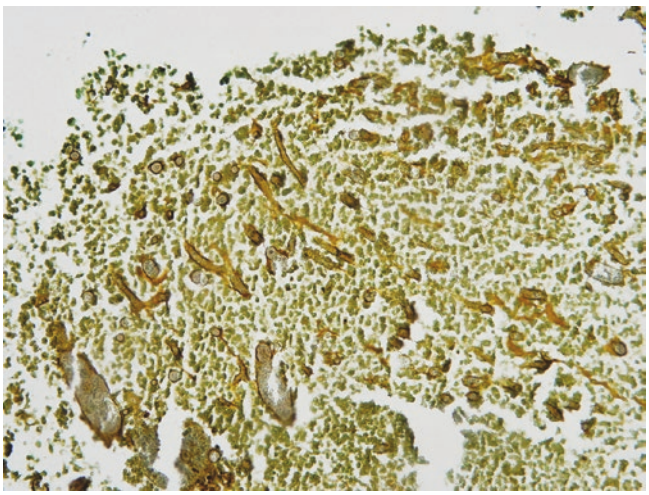


Fig. A.27 Histopathologic examination with GMS stain of pituitary tissue demonstrating broad, nonseptate hyphae with acute or wide-angle branching

Instructive Case 20

A 30-year-old man with known HIV infection presented with a 4-week history of increasing dyspnea on exertion, tachypnea, and fever. His chest radiograph revealed diffuse alveolar infiltrates and blood gas, hypoxemia. He had been noncompliant with this antiretroviral therapy and was profoundly lymphopenic. *Pneumocystis* pneumonia was confirmed by bronchoalveolar lavage.

The patient was treated with trimethoprim-sulfamethoxazole (TMP-SMX) and his antiretroviral treatment restarted. On TMP-SMX treatment he showed a gradual improvement, but at 4 weeks after having starting these therapies his respiratory symptoms recurred and chest radiograph found new diffuse interstitial infiltrates. *Pneumocystis* organisms were not visualized on repeat bronchoalveolar lavage. A recheck of his lymphocyte subsets demonstrated his CD4 lymphocyte count had risen to 300 cells/μL.

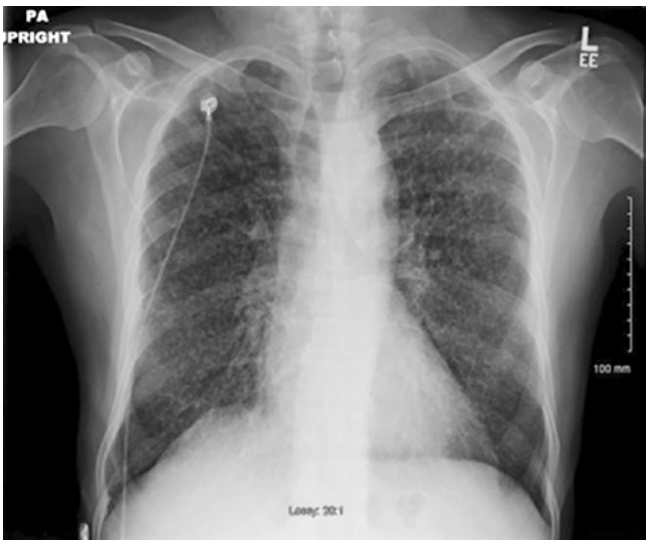


Fig. A.28 Chest radiograph

Question

1. What is the most likely diagnosis for the patients' sudden deterioration after responding to initial therapy?
   A. Bacterial superinfection.
   B. Relapse of *Pneumocystis* pneumonia due to infection with TMP-SMX resistant *Pneumocystis.*
   C. Immune restitution disease (IRD)/immune reconstitution inflammatory syndrome (IRIS).
   D. Stevens-Johnson syndrome secondary to TMP-SMX.

Instructive Case 21

A 68-year-old Caucasian man with polycystic renal disease presented with a chief complaint of swelling of his left lower extremity. His past history included progressive renal failure treated with renal transplantation. The patient was admitted for a diagnosis of cellulitis and placed on vancomycin and piperacillin/tazobactam. When ultrasound revealed deep venous thrombosis, antibiotics were discontinued and anticoagulation commenced. Chest radiograph was abnormal but no initial respiratory symptoms were noted (Fig. A.28). He subsequently developed progressive respiratory symptoms and radiographic changes and was transferred to the ICU.

Bronchoscopy revealed lymphocytic and eosinophilic cellularity and culture grew *C. immitis*. His antirejection drugs were stopped and lipid amphotericin B therapy was started. The patient gradually improved. When his dialysis graft became nonfunctional after one month of therapy, he was changed to oral posaconazole. He is currently on hemodialysis and fluconazole therapy and doing well. No pretransplantation evaluation of the donor or recipient for coccidioidomycosis was performed.

Instructive Cases Discussion

Instructive Case 1

Answers: 1. **B**, 2. **C**

Discussion, Question 1

Amphotericin B-induced nephrotoxicity occurs primarily through two mechanisms: (1) constriction of afferent arterioles leading to direct decreases in GFR (glomerular toxicity), and (2) direct damage to the distal tubules (tubular toxicity) (answer B), which in turn can lead to glomerular feedback that further cause constriction of the afferent arterioles. Tubular toxicity of amphotericin B is essentially limited to the distal tubules and most commonly evident as hypokalemia (answer B). It occurs in the majority of patients receiving amphotericin B and may require up to 15 mmol of supplemental potassium per hour. Amphotericin B-induced hypokalemia is not associated with increased plasma aldosterone or renin levels and appears to result from increased permeability of the distal tubular cells due to direct toxic effects of amphotericin B. Although lipid amphotericin B formulations reduce glomerular toxicity, they do not eliminate distal tubular toxicity. Decreases in serum potassium and magnesium caused by distal tubular toxicity frequently precede decreases in glomerular filtration rate (increases in serum creatinine, blood urea nitrogen) during amphotericin B therapy, particularly in patients receiving lipid amphotericin B formulations. Afferent arteriole constriction (answer A) would be more specifically associated with decreases in glomerular filtration (decrease in serum creatinine). Suppression of erythropoietin synthesis (answer C) is a more chronic effect of amphotericin B and manifests primarily as normochromic, normocytic anemia. Amphotericin B has not been shown to directly damage pancreatic islet cells (answer D).

Discussion, Question 2

Nephrotoxicity is the dose-limiting toxicity of amphotericin B therapy. All of the answers are potential approaches that have been applied to prevent or manage the development of nephrotoxicity during administration of a lipid formulation of amphotericin B (answer D). Decreasing the daily dosing of amphotericin B (answer A) from 7.5 mg/kg/day to 5.0 mg/kg/day may attenuate the nephrotoxicity of liposomal amphotericin B. Isavuconazole is licensed for the treatment of mucormycosis and is an appropriate alternative to liposomal amphotericin B. Saline loading (answer C) can reduce tubular-glomerular feedback and delay the onset of glomerular toxicity or reduce the severity of azotemia.

Instructive case 1 contributed by R. E. Lewis and T.J. Walsh.

Instructive Case 2

Discussion

This case is somewhat unusual in that the site of inoculation of *Sporothrix schenckii* had received a skin graft, and thus the lesions were atypical. They arose in the grafted area and contributed to loss of portions of the graft. The lack of response to antistaphylococcal antibiotics was a clue that this was not a typical bacterial infection. The possibility of sporotrichosis was raised by the Infectious Diseases consultant because of the proximal spread of nodules and the exposure to soil during the original accident. Biopsy confirmed this suspicion when the cultures yielded a mold. The fact that no organisms were seen on the biopsy is not unusual.

Fluconazole was used because an experimental protocol was available at the time, and the patient had no insurance and was unable to purchase other antifungal agents. The response to fluconazole was adequate, but slower than usually noted with itraconazole, which is the treatment of choice for sporotrichosis.

Instructive case 2 contributed by C. A. Kauffman.

Instructive Case 3

Beware of "Steroid-responsive pneumonia" with or without eosinophils.

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections* , https://doi.org/10.1007/978-3-031-35803-6

Discussion

This case demonstrates that coccidioidal pneumonia may present as community-acquired pneumonia. However, it is protean in its presentation and any pneumonia (with exposure in endemic areas) that fails initial antimicrobial therapy should be evaluated for the possibility of *Coccidioides*.

Instructive case 3 contributed by R. H. Johnson and A. Heidari.

Instructive Case 4

Discussion

This is an example of the juvenile type paracoccidioidomycosis.

Instructive case 4 contributed by A. Restrepo, A. M. Tobón, and C. A. Agudelo.

Instructive Case 5

Discussion

This case is instructive for several reasons. The patient's past medical history is significant for a long history of immunosuppression due to long-term corticosteroids and his diabetes mellitus. Both of these conditions predispose the patient to an increased risk of fungal infections because of alterations in cell-mediated immunity. The case demonstrates the increasing incidence and the capacity of previously nonpathogenic yeast to produce invasive infection. In addition, the presentation of the patient with nonspecific signs and symptoms is not uncommonly seen in many invasive fungal infections. In fact, the problem with the diagnosis of invasive fungal infections is that there are no "classic or pathognomonic manifestations"; this makes the diagnosis difficult to establish and creates a delay in the initiation of appropriate antifungal therapy. In this case, the aspiration of the infected knee demonstrated several organisms (gram-negative bacilli and yeast), thus assisting with the diagnosis. However, *Candida*, not *Trichosporon*, would have been the more common cause of infection in this patient. It is not until the laboratory identifies the organisms that the true diagnosis is established. Although there are no clinical trials establishing the best antifungal agent for *Trichosporon* species, the azoles (fluconazole, voriconazole) have been shown to have in vitro activity. In this case in vitro susceptibility results demonstrated that both fluconazole and itraconazole had good activity. Although there are no established breakpoints for the echinocandins, the MIC of 2.0 μg/mL for caspofungin appears to be within the "standard" ranges described for clinical activity against *Candida* species. Furthermore, although there are some in vitro and animal studies demonstrating additive or synergistic activity with the combination of echinocandins and azoles, the use of combination antifungal therapy has not been demonstrated to be any better than monotherapy.

Instructive case 5 contributed by J. A. Vazquez.

Instructive Case 6

Blastomycosis in AIDS.

Discussion

1. Blastomycosis in AIDS patients is more likely to be multiorgan disease with CNS involvement being noted in up to 40% of patients. Routine MRI or CT scans should definitely be performed in any patient with severe end-stage AIDS whether or not they have neurologic signs or symptoms.
2. Wet preparations of skin lesions allowed a presumptive clinical diagnosis and early initiation of amphotericin B therapy in this patient.
3. Central nervous system disease in end-stage AIDS patients should be treated with a full course of amphotericin B, e.g., 1.5–2.5 g.
4. Fluconazole at high doses (800 mg/day) may be a reasonable substitute in patients intolerant to amphotericin B or as step down therapy in patients who have responded to initial treatment with amphotericin B.
5. In immunosuppressed patients, chronic suppressive therapy with fluconazole should be considered.

Instructive case 6 contributed by S. W. Chapman and D. C. Sullivan.

Instructive Case 7

Discussion

This case represents acute pulmonary histoplasmosis misdiagnosed as sarcoidosis. This case illustrates the importance of thorough testing to exclude histoplasmosis before beginning immunosuppressive treatment for sarcoidosis. While there are clinical features that help to distinguish sarcoidosis from histoplasmosis, differentiation requires laboratory testing to exclude histoplasmosis. Administration of corticosteroids resulted in transient clinical improvement, followed by progression with worsening pulmonary disease and progressive dissemination. Failure to demonstrate yeast resembling *H. capsulatum* on the initial bronchoscopy resulted in a mistaken diagnosis of sarcoidosis. Of note is that cytology and culture of respiratory secretions are often negative in acute histoplasmosis, and cannot be used to exclude the diagnosis.

Additional testing should include serology for antibodies to *H. capsulatum*, and tests for *Histoplasma* antigen in urine and respiratory secretions. Serology is often negative during the first month after exposure, but positive thereafter. Antigen may be detected in the urine or bronchoscopy specimen of 75% of cases during the acute illness and before antibodies have appeared. Corticosteroids for sarcoidosis should not be given without thorough evaluation to exclude histoplasmosis. Of note is that fungal cultures require up to 4 weeks of incubation for isolation of *H. capsulatum*, during which corticosteroids should be withheld except in severe cases. Tests for antigen and antibody, and cytology and histopathology on tissues including bronchial washing or bronchoalveolar lavage, lung and bone marrow should be performed and may provide early evidence for histoplasmosis, but do not exclude the diagnosis.

If cytology, histopathology, antigen testing, and serology are negative and corticosteroids are required for severe sarcoidosis, itraconazole may be given while waiting for culture results in selected patients in whom the diagnosis of histoplasmosis is suspected based on epidemiologic grounds.

Instructive case 7 contributed by L. J. Wheat and N. G. Conger.

Instructive Case 8

Discussion

Subcutaneous phaeohyphomycosis is among the most common manifestations of disease due to dematiaceous fungi. It is seen in both immunocompetent and immunocompromised individuals and is not usually associated with dissemination, though the risk of dissemination is higher in immunosuppressed patients. Complete excision alone has been reported as a successful therapy, particularly in immunocompetent patients. In immunocompromised patients, antifungal therapy is often given after surgical excision to reduce the risk of dissemination. However, itraconazole and voriconazole both have significant interactions with immunosuppressive agents such as tacrolimus and sirolimus, and combined use of these drugs requires close monitoring and commonly adjustment of the immunosuppressive agents.

Instructive case 8 contributed by S. G. Revankar.

Instructive Case 9

Discussion

This case demonstrates that pleural pulmonary coccidioidomycosis may present as hydropneumothorax secondary to a ruptured coccidioidal cavity.

Instructive case 9 contributed by R. H. Johnson and A. Heidari.

Instructive Case 10

Discussion

Nodular pulmonary infiltrates in neutropenic patients are often caused by pathogenic fungi. Mycological identification is critical for proper management. This case illustrates that pulmonary mucormycosis in a neutropenic host may be associated with fever but a paucity of other findings on initial presentation. A BAL is often negative and more invasive procedures, such as a fine needle aspiration, may be necessary to establish a diagnosis. During neutropenia, pulmonary mucormycosis may progress rapidly, despite amphotericin B therapy. Surgery has an important role in these patients, as it may be the only way of controlling this angioinvasive infection. Granulocyte transfusions may have a role to gain time until neutropenia resolves. Ultimately, however, recovery from these infections is often contingent upon recovery of myelopoiesis. In the case presented, control of the pulmonary infection was achieved with combined medical and surgical intervention. Unfortunately, the patient ultimately succumbed to complications of his hairy cell leukemia.

Instructive case 10 contributed by Charalampos Antachopoulos, MD, Juan C. Gea-Banacloche, MD, Emmanuel Roilides, MD, PhD, and Thomas J. Walsh, MD, PhD (Hon).

Instructive Case 11

Discussion

This patient is an example of chronic, adult multifocal paracoccidioidomycosis.

Instructive case 11 contributed by A. Restrepo, A. M. Tobón, and C. A. Agudelo.

Instructive Case 12

Answer: D

Discussion

Unpredictable, low-frequency idiosyncratic liver failure with azoles occurs on a background of a much higher frequency of mild asymptomatic liver injury. Mild liver injury is exacerbated by concomitant medications or dominated by other disease states (as in this case with the graft versus host disease that often affects the liver). Because mild liver injury is generally reversible and transient, immediate discontinuation of voriconazole is not necessary (answers A or B). In clinical trials, voriconazole was continued in the majority of patients with elevated serum transaminases until they reached greater than 3 times the upper limit of normal (which has not

been reached in this patient). Because the patient will likely receive corticosteroids for the graft versus host disease reactivation, continuation of voriconazole and monitoring of liver function tests with the initiation of steroid therapy (answer D) would be the most reasonable approach. Although reduction of the voriconazole dose is an option (answer C), reducing antifungal intensity in an immunocompromised patient with active graft versus host disease and receiving steroids is undesirable. Many clinicians would potentially add a second antifungal agent in this patient if she had other signs of infection (i.e., pulmonary nodules in lung).

Instructive case 12 contributed by R. E. Lewis.

Instructive Case 13

Discussion

The most common bone disease secondary to coccidioidomycosis is osteomyelitis of the spine. Persistent spine pain should be evaluated for malignancy and infectious diseases. Coccidioidal infection most often presents as progressive pain with evidence of discitis.

Instructive case 13 contributed by R. H. Johnson and A. Heidari.

Instructive Case 14

Discussion

For induction therapy, she had received combination therapy with amphotericin B in a lipid formulation and a reduced dose of flucytosine because of kidney dysfunction. Her clinical response did not require repeated lumbar punctures to control raised intracranial pressure because symptoms did not worsen and actually improved. Her response to antifungal combination therapy was appropriate with a negative CSF culture at the end of 2 weeks of induction therapy.

Case continued

Patient did relatively well on her suppressive fluconazole therapy at 200 mg/day for 4 months (dosed for reduced renal function) when she developed severe headaches and a MRI scan showed diffuse supra- and infratentorial leptomeningeal enhancement. At that time CSF cryptococcal polysaccharide antigen was 1:16, white blood cell count was 100 cells/μl, and cultures negative. After 2 weeks of AmBisome at 5 mg/kg/d and no improvement, the patient was continued on fluconazole and a 6- week dexamethasone taper was begun with immediate improvement in symptoms. Tacrolimus and mycophenolate were stopped and the patient was started on dialysis. One week after stopping the 6-week taper of corticosteroids, her headaches returned and a 4- month steroid taper was begun. She improved and was eventually weaned off corticosteroids and now has received suppressive fluconazole for 1–2 years and is doing well awaiting a new transplant.

Discussion

In this case the patient developed cryptococcal meningitis after receiving severe therapeutic immunosuppression in an attempt to save her renal transplant from rejection. She initially responded to potent antifungal combination therapy, which was adjusted to renal dysfunction. Initially she did well on this suppressive therapy, but as she completely lost kidney function and immunosuppressive therapy was reduced she again developed meningeal symptoms and signs, but the work-up did not reveal evidence of an ongoing viable yeast infection. It was then decided that this may represent Immune Reconstitution Inflammatory Syndrome (IRIS) and she was started on corticosteroids which improved and eventually resolved her symptoms.

This case illustrates the dynamic relationship between the immune system and cryptococcosis. Although there are standardized, well-studied antifungal treatment regimens, there are clearly times when clinical judgment must be used regarding management. Currently management of increased intracranial pressure and IRIS is performed without the luxury of guidance from robust evidence-based studies.

Instructive case 14 contributed by M. Chayakulkeeree and J. R. Perfect.

Instructive Case 15

Blastomycosis presenting with ARDS.

Discussion

1. Patients presenting with severe pulmonary disease, whether miliary or ARDS, have a high rate of mortality ($\geq 50\%$). All patients presenting in this fashion should be initially treated with amphotericin B.
2. Life-threatening pulmonary disease may be seen in non-immunocompromised patients. Hence, blastomycosis must be considered in any patient living in or with recent travel to the endemic area and who presents with severe or overwhelming pneumonia.
3. Most patients who die do so within the first week of therapy emphasizing that amphotericin B therapy should be initiated as soon as possible after diagnosis.
4. Cytology may have a higher diagnostic yield than expectorated sputum examined under wet preparation.

Instructive case 15 contributed by S. W. Chapman and D. C. Sullivan.

Instructive Case 16

Discussion

Fibrosing mediastinitis [FM] results from excessive scarring around the hilar and mediastinal lymph nodes. This scar tissue extends from the lymph nodes to invade important nearby structures, such as the pulmonary arteries or veins, bronchial arteries, vena cava, trachea, main stem and lobar or segmental bronchi, esophagus, pericardium, and even the heart. FM represents a scarring response to a prior episode of histoplasmosis rather than an active and progressive infection.

The severity of the illness depends on the extent of the scarring and the specific structures that are involved. In many cases the consequences are mild and non-progressive, causing minimal or no limitation to function, and requiring no consideration for therapy. In others, symptoms may be more severe, or even disabling, prompting consideration of the treatment options. In those with extensive involvement in both lungs, the illness is progressive and eventually fatal in nearly half of cases.

Medical treatment with antifungal drugs that are used for the treatment of other types of histoplasmosis is not effective in patients with fibrosing mediastinitis, because the manifestations of FM are caused by the scar tissue, not by active infection, and scar tissue is not affected by any medical treatments.

Minimally invasive procedures to open the blockages are useful in some cases. These procedures are relatively safe and sometimes effective, although the long-term results are not fully understood. The largest experience has been with stenting of the occluded blood vessels. Stenting of obstructed airways is not felt to be as useful, and there is little experience with this procedure. Stenting or dilatation of obstructed vessels is not always successful because the fibrotic tissue may be as hard as stone, preventing passage of a small wire past the blockage. Active bleeding may be stopped by embolization of the involved blood vessel.

Surgical correction of the obstructed blood vessel or airway also is not often possible, and carries a high operative mortality. Thus, surgery should be reserved for severe cases and only then after less risky procedures are tried. The operative mortality is at least 20% overall, but can range from 50 to 75% in patients who undergo total removal of the involved lung (pneumonectomy). Of note is that the operative mortality may be even higher if the surgeon is not experienced with treatment of fibrosing mediastinitis. The reason that the mortality is so high is that the scar tissue is like cement, encasing the blood vessels and airways and obliterating tissue planes. Vital structures are often damaged while attempting to remove the scar tissue, causing bleeding or airway leaks. Death is caused by uncontrollable bleeding, respiratory failure, infection, and other postoperative complications. The indications for surgery are not well described, but at a minimum should include patient limitations severe enough to justify the risk of the known operative mortality. Surgical indications might include severe and recurrent bleeding not responsive to embolization, recurrent pneumonias that are not preventable by antibiotic prophylaxis, or respiratory failure. Surgery should only be conducted by surgeons who are experienced with fibrosing mediastinitis.

Instructive case 16 contributed by L. J. Wheat and N. G. Conger.

Instructive Case 17

Discussion

This patient is an example of the chronic unifocal pulmonary paracoccidioidomycosis.

Instructive case 17 contributed by A. Restrepo, A. M. Tobón, and C. A. Agudelo.

Instructive Case 18

Discussion

This case illustrates that chronic meningitis including coccidioidal disease may present with an increased intracranial pressure with or without radiographic changes. The increased pressure requires its own separate management plan.

Instructive Case 18 contributed by R. H. Johnson and A. Heidari.

Instructive Case 19

Cutaneous/Locally Invasive Mucormycosis.

Discussion

This case illustrates primary cutaneous mucormycosis with direct invasiveness of adjacent tissues. This entity is uncommon and represents less than 5% of all infections due to Mucorales. The case demonstrates the direct invasion via the scalp, following the craniotomy scar and subsequently leading to direct invasion of pituitary gland tissue. This case also demonstrates the difficulty in establishing an early definitive diagnosis. Although the infection was initially a superficial wound infection the diagnosis was not made until the size of the necrotic lesion had increased by about 200% and a biopsy was performed. Furthermore, it also demonstrates the high morbidity and mortality associated with a delayed diagnosis and the resultant delays in appropriate and adequate management. Although in this case complete excision alone would probably have resulted in a cure early on, most cases require

wide and aggressive surgical removal of all necrotic tissue, along with appropriate antifungal therapy. The important reminder in this case is to always maintain a broad differential diagnosis in patients at high risk of developing an invasive fungal infection.

Instructive case 19 contributed by J. A. Vazquez.

Instructive Case 20

Answer: C

Discussion

Immune restitution disease, also called immune reconstitution inflammatory syndrome (IRIS), is an acute inflammatory insult to the host, most often to the lungs. The pathophysiology is not fully understood but it is felt to represent an antigen-driven immune response initiated by a recovering immune system as a result of treatment in patients with AIDS. It is seen most often in the setting of newly diagnosed AIDS presenting with a pulmonary opportunistic infection. The role of retreatment of the OI has not been addressed, but often there is no evidence of ongoing infection. Because the pathogenesis is felt to involve the return of pathogen-specific T cells, treatment with corticosteroids has been suggested.

Symptomatic *Pneumocystis* pneumonia coinciding with engraftment following bone marrow transplant is sometimes considered as a manifestation of IRIS but in reality, this should be considered as a distinct entity, even though it may appear clinically similar. In this latter case the inflammatory injury is an appropriate response of a newly functioning immune system to an active infection with *Pneumocystis*.

Instructive case 20 contributed by F. Gigliotti and T.W. Wright.

Instructive Case 21

Discussion

This case demonstrates that immunosuppressive therapy and transplantation may result in pulmonary, miliary, or disseminated coccidioidomycosis. The infection may be reactivation of prior known or unknown coccidioidal infection in the recipient or from the donor allograft. In the proper endemic setting it is probably cost effective to screen all donors and recipients for prior coccidioidal disease.

Instructive case 21 contributed by R. H. Johnson and A. Heidari.

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D. R. Hospenthal et al. (eds.), *Diagnosis and Treatment of Fungal Infections*, https://doi.org/10.1007/978-3-031-35803-6

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