Fungal Infection

DIAGNOSIS AND MANAGEMENT

Fourth Edition

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DIAGNOSIS AND MANAGEMENT

FOURTH EDITION

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Preface to the fourth edition

In the 9 years that have elapsed since the third edition of this book was published, there have been a number of significant developments in the diagnosis and management of fungal infections. In developed countries, the ongoing use of combination antiretroviral treatment regimens has led to a sustained reduction in the incidence of opportunistic fungal infections among persons with the acquired immunodeficiency syndrome. In contrast, in resource-limited settings where these drugs are unavailable, the burden of these diseases is large and increasing. Other developments in medical practice that have led to the emergence of new populations at risk for fungal infections include the increasing use of aggressive therapeutic technologies in intensive care units, and the introduction of novel immunosuppressive regimens among patients undergoing transplants or treatment for malignancies. On the other hand, new methods of diagnosis have been introduced and new antifungal agents in the azole and echinocandin classes have been licensed for use. Unfortunately, these developments have been offset by the emergence of resistance to several groups of antifungal drugs.

As in the past, we have tried to include as many of these developments as possible without, we hope, confusing what is intended to be a concise introduction to the subject. For this fourth edition, the structure of the book remains unchanged, but extensive revision has been undertaken to most of the chapters. Some new chapters have been introduced and others deleted. Another feature we have retained from the third edition is a list of further reading at the end of each chapter. These lists are not by themselves all embracing, but rather are intended to highlight some of the most important papers that we have drawn upon for the information provided in the text.

We have made every effort to describe generally accepted practices and to ensure that our drug and dosage recommendations are accurate and in agreement with current guidelines and standards at the time of going

to press. It should be noted that the formulations and usages of the different drugs described do not necessarily have the specific approval of the regulatory authorities of all countries. Because dosage regimens can be modified in the light of new clinical research findings and changes in government regulations, readers are advised to check the most current product information provided by the manufacturer of each drug to be administered to verify the recommended dose, the method and duration of administration, and additional warnings and contraindications. This is of the utmost importance when the recommended drug herein is a new or an infrequently used agent. It is the responsibility of the treating physician, relying on experience and knowledge of the patient, to determine dosages and treatment strategies for each individual.

Malcolm D. Richardson
David W. Warnock
November 2011

Preface to the first edition

Fungal infections are assuming greater importance, largely because of their increasing incidence among transplant patients and other immunocompromised individuals, including those with AIDS. As a result, clinicians and microbiologists alike need to be familiar with the clinical presentation and methods for the diagnosis of these infections, as well as the current treatment choices.

In this book, we have attempted to provide a succinct account of the clinical manifestations, laboratory diagnosis and management of fungal infections found in European, American and Australasian practices. The book covers problems encountered both in hospitals and general practice, and is designed to permit clinicians to make the best use of the various laboratory investigations available. Emphasis is placed on clinical presentation, specimen collection, interpretation of laboratory findings, and choice of treatment regimen. In general, the length of the chapters reflects the frequency or the importance of the clinical problem, or both.

We have designed this book to facilitate rapid information retrieval. Our reading list of established literature has been carefully selected to permit efficient access to specific aspects of fungal infections and has been annotated to guide the reader.

We hope this book will be of interest to medical students, junior hospital medical staff, hospital specialists and general practitioners. In particular, it should appeal to microbiologists, infectious disease specialists, dermatologists, haematologists, genitourinary medicine specialists, oncologists and intensive-care staff.

Malcolm D. Richardson
David W. Warnock
March 1993

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CHAPTER 1

Introduction

The last several decades have seen unprecedented changes in the pattern of fungal infections in humans. These diseases have assumed a much greater importance because of their increasing incidence in persons with the acquired immunodeficiency syndrome (AIDS), in recipients of solid organ or haematopoietic stem cell transplants (HSCT), in persons with haematological malignancies and in other debilitated or immunocompromised individuals. Although gains have been made in the treatment and prevention of fungal disease, major changes in health care practices have resulted in the emergence of new at-risk populations.

1.1 The nature of fungi

The fungi form a large, diverse group of organisms, most of which are found as saprophytes in the soil and on decomposing organic matter. They are eukaryotic, but differ from other groups, such as plants and animals, in several major respects. First, fungal cells are encased within a rigid cell wall, mostly composed of polysaccharides (glucan, mannan), chitin and glycoproteins in various combinations. This feature contrasts with animals, which have no cell walls, and plants, which have cellulose as the major cell wall component. Second, fungi are heterotrophic. This means that they are lacking in chlorophyll and therefore require preformed organic carbon compounds for their nutrition. Fungi obtain their nourishment by secreting enzymes for external digestion and by absorbing the released nutrients through their cell wall. Third, fungi are simpler in structure than plants or animals. There is no division of cells into organs or tissues. The basic structural unit of fungi is either a chain of tubular, filament-like cells

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(termed hypha) or an independent single cell (termed yeast). Fungal cell differentiation is no less sophisticated than is found in plants or animals, but it is different. Many fungal pathogens of humans and animals change their growth form during the process of tissue invasion. These dimorphic pathogens usually change from a multicellular hyphal form in the natural environment to a budding, single-celled yeast form in tissue.

In most multicellular fungi, the vegetative stage consists of a mass of branching hyphae, termed mycelium. Each individual hypha has a rigid cell wall and increases in length as a result of apical extension with mitotic cell division. In most fungi, the hyphae are septate, with more or less frequent cross walls. In the more primitive fungi, the hyphae usually remain aseptate (without cross walls). Fungi that exist in the form of microscopic multicellular mycelium are commonly called moulds.

Many fungi exist in the form of independent single cells. Most yeasts propagate by an asexual process called budding, in which the cell develops a protuberance from its surface. The bud enlarges and may become detached from the parent cell, or it may remain attached and itself produce another bud. In this way, a chain of cells may be produced. Under certain conditions, continued elongation of the parent cell before it buds results in a chain of elongated cells, termed pseudohypha. Some yeasts reproduce by fission of the cells. Yeasts are neither a natural nor a formal taxonomic group, but are a growth form shown in a wide range of unrelated fungi.

Moulds reproduce by means of microscopic propagules called either conidia or spores. Many fungi produce conidia that result from an asexual process (involving mitosis only). Except for the occasional mutation, these spores are identical to the parent. Asexual conidia are generally short-lived propagules that are produced in enormous numbers to ensure dispersion to new habitats. Many fungi are also capable of sexual reproduction (involving meiosis). Some species are self-fertile (homothallic) and able to form sexual structures within individual colonies. Most, however, are heterothallic and do not form their sexual structures unless two different mating strains come into contact. Meisosis then leads to the production of sexual spores. In some species, the sexual spores are borne singly on specialized generative cells and the whole structure is microscopic in size. In other cases, however, the spores are produced in millions in 'fruiting bodies' such as mushrooms. Many fungi can produce more than one type of spore, depending on the growth conditions, the precise method of spore production and type(s) of spore produced being

unique to each species. Sexual reproduction and its accompanying structures form the main basis for classification of the fungi.

1.2 Classification and nomenclature of fungi and fungal diseases

The fungal kingdom is one of the six kingdoms of life. It is organized in a hierarchical manner and is currently divided into seven phyla, which include the Ascomycota and Basidiomycota. The phylum name Zygomycota is no longer accepted because of its polyphyletic nature. In its place, the phylum Glomeromycota and four subphyla, including the Mucoromycotina and Entomophthoromycotina, have been created pending further resolution of taxonomic questions. Historically, fungal classification has largely been based on morphological features, in particular the method of sexual spore production. In some fungi, however, the asexual stage (termed the anamorph) has proved so successful as a means of rapid dispersal to new habitats, that the sexual stage (termed the teleomorph) has diminished or even disappeared. In these fungi, the shape of the asexual spores and the arrangement of the spore-bearing structures have been of major importance in classification and identification. With the advent of DNA sequence analysis, fungal species are now defined as groups of organisms with concordant sequences at multiple different genetic loci, rather than organisms that share common morphological characteristics or organisms that can mate with one another. Even in the absence of the sexual stage, it is now often possible to assign asexual, anamorphic or mitosporic fungi to genera within the phyla Ascomycota or Basidiomycota on the basis of DNA sequence analysis.

The scientific names of fungi are subject to the International Botanical Code of Nomenclature, a convention that dates from the time when biologists regarded these organisms as 'lower plants'. In general, the correct name for any species is the earliest name published in line with the requirements of the Code. Any later names are termed synonyms. To avoid confusion, however, the Code allows for certain exceptions. The most significant of these is when an earlier generic name has been overlooked, a later name is in general use, and a reversion to the earlier name would cause problems. Another reason for renaming a fungus is when new research necessitates the transfer of a species from one genus to another, or establishes it as the type of a new genus. Such changes are quite in order, but with the provision that the specific epithet should remain unchanged.

Many fungi bear two names, one designating their sexual stage and the other their asexual stage. Often this is because the anamorphic and teleomorphic stages were described and named at different times without the connection between them being recognized. The Code of Nomenclature permits this practice, and while the name of the teleomorph takes precedence and covers both stages, the name given to an anamorph may be used as appropriate. Thus, it is permissible to refer to a fungus by its asexual designation if this is the stage that is usually obtained in culture.

Unlike the names of fungi, the names of fungal diseases are not subject to strict international control. Their usage tends to reflect local practice. One popular method has been to derive disease names from the generic names of the causal organisms: for example, aspergillosis, cryptococcosis, histoplasmosis, etc. However, if the fungus changes its name, then the disease name has to be changed as well. For example, the term zygomycosis has been used for decades to describe infections caused by members of the class Zygomycetes. With the recent abolition of this class, the more precise terms mucormycosis and entomophtoromycosis have begun to supplant zygomycosis to describe diseases caused by species belonging to the orders Mucorales and Entomophthorales, respectively (see Chapters 13 and 20).

In 1992, a sub-committee of the International Society for Human and Animal Mycology recommended that the practice of forming disease names from the names of their causes should be avoided and that, whenever possible, individual diseases should be named in the form 'pathology A due to (or caused by) fungus B'. This recommendation was not intended to apply to long-established disease names, such as aspergillosis, rather it was intended to offer a more flexible approach to nomenclature.

There is also much to be said for the practice of grouping together mycotic diseases of similar origins under single headings. One of the broadest and most useful of these collective names is the term 'phaeohyphomycosis', which is used to refer to a range of superficial, subcutaneous and systemic infections caused by any brown-pigmented mould that adopts a septate hyphal form in host tissue (see Chapter 25). The number of organisms implicated as aetiological agents of phaeohyphomycosis has increased from 16 in 1975 to more than 250 at the present time. Often these fungi have been given different names at different times, and the use of the collective disease name has helped to reduce the confusion in the literature. The term 'hyalohyphomycosis' is another collective name that is increasing in usage. This term is used to describe infections caused by colourless (hyaline) moulds that adopt a septate hyphal form in tissue (see Chapter 23). To date, more than 70 different organisms have been

implicated, including a number of important emerging fungal pathogens, such as *Fusarium* species, that are not the cause of otherwise-named diseases, such as aspergillosis.

1.3 Fungi as human pathogens

There are at least 100,000 named species of fungi. However, fewer than 500 have been associated with human disease, and no more than 100 are capable of causing infection in otherwise normal individuals. The remainder is only able to produce disease in hosts that are debilitated or immunocompromised in some way. Most human infections are caused by fungi that grow as saprophytes in the environment and are acquired through inhalation, ingestion or traumatic implantation. Some yeasts are human commensals and cause endogenous infections when there is some imbalance in the host. Many fungal diseases have a worldwide distribution, but some are endemic to specific geographical regions, usually because the aetiological agents are saprophytes restricted in their distribution by environmental conditions.

Fungal infections can be classified into a number of broad groups according to the initial site of infection. Grouping the diseases in this manner brings out clearly the degree of parasitic adaptation of the different groups of fungi and the way in which the site affected is related to the route by which the fungus enters the host.

1.3.1 The superficial mycoses

These are infections limited to the outermost layers of the skin, the nails and hair, and the mucous membranes. The principal infections in this group are the dermatophytoses and superficial forms of candidosis. These diseases affect millions of individuals worldwide, but there are regional variations. They are readily diagnosed, and usually respond well to treatment.

The dermatophytes are limited to the keratinized tissues of the epidermis, hair and nail. Most are unable to survive as free-living saprophytes in competition with other keratinophilic organisms in the environment and thus are dependent on passage from host to host for their survival. These obligate pathogens seem to have evolved from unspecialized saprophytic forms. In the process, most are now no longer capable of sexual reproduction and some are even incapable of asexual reproduction. In general, these organisms have become well adapted to humans, evoking little or no inflammatory reaction from the host. Only dermatophyte infections are truly contagious.

The aetiological agents of candidosis, like the dermatophytes, are largely dependent on the living host for their survival, but differ from them in the manner by which this is achieved. These organisms, of which *Candida albicans* is the most important, are normal commensal inhabitants of the human digestive tract or skin. Acquisition of these organisms from another host seldom results in overt disease, but rather results in the setting-up of a commensal relationship with the new host. These organisms do not produce disease unless some change in the circumstances of the host lowers its natural defences. In this situation, endogenous infection from the host's own reservoir of the organism may result in mucosal, cutaneous or systemic infection.

Other common superficial infections include pityriasis versicolor, a mild and often recurrent infection of the stratum corneum, caused by lipophilic yeasts of the genus *Malassezia*. These organisms are skin commensals. Disease is probably related to host and environmental factors. Pityriasis versicolor is most common in hot, humid tropical climates.

1.3.2 The subcutaneous mycoses

These are infections of the dermis, subcutaneous tissues and adjacent bones that generally show slow localized spread. They usually result from the traumatic implantation of saprophytic fungi from soil or vegetation. More widespread dissemination of the infection, through the blood or lymphatics, is uncommon, and usually only occurs if the host is in some way debilitated or immunocompromised. The principal subcutaneous mycoses are mycetoma, sporotrichosis, phaeohyphomycosis and chromoblastomycosis. These infections are most frequently encountered among the rural populations of the tropical and sub-tropical regions of the world, where individuals go barefoot and wear the minimum of clothing.

1.3.3 The systemic mycoses

Deep-seated fungal infections usually originate in the lungs, but may spread to many other organs. These infections are most commonly acquired as a result of inhaling spores of organisms that grow as saprophytes in the environment, or as pathogens on plants.

The organisms that cause systemic fungal infection can be divided into two distinct groups: the true pathogens and the opportunists. The first of these groups is comprised of a handful of organisms, mostly dimorphic fungi that are able to invade and develop in the tissues of a normal host with no recognizable predisposition. The principal diseases are blastomycosis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis. The second group, the opportunists, consists of less virulent and less

well-adapted organisms that are only able to invade the tissues of an immunocompromised host. Although new species of fungi are regularly being identified as causes of disease in immunocompromised patients, five diseases still account for most reported infections: aspergillosis, candidosis, cryptococcosis, mucormycosis and pneumocystosis.

In many instances, infections with true pathogenic fungi are asymptomatic or mild and of short duration. Most cases occur in geographical regions where the aetiological agents are found in nature and follow inhalation of spores that have been released into the environment. Individuals who recover from these infections may enjoy marked and lasting resistance to reinfection, while the few patients with chronic or residual disease often have a serious underlying illness.

In addition to their well-recognized manifestations in otherwise normal persons, infections with true pathogenic fungi have emerged as important diseases in immunocompromised individuals. Histoplasmosis and coccidioidomycosis, for instance, have been recognized as AIDS-defining illnesses. Both diseases have been seen in significant numbers of human immunodeficiency virus (HIV)-infected persons throughout North and South America. In immunocompromised individuals, infections with true pathogenic fungi are often life-threatening and unresponsive to antifungal drugs, or relapse following discontinuation of treatment.

Opportunistic fungal infections occur in individuals who are immunosuppressed as a result of an underlying illness or their treatment. In most cases, infection results in significant disease. Resolution of the infection does not confer protection, and reinfection or reactivation may occur if host resistance is again lowered. In contrast to the restricted geographical distribution of most of the true pathogenic fungi, many opportunistic fungi are ubiquitous in the environment worldwide, being found in the soil, on decomposing organic matter and in the air. These infections are associated with high case fatality rates, but estimates of their incidence are thought to be quite conservative in comparison with their true magnitude, because many cases go undiagnosed or unreported.

1.4 The changing pattern of fungal infection

Over the past few decades, major advances in health care have led to an unwelcome increase in the number of life-threatening infections due to true pathogenic and opportunistic fungi. These infections are being seen in ever increasing numbers, largely because of the increasing size of the population at risk. This population includes persons with HIV infection, transplant recipients, cancer patients and other individuals receiving

immunosuppressive treatment. Among patients undergoing transplants or treatment for malignancies, novel and more intensive regimens have resulted in more profound levels of immunosuppression that are sustained for longer periods. Likewise, the increasing use of invasive monitoring and aggressive therapeutic technologies in intensive care units has resulted in improved survival of individuals with life-threatening illnesses, but has also contributed to an increase in the number of persons at risk for fungal infections. Other developments in medical practice that have led to significant changes in the incidence of invasive fungal diseases among the different groups of at-risk patients include the increasing use of triazole antifungal agents for treatment and chemoprophylaxis, and the widespread use of amphotericin B for empirical treatment of suspected fungal infection.

In addition to the rise in prevalence of opportunistic fungal infections due to such well-recognized organisms as *Aspergillus fumigatus*, *C. albicans* and *Pneumocystis jirovecii*, an ever increasing number of fungi, hitherto regarded as harmless saprophytes, are being reported as the cause of serious or lethal infection in immunocompromised individuals. For instance, *Fusarium* species, long recognized as a cause of nail and corneal disease, are now well documented as the aetiological agents of lethal invasive infections in neutropenic cancer patients and HSCT recipients. The emergence of these organisms as significant pathogens has important implications for diagnosis and management, not only because the clinical presentation can mimic a more common disease, aspergillosis, but also because the organisms are usually resistant to amphotericin B, the drug of choice for empirical treatment of suspected fungal infections in febrile neutropenic patients.

There has also been a marked increase in the incidence of several of the fungal diseases that are endemic in North America, in particular histoplasmosis and coccidioidomycosis. Urban development and changing land use in the endemic regions have contributed to this trend, as has the seasonal migration of previously unexposed populations from non-endemic regions to the desert South West. Many of these migrants are older, have underlying chronic illness and debilitation, and consequently are at greater risk of developing the more serious forms of coccidioidomycosis. In addition, there is evidence that the increase in reported cases of this disease may be linked to changing climatic conditions.

Increased international travel has also led to a rise in the number of reported outbreaks and sporadic cases of histoplasmosis and coccidioidomycosis among individuals who normally reside in places far distant from the regions where these diseases are endemic. The largest number

of travel-related mycoses has been reported from US residents, many of whom have acquired an infection while visiting an endemic region within North or Central America or, less commonly, in South America, Africa or Asia. Travel-related fungal infections have also been reported among international visitors to North America, or to countries in Latin America, Africa and Asia. Most of these infections have occurred among persons returning to European countries, Australia or Japan. However, with increasing numbers of visitors and immigrants to the United States from Asia, travel- and migration-related infections are now being reported from regions such as the Indian sub-continent.

In many respects, the current pattern of invasive fungal disease in developing countries is quite different from that seen in developed countries. In the industrialized world, opportunistic fungal infections predominantly occur in the context of aggressive immunosuppressive therapies. Throughout the developed world, the widespread use of combination antiretroviral treatment regimens has led to a marked reduction in the rates of AIDS-associated opportunistic infections. In contrast, in many resource-poor countries in sub-Saharan Africa and parts of Asia, the burden of fungal diseases among those with HIV infection is large and increasing. According to recent estimates, around 958,000 cases of cryptococcal meningitis occur worldwide in persons with HIV infection each year. The region with the greatest number of cases is sub-Saharan Africa, with 720,000 cases, followed by South and South East Asia with 120,000 cases. The disease is one of the leading causes of infection-related mortality in sub-Saharan Africa, with around 500,000 deaths each year. Moreover, cryptococcosis is estimated to cause more deaths in this region than diseases such as tuberculosis, which are more common in the population.

1.5 New directions in diagnosis

Among the many challenges in dealing with opportunistic invasive fungal diseases, none is more critical than early diagnosis of these infections. This is essential to reduce the high case fatality rates of these diseases in immunocompromised individuals. There has been some progress in the field of diagnosis in recent years, but this has largely been due to the increased use of computed tomographic scanning and other imaging procedures. Laboratory methods for the diagnosis of fungal infections continue to be updated, but still depend, for the most part, on isolation of the fungus in culture, on its microscopic detection in clinical material and on the detection of a serological response to the pathogen (see Chapter 2).

Nevertheless, the search for more rapid, sensitive and specific non-culture-based tests is continuing.

New approaches to the diagnosis of invasive fungal infections include the detection of fungal cell wall components or metabolites and the detection of fungal DNA in clinical specimens. However, despite much recent progress, the goal of developing simple, rapid and cost-effective clinical tests for the diagnosis of invasive fungal infections remains elusive. New diagnostic procedures based on the detection of fungal DNA are presently being developed, but have not yet had a significant impact in most clinical laboratories, largely because they have not been standardized and validated. Only a few of these methods are commercially available.

1.6 New directions in treatment and prevention

The rising prevalence of invasive fungal infections has brought about an increased use of existing antifungal agents and has stimulated research for new ones. The last two decades have seen the introduction of an important new class of antifungal agents (the echinocandins), the expansion of an established class of agents (the azoles) and the development of novel methods for delivering established agents (lipid-based formulations of amphotericin B). The new drugs that have been introduced have changed the standards of care for the treatment of many invasive fungal infections, particularly aspergillosis and candidosis, but some problems remain (see Chapter 3). There are now few life-threatening infections for which there is no effective treatment, and there are many for which there are several reliable therapeutic options. On the other hand, triazole and echinocandin resistance, an uncommon clinical problem at present, is of concern and requires more rapid approaches to detection and continued surveillance.

Although opportunistic fungal infections in persons with AIDS are no longer a major problem in developed countries, the burden of these diseases is continuing to increase in many developing countries with large HIV epidemics. Throughout the developed world, the use of combination antiretroviral treatment has proved to be the most effective method of preventing all opportunistic infections in persons with AIDS. Because these drugs are seldom available in resource-limited countries, other measures are needed to prevent diseases such as cryptococcosis and penicilliosis. In this respect, antifungal chemoprophylaxis is currently the most promising of the potential prevention strategies.

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CHAPTER 2

Laboratory diagnosis of fungal infection

2.1 Introduction

As with other microbial infections, the diagnosis of fungal infections depends upon a combination of clinical observation and laboratory investigation. Superficial fungal infections often produce characteristic lesions that suggest a fungal diagnosis, but it is not unusual to find that the appearance of lesions has been modified and rendered atypical by previous treatment. In most situations where invasive fungal infection is entertained as a diagnosis, the clinical presentation is non-specific and can be caused by a wide range of infections, underlying illness or complications of treatment. Cultures may be negative, particularly in the early stages of infection. Nor can radiological or other diagnostic imaging methods be relied upon to distinguish fungal infection from other causes of disease.

Laboratory tests can help in establishing or confirming the diagnosis of a fungal infection, in providing objective assessments of response to treatment and in monitoring resolution of the infection. The successful laboratory diagnosis of fungal infection depends in major part on the collection of appropriate clinical specimens for investigation. It is also dependent on the selection of appropriate transport conditions and microbiological test procedures. These differ from disease to disease and depend on the site of infection as well as the presenting symptoms and clinical signs. Interpretation of the results can sometimes be made with confidence, but at times the findings can be unhelpful or even misleading. It is in these situations

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that close liaison between the clinician and the laboratory is particularly important.

In neutropenic patients and transplant recipients, invasive fungal infection often presents as persistent fever that fails to respond to broad-spectrum antibacterial treatment. The successful management of these patients often depends on the prompt initiation of empirical antifungal treatment without waiting for formal confirmation of the diagnosis. It is essential that these high-risk individuals be subjected to frequent microbiological surveillance for fungal infection.

2.2 Collection of specimens

To establish or confirm the diagnosis of suspected fungal infection, it is essential for the clinician to provide the laboratory with adequate specimens for investigation. Inappropriate collection, storage or processing of specimens can result in a missed diagnosis. Moreover, to ensure that the most appropriate laboratory tests are performed, it is essential for the clinician to indicate that a fungal infection is suspected and to provide sufficient background information.

Many of the specimen collection and transport guidelines for mycology are similar to those for bacteriology. On those occasions where they differ, it is important to communicate that information to clinicians. One such difference is sample volume: the amount of material required for fungal culture is often greater than that needed for bacterial culture. This is because several types of specimens, including body fluids or respiratory secretions, need to be concentrated or pre-treated prior to plating to maximize recovery of fungi.

In addition to specifying the source of the specimen and its time of collection, it is important to provide information on any underlying illness, recent travel or previous residence abroad, any animal contacts and the patient's occupation if considered relevant. This information will help the laboratory to anticipate which fungal pathogens are most liable to be involved and permit the selection of the most appropriate test procedures. In addition, the laboratory *must* be informed if there are particular risks associated with the handling of the specimen; for instance, if the patient has hepatitis or human immunodeficiency virus (HIV) infection.

With the exception of skin, hair and nails, specimens for mycological examination should be collected and transported to the laboratory in leak-proof sterile containers appropriate to the type of material being investigated. All specimen containers should be clearly labelled.

2.2.1 Skin, nails and hair

Skin, nails and hair should be collected into folded squares of black paper (about 10 cm × 10 cm). The use of paper permits the specimen to dry out, which helps to reduce bacterial contamination and also provides a convenient means of storing specimens for long periods (12 months or longer). Several commercial transport package systems are available (MycoTrans, Biggar, Lanarkshire, UK; Dermaco, Toddington, Bedfordshire, UK). Superficial lesions should be cleaned with 70% alcohol prior to sampling, as this will improve the chances of detecting fungus on microscopic examination, as well as reducing the likelihood of bacterial contamination of cultures. Prior cleaning is essential if ointments, creams or powders have been applied to the lesion.

Material should be collected from cutaneous lesions by scraping outwards from the margin of the lesion with a blunt scalpel. If there is minimal scaling, it is helpful to use clear adhesive tape, or adhesive skin sampling discs, to remove material for examination. The Sellotape strip or disc should be pressed against the lesion, peeled off and placed (adhesive side down) on a clean glass microscope slide for transportation to the laboratory.

It is often helpful to use a Wood's light to select infected scalp hairs for laboratory investigation. If none of the hairs give the green fluorescence, which is a feature of some forms of dermatophyte scalp infection, a search should be made for lustreless hairs or stumps, and for hairs broken off at follicle mouths. Hairs should be plucked from the scalp with forceps. Cut hairs without roots are unsuitable for mycological investigation because the infection is usually confined near or below the surface of the scalp.

Another method that is useful for collection of adequate material from patients with inconspicuous scalp lesions is to brush the scalp with a plastic massage pad, which is then pressed into the surface of an agar plate. The pad should be sterilized in 1% chlorhexidine for 1 hour and rinsed in sterile water before being reused.

Nail specimens should be taken from any discoloured, dystrophic or brittle parts of the nail. Specimens should be cut as far back as possible from the edge of the nail and should include the full thickness of the nail because some fungi are confined to the lower parts. If the nail is thickened, scrapings can also be taken from beneath it.

2.2.2 Mucous membranes

Although scrapings from oral lesions are better than swabs for diagnosis of oral infections, the latter are more frequently used, mainly because they are more convenient for transporting material to the laboratory. Swabs

should be either moistened with sterile water or saline prior to taking the sample or sent to the laboratory in transport medium.

For vaginal infections, swabs should be taken from discharge in the vagina and from the lateral vaginal wall. Swabs should be sent to the laboratory in a transport medium.

2.2.3 Ear

Scrapings of material from the ear canal are to be preferred, although swabs can also be used.

2.2.4 Eye

Material from a corneal ulcer with a suspected fungal cause should be collected by scraping the ulcer with a sterile platinum spatula. The entire base of the ulcer as well as the edges should be sampled. Because the amount of material that can be obtained will be small, it is best transferred to an agar plate for culture and to a glass slide for microscopic examination at the bedside. The plate should be marked to indicate the point of inoculation before being sent to the laboratory. Swabs are not suitable for sampling corneal lesions.

In patients with suspected fungal endophthalmitis, vitreous humour should be collected whenever possible. Vitreous humour specimens that have been diluted by the irrigating solution should be concentrated by centrifugation before the sediment is examined in the laboratory.

2.2.5 Blood

Blood culture should be performed in all cases of suspected deep fungal infection. Isolation of fungi from blood depends on a number of factors, including the volume of blood sampled, the blood-to-broth ratio, the number of samples collected and the method of processing (see Section 2.6.5). The volume of blood is the most critical factor: in adults, 20–30 mL per culture, divided between two bottles, is recommended for highest recovery and the shortest time to detection. In infants and children, total blood volumes based on weight are recommended. Culture of arterial blood should be considered if venous blood cultures are unsuccessful in a patient with suspected deep mycosis.

2.2.6 Cerebrospinal fluid

Cerebrospinal fluid (CSF) specimens of 3–5 mL are ideal, but are often smaller than this. Samples can be centrifuged and the supernatant fluid used for serological tests. The sediment can be cultured, but it is also useful for microscopic examination.

2.2.7 Urine

In non-catheterized patients, fresh mid-stream specimens of urine are adequate for mycological investigation, provided care is taken to ensure that vaginal or perineal infection does not lead to contamination. In infants, suprapubic aspiration is the best method of urine collection. Urine samples should be centrifuged and the sediment processed for microscopic examination and culture. Quantification of organisms as performed in bacteriology is not useful. The supernatant can be tested for fungal antigens.

Patients with blastomycosis or cryptococcosis may have prostatic infection, and it is therefore important to collect urine specimens following prostatic massage. The specimen should be centrifuged and the sediment cultured. Other disseminated infections that can be diagnosed on the basis of a positive urine culture include coccidioidomycosis and histoplasmosis.

2.2.8 Other fluids

Chest, abdominal and joint fluids, whether aspirated or drained, should be collected into sterile containers that include a small amount of sterile heparin (diluted 1:1000) to prevent blood clotting. The specimens should be centrifuged and the sediment cultured. Drain fluid from patients on continuous peritoneal dialysis should be collected in a sterile container without heparin.

2.2.9 Lower respiratory tract specimens

Fresh, early morning samples of sputum are ideal. These should be collected in sterile containers and processed within 2 hours of collection. If delay in processing is unavoidable, specimens must be stored at 4°C. If the patient does not have a productive cough, a sputum sample may be induced by introducing nebulized saline into the bronchial tree. It is recommended that at least three samples of sputum be submitted for microscopic examination and culture whenever a fungal infection is suspected: 24-hour collections of sputum are not suitable for mycological investigation. Viscous lower respiratory tract specimens should be pre-treated before processing. Lysis with mucolytic agents, such as *N*-acetyl-L-cysteine or dithiothreitol, followed by centrifugation and plating of the sediment improves the recovery of many fungi.

In immunocompromised patients, the most useful procedures for collection of lower respiratory tract specimens are bronchoalveolar lavage (BAL) or a bronchial wash. These procedures are carried out with a fibre-optic bronchoscope and provide good material for microscopic

examination and culture. Specimens should be centrifuged and the sediment examined.

Percutaneous needle biopsies are useful in patients with focal lung disease, in particular those with peripheral lesions that are not accessible to a bronchoscope. Large needles are better than fine needles, and the procedure should be carried out under radiological guidance. Specimens should be processed for microscopic examination and culture.

2.2.10 Pus

If possible, swabs should not be used to collect material from draining abscesses or ulcers. If a swab must be used, then material should be taken from as deep as possible within the lesion. Pus from undrained subcutaneous abscesses or sinus tracts should be aspirated with a sterile needle and syringe. If grains are visible in the pus (as in mycetoma), these should be collected. In mycetoma, if the crusts at the opening of sinus tracts are lifted, grains can often be found in the pus underneath.

2.2.11 Bone marrow

These specimens are useful for making the diagnosis in a number of deep fungal infections, including histoplasmosis, cryptococcosis, paracoccidioidomycosis and penicilliosis. About 0.5 mL (children) to 3 mL (adults) of aspirated material should be collected into a sterile container that includes a small amount of sterile heparin (diluted 1:1000). Because lysis-centrifugation enhances the recovery of *Histoplasma capsulatum* and other moulds, Isolator tubes (Wampole Laboratories, Cranbury, New Jersey, USA) should be the method of choice for these organisms.

2.2.12 Tissue

Tissue specimens should be placed in sterile saline, *not* in formalin. If possible, material should be obtained from both the middle and the edge of lesions. Total excision of small cutaneous, subcutaneous or mucosal lesions is often possible. With the exception of *H. capsulatum*, fungi present in tissue are best recovered when the specimen is minced and not ground.

2.2.13 Medical devices

A wide range of medical devices, including contact lenses, replacement joints, stents and surgical implants, may be submitted for fungal culture. Most should be submitted in a sterile container and transported and stored at room temperature.

2.3 Specimens for serological tests

Serological tests for dimorphic fungal pathogens are much more helpful if paired or sequential specimens are collected. Blood, CSF, urine and other biological fluids for serological testing should be collected into glass or plastic tubes without anticoagulants; 5–10 mL is usually sufficient.

2.4 Specimens for antifungal drug level determinations

The concentrations of antifungal drugs are measured for two principal reasons: to ensure that adequate drug concentrations are attained and to ensure that concentrations that could cause unpleasant or even harmful side effects are avoided.

Blood and other biological fluids should be collected into glass or plastic tubes without anticoagulants; 5–10 mL is usually sufficient. Care should be taken to ensure that specimens are taken at the most appropriate times: samples should be collected just before a dose is due and/or around the expected time of peak blood concentrations (see Chapter 3).

2.5 Transport of specimens

Apart from specimens of skin, nails and hair, which can often be stored for weeks or even months before processing, specimens for mycological investigation must be processed as soon as possible after collection. Delay may result in the death of fastidious organisms, overgrowth of bacterial contaminants, and/or multiplication in the number of organisms present. If specimens cannot be delivered to the laboratory within 2 hours, they should be stored at 4°C.

Specimens mailed to laboratories must be packaged and labelled according to the guidelines laid down for the transport of biological material by the relevant postal authorities. Metal canisters are now recommended for packaging of certain hazardous materials, such as specimens from HIV-infected persons. Plastic petri dishes are unsuitable for sending through the mail. The specimen container or culture should be sealed within a plastic bag before packaging so that any breakage and subsequent spillage is contained. The sender's name should be clearly marked on the outside of the package so that they may be contacted for instructions should a problem arise.

2.6 Interpretation of laboratory test results

Interpretation of the results of laboratory tests can sometimes be made with confidence, but at times the findings may be unhelpful or even

misleading. The investigations available include direct microscopic examination, histopathology, culture and serological tests. The choice of appropriate tests differs from one disease to another and depends on the site of infection as well as the presenting symptoms and clinical signs. It must always be appreciated that every laboratory test has its limitations and that negative results can be obtained, which may lead to unjustified exclusion of a mycological diagnosis.

2.6.1 Direct microscopic examination

The direct microscopic examination of clinical material is one of the simpler and most helpful procedures for the laboratory diagnosis of fungal infection. Various methods can be used: unstained wet-mount preparations may be examined by light-field, dark-field or phase-contrast illumination, or dried smears can be stained and examined. Chemical brighteners, such as Calcofluor white (Sigma-Aldrich, St. Louis, Missouri, USA), a compound that stains the fungal cell wall, can be helpful in revealing fungal elements in wet mounts of sputum, skin and other clinical materials when examined under a fluorescence microscope. Most fungi can be visualized with the routine stains used for cytological preparations, including Giemsa, Papanicolaou and Wright's stains.

Direct microscopic examination is most useful in the diagnosis of superficial and subcutaneous fungal infections. Recognition of fungal elements in skin scrapings, hair or nail specimens can provide a reliable indication of the mycosis involved, whether it be dermatophytosis, candidosis or pityriasis versicolor. In certain situations, direct microscopic examination of fluids or other clinical material can establish the diagnosis of a deep mycosis. Instances include the detection of encapsulated cells of *Cryptococcus* species in CSF or *H. capsulatum* cells in peripheral blood smears. More often, however, only a tentative diagnosis of deep fungal infection can be made on the basis of microscopic examination. Nevertheless, microscopic examination can help to determine whether an organism recovered later in culture is a contaminant or a pathogen and to assist the laboratory in selecting the most appropriate culture conditions to recover organisms visualized on direct smear.

2.6.2 Histopathological examination

Histopathological examination of tissue sections is one of the most reliable procedures for the diagnosis of subcutaneous and deep-seated fungal infections. However, as with culture, it may be difficult to obtain a tissue biopsy specimen from a deep site, such as the lung, in a critically ill patient. The ease with which a fungal pathogen can be recognized in tissue is dependent not only on its abundance but also on the distinctiveness of

its appearance. Histopathology can define the diagnostic significance of a positive fungal culture. It can also provide a rapid presumptive diagnosis while the results of fungal culture are awaited, and it may be the only means of establishing a diagnosis if cultures are not performed.

Histopathological examination of biopsies, surgical resection specimens and autopsy specimens should always begin with hematoxylin and eosin staining. However, many fungi stain poorly and this method alone may be insufficient to reveal fungal elements in tissue. There are a number of special stains for detecting and highlighting fungi, and the clinician should request these if a fungal infection is suspected. Methenamine-silver (Grocott or Gomori) and periodic acid-Schiff staining are among the most widely used procedures for specific staining of the fungal cell wall. In addition, mucicarmine and Fontana-Masson stains (for mucin and melanin, respectively) are useful for identification of *Cryptococcus* species and dematiaceous moulds that may not produce abundant melanin.

It should be appreciated that these staining methods, although useful in revealing the presence of fungal elements in tissue, seldom permit the precise fungal genus involved to be identified. There are few instances where morphological characteristics are specific. For example, the detection of fine, non-pigmented (hyaline), septate hyphae with acute-angle branching is consistent with *Aspergillus* infection, but it is also characteristic of a large number of less-common organisms, including species of *Fusarium*, *Paecilomyces* and *Scedosporium*. The Mucorales, on the other hand, have wide, irregular hyaline hyphae with few or no septa and open-angle branching. Likewise, the detection of yeasts seldom permits a specific diagnosis. Small, narrow-based, budding yeasts are consistent with *H. capsulatum*. However, small variants of *Blastomyces dermatitidis*, *Candida glabrata*, capsule-deficient *Cryptococcus* species, endospores of *Coccidioides* species, *Penicillium marneffei* and *Pneumocystis jirovecii* may appear similar. Broad-based, budding yeasts are consistent with *B. dermatitidis*, but endospores of *Coccidioides* species and conidia of *Aspergillus* species may appear similar.

In order to avoid misinterpretation, pathologists should describe the fungal elements observed in the tissue sample and refrain from trying to offer a specific diagnosis. The report should describe the morphological characteristics of the fungus and should state whether or not there is invasion of tissues or vessels, the amount of fungal elements observed and the host reaction to the infection (inflammation, necrosis, haemorrhage). The comment section of the report should name the fungi most frequently associated with the observed morphology, as well as other organisms that need to be included in the differential diagnosis.

2.6.3 Immunohistochemistry

To overcome the limitations of conventional histochemical stains, a number of immunohistochemical methods have been developed for the detection and identification of fungal targets (antigens) in tissue. Immunoperoxidase and immunofluorescent staining reagents, both monoclonal and polyclonal, have been developed and tested for some fungi; however, there are few commercially available reagents. These include reagents for *Aspergillus* species and the Mucorales (AbD Serotec, MorphoSys US Inc., Raleigh, North Carolina, USA). Immunohistochemical staining of tissue sections can facilitate the identification of atypical fungal elements and the detection of small numbers of organisms. It can also assist with the diagnosis of mixed infections. However, careful evaluation and validation of reagents and tests must be performed before results can be used for patient care purposes.

2.6.4 In situ hybridization

In situ hybridization involves the use of DNA probes to detect the presence of specific nucleic acids of the fungal agent. A tissue section is prepared and the hybridization is performed directly on the slide. After hybridization, unbound probe is removed and the probe is detected in various ways. Most frequently, the probes used to detect fungi in situ have been genus specific, have been targeted against organism-specific ribosomal DNA (18S, 28S or 5.8S) and have been labelled with digoxigenin. In most published reports, in situ hybridization has been performed in cases where fungal elements have first been detected with routine histochemical stains, and the hybridization assay has been used to confirm the identity of the aetiological agent. Probes for in situ hybridization assays in tissues are not commercially available at this time.

2.6.5 Culture

Although culture often provides the definitive diagnosis of a fungal infection, it also has some limitations. Chief amongst these is failure to recover the organism. This may be due to inadequate specimen collection or delayed transport of specimens. Incorrect isolation procedures or inadequate periods of incubation are other important factors. It is essential for the clinician to inform the laboratory if a particular fungal infection is suspected and provide sufficient information to permit the most appropriate culture procedures to be followed. The isolation and identification of moulds and yeasts can take several weeks. In such unavoidable instances, the result may become available too late either to help with the diagnosis

or with the choice of treatment. Nevertheless, culture should always be attempted so that a definitive diagnosis can be obtained.

Isolation in culture will permit most pathogenic fungi to be identified. Most of these organisms are not fastidious in their nutritional requirements and will grow on the media used for bacterial isolation from clinical material. However, growth on these media can be slow, and development of the spores and other structures used in fungal identification can be poor. For these reasons, most laboratories use several different culture media and incubation conditions for recovery of fungal agents. Most laboratories use a medium, such as glucose peptone (Sabouraud's dextrose) agar or malt agar, that will recover most common fungi. However, certain fastidious organisms such as yeast-phase *H. capsulatum* will not grow on these substrates and require the use of richer media, such as brain–heart infusion agar. A variety of chromogenic agars that incorporate multiple chemical dyes in a solid medium are available commercially for the detection and preliminary identification of *Candida* species. Chromogenic media can be helpful in detecting the presence of mixed cultures. The laboratory should be made aware of the particular fungal agent(s) that are suspected in a given sample so that the most appropriate media can be included.

Many clinical specimens submitted for fungal culture are contaminated with bacteria, and it is essential to add antibacterial antibiotics to fungal culture media. Media containing chloramphenicol are commercially available. However, gentamicin, vancomycin and other antimicrobial agents are increasingly being used to suppress the growth of bacteria resistant to older agents. If dermatophytes or dimorphic fungi are being isolated, cycloheximide (actidione) should be added to the medium to prevent overgrowth by faster growing fungi.

The optimum growth temperature for most pathogenic fungi is around 30°C. Material from patients with a suspected superficial infection should be incubated at 25–30°C, because most dermatophytes will not grow at higher temperatures. Material from subcutaneous or deep sites should be incubated at two temperatures: 25–30°C and 37°C. This is because a number of important pathogens, including *H. capsulatum*, *B. dermatitidis* and *Sporothrix schenckii*, are thermally dimorphic and the change in their growth form, depending on the incubation conditions, is useful in identification. At 25–30°C, these organisms develop as moulds on glucose peptone agar, but at higher temperatures on an enriched medium, such as brain–heart infusion agar, these organisms grow as budding yeasts. Some pathogenic fungi grow slowly in culture and require plates to be held for up to 2 weeks, and in some case up to 4 weeks, before being discarded as

negative. However, many common pathogenic fungi, such as *Aspergillus fumigatus* and *Candida albicans*, will produce identifiable colonies within 1–3 days. Cultures should be examined at frequent intervals (at least three times weekly) and appropriate sub-cultures made, particularly from blood-enriched media on which fungi often fail to sporulate.

A wide range of manual, biphasic, automated and continuously monitored systems for blood culture are now available. However, no single commercial system or culture medium can detect all potential blood pathogens. If a manual system is employed, isolation rates are higher when the medium is vented and aerated, and biphasic media, incorporating both agar and broth phases, are used. Many automated and continuously monitored systems are available, and several have special culture media to enhance fungal growth. Even without specific fungal media, these systems are able to recover *Candida*, *Cryptococcus* and *Trichosporon* species as efficiently as manual or lysis-centrifugation methods. However, unless specialized media are used, clinicians should not expect automated systems to detect moulds.

The lysis-centrifugation method (Isolator, Wampole Laboratories) is currently the most sensitive procedure for isolation of moulds and dimorphic fungi, such as *H. capsulatum*, from blood, but it is more expensive and labour-intensive than other methods. With this procedure, 10 mL of blood is collected into a tube containing chemicals that lyse red blood cells and inactivate antimicrobial substances in blood. The tube is centrifuged and the sediment is then inoculated onto appropriate culture media. Modern, automated, continuously monitored systems have largely replaced the lysis-centrifugation method for the detection of *Candida* species.

It is important to recognize that growth of an organism in culture does not necessarily establish its role as a pathogen. Only if the organism is identified as an unequivocal pathogen, such as *Trichophyton rubrum* or *H. capsulatum*, can the diagnosis be firmly established. If, however, an opportunistic organism such as *A. fumigatus* or *C. albicans* is recovered, its isolation may have no clinical relevance unless there is additional evidence of its involvement in a pathogenic process. In this situation, culture results should be compared with those of microscopic examination. Isolation of opportunistic fungal pathogens from sterile sites, such as blood or CSF, often provides reliable evidence of significant infection, but their isolation from material such as pus, sputum or urine must be interpreted with caution. Attention should be given to the amount of fungus isolated and further investigations undertaken.

Many unfamiliar moulds have been reported as occasional causes of lethal systemic infection in immunocompromised patients. No isolate

should be dismissed as a contaminant without careful consideration of the clinical condition of the patient, the site of isolation, the method of specimen collection, the amount of organisms recovered and the likelihood of contamination. However, it is notable that, in one report, only 24% of unusual moulds isolated from sterile body sites were shown to be responsible for significant clinical disease.

2.6.6 Fungal identification

Most fungi can be identified after growth in culture. Classical phenotypic identification methods for moulds are largely based on a combination of macroscopic and microscopic morphologic characteristics, as well as biochemical and physiological tests. Useful macroscopic characteristics include colonial form, surface colour and pigmentation. Because identification is usually dependent on visualization of the spore-bearing structures, identification is usually dependent on the ability of the organism to sporulate. Moulds that fail to sporulate in culture are often impossible to identify, and it is therefore important to select culture conditions that favour sporulation.

Historically, dimorphic fungi, such as *H. capsulatum* and *B. dermatitidis,* were identified by observing the conversion of mycelial growth at 25°C to yeast-like growth at 37°C. However, development of DNA probe-based tests (Accuprobe; GenProbe Inc., San Diego, California, USA) has enabled these pathogens to be rapidly identified using only a small amount of mycelial material.

Yeasts are usually identified on the basis of their morphological and biochemical characteristics. Useful morphologic characteristics include the colour of the colonies, the size and shape of the cells, the presence of a capsule around the cells, the production of hyphae or pseudohyphae and the production of chlamydospores. Useful biochemical tests include the assimilation and fermentation of sugars, and the assimilation of nitrate and urea. Most yeasts associated with human infections can be identified using one of the numerous commercial identification systems that are based on the differential assimilation of various carbon compounds. A number of simple rapid tests have been devised for the presumptive identification of some of the most important human pathogens. Foremost among these are the serum germ tube test for *C. albicans*, which can be performed in less than 3 hours, the urease test for *Cryptococcus* species and the rapid trehalose test for *C. glabrata*. Several rapid commercial test panels are also available, most of which are more accurate in the identification of the common, rather than unusual, yeast pathogens.

2.6.7 Molecular identification

Many clinical laboratories today employ DNA sequencing as part of their routine protocol for fungal identification. Molecular identification is useful when an isolate fails to sporulate in culture, displays atypical morphological characteristics, or requires prolonged incubation or incubation on specialized media in order to sporulate, or if the phenotypic results are non-specific or confusing. The US Clinical and Laboratory Standards Institute has recently issued guidelines establishing interpretive criteria for identifying fungi using DNA sequencing. With the advent of molecular identification, it has become apparent that some of the most frequently isolated species are groups or complexes of species and that there are many cryptic species.

Several regions within the fungal genome have been evaluated as potential targets for species identification, but the most widely used has been the ribosomal DNA (rDNA) gene complex. The 18S, 5.8S and 28S regions of this complex are separated by the more variable internal transcribed spacer (ITS) 1 and 2 regions. The ITS1 and 2 regions, together with the D1/D2 domains of the 28S ribosomal subunit, have been shown to contain sufficient sequence heterogeneity to provide differences at the species level. Ribosomal genes exist in 50–500 copies per cell and so are detected with better sensitivity than a single-copy gene. In some cases, ITS sequencing does not provide sufficient resolution, so that single-copy targets such as β-tubulin, elongation factor 1-α, calmodulin or other loci are used for identification.

Most methods of species identification have exploited the enormous resolving power of the polymerase chain reaction (PCR). With PCR, as little as a few picograms of input DNA can be amplified so that after 30–40 cycles, the resulting product can be visualized on an agarose gel or detected using chemiluminescence, spectrophotometry or a laser instrument.

The reliability of the molecular identification is, of course, dependent upon the reliability of the database with which comparisons are made. Identification of unknown fungal isolates can be made by comparing a partial DNA sequence of that organism with sequences in a central database such as GenBank, operated by the US National Library of Medicine, or the CBS database operated by the Centraalbureau voor Schimmelcultures in the Netherlands. GenBank contains thousands of DNA sequences from medically important as well as saprophytic fungi. Any investigator can submit a new DNA sequence to GenBank for no cost, thus continually expanding the number of sequences in the database. The main disadvantage of the GenBank database is the lack of

curatorial control. Incorrect entries are not challenged, and phylogenetic changes in genus/species names are not always made. The more restricted CBS databases contain sequences from isolates whose identification has been well validated phenotypically and taxonomically. The MicroSeq D2 LSU fungal sequencing system (Applied Biosystems, Warrington, UK) is a commercially available fungal identification system that provides reagents for sequencing the D2 region of the large ribosomal subunit, identification and analysis software, and a library of fungal sequences.

2.6.8 Serological tests

Serological testing often provides the most rapid means of diagnosing a fungal infection. The majority of tests are based on the detection of antibodies to specific fungal pathogens, although tests for fungal antigens are now becoming more widely available. At their best, individual serological tests can be diagnostic, e.g. tests for antigenaemia in cryptococcosis and histoplasmosis. In general, however, the results of serological testing are seldom more than suggestive or supportive of a fungal diagnosis. These tests must be interpreted with caution and considered alongside the results of other clinical and laboratory investigations.

Tests for antibodies have proved useful in diagnosing endemic fungal diseases, such as histoplasmosis and coccidioidomycosis, in immunocompetent persons. In these individuals, the interval between exposure and the development of symptoms (2–6 weeks) is usually sufficient for a humoral response to develop. Tests for fungal antibodies in patients with endemic fungal diseases are most helpful when paired serum specimens (acute and convalescent) are obtained so that it can be determined whether titres are rising or falling. Tests for detection of *Aspergillus* antibodies are also important in the diagnosis of allergic bronchopulmonary aspergillosis and the different forms of chronic pulmonary aspergillosis (see Chapter 10). Tests for antibodies are much less useful in immunocompromised persons, many of whom are incapable of mounting a detectable humoral response to infection.

In this situation, serological tests for detecting fungal antigens can be helpful. Antigen detection is an established procedure for the diagnosis of aspergillosis, blastomycosis, candidosis, coccidioidomycosis, cryptococcosis and histoplasmosis, and similar tests are currently being evaluated for several other invasive fungal infections. Antigen detection has been performed on a wide range of clinical specimens, including serum, CSF, urine and BAL fluid. Antigen testing is included within the consensus definitions for diagnosing invasive fungal infections issued by the European Organization for Research and Treatment of Cancer (EORTC)

and the US National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) and may be considered part of the expected standard of care for haematology patients at risk of fungal infection.

Antigen detection methods are complicated by several important factors. First, antigen is often released in minute amounts from fungal cells, necessitating the use of highly sensitive test procedures to detect low amounts circulating in serum. Second, antigen is often cleared very rapidly from the circulation, necessitating frequent collection of samples. Third, antigen is often bound to circulating IgG, even in immunocompromised individuals, and therefore steps must be taken to dissociate these complexes before antigen can be detected.

Numerous tests are available for the detection of fungal antibodies. Immunodiffusion (ID) is a simple, specific and inexpensive method, but it is insensitive, and this reduces its usefulness as a screening test. It is, however, useful for diagnosis and monitoring of treatment in patients with allergic bronchopulmonary or chronic pulmonary aspergillosis. Complement fixation (CF) is more sensitive, but more difficult to perform and interpret than ID. However, CF remains an important test for several endemic fungal diseases, including histoplasmosis and coccidioidomycosis. Latex agglutination is a simple, but insensitive method that can be used for detection of antibodies or antigens. It has proved most useful for detection of the polysaccharide capsular antigens of *Cryptococcus* species that are released in large amounts in most patients with cryptococcosis. Commercially available enzyme-linked immunosorbent assay (ELISA) kits for the detection of galactomannan and mannan, respectively, are widely used for the detection of *Aspergillus* and *Candida* antigens and demonstrate good specificity but variable sensitivity.

2.6.9 β-D-glucan detection

(1-3)-β-D-glucan is a major cell wall polysaccharide component of many fungi, including *Aspergillus* species, *Candida* species and *P. jirovecii*. However, it is not found in *Cryptococcus* species or the Mucorales. β-D-glucan can be detected in serum during fungal infection by using a chromogenic variant of the limulus amoebocyte lysate assay. Several methods for β-D-glucan detection are commercially available, including the Fungitell (or Glucatell) Assay (Associates of Cape Cod, East Falmouth, Massachusetts, USA), the Fungi-Tec G-Test (Seikagaku Corporation, Tokyo, Japan) and the Wako β-Glucan Assay (Wako Pure Chemical Industries, Osaka, Japan). These assays differ in many respects, including the measurement method (colourimetric or turbidimetric), the horseshoe crab species used, the type of β-D-glucan used as standard, the pre-treatment method and the

cut-off value used to define a positive result. Performance comparisons of the different commercial methods have been limited and inconclusive.

In North America and Europe, the most frequently used method for β-D-glucan detection is the Fungitell assay. This has been approved by the US Food and Drug Administration (FDA) for the presumptive diagnosis of invasive fungal infections, including aspergillosis, candidosis, *Fusarium* infection and trichosporonosis. Levels of (1-3)-β-D-glucan have also been reported to be elevated in patients with *Pneumocystis* pneumonia, but the assay is not currently approved for this indication. β-D-glucan testing is now included in the EORTC-MSG consensus definitions for diagnosis of invasive fungal disease.

Although a positive β-D-glucan test result does not identify the aetiological agent, a recent meta-analysis has indicated that it can be used to discriminate between patients with an invasive infection, such as aspergillosis or candidosis, and patients without invasive fungal disease, thereby allowing the earlier initiation of antifungal treatment. Further work is needed to determine the optimum sampling interval for patients at risk, the optimum cut-off value and the influence of concurrent bacteraemia on test performance.

Despite its diagnostic potential, the β-D-glucan test has been associated with false-positive results among patients who have recently received albumin or immunoglobulin products or undergone haemodialysis with cellulose membranes and among those exposed to other sources of glucan, such as certain antibiotics (including amoxycillin-clavulanate, ertapenem, piperacillin-tazobactam and trimethoprim-sulfamethoxazole). As these treatments are common among patients receiving intensive care, the β-D-glucan test should be used with caution in this setting. The test also requires endotoxin-free dedicated glassware to reduce laboratory contamination.

2.7 Molecular diagnosis of fungal infection

DNA-based methods for the detection of pathogenic fungi in clinical samples have been extensively investigated and are now beginning to be marketed in some countries. Numerous methods have been developed and evaluated for the detection of fungal DNA sequences in blood (serum, plasma and whole blood), CSF, respiratory tract fluids, tissue, and other clinical samples, but as yet there are no agreed standards. Most of these molecular diagnostic tests are PCR based, to take advantage of the enormous increase in sensitivity offered by the manifold amplification of PCR targets as well as the specificity offered by appropriate primer and

probe design. Critical factors influencing fungal DNA detection in clinical samples are the correct processing of specimens, DNA extraction protocol, PCR design (molecular target selection and amplicon specification method) and quality control requirements. One of the most significant limitations of PCR-based diagnostic tests for fungal infection is the potential for reagent and specimen contamination from various sources, particularly the environment.

The success of any nucleic acid-based method for diagnosis of fungal infection is dependent on efficient fungal cell lysis and DNA purification. Fungal DNA has been isolated from a wide range of clinical specimens using both manual and automated extraction platforms, but there are wide variations in performance between different procedures. Fully automated, high-throughput, nucleic acid extraction systems are now commonplace in clinical laboratories and have reduced the risk of cross-contamination. However, fungal target cell lysis (when whole blood is used) and fungal disruption must be performed prior to automated steps.

Several regions within the fungal genome have been evaluated as potential targets for detection, but the most widely used target has been the multi-copy rDNA gene complex. This section of the genome includes the relatively conserved regions of the 18S, 5.8S and 28S genes and the more variable ITS regions. In general, universal primers are used to amplify the initial target and then species-specific probes are used to identify the organism. Numerous methods for detection of the presence of the amplified targets have been evaluated, including Southern blot hybridization, nested PCR and PCR-ELISA. More recent developments in molecular diagnostics have included the use of real-time PCR methods in which thermocycling is combined with fluorescence monitoring of the amplified product during its generation. With pan-fungal primers and species- or genus-specific hybridization probes, these techniques permit quantification of the amounts of fungal DNA that are present in clinical samples, thus allowing the microbial load to be measured. Real-time PCR can also be used to detect point mutations that result in drug resistance and through multiplexing or melt curve analysis differentiate between species or genera in a single reaction. These methods have improved turnaround times and reduced the risk of contamination by eliminating the need for post-amplification handling of samples.

Despite recent progress, the goal of developing rapid and cost-effective tests for the molecular diagnosis of acute life-threatening fungal infections remains elusive. Although numerous research laboratories now offer 'in-house' procedures for molecular detection of fungal infection from tissue specimens or from body fluids, the sensitivity, specificity and predictive

value of these tests have not always been thoroughly investigated. The lack of standardization and near absence of any evaluated commercial PCR assays means PCR-based methods are not currently included within the EORTC-MSG definitions.

To date, the LightCycler SeptiFast System (Roche Molecular Diagnostics, Pleasanton, California, USA) is the only commercial PCR platform for the detection of bacterial and fungal pathogens. This system employs multiplex real-time PCR to detect 25 different organisms, including *A. fumigatus*, *C. albicans*, *C. glabrata*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis*. In an initial evaluation, the SeptiFast System appeared more sensitive than blood culture for fungal pathogens. The results of ongoing studies are awaited with interest.

In recent years, great efforts have been made to improve the standardization and quality control of molecular methods for the diagnosis of invasive fungal disease. A multi-centre effort involving many laboratories worldwide is under way to standardize *Aspergillus* DNA detection using PCR-based assays by a Working Group entitled European *Aspergillus* PCR Initiative of the International Society for Human and Animal Mycology. With respect to nucleic acid detection methods for other invasive fungal diseases, there are fewer data to support their use. In general, the results are promising but larger scale multi-centre evaluations with standardized methods are needed to demonstrate the relevance of these assays.

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CHAPTER 3

Antifungal drugs

3.1 Introduction

In comparison with the number of antibacterial drugs available, there are far fewer antifungal compounds. There are four major families of compounds in widespread clinical use: the allylamines, the azoles, the echinocandins and the polyenes. In addition, there is a miscellaneous group of compounds, such as flucytosine and griseofulvin, which do not belong to one of the major families. Each of these classes of antifungal agent has advantages and limitations related to its spectrum of activity, pharmacokinetics, mode of administration, drug interactions and toxicity profile.

This chapter reviews the principal antifungals in current use for superficial, subcutaneous and deep-seated fungal infections. Agents that are currently in development will not be discussed.

3.2 Allylamines

The allylamines are a group of synthetic antifungal compounds effective in the topical and oral treatment of dermatophytosis. Two drugs, terbinafine and naftifine, are licenced for clinical use. Naftifine is only available as a topical preparation.

3.2.1 Mechanism of action

The allylamines inhibit the action of squalene epoxidase, a crucial enzyme in the formation of ergosterol, the principal sterol in the membrane of

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susceptible fungal cells. The consequent accumulation of squalene leads to membrane disruption and cell death.

3.3 Terbinafine

Terbinafine is a lipophilic drug that is available for oral and topical administration.

3.3.1 Spectrum of activity

Terbinafine is effective against several groups of pathogenic fungi, including dermatophytes (*Epidermophyton floccosum*, *Microsporum* and *Trichophyton* species) and many dematiaceous moulds. It has some activity against *Aspergillus* species, *Candida* species and some dimorphic fungi (*Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Penicillium marneffei* and *Sporothrix schenckii*).

3.3.2 Acquired resistance

Resistance has rarely been reported among dermatophytes even after prolonged exposure.

3.3.3 Pharmacokinetics

About 5% of a given dose of terbinafine is absorbed following topical application. The drug is well absorbed (70–80%) after oral administration, peak serum concentrations in the region of 1 mg/L being attained 2 hours after a single 250-mg dose. Levels increase in proportion to dosage up to at least 750 mg. Terbinafine is >99% bound to plasma proteins in humans. Administration of the drug with food does not affect absorption.

Terbinafine is rapidly and extensively distributed to body tissues. It appears in the stratum corneum within a few hours of oral administration being commenced, as a result of diffusion through the dermis and epidermis and secretion in sebum. Diffusion from the nail bed is the major factor in its rapid penetration of nails. Terbinafine has been found to persist in nail for long periods after cessation of treatment.

Terbinafine undergoes first-pass metabolism and is rapidly and extensively metabolized by at least seven hepatic cytochrome P-450 (CYP) enzymes. None of the 15 identified metabolites is active. About 80% of an administered dose is eliminated as metabolites in the urine and the remainder is excreted with the bile. The drug has an elimination half-life of 17 hours, but this is prolonged in patients with hepatic or renal impairment.

3.3.4 Pharmaceutics

Terbinafine hydrochloride is available as tablets and oral granules, and in a number of topical forms.

3.3.5 Therapeutic use

Topical terbinafine is effective against cutaneous dermatophytosis and candidosis, as well as pityriasis versicolor. Oral terbinafine is the drug of choice for dermatophytosis of the skin and nails where topical treatment is considered inappropriate or has failed. It is not as effective as itraconazole for fungal nail infections (onychomycosis) involving non-dermatophytes (see Chapter 7). Terbinafine is also licenced for the treatment of tinea capitis in children. Terbinafine has sometimes proven effective for chromoblastomycosis, phaeohyphomycosis, pythiosis and sporotrichosis, but it is not licenced for these indications. Oral terbinafine is ineffective against pityriasis versicolor.

3.3.6 Mode of administration

Topical terbinafine should be applied morning and/or evening for 2–4 weeks depending on the site of infection. Tinea pedis should be treated for 2 weeks, and tinea corporis and cruris for 2–4 weeks. Cutaneous candidosis and pityriasis versicolor should be treated for 2 weeks.

Terbinafine oral granules can be used to treat tinea capitis in children aged 4 years or older. The recommended dose is 250 mg/day for patients weighing more than 35 kg, 187.5 mg/day for those weighing 25–35 kg and 125 mg/day for those weighing less than 25 kg. The drug should be taken once daily with food, and treatment continued for 6 weeks.

The usual adult dose of oral terbinafine is 250 mg/day, but this should be halved in patients with impaired hepatic or renal function (creatinine clearance less than 50 mL/min or serum creatinine concentration >300 μmol/mL). The duration of treatment will depend upon the site and extent of dermatophyte infection. Tinea pedis should be treated for 2–6 weeks, and tinea corporis and cruris for 2–4 weeks. Fingernail infection can often be cured with treatment periods of 6 weeks, but some patients with toenail infection require treatment for 12 weeks or longer. Pulsed treatment (in which a week of treatment is alternated with 3 weeks without treatment) has given good results in nail infections. The regimen consists of 250 mg twice daily for one week each month for 3–4 months.

3.3.7 Drug interactions

Although terbinafine is metabolized by the human hepatic cytochrome P-450 system, it does not inhibit most CYP enzymes at clinically relevant concentrations. Terbinafine is a potent competitive inhibitor of CYP-2D6,

and its concomitant administration with drugs that are predominantly metabolized by this enzyme system should be carefully monitored. Blood concentrations of terbinafine are reduced following its concomitant administration with drugs, such as rifampicin, that induce the hepatic cytochrome P-450 system. Blood levels of terbinafine are increased if it is given with drugs, such as cimetidine, that inhibit the cytochrome P-450 system. Terbinafine increases the clearance of cyclosporin by 15%, but its clearance is unaffected by cyclosporin.

3.3.8 Toxicity and adverse reactions

Terbinafine produces few adverse reactions. The commonest side effects are diarrhoea, dyspepsia, nausea and abdominal pain. Skin rashes and pruritus have also been reported. Isolated cases of Steven-Johnson syndrome have occurred with oral terbinafine. Taste disturbance and taste loss have been reported, but these usually resolve within several weeks once the drug is stopped.

There have been rare cases of liver failure and death among persons taking terbinafine for nail infections. The drug is not recommended for patients with chronic or active liver disease.

3.4 Other allylamine compounds for topical administration

3.4.1 Naftifine

Naftifine is used for the treatment of the dermatophytoses, including tinea corporis, tinea cruris and tinea pedis. The dosage and duration of treatment varies according to the condition being treated.

3.5 Azoles

This large group of synthetic agents contains many compounds that are effective in the topical treatment of dermatophytosis and superficial forms of candidosis: a number of these drugs are available for systemic administration.

3.5.1 Mechanism of action

Azole compounds inhibit a fungal cytochrome P-450-dependent enzyme, lanosterol 14 α-demethylase (Erg11p or CYP51p depending on nomenclature), which is responsible for the conversion of lanosterol to ergosterol, the principal sterol in the membrane of susceptible fungal cells. This results in accumulation of 14 α-methyl sterols and depletion of ergosterol with subsequent disruption of cell membrane structure and function. The

activity is essentially fungistatic, but some of the newer triazoles exert fungicidal effects at therapeutic concentrations.

3.5.2 Acquired resistance

Molecular analysis of azole drug resistance has focused on *Candida albicans*, but studies have also been done on *Candida glabrata* and *Candida krusei*, as well as *Aspergillus fumigatus* and *Cryptococcus neoformans*. Several molecular mechanisms of resistance have been described, and the development of clinical resistance is seldom the result of a single alteration.

In *C. albicans*, resistance mechanisms include over-expression of a number of efflux pump genes, including the ATP-binding cassette (ABC) transporter genes, *CDR1* and *CDR2*, and the major facilitator gene, *MDR1*. Over-expression of these genes leads to reduced drug accumulation. ABC pumps associated with azole resistance have also been identified in other *Candida* species and *C. neoformans*. In *A. fumigatus*, over-expression of both ABC and major facilitator pumps has been associated with decreased intracellular levels of azole drugs, leading to resistance.

The target enzyme of the azole antifungals is lanosterol 14 α-demethylase, the product of the *ERG11* (or *cyp51A*) gene. Several alterations in this gene have been associated with resistance in *C. albicans*, including point mutations in the coding regions and over-expression of the gene. Point mutations in *ERG11* result in structural alteration of the enzyme and lead to reduced binding with azole antifungals. Point mutations in the *cyp51A* gene of *A. fumigatus* are responsible for most instances of resistance. Point mutations have also been reported in *C. neoformans*.

Increasing rates of triazole resistance have been found since 2004 in patients with invasive aspergillosis in the Netherlands and United Kingdom, with 20% of patients in Manchester in 2009 having triazole-resistant strains. Isolated resistance to itraconazole is commonly observed, but cross-resistance to posaconazole and/or voriconazole occurs in more than 50% of instances. Azole-resistant *A. fumigatus* strains have been isolated from patients who have received azole treatment, from individuals who have not been exposed and from the environment in several European countries.

3.5.3 Drug interactions

Most azole antifungal agents are extensively metabolized by the human hepatic cytochrome P-450 enzyme system and are potent inhibitors of CYP-3A4; some also inhibit CYP-2C9 and CYP-2C19. Administration of an azole with other drugs that are metabolized by these enzymes can result in increased concentrations of the azole, the interacting drug, or both (Table 3.1). When the azole is discontinued, the change in metabolism that

Table 3.1 Drug interactions involving triazole antifungals

Drug	*Decreased serum concentration of azole*	*Increased serum concentration of co-administered drug*
Fluconazole	Antibacterials (rifampicin)	Analgesics (alfentanil, fentanyl, ibuprofen)
		Antibacterials (rifabutin)
		Antidepressants (amitriptyline)
		Antiepileptics (phenytoin)
		Antihistamines (astemizole, terfenadine*)
		Antihypertensives (losartan)
		Antineoplastics (cyclophosphamide)
		Benzodiazepines (diazepam, midazolam, triazolam)
		Calcineurin inhibitors (cyclosporin, tacrolimus)
		Cisapride*
		Oral hypoglycaemics (glimepiride, glipizide, glyburide, tolbutamide)
		Protease inhibitors (nelfinavir)
		Proton pump inhibitors (omeprazole)
		Statins (fluvastatin)
		Theophylline
		Warfarin
Itraconazole	Antibacterials (isoniazid, rifabutin, rifampicin)	Analgesics (alfentanil, fentanyl, levacetylmethadol*)
	Antiepileptics (carbamazepine, phenobarbital, phenytoin)	Antiarrhythmics (digoxin, disopyramide, dofetilide*, quinidine*)
	NNRTI (nevirapine)	Antibacterials (rifabutin)
	Protease inhibitors (indinavir, ritonavir)	Antiepileptics (phenytoin)
		Antihistamines (astemizole*, terfenadine*)
		Antihypertensives (celiprolol)
		Antimalarials (halofantrine)
		Antineoplastics (busulfan, cyclophosphamide, docetaxel, vincristine)
		Antipsychotics (bromperidol, haloperidol, pimozide*, risperidone)
		Anxiolytics (buspirone)
		Benzodiazepines (diazepam, midazolam*, triazolam*)

(*Continued*)

Table 3.1 (*Continued*)

Drug	*Decreased serum concentration of azole*	*Increased serum concentration of co-administered drug*
		Cabergoline
		Calcineurin inhibitors (cyclosporin, sirolimus, tacrolimus)
		Calcium channel blockers (amlodipine, felodipine, nifedipine, nisoldipine*, verapamil)
		Cilostazol
		Cimetidine
		Cisapride*
		Corticosteroids (budesonide, dexamethazone, fluticasone, methylprednisolone)
		Eletriptan
		Ergot alkaloids*
		Loperamide
		NNRTI (nevirapine)
		Oral hypoglycaemics (glipizide, glyburide, repaglinide, tolbutamide)
		Protease inhibitors (indinavir, ritonavir, saquinavir)
		Ropivacaine
		Statins (atorvastatin, lovastin*, simvastatin*)
		Trimetrexate
		Warfarin
Posaconazole	Antibacterials (rifampicin, rifabutin)	Antibacterials (rifabutin)
	Antiepileptics (carbamazepine, phenobarbital, phenytoin)	Antiarrhhythmics (digoxin, quinidine*)
	Cimetidine	Antiepileptics (phenytoin)
	NNRTI (efavirenz)	Antihistamines (astemizole*, terfenadine*)
	Proton pump inhibitors (esomeprazole)	Antimalarials (halofantrine*)
		Antineoplastics (vinblastine, vincristine)
		Antipsychotics (pimozide*)
		Benzodiazepines (midazolam)
		Calcineurin inhibitors (cyclosporin, sirolimus*, tacrolimus)
		Calcium channel blockers (diltiazem, nifedipine, nisoldepine, verapamil)

Table 3.1 (*Continued*)

Drug	*Decreased serum concentration of azole*	*Increased serum concentration of co-administered drug*
		Cisapride*
		Ergot alkaloids*
		Oral hypoglycaemics (glipizide)
		Protease inhibitors (atazanavir, ritonavir)
		Statins (atorvastatin, lovastatin, simvastatin)*
Voriconazole	Antibacterials (rifabutin*, rifampicin*)	Analgesics (alfentanil, diclofenac fentanyl, ibuprofen, methadone, oxycodone, sufentanil)
	Antiepileptics (carbamazepine*, phenobarbital*, phenytoin)	Antiarrhhythmics (quinidine*)
		Antibacterials (rifabutin*)
	NNRTI (efavirenz)	Antiepileptics (phenytoin)
		Antihistamines (astemizole*, terfenadine*)
	Protease inhibitors (amprenavir, ritonavir*, saquinavir)	Antineoplastics (vinblastine, vincristine)
		Antipsychotics (pimozide*)
		Benzodiazepines (alprazolam, diazepam, midazolam, triazolam, zolpidem)
		Calcineurin inhibitors (cyclosporin, sirolimus*, tacrolimus)
		Calcium channel blockers (felodipine)
		Cisapride*
		Ergot alkaloids*
		NNRTI (efavirenz)
		Oral hypoglycaemics (glipizide, glyburide, tolbutamide)
		Protease inhibitors (amprenavir, nelfinavir, ritonavir*, saquinavir)
		Proton pump inhibitors (omeprazole)
		Statins (lovastatin)
		Warfarin

This list is not all-inclusive. Readers are advised to check the manufacturers' prescribing information to see whether additional contraindications for drug use have been introduced.
NNRTI: non-nucleoside reverse transcriptase inhibitors.
*Co-administration is contraindicated.

occurs may necessitate upwards or downwards adjustment of the dosage of the other drug. Because of the risk of serious adverse cardiovascular events, systemic azoles are contraindicated in patients receiving cisapride, astemizole or terfenadine (these drugs are no longer available in most countries). Administration of an azole with drugs that are potent inducers of the human cytochrome P-450 enzyme system results in a marked reduction in blood levels, especially with itraconazole and ketoconazole.

3.6 Fluconazole

Fluconazole is a bis-triazole compound that is available for oral and parenteral administration.

3.6.1 Spectrum of activity

Fluconazole is most active against most *Candida* species and *Cryptococcus* species. However, *C. krusei* is intrinsically resistant. The spectrum of activity also includes *B. dermatitidis*, *Coccidioides* species and *H. capsulatum*. Fluconazole is active against dermatophytes, but has no activity against most other moulds, including *Aspergillus* species, *Fusarium* species and the Mucorales.

3.6.2 Acquired resistance

There have been few reports of resistance developing in *C. albicans* during short-term fluconazole treatment in patients with mucosal or deep-seated forms of candidosis. In contrast, many strains of *C. glabrata* rapidly become resistant during fluconazole treatment. In persons with the acquired immunodeficiency syndrome (AIDS), resistant strains of *C. albicans* have appeared following repeated courses of low-dose fluconazole treatment for oral or oesophageal infection. Many of these fluconazole-resistant *C. albicans* strains appear to be cross-resistant to other azoles. This is an uncommon problem at present (see Chapter 5). There are a few reports of resistant strains of *C. neoformans* from AIDS patients with relapsed infection following long-term maintenance treatment with fluconazole.

3.6.3 Pharmacokinetics

Oral administration of fluconazole leads to rapid and almost complete absorption of the drug. Identical serum concentrations are attained after oral and parenteral administration, indicating that first-pass metabolism of the drug does not occur. Blood concentrations increase in proportion to dosage over a wide range of dose levels. Two hours after a single

50-mg oral dose, serum concentrations in the region of 1 mg/L can be anticipated, but after repeated dosing this increases to about 2–3 mg/L. The steady state is reached within 5–10 days by giving oral doses of 50–400 mg once daily. Administration of the drug with food does not affect absorption.

Oral or parenteral administration of fluconazole results in rapid and widespread distribution of the drug. The volume of distribution in adults is 0.6–0.8 L/kg, and this is similar to total body fluid volume. Unlike other azole antifungals, fluconazole is only 10% bound to plasma proteins, but this can increase up to 23% in patients with chronic renal failure. Levels of the drug in most tissues and fluids usually exceed 50% of the simultaneous blood concentration.

Unlike other azole antifungals, such as itraconazole and voriconazole, fluconazole is not extensively metabolized by the hepatic cytochrome P-450 enzyme system. Metabolism by the CYP-3A4 enzyme system accounts for only 11% of the total drug excreted. About 80% of a given dose is eliminated as unchanged drug in the urine. The drug is cleared by glomerular filtration, but there is significant tubular reabsorption. Fluconazole has a serum half-life of about 25–30 hours in adults, but this is prolonged in renal failure, necessitating adjustment of the dosage regimen in patients with glomerular filtration rates below 50 mL/min. In children, the volume of distribution and clearance rates are increased and the half-life is considerably shorter (15–25 hours). In infants, fluconazole has a prolonged half-life of 55–90 hours.

Fluconazole is removed during haemodialysis and, to a lesser extent, during peritoneal dialysis. A 3-hour haemodialysis session will reduce the blood concentration by about 50%.

3.6.4 Pharmaceutics

Fluconazole is available in oral and parenteral forms: as tablets, oral suspension and an intravenous infusion. The drug is supplied for parenteral administration at a concentration of 2 mg/mL in 0.9% sodium chloride solution. Dosing recommendations are identical for all dosage forms.

3.6.5 Therapeutic use

Fluconazole is extensively used for the treatment of mucosal and cutaneous forms of candidosis. It is also effective in various forms of dermatophytosis and pityriasis versicolor.

It is an effective drug for treatment of invasive forms of candidosis and is the preferred initial treatment for those with *Candida parapsilosis* infection. However, fluconazole should not be used in patients with

C. glabrata or *C. krusei* infection. It has proved to be an effective prophylactic treatment against candidosis in haematopoietic stem cell transplant (HSCT) recipients. However, fluconazole is ineffective in aspergillosis and mucormycosis.

Fluconazole is a useful drug in acute cryptococcal meningitis, but it should not be used as first-line treatment in persons with AIDS unless there are particular reasons for withholding amphotericin B. However, it is more effective and better tolerated than amphotericin B as maintenance treatment to prevent relapse of cryptococcosis in patients with AIDS.

Fluconazole is the drug of choice for patients with coccidioidal meningitis. However, it must be continued for life to prevent relapse.

3.6.6 Mode of administration

As absorption following oral administration is good, this is the preferred method of administration. If the patient cannot take the drug by mouth, the intravenous solution can be used. This should be infused at a maximum rate of 200 mg/hour. In general, a loading dose of twice the daily dose is recommended on the first day of treatment to result in serum concentrations close to steady state by the second day of treatment.

Vaginal candidosis can be treated with a single 150-mg oral dose of fluconazole. Oropharyngeal candidosis should be treated with 200 mg on the first day followed by 100 mg/day for at least 2 weeks. Oesophageal candidosis should be treated with 200 mg on the first day followed by 100 mg/day for at least 3 weeks; treatment should be continued for at least 2 weeks following resolution of symptoms.

The recommended dose for adult patients with cryptococcosis or invasive forms of candidosis is 6 mg/kg per day (400 mg/day in a 70-kg patient). However, some clinicians have used higher dosages in life-threatening infections. The duration of treatment will differ from patient to patient depending upon the nature and extent of the infection and the underlying illness.

The recommended dose for children is 3 mg/kg for oropharyngeal and oesophageal candidosis, and 6–12 mg/kg for cryptococcosis or invasive forms of candidosis. Because of its more rapid clearance in children, fluconazole should be administered at 12-hour intervals for the treatment of life-threatening infections.

Long-term maintenance treatment with fluconazole to prevent relapse in AIDS patients with cryptococcosis should be administered at a dosage of 200 mg/day. To reduce the risk of invasive candidosis in neutropenic

HSCT recipients, prophylactic treatment with fluconazole should be given at a dosage of 400 mg once daily.

Patients with renal impairment should be given the normal dose for the first 48 hours of treatment. Thereafter, in persons with a creatinine clearance of 21–40 mL/min, the dosage interval should be doubled to 48 hours or the dose halved. Persons with a clearance of 10–20 mL/min require a 72-hour interval between doses.

Patients receiving regular haemodialysis require a full dose of fluconazole after each dialysis session.

3.6.7 Drug interactions

Fluconazole is a potent inhibitor of CYP-2C9 and CYP-2C19, and coadministration with other drugs that are metabolized by these enzyme systems should be avoided (Table 3.1). Fluconazole is a much less potent inhibitor of CYP-3A4 than other azole antifungals, including itraconazole and ketoconazole.

Concomitant administration of fluconazole with drugs, such as rifampicin, that are potent inducers of the human cytochrome P-450 enzyme system leads to a less marked reduction in the blood levels of the antifungal agent than is seen with other azoles. However, depending on clinical circumstances, consideration should be given to increasing the dose of fluconazole when it is administered with rifampicin.

3.6.8 Toxicity and adverse reactions

Fluconazole is one of the least toxic and best tolerated azole drugs, and side effects during treatment are rare. The commonest patient complaints are headache, hair loss and loss of appetite, but these seldom necessitate discontinuation of treatment in patients receiving up to 400 mg/day. Transient asymptomatic elevations of serum transaminase levels are quite common in AIDS patients treated with the drug, and treatment should be discontinued in patients who have test findings indicative of progressive or persistent hepatic dysfunction. There have been rare cases of serious hepatic reactions during fluconazole treatment, including hepatitis, cholestasis and fulminant hepatic failure. Occasional fatal hepatic reactions have occurred, particularly in patients with serious underlying medical conditions, such as AIDS or malignancies.

Fatal exfoliative skin rashes (Stevens-Johnson syndrome) have been reported in a few patients with AIDS or cancer, but the causal relationship with fluconazole is unclear because of the concomitant administration of other drugs.

3.7 Itraconazole

Itraconazole is a lipophilic triazole drug available for oral or parenteral administration.

3.7.1 Spectrum of activity

Itraconazole has a broad spectrum of activity, including *Aspergillus* species, *Candida* species, many dematiaceous moulds, dermatophytes and dimorphic fungi (*B. dermatitidis*, *Coccidioides* species, *H. capsulatum*, *P. brasiliensis, P. marneffei* and *S. schenckii*). It has modest activity against *Cryptococcus* species, but is ineffective against *Scedosporium* species and the Mucorales.

3.7.2 Acquired resistance

This is rare, but ketoconazole-resistant *C. albicans* strains from patients with chronic mucocutaneous candidosis have been cross-resistant to itraconazole, as have many fluconazole-resistant *C. albicans* strains from AIDS patients with chronic relapsing oropharyngeal candidosis. Itraconazole-resistant strains of *A. fumigatus* have been isolated from patients who have received azole treatment, as well as from individuals who have not been exposed, and from the environment in several European countries.

3.7.3 Pharmacokinetics

Absorption of itraconazole from the gastrointestinal tract is slow and incomplete (about 30% with capsules), but is improved if the drug is given with food or an acidic cola beverage. Oral administration of a single 100-mg capsule will produce peak serum concentrations between 0.1–0.2 mg/L about 2–4 hours later. Higher concentrations are obtained after repeated dosing, but there is marked variation between individuals. As with ketoconazole, there is a disproportionate increase in blood levels with increasing oral dosage. The steady state is reached within 2 days following parenteral administration of 200 mg of itraconazole twice daily. Serum concentrations are markedly reduced when gastric acid production is impaired.

Much higher blood concentrations (up to 1–1.5 mg/L) have been attained in AIDS patients and neutropenic individuals following administration of a 5 mg/kg dose of the oral solution formulation of itraconazole for 1–2 weeks. This formulation is better absorbed if given without food. However, even in the presence of food, higher serum concentrations are achieved with the oral solution than with the capsule.

Itraconazole is highly lipophilic and has a large volume of distribution (11 L/kg). It is >99% bound to plasma proteins and, as a result, concentrations of the drug in body fluids such as cerebrospinal fluid (CSF) are minimal. In contrast, drug concentrations in tissues, such as lung, liver and bone, are two to three times higher than in serum. High concentrations of itraconazole are also found in the stratum corneum as a result of drug secretion in sebum. Itraconazole has been found to persist in the skin for 2–4 weeks after the end of a 4-week course of treatment. It persists in toenails for up to 6 months after the end of a 3-month course of treatment, but levels in the fingernails decline about 3 months after the end of treatment.

Itraconazole is extensively metabolized by the human hepatic cytochrome P-450 CYP-3A4 enzyme system. Most of the metabolites are inactive and are excreted with the bile (55%) and urine (40%). However, the major metabolite, hydroxyitraconazole, is bioactive and has a similar spectrum of activity as the parent compound. In the steady state, this metabolite is found at serum concentrations about twofold higher than those of the parent drug. The serum half-life of itraconazole is about 20–30 hours, increasing to 40 hours after prolonged dosing. Less than 0.03% of an administered dose of itraconazole is excreted unchanged in the urine, but up to 18% is eliminated in faeces as unchanged drug. About 80–90% of the intravenous carrier, hydroxypropyl-β-cyclodextrin, is excreted unchanged in the urine.

No adjustment of dosage is required in hepatic or renal failure, or during haemodialysis or peritoneal dialysis.

3.7.4 Pharmaceutics

Itraconazole is available in oral and parenteral forms: as oral capsules, oral solution and an intravenous infusion. The major limitation of the capsule formulation is its highly variable acid-dependent absorption that often leads to sub-therapeutic concentrations in patients with elevated gastric pH. The oral solution is associated with gastrointestinal intolerance. The parenteral formulation avoids these problems, but is not widely used.

The drug is supplied for parenteral administration as 25-mL solution containing 250 mg itraconazole and 400 mg hydroxypropyl-β-cyclodextrin. The drug solution is diluted with 50 mL 0.9% sodium chloride solution (for injection) prior to infusion. After reconstitution, the diluted drug solution can be stored in a refrigerator (2–8°C) or at room temperature (15–25°C) for up to 48 hours, provided it is protected from direct light. If there are signs of precipitation or discolouration, the infusion

solution must be discarded. Intravenous itraconazole must not be infused with other drugs.

3.7.5 Therapeutic use

Itraconazole can be used to treat various superficial fungal infections, including the dermatophytoses, pityriasis versicolor, and mucosal and cutaneous forms of candidosis. It is effective in chromoblastomycosis, paracoccidioidomycosis and sporotrichosis. It has also proven useful for certain forms of aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, *P. marneffei* infection and phaeohyphomycosis. Itraconazole has been used as maintenance treatment to prevent relapse in AIDS patients with histoplasmosis or *P. marneffei* infection. However, it is less effective than fluconazole as maintenance treatment in AIDS patients with cryptococcosis.

3.7.6 Mode of administration

Vaginal candidosis can be treated with two 200-mg oral doses of itraconazole taken 6–8 hours apart. In non-compromised individuals, oropharyngeal candidosis can be treated with 100 mg/day for 2 weeks, but AIDS and neutropenic patients often require 200–400 mg/day because of impaired oral absorption. Administering the oral solution formulation (200–400 mg/day for 1–2 weeks) often results in improved absorption.

The recommended dosage for patients with cutaneous dermatophytoses is 100 mg/day, but the duration of treatment depends on the site of infection. Tinea corporis or tinea cruris should be treated for 2 weeks, but tinea pedis and tinea manuum require treatment for 4 weeks. Pulsed treatment (in which a week of treatment is alternated with 3 weeks without treatment) has given good results in nail infections. Two pulses of itraconazole (400 mg/day) are recommended for fingernail infections, but toenails require three or more pulses of treatment. Itraconazole is also available for continuous treatment of nail infection and a dosage of 200 mg/day for 3 months is recommended.

The recommended dosage for patients with pityriasis versicolor is 200 mg/day for 1 week.

The recommended oral dose of itraconazole for patients with invasive fungal infections, such as aspergillosis, blastomycosis and histoplasmosis, is 200–400 mg/day in adults or 5 mg/kg per day in children. It is often recommended that a loading dose of 600 mg/day for 3 days should be given at the start of treatment. Impaired absorption in AIDS and neutropenic patients will lead to low blood levels, and higher dosages, or substitution

of the intravenous formulation, may be required to ensure adequate serum concentrations. Absorption of itraconazole is reduced if it is given together with compounds that reduce gastric acid secretion: these drugs should be administered at least 2 hours after the intake of itraconazole. Oral dosages above 200 mg/day should be given as two divided doses.

The recommended intravenous dose of itraconazole is 200 mg at 12-hour intervals for four doses followed by 200 mg/day for up to 2 weeks. Each dose should be infused over a 1-hour period. Parenteral administration of itraconazole should be avoided in individuals with moderate or severe renal impairment (creatinine clearance <30 mL/min) because of accumulation of the intravenous carrier, hydroxypropyl-β-cyclodextrin.

The recommended oral dose of itraconazole for long-term maintenance treatment to prevent relapse in AIDS patients with histoplasmosis or *P. marneffei* infection is 200 mg/day.

3.7.7 Drug interactions

Absorption of itraconazole is reduced if it is given together with drugs that reduce gastric acid secretion, such as antacids, H2-antagonists, and proton pump inhibitors.

Itraconazole and its major metabolite, hydroxyitraconazole, are potent inhibitors of CYP-3A4, and it should not be given with drugs metabolized by this enzyme system (Table 3.1). Concomitant administration of itraconazole with drugs that induce the cytochrome P-450 system results in a marked reduction in blood levels of the azole agent.

3.7.8 Toxicity and adverse reactions

Most side effects associated with itraconazole are mild and reversible. The most frequently reported adverse events are headache, loss of appetite, nausea, abdominal discomfort, diarrhoea, skin rashes and transient asymptomatic elevations of liver enzymes. Rare, but serious, side effects include Steven-Johnson syndrome, reversible idiosyncratic hepatitis and hypokalemia. Gastrointestinal intolerance is more common with oral solution than with capsules and is sometimes severe enough to necessitate discontinuation of treatment.

Intravenous itraconazole has been associated with congestive heart failure. Neither intravenous nor oral itraconazole should be used to treat infections in patients with evidence of ventricular dysfunction unless the expected benefit clearly exceeds the risk. There have been rare cases of liver failure and death among persons taking itraconazole, including some within the first week. The drug is best avoided in patients with liver

disease and those with elevated liver enzymes, unless the expected benefit outweighs the risk. If signs of liver disease develop during treatment, the drug should be discontinued and liver function tests performed.

3.8 Ketoconazole

Ketoconazole is a dioxolane imidazole drug formulated for oral or topical use. It is the only antifungal imidazole still available for systemic administration, but its main use is now as a topical agent.

3.8.1 Spectrum of activity

Ketoconazole is active against dermatophytes and dimorphic fungi (*B. dermatitidis*, *Coccidioides* species, *H. capsulatum*, *P. brasiliensis* and *S. schenckii*). It is also active against *Candida* species and *Cryptococcus* species, but ineffective against *Aspergillus* species and the Mucorales.

3.8.2 Acquired resistance

Acquired resistance is rare, but resistant *C. albicans* strains have been recovered from patients given long-term treatment for chronic mucocutaneous candidosis and AIDS patients with chronic relapsing oropharyngeal or oesophageal candidosis.

3.8.3 Pharmacokinetics

Ketoconazole is erratically absorbed after oral administration. Absorption is favoured by an acid pH. Food delays absorption but does not significantly reduce the peak concentration. Two hours after a 400-mg dose, serum concentrations in the region of 5–6 mg/L can be anticipated, but there is much variation among individuals. There is a disproportionate increase in blood levels with increasing dosage.

As a result of high protein binding (>95%), the unbound concentrations of ketoconazole in body fluids are low. Penetration into CSF is poor and unreliable, although effective concentrations have been recorded with high doses (1200 mg) in some cases of meningitis. Less than 5% of an oral dose is excreted unchanged in the urine.

Ketoconazole is extensively metabolized by the hepatic cytochrome P-450 CYP-3A4 enzyme system. None of the metabolites are active. The half-life appears to be dose-dependent. There is an initial half-life of 1–4 hours and an elimination half-life ranging from 6 to 10 hours.

3.8.4 Pharmaceutics

Ketoconazole is available in a number of oral and topical forms.

3.8.5 Therapeutic use

Until the introduction of fluconazole and itraconazole, ketoconazole was regarded as the drug of choice for chronic mucocutaneous candidosis and an effective alternative oral treatment for certain forms of blastomycosis, histoplasmosis and paracoccidioidomycosis. However, prolonged administration of high dosage was often required and later relapse was a common problem. It is best avoided as an oral treatment for dermatophytosis or cutaneous or vaginal candidosis, because of its possible effects on the liver and on steroid metabolism. However, it is a useful topical drug for dermatophytosis, cutaneous candidosis, pityriasis versicolor and seborrhoeic dermatitis.

3.8.6 Mode of administration

Topical ketoconazole cream should be applied morning and evening, and treatment should be continued for 48 hours after all symptoms and signs have cleared. Pityriasis versicolor and seborrhoeic dermatitis can be treated with ketoconazole shampoo.

The usual adult oral dose is 200–400 mg/day, depending on the infection being treated. In children, a dose of 3 mg/kg can be used. The duration of treatment is usually 2 weeks, but this will depend upon the nature of the infection.

3.8.7 Drug interactions

Absorption of ketoconazole is reduced if it is given together with drugs that reduce gastric acid secretion, such as antacids, H2-antagonists and proton pump inhibitors.

Ketoconazole is a potent inhibitor of CYP-3A4, and its administration with other drugs metabolized by this system can result in potentially dangerous levels of the azole, the interacting drug, or both. Oral ketoconazole should not be co-administered with astemizole, cisapride, or terfenadine. Concomitant administration with rifampicin or phenytoin results in a marked reduction in blood levels of the azole agent.

3.8.8 Toxicity and adverse reactions

Unwanted effects include loss of appetite, abdominal pain, nausea, vomiting, headache, skin rashes and pruritus. Transient abnormalities of hepatic enzymes are common with oral ketoconazole, but serious liver damage is idiosyncratic and rare, occurring in between 1:10,000 and 1:15,000 patients treated for longer than 2 weeks. Most cases have been reported in patients treated for onychomycosis or chronic recalcitrant dermatophytosis. In most instances, hepatic damage is reversible when the drug is

discontinued. Liver function tests should be performed before starting treatment and at monthly intervals thereafter, particularly in patients on prolonged treatment or in those receiving other hepatotoxic drugs.

High doses of ketoconazole (over 800 mg/day) inhibit human adrenal and testicular steroid synthesis, with clinical consequences such as alopecia, gynaecomastia and impotence. High dose ketoconazole should be avoided in conditions associated with hypoadrenalism: these include AIDS, histoplasmosis, paracoccidioidomycosis and tuberculosis.

3.9 Posaconazole

Posaconazole is a second-generation triazole compound that is currently only available as an oral suspension. Posaconazole is highly lipophilic and has a chemical structure similar to that of itraconazole.

3.9.1 Spectrum of activity

Posaconazole is highly active against *Aspergillus* species, *Candida* species, *Cryptococcus* species and *Trichosporon* species. It has potent activity against dimorphic fungi (*B. dermatitidis*, *Coccidioides* species, *H. capsulatum*, *P. marneffei* and *S. schenckii*). It is less active against *Fusarium* species and *Scedosporium* species, but appears to be effective against many dematiaceous moulds. Unlike other azole antifungals, posaconazole has significant activity against the Mucorales.

3.9.2 Acquired resistance

Acquired resistance has not been reported, but some itraconazole-resistant strains of *A. fumigatus* show reduced susceptibility to posaconazole.

3.9.3 Pharmacokinetics

Posaconazole is absorbed slowly after oral administration, with peak serum concentrations being achieved around 3–4 hours after ingestion. Maximum blood levels are about fourfold lower when the drug is given under fasting conditions, compared with when it is given with a high-fat meal. Four hours after a single 200-mg dose administered with a high-fat meal, serum concentrations in the region of 0.5 mg/L can be anticipated. Blood concentrations increase in proportion to dosage up to 800 mg when taken with a high-fat meal. However, no further increases are observed when the dose is increased from 800 to 1200 mg. Administration of an 800-mg dose of the drug in four divided doses (at 6-hour

intervals) leads to a two- to threefold increase in serum concentrations, compared with a 400-mg dose (at 12-hour intervals). The steady state may not be achieved for 7–10 days after starting treatment.

Posaconazole has a large volume of distribution (about 1800 L), indicating extensive distribution and penetration into tissues. The drug is strongly bound to plasma proteins (>98%). Less than 0.2% of a given dose is excreted as unchanged drug in the urine.

Posaconazole is not as extensively metabolized by the hepatic cytochrome P-450 enzyme system as other triazole antifungals. More than 75% of an administered dose is eliminated in the faeces, predominantly as unchanged drug (66% of the dose). The remainder is excreted as glucuronidated derivatives in the urine. The drug has a long elimination half-life of about 35 hours.

Posaconazole is a substrate for intestinal P-glycoprotein, an adenosine triphosphate-dependent plasma membrane transporter responsible for drug efflux from cells. Multiple peaks in posaconazole blood concentrations have been observed, suggesting that effluxed drug is reabsorbed into the systemic circulation.

Posaconazole is not removed by haemodialysis.

3.9.4 Pharmaceutics

Posaconazole is available as a suspension for oral administration (40 mg/mL). Oral tablet and intravenous formulations are currently in development.

3.9.5 Therapeutic use

The lack of an intravenous formulation of posaconazole is a major disadvantage when treating seriously ill patients. In the EU, posaconazole has been licenced for the treatment of oropharyngeal candidosis, as well as for salvage treatment of invasive aspergillosis, coccidioidomycosis, chromoblastomycosis, *Fusarium* infections, and mycetoma. It has also been approved for prophylaxis of invasive fungal infections in high-risk patients, such as HSCT recipients with graft-versus-host disease (GVHD) and neutropenic patients with haematological malignancies. In the United States, the drug has been approved for the treatment of oropharyngeal candidosis, as well as for prophylaxis of invasive aspergillosis and candidosis. Other indications for which posaconazole has proved effective, but is not currently licenced, include coccidioidomycosis, histoplasmosis, phaeohyphomycosis and infections caused by the Mucorales.

3.9.6 Mode of administration

Each dose of posaconazole suspension should be administered with a full meal or with a liquid nutritional supplement in patients who cannot eat a full meal. For patients who cannot eat a full meal or tolerate an oral nutritional supplement, alternative antifungal treatments should be considered.

The recommended dose for adult patients with oropharyngeal candidosis is 100 mg (2.5 mL twice daily on the first day followed by 100 mg (2.5 mL) once daily for 13 days. Patients with oropharyngeal infection refractory to itraconazole and/or fluconazole should receive 400 mg (10 mL) twice daily. The duration of treatment will differ from patient to patient and depend on the nature of the infection and the clinical response.

The recommended dose of posaconazole for salvage treatment of invasive fungal infections is 200 mg (5 mL) administered four times daily. For prophylaxis of invasive fungal infections, the adult dose is 200 mg (5 mL) given three times daily. The dosage in children is not established.

No dosage adjustment is required in patients with hepatic impairment, but continued monitoring is recommended. Because renal elimination is minimal, no adjustment of dose is required for patients with renal impairment. Because of variable serum concentrations, individuals with severe renal impairment should be monitored for breakthrough invasive fungal infections.

3.9.7 Drug interactions

Absorption of posaconazole is reduced if it is given together with H2-receptor antagonists, such as cimetidine, or proton pump inhibitors, such as esomeprazole.

Posaconazole has no significant effect on most cytochrome P-450 enzyme systems involved in drug metabolism (including CYP-2C19 and CYP-2C9). However, it is a potent inhibitor of CYP-3A4, and concomitant administration may lead to increased serum concentrations of drugs metabolized by this enzyme system (Table 3.1). When posaconazole is discontinued, dosage of the other drugs may need to be adjusted. Posaconazole is metabolized by glucouronidation and is a substrate for P-glycoprotein efflux. Inhibitors of these pathways, such as cyclosporin, quinidine and verapamil, may increase posaconazole concentrations. Blood levels of posaconazole may be significantly reduced by its concomitant administration with drugs that induce these pathways, such as rifamycin antibiotics and certain antiepileptics (Table 3.1).

3.9.8 Toxicity and adverse reactions

Posaconazole is usually well tolerated, even among patients receiving the drug for longer than 6 months. The most frequent side effects have been gastrointestinal symptoms and headache. Mild to moderate, transient abnormalities of liver enzymes have also been reported. These do not appear be associated with higher serum concentrations of the drug and seem to occur less frequently than with other triazole drugs. Rare cases of cholestasis or hepatic failure (including fatalities) have also been reported during treatment with posaconazole, and it is advisable to discontinue the drug if signs of liver disease appear.

3.10 Voriconazole

Voriconazole is a second-generation triazole compound available for oral or intravenous administration. Its chemical structure is similar to that of fluconazole.

3.10.1 Spectrum of activity

Voriconazole is highly active against *Aspergillus* species, *Fusarium* species and *Scedosporium* species, as well as *Candida* species, *Cryptococcus* species and *Trichosporon* species. It has potent activity against dimorphic fungi (*B. dermatitidis*, *Coccidioides* species, *H. capsulatum* and *P. marneffei*) and many dematiaceous moulds, but appears to be ineffective against the Mucorales.

3.10.2 Acquired resistance

Acquired resistance is rare, but some fluconazole-resistant strains of *Candida* species show reduced susceptibility to voriconazole, as do some itraconazole-resistant strains of *A. fumigatus*.

3.10.3 Pharmacokinetics

Voriconazole is rapidly and almost completely absorbed after oral administration (about 96%). Two hours after a single 400-mg dose, serum concentrations in the region of 2 mg/L can be anticipated. In adults, there is a disproportionate increase in blood levels with increasing oral and parenteral dosage, due to saturable first-pass metabolism. In contrast, in children, increases in dosage produce proportional changes in drug levels, and clearance of the drug is more rapid. The steady state is achieved 5–7 days after starting treatment, but can be reached in 24 hours by giving a loading dose. Absorption of the drug is reduced with high-fat meals, but is not affected by changes in gastric pH.

Voriconazole has a large volume of distribution (about 4.6 L/kg) with a plasma protein binding of about 58%. Voriconazole is extensively distributed into tissues and penetrates well into CSF: levels are around 30–60% of the simultaneous blood concentration. Voriconazole concentrations in brain tissue are higher than those in the CSF. Less than 2% of a given dose is excreted as unchanged drug in the urine.

Voriconazole is extensively metabolized by several different hepatic cytochrome P-450 enzymes, primarily CYP-2C19, with CYP-2C9 and CYP-3A4 being involved to a much lesser extent. None of the metabolites is active. More than 80% of an administered dose is eliminated as metabolites in the urine. The elimination half-life is dose-dependent and is about 6–9 hours at a 3 mg/kg parenteral dose or 200-mg oral dose.

As a result of a point mutation in the gene encoding the CYP-2C19 enzyme, some persons are poor metabolizers while others are extensive metabolizers. About 3–5% of Caucasians and 15–20% of non-Indian Asians are poor metabolizers. Voriconazole blood concentrations are as much as two- to fourfold lower in individuals who metabolize the drug more extensively.

Voriconazole is not removed by haemodialysis.

3.10.4 Pharmaceutics

Voriconazole is available in oral and parenteral forms, as oral tablets, oral suspension and intravenous infusion.

The drug is supplied for intravenous administration in lyophilized form in 200-mg amounts. It is reconstituted in 19 mL sterile water (for injection) to give an extractable volume of 20 mL concentrated solution containing 10 mg/mL voriconazole. The drug solution is further diluted with 5% glucose (for injection) or 0.9% sodium chloride (for injection). The reconstituted drug can be stored in a refrigerator (2–8°C) for up to 24 hours. If there are signs of precipitation or discolouration, the infusion solution must be discarded. Other medications for injection should not be added to a voriconazole infusion.

3.10.5 Therapeutic use

In the EU, voriconazole has been approved for the treatment of invasive aspergillosis and has become the drug of choice for these infections. It is also licenced for the treatment of candidemia in non-neutropenic patients, for the treatment of serious invasive infections caused by *Candida* species that are resistant to fluconazole and for the treatment of infections caused by *Fusarium* species and *Scedosporium* species. In the United States, voriconazole has been approved for similar indications, as well

as for oesophageal candidosis. Other indications for which voriconazole has proved effective, but is not currently licenced, include coccidioidomycosis, histoplasmosis, paracoccidioidomycosis, *P. marneffei* infection and phaeohyphomycosis. Voriconazole is not currently licenced for the prophylaxis of fungal infection or for the treatment of persistently febrile patients with low neutrophil numbers who have suspected, but unproven, fungal infection.

3.10.6 Mode of administration

Intravenous treatment with voriconazole should be initiated with two loading doses of 6 mg/kg 12-hour apart, followed by 4 mg/kg at 12-hour intervals. Each dose should be infused at a maximum rate of 3 mg/kg per hour over a 1–2-hour period. The concentration of the infusion solution should not exceed 5 mg/mL.

Once the patient can tolerate oral medication, intravenous administration can be discontinued. Patients who weigh more than 40 kg should receive an oral dose of 200 mg at 12-hour intervals. Adult patients who weigh less than 40 kg should receive 100 mg at 12-hour intervals. If treatment is initiated with oral voriconazole, patients who weigh more than 40 kg should receive two loading doses of 400 mg at 12-hour intervals. Adult patients who weigh less than 40 kg should receive two loading doses of 200 mg at 12-hour intervals. If the patient response is inadequate, the oral dose can be increased to 300 mg twice daily (or 150 mg twice daily for patients weighing less than 40 kg). Oral voriconazole should be taken at least 1 hour before, or 1 hour after a meal. The duration of treatment will differ from patient to patient and depend on the nature of the infection and the clinical response.

If a patient is unable to tolerate treatment with voriconazole, the intravenous maintenance dose can be reduced to 3 mg/kg at 12-hour intervals. The oral dose can be reduced in 50-mg steps to a minimum of 200 mg twice daily (100 mg twice daily in patients weighing less than 40 kg).

Because of the accelerated clearance of voriconazole in children, the dose may need to be increased. A maintenance dose of 7 mg/kg per day has been recommended in order to attain blood concentrations comparable to those of adults. Loading regimens have not been adequately studied in children.

It is recommended that standard loading dose regimens be used, but that the maintenance dose is halved in patients with mild to moderate hepatic cirrhosis. Voriconazole has not been studied in patients with severe cirrhosis, but has been associated with elevations in liver enzymes

and clinical signs of liver damage. It should not be used in patients with severe hepatic impairment unless the benefit outweighs the potential risk.

No adjustment of oral dose is required for patients with renal impairment. However, like itraconazole, parenteral administration of voriconazole should be avoided in individuals with moderate or severe renal impairment (creatinine clearance <50 mL/min) because of accumulation of the intravenous carrier, sulphobutyl ether-β-cyclodextrin. Whether this accumulation results in significant adverse effects in humans is not yet clear. Nonetheless, parenteral voriconazole should not be used in patients with renal impairment, unless the benefit of intravenous treatment outweighs the potential risk. Serum creatinine concentrations should be monitored in these patients, and if increases occur, oral treatment should be substituted.

3.10.7 Drug interactions

Absorption of voriconazole is not reduced if it is given together with drugs that reduce gastric acid secretion.

Voriconazole inhibits CYP-2C9 and CYP-2C19 and co-administration with other drugs that are metabolized by these enzymes should be avoided (Table 3.1). It is a less potent inhibitor of CYP-3A4 than are itraconazole or posaconazole. Blood levels of voriconazole are significantly reduced by administration with drugs that induce hepatic cytochrome P-450 enzyme activity.

3.10.8 Toxicity and adverse reactions

The most frequently reported adverse events have been nausea, vomiting, skin rashes and transient abnormalities of liver enzymes. In addition, about 21% of patients treated with voriconazole experience abnormal vision, colour vision change and/or photophobia. These disturbances usually occur during the first week of therapy, are generally mild, and disappear with continued treatment in most patients. The mechanism of action is unclear, but there is some evidence that visual disturbances may be associated with higher serum concentrations or dosages of the drug. Visual function, including visual acuity, visual field and colour perception, should be monitored if voriconazole treatment is continued for longer than 4 weeks.

Rare, but serious, adverse reactions include Stevens-Johnson syndrome, hepatitis, cholestasis and fulminant hepatic failure (including fatalities). Voriconazole should only be used in patients with severe hepatic impairment if the benefit outweighs the potential risk. In such cases, monitoring of liver function is essential.

3.11 Other imidazole compounds for topical administration

3.11.1 Bifonazole

Bifonazole is used for the treatment of dermatophytosis and pityriasis versicolor. The dosage and duration of treatment varies according to the condition being treated.

3.11.2 Butoconazole

Butoconazole is used for the treatment of vaginal candidosis. Pessaries (100 mg) should be inserted high in the vagina for 3 consecutive days.

3.11.3 Clotrimazole

Clotrimazole is used for the treatment of dermatophytosis and oral, cutaneous and genital candidosis. For oral candidosis, oral troches (10 mg) should be dissolved in the mouth five times daily for 2 weeks or longer. For vaginal candidosis, pessaries (500, 200 or 100 mg) should be inserted high in the vagina for 1, 3 or 6 consecutive days, respectively. For fungal skin infections, dosage and duration of treatment varies according to the condition being treated.

3.11.4 Econazole nitrate

Econazole nitrate is used for the treatment of dermatophytosis and oral, cutaneous and genital candidosis. It has also been used to treat corneal infections. For vaginal candidosis, pessaries (150 mg) should be inserted high in the vagina for 3 consecutive days. For fungal skin infections, dosage and duration of treatment varies according to the condition being treated.

3.11.5 Fenticonazole nitrate

Fenticonazole nitrate is used for the treatment of vaginal candidosis. Pessaries (600 or 200 mg) should be inserted high in the vagina for 1 or 3 consecutive days, respectively.

3.11.6 Isoconazole nitrate

Isoconazole nitrate is used for the treatment of dermatophytosis and cutaneous and vaginal candidosis. For vaginal candidosis, pessaries (600 mg as a single dose) should be inserted high in the vagina. For fungal skin infections, dosage and duration of treatment varies according to the condition being treated.

3.11.7 Miconazole nitrate

Miconazole nitrate is used for the topical treatment of dermatophytosis, pityriasis versicolor, and oral, cutaneous and genital candidosis. It is no longer available for intravenous use in invasive fungal infections. For vaginal candidosis, pessaries (200 or 100 mg) should be inserted high in the vagina for 7 or 14 consecutive days. For oral candidosis, oral gel (125 mg) should be placed in the mouth four times daily. For fungal skin infections, dosage and duration of treatment varies according to the condition being treated.

3.11.8 Oxiconazole

Oxiconazole is used for the treatment of dermatophytosis and cutaneous candidosis. The dosage and duration of treatment varies according to the condition being treated.

3.11.9 Sertaconazole nitrate

Sertaconazole nitrate is used for the treatment of dermatophytosis and vaginal candidosis. The dosage and duration of treatment varies according to the condition being treated.

3.11.10 Sulconazole nitrate

Sulconazole nitrate is used for the treatment of dermatophytosis and cutaneous candidosis. The dosage and duration of treatment varies according to the condition being treated.

3.11.11 Terconazole

Terconazole is used for the treatment of dermatophytosis and cutaneous and genital candidosis. For vaginal candidosis, pessaries (80 mg) should be inserted high in the vagina for 3 consecutive days. For fungal skin infections, dosage and duration of treatment varies according to the condition being treated.

3.11.12 Tioconazole

Tioconazole is used for the treatment of the dermatophytoses (including nail infections) and cutaneous and genital candidosis. For vaginal candidosis, pessaries (300 mg as a single dose) should be inserted high in the vagina. For fungal skin and nail infections, dosage and duration of treatment varies according to the condition being treated.

3.12 Echinocandins

The echinocandins are a new class of semisynthetic lipopeptide antifungal agents that target the fungal cell wall. Three agents of this type (anidulafungin, caspofungin and micafungin) have been licenced for use in serious fungal infections. Because of their high molecular weight and low oral bioavailability, these drugs are only available as intravenous preparations.

3.12.1 Mechanism of action

The echinocandins disrupt fungal cell wall synthesis by inhibiting the enzyme (1-3)-β-D-glucan synthase. This results in inhibition of the formation of (1-3)-β-D-glucan, an integral polysaccharide component of the cell wall of susceptible fungi. Inhibition leads to osmotic lysis of the cell and eventual cell death. Echinocandins bind to FKSp, the major subunit of (1-3)-β-D-glucan synthase, which is encoded by three *FKS* genes in *Candida* species. The echinocandins are fungicidal for *Candida* species, but fungistatic for *Aspergillus* species.

3.12.2 Spectrum of activity

The echinocandins have a limited spectrum of activity. They are highly active against a broad range of *Candida* species, including fluconazole-resistant strains. However *C. parapsilosis*, *Candida lusitaniae* and *Candida guilliermondii* appear to be less susceptible. The echinocandins are also active against *Aspergillus* species, including those that are intrinsically resistant to amphotericin B. The echinocandins are ineffective against fungi that lack a significant amount of (1-3)-β-D-glucan in their cell wall, including *Cryptococcus* species and *Trichosporon* species, as well as *Fusarium* species and the Mucorales. These drugs are also effective against the cyst stage, but not the trophozoite form of *Pneumocystis jirovecii*.

3.12.3 Acquired resistance

This is uncommon at present, but resistant strains of several *Candida* species have been recovered from patients failing caspofungin or micafungin treatment. Resistance has been associated with acquisition of mutations in the *FKS1* and/or *FKS2* genes that led to amino acid substitutions within the FKS1p and FKS2p subunits of (1-3)-β-D-glucan synthase. Echinocandin resistance is usually only observed following prolonged therapy.

3.12.4 Drug interactions

The echinocandins do not interact with the human hepatic cytochrome P-450 system and their use has been associated with very few significant drug interactions.

3.13 Anidulafungin

Anidulafungin is derived from a fermentation product of the fungus *Aspergillus nidulans*.

3.13.1 Pharmacokinetics

Serum concentrations of anidulafungin increase in proportion to dosage. The steady state is achieved on the first day after a loading dose (twice the daily maintenance dose). Following a single 200-mg loading dose and a 100-mg parenteral maintenance dose, administered at a rate of 1.1 mg/min, peak serum concentrations of anidulafungin in the region of 7 mg/L can be anticipated. Anidulafungin has a volume of distribution of 30–50 L that is similar to total body fluid volume. It is strongly bound to plasma proteins (>99%), and as a result, levels in body fluids, such as CSF, are negligible.

Unlike caspofungin and micafungin, anidulafungin is not metabolized by the liver, but undergoes slow non-enzymatic degradation in the blood to an inactive peptide breakdown product that is enzymatically degraded and excreted in the bile and faeces. About 30% of a given dose is eliminated in the faeces, of which less than 10% is unchanged drug. Less than 1% of a dose is excreted in the urine. The drug has a half-life of around 24 hours, which is longer than that of caspofungin or micafungin.

Anidulafungin is not cleared by haemodialysis.

3.13.2 Pharmaceutics

Anidulafungin is only available for parenteral administration. The drug is supplied in lyophilized form in 100-mg amounts and is reconstituted in 30 mL 20% dehydrated alcohol in water (for injection). The reconstituted drug solution is further diluted by adding 250 mL 5% glucose (for injection) or 0.9% sodium chloride (for injection). The reconstituted solution must be used within 24 hours, during which time it can be stored at room temperature (25°C, range 15–30°C). Other medications for injection should not be added to an anidulafungin infusion.

3.13.3 Therapeutic use

In the EU, anidulafungin is approved for the treatment of invasive candidosis in non-neutropenic adult patients. It has not been evaluated in

sufficient numbers of neutropenic patients to determine its effectiveness in that group. In the United States, anidulafungin is licenced for the treatment of oesophageal candidosis, candidaemia and some invasive forms of candidosis.

3.13.4 Mode of administration

Patients with invasive forms of candidosis should receive a single 200-mg loading dose on the first day, followed by 100 mg/day thereafter. Those with oesophageal candidosis should receive a 100-mg loading dose on the first day, followed by 50 mg/day for at least 2 weeks. The rate of infusion should not exceed 1.1 mg/min. More rapid infusions may result in more frequent histamine-related reactions. The duration of treatment will differ from patient to patient and depend on the extent of disease and the clinical response. In general, treatment of invasive candidosis should be continued for at least 2 weeks following the last positive culture.

No dosage adjustment is required in patients with renal or hepatic impairment, patients using other medications or those in other special populations.

3.13.5 Drug interactions

No clinically significant interactions have yet been reported with drugs likely to be co-administered with anidulafungin.

3.13.6 Toxicity and adverse reactions

Anidulafungin is well tolerated. The most notable, but uncommon, event reported is a histamine-mediated infusion-related reaction. However, this is infrequent when the rate of infusion does not exceed 1.1 mg/min. Occasional injection site reactions, including phlebitis and thrombophlebitis have been noted. Transient elevations of liver enzymes have been reported. Isolated cases of significant hepatic dysfunction, hepatitis or worsening liver failure have also been reported, although it is not clear if these were drug related.

3.14 Caspofungin

Caspofungin is derived from a fermentation product of the fungus *Glarea lozoyensis*.

3.14.1 Pharmacokinetics

Blood concentrations increase in proportion to dosage. One hour after a single 70-mg parenteral dose, administered over a 1-hour period, serum concentrations of caspofungin in the region of 10 mg/L can be anticipated.

Less than 10% of the dose remains in the blood 36–48 hours after administration. Most of the remainder is distributed to the tissues (about 92% of the dose), with the highest concentrations being found in the liver. Caspofungin has a volume of distribution of 0.15 L/kg and is strongly bound to plasma proteins (97%). As a result, levels in body fluids, such as CSF, are negligible.

There is little excretion or metabolism of caspofungin during the first 30 hours after administration. The drug is slowly metabolized by the liver through non-enzymatic peptide hydrolysis and *N*-acetylation, and the inactive metabolites are excreted in the bile and faeces. About 1% of a dose is excreted unchanged in the urine. The drug has a half-life of about 9–11 hours.

Caspofungin is not removed by haemodialysis.

3.14.2 Pharmaceutics

Caspofungin (as acetate) is only available for parenteral administration. The drug is supplied in lyophilized form in 50- and 70-mg amounts and is reconstituted in 10.5 mL water (for injection). The reconstituted drug solution is further diluted by adding 10 mL to 250 mL 0.9% sodium chloride (for injection). The infusion solution may be stored at room temperature (up to 25°C) for up to 24 hours, or in a refrigerator (2–8°C) for up to 48 hours. Other medications for injection should not be added to a caspofungin infusion.

3.14.3 Therapeutic use

In the EU, caspofungin is approved for the treatment of invasive candidosis in adults and children, for the salvage treatment of aspergillosis in patients who have failed to respond to, or are intolerant of other antifungal agents, and for empirical treatment of presumed fungal infections in febrile neutropenic patients. It has similar indications in the United States, but is also licenced for the treatment of oesophageal candidosis.

3.14.4 Mode of administration

A single 70-mg loading dose of caspofungin should be administered on the first day, followed by 50 mg daily thereafter. Each dose should be infused over a 1-hour period. The duration of treatment will differ from patient to patient and depend on the extent of disease and the clinical response. In general, treatment of invasive candidosis should be continued for at least 2 weeks after the last positive culture.

No dosage adjustment is required in adult patients with mild hepatic impairment. However, a dose reduction to 35 mg following the 70 mg

loading dose is recommended for adults with moderate hepatic impairment. There is no clinical experience in adults with severe liver disease. No dosage adjustment is necessary for patients with renal impairment.

3.14.5 Drug interactions

Caspofungin has few documented interactions. Initial data indicated that an interaction occurred when it was co-administered with cyclosporin and this resulted in transaminase elevations of two to three times the upper limit of normal, resolving when both drugs were discontinued. In addition, caspofungin serum concentrations were increased, but there was no effect on cyclosporin pharmacokinetics. More recent data, however, suggest that this effect is not consistent in patients receiving the drugs. Moreover, elevations of liver enzymes are common when caspofungin is used alone. Co-administration of caspofungin with cyclosporin is not recommended unless the expected benefit outweighs the risk. If the drugs are used together, close monitoring of liver enzymes is recommended. Caspofungin interacts with tacrolimus, reducing its serum concentrations. Tacrolimus levels should be monitored and the dosage adjusted if required.

Caspofungin blood concentrations are reduced when it is co-administered with rifampicin. Concurrent administration with other inducers of drug clearance, such as efavirenz, nevirapine, phenytoin, dexamethasone or carbamazepine, has also led to reductions in caspofungin concentrations. Patients receiving concurrent treatment with these drugs may require a 70-mg daily dose of caspofungin.

3.14.6 Toxicity and adverse reactions

Caspofungin is well tolerated. The most notable, but uncommon, event reported is a histamine-mediated infusion-related reaction. This can be relieved by slowing the rate of infusion or pre-medicating with an antihistamine, such as diphenhydramine. Occasional cases of infusion-related pain and phlebitis at the infusion site have been reported, and these appear to be more frequent than with anidulafungin or micafungin. Transient mild elevations of liver enzymes are common. Rare cases of significant hepatic dysfunction, hepatitis or worsening liver failure have also been reported, although it is not clear if these were drug related.

3.15 Micafungin

Micafungin is derived from a fermentation product of the fungus *Coleophoma empetri*.

3.15.1 Pharmacokinetics

Like anidulafungin and caspofungin, serum concentrations of micafungin increase in proportion to dosage. The steady state is reached within 4–5 days, at which time peak serum concentrations in the region of 5 or 15 mg/L can be anticipated after an intravenous dose of 50 or 150 mg, respectively. Micafungin has a volume of distribution of 0.4 L/kg and is strongly bound to plasma proteins (99%). As a result, levels in body fluids, such as CSF, are negligible.

Micafungin is metabolized by the liver and the inactive metabolites are excreted in the faeces (71%) and urine (12%). Less than 1% of a dose is eliminated as unchanged drug in the urine. Micafungin has a half-life of 10–17 hours.

Micafungin is not cleared by haemodialysis.

3.15.2 Pharmaceutics

Micafungin (as sodium) is only available for parenteral administration. The drug is supplied in lyophilized form in 50- and 100-mg amounts and is reconstituted in 5 mL 0.9% sodium chloride (for injection) or 5% glucose (for injection). The reconstituted drug solution is further diluted by adding 100 mL 0.9% sodium chloride (for injection) or 5% glucose (for injection). The infusion solution must be used within 24 hours, during which time it should be protected from light. It can be stored at room temperature (25°C). Other medications for injection should not be added to a micafungin infusion.

3.15.3 Therapeutic use

In the EU, micafungin is approved for the treatment of oesophageal candidosis in adults and for invasive candidosis in adults and children, including neonates. In the United States, the drug is approved for the treatment of oesophageal candidosis, candidaemia and certain invasive forms of candidosis in adults. Micafungin is also licenced for the prophylaxis of *Candida* infections in HSCT recipients in the EU and United States.

3.15.4 Mode of administration

Unlike anidulafungin and caspofungin, a loading dose is not required. The recommended dosage of micafungin for patients with invasive candidosis is 100 mg/day, while those with oesophageal candidosis should receive 150 mg/day. The drug should be infused over a 1-hour period. More rapid infusions may result in more frequent histamine-related reactions. The recommended dose of micafungin for prophylaxis is 50 mg/day. In general, treatment of invasive candidosis should be continued for at least 1 week after two sequential negative blood cultures have been obtained

and after resolution of clinical signs and symptoms of infection. Treatment of oesophageal infection should be continued for 1 week after resolution of clinical signs.

No dosage adjustment is required in patients with severe renal impairment or mild to moderate hepatic impairment. The effect of severe hepatic impairment on micafungin pharmacokinetics has not been studied, and its use in these patients is not recommended.

3.15.5 Drug interactions

Although micafungin is not a major substrate for the cytochrome P-450 enzyme system, it does have some interactions that appear to be mediated by this mechanism. Micafungin appears to inhibit the cytochrome P-450-3A4 metabolism of itraconazole, nifedipine and sirolimus, and may increase blood concentrations of these drugs. Unlike caspofungin, the clearance of micafungin is unchanged when it is co-administered with rifampicin.

3.15.6 Toxicity and adverse reactions

Micafungin is well tolerated. Occasional histamine-mediated infusion-related reactions have been reported, but these are infrequent when the drug is infused over a 1-hour period. Injection site reactions have been noted, but tend to occur more often in patients receiving micafungin by peripheral intravenous administration. Isolated cases of significant hepatic dysfunction, hepatitis and liver failure, including fatalities, have occurred. Patients who develop abnormal liver function tests during micafungin treatment should be carefully monitored and the drug discontinued, unless the expected benefit outweighs the risk.

3.16 Polyenes

Around 100 polyene antibiotics have been described, but few have been developed for clinical use, largely because of problems with stability, oral bioavailability and toxicity. The polyenes are large molecules that consist of a closed macrolide lactone ring. One side of the ring is composed of a rigid lipophilic chain with a variable number of conjugated double bonds, and on the opposite side, there are a similar number of hydroxyl groups. Thus, the molecule is amphipathic, and this feature of its structure is thought to be important in its mechanism of action.

3.16.1 Mechanism of action

The polyenes binds to sterols, principally ergosterol, in the membranes of susceptible fungal cells, causing impairment of membrane barrier function, leakage of cell constituents, metabolic disruption and cell death.

In addition to its membrane permeabilizing effects, amphotericin B can cause oxidative damage to fungal cells through a cascade of oxidative reactions linked to lipoperoxidation of the cell membrane. More recently, however, an antioxidant effect of amphotericin B has been observed, and thus the relevance of pro-oxidant effects remains unclear. Mammalian cell membranes also contain sterols, and it has been suggested that non-selective binding of amphotericin B to cholesterol in mammalian cell membranes accounts for some of the toxic side effects seen in humans.

3.17 Amphotericin B

Amphotericin B is derived from a fermentation product of *Streptomyces nodosus*.

The conventional micellar suspension formulation of this drug (amphotericin B deoxycholate) is often associated with unpleasant infusion-related reactions and treatment-limiting toxic side effects, particularly renal damage. This problem led to the development of three lipid-based formulations of the drug: liposomal amphotericin B (AmBisome), in which the drug is encapsulated in small phospholipid-containing liposomes; amphotericin B lipid complex (ABLC), in which it is complexed with phospholipids to form much larger ribbon-like structures; and amphotericin B colloidal dispersion (ABCD), in which the drug is complexed with cholesterol sulphate to form small lipid discs. These formulations possess the same broad spectrum of activity as the micellar suspension, but appear to be less nephrotoxic because of their altered pharmacological distribution.

3.17.1 Spectrum of activity

Amphotericin B has a broad spectrum of activity, including many *Aspergillus* species, *Candida* species, *Cryptococcus* species and the Mucorales. The drug is also effective against dimorphic fungi (*B. dermatitidis*, *Coccidioides* species, *H. capsulatum*, *P. brasiliensis*, *P. marneffei* and *S. schenckii*). *C. lusitaniae* and *C. krusei* may be less susceptible, while *Aspergillus terreus*, some *Fusarium* species, *Scedosporium* species and *Trichosporon* species are innately resistant.

3.17.2 Acquired resistance

This is rare, but resistant strains of several *Candida* species with alterations in the cell membrane, including reduced amounts of ergosterol, have been isolated following prolonged treatment. There are a few reports

of resistant strains of *C. neoformans* isolated from AIDS patients with relapsed infection.

3.17.3 Pharmacokinetics

3.17.3.1 Conventional formulation

Parenteral administration of a 1 mg/kg dose of the conventional formulation of the drug will produce maximum serum concentrations of 1–2 mg/L. Less than 10% of the dose remains in the blood 12 hours after administration and more than 95% of this is protein-bound. Most of the remainder can be found in the liver (up to 40% of the dose), lungs (up to 6%) and kidneys (up to 2%). Levels in CSF are less than 5% of the simultaneous blood concentration. Amphotericin B deoxycholate has a large volume of distribution (3–5 L/kg) and binds to tissues for prolonged periods of time, re-entering the circulation slowly from these storage sites.

Amphotericin B deoxycholate is mostly excreted as unchanged drug in the faeces (42%) and urine (21%). No metabolites have been identified. The drug is cleared very slowly, with the conventional formulation having a terminal half-life of about 127 hours.

Blood concentrations are unchanged in hepatic or renal failure. Likewise, haemodialysis does not influence blood levels unless the patient is hyperlipemic, in which case there is some drug loss due to adherence to the dialysis membrane.

3.17.3.2 Lipid-based formulations

The pharmacokinetics of the different lipid-based formulations of amphotericin B is quite diverse. Large structures, such as ABLC, are rapidly removed from the blood, but smaller liposomes remain in the circulation for much longer periods.

The pharmacokinetics of amphotericin B after parenteral administration of AmBisome are non-linear, and there is a disproportionate increase in serum concentrations when the dose is increased from 1 to 5 mg/kg per day. Maximum levels of around 80 mg/L have been attained after administration of a 5 mg/kg dose for 1 week. AmBisome has a lower volume of distribution (0.1 L/kg) than the other lipid formulations. Administration of AmBisome results in much higher drug concentrations in the liver and spleen than are achieved with conventional amphotericin B. Levels in renal tissue are much lower than those obtained with equivalent amounts of the conventional formulation. Less than 10% of a given dose of AmBisome appears as unchanged drug in the faeces and urine within 1 week. This formulation has a half-life of 6–7 hours.

The maximum serum concentrations obtained after parenteral administration of ABLC are lower than after administration of equivalent amounts of conventional amphotericin B due to more rapid distribution of the drug to tissue. Maximum levels have ranged from 1 to 2 mg/L after administration of a 5 mg/kg dose for 1 week. The pharmacokinetics of amphotericin B after parenteral administration of ABLC are non-linear. The volume of distribution and clearance from blood increase as dosage is increased, resulting in less-than-proportional increases in serum concentrations over a dosage range from 0.6 to 5 mg/kg per day. Administration of ABLC results in much higher drug concentrations in the liver, spleen and lungs than are achieved with conventional amphotericin B. Levels in renal tissue are much lower than those obtained with equivalent amounts of the conventional formulation. This formulation has a half-life of about 170 hours.

Maximum serum concentrations of about 3 mg/L have been obtained following a 5-mg/kg dose of ABCD, but levels in the blood decline soon after the end of the infusion due to rapid distribution of the drug to tissue. Administration of ABCD results in much higher drug concentrations in the liver and spleen than are achieved with conventional amphotericin B. Levels in renal tissue are much lower than those obtained with equivalent amounts of the conventional formulation, but higher than those in patients treated with liposomal amphotericin B. This formulation has a half-life of about 28 hours.

The effect of renal or hepatic impairment on the disposition of amphotericin B after administration of the lipid-based formulations of the drug has not been studied.

3.17.4 Pharmaceutics

Amphotericin B is available in oral, topical and parenteral forms.

3.17.4.1 Conventional formulation

Amphotericin B is supplied for parenteral administration in lyophilized form in 50-mg amounts together with 41 mg sodium deoxycholate (which acts as a dispersing agent) and a sodium phosphate buffer. The addition of 10 mL sterile water gives a clear micellar suspension. This is further diluted with 490 mL 5% dextrose solution prior to injection to give a final drug concentration of 100 mg/L. The dextrose solution should have a pH of 4.2 or greater to prevent precipitation of the drug. The diluted drug should be used within 24 hours, but does not need to be protected from light. Other preparations for injection should not be added to an amphotericin B infusion. If there are signs of precipitation, the infusion must be discarded.

3.17.4.2 Lipid-based formulations

AmBisome is supplied for parenteral administration in lyophilized form in 50-mg amounts and is first reconstituted in 12 mL sterile water (for injection) to give a drug concentration of 4 mg/mL. The drug solution is filter sterilized and further diluted with 5% dextrose (for injection) to give a final drug concentration in the range of 1–2 mg/mL amphotericin B. Lower concentrations (0.2–0.5 mg/mL) may be appropriate for infants and small children to provide sufficient volume for infusion. The reconstituted drug in water can be stored in a refrigerator (2–8°C) for up to 24 hours before dilution with 5% dextrose solution. Infusion of the drug should be commenced within 6 hours of dilution with 5% dextrose solution.

ABLC is supplied for parenteral administration as a sterile suspension in 100-mg amounts, which must be filter sterilized and diluted before use with 5% dextrose (for injection) to a final infusion concentration of 1 mg/mL. For children and patients with cardiovascular disease, the drug may be diluted to a final infusion concentration of 2 mg/mL. The final infusion volume should be about 500 mL (250 mL in children). The diluted suspension can be stored in a refrigerator (2–8°C) for up to 48 hours, and at room temperature for an additional 6 hours, before infusion.

ABCD is supplied for parenteral administration in lyophilized form in 50- or 100-mg amounts and is first reconstituted in 10 or 20 mL sterile water (for injection) to give a drug concentration of 5 mg/mL. The drug solution is then diluted eightfold with 5% dextrose (for injection) to give a final concentration of 0.625 mg/mL amphotericin B. The reconstituted drug in water can be stored in a refrigerator for up to 24 hours prior to dilution with 5% dextrose solution. After further dilution with 5% dextrose solution, the drug should be stored in a refrigerator (2–8°C) and used within 24 hours.

To avoid precipitation, AmBisome, ABLC and ABCD must not be reconstituted or diluted with saline and should not be mixed with other drugs. Existing lines must be flushed with 5% dextrose solution prior to the infusion or, if this is not feasible, a separate line should be used.

3.17.5 Therapeutic use

Topical amphotericin B preparations can be used to treat mucosal and cutaneous forms of candidosis.

Parenteral amphotericin B remains an important drug for many serious fungal infections, including aspergillosis, blastomycosis, candidosis, coccidioidomycosis, cryptococcosis, histoplasmosis, mucormycosis and paracoccidioidomycosis. However, administration of the conventional

formulation of the drug is associated with harmful side effects and unpleasant reactions that often preclude adequate dosing and can lead to treatment failure.

Clinical experience to date with lipid-based preparations of amphotericin B is now sufficient to indicate that these agents are no less active than conventional amphotericin B, and for some infections, such as AIDS-associated cryptococcal meningitis, they are more effective. AmBisome and ABLC are better tolerated than conventional amphotericin B and higher doses can be given over shorter periods with fewer toxic reactions. However, use of ABCD has been limited by a higher incidence of infusion-related reactions.

AmBisome is licenced for the treatment of cryptococcal meningitis in persons with AIDS, as well as for the empirical treatment of presumed fungal infection in febrile neutropenic patients. AmBisome, ABLC and ABCD are approved for the salvage treatment of aspergillosis, candidosis and cryptococcosis in patients who have failed to respond to conventional amphotericin B, or who have developed severe side effects to conventional amphotericin B or in whom conventional amphotericin B is contraindicated because of renal impairment. In the United States, ABCD is only licenced for the salvage treatment of aspergillosis.

The conventional parenteral formulation of amphotericin B has been instilled into a number of sites, including the bladder, peritoneum and joints. However, intraperitoneal infusion of the drug is painful and has been associated with adhesion formation.

3.17.6 Mode of administration

The dose and duration of topical treatment will differ from patient to patient and depend on the nature and extent of infection. The usual adult dose of the oral suspension for oral forms of candidosis is 1–2 mL (100–200 mg) at 6-hour intervals. As the drug is not absorbed, the success of treatment depends on maintaining an adequate concentration in the mouth for as long as possible. The recommended dosage of the oral suspension for infants and children is 1 mL (100 mg) at 6-hour intervals.

3.17.6.1 Conventional formulation

Most patients with deep fungal infection are treated with 1–2 g of amphotericin B over 6–10 weeks, but this will differ from person to person, depending upon the nature and extent of the infection and the underlying illness. In adults with normal renal function, the usual daily dose is between 0.6 and 1 mg/kg.

It is prudent to give an initial test dose of 1 mg of amphotericin B deoxycholate in 50 mL dextrose solution to all patients before initiating treatment because occasional individuals will have an idiosyncratic reaction of severe hypotension or an acute anaphylactoid reaction. This should be administered over 1–2 hours (0.5 mg in children weighing less than 30 kg), with general clinical observation and monitoring of temperature, pulse and blood pressure at 30-minute intervals. In the few patients with a reaction, the test infusion should be discontinued, supportive treatment including hydrocortisone should be given, and a repeat test dose administered over a longer period. If the test dose is well tolerated, there can be progression to larger doses. Several dosage regimens are available (see Tables 3.2 and 3.3).

In patients who are not immunocompromised or suffering a serious life-threatening infection, optimum dosage is best achieved through gradual augmentation of the dose. The dose can be increased up to 1 mg/kg, but individual infusions should not contain more than 50 mg of the drug.

In immunosuppressed patients and others with a serious infection, the dose of amphotericin B should be increased as rapidly as the patient's

Table 3.2 Regimens for rapid escalation of amphotericin B dosage

Time infusion started (h)	*Duration of infusion (h)*	*Dosage (mg)*	*Volume of solution 1 (mL)*	*Volume of solution 2 (mL)*
0	2	1	10	40
4	6	24	240	760
16	6	25	250	750
40	6	50	500	500
(Then at 24-h intervals, dose not to exceed 50 mg or 1.0 mg/kg per infusion, whichever is the lesser.)				
0	2	1	10	40
2	6	9	90	360
12	6	10	100	400
24	6	20	200	300
48	6	30	300	700
72	6	40	400	600
96	6	50	500	500
(Then at 24-h intervals, dose not to exceed 50 mg or 1.0 mg/kg per infusion, whichever is the lesser)				

Solution 1: amphotericin B at 100 mg/L in 5% dextrose solution; solution 2: 5% dextrose solution.

Table 3.3 Regimen for gradual escalation of amphotericin B dosage

Time infusion started (h)	*Duration of infusion (h)*	*Dosage (mg)*	*Volume of solution 1 (mL)*	*Volume of solution 2 (mL)*
0	2	1	10	40
2	6	9	90	360
24	6	10	100	400
48	6	20	200	300
72	6	30	300	700
96	6	40	400	600
120	6	50	500	500

(Then at 24-h intervals, dose not to exceed 50 mg or 1.0 mg/kg per infusion, whichever is the lesser.)

Solution 1: amphotericin B at 100 mg/L in 5% dextrose solution; solution 2: 5% dextrose solution.

tolerance of the drug will allow. Rapid augmentation of the dose carries a greater risk of acute renal failure, but immunosuppressed patients often tolerate these regimens well.

Four-hour infusions appear to be as well tolerated as the more traditional 6-hour infusion period in patients with normal renal function. However, it is not advisable to give the infusion over less than 4 hours.

After 2 weeks of treatment, blood concentrations become stable, tissue levels begin to accumulate and it becomes possible to administer the drug at 48- or 72-hour intervals. The maximum dose can then be increased from 1 to 1.5 mg/kg.

Intrathecal administration of amphotericin B is often associated with side effects and complications, and oral triazole treatment has largely replaced this form of management in patients with fungal meningitis. Intracisternal injection is to be preferred to lumbar intrathecal administration, because of the risk of chronic lumbar arachnoiditis, but even with this, the meninges become damaged and a subcutaneous reservoir may be required to deliver the drug to the CSF. Injections should be given two or three times per week, with the dose increased from 0.025 mg as tolerated to 0.25–1 mg.

3.17.6.2 Lipid-based formulations

It is not possible to define the total dosage requirements and duration of treatment needed to cure particular fungal infections. For this reason, the dosage of lipid-based formulations of amphotericin B should be adjusted to the individual requirements of each patient. It is prudent to give an

initial test dose of 1 mg to all patients (0.5 mg in children weighing less than 30 kg) because of the risk of acute anaphylactoid reactions.

The recommended dose of AmBisome for patients with systemic fungal infections is 3–5 mg/kg per day. For the empiric treatment of suspected fungal infection in febrile neutropenic patients, the recommended dose is 3 mg/kg per day. The dose is increased to 6 mg/kg per day in HIV-infected patients with cryptococcal meningitis. The liposomal formulation should be infused over a 2-hour period; if this is well tolerated, the infusion time can be reduced to 1 hour. If the patient experiences discomfort during the infusion, the duration of infusion may be increased. AmBisome has been administered to individual patients for up to 3 months, in a cumulative dosage of 15 g without significant toxic side effects. However, a cumulative dosage of 1–3 g given over 3–4 weeks has been more typical.

The recommended dosage of ABLC is 5 mg/kg per day and this should be infused at a rate of 2.5 mg/kg per hour. This formulation of amphotericin B has been administered to individual patients for up to 11 months, to a cumulative dosage of 50 g without significant toxic side effects.

It is usual to begin treatment with ABCD with a dose of 1 mg/kg per day, increasing to the recommended daily dose of 3–4 mg/kg as required. This formulation is infused at a rate of 1 mg/kg per hour.

3.17.7 Drug interactions

Amphotericin B can augment the nephrotoxic effects of many other drugs, including aminoglycoside antibiotics, cidofovir, foscarnet, pentamidine, cyclosporin, tacrolimus and cisplatin. These drugs should be co-administered with caution, and renal function monitored. Individuals receiving concurrent treatment with other nephrotoxic drugs are less likely to have renal dysfunction when receiving lipid-based formulations of amphotericin B. Concurrent use of amphotericin B and corticosteroids or digitalis glycosides may induce hypokalaemia. If given together, serum potassium levels should be monitored.

Amphotericin B and flucytosine have an additive or synergistic effect when used in combination against *Candida* species or *C. neoformans*. However, the nephrotoxic effects of amphotericin B can impair the excretion of flucytosine when these drugs are given together.

3.17.8 Toxicity and adverse reactions

3.17.8.1 Conventional formulation

Amphotericin B deoxycholate causes infusion-related reactions, including fever, chills, rigors, myalgias, arthralgias and hypotension in about 70% of those being treated. These unpleasant reactions differ from patient to

patient, but usually begin 1–3 hours after starting the infusion and last for about an hour. They are most common during the first week of treatment and often diminish thereafter. These reactions can be prevented or lessened by either slowing the rate of infusion or by giving 25 mg of parenteral hydrocortisone just before the infusion is started or 25–50 mg during it if a reaction occurs. Other useful pre-medications include diphenhydramine, meperidine, acetaminophen, and ibuprofen. Nausea and vomiting are less frequent side effects and, just as with fever and rigors, often diminish as treatment proceeds. Pre-medication with antiemetics is helpful.

Local phlebitis from intravenous administration of the conventional formulation of amphotericin B is common. If the drug is given through a peripheral vein, the infusion site should be changed for each dose. Phlebitis can be prevented or ameliorated by slowing the rate of the infusion or by adding a small amount of heparin (500–1000 units/L) to the solution.

The most serious toxic effect of amphotericin B is renal tubular damage. This is reported in 15–80% of patients, depending on the population studied. Most patients receiving the conventional formulation of the drug suffer some impairment of renal function, but it occurs most frequently in individuals given more than 0.5 mg/kg per day. Other risk factors include concomitant treatment with other nephrotoxic drugs and elevated baseline serum creatinine. Infants and children are less susceptible to the nephrotoxic effects of amphotericin B. Most cases of amphotericin B-induced nephrotoxicity are mild to moderate in nature, and renal function will return to almost normal levels in most patients several months after treatment has ceased. Severe nephrotoxicity is uncommon, but it too is often reversible. However, irreversible renal failure can occur.

Renal damage can be reduced or prevented by careful monitoring during treatment. In the stable patient, renal function should be measured twice weekly and the treatment interrupted or the dosage modified if the serum creatinine concentration exceeds 250 μmol/L. The risk of amphotericin B-induced renal impairment can be reduced by pre- and post-infusion hydration and sodium repletion with 500 mL saline, provided the clinical status of the patient will allow sodium loading.

Amphotericin B also causes renal wasting of potassium and magnesium due to renal tubular damage and this can reach symptomatic proportions. Serum concentrations of potassium and magnesium should be monitored at regular intervals. Their loss can be reduced by administering 10–20 mg of oral amiloride per day, but intravenous electrolyte supplements must be given if low levels are seen.

Patients treated for more than 2 weeks often develop a mild normochromic, normocytic anaemia. Blood transfusion may be of benefit, but is not usually required.

Pulmonary reactions, with acute dyspnoea, hypoxaemia and interstitial infiltrates, can occur when treatment with amphotericin B is combined with granulocyte transfusion. For this reason, it is advisable to separate the infusion of the drug from the time of granulocyte transfusion.

3.17.8.2 Lipid-based formulations

The lipid-associated formulations all lower the risk of amphotericin B-induced nephrotoxicity. Infusion-related adverse events, such as fever, rigors and hypotension, are also less common after administration of AmBisome or ABLC. Infusion-related reactions, such as hypoxia, are more common with ABCD treatment than with the other lipid-based formulations, and these have limited its clinical usefulness.

The immediate side effects that occur in response to AmBisome differ from those reported following administration of conventional formulation. Published reports have described a distinctive triad of infusion-related side effects (chest pain, dyspnoea and hypoxia; severe abdominal, flank or leg pain; vasodilation and urticaria) associated with AmBisome. These reactions, which have been reported in about 20% of patients, develop within a few minutes of the start of the infusion and can be severe. Slowing the infusion appears to have no effect, but symptoms disappear rapidly when the infusion is interrupted or intravenous diphenhydramine (1 mg/kg) is administered. Similar reactions have been documented with liposomal preparations of other drugs, which suggests that it is the liposome rather than the amphotericin B component that is responsible for these side effects. They can be prevented by pre-medication with diphenhydramine 30 minutes before the first infusion of AmBisome.

Infusion-related hypoxia has been reported in up to 25% of patients treated with ABCD and up to 20% treated with ABLC, but is usually reversible. Infusion-related reactions can be attenuated or prevented by pre-medication with acetaminophen, diphenhydramine, meperidine or hydrocortisone. Patients intolerant of one lipid-based formulation of amphotericin B may tolerate another well.

In addition to infusion-related reactions, patients treated with lipid-based formulations of amphotericin B can also experience renal failure, abnormalities of liver function tests, and severe hypersensitivity reactions, which may be exacerbated when changing from one formulation to another. However, these toxic side effects are less frequent and/or less intense when compared to conventional amphotericin B.

Nephrotoxicity is less common with all three lipid-based formulations of amphotericin B than with the conventional preparation. In comparative trials, the respective rates of renal damage (defined as twice baseline serum creatinine concentrations) have been as follows: ABCD, about 15%;

AmBisome, about 20%; ABLC, about 25%; and conventional amphotericin B, 30–50%. Administration of lipid-based formulations has been reported to stabilize, or even improve, renal function in patients with pre-existing renal impairment following conventional amphotericin B treatment, even when the dose is increased. Renal function should be monitored at regular intervals, particularly in patients receiving other nephrotoxic drugs.

Other adverse events associated with lipid-based formulations of amphotericin B have included elevations in liver transaminases, alkaline phosphatase, and serum bilirubin concentrations. Abnormalities of liver enzymes have been noted in 25–50% of patients treated with AmBisome, but these findings are reversible without drug discontinuation in most patients.

3.18 Other polyene compounds for topical administration

3.18.1 Natamycin

Natamycin is used for the topical treatment of oral and vaginal candidosis. It has also been used to treat corneal infections.

3.18.2 Nystatin

Nystatin is used for the topical treatment of oral, oesophageal, gastrointestinal, genital and cutaneous forms of candidosis. It has also been used to treat corneal infections and as gastrointestinal prophylaxis.

3.19 Flucytosine

Flucytosine (5-fluorocytosine) is a synthetic fluorinated analogue of cytosine.

3.19.1 Mechanism of action

Flucytosine is the only available antifungal agent that acts as an antimetabolite. It disrupts pyrimidine metabolism and thus the synthesis of DNA, RNA and proteins within susceptible fungal cells. The drug is transported into these cells by cytosine permeases and there converted by cytosine deaminase to 5-fluorouracil (5-FU). Fungi lacking the enzyme cytosine deaminase are intrinsically resistant to flucytosine. Two mechanisms then account for the antifungal activity. The first involves the conversion of 5-FU into 5-fluorouridine triphosphate, which is incorporated into fungal RNA in place of uridylic acid, with resulting inhibition of protein synthesis. The second mechanism involves the conversion of 5-FU to

5-fluorodeoxyuridine monophosphate, which blocks the enzyme thymidylate synthetase, causing inhibition of DNA synthesis.

3.19.2 Spectrum of activity

Flucytosine has a limited spectrum of activity. It includes *Candida* species, *Cryptococcus* species and some dematiaceous moulds causing chromoblastomycosis. Intrinsic resistance to flucytosine is uncommon among *Candida* species, only occurring in around 2–3% of isolates.

3.19.3 Acquired resistance

Induction of resistance during monotherapy with flucytosine is a common problem, and the drug is usually given in combination with other antifungal agents, including amphotericin B and fluconazole. The most common cause of resistance appears to be loss of the enzyme uridine monophosphate pyrophosphorylase.

3.19.4 Pharmacokinetics

Flucytosine is rapidly and almost completely absorbed after oral administration. In adults with normal renal function, an oral dose of 25 mg/kg given at 6-hour intervals will produce peak serum concentrations at 1–2 hours of 70–80 mg/L and trough concentrations of 30–40 mg/L. Absorption is slower in persons with impaired renal function but peak serum concentrations are higher. There is slight accumulation of the drug during the first 4 days of treatment, but thereafter peak serum concentrations remain almost constant. Peak levels are reached in a shorter period of 1–2 hours in persons who have received several previous doses of the drug. The protein binding of flucytosine is low (about 12%), resulting in high levels of circulating unbound drug.

Flucytosine is widely distributed, with levels in most tissues and fluids usually exceeding 50% of the simultaneous blood concentration. The main means of elimination is renal excretion of the unchanged drug. Flucytosine is excreted by means of glomerular filtration without significant tubular reabsorption. About 90% of an administered dose appears in the urine as intact drug and concentrations of 1000 mg/L are not unusual in persons with normal renal function. The serum half-life is between 3 and 6 hours, but may be greatly extended in renal failure, necessitating modification of the dosage regimen.

Less than 1% of an administered dose of flucytosine is metabolized in man. The drug is deaminated to 5-FU or dihydrofluorouracil and this could account for the myelotoxic effects associated with high serum

concentrations. The remainder of the compound is excreted unchanged in the urine.

3.19.5 Pharmaceutics

In the United States, flucytosine is available as oral tablets; elsewhere it is also available as an infusion for parenteral administration. The latter is supplied in 250-mL amounts containing 10 mg/mL in aqueous saline solution.

3.19.6 Therapeutic use

Because of the risk of resistance, flucytosine is rarely administered as a single drug. Its principal use is in combination with amphotericin B in the treatment of cryptococcosis and invasive forms of candidosis. Combination treatment with fluconazole has been shown to be effective in AIDS-associated cryptococcal meningitis.

3.19.7 Mode of administration

As absorption following oral administration is good, this is the preferred method of administration. If the patient cannot take the drug by mouth, the intravenous solution should be used. This can be administered through a venous catheter or as an intraperitoneal infusion. The drug should be infused over a 20–40 minutes period, provided this is balanced with the fluid requirements of the patient. Twice-weekly blood counts (total white cells and platelets) should be performed.

In adults with normal renal function, the usual starting dose of flucytosine is 50–150 mg/kg given as four divided doses at 6-hour intervals. If renal function is impaired, an initial dose of 25 mg/kg should be given, but the subsequent dose and interval should be adjusted so as to produce peak serum concentrations of 70–80 mg/L and trough concentrations of 30–40 mg/L. The drug accumulates in renal failure, necessitating modification of the dose regimen (see Table 3.4). The half-life of flucytosine is prolonged in small infants and the drug should be administered at 12- or 24-hour intervals.

3.19.8 Drug interactions

The antifungal activity of flucytosine is competitively inhibited by cytarabine (cytosine arabinoside), and the two drugs should not be administered together. Nephrotoxic drugs, such as amphotericin B, decrease the elimination of flucytosine, and blood levels of the latter drug should be monitored when these compounds are given together. Flucytosine is myelosuppressive (explained later) and should be used with caution in

Table 3.4 Regimens for administration of flucytosine in renal impairment

Creatinine clearance (mL/min)	*Individual dosage (mg/kg)*	*Dosage interval (h)*
40	25–37.5	6
40–20	25–37.5	12
20–10	25–37.5	24
<10	25–37.5	>24*

Renal function is considered to be normal when creatinine clearance is greater than 40–50 mL/min or concentration of creatinine in serum is less than 180 μmol/L; concentration of creatinine in serum is not reliable unless renal function is stable.
*Dosage interval must be based on serum drug concentration measurement at frequent intervals.

patients receiving other drugs, such as zidovudine and ganciclovir, that could enhance its immunosuppressive side effects.

3.19.9 Toxicity and adverse reactions

The most frequent side effects of flucytosine include nausea, diarrhoea, vomiting and abdominal pain. Serious adverse reactions include myelosuppression and hepatoxicity; these are more likely to occur if excessively high serum concentrations (>100 mg/L) are maintained. The effect is usually reversible if treatment is discontinued.

Elevated transaminase levels develop in some patients, but usually return to normal after the drug is discontinued. Rare cases of liver necrosis leading or contributing to death have been reported.

3.20 Griseofulvin

Griseofulvin is an antifungal antibiotic derived from a number of *Penicillium* species. It was the first oral drug for treatment of dermatophytosis.

3.20.1 Mechanism of action

Griseofulvin is a fungistatic drug that binds to microtubular proteins and inhibits fungal cell mitosis. It also acts as an inhibitor of nucleic acid synthesis.

3.20.2 Spectrum of activity

The spectrum of useful activity is restricted to dermatophytes (*E. floccosum*, *Microsporum* species and *Trichophyton* species).

3.20.3 Acquired resistance

Treatment failure attributable to the development of griseofulvin resistance is a rare problem in patients with dermatophytosis.

3.20.4 Pharmacokinetics

Absorption of griseofulvin from the gastrointestinal tract differs between individuals and is dependent on drug formulation, but is improved if the drug is given with a high-fat meal. Individual patients tend to achieve consistently high or low serum concentrations. Four hours after a single 1000-mg dose, blood levels in the region of 0–3.75 mg/L can be anticipated.

Griseofulvin appears in the stratum corneum within 4–8 hours of oral administration, as a result of drug secretion in perspiration. However, levels begin to fall soon after the drug is discontinued, and within 48–72 hours, it can no longer be detected. The mechanisms by which griseofulvin are delivered to the hair and nails are not well understood: deposition in newly formed cells is thought to be the major factor.

Griseofulvin is metabolized by the liver to 6-desmethyl griseofulvin, which is excreted in the urine. The drug has an elimination half-life from 9 to 21 hours. Less than 1% of a given dose appears in the urine in unchanged form.

3.20.5 Pharmaceutics

Griseofulvin is available as oral tablets and oral suspension.

3.20.6 Therapeutic use

Newer oral agents are often preferred for nail infections, but griseofulvin remains a useful second-line drug for moderate to severe dermatophytosis of the skin and scalp hair, where topical treatment is considered inappropriate or has failed.

3.20.7 Mode of administration

The recommended dose for children with tinea capitis is 20–25 mg/kg per day of the microsize formulation or 10–15 mg/kg per day of the ultramicrosize formulation. The normal dosage for nail infections in adults is 500–1000 mg/day; in children, the recommended dose is 10 mg/kg per day. The drug should be taken with a meal.

The duration of treatment will differ from patient to patient and depend upon the nature and extent of the infection. Children with tinea capitis should be treated for a period of 6–8 weeks. Some patients with *M. canis* scalp infection require treatment for up to 12 weeks. The drug should be

given for 6–9 months in fingernail and 12–18 months in toenail infections. Long-term cure rates for fingernail infections are around 70%, but griseofulvin is a disappointing drug in toenail disease where cure rates of only 30–40% can be expected.

3.20.8 Drug interactions

Absorption of griseofulvin is reduced in persons receiving concomitant treatment with phenobarbital. Griseofulvin has been shown to stimulate the metabolism of oral anticoagulants, oral contraceptives and cyclosporin, and may reduce their effectiveness.

3.20.9 Toxicity and adverse reactions

In most cases, prolonged courses and high doses of griseofulvin are well tolerated. Adverse effects occur in around 15% of patients, and include headaches, nausea, vomiting and abdominal discomfort. Urticarial reactions and erythematous rashes occur in occasional patients. The drug is contraindicated in patients with liver disease as hepatic function may deteriorate and the risk of other side effects is increased.

3.21 Other miscellaneous compounds for topical administration

In addition to the antifungal compounds described so far, a number of other drugs are available for topical use. Not all the preparations listed in the following sections are available in all countries.

3.21.1 Amorolfine hydrochloride

Amorolfine hydrochloride is a synthetic morpholine compound that inhibits fungal ergosterol biosynthesis. It can be used to treat tinea corporis, tinea cruris and tinea pedis, as well as localized distal onychomycosis. For fungal skin infections, amorolfine should be applied once daily for at least 2–3 weeks (up to 6 weeks for tinea pedis). For fingernail infections, amorolfine nail solution should be applied once or twice weekly for at least 6 months. Toenail infections should be treated for 9–12 months.

3.21.2 Butenafine hydrochloride

Butenafine hydrochloride is a synthetic benzylamine compound that acts as an ergosterol biosynthesis inhibitor. It is used for the treatment of tinea corporis, tinea cruris and tinea pedis. The dosage and duration of treatment varies according to the condition being treated.

3.21.3 Ciclopiroxolamine

Ciclopiroxolamine is a synthetic pyridinone compound used in tinea corporis, tinea cruris, tinea pedis, onychomycosis, cutaneous candidosis and pityriasis versicolor. For fungal skin infections, it should be applied twice daily for at least 2–4 weeks. For nail infections, the solution should be applied once daily for up to 12 months.

3.21.4 Haloprogin

Haloprogin is a halogenated phenolic compound that is effective in tinea corporis, tinea cruris, tinea pedis and pityriasis versicolor. The dosage and duration of treatment varies according to the condition being treated.

3.21.5 Tolnaftate

Tolnaftate is a thiocarbamate compound used in the treatment of tinea cruris and tinea pedis. The dosage and duration of treatment varies according to the condition being treated.

3.22 Prophylactic treatment for prevention of fungal infection

Because the timely and reliable diagnosis of fungal infections continues to be difficult, antifungal drug prophylaxis is often appropriate among high-risk individuals, such as patients with prolonged and severe neutropenia, allogeneic HSCT recipients, solid organ transplant recipients, critical-care patients and those with advanced HIV infection.

In several large clinical trials, fluconazole prophylaxis was found to reduce the incidence of superficial and deep forms of candidosis among HSCT recipients during the period prior to engraftment. As a result, its use (at a dosage of 400 mg/day during the period of neutropenia) has been endorsed in published guidelines for prevention of infection in allogeneic transplant recipients. Issues that remain to be resolved about fluconazole prophylaxis are the optimal duration (halting at the time of engraftment or continuing until the risk of GVHD has abated) and its role in autologous transplant recipients, non-ablative transplant recipients and in those with acute or chronic GVHD. Fluconazole prophylaxis has been associated with an increase in colonization and infection with *C. glabrata* and *C. krusei*, and it offers no protection against infection with *Aspergillus* species or other less common moulds.

Itraconazole has been evaluated as prophylaxis in several clinical trials, but conclusions regarding its effectiveness have been limited, because the risk for aspergillosis was too low in the populations studied. In most trials,

it has been found to be as effective as fluconazole for the prevention of candidosis.

Until recently, almost all trials of antifungal prophylaxis among allogeneic HSCT recipients have focused on the neutropenic period following transplantation. Two recent trials comparing fluconazole with itraconazole oral solution have extended the period of administration of antifungal treatment from the time of conditioning through to the period when acute GVHD is likely to occur. Although long-term itraconazole prophylaxis was associated with fewer cases of proven invasive fungal infections than fluconazole, survival rates were similar. However, itraconazole solution was associated with a higher incidence of gastrointestinal intolerance, dose-limiting toxicities and drug–drug interactions.

Two large prospective studies that were designed to evaluate the prophylactic use of posaconazole (at a dosage of 200 mg three times daily) have been reported. In the first trial, posaconazole was superior to either fluconazole or itraconazole for the prevention of invasive fungal infections in patients with prolonged neutropenia due to treatment of acute myeloid leukaemia. In the second trial, posaconazole was compared with fluconazole as prophylaxis against invasive fungal infections in HSCT recipients who were also receiving treatment for GVHD. Posaconazole was as effective as fluconazole in preventing all fungal infections and was superior in preventing invasive aspergillosis. There were fewer deaths in the posaconazole group, and the drug was well tolerated and relatively safe.

A large multicentre comparison of voriconazole (at a dosage of 200 mg twice daily) with fluconazole for the prevention of invasive fungal infection after myeloablative allogeneic HSCT has recently been published. In contrast to earlier prophylaxis trials, fungal free survival (FFS), rather than invasive fungal infection, incidence was chosen as the primary endpoint. FFS rates were similar in the two treatment groups at 6 months. Relapse-free and overall survival rates were also similar. There was a trend to fewer *Aspergillus* infections and less empirical antifungal drug use in the voriconazole group. The rates of adverse events were similar in both groups.

Micafungin was compared with fluconazole as prophylaxis in neutropenic patients undergoing HSCT. Both drugs were equally effective in preventing candidosis, and micafungin showed a non-significant trend towards a decreased incidence of aspergillosis. The major limitation of the echinocandins is the requirement for intravenous administration, making their long-term use as prophylaxis difficult.

Fluconazole prophylaxis (at a dosage of 400 mg/day) also appears to be effective in preventing candidosis among some high-risk non-neutropenic

patient populations. These include surgical patients with recurrent gastrointestinal perforations or anastomotic leakages, liver and pancreatic transplant recipients, and low-birth-weight infants. The need for prophylaxis in these different groups should be assessed on the basis of institutional rates of invasive candidosis and should be targeted towards high-risk patients, such as infants whose birth weight is <1000 g.

Prophylaxis with trimethoprim-sulphamethoxazole is the standard of care for prevention of *P. jirovecii* infection in both HIV-infected and -uninfected immunocompromised patients. Several clinical trials, performed in developed countries in the 1990s, demonstrated that long-term prophylaxis with either fluconazole or itraconazole reduced the risk for development of cryptococcosis in persons with HIV infection, but did not provide an overall survival benefit. As a result, routine prophylaxis with these drugs was never recommended. In developing countries, where cryptococcosis has a higher incidence and a higher case-fatality rate, clinical trials may be more likely to demonstrate a benefit for antifungal prophylaxis in preventing cryptococcosis and other opportunistic fungal infections among HIV-infected persons.

3.23 Empirical treatment of suspected fungal infection in the neutropenic patient

Empirical treatment can be defined as the administration of an antifungal agent to a neutropenic patient with persistent fever that is without an identified source and is unresponsive to 4–7 days of treatment with appropriate antibacterial agents. The objective of this approach is to ensure that all patients with a possible invasive fungal infection receive appropriate treatment early in the course of the disease. Although it has become the standard practice of care worldwide and has been endorsed in consensus guidelines, this approach has resulted in significant over-treatment and expenditure (given the low positive predictive value of persistent or relapsing fever for diagnosing an invasive fungal infection).

Two randomized prospective clinical trials in the 1980s first showed that empirical treatment with amphotericin B was associated with a trend towards fewer invasive fungal infections in antibiotic-treated neutropenic patients with persistent fever. In the mid-1990s, several large clinical trials in which composite endpoints (including resolution of fever, survival and prevention of infection) were used indicated that lipid formulations of amphotericin B are as effective, but safer than the conventional preparation. More recently, a large randomized multicentre trial with composite endpoints indicated that caspofungin was at least as effective as liposomal amphotericin B for empirical treatment of persistently febrile patients

with neutropenia. In addition, caspofungin proved to be significantly better tolerated. Another large multicentre trial indicated that although the number of patients discontinuing voriconazole treatment because of toxic effects was similar to the number discontinuing liposomal amphotericin B, the number discontinuing treatment because of a lack of efficacy was significantly lower among the group receiving polyene treatment.

3.24 Pre-emptive antifungal treatment

There are many causes of fever in neutropenic patients (e.g. bacterial infections, transfusion reactions, drug reactions, tissue necrosis and growth factors). Thus, although fever in a neutropenic patient should prompt a meticulous evaluation, it has been argued that it should no longer be used as the sole criterion for initiation of empirical antifungal treatment. The term 'pre-emptive antifungal treatment' has been introduced to describe a new approach that is similar to empirical treatment in that the objective is to treat a suspected early infection. However, this approach makes use of new non-culture-based diagnostic methods, such as antigen tests, detection of (1-3)-β-D-glucan and detection of fungal DNA, in conjunction with modern imaging procedures, such as high-resolution CT scanning, to select those febrile neutropenic patients at greatest risk for developing an invasive fungal infection. Prospective studies that compare competing diagnostic and treatment algorithms are now being conducted to delineate the optimum screening strategies for particular patient populations. These studies should permit us to determine the impact of more targeted approaches, such as pre-emptive treatment, both in terms of clinical outcome and cost-effectiveness.

Pre-emptive antifungal treatment may also be useful in cryptococcosis. Most patients with cryptococcal meningitis in sub-Saharan Africa present late in the course of disease, often with very low CD4 T-lymphocyte counts and with signs of advanced infection; survival rates are poor. Pre-emptive treatment may be beneficial among asymptomatic or mildly symptomatic individuals with a positive serum cryptococcal antigen test. Identifying antigen-positive persons with few or no symptoms would allow for early antifungal treatment with an oral azole, such as fluconazole, which is widely available and inexpensive.

3.25 Combination antifungal treatment of invasive fungal infections

To date, the benefits of combination antifungal treatment have been difficult to prove for invasive fungal infections other than cryptococcal

meningitis. However, several factors have stimulated interest in evaluating the potential of drug combinations for difficult-to-treat infections such as aspergillosis. These include the recent introduction of a new class of antifungal agents (the echinocandins), the development of extended-spectrum triazoles (such as posaconazole and voriconazole) and much better tolerated lipid formulations of amphotericin B. The potential advantages of combination treatment are several: broader spectrum of action, additive or synergistic effects when several agents with different mechanisms of action are used together, lowered risk of drug resistance emerging during treatment and reduced toxicities if doses of single agents can be reduced when given in combination while preserving clinical effectiveness. The disadvantages of combination treatment include increased cost, increased toxicities when high dosages of several drugs are used together, increased potential for drug–drug interactions, and potential for antagonistic interactions between the different agents.

Substantial in vitro and in vivo animal model data suggest that the most promising drug combinations are an extended-spectrum triazole plus an echinocandin and a polyene plus an echinocandin. Both these combinations exploit the different mechanisms of action of the individual agents. Clinical reports of combination treatment of invasive aspergillosis are beginning to accumulate, but at this time consist of retrospective evaluations and prospective, non-comparative trials. To date, the response rates reported in most published combination treatment studies do not suggest large gains over single agent treatment. Furthermore, these reports have been notable for the lack of a sustained survival advantage following combination treatment. Nonetheless, the clinical use of combination treatment for difficult-to-treat infections, such as aspergillosis, appears to be increasing, despite the absence of a definitive trial demonstrating its benefit.

3.26 Laboratory monitoring

3.26.1 Antifungal drug susceptibility testing

As with antibacterial drugs, tests designed to ascertain the minimum amount of an antifungal compound needed to inhibit the growth of fungal strains in culture (minimum inhibitory concentration or MIC) are often assumed to be the most dependable means of determining the relative effectiveness of different antifungal agents and of detecting the development of drug-resistant strains. In addition, it is often assumed that the clinical outcome of treatment can be predicted from the results of in vitro testing of a patient's isolate against a panel of potentially useful agents. Such an approach has become more reasonable with the development of reliable and

reproducible reference procedures for in vitro testing of antifungal agents and the demonstration of correlations with clinical outcome, at least for some forms of candidosis. However, the pitfalls of assuming a correlation between the results of susceptibility testing of antifungal drugs and organisms in vitro and outcome in vivo should not be underestimated.

Two independent standards for susceptibility testing of *Candida* species have been devised and validated: the US Clinical and Laboratory Standards Institute (CLSI) method and the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) method. The CLSI has developed reference methods for broth macrodilution and microdilution susceptibility testing for *Candida* and *Cryptococcus* species, as well as an agar disc diffusion method. The CLSI established interpretive breakpoints for the triazole agents fluconazole, itraconazole and voriconazole, and the echinocandins, anidulafungin, caspofungin and micafungin, to be applied to all *Candida* species.

The EUCAST has also developed a broth microdilution method for *Candida* species and established species-specific breakpoints for fluconazole, itraconazole and voriconazole to ensure a more sensitive means of detecting the emergence of resistance. Currently, EUCAST breakpoints have been developed and validated only for *C. albicans*, *C. tropicalis* and *C. parapsilosis*. Although good agreement has been documented between the CLSI and EUCAST broth microdilution methods, CLSI breakpoints should not be used to interpret MICs obtained by the EUCAST method. The latter are consistently lower, and falsely susceptible results could be reported.

The CLSI and EUCAST have also developed standardized broth microdilution methods for the testing of conidium-forming moulds, including *Aspergillus* species, *Fusarium* species and *Scedosporium* species. However, no interpretive breakpoints have been established for any species of mould.

Antifungal susceptibility testing is often helpful for isolates of *Candida* species (particularly for species other than *C. albicans*) from deep sites, but not at present for other fungi or settings. Testing of oropharyngeal isolates of *Candida* species from patients who have failed to respond to standard azole treatment can help to distinguish failures due to drug resistance from other causes. With mould isolates, susceptibility testing is currently most useful for detecting resistance or outliers based on wild-type distributions. The CLSI and EUCAST have proposed identical epidemiological cutoffs for itraconazole, posaconazole and voriconazole with *A. fumigatus*. This is an important development that will enhance surveillance of resistance and facilitate the eventual development of interpretive breakpoints.

3.26.2 Monitoring of blood concentrations of antifungal drugs

Many antifungal drugs show marked variation in blood concentrations due to inconsistent absorption, metabolism, elimination or interaction with concomitant medications. Therapeutic monitoring of blood levels is a valuable tool for assessing whether adequate concentrations are being achieved at the site of infection and can be important in optimizing drug choice and dosing decisions.

Validated analytical methods have been developed for each of the antifungal drugs in common use. Both microbiological and chromatographic methods have been developed. In most cases, these methods give similar results. An exception to this rule is the triazole antifungal itraconazole, where microbiological results are two or three times higher than those observed with high-performance liquid chromatography. This is due the presence in samples of the active metabolite, hydroxyitraconazole.

Serum concentrations of fluconazole are predictable from dosing and organ function, and routine monitoring of drug levels is not required. Excessive concentrations have not been associated with unwanted side effects. Concentrations are unchanged in persons with AIDS and in HSCT recipients, and the reduction in concentration following concomitant administration with rifampicin is smaller than that seen with other azole antifungals.

Absorption of the capsule formulation of itraconazole after oral administration shows marked variation between individuals. Low blood concentrations can be anticipated in persons with AIDS and HSCT recipients, as well as in patients receiving concomitant treatment with rifampicin. Because low concentrations of itraconazole are often predictive of treatment failure, measurement of blood levels is advisable in situations where the drug is used to treat or prevent serious invasive fungal infections, in patients in whom poor absorption or drug interactions are anticipated and when there is treatment failure or relapse. Serum concentrations of itraconazole should be determined only after the patient has reached the steady state, typically after 1–2 weeks. For prophylaxis, a target trough concentration of >0.5 mg/L has been proposed; for treatment, a trough of >1–2 mg/L is recommended. HPLC is the preferred method for determining itraconazole concentrations because microbiological methods detect the active metabolite, hydroxyitraconazole, in addition to the drug itself.

Voriconazole serum concentrations are highly variable, largely due to differences in the rate of metabolism between individuals, and it may be beneficial to monitor blood levels. For prophylaxis, a target trough concentration of >0.5 mg/L has been proposed; for treatment, a trough of

1–2 mg/L has been suggested. To avoid toxicity, trough concentrations of 5–6 mg/L should not be exceeded.

Variable levels of posaconazole have been noted in patients receiving treatment. As with itraconazole and voriconazole, there appears to be a relationship between posaconazole trough concentrations and clinical response, and measurement of drug levels may therefore be useful. For prophylaxis, a target trough concentration of >0.5 mg/L has been proposed; for treatment, a trough of >0.5–1.5 mg/L has been suggested.

There is no established relationship between efficacy and toxicity of the echinocandins or amphotericin B and serum concentrations of these drugs. For this reason, routine monitoring of blood levels during treatment is not indicated.

Regular monitoring of serum concentrations of flucytosine is advisable in all patients to reduce the risk of hepatotoxicity and haematological toxicity. This is essential when there is renal impairment or when the drug is given in combination with amphotericin B. Levels should be determined twice weekly or more frequently if renal function is changing. To avoid toxicity, a peak concentration of 100 mg/L should not be exceeded. In contrast, there are few data relating efficacy to blood levels for flucytosine. A reasonable goal is to maintain a trough concentration of >20–25 mg/L. Blood should be taken just before a dose of flucytosine is due and 2 hours after an oral dose or 30 minutes after an intravenous dose. In patients with renal impairment, peak concentrations tend to occur later after oral dosing.

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CHAPTER 4

Dermatophytosis

4.1 Introduction

The term dermatophytosis is used to describe infections of the skin, hair and nails due to a group of related filamentous fungi, the dermatophytes, which are also known as the ringworm fungi. These infections are among the most common causes of skin disease. They can be difficult to diagnose, however, and are often mistaken for other disorders. With the exception of nail infections, dermatophytosis responds rapidly and can be managed satisfactorily if treated correctly.

The clinical presentation of these infections depends on several factors, including the site of infection, the immunological response of the host and the species of infecting fungus. In most forms of dermatophytosis, the fungus is confined to the superficial stratum corneum, nails and hair. However, deeper infection involving the dermis can occur, as in kerion, and this can result in the formation of suppurative lesions.

Infections caused by dermatophytes are named according to the anatomical site(s) involved: tinea capitis (scalp), tinea corporis (trunk, legs and arms), tinea cruris (groin), tinea pedis (feet), tinea manuum (hands) and tinea unguium (nails). The term tinea facei is sometimes used to describe a subset of tinea corporis affecting only the skin of the face, while tinea barbae refers to dermatophyte infections of beard and moustache areas of the face. The term tinea incognito is used to describe infections that do not show any of the usual characteristic features of dermatophytosis, often because of the inappropriate use of topical corticosteroids. In developed countries with a temperate climate, tinea pedis is the most common

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clinical form of dermatophytosis. By contrast, in the tropics, tinea capitis and tinea corporis are the most prevalent.

4.2 The causal organisms and their habitat

There are three closely related genera of dermatophytes, *Trichophyton*, *Microsporum* and *Epidermophyton*. Of the 30 or so species that are recognized at present, some are worldwide in distribution, but others are restricted to particular continents or regions. About 10 species are common causes of human infection.

The dermatophytes are termed geophilic, zoophilic or anthropophilic depending upon whether their normal habitat is the soil, an animal or man. Members of all three groups can cause human infection, but their different natural reservoirs have important epidemiological implications in relation to the acquisition, site and spread of human infection.

Although the geophilic group of dermatophytes can cause infection in both animals and humans, their normal habitat is the soil. Members of the anthropophilic and zoophilic groups are thought to have evolved from these and other keratinophilic soil-inhabiting fungi, different species having adapted to different natural hosts. Individual members of the zoophilic group are often associated with a particular animal host, for instance *Microsporum canis* with cats and dogs, *Trichophyton mentagrophytes* with rodents and *Trichophyton verrucosum* with cattle. However, these organisms can also spread to humans.

Those dermatophytes for which humans are the usual host are termed anthropophilic. These species can be divided into those that are common causes of scalp infections, for instance *Microsporum audouinii* and *Trichophyton tonsurans*, and those that cause foot and nail infections, for instance *Epidermophyton floccosum*, *Trichophyton interdigitale* and *Trichophyton rubrum*. The anthropophilic species are the most highly specialized group of dermatophytes. They rarely infect other animals and often show a strong preference for a particular body site, only occasionally being found in other regions. For instance, *M. audouinii* infects scalp hair, but is seldom found on the skin.

The dermatophytes are keratinophilic fungi that are normally found growing only in the dead keratinized tissue of the stratum corneum of the skin, within and around the scalp hair, and in the nails. In these tissues, dermatophyte growth is restricted to the production of hyphae, which branch and segment into chains of spores called arthroconidia (or arthrospores). Arthroconidia are the main means of dissemination and propagation of the fungus. Although arthroconidia are common in skin

and in nail, hyphae may be present without arthroconidia formation, irrespective of the species involved. In hair, the type and extent of invasion varies according to the species of dermatophyte, and this affects the clinical presentation. The hyphae and arthroconidia of some species of dermatophytes remain strictly within the hair shaft (endothrix), while others form a sheath of arthroconidia around the outside of the shaft (ectothrix). In endothrix infection, the hair shaft is almost completely destroyed and breaks off at, or below, the mouth of the follicle. In ectothrix infection, the hair remains sufficiently robust to stay intact to a length of 2–3 mm above the mouth of the follicle. The variations in the form of hair invasion are not specific; however, as with infections of the skin and nail, culture is required to identify the species of fungus causing the infection.

4.3 Epidemiology

Unlike most other fungal infections, dermatophytosis is a contagious, host-to-host transmissible disease of humans and animals. Infection with an anthropophilic dermatophyte is acquired by direct or indirect contact with an infected individual. Indirect transfer may occur via the floors of communal bathing facilities, or on shoes, clothing, brushes, towels, bedding and other fomites. Dermatophyte arthroconidia can remain viable in the environment for long periods of time and the interval between deposition and transfer to another host may be considerable.

Infection with a zoophilic *Microsporum* and *Trichophyton* species is often the result of direct animal-to-human contact. These dermatophytes may then be transmitted among humans to a limited extent. Infection can also occur as a result of environmental contamination by an infected domestic pet, or when buildings, gates, fence posts or implements are contaminated by farm animals. Infection with geophilic dermatophytes, such as *Microsporum gypseum*, is uncommon and involves transmission of a soil-borne inoculum to humans or other animals. Occasional outbreaks of human disease, originating from contaminated soil, have been reported.

Dermatophytosis is among the most common dermatological conditions in industrialized countries. However, because many cases are never brought to medical attention, reliable incidence and prevalence figures are difficult to obtain. The US population, at large, has an estimated 10–20% lifetime risk of acquiring a dermatophyte infection. Furthermore, dermatophytosis has been estimated to account for around 4 million physician visits in the United States each year between 1995 and 2004. Infections of the scalp, trunk and limbs, feet and nails each accounted for about 20%

of the total; more than 70% of visits for nail infections occurred among those older than 45 years.

The prevalence of specific dermatophyte species differs from one geographic location to another and has varied over time, due to factors such as changing social conditions and standards of hygiene, movement of populations and the introduction of new treatments. In Northern Europe, *T. rubrum* and *T. interdigitale* are predominant, accounting for 80–90% of dermatophytes isolated. In contrast, in most of central and southern Europe and in parts of South America, *M. canis* is the principal dermatophyte isolated from human infections. In North America, *T. rubrum* and *T. tonsurans* are the principal aetiological agents, with recent reports indicating that the latter species accounts for almost 50% of dermatophytes isolated. *Trichophyton violaceum* is common in eastern and southern Europe, North Africa, and parts of India.

4.4 Laboratory diagnosis of dermatophytosis

Superficial fungal infections, such as dermatophytosis, may present with characteristic lesions, but where this is not the case, laboratory investigations are essential for correct diagnosis and management. Laboratory confirmation of a suspected dermatophyte infection should always be obtained before committing a patient to a course of treatment that will last for several weeks, may incur substantial cost and have potential side effects. In addition, isolation and identification of the aetiological agent can assist in controlling infections due to household pets or other domesticated animals where there is an ongoing source of inoculum.

A laboratory diagnosis may be made in two ways: by detection of the fungus in a specimen in clinical material through direct microscopic examination and by isolation of the aetiological agent in culture. Microscopy and culture should both be attempted on all occasions. If, however, there is insufficient clinical material for both procedures, the former should be performed.

It has been estimated that as much as 50% of suspicious material sampled from infected persons may not contain any fungus. It is important, therefore, to employ the correct procedures when taking specimens of skin, nails or hair for laboratory investigation (see Chapter 2). It is essential to provide sufficient details about the patient. In addition to specifying age, gender and the body site from which the specimen was taken, details on any underlying illness, drug treatment, contact with animals, recent travel and previous residence abroad should also be given.

The detection of fungal hyphae and/or arthroconidia by direct microscopic examination of clinical material is often sufficient for the diagnosis of a dermatophyte infection but, apart from hair specimens, gives no indication as to the species involved. With hair, the size and disposition of the arthroconidia can indicate which group of species is involved. Most specimens can be examined satisfactorily in wet mounts after partial digestion with 10–20% potassium hydroxide solution. Detection of fungal elements may be enhanced by the use of fluorescent brighteners, such as Calcofluor white (Sigma-Aldrich, St. Louis, Missouri, USA), that bind to fungal cell walls.

Culture is a more reliable method of diagnosis than microscopic examination. Because it permits the species of dermatophyte to be determined, it can provide information as to the source of the infection and aid in the selection of the most appropriate form of treatment. The results of culture can be positive even if microscopic examination is negative, but it is more common for microscopic examination to be positive while culture is negative.

Skin, nail and hair specimens can be cultured on glucose peptone (Sabouraud's dextrose) or malt agar supplemented with chloramphenicol to reduce bacterial growth and cycloheximide (actidione) to suppress moulds other than dermatophytes. Most dermatophyte cultures can be identified after 1–2 weeks' incubation at 28–30°C. It is not unusual to isolate moulds other than dermatophytes from abnormal skin and nails. In many cases, these are casual, transient contaminants and direct microscopic examination of clinical material is negative. However, certain moulds are capable of causing infection, and when this is so, it is important that their significance is recognized. These infections are described in Chapters 6 and 7.

Numerous nucleic acid amplification methods have been described for the direct detection of dermatophyte DNA in skin and nail specimens. To date, none has become established as a routine diagnostic procedure. Several regions within the dermatophyte genome have been evaluated as potential targets for detection, but most effort has focused on the internal transcribed spacer regions of the ribosomal DNA gene complex.

4.5 Tinea capitis

4.5.1 Definition

The term tinea capitis is used to refer to dermatophyte infections of the hair of the scalp and scalp skin. Individuals who have neither symptoms nor signs, but from whose scalps dermatophytes can be isolated, are described as 'carriers'.

4.5.2 Geographical distribution

The condition is worldwide in distribution, but is most prevalent in Africa, Asia and southern and eastern Europe, where it is the most common form of dermatophytosis. Favus used to be worldwide in distribution, but is now confined to North Africa, the Middle East and parts of southern and eastern Europe.

4.5.3 Causal organisms

Tinea capitis is caused by a number of *Microsporum* and *Trichophyton* species. The predominant aetiological agents of anthropophilic tinea capitis differ from one region to another and can change within a particular region over time. *T. tonsurans* is now predominant in the United States, the United Kingdom and Brazil, where it accounts for 50–90% of cases of tinea capitis. Other causes of anthropophilic tinea capitis include *T. violaceum*, which is predominant in the Middle East and southern Asia, and *M. audouinii* and *Trichophyton soudanense*, which are important in parts of Africa.

The most common zoophilic cause of tinea capitis is *M. canis,* which is spread from cats and dogs. Sporadic cases of *M. canis* infection occur worldwide. *M. canis* has declined in importance as a cause of tinea capitis in the United Kingdom, but it remains the principal dermatophyte isolated from human infections in most of central and southern Europe. *T. verrucosum*, which is acquired from cattle, has also declined in relative importance as a cause of tinea capitis in the United Kingdom. Among the other less common causes of zoophilic tinea capitis are *Trichophyton equinum* (acquired from horses) and *T. mentagrophytes* (acquired from rodents).

The anthropophilic dermatophyte *Trichophyton schoenleinii* is the sole aetiological agent of favus, although infections with other dermatophytes, such as *M. gypseum*, *T. verrucosum* and *T. violaceum*, can sometimes produce somewhat similar lesions. Although *T. schoenleinii* is anthropophilic, favus is less contagious than other forms of tinea capitis due to *Microsporum* or other *Trichophyton* species. It is usually contracted in childhood and may persist into adult life. Debilitated or malnourished children, or children suffering from a chronic disease such as tuberculosis, are more susceptible to this infection. Instances have been reported in which several generations of the same family were affected.

4.5.4 Epidemiology

Tinea capitis due to anthropophilic *Microsporum* and *Trichophyton* species is a contagious disease that remains endemic in many resource-limited

countries because of poor hygiene, overcrowding and lack of affordable treatment. Having declined in prevalence since 1960, the number of cases of anthropophilic tinea capitis has increased since 1980 among urban populations in many European countries, possibly as a result of increased immigration from North Africa and the Caribbean islands. The largest increases have been noted with *T. tonsurans* and *T. violaceum* in the United Kingdom, and with *M. audouinii* and *T. soudanense* in France.

Anthropophilic tinea capitis is primarily a disease of children, being more common in males than females, and most prevalent between 6 and 10 years of age. The disease seldom persists beyond the age of 16. Large outbreaks can occur in schools or other places where children are congregated. Spread is thought to be mediated through direct contact, although indirect spread through contaminated fomites is sometimes a factor in the dissemination of infection. Little is known about the risk factors for anthropophilic tinea capitis. Among those that have been suggested are overcrowding within households or schools, use of shared brushes and combs, social and cultural factors, and ethnic group. Although the current spread of *T. tonsurans* in the United States and Europe is most often seen in black communities, this infection also occurs among children from other ethnic backgrounds.

Sporadic cases of tinea capitis due to zoophilic species of *Microsporum* and *Trichophyton* occur worldwide. However, these organisms are seldom responsible for more than minor outbreaks of human infection. Household pets are a common source of *M. canis* infection, but feral cats are another prolific source.

There are few reliable figures for the incidence or prevalence of tinea capitis. In the United States, *T. tonsurans* infection has been estimated to occur in 3–13% of school-aged children, with carriers occurring in as many as 34% of household contacts. In a recent prospective, cross-sectional surveillance of more than 10,000 children aged 6–11 from one large metropolitan setting in the United States, 6.6% were found to have *T. tonsurans* infection of the scalp. Infection rates at participating schools ranged from 0 to 19%, but exceeded 30% among the youngest children in several schools. Black children demonstrated the highest rates of infection (13%), while infection rates for Hispanic (1.6%) and white children (1.1%) were markedly lower.

4.5.5 Clinical manifestations

The clinical manifestations of tinea capitis are varied and can range from mild scaling (similar to seborrhoeic dermatitis) and hair loss to

widespread alopecia or, less commonly, to a highly inflammatory suppurating lesion termed a kerion. The latter condition is usually caused by infection with a zoophilic dermatophyte. A kerion may be limited in extent, but a large confluent lesion can develop, which involves most of the scalp. If diagnosis and treatment are delayed, kerion may cause a scarring alopecia.

M. canis and *M. audouinii* invade the scalp hair in a distinctive manner. The lesions consist of one or more discrete, round or oval, erythematous patches of scaling and hair loss (2–6 cm in diameter). These may extend with time to involve the entire scalp. The hairs in these lesions are all parasitized and most of them are broken about 2–3 mm above the surface of the scalp. The hair surface is covered with a dense mass of small (2–3 μm diameter) arthroconidia (ectothrix infection). Infected hairs show green fluorescence under Wood's light.

T. tonsurans, *T. violaceum* and *T. soudanense* infections are acquired from infected humans. The typical lesions are irregular, erythematous patches of scaling (0.5–1 cm in diameter). The lesions are not well demarcated. The affected hairs often break off at the scalp surface, leading to alopecia and giving a black-dot appearance in dark-haired patients. Other hairs are broken off 1–2 mm from the scalp surface and are often obscured under a layer of scales. Infected hairs are filled with arthroconidia (endothrix infection) and do not fluoresce under Wood's light.

T. mentagrophytes and *T. verrucosum* infections of the scalp are acquired from infected animals. The typical lesions are single and are erythematous and pustular. Kerion formation is common. Infected hairs are covered with chains of large arthroconidia but do not fluoresce under Wood's light.

The main clinical manifestations of favus are the formation of crusted, inflamed patches on the scalp, with permanent hair loss due to follicular scarring. The scalp itches and gives off a foetid odour. The crusts (scutula) develop around the follicular openings and can fuse to cover large areas of the scalp. Longstanding favus can lead to permanent diffuse patches of alopecia. Although infected, the hairs tend not to break off and can grow to normal length. Infected hairs give off a dull green fluorescence under Wood's light.

Both adults and children living with cases of anthropophilic tinea capitis have been found to harbour organisms without showing symptoms. The 'carrier state' occurs primarily in adults. The patient usually remains asymptomatic, but the infection may present as a slight scaling resembling dandruff or seborrhoeic dermatitis.

4.5.6 Differential diagnosis

Tinea capitis is often difficult to distinguish from other causes of scaling (such as psoriasis and seborrhoeic dermatitis) or hair loss (such as alopecia areata and discoid lupus erythematosus). For this reason, Wood's light examination and laboratory tests should be performed in any patient with scaling scalp lesions or hair loss of undetermined origin. A kerion can be easily misdiagnosed as a bacterial abscess, and then inappropriately incised, drained and treated with antibiotics. Such treatment has no effect on the fungus. The longer the infection remains undiagnosed, the greater the risk of scarring alopecia. It is important that any pustular eruption, particularly of the scalp, is recognized as a possible fungal infection and investigated accordingly.

Other diseases that may cause a similar clinical picture to that of tinea capitis include atopic dermatitis, bacterial folliculitis, lichen planus, and trichotillomania.

4.5.7 Essential investigations and their interpretation

Specimens from the scalp should include hair roots, the contents of plugged follicles and skin scales. Except for favus, the distal portion of infected hair seldom contains any fungus. For this reason, cut hairs without roots are unsuitable for mycological investigation. One method that is useful for collecting material from the scalp is hair brush sampling.

Hairs infected with *M. audouinii* or *M. canis* produce a brilliant green fluorescence under Wood's light in a darkened room. However, in recent infections, or at the spreading margin of lesions, the fluorescent part of the hair may not yet have emerged from the follicle, and fluorescence can only be detected after the hair is plucked. *T. schoenleinii* causes a pale dull green fluorescence of infected hair. The fluorescent hairs tend to be long, in contrast to the short hair stumps characteristic of *Microsporum* infection. It is important to remember that creams and ointments applied to scalp lesions, as well as host tissue and exudates, can produce a pale bluish or purplish fluorescence under Wood's light.

Direct microscopic examination of infected material should reveal arthroconidia of the dermatophyte located outside (ectothrix) or inside (endothrix) the affected hair. The arthroconidia can be either small (2–4 μm in diameter) or large (up to 10 μm in diameter) in size. Skin scales will contain hyphae and arthroconidia. In *T. schoenleinii* infection (favus), loose chains of arthroconidia and air spaces are seen within the affected hairs. The scutulum (crust) consists of mycelium, neutrophils and epidermal cells.

Isolation of the aetiological agent in culture will permit the species of dermatophyte involved to be determined. This will provide information as to the source of the infection and aid the selection of appropriate treatment.

4.5.8 Management

If possible, specimens for mycological examination should be taken before starting treatment. Tinea capitis always requires systemic treatment because topical antifungal agents do not penetrate the hair follicles. Topical treatment may be used as an adjunct to systemic antifungal drugs.

An analysis of 21 randomized controlled clinical trials that evaluated systemic antifungal treatment in patients under the age of 18 indicated that newer agents, including terbinafine, itraconazole and fluconazole, are similar in efficacy to griseofulvin for tinea capitis caused by *Trichophyton* species. Although they may be more expensive, newer agents may be preferred because shorter treatment durations may improve treatment compliance. There was insufficient evidence on the use of systemic treatments in children with *Microsporum* infections to establish whether the newer agents were more efficacious than griseofulvin. Not all treatments for tinea capitis are available in paediatric formulations, but the drugs all appear to be reasonably safe (see Chapter 3).

Griseofulvin is safe in children and highly effective in the treatment of most forms of tinea capitis. The recommended dose is 20–25 mg/kg per day of the microsize formulation or 10–15 mg/kg per day of the ultramicrosize formulation. This should be given for a period of 6–8 weeks. The drug should be taken with a meal. Many patients require treatment for more than 8 weeks and the general rule is to treat the individual until the hair starts regrowing and fungal culture is negative. Infection with *M. canis* usually requires a longer duration of treatment compared with *T. tonsurans*.

Terbinafine is available in tablet form for treatment of tinea capitis. The recommended adult dose is 250 mg/day. The recommended paediatric dosage is 62.5 mg/day for children of less than 20 kg, 125 mg/day for those of 20–40 kg and 250 mg/day for those of more than 40 kg. Treatment should be continued for at least 4 weeks. Higher dosages, or more prolonged treatment, may be required for *M. canis* infections.

The oral granule formulation of terbinafine is licenced for the treatment of tinea capitis in children aged 4 years or older. The recommended dose is 250 mg/day in patients weighing more than 35 kg, 187.5 mg/day in those weighing 25–35 kg and 125 mg/day in those weighing less than

25 kg. The drug should be taken once daily with food, and treatment continued for 6 weeks. In a large randomized trial, the oral granule formulation of terbinafine was found to be superior in treating *T. tonsurans*, similar against *T. violaceum*, and less efficacious against *M. canis* when compared with griseofulvin oral suspension.

Although it is not currently licenced for this indication, several clinical trials have indicated that itraconazole is effective in paediatric tinea capitis. The usual dosage regimen is 5 mg/kg for 4 weeks. Pulse-dosing with itraconazole (in which 1 week of treatment at a dosage of 5 mg/kg per day is given each month for 2–4 months) also appears to be effective. High cure rates have been achieved in children with *T. tonsurans* infections when 1–3 pulses were used. Longer periods of treatment may be required for *M. canis* infections.

Fluconazole is licenced for use in children (although not for dermatophytosis) and is a useful alternative treatment for tinea capitis. The doses that have been used have ranged from 1.5 to 6 mg/kg per day for 4–6 weeks. There is some evidence to suggest that fluconazole (4 weeks) is as effective as griseofulvin (6 weeks) against *Trichophyton* infections.

Although topical antifungal agents do not have a role in the treatment of tinea capitis, they can be used as an adjunct to oral agents during the initial stages of treatment. Both ketoconazole (2%) and selenium sulphide (1–2%) shampoos have been found to reduce scalp carriage and shedding of organisms if used on a daily basis. Topical antifungal treatment is also recommended for asymptomatic carriers.

Oral antifungal treatment and the use of an antifungal shampoo decrease the risk of transmission, and most experts consider that children with tinea capitis can return to school once treatment has been initiated. In most cases, the child will have been at school for some time before the infection was detected and exclusion will be too late to prevent its spread to other children.

4.5.9 Prevention

To prevent spread or recurrence of anthropophilic infections, it is important to screen all members of infected households for tinea capitis. Contacts of infected children who are asymptomatic carriers can be an ongoing source of infection. These individuals should use selenium sulphide or ketoconazole shampoo every day, or every other day, until the patient who is being treated is cured. Contacts do not require oral antifungal treatment. It is also important to ensure that inanimate objects, such as combs, brushes and other hair accessories, are discarded or disinfected. The former is preferable.

Tinea capitis in schools presents a serious public health problem. It is important that children, parents and staff are informed about the infection and are reassured that the disease is treatable and not a sign of neglect. If more than two children in a class are infected, it is recommended that the remainder be screened after parental consent is obtained. Thereafter, carriers and infected children should be treated as described. Contacts without scalp infection but with cutaneous lesions can be treated with topical antifungal agents.

To prevent spread or recurrence of zoophilic tinea capitis, it is important to identify potential sources of infection and, in the case of household pets, take the animal for treatment of suspected dermatophytosis. *M. canis* infection in cats and dogs can often be detected with Wood's light examination. It is more difficult to detect and eliminate *T. verrucosum* infection in cattle, because infected hairs are not fluorescent and because the fungus can survive for long periods on hairs and skin scales that have been deposited on the walls of buildings and gates. Fungicidal washes have sometimes been effective in controlling the spread of this infection.

4.6 Tinea corporis

4.6.1 Definition

The term tinea corporis is used to refer to dermatophyte infections of the trunk, legs and arms, but excluding the groin, hands and feet.

4.6.2 Geographical distribution

The condition is worldwide in distribution, but is most prevalent in tropical and sub-tropical regions.

4.6.3 Causal organisms

Tinea corporis is caused by *E. floccosum* and many species of *Trichophyton* and *Microsporum*.

Tinea imbricata is a chronic infection of the glabrous skin caused by the anthropophilic dermatophyte *Trichophyton concentricum*. The disease is endemic to the Pacific Islands and Melanesia, but there have been occasional reports from South East Asia and Central and South America.

4.6.4 Epidemiology

Tinea corporis commonly occurs following contact with infected household pets or farm animals, but occasional cases result from contact with wild mammals or contaminated soil. Human-to-human spread of infection with geophilic or zoophilic species is unusual. *M. canis* is a frequent

cause of human infection, and *T. verrucosum* infection is sometimes seen in rural districts. Infection with anthropophilic species, such as *E. floccosum* or *T. rubrum,* often follows spread from another infected body site, such as the feet. Tinea corporis caused by *T. tonsurans* is sometimes seen in children with tinea capitis and their close contacts.

4.6.5 Clinical manifestations

Tinea corporis may affect any body site, but infections with zoophilic species are more likely to occur on exposed parts, such as the face, neck and arms. Patients may complain of mild pruritus and this may be exacerbated after exposure to the sun. The clinical manifestations are variable, depending on the species of fungus involved and the extent of progression, but in typical cases, round scaling lesions that are dry, erythematous and clearly circumscribed are seen. The fungus is more active at the margin of the lesions and hence this is more erythematous than the middle, which tends to heal earlier. As the first ring of advancing infection continues to spread outwards, it may become surrounded by one or more concentric rings or arcuate patterns. Adjacent lesions may fuse, producing gyrate patterns. In some instances, particularly when a zoophilic dermatophyte is involved, the lesion may be markedly inflamed and even pustular.

The lesions of tinea corporis are often more extensive, but less obvious, in immunosuppressed individuals. Patients with tinea corporis often have concurrent dermatophytosis of the scalp, beard or nails.

Tinea imbricata is characterized by the development of homogenous sheets or concentric rings of scaling. Lesions typically begin on the face and then spread to cover large parts of the affected person.

4.6.6 Differential diagnosis

Tinea corporis can be difficult to distinguish from other causes of erythematous, scaling skin lesions, such as atopic dermatitis, eczema, impetigo, lichen planus, pityriasis rosea, pityriasis versicolor, psoriasis, seborrhoeic dermatitis and syphilis. For this reason, laboratory tests should be performed in any patient with skin lesions of undetermined origin.

4.6.7 Essential investigations and their interpretation

Material for mycological investigation should be collected from the raised border of the lesion by scraping outwards with a blunt scalpel held perpendicular to the skin. If vesicles are present, the entire top should be submitted for examination.

Direct microscopic examination of infected material in 10–20% potassium hydroxide should reveal the branching hyphae characteristic of a dermatophyte infection.

Isolation of the aetiological agent in culture will permit the species of fungus involved to be determined. This will provide information as to the source of the infection and aid the selection of appropriate treatment.

4.6.8 Management

Topical antifungal preparations are the treatment of choice for localized lesions. Numerous imidazole compounds (including bifonazole, clotrimazole, econazole nitrate, isoconazole nitrate, miconazole nitrate, oxiconazole, sertaconazole nitrate, sulconazole nitrate, terconazole and tioconazole) and two allylamine compounds (naftifine and terbinafine) are available in different countries in a number of topical formulations. Other topical products used for treating tinea corporis include amorolfine hydrochloride, butenafine hydrochloride and ciclopiroxolamine. These drugs should be applied once or twice daily for 2–4 weeks. To prevent relapse, treatment should be continued for at least 1 week after the lesions have cleared and the medication should be applied at least 3 cm beyond the advancing margin of the lesion.

If the lesions are extensive or follicular structures are involved and the patient fails to respond to topical preparations, oral treatment is usually indicated. Itraconazole (100 mg/day for 2 weeks), fluconazole (150–300 mg once weekly for 2–4 weeks) and terbinafine (250 mg/day for 2–4 weeks) have all been reported to be effective.

It is important to avoid topical corticosteroids in the management of tinea corporis. While these agents will reduce itching, their use may inhibit the host response, promoting an exacerbation of inflammatory symptoms. Topical steroids, including preparations that contain a combination of a corticosteroid and an antifungal agent, should be avoided in the treatment of immunocompromised or diabetic patients.

4.6.9 Prevention

To prevent spread or recurrence of zoophilic tinea corporis, it is important to identify potential sources of infection and, in the case of household pets, take the animal for treatment of suspected dermatophytosis. Infection with anthropophilic species, such as *T. rubrum*, sometimes follows spread from another infected body site, such as the scalp, feet, hands or nails. To prevent reinfection, these sites should be examined and treated if dermatophytosis is present. Tinea corporis can also result from close

body contact with other infected individuals, who should be identified and treated if possible.

4.7 Tinea cruris

4.7.1 Definition

The term tinea cruris is used to refer to dermatophyte infections of the groin, perianal and pubic region.

4.7.2 Geographical distribution

The condition is worldwide in distribution.

4.7.3 Causal organisms

The dermatophytes most often encountered in tinea cruris are the anthropophilic species, *T. rubrum* and *E. floccosum*.

4.7.4 Epidemiology

Tinea cruris is a common form of dermatophytosis. It is more prevalent in men than women. It usually occurs between the ages of 18 and 60, but is most common between the ages of 18 and 25 and between 40 and 60. The infection often follows spread from another infected site, such as the feet or nails, in the same individual. It may also be transmitted from person to person through direct contact, or through indirect contact via contaminated fomites, such as towels, clothing and bed linen. Tinea of the groin is a highly contagious condition and outbreaks of infection have sometimes occurred in schools and other closed groups, such as sports teams.

Maceration and occlusion of the skin in the groin give rise to warm moist conditions that favour the development of the infection. Other predisposing factors for tinea cruris include hyperhidrosis and diabetes mellitus.

4.7.5 Clinical manifestations

Tinea cruris usually presents as one or more rapidly spreading, erythematous lesions with central clearing on the inside of the thighs. The lesions, which tend to coalesce, have a raised erythematous border that encloses a brown area of scaling. Patients often complain of intense pruritus. Scratching may result in small satellite lesions that sometimes fuse with the primary lesion altering its outline. The infection may spread from the inside of the thigh to the scrotum, penis, natal cleft, and gluteal folds, as well as to the anterior and posterior aspects of the thighs. Localized scrotal infection is quite common: the clinical signs are often inconspicuous.

Tinea of the groin may spread to other skin folds, particularly the axillae, which may also be the primary site of infection. Interdigital infection of the hands or feet may also develop secondarily from the groin infection. Infection of the toe clefts may precede the development of tinea cruris.

4.7.6 Differential diagnosis

Tinea cruris can be difficult to distinguish from other causes of erythematous groin lesions, such as bacterial and *Candida* intertrigo, erythrasma, psoriasis and seborrhoeic dermatitis. Women with a crural dermatosis are often infected with *Candida*, whereas men are more likely to have a dermatophyte infection. For these reasons, laboratory tests should be performed in any patient with groin lesions of undetermined origin.

4.7.7 Essential investigations and their interpretation

Direct microscopic examination of infected material should reveal the branching hyphae characteristic of a dermatophyte infection. Isolation of the aetiological agent in culture will permit the species of fungus involved to be determined.

4.7.8 Management

Topical antifungal preparations are the treatment of choice for tinea cruris. Numerous imidazole compounds (including bifonazole, clotrimazole, econazole nitrate, isoconazole nitrate, miconazole nitrate, oxiconazole, sertaconazole nitrate, sulconazole nitrate, terconazole and tioconazole) and two allylamine compounds (naftifine and terbinafine) are available in different countries in a number of topical formulations. Other topical products used for treating tinea cruris include amorolfine hydrochloride, butenafine hydrochloride and ciclopiroxolamine. These drugs should be once or twice daily for at least 2 weeks. To prevent relapse, treatment should be continued for at least 1 week after the lesions have cleared.

If a patient has extensive lesions, or fails to respond to topical treatment, or has tinea pedis as well, oral treatment is usually indicated. Itraconazole (100 mg/day for 2 weeks), fluconazole (150–300 mg once weekly for 2–4 weeks) and terbinafine (250 mg/day for 2–4 weeks) have all been reported to be effective.

Tinea cruris recurs in about 20–25% of patients. If this happens, patients should be given further antifungal treatment and advice about non-pharmacological control measures should be repeated.

4.7.9 Prevention

To prevent reinfection following treatment, the patient should be advised to dry the groin thoroughly after bathing and to use separate towels to dry the groin and the rest of their body. The feet should be examined and treated if tinea pedis is present. Occlusive or synthetic garments should be avoided. If the patient is obese, weight loss may be of benefit by reducing chafing and sweating.

4.8 Tinea pedis

4.8.1 Definition

The term tinea pedis is used to refer to dermatophyte infections of the feet. These infections often involve the interdigital spaces, but chronic diffuse desquamation can affect the entire sole.

4.8.2 Geographical distribution

The condition is worldwide in distribution.

4.8.3 Causal organisms

The anthropophilic dermatophytes *T. rubrum* and *T. interdigitale* are the commonest causes of tinea pedis in Europe and North America. *E. floccosum* is an uncommon cause of tinea pedis.

4.8.4 Epidemiology

Tinea pedis is a contagious condition and is easily spread from person to person. Transfer within households has been reported, but the main spread occurs in communal baths and showers. The infection is usually acquired by walking barefoot on contaminated floors. Tinea pedis is a modern affliction, associated with the wearing of occlusive footwear. Heat and moisture are essential for the growth of the fungus, and it is notable that infections in temperate climates tend to be more common during the summer months.

Tinea pedis is more commonly seen in men than women. It often begins in late childhood or adolescence and is most frequent between the ages of 25 and 50. The disease is most prevalent among individuals frequenting swimming pools and fitness centres, involved in sporting activities, or living in closed communities (such as residential schools and prisons) where bathing facilities are shared. Tinea pedis has been found to affect about 15–20% of adult males. Among industrial workers such as coal miners, who are exposed on a daily basis to infection, the prevalence of infection

is much higher. It has been estimated that about one-third of patients with tinea pedis also have a fungal nail infection.

4.8.5 Clinical manifestations

Tinea pedis may be unilateral, but bilateral involvement is more common. Three main clinical forms may be distinguished: acute or chronic interdigital infection, chronic hyperkeratotic (moccasin or dry type) infection of the soles and lateral borders of the feet, and vesicular (inflammatory) infection of the instep and sole. Occasional patients develop an acute ulcerative infection of the soles. It is not unusual for patients to develop a combined clinical presentation involving two or even three of these forms of tinea pedis.

Acute or chronic interdigital infection is the commonest form of tinea pedis and is characterized by itching, peeling, maceration and fissuring of the toe webs. The skin beneath the whitish build-up of debris may appear red and weeping. A foul odour is sometimes present. The cleft between the fourth and fifth toes is most often involved, but the infection may spread to adjacent areas of the feet, including the toenails. In a patient with supposed chronic tinea pedis, an absence of nail involvement makes the diagnosis of dermatophytosis questionable.

Chronic hyperkeratotic infection is characterized by areas of pink, mildly erythematous skin covered with fine white scaling. Vesicles and pustules are absent. Hyperkeratosis is usually limited to the heels, soles and lateral borders of the feet. The distribution of the infection may be patchy or involve the entire weight-bearing surface, in which case the disease is termed 'moccasin' or 'dry type' tinea pedis. The condition is usually bilateral and may be asymptomatic.

Vesicular or vesicobullous tinea pedis is characterized by the development of vesicles, usually beginning on the sole, the instep and the interdigital clefts. The eruptions vary in size, may be isolated or coalesce into vesicles or bullae, and are initially filled with a clear fluid. After rupturing, the lesions dry, leaving a ragged ring-like border. The disease may resolve without treatment, but often recurs.

Ulcerative tinea pedis is characterized by maceration and ulceration of large areas of the soles. White hyperkeratosis and strong odour are common. Bacterial superinfection, usually with gram-negative organisms, is frequent and should be taken into account in treating this condition.

In certain parts of the world, concomitant mould, *Candida* and/or bacterial infection is relatively common in patients with tinea pedis. These conditions usually represent secondary infection following fissuring or

maceration of a toe cleft. The secondary infection may induce inflammation and further maceration.

4.8.6 Differential diagnosis

The symptoms and clinical signs of tinea pedis can be difficult to distinguish from those of a number of other infectious causes of toe web intertrigo, such as bacterial or *Candida* infection. Non-infectious conditions that resemble tinea pedis, include contact dermatitis, dyshidrotic eczema and psoriasis.

Candidosis most often presents as mild interdigital erosion and maceration. It sometimes occurs in patients with diabetes mellitus and is more common in hot climates. It often occurs in conjunction with a dermatophyte infection. Other moulds that produce lesions indistinguishable from tinea pedis include *Neoscytalidium dimidiatum* (see Chapter 6).

Dermatophytosis of the feet is often associated with itching. In contrast, gram-negative bacterial infection tends to produce more painful inflammatory lesions, often with marked erosion of the skin. The clinical appearance of erythrasma (*Corynebacterium minutissimum* infection) is difficult to distinguish from interdigital tinea pedis.

Laboratory tests should be performed in any patient with foot lesions of undetermined origin.

4.8.7 Essential investigations and their interpretation

Direct microscopic examination of infected material should confirm a clinical diagnosis of dermatophyte infection. It is sometimes possible to distinguish a yeast infection from tinea pedis. Isolation of the aetiological agent in culture will permit the species of fungus involved to be determined. Media containing cycloheximide (actidione) should not be used if infection with *N. dimidiatum* is suspected.

Wood's light examination of the lesion should be performed to establish whether the patient has erythrasma. The coral red fluorescence characteristic of this condition, does not, however, exclude coexistent tinea pedis.

4.8.8 Management

Interdigital tinea pedis will often respond to topical treatment with an imidazole compound, such as bifonazole, clotrimazole, econazole nitrate, isoconazole nitrate, miconazole nitrate, oxiconazole, sertaconazole nitrate, sulconazole nitrate, terconazole or tioconazole, or an allylamine, such as naftifine hydrochloride or terbinafine. Other topical products used for treating tinea pedis include amorolfine hydrochloride, butenafine hydrochloride and ciclopiroxolamine. Terbinafine should be applied to the

toe clefts and other affected sites morning and evening for at least 2 weeks. Imidazoles often need to be applied for up to 4 weeks, and amorolfine for up to 6 weeks. The patient may also benefit by applying the cream to the soles to help prevent the infection from spreading. The recurrence rate following topical treatment is quite high, and chronic infection with minor scaling that persists despite treatment is not uncommon. Exacerbations of previous infection may also occur.

Mixed fungal and bacterial infections of the feet are common. For this reason, topical antifungal preparations that are effective against dermatophytosis and candidosis, and which possess some antibacterial action (such as econazole nitrate, miconazole nitrate, sulconazole nitrate, naftifine hydrochloride and ciclopiroxolamine) are often recommended.

If the disease is extensive, involving the sole and dorsum of the foot, or there is acute inflammation, oral treatment with terbinafine (250 mg/day for 2–6 weeks), itraconazole (100 mg/day for 4 weeks, or 200 mg/day for 2–4 weeks, or 400 mg/day for 1 week) or fluconazole (150 mg once weekly for 2–6 weeks) should be given in addition to topical treatment (which should be continued for 8 weeks or longer). However, relapse is common.

Chronic tinea pedis is often associated with infection of the nails. Inadequate treatment of onychomycosis may result in reinfection of the feet. Chronic tinea pedis may warrant the use of oral antifungal treatment, particularly if topical regimens have failed.

Tinea pedis is a chronic condition that seldom resolves if left untreated. Exacerbations, which tend to occur in the summer, alternate with partial remissions. Nevertheless, the prognosis in general remains benign.

4.8.9 Prevention

It is important to inform the patient of measures that can help to control the infection or prevent reinfection. These include use of antibacterial soaps; daily bathing of the feet, followed by thorough drying of the toes and interdigital spaces; liberal application of antifungal powders to the feet after bathing; wearing of cotton socks to absorb sweat; frequent changing of socks; application of antifungal powders to footwear; avoidance of occlusive footwear that increases sweating and wearing of protective footwear in hotels, changing rooms, gymnasiums and other public facilities.

Educating infected individuals not to expose others to their infection by not walking barefoot on the floors of communal changing rooms and by avoiding public baths and showers can help to reduce the spread of tinea pedis. Frequent hosing of the floors of public baths and the

discouraging of antifungal foot dips near communal baths are helpful preventive measures.

4.9 Tinea manuum

4.9.1 Definition

The term tinea manuum is used to refer to dermatophyte infections of one or both hands.

4.9.2 Geographical distribution

The condition is worldwide in distribution.

4.9.3 Causal organisms

The anthropophilic dermatophytes *T. rubrum* and *T. interdigitale* are the commonest causes of tinea manuum. Less commonly, the condition is caused by zoophilic dermatophytes, such as *M. canis* and *T. verrucosum*, or geophilic dermatophytes, such as *M. gypseum*.

4.9.4 Epidemiology

Dermatophytosis of the hands can be acquired as a result of contact with another person, with an animal or with soil, either through direct contact or via a contaminated object such as a towel or gardening tool. Manual work, profuse sweating and existing inflammatory conditions, such as contact eczema, are predisposing factors. Autoinoculation from another site of infection can also occur. In 'two feet, one hand' syndrome, tinea manuum develops on the hand used to excoriate the infected feet or nails.

4.9.5 Clinical manifestations

Tinea manuum is usually unilateral, the right hand being more commonly affected and should be suspected where a dry scaling eruption of one palm is seen. Tinea manuum often coexists with tinea pedis. Lesions on the dorsum of the hand or in the interdigital spaces appear similar to those of tinea corporis. They have a distinct margin and central clearing may occur.

Two clinical forms of palmar infection may be distinguished: the dyshidrotic or eczematoid form and the hyperkeratotic form. In the former condition, periods of partial remission intervene between successive exacerbations. In contrast, the hyperkeratotic form is chronic and spontaneous healing does not occur. It is not unusual for one form to turn into the other.

The dyshidrotic form of tinea manuum is characterized in the acute stage by vesicles that tend to appear in an annular or segmental pattern. These are localized to the edges of the hand, to the lateral and palmar aspects of the fingers or to the palm itself where the vesicles are rather larger, tense, often single and contain a clear viscous fluid. Removal of the top of the vesicles exposes a pinkish-red weeping surface with fine scaling margins. Pruritus, formication and burning are common symptoms.

The hyperkeratotic form of tinea manuum is a sub-acute or chronic condition. It begins as a succession of adjacent vesicles that desquamate. This results in a reddened scaling lesion that is round or irregular in outline and enclosed by a thick white squamous margin from which extensions run straight towards the centre. Once the chronic stage is reached, the disease involves most or all of the palm and fingers. The dry hyperkeratosis, with underlying erythema, readily causes fissuring in the palmar creases. The hand has a mealy appearance because of the furfuraceous scales that remain adherent to the horny layer. This is thickened and black in the creases.

4.9.6 Differential diagnosis

Tinea manuum must be distinguished from other forms of dyshidrosis. This condition, whatever its origin, is usually bilateral or even symmetrical. In its typical form, clear vesicles are grouped on the lateral and volar aspects of the fingers as well as on the palm. There is little or no inflammation of the base. Dyshidrotic eczema is usually bilateral, but mycological examination is often required to distinguish it and other conditions (such as contact dermatitis and psoriasis) from tinea manuum.

4.9.7 Essential investigations and their interpretation

Direct microscopic examination of infected material, such as vesicle tops, contents and skin scales, should reveal the branching hyphae characteristic of a dermatophyte infection. Isolation of the aetiological agent in culture will permit the species of fungus involved to be determined.

4.9.8 Management

Topical treatment with an imidazole compound, such as bifonazole, clotrimazole, econazole nitrate, isoconazole nitrate, miconazole nitrate, oxiconazole, sertaconazole nitrate, sulconazole nitrate, terconazole or tioconazole, or an allylamine, such as naftifine hydrochloride or terbinafine, will often suffice. Other topical products used for treating tinea manuum include butenafine hydrochloride and ciclopiroxolamine. In cases that fail

to respond to topical treatment, oral terbinafine (250 mg/day for 2–6 weeks), or itraconazole (100 mg/day for 4 weeks) should be prescribed.

4.9.9 Prevention

To prevent spread or recurrence of zoophilic tinea manuum, it is important to identify potential sources of infection and, in the case of household pets, take the animal for treatment of suspected dermatophytosis. Infection with anthropophilic species, such as *T. rubrum*, often follows spread from another infected body site, such as the feet, groin or nails. To prevent reinfection these sites should be examined and treated if dermatophytosis is present.

4.10 Tinea unguium

4.10.1 Definition

The term tinea unguium is used to describe dermatophyte infections of the fingernails or toenails. Onychomycosis is a less specific term used to describe fungal disease of the nails. In addition to dermatophytes, it can be caused by *Candida* species (see Chapter 5) as well as by a number of other moulds (see Chapter 7).

4.10.2 Geographical distribution

The condition is worldwide in distribution.

4.10.3 Causal organisms

There is wide geographical and racial variation in the aetiological agents of onychomycosis, but in the United Kingdom, 85–90% of nail infections are due to dermatophytes and about 5% are due to non-dermatophyte moulds. In North America, dermatophytes account for about 60% of nail infections, with non-dermatophyte moulds and *Candida* species representing about 20% each. The most commonly implicated dermatophyte is the anthropophilic species, *T. rubrum*, followed by *T. interdigitale*. Zoophilic species are seldom involved and usually only in fingernail infections.

4.10.4 Epidemiology

Onychomycosis is among the most common nail disorders in adults, accounting for 15–40% of all nail diseases. Onychomycosis is most prevalent in older adults but, because of the limited number of large-scale studies, the actual incidence of the condition is difficult to assess. Moreover, many reports do not distinguish between dermatophytosis and other forms of onychomycosis, or between infections of the finger and toenails. It has

been estimated that onychomycosis occurs in about 3% of the adult population in the United Kingdom, but a recent report revealed that 14% of North American adults who visited medical clinics for other diseases had onychomycosis.

Many risk factors for onychomycosis have been identified. They include male gender, increasing age, peripheral vascular disease and hyperhidrosis. Fungal nail disease is more prevalent in individuals with other nail problems, in persons with immunosuppressive conditions such as diabetes mellitus or human immunodeficiency virus (HIV) infection and in those taking immunosuppressive medications. Tinea unguium is associated with tinea pedis in up to one-third of cases. The difference between the incidence of onychomycosis in men and women might be a reflection of the degree to which individuals are concerned about the appearance of their nails. Likewise the higher incidence of onychomycosis in older individuals could be due to the greater likelihood of younger patients to seek treatment at an earlier stage.

4.10.5 Clinical manifestations

There are four recognized clinical patterns of dermatophyte onychomycosis: distolateral subungual onychomycosis, superficial white onychomycosis, proximal subungual onychomycosis and total dystrophic onychomycosis. Progression of disease can lead to overlap of these presentations.

Tinea unguium of the toenails is usually secondary to tinea pedis, while fingernail infection often follows tinea manuum, tinea capitis or tinea corporis. Tinea unguium may involve a single nail, more than one nail, both finger and toenails, or in exceptional circumstances, all of them. The first and fifth toenails are more frequently affected, probably because footwear causes more damage to these nails. Dermatophyte infection of the fingernails occurs in a similar pattern to the toenails, but is much less common. Fingernail infections are usually unilateral. It is very rare to see a patient with fingernail infection without toenail involvement.

Distolateral subungual disease is the most common presentation of dermatophyte nail infection. The fungus invades the nail and nail bed by penetrating the distal or lateral margins. The affected nail becomes thickened and discoloured, with a varying degree of onycholysis (separation of the nail plate from the nail bed), although the nail plate is not initially affected. The infection may be confined to one side of the nail or spread to involve the whole of the nail bed. In time, the nail plate becomes friable and may break up. Toenails are more commonly affected than fingernails.

In superficial white onychomycosis, the infection begins at the superficial layer of the nail plate and spreads to the deeper layers. Crumbling white lesions appear on the nail surface, particularly the toenails. These gradually spread until the entire nail plate is involved. This condition is most commonly seen in children and is usually due to *T. interdigitale* infection.

Most cases of proximal subungual onychomycosis involve the toenails. This infection originates in the proximal nail fold, with subsequent penetration into the newly forming nail plate. The distal portion of the nail remains normal until late in the course of the disease. *T. rubrum* is the usual cause. Although proximal subungual onychomycosis is the least common presentation of dermatophyte nail infection in the general population, it is common in persons with the acquired immunodeficiency syndrome (AIDS) and has sometimes been considered a useful marker of HIV infection. In AIDS patients, the infection often spread rapidly from the proximal margin and upper surface of the nail to produce gross white discolouration of the plate without obvious thickening.

These different clinical forms of nail disease may all eventually lead to total dystrophic onychomycosis, in which the whole of the nail bed and nail plate is involved. The pattern of infection is variable. In occasional cases, pockets of tightly packed hyphae develop in the subungual space leading to a dense white lesion (termed a dermatophytoma) visible beneath the nail. The type of infection can be resistant to antifungal treatment without prior removal of the lesion. This appearance is most often seen in the great toenail. In total dystrophic onychomycosis, the infected nail generally begins to lift up from the nail bed due to an accumulation of debris (hyperkeratosis) under the nail. The nail becomes thickened and yellow or brown in colour. The nail plate may crumble, beginning at the free end. The infection may be confined to only one nail, but more commonly, several nails, on one or both feet are affected. Many patients have concurrent interdigital or moccasin tinea pedis, and some may also have tinea cruris. Most patients complain about nail discomfort, particularly when cutting, and many may experience pain during activities, such as running and jogging.

4.10.6 Differential diagnosis

The clinical signs of tinea unguium are often difficult to distinguish from those of a number of other infectious causes of nail damage, such as *Candida*, mould or bacterial infection. Unlike dermatophytosis, candidosis of the nails (see Chapter 5) usually begins in the proximal nail plate, and nail fold infection (paronychia) is also present. Mould infections of nails

are described in Chapter 7. Bacterial infection, particularly when due to *Pseudomonas aeruginosa*, tends to result in green or black discolouration of nails. Sometimes bacterial infection can coexist with fungal infection and may require treatment in its own right.

Many other non-infectious conditions can produce nail changes that mimic onychomycosis, but the nail surface does not usually become soft and friable as in a fungal infection. Non-fungal causes of nail dystrophies include chronic trauma, psoriasis, onycholysis, onychogryphosis, subungual malignant melanoma and lichen planus.

4.10.7 Essential investigations and their interpretation

Laboratory confirmation of a clinical diagnosis of tinea unguium should be obtained before starting treatment. This is important for several reasons: to eliminate non-fungal dermatological conditions, to detect mixed infections, and to diagnose patients with less responsive forms of onychomycosis, such as toenail infections due to *T. rubrum*. It is also helpful to repeat cultures after treatment has started to ensure that the infection is responding to treatment.

It is not uncommon to obtain negative results from culture of nail specimens from patients with dermatophytosis. One reason for this is that good specimens are often difficult to obtain. Because dermatophyte infection is a disease of the nail bed, subungual material will often give helpful positive culture results. The following methods are recommended. If distal subungual lesions are present, take scrapings or clippings from under the distal edge of the nail. If there is superficial white onychomycosis, take scrapings from the diseased nail surface. If there is proximal subungual involvement, a nail drill or scalpel may be used to obtain material.

Direct microscopic examination of infected nail material should confirm a clinical diagnosis of fungal infection. It is sometimes possible to distinguish *Candida* infection or infection due to moulds, such as *Scopulariopsis brevicaulis,* from tinea unguium.

Isolation of the aetiological agent in culture will permit the species of dermatophyte involved to be determined. It is essential to inform the laboratory if nail material is suspected of being infected with non-dermatophyte moulds, so that duplicate plates with and without cycloheximide (actidione) can be inoculated. The results of culture can be positive even if microscopic examination is negative, but it is more common for microscopic examination to be positive while culture is negative.

4.10.8 Management

Tinea unguium is a difficult condition to treat and has a high rate of recurrence. Finger nails are easier to treat than toenails, due to the faster

rate of growth, and recommended treatment durations are shorter. If the affected nail has been injured or shows other abnormal growth patterns, outgrowth may be slow and the nail may never regain a normal appearance even though the infection may be eradicated.

In general, onychomycosis should be treated with an oral antifungal agent. However, localized distal nail disease and superficial white onychomycosis can sometimes be treated with topical antifungals, such as amorolfine hydrochloride, ciclopiroxolamine or tioconazole solutions. Amorolfine 5% solution should be applied once or twice weekly, while tioconazole 28% solution should be applied twice daily. Neither of these agents is available in the United States. Amorolfine treatment must be continued for at least 6 months for success with fingernails and 9–12 months for toenails. Tioconazole solution should be applied for 6–12 months. Ciclopiroxolamine 8% solution must be applied once daily for up to 12 months. Response rates are low compared with oral agents used in nail infections, and topical agents are usually reserved for distal infections of limited extent. Combining topical treatment with debridement of the upper surface of the infected nail may increase the likelihood of treatment success.

The oral antifungal agents, terbinafine, itraconazole and fluconazole, are effective in onychomycosis; terbinafine and itraconazole are licenced for use in adults with this indication. Long-term cure rates with these agents in extensive or proximal subungual onychomycosis approach 50% in most trials, but reported success rates appear lower in patients over 65 years of age. In randomized clinical trials, terbinafine appears superior, although the differences between the two drugs have shown substantial variations between trials. Both terbinafine and itraconazole have been found to persist in the nail tissue for prolonged periods after cessation of treatment. Drug interactions are more common with itraconazole than terbinafine (see Chapter 3).

The allylamine terbinafine is now the treatment of choice for patients with dermatophytosis of the fingernails or toenails. However, it is not appropriate for *Candida* nail infections, non-dermatophyte mould infections or mixed nail infections. Oral treatment with 250 mg/day for 6 weeks is often sufficient to cure a fingernail infection, but toenails require treatment for 12–16 weeks or longer. Treatment with oral terbinafine will also clear associated cutaneous lesions without the need for additional topical treatment. Pulsed treatment with terbinafine (in which a week of treatment is alternated with three weeks without treatment) is at least as effective as continuous treatment while offering potential improvements in patient compliance and cost-effectiveness. The regimen consists of 250 mg twice daily for one week each month for 3–4 months. Terbinafine (250 mg/day

for 3 months) has proved to be effective in HIV-infected individuals with tinea unguium.

Itraconazole is another effective treatment for dermatophyte nail infection. This drug persists in nail for at least 6 months and pulsed treatment has given encouraging results. Two pulses of treatment with 200 mg twice daily are recommended for success with fingernails and three pulses of treatment (or more) for toenail infections. Itraconazole is also available for continuous treatment, and a dosage of 200 mg/day for 3 months is the most appropriate for severe nail disease.

Fluconazole has proven to be less effective than terbinafine or itraconazole for onychomycosis. The recommended regimen is 150–450 mg once weekly for 6–9 months in toenail infection or 3 months in fingernail infection.

Oral griseofulvin is no longer regarded as a first-line choice for toenail infections, but it works quite well in fingernail infections if given over a 6–9-month period. The usual adult dose is 500–1000 mg/day; in children the recommended dose is 10 mg/kg per day. The drug must be taken until the affected nail has fully grown out. With griseofulvin, about 70% of fingernail infections can be cured, as compared with only 20–40% in toenail infections.

Nail debridement may be required in individuals receiving oral or topical treatment, particularly where the nail is too thick for the patient to cut or where the infection presents as a dermatophytoma or lateral infection. A recent clinical trial showed that older patients who received aggressive debridement in conjunction with a 12-week course of oral terbinafine showed higher rates of clinical improvement than did subjects who did not undergo debridement. Care must be taken not to damage the underlying skin during debridement, particularly in individuals, such as diabetics, who are susceptible to lower limb complications.

Although whole or partial nail plate avulsion is useful in some dermatophyte nail infections, this painful and disfiguring surgical procedure is best reserved for those rare instances where there are either contraindications to the use of oral antifungals or a drug-resistant organism is present. An alternative approach is chemical avulsion through the application of 40% urea paste to the nail under occlusion for 4–7 days. The infected nail can then be excised, allowing the underlying surface to be treated with a topical antifungal agent.

4.10.9 Prevention

Many individuals with onychomycosis are unaware of their fungal infection and some believe that dystrophic nails are simply part of the ageing

process. Patients should be made aware that the disease is contagious, and can be spread to those around them. Individuals with untreated or partially treated plantar or interdigital tinea pedis should be informed of the risk of developing onychomycosis.

Measures that can help to control nail infection or prevent reinfection include application of antifungal powders to the feet after bathing, wearing absorbent cotton socks, frequent changing of socks, application of antifungal powders to footwear, avoidance of occlusive footwear that increases sweating and wearing protective footwear in hotels, changing rooms, gymnasiums and other public facilities. It is also important to keep the nails as short as possible and to avoid sharing nail clippers with other household members.

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CHAPTER 5

Superficial candidosis

5.1 Definition

The term candidosis (candidiasis) is used to refer to infections caused by yeasts belonging to the ascomycetous genus *Candida*. These opportunist pathogens can cause acute or chronic invasive infection in immunocompromised or debilitated individuals (see Chapter 11), but are more often seen causing mucosal, cutaneous or nail infection.

5.2 Geographical distribution

These conditions are worldwide in distribution.

5.3 The causal organisms and their habitat

Although *Candida albicans* is the most important cause of superficial forms of candidosis, many other members of the genus are recognized as human pathogens. Most of these organisms are dimorphic, growing as budding yeast cells, pseudohyphae, and/or true hyphae. Typically, *Candida glabrata* (which used to be classified as *Torulopsis glabrata*) produces only yeast cells, and only *C. albicans* forms true hyphae in tissues.

Candida species can be recovered from the mouth and gastrointestinal tract of a substantial proportion (30–50%) of the normal adult population and from the genital tract of about 20% of normal women. *C. albicans* accounts for 60–80% of isolations from the mouth and 80–90% from the genital tract. *C. albicans* is seldom recovered from normal skin, being much less prevalent than *Candida parapsilosis* and *Candida guilliermondii*,

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but it is a frequent colonizer of moist or damaged skin and nails. Unlike *C. albicans*, *Candida tropicalis*, *C. parapsilosis* and a number of other pathogenic members of this large genus can sometimes be recovered from the environment.

5.4 Epidemiology

In most cases, superficial *Candida* infection is derived from the individual's own endogenous reservoir in the mouth, gastrointestinal tract, lower genital tract, or skin. In some cases, however, transmission of organisms from person to person can occur. For instance, neonatal oral candidosis is more common in infants born of mothers with vaginal candidosis, which suggests that infection occurs when the infant takes in some of the vaginal contents during parturition. The hands of mothers and healthcare workers are another potential source of neonatal infection.

Individuals colonized with *Candida* species possess numerous complicated and often interdependent mechanisms to prevent the organism from establishing an infection. Efficient protection is believed to involve both humoural and cell-mediated immunological mechanisms. Non-specific mechanisms are also important, but it is well recognized that the contribution of particular elements to protection against mucosal, cutaneous and deep-seated forms of candidosis is different. Even trivial impairments of these mechanisms are often sufficient to allow *C. albicans*, the most pathogenic member of the genus to establish a cutaneous or mucosal infection. More serious impairment of the host can lead to life-threatening invasive infection, often with less pathogenic organisms, such as *C. parapsilosis*.

Most cases of oropharyngeal candidosis are caused by *C. albicans*. However, several other members of the genus, including *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *Candida krusei*, have sometimes been associated with oropharyngeal disease. Both general and local predisposing factors are important in the development of oropharyngeal candidosis. Debilitated and immunosuppressed individuals, such as persons with diabetes mellitus, persons receiving corticosteroids, recipients of haematopoietic stem-cell transplants and persons with human immunodeficiency virus (HIV) infection, are more susceptible to oropharyngeal candidosis. Local factors, such as xerostomia and trauma from unhygienic or ill-fitting dentures, are also important. Local tissue damage is also a critical factor in the development of cutaneous forms of candidosis. Most infections occur in moist, occluded sites and follow maceration of tissue.

Prior to the introduction of combination antiretroviral treatment, oropharyngeal candidosis was the most common opportunistic infection seen in persons with HIV infection. Over 80% of untreated, HIV-infected individuals developed candidosis at some time during their illness. Furthermore, oropharyngeal candidosis occurred in about 60% of those with a CD4 T-lymphocyte count of less than 100–200 cells per μL, more than half of whom experienced recurrent infection. Recent data indicate that the prevalence of oropharyngeal (and oesophageal) candidosis has fallen since the widespread introduction of new antiretroviral therapies, including protease inhibitors, perhaps by as much as 50–60%. The development of this condition is often one of the earlier clinical manifestations of HIV infection in asymptomatic individuals and is a reliable marker of disease progression.

About 20% of women of childbearing age harbour *Candida* species in the lower genital tract without symptoms or clinical signs of vulval or vaginal infection. Several host, behavioural and genetic factors have been associated with increased rates of asymptomatic colonization, including uncontrolled diabetes mellitus, recent antibiotic use, use of high-oestrogen-content oral contraceptives and orogenital sex. Vaginal colonization with *Candida* species is more common among women who are genetic and phenotypic non-secretors of blood group antigens. Colonization is also more prevalent among HIV-infected women than among HIV-negative high-risk behaviour women, but does not appear to increase with progressive decline in CD4 cell counts.

Up to 85% of cases of symptomatic vaginitis are due to *C. albicans*. Most of the remainder is due to *C. glabrata* (5–10% of cases) or *C. tropicalis* (less than 5% of cases). It is seldom possible for a precipitating factor to be identified to account for the transformation from asymptomatic colonization to infection. However, symptomatic vaginitis is much more common in pregnant women. Moreover, a significant proportion of women with chronic or recurrent candidosis first present with this infection while pregnant. Symptomatic vaginitis is also more common among women with uncontrolled diabetes mellitus, a disorder that has been implicated as a predisposing factor in other superficial forms of candidosis. The lower prevalence of vaginal candidosis after menopause emphasizes the hormonal dependence of the infection. Most cases of the infection in older women are associated with uncontrolled diabetes mellitus or the use of exogenous oestrogen replacement treatment. Vaginal candidosis is often seen during or after use of broad-spectrum antibiotics. However, aberrations of iron metabolism and low-oestrogen oral contraception are no longer regarded as significant risk factors. Precipitating factors for *C. glabrata*

infection include diabetes mellitus, older age, and previous use of azole antifungal agents.

Chronic mucocutaneous candidosis is a rare condition that occurs in individuals with underlying endocrinological disorders or inherited defects in the cell-mediated immunological response. The infection is seen in individuals with disorders in which T-lymphocyte activation is impaired or production of T-cell factors needed for macrophage activation is subnormal. These defects are often specific to *C. albicans*, but some patients have more profound defects that involve the T-cell-mediated response to other organisms as well. Patients with chronic mucocutaneous candidosis seldom develop deep-seated infection, despite their widespread or generalized cutaneous or mucosal lesions.

5.5 Clinical manifestations

5.5.1 Oropharyngeal candidosis

Oropharyngeal candidosis can be classified into a number of distinct clinical forms: pseudomembranous candidosis, erythematous (or atrophic) candidosis and hyperplastic (or hypertrophic) candidosis.

Pseudomembranous candidosis (thrush) is an acute infection, but it can recur in patients using steroid inhalers and in immunocompromised individuals. It is the most common form of candidosis in persons with the acquired immunodeficiency syndrome (AIDS). It is also seen in neonates and among terminally ill patients, in particular those with serious underlying disorders, such as leukaemia and other malignancies.

Acute pseudomembranous candidosis presents as white raised lesions that appear on the surfaces of the buccal mucosa, tongue, hard and soft palate, and tonsils. If left untreated, these can develop to form confluent plaques. The lesions are often painless, although mucosal erosion and ulceration may occur. The infection may spread to involve the throat, giving rise to severe dysphagia. In HIV-infected individuals, the lesions of the pseudomembranous form are persistent and often spread to affect all parts of the mouth.

It is important to distinguish this condition from hyperplastic candidosis (oral leukoplakia). The simplest test is to determine whether the white pseudomembrane can be dislodged. If it can be wiped off to reveal an eroded, erythematous and sometimes bleeding base, then this is diagnostic for pseudomembranous candidosis.

Erythematous (atrophic) candidosis is often associated with broad-spectrum antibiotic treatment, chronic corticosteroid use and with HIV infection. It can arise as a consequence of persistent pseudomembranous

candidosis when pseudomembranes are shed or, in HIV infection, may precede pseudomembranous candidosis. It can affect any part of the oral mucosa and manifests as a flat red lesion, usually on the palate or dorsum of the tongue. If the tongue is affected, the dorsum is depapillated, shining and smooth. Tongue movement is restricted and swelling results in trauma to the lateral borders if a natural dentition is present. The mouth is often so tender that the patient finds it difficult to tolerate solid food and consumption of hot or cold liquids causes severe pain.

Hyperplastic candidosis (or *Candida* leukoplakia) is an important condition because the lesions can undergo malignant transformation. About 5% of all oral leukoplakias become malignant, but for *Candida* leukoplakias, the figure is 15–20%. It remains unclear whether this condition is a hyperplastic lesion superinfected with *C. albicans* or the converse. The lesions of hyperplastic candidosis usually occur on the inside surface of one or both cheeks or, less commonly, on the tongue. The lesions are usually asymptomatic and the condition is often associated with smoking or local trauma due to dental neglect. The lesions range from small translucent white areas to large dense opaque plaques. Lesions that contain both red erythroplakic and white leukoplakic areas must be regarded with great suspicion as malignant change is often present. In contrast to the pseudomembranous form of oral candidosis, the lesions cannot be rubbed from the surface of the buccal mucosa.

In addition to the three major forms of oropharyngeal candidosis, there are several other lesions associated with oral *Candida* infection. Chronic atrophic candidosis (or denture stomatitis) is usually associated with oral prostheses, occurring in up to 60% of denture wearers. It is most prevalent in individuals who do not remove and sterilize their dentures overnight. Apart from soreness, the condition is usually asymptomatic, often being discovered only when a new prosthesis is required. The only presenting complaint may be associated angular cheilitis. Lower dentures are seldom involved. The characteristic presenting signs are chronic mucosal erythema and oedema of the portion of the hard palate that comes into contact with the fitting surface of the upper denture. The condition is mainly due to the overgrowth of *C. albicans* beneath the dentures. The mucosa beneath the dentures is rarely involved.

Angular cheilitis often develops in association with other forms of oropharyngeal candidosis, in particular denture stomatitis, but it may occur without signs of other oral disease. The condition has been reported in up to 20% of HIV-infected persons. Angular cheilitis is most common in patients with moist, deep folds at the corners of their mouth. These angular folds are often due to overclosure of the mouth in individuals who do

not wear their dentures on a regular basis or who have old worn dentures. The characteristic presenting signs are soreness, erythema and fissuring at the corners of the mouth.

5.5.2 Vaginal candidosis

Vaginal candidosis is a common condition, and while most patients respond well to treatment, in some, the infection is recurrent and others have persistent symptoms that fail to respond to treatment. Up to 75% of all women will suffer at least one episode of this condition during their lifetime, with 40–50% suffering a further episode. *C. albicans* is the most important cause of vaginal candidosis, accounting for up to 85% of cases. *C. glabrata* accounts for about 5–10% of cases. Infections with this species are apt to be milder, but they should not be ignored as *C. glabrata* is often resistant to azole antifungal treatment.

Most women with vaginal candidosis complain of intense vulval and vaginal pruritus and burning with or without vaginal discharge. The condition is often abrupt in onset and, in women who are not pregnant, it tends to begins during the week before menstruation. Some women complain of recurrent or increasing symptoms preceding each menstrual period. Pruritus is often more intense when the patient is warm in bed or after a bath. Dysuria and dyspareunia are common.

Vulval erythema with fissuring is the most common clinical finding. This is often localized to the mucocutaneous margins of the vaginal introitus, but it can spread to affect the labia majora. Perineal intertrigo with vesicular or pustular lesions may be present. Vaginitis with discharge is another common clinical finding. The classical sign of florid vaginal candidosis in the pregnant woman is the presence of thick white adherent plaques on the vulval, vaginal or cervical epithelium. This is a useful sign in the non-pregnant patient as well. Often, the discharge is thick and white and contains curds, but it can be thin or even purulent. Vulvitis may be present without a concomitant vaginal infection.

Vaginal candidosis is but one of a number of causes of vaginal discharge and must be distinguished from other conditions such as bacterial vaginosis, trichomoniasis, and chlamydial and gonococcal infections. Other causes of mucosal pruritus include herpes infections, contact dermatitis, psoriasis and allergies (including reactions to topical antifungals).

It is important to remember that some patients will have more than one genital infection. Simultaneous infection with *C. albicans* and *Trichomonas vaginalis* is, however, unusual. Candidosis is also less common among women with non-specific genital infection, among contacts of men with non-specific urethritis and among women with bacterial vaginosis.

5.5.3 Penile candidosis

In men, genital candidosis usually presents as a balanitis or balanoposthitis. Patients often complain of soreness or irritation of the glans penis; less commonly, there is a subpreputial discharge. Maculopapular lesions with diffuse erythema of the glans penis are often present; on occasion, there is oedema and fissuring of the prepuce. Itching, scaling, cutaneous lesions are sometimes found on the penis or in the groin.

Men with insulin-dependent diabetes may present with an acute fulminating oedematous form of balanoposthitis with ulceration of the penis and fissuring of the prepuce. White plaques can be found on gentle retraction of the prepuce.

About 20% of male contacts of women with vaginal candidosis complain of soreness and itching of the glans penis soon after intercourse and lasting for 24–48 hours. Men who have a penile catheter inserted long-term or those using Paul's tubing often develop chronic or recurrent penile candidosis.

The diagnosis should not be made on clinical grounds alone as there are other causes of balanitis and balanoposthitis. Specimens for mycological investigation should be taken from the coronal sulcus and subpreputial sac. Patients should be investigated for diabetes mellitus.

5.5.4 Cutaneous candidosis

C. albicans is the most important cause of cutaneous candidosis. The lesions tend to occur in the folds of the skin, such as the groin and the intergluteal folds, where maceration and occlusion give rise to warm moist conditions. Lesions can also arise in small folds, such as the interdigital spaces between the fingers. The infection is more common in overweight or diabetic individuals.

The initial lesions of superficial cutaneous candidosis (intertrigo) are papules or vesicopustules that later enlarge and become confluent. The larger lesions are erythematous and have an irregular margin. The main lesion is often surrounded by numerous small papulopustules, termed satellite lesions. Soreness and itching are common symptoms. Application of topical steroids will alter the appearance of the lesions, making them difficult to distinguish from those of dermatophytosis.

Candida infection of the toe webs is difficult to distinguish from a dermatophyte infection; indeed, most cases occur in association with dermatophytosis. Candidosis of the finger webs (sometimes termed erosio interdigitalis blastomycetica) presents as white fissures in the interdigital folds, with surrounding erythema and maceration. It is often uncomfortable and may be painful. This condition is not common and is usually

seen in individuals whose occupations necessitate frequent immersion of the hands in water and who are also subject to interdigital trauma. Interdigital candidosis is often associated with onychia and paronychia of the same hand.

In infants with the uncommon condition, congenital cutaneous candidosis, discrete vesicopustules, often on an erythematous base, are present at birth or appear soon thereafter. The lesions are most often seen on the face and trunk and may spread rapidly to involve the whole surface in about 24 hours. This condition is thought to result from intrauterine or interpartum infection; maternal vaginal candidosis is found in over 50% of cases. The pustules do not persist for very long and desquamate prior to spontaneous resolution of the lesions. In premature or low-birth-weight infants, or infants with prolonged rupture of membranes, congenital cutaneous candidosis can become invasive, leading to acute disseminated candidosis (see Chapter 11).

The precise role of *C. albicans* in the evolution of rashes on the buttocks and in the perianal region of infants, associated with wearing napkins (diapers), remains unclear. This condition should not be considered a primary *Candida* infection as it is preceded by an irritant dermatitis.

Other cutaneous forms of candidosis include the erythematous, macronodular lesions seen in about 10% of neutropenic individuals with disseminated candidosis, and the purulent follicular and nodular cutaneous lesions seen in drug abusers (see Chapter 11).

5.5.5 *Candida* nail infection

Candida infection accounts for 5–10% of all cases of onychomycosis. Three forms of infection are recognized: infection of the nail folds (or *Candida* paronychia), distal nail infection and total dystrophic onychomycosis. The last is a manifestation of chronic mucocutaneous candidosis. Nail and nail fold infections with *Candida* are more common in women than men. Fingernails are more commonly affected than toenails. These infections often occur in individuals whose occupations necessitate repeated immersion of the hands in water and the nails affected tend to be those of the dominant hand. The fourth and fifth fingers are involved less frequently than thumbs and middle fingers. Among the various species implicated, *C. albicans* and *C. parapsilosis* are the most common.

Candida paronychia usually starts in the proximal nail fold, but the lateral margins are sometimes the first site to be affected. The periungual skin becomes swollen, erythematous and painful, and a prominent gap often develops between the fold and the nail plate. Nail plate involvement often follows, infection usually commencing in the proximal section. White,

green or black marks appear in the proximal and lateral portions of the nail and then in the distal parts. The nail becomes more opaque, and transverse or longitudinal furrowing or pitting occurs. The nail becomes friable and may become detached from its bed. Unlike dermatophyte infections, pressure on and movement of the nail is painful. Bacterial superinfection is common and it is often difficult, if not impossible, to determine which organism is the cause of the nail damage.

Distal *Candida* nail infection presents as onycholysis and subungual hyperkeratosis. It is often difficult to distinguish from dermatophytosis, but the degree of nail damage tends to be less than that seen in dermatophyte infections. Moreover, finger nails are nearly always involved, while 80% of dermatophyte infections affect the toenails. Distal nail infection with *Candida* is uncommon and nearly all patients with this condition suffer from Raynaud's phenomenon or some other underlying vascular problem.

In patients with chronic mucocutaneous candidosis, the organism invades the nail plate from the outset, causing gross thickening and hyperkeratosis. This condition is often referred to as total dystrophic onychomycosis.

5.5.6 Chronic mucocutaneous candidosis

The term chronic mucocutaneous candidosis is used to describe a group of uncommon conditions in which individuals with congenital immunological or endocrinological disorders develop persistent or recurrent mucosal, cutaneous or nail infections with *C. albicans*. The disease often appears within the first 3 years of life. The mouth is the first site to be involved, but lesions then appear on the scalp, trunk, hands and feet. The nails and sometimes the whole of the fingertips are affected.

Four childhood forms of chronic mucocutaneous candidosis are recognized. Two forms are inherited: one as an autosomal dominant trait and the other as an autosomal recessive trait. The third form is associated with a range of endocrine disorders. The most common is hypoparathyroidism, but hypoadrenalism and hypothyroidism also occur. The fourth form has no recognized inheritance pattern and is not associated with endocrinopathies. In some forms of chronic mucocutaneous candidosis, there is an enormous proliferation of epidermal cells that results in disfiguring hyperkeratotic lesions on the mucous membranes, skin and nails.

Adult forms of chronic mucocutaneous candidosis usually occur in association with thymoma or with systemic lupus erythematosus. Like the childhood forms, this disease is characterized by recalcitrant infections of mucous membranes, nails and skin.

5.6 Essential investigations and their interpretation

The clinical manifestations of oropharyngeal candidosis are often characteristic, but can be confused with other disorders. For this reason, the diagnosis should be confirmed by demonstration of the various morphological forms of the fungus in smears prepared from swabs or scrapings of lesions and its isolation in culture. As *Candida* species are normal commensals in the mouth, their isolation alone cannot be considered diagnostic of infection. Swabs should be moistened with sterile water or saline prior to taking the specimen, or it sent to the laboratory in transport medium.

The diagnosis of vaginal candidosis depends on a combination of typical symptoms and signs and the demonstration of the fungus in smears or its isolation in culture. The latter is much more sensitive and reliable (about 90%) than the former (about 40%). Swabs should be taken from discharge in the vagina and from the lateral vaginal wall, and sent to the laboratory in transport medium.

Intertriginous candidosis is often difficult to diagnose if the lesions are other than typical in appearance. Isolation of *Candida* species from swabs or scrapings is of dubious significance, because the organisms are frequent colonizers of a range of cutaneous lesions. Microscopic demonstration of the organism in scrapings of lesions is much more significant.

The diagnosis of nail fold infections rests in part on the characteristic clinical appearance. However, microscopic examination and culture is needed to confirm the diagnosis. Material can be taken from the swollen periungual nail wall or from under the nail fold using a disposable microbiological loop or moistened swab. Pus can be obtained from under the nail fold by applying light pressure. Microscopic demonstration or isolation of the fungus from nail can be difficult with proximal lesions, but material from a distal or lateral lesion, together with subungual debris, will often reveal the diagnosis. Isolation of *C. albicans* from nails is seldom significant unless the organism is seen on microscopic examination.

5.7 Management

5.7.1 Oropharyngeal candidosis

Immunocompetent patients with uncomplicated oropharyngeal candidosis respond to topical treatment with nystatin, amphotericin B or an imidazole. In infants, pseudomembranous candidosis can be treated with nystatin oral suspension (100,000 units/mL) or amphotericin B oral suspension (100 mg/mL). This should be dropped into the mouth after each feed or at 4- to 6-hour intervals. In most cases, the lesions will clear within 2 weeks. Older children and adults with the pseudomembranous form

of oral candidosis can be treated with clotrimazole troches (one 10 mg troche five times daily), or nystatin or amphotericin B oral suspension (1 mL at 6-hour intervals for about 2–3 weeks), or miconazole oral gel (250 mg at 6-hour intervals). It is essential that any medication should be retained in the mouth for as long as possible. Treatment should be continued for at least 48 hours after all lesions have cleared and symptoms have disappeared.

In HIV-infected persons and other immunocompromised individuals with oropharyngeal candidosis, the relapse rate with topical antifungal treatment is high, and now that safe oral agents are available, these are to be preferred. In addition to antifungal treatment, patients must be given careful instruction in oral hygiene. Oral fluconazole (100–200 mg/day for 7–14 days) has been found to be more effective than itraconazole (200–400 mg/day for 14 days) or ketoconazole (200–400 mg/day for 14 days) and is considered the drug of choice for controlling oropharyngeal candidosis in HIV-infected individuals.

The oral solution formulation of itraconazole (200 mg/day for 7–14 days) is better absorbed than the capsules and has proved as effective as oral fluconazole. However, it is less well tolerated (see Chapter 3).

In clinical trials, oral posaconazole has proved as effective as fluconazole in the treatment of oropharyngeal candidosis in HIV-infected patients and has been licenced for this indication. In addition, posaconazole was more successful than fluconazole in sustaining clinical success after treatment was discontinued. The recommended dose of posaconazole is 100 mg (2.5 mL) twice daily on the first day followed by 100 mg (2.5 mL) once daily for 13 days. The duration of treatment will differ from patient to patient and depend on the nature of the infection and the clinical response.

Most immunocompromised patients with oropharyngeal candidosis will respond initially to oral antifungal treatment. Prior to the introduction of highly active antiretroviral therapy (HAART), up to 60% of patients with AIDS relapsed within 3 months of the successful completion of azole treatment for oropharyngeal candidosis. However, the use of HAART has been associated with a dramatic decline in the rates of oral colonization and symptomatic infection; the relapse rate has also decreased. In HIV-infected individuals, it is important to recognize that recurrence does not necessarily denote the development of azole-drug-resistant strains of *C. albicans*, nor does it imply that the recurrent episode will be unresponsive to standard treatment. Other reasons for treatment failure or recurrence include patient non-compliance, immunosuppression, drug interactions and reduced drug absorption. Antifungal drug susceptibility testing has been shown to be predictive of clinical response to azole agents.

Management of oropharyngeal candidosis in HIV-infected patients from whom fluconazole-resistant strains of *C. albicans* have been isolated is difficult. In the first instance, higher dosages of fluconazole (400–800 mg/day) should be tried, but the benefit is usually transient. If this is unsuccessful, either itraconazole solution at a dosage of 200 mg daily or posaconazole suspension at a dosage of 400 mg (10 mL) twice daily for 3 days, followed by 400 mg daily for up to 4 weeks, can be prescribed. Voriconazole at a dosage of 200 mg twice daily or amphotericin B oral suspension (1 mL four times daily) are recommended when treatment with other agents has failed.

As a last resort, patients with unresponsive oropharyngeal candidosis can usually be managed with parenteral amphotericin B (conventional formulation or lipid-based preparation) or an echinocandin. In clinical trials, anidulafungin, caspofungin and micafungin have proved as effective and safe as oral fluconazole for the treatment of oropharyngeal candidosis in patients with HIV infection. Patients with otherwise unresponsive *Candida* infection can be managed with intravenous caspofungin (50 mg/day) or micafungin (150 mg/day); a loading dose is not required. Treatment with anidulafungin should be initiated with an intravenous loading dose of 100 mg on the first day, followed by 50 mg/day. Treatment should be given for a minimum of 2 weeks and should be continued for a minimum of 1 week after resolution of symptoms.

Chronic atrophic candidosis should be treated with topical antifungal agents, such as nystatin, amphotericin B or an imidazole. Patients should be instructed to remove and place their dentures in a sterilizing solution overnight. When the condition is resolved, a new prosthesis is usually required. Angular cheilitis should be treated with a topical antifungal preparation containing a steroid and perhaps an antibacterial agent as well. It is also important to correct the reason for overclosure of the mouth: a replacement denture may be required.

5.7.2 Vaginal candidosis

Most patients with vaginal candidosis respond to topical treatment with nystatin or an imidazole. Nystatin requires a longer treatment period and has a lower cure rate (75–80%) than the topical azoles (85–90%). However, it is often useful in women whose condition has failed to respond to azole treatment. Topical therapy is preferred in pregnancy whenever possible. Treatment of vaginal candidosis in HIV-infected women is identical to that recommended for HIV-negative women.

If a patient is to be treated with nystatin, one or two vaginal pessaries (100,000 units each) should be inserted high in the vagina at bedtime for 14 consecutive nights, regardless of an intervening menstrual

period. If vulvitis is a problem, nystatin cream should also be applied for 2 weeks.

Numerous imidazole compounds (including butoconazole, clotrimazole, econazole nitrate, fenticonazole nitrate, isoconazole nitrate, miconazole nitrate, sertaconazole nitrate, terconazole and tioconazole) are available in different countries in a number of topical formulations for the treatment of vaginal candidosis (see Chapter 3). These drugs give higher cure rates than nystatin with shorter courses of treatment and all of them have a similar low relapse rate. These drugs are safe, and side effects after topical application are uncommon. Treatment times range in duration from 1 to 7 days. Shorter regimens (with higher antifungal dosages) achieve better patient compliance, but treatment courses of less than six nights should be reserved for first episodes. The management of vaginal candidosis in pregnant women is more difficult, because the clinical response tends to be slower and recurrence is more frequent. Most topical agents are effective, especially when prescribed for longer periods of 1–2 weeks.

Itraconazole and fluconazole have been licenced for the short-term oral treatment of vaginal candidosis. These drugs are more expensive than topical preparations, but patient compliance is improved. Fluconazole is given as a single 150 mg dose, and itraconazole is given as two doses of 200 mg 8 hours apart with food. Oral treatment appears to be at least as effective as, if not superior to, topical treatment.

Women with recurrent vaginal candidosis (four or more episodes within 12 months) present a difficult management problem. These patients often suffer from depression and many develop psychosexual problems as a result of their illness. It is essential to make a correct diagnosis and to ensure that the patient avoids potential precipitating factors, though these may not be obvious. Other diagnoses include herpes infection, allergic reactions and bacterial vaginosis. Physical examination, investigations to exclude diabetes mellitus and mycological investigation are essential and, if possible, should be performed when the patient has symptoms but has had no treatment.

There is no need to investigate oral or intestinal colonization with *C. albicans* in women with recurrent vaginal candidosis. Trials have demonstrated that oral nystatin treatment, given to reduce intestinal colonization with *C. albicans*, fails to prevent recurrence of symptoms of vaginal infection. The role of sexual transmission in vaginal infection is unknown, and topical or oral treatment of the male partner does not seem to prevent recurrence in the woman. In most cases, symptomatic recurrence is thought to result from vaginal relapse after inadequate treatment of a previous episode.

Once the diagnosis is confirmed and following efforts to exclude a reversible, treatable underlying cause, an induction and long-term maintenance regimen with an azole antifungal agent has become the standard of care in most patients. Many women with recurrent candidosis can be managed with intermittent prophylactic treatment with a single dose or multiple doses of topical or oral antifungals given to prevent symptomatic episodes. Local treatment with clotrimazole (500 mg as a single dose) at 2- or 4-week intervals has been shown to suppress symptoms even if mycological cure is not achieved. However, the best results have been obtained with 10–14 days of induction treatment with a topical or oral azole, followed by once-weekly doses of oral fluconazole (150 mg). After symptoms have been suppressed for 6 months, regular treatment can be discontinued to allow the patient to be reassessed. Many women do not revert to the previous pattern of frequent recurrence.

Although antifungal drug resistance does sometimes have a role in treatment failure, other factors such as allergic reactions or poor compliance are much more common reasons for a poor response. Nevertheless, drug resistance should be considered if organisms other than *C. albicans* are isolated from women with recurrent candidosis. By comparison with *C. albicans*, isolates of *C. glabrata* are much less susceptible to fluconazole and other azoles. Women with recurrent *C. glabrata* infection can sometimes be managed with nystatin or topical boric acid treatment (600 mg/day for 14 days).

5.7.3 Penile candidosis

Genital candidosis in men should be treated with saline washes or local applications of an antifungal cream. Nystatin should be applied morning and evening for at least 2 weeks. Clotrimazole, miconazole nitrate or econazole nitrate creams should be applied morning and evening for at least 1 week. The female contacts should also be investigated. Men who fail to respond to treatment should be investigated for other infectious or non-infectious causes of their condition.

5.7.4 Cutaneous candidosis

Most patients with cutaneous candidosis respond to topical treatment with terbinafine or an imidazole, applied for 2–4 weeks. However, relapse is common if any underlying problem is not controlled. If infection is associated with an underlying skin disease, such as flexural eczema, treatment with a combination preparation containing an azole agent together with hydrocortisone is often helpful.

The prognosis for congenital cutaneous candidosis in healthy normal-birth-weight term infants is good, and spontaneous cure often occurs after several weeks. The use of topical antifungal agents, such as nystatin or an imidazole, will hasten the cure. Premature or low-birth-weight infants, or infants with prolonged rupture of membranes, may require systemic antifungal treatment. Conventional amphotericin B is well tolerated in infants and has been used successfully at a dose of 0.5–1 mg/kg per day (see Chapter 11).

In infants, napkin dermatitis with associated *Candida* infection can be treated with combination topical preparations. It is advisable to use preparations containing hydrocortisone, rather than more potent steroids, because of the risk of absorption. Mothers of affected infants should be advised of the basic irritant cause of the problem.

5.7.5 *Candida* nail infection

Topical treatment with an imidazole or terbinafine will often cure *Candida* paronychia when this is confined to the nail folds. The antifungal cream or lotion should be applied morning and evening for up to 6 months. Measures to reduce maceration of the nail folds should be incorporated into the management of such cases. If patients have developed proximal nail damage, then oral antifungal treatment is usually required.

Localized distal nail infection can sometimes be treated with topical antifungals, such as amorolfine hydrochloride, ciclopiroxolamine or tioconazole solutions. Amorolfine 5% solution should be applied once or twice weekly, while tioconazole 28% solution should be applied twice daily. Ciclopiroxolamine 8% solution must be applied once daily. Combining topical treatment with debridement of the upper surface of the infected nail may increase the likelihood of treatment success.

Nail plate infection seldom responds to topical treatment. In this situation, itraconazole is the most appropriate oral treatment. This drug persists in nail for at least 6 months and pulsed treatment has given encouraging results. Two pulses of treatment with 200 mg twice daily for 1 week per month are recommended for success with fingernails. Infection of the toenails is much less common, but three or four pulses of treatment should be sufficient. Itraconazole is also available for continuous treatment and a dosage of 200–400 mg/day for 6 weeks is recommended for fingernail infections.

5.7.6 Chronic mucocutaneous candidosis

In most patients, oral and cutaneous lesions will respond to short courses of antifungal treatment. Much longer courses of treatment are needed to

clear nail infections. However, the improvement is often transient, and the infection will recur unless the underlying immunological defect is corrected.

Oral treatment with ketoconazole led to a marked improvement in the condition of a substantial number of patients with chronic mucocutaneous candidosis, but protracted treatment was required to sustain remission, and this led to the development of drug resistance in some cases. Itraconazole and fluconazole have now superseded ketoconazole. These drugs are no more effective than the older imidazole, but they are probably safer for long-term use. The required dosages are similar to those used for other forms of mucocutaneous candidosis.

5.8 Prevention

Among the prevention measures that have been shown to be effective in oropharyngeal candidosis are improved oral hygiene, smoking cessation and the avoidance of non-essential antibiotic and steroid use. Avoidance of broad-spectrum antibiotics has also proved effective in some women with vaginal candidosis, but many of those affected have no obvious predisposing factors. Most cases of cutaneous candidosis affect moist occluded sites and follow maceration of the tissue. Prevention can sometimes be achieved by careful drying.

There have been numerous attempts to develop chemoprophylaxis regimens that will reduce oral colonization with *Candida* species and help prevent the development of oropharyngeal candidosis in neutropenic individuals. There have, however, been no convincing demonstrations that either nystatin or amphotericin B oral suspensions or tablets prevent mucosal forms of candidosis in this setting. In large randomized trials among neutropenic cancer patients and transplant recipients, chemoprophylaxis with fluconazole (50–200 mg/day) has been shown to reduce the incidence of oral colonization and infection with all *Candida* species, apart from *C. glabrata* and *C. krusei*. The use of fluconazole prophylaxis (at a higher dosage of 400 mg/day) has been endorsed in published guidelines for prevention of invasive candidosis in high-risk individuals, such as allogeneic stem-cell and liver transplant recipients, critically ill surgical patients and patients with chemotherapy-induced neutropenia (see Chapter 11).

There have also been a few trials of azole chemoprophylaxis for the prevention of superficial forms of candidosis in persons with advanced HIV infection. Oral fluconazole (50–200 mg/day, or 150–200 mg once weekly) has been shown to reduce the risk for oropharyngeal, oesophageal and

vaginal candidosis, but colonization is not eliminated and infection still recurs. Routine prophylaxis is not recommended because acute treatment is highly effective, prophylaxis can lead to disease caused by azole-resistant *Candida* species, prophylactic agents can produce drug interactions and prophylaxis is expensive. The most effective method of preventing mucocutaneous candidosis in HIV-infected individuals is the reversal of the underlying immunosuppression with antiretroviral treatment.

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CHAPTER 6

Other cutaneous fungal infections

6.1 Pityriasis versicolor

6.1.1 Definition

Pityriasis versicolor (tinea versicolor) is a common, mild, but often recurrent infection of the stratum corneum due to lipophilic yeasts of the genus *Malassezia*. Less frequently, these organisms cause serious systemic infection in low-birth-weight infants and other immunocompromised and debilitated individuals (see Chapter 26).

6.1.2 Geographical distribution

The disease is worldwide in distribution, but is much more prevalent in tropical and sub-tropical regions.

6.1.3 The causal organisms and their habitat

Malassezia species form part of the normal microbial flora of the skin of humans and other warm-blooded animals. Until recently, only three *Malassezia* species were recognized: these were two lipid-dependent species, *Malassezia furfur* and *Malassezia sympodialis,* and one non-obligate lipophile, *Malassezia pachydermatis*. Based on molecular analysis, the *M. furfur* species complex has been progressively enlarged to include *M. sympodialis*, and seven new taxa named *Malassezia globosa, Malassezia obtusa, Malassezia restricta*, *Malassezia slooffiae*, *Malassezia dermatis*, *Malassezia japonica* and *Malassezia yamatoensis*. Some additional members of the complex have been reported only from animals. The dominant species associated with pityriasis versicolor are *M. globosa*,

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M. sympodialis and occasionally *M. furfur* and *M. slooffiae*. These species are difficult to distinguish, and it is usually sufficient to refer to organisms associated with dermatological conditions as members of the *M. furfur* complex. *M. pachydermatis*, which causes ear infections and seborrhoeic dermatitis in animals and occasional invasive infections in humans (see Chapter 26), is not considered a member of the *M. furfur* complex. It is not lipid-dependent and is easily distinguished from *M. furfur* complex members by its ability to grow on routine mycological media.

6.1.4 Epidemiology

Infection with *Malassezia* species is nearly always endogenous in origin. The prevalence of skin colonization with these organisms is dependent upon age, anatomical site and, to a lesser degree, race. The incidence of skin colonization rises from around 25% in children to almost 100% in adolescents and adults. The density of colonization in post-pubertal individuals is greater in anatomical sites that contain pilosebaceous glands; *Malassezia species* have been isolated from 100% of samples from the backs of adults, but from only 75% taken from the face and scalp. It is thought that colonization with *Malassezia* species primarily occurs at the time of puberty when the sebaceous glands become active and the concentration of lipids on the skin increases.

The precise conditions that lead to the development of pityriasis versicolor and other forms of superficial *Malassezia* infection (see Section 6.2) have not been defined, but host and environmental factors both appear to be important. The lesions of pityriasis versicolor and seborrhoeic dermatitis have a predilection for sites well supplied with sebaceous glands, such as the chest, back and upper arms, and it has been shown that patients with the latter condition have higher concentrations of lipids on their skin than do other individuals. Instances where non-cohabiting members of the same family have developed pityriasis versicolor suggest a genetic predisposition. The increased incidence of *Malassezia* folliculitis and seborrhoeic dermatitis in persons with the acquired immunodeficiency syndrome (AIDS) and those receiving corticosteroid or other immunosuppressive treatment suggests that the relationship between *Malassezia* species and the immune system is important.

Pityriasis versicolor is worldwide in distribution, but is most prevalent in hot, humid tropical and sub-tropical climates, where 30–40% of the adult population may be affected. In temperate climates, the disease affects 1–4% of the adult population, but is most common during the warmer, more humid summer months. *Malassezia* folliculitis is also more

prevalent in tropical countries, and in temperate regions, it is more common during the summer months.

Human-to-human transmission of *Malassezia* species is possible, either through direct contact or via contaminated clothing or bedding. In practice, however, infection is endogenous in most cases and spread between persons is uncommon.

6.1.5 Clinical manifestations

Pityriasis versicolor is a disfiguring but otherwise harmless condition. The characteristic lesions consist of patches of fine scaling, particularly on the trunk and upper portions of the arms. Less commonly, pityriasis versicolor affects the face, scalp and other sites. Facial lesions typically occur on the forehead and are more common in children than in adults. The lesions vary in colour and may be hypopigmented (white) or hyperpigmented (red, pink, brown or black). They may become confluent and progress to cover large areas of the trunk and limbs. Most patients are asymptomatic, but mild pruritus occurs in some cases.

In light-skinned subjects, the affected skin may appear darker than normal. The lesions are light pink in colour, but grow darker, turning a pale brown shade. In dark-skinned or tanned individuals, the affected skin loses colour and becomes depigmented. The same patient may have lesions of different shades, the colours depending on the thickness of the scales, the severity of the infection and the inflammatory reaction of the dermis, and in particular, the amount of exposure to sunlight, which may vary from one lesion to another. The disease is exacerbated by sunlight and sweating.

The clinical manifestations of pityriasis versicolor in immunocompromised persons are similar to those seen in normal individuals. However, the lesions are usually more extensive.

In most cases, the lesions show a pale yellow fluorescence under Wood's light, permitting the extent of the disease to be judged, and enabling erythrasma (coral red fluorescence) and tinea corporis (no fluorescence) to be excluded.

6.1.6 Differential diagnosis

Hyperpigmented lesions must be distinguished from a number of conditions, including erythrasma, naevi, seborrhoeic dermatitis, pityriasis rosea and tinea corporis. Hypopigmented lesions can be confused with pityriasis alba and vitiligo.

6.1.7 Essential investigations and their interpretation

Material for direct microscopic examination should be obtained by taking scrapings from the scaling edge of a lesion. Pityriasis versicolor lesions contain a mixture of budding yeast cells, typical of the organism observed in normal skin sites, and numerous short, broad unbranched hyphae. These hyphae, which are thought to be the same organism in its pathogenic phase, are not seen at unaffected skin sites or in culture. Direct microscopic examination of scrapings in 10–20% potassium hydroxide solution is sufficient to permit the diagnosis of pityriasis versicolor if clusters of round or oval budding cells and short hyphae are seen.

Because *Malassezia* species are part of the normal cutaneous flora, their isolation in culture does not contribute to diagnosis. Besides, with the exception of *M. pachydermatis*, these organisms cannot be isolated on routine mycological media unless lipid is added.

6.1.8 Management

If left untreated, pityriasis versicolor will persist for long periods. Most patients respond to topical treatment, but up to 60% relapse within 12 months. Oral treatment is indicated in patients with extensive or recalcitrant lesions.

There are numerous topical agents that can be used to treat pityriasis versicolor. Selenium sulphide 2–5% shampoo should be applied daily for one week and then on the first and third days of the month for the subsequent 6 months. It should be left on for 5–10 minutes and then washed off. Ketoconazole 2% shampoo should be applied once daily for 3–5 days. It should be left in contact with the lesions for 3–5 minutes before being rinsed off.

Topical imidazoles, such as bifonazole, clotrimazole, econazole nitrate, ketoconazole, miconazole nitrate and sulconazole nitrate, should be applied morning and evening for 4–6 weeks. Topical terbinafine should be applied to the lesions once or twice daily for 1–2 weeks. Pityriasis versicolor is often a difficult disease to clear and topical preparations may need to be reused at intervals to ensure that the infection is eradicated.

Oral antifungal treatment should be reserved for patients with extensive lesions or recalcitrant infection that is unresponsive to topical treatment. Both itraconazole (200 mg once daily for 1 week) and ketoconazole (200–400 mg once daily for 2 weeks) are effective treatments. A 400-mg dose of itraconazole given once monthly for 6 months is

effective in preventing relapse. Oral terbinafine is inactive in patients with pityriasis versicolor.

6.2 Other *Malassezia* infections

In addition to pityriasis versicolor, two other cutaneous diseases are associated with *Malassezia* species. These are *Malassezia* folliculitis and seborrhoeic dermatitis.

6.2.1 *Malassezia* folliculitis

There are three main forms of this chronic disorder, in which a cutaneous folliculitis is associated with *Malassezia* species, principally *M. globosa* and *M. restricta*. The first, which is most common in young adults, is a folliculitis on the back, chest, or upper arms that consists of small, scattered, itching, erythematous follicular papules that slowly enlarge and become pustular. These often appear after sun exposure, or antibiotic or immunosuppressive treatment. These patients do not usually have seborrhoeic dermatitis.

In the second form, which is seen in some patients with seborrhoeic dermatitis, there are numerous small follicular papules over the upper and lower chest and back. The rash is more florid and is particularly marked on the back. The third form, which is seen in persons with AIDS, consists of multiple itching follicular papules and pustules on the trunk and face. This form is very similar to the second form, and the patients usually have severe seborrhoeic dermatitis.

Treatment with a topical imidazole or selenium sulphide is often effective, but oral treatment with ketoconazole (200 mg/day for 1–2 weeks) may be required in patients with extensive or recalcitrant lesions. To prevent recurrence, maintenance treatment may be given once or twice per week.

6.2.2 Seborrhoeic dermatitis

Seborrhoeic dermatitis is a chronic, relapsing, scaling dermatosis of the face, scalp and trunk that has many causes. It affects 1–3% of adults, although it is more frequent in men than women. Dandruff (scalp scaling) is the mildest form of seborrhoeic dermatitis and affects 5–10% of the population.

The role of *Malassezia* species (including *M. globosa, M. restricta* and, to a lesser extent, *M. furfur, M. obtusa, M. slooffiae* and *M. sympodialis*) in the pathogenesis of seborrhoeic dermatitis remains controversial and is

based on the fact that most cases respond to topical or oral treatment with an azole antifungal. Improvement is associated with disappearance of the organisms, and relapse with recolonization. While it is improbable that invasion of the epidermis is responsible for the development of seborrhoeic dermatitis, an indirect disease mechanism such as sensitization is possible.

Seborrhoeic dermatitis is a common and troublesome problem, estimated to occur in up to 80% of persons with human immunodeficiency virus (HIV) infection. The lesions take the form of an erythematous scaling rash on the scalp, face, ears, chest and the upper part of the back. Scaling of the eyelid margins and around the nasal folds is a common presentation. Itching is common on the scalp. In HIV-infected persons, the onset of seborrhoeic dermatitis is often an early sign of CD4 T-lymphocyte cell suppression. Onset may be sudden and the rash is often more extensive than in other individuals.

The clinical signs and distribution of the lesions are typical, and mycological investigation is not required.

Milder forms of seborrhoeic dermatitis can usually be managed with topical ketoconazole 2% cream with or without a topical corticosteroid. Patients with inflamed lesions often benefit from topical application of combined hydrocortisone 1% and antifungal drugs. Relapse is common, but retreatment as required is the simplest method of management. Ketoconazole shampoo, used twice per week for 2–4 weeks, is an effective treatment for seborrhoeic dermatitis and dandruff of the scalp. Thereafter, it should be used at 1- or 2-week intervals to prevent recurrence. Other topical agents that are effective include terbinafine, butenafine and ciclopiroxolamine, and the immunomodulators, pimecrolimus and tacrolimus.

Oral treatment with ketoconazole (200 mg/day for 1–2 weeks) should be reserved for patients not responding to topical treatment and those with severe or extensive lesions, such as persons with AIDS. Oral itraconazole and terbinafine are also effective.

6.3 Piedra

The term piedra refers to two uncommon fungal infections in which firm, irregular nodules, composed of fungal elements, are formed on and along the hair shafts. These two disorders are distinguished according to the colour of the nodules and the aetiological agent. White piedra is less common than black piedra.

6.4 White piedra

6.4.1 Definition

The term white piedra is used to refer to an uncommon, asymptomatic fungal infection of scalp, facial or pubic hair in which soft greyish-white nodules are formed along the hair shafts.

6.4.2 Geographical distribution

The condition is worldwide in distribution, but is more common in tropical and sub-tropical regions.

6.4.3 The causal organisms and their habitat

The disease is caused by members of the basidiomycetous yeast genus *Trichosporon*. Based on molecular analysis, the taxonomy of the genus has been progressively modified to the point where more than 30 species are now recognized. *Trichosporon inkin* is the principal cause of white piedra of the pubic hair, while *Trichosporon ovoides* has been associated with piedra of the scalp hair. Other species are associated with systemic infections in immunocompromised individuals (see Chapter 26). As with the *M. furfur* complex, these species are difficult to distinguish, and it is usually sufficient to describe isolates from dermatological specimens simply as members of the genus *Trichosporon*.

These organisms have a widespread natural distribution, being found in soil and water and on plants. They are also common commensal inhabitants of the skin and gastrointestinal tract in humans.

6.4.4 Epidemiology

Trichosporon species form part of the normal cutaneous flora of humans, and most cases of white piedra are endogenous in origin. Shared cosmetics or lotions may serve to spread the infection from person to person. The disease affects both men and women of all ages, but is most common in young adults. The most important predisposing factors are humidity, hyperhidrosis and poor personal hygiene.

6.4.5 Clinical manifestations

The presence of irregular, soft, white or light brown nodules, about 1.0–1.5 mm in diameter, along the hairs is characteristic of white piedra. The nodules, which are mainly located on the distal half of the hair shaft, are adherent to the hairs but can usually be detached. The uncovered part of the hair shafts and the underlying skin appear unaffected. No

broken hairs are seen. The infection is more commonly found on the hairs of the beard and moustache. Less frequently, it affects the scalp, axillae and genital region. Usually, only localized parts of the scalp are involved. In other sites, however, there is a more widespread distribution of nodules.

6.4.6 Differential diagnosis

White piedra of the genital hair can be confused with pediculosis and trichomycosis axillaris.

6.4.7 Essential investigations and their interpretation

Direct microscopic examination of epilated hairs mounted in 10–20% potassium hydroxide solution will reveal that the nodules consist of septate hyphae, arthroconidia and blastospores.

Hairs should be inoculated on to the surface of glucose peptone (Sabouraud's dextrose) agar plates, supplemented with chloramphenicol to reduce bacterial growth, and incubated at 25–30°C. The isolation medium should not contain cycloheximide (actidione), as this is inhibitory to some *Trichosporon* species. Identifiable white- to cream-coloured colonies will appear within 2–3 days. These consist of hyaline mycelium, which is septate and fragmented into rectangular, oval or round arthrospores. Numerous budding blastospores are also present. Identification can be confirmed by biochemical tests.

6.4.8 Management

Treatment is difficult. Shaving or clipping the hairs of the affected region is usually sufficient to clear the infection, but relapse is common. Topical treatment with an imidazole or ciclopiroxolamine has been reported to be effective, as has the use of ketoconazole 2% shampoo. Treatment with a combination of oral itraconazole (100 mg/day for 1 month) and ketoconazole 2% shampoo (for 2 months) or a topical azole cream (for 2–3 months) may be effective in patients for whom shaving the scalp hair is unacceptable.

6.5 Black piedra

6.5.1 Definition

The term black piedra is used to refer to an uncommon, asymptomatic infection of the scalp hair in which small, dark brown or black, hard, firmly adherent nodules are formed on the distal portion of the hair shafts.

6.5.2 Geographical distribution

The disease is most frequently seen in the humid tropical regions of South and Central America, South East Asia, Africa and the South Pacific islands.

6.5.3 The causal organism and its habitat

The disease is caused by *Piedraia hortae*, a mould that penetrates the cuticle, but does not invade the hair shaft itself. The natural habitat of *P. hortae* is thought to be soil and vegetation.

6.5.4 Epidemiology

Black piedra affects young adults of both sexes with a slight predominance among men. There are reports of epidemics in families and communities. The infection is spread by the common use of combs, hairbrushes or utensils used for washing the hair. Certain hygienic habits, such as the application of plant oils to the scalp hair, seem to predispose to the condition.

6.5.5 Clinical manifestations

The disease is most commonly found on the scalp, but it also occurs occasionally at other sites, including the beard or moustache and pubic region. Affected hairs show from 4 to 8 (or more) firmly attached nodules, 1–2 mm in diameter. These are oval or elongated, hard, dark brown to black in colour, and may surround the hair. The uncovered part of the hair shafts and the underlying skin appear unaffected. Broken hairs are sometimes seen.

6.5.6 Differential diagnosis

Black piedra can be confused with trichorrhexis nodosa and trichonodosis, but mycological examination will always confirm the diagnosis.

6.5.7 Essential investigations and their interpretation

Direct microscopic examination of the nodules in 10–20% potassium hydroxide solution will reveal a packed mass of brown-pigmented branching hyphae surrounding round to oval asci, each of which holds 2–8 colourless ascospores.

Hair fragments should be implanted in the usual media and incubated at room temperature. Identifiable dark brown to black colonies will appear after 2–3 weeks. They have a folded surface with a flat margin. Microscopy of a direct mount reveals only thick-walled, septate, branched, dark-pigmented hyphae.

6.5.8 Management

The simplest treatment is to cut the affected hairs. Topical treatment with benzoic acid or salicylic acid, or with an imidazole cream is also often effective. However, relapse is a common problem, regardless of the treatment used.

6.6 Tinea nigra

6.6.1 Definition

The term tinea nigra is used to refer to a rare, chronic infection of the stratum corneum due to *Hortaea werneckii*, a dematiaceous (brown-pigmented) mould. This condition often affects the palms. Less commonly, it involves the soles.

6.6.2 Geographical distribution

The disease has a worldwide distribution, but is more common in the tropical and sub-tropical regions of Central and South America, Africa and Asia. Occasional cases have been acquired in the coastal regions of the southeastern United States.

6.6.3 The causal organism and its habitat

H. werneckii (formerly *Phaeoannellomyces werneckii* or *Exophiala werneckii*) is a saprophytic mould found in the soil, sewage, wood and decomposing vegetation.

6.6.4 Epidemiology

Human infection is thought to follow traumatic inoculation. Tinea nigra is most common in children and young adults, but it can occur in individuals of any age. It has a predilection for individuals who are hyperhidrotic. Although familial infections have been reported, the condition does not appear to be contagious.

6.6.5 Clinical manifestations

Tinea nigra occurs most commonly on the palms of the hands and soles of the feet. Less frequently, it involves other sites, such as the neck and chest. Infection of the hands or feet is usually unilateral.

The lesion consists of one or several flat, light brown to black, non-scaling patches with a well-defined edge. Inflammation is absent. The lesions, which are very small at first, expand slowly over a period of weeks to months and may become confluent. The pigmentation is irregularly

distributed over larger lesions. The disease is asymptomatic and may remain undiagnosed for a long time.

6.6.6 Differential diagnosis

Tinea nigra must be differentiated from naevi, from malignant melanoma and from chemical stains (such as silver nitrate).

6.6.7 Essential investigations and their interpretation

Direct microscopic examination of scrapings from the margin of a lesion in 10–20% potassium hydroxide solution will reveal irregular, branched, septate, brown hyphae and occasional clusters of budding yeast cells.

Scrapings should be inoculated on to glucose peptone (Sabouraud's dextrose) agar plates and incubated at 25–28°C. Identifiable olive to greenish black, moist colonies of *H. werneckii* should appear within 2–3 weeks.

6.6.8 Management

Many topical agents have proved effective, including benzoic acid, salicylic acid and 10% thiabendazole solution. Topical antifungals, such as clotrimazole, econazole nitrate, miconazole nitrate, oxiconazole, terbinafine and ciclopiroxolamine, have also been successful. Most lesions disappear within 2–4 weeks, but treatment should be continued for at least 3 weeks to avoid recurrence.

6.7 *Neoscytalidium* infection

The brown-pigmented (dematiaceous) mould *Neoscytalidium dimidiatum* (formerly named *Scytalidium dimidiatum* or *Hendersonula toruloidea*) is a tropical plant pathogen that can also cause human infection of the palms, soles and nails (see Chapter 7). Non-pigmented mutants of *N. dimidiatum* were formerly referred to as *Scytalidium hyalinum*.

Although most of the reported cases of these infections have been described in immigrants to Europe and North America from the tropics and sub-tropics, cases have also been seen in West Africa and South East Asia. The geographic range, as defined by immigrants with the infection, is large: it includes most of the Caribbean islands, parts of South America, Africa, the Indian sub-continent, South East Asia and the West Pacific islands. Unlike dermatophytosis, these mould infections are not contagious.

The clinical signs of *Neoscytalidium* infection are identical to those of *Trichophyton rubrum* infection. In a recent report on more than 300 patients seen in France, 65% of patients had cutaneous lesions and 35% had nail

infections. The feet were involved in more than 90% of cases. The skin and nail lesions are indistinguishable from those observed in *T. rubrum* infection. The soles show hyperkeratosis, scaling or both, and the toe clefts show scaling or maceration. Palm lesions are usually unilateral and show scaling and, more rarely, hyperkeratosis. Itching is minimal.

Direct microscopic examination of scrapings shows fungal hyphae and arthroconidia, which may be difficult to distinguish from those of a dermatophyte. *N. dimidiatum* will grow on glucose peptone agar, but is inhibited if cycloheximide (actidione) is incorporated in the medium. Therefore, it is essential to inform the laboratory if skin material is suspected of being infected with *N. dimidiatum*, so that duplicate plates with and without cycloheximide can be inoculated.

Neoscytalidium infections are difficult to treat. The mould is resistant to the modern treatments for cutaneous fungal infection. However, benzoic acid can sometimes be used to treat these infections. Where available, amphotericin B dermatological lotion can cure infection of the toe clefts. However, it is not effective for sole, palm or nail infections.

6.8 *Alternaria* infection

Members of the genus *Alternaria* are common soil organisms and important plant pathogens. These dematiaceous moulds are commonly found in damp environments in buildings and have been implicated as occasional causes of subcutaneous and paranasal sinus phaeohyphomycosis (see Chapter 25). *Alternaria infectoria* and *Alternaria alternata* are the most frequent causes of human infection; in many case reports, however, identification to the species level was not reported.

Most cases of cutaneous *Alternaria* infection have occurred in immunocompromised individuals, such as solid organ transplant recipients or persons receiving systemic corticosteroid treatment. Unlike dermatophytosis, *Alternaria* infections are not contagious.

The clinical manifestations of cutaneous *Alternaria* infection can range from shallow-based non-healing ulcers that evolve from nodules, to verrucous lesions and erythematous confluent scaling lesions. Microabscesses and/or well-formed granulomata may also be present. The arms and legs are the most frequent sites of infection.

Because *Alternaria* species are common culture contaminants, detection of fungal elements during direct microscopic examination of specimens or in tissue sections is important to establish the clinical significance of a positive culture. The organisms appear as distorted brown-pigmented, branching hyphae.

Anecdotal case reports suggest that itraconazole (100–600 mg/day for 2–6 months) is usually an effective treatment for cutaneous *Alternaria* infection. In some instances, discontinuation of steroid treatment, or a reduction in dosage, has been sufficient to resolve the infection.

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CHAPTER 7

Mould infections of nails

7.1 Definition

The term onychomycosis is used to describe infection of the nails due to fungi. Nail infections due to dermatophyte fungi (tinea unguium) and *Candida* species have been described in Chapters 4 and 5. This chapter deals with infections caused by other less common moulds.

7.2 Geographical distribution

The disease is worldwide in distribution.

7.3 The causal organisms and their habitat

Various filamentous fungi other than dermatophytes have been isolated from abnormal nails. Often, these are casual, transient contaminants, and direct microscopic examination of nail clippings and scrapings is negative. However, certain environmental moulds that are found in soil or plant material are capable of causing nail infection, and when this is, so it is important that their significance is recognized. Unlike dermatophytes, these moulds are not keratinolytic, and they are generally considered to be secondary invaders rather than primary pathogens of the nail plate.

There is wide geographical variation in the causative organisms, but *Scopulariopsis brevicaulis*, a ubiquitous soil fungus, is the most common cause of non-dermatophyte nail infection. *Neoscytalidium dimidiatum* (which used to be named *Scytalidium dimidiatum* or *Hendersonula toruloidea*) has been isolated from diseased nails as well as from skin infections

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of the hand and foot (see Chapter 6) in patients from the tropics. Other causes of nail infection include *Acremonium* species, *Aspergillus* species, *Fusarium* species and *Onychocola canadensis*.

7.4 Epidemiology

Mould infections of nails have been reported in all age groups, but are most prevalent in older individuals Men are more commonly affected than women, and toenails are more frequently involved than fingernails. The prevalence of mould infection of the nails is difficult to assess from published work, because many reports do not distinguish between dermatophytosis and other forms of onychomycosis. However, it has been estimated that non-dermatophyte moulds account for about 5% of cases of onychomycosis diagnosed in the United Kingdom and around 20% of cases diagnosed in North America. Unlike dermatophytosis, these mould infections are not contagious, but many of them will not respond to the standard treatments for dermatophyte or *Candida* onychomycosis.

Many risk factors for onychomycosis have been identified. They include increasing age, occulusive footware, local trauma, peripheral vascular disease, hyperhidrosis and psoriasis. Mould infections of nails are more prevalent in individuals with other nail problems, in persons with immunosuppressive conditions such as diabetes mellitus or human immunodeficiency virus (HIV) infection, and in those taking immunosuppressive medications.

7.5 Clinical manifestations

With the exception of *Neoscytalidium* infection, non-dermatophyte moulds usually occur as secondary invaders in nails that have previously been diseased or traumatized. This may account for the fact that these infections often affect only one nail. The toenails, especially the big toenail, are more frequently affected than the fingernails.

Non-dermatophyte moulds have been implicated as occasional causes of several forms of fungal nail infection, particularly proximal subungual onychomycosis, distolateral subungual onychomycosis and, less commonly, superficial white onychomycosis. In proximal subungual onychomycosis, the infection originates in the proximal nail fold, with subsequent penetration into the newly forming nail plate. The distal portion of the nail remains normal until late in the course of the disease, but fungal invasion of the proximal nail fold leads to the development of a yellow-white discolouration. In contrast to dermatophyte nail infection,

onychomycosis due to *Aspergillus*, *Fusarium* or *Scopulariopsis* infection is often associated with periungual inflammation that may be quite marked and painful. In some cases, this is associated with a purulent discharge.

In distolateral subungual onychomycosis, the fungus invades the nail and nail bed by penetrating the distal or lateral margins. The affected nail becomes thickened and discoloured, with a varying degree of onycholysis (separation of the nail plate from the nail bed). Nail infection with *N. dimidiatum* often presents as distolateral subungual onychomycosis and can lead to total dystrophic onychomycosis. The lesions are indistinguishable from those observed in *Trichophyton rubrum* infection. Paronychia is not infrequent with *N. dimidiatum* infection of the fingernails and may be confused with *Candida* infection.

In superficial white onychomycosis, the infection begins at the superficial layer of the nail plate and spreads to the deeper layers. Crumbling white lesions appear on the nail surface and gradually spread until the entire nail plate is involved. In HIV-infected individuals, mould infection of nails mostly presents as distolateral subungual onychomycosis or total dystrophic onychomycosis of the toenails.

A non-dermatophyte mould should be suspected to be the aetiological agent of onychomycosis when previous antifungal treatment has failed on several occasions, direct microscopic examination has been positive but no dermatophyte has been isolated and there is no sign of associated skin infection.

7.6 Differential diagnosis

Mould infections of nails have few specific clinical features. For this reason, mycological and histological examinations should be performed on any patient with nail lesions of undetermined origin.

7.7 Essential investigations and their interpretation

Laboratory confirmation of a clinical diagnosis of onychomycosis must be obtained before treatment is commenced. Methods for collecting nail specimens are as detailed in Chapter 2. It is essential to inform the laboratory if nail material is suspected of being infected with a non-dermatophyte mould, so that duplicate plates with and without cycloheximide (actidione) can be inoculated.

Isolation of a mould is not a sufficient reason for ascribing a pathogenic role to it without further investigation. Moulds are ubiquitous and their aetiological role must be assessed whenever they are isolated from nail

material. The fungus must be seen on direct microscopic examination and the mould must be isolated in pure culture without the simultaneous appearance of a dermatophyte. Repeated isolation of the same mould indicates with some certainty its involvement in nail infection. It is sometimes possible to distinguish infections with *S. brevicaulis* from tinea unguium on microscopic examination: the characteristic, roughened thick-walled oval conidia of this mould are often present in infected nails.

If an environmental mould has been found on mycological investigation, the relationship between it and the nail plate can be determined with the aid of histological sections.

7.8 Management

Onychomycosis is more prevalent among older individuals, many of whom have specific risk factors for a poor response to treatment. These include dystrophic nails, slow growth of nails, and an increased prevalence of peripheral vascular disease and diabetes mellitus. Older patients with diabetes should be treated for onychomycosis to prevent secondary bacterial infections and subsequent complications. Mould infections of nails are difficult to cure, and even if the mould can be eradicated, this seldom leads to normal nail growth.

Localized distal nail disease and superficial white onychomycosis can sometimes be treated with topical antifungals, such as amorolfine hydrochloride, ciclopiroxolamine, or tioconazole solutions. Amorolfine 5% solution should be applied once or twice weekly, while tioconazole 28% solution should be applied twice daily. Amorolfine treatment must be continued for at least 6 months for success with fingernails and 9–12 months for toenails. Tioconazole solution should be applied for 6–12 months. Ciclopiroxolamine 8% solution must be applied once daily for up to 12 months. Combining topical treatment with debridement of the upper surface of the infected nail may increase the likelihood of treatment success.

Proximal nail disease or severe nail bed involvement may respond to topical treatment with terbinafine and ciclopiroxolamine if this is preceded by chemical or surgical avulsion of the diseased nail. Chemical avulsion with 40% urea paste is now preferred to surgical avulsion, as the latter has some potential to alter the nail bed and may also be painful for the subject to undergo. Removal of the infected nail will eliminate most of the invading fungi and allow the underlying surface to be treated.

Oral treatment with terbinafine (250 mg/day for 12 weeks or longer) or itraconazole (200 mg/day twice daily for one week each month

repeated for three months) has usually been effective in patients with proximal or distolateral subungual onychomycosis of the toenails caused by *Aspergillus* species. It is also possible to cure many patients with *Scopulariopsis* or *Acremonium* nail infections. However, these drugs have been less successful in *Fusarium* infections, and there appears to be no effective antifungal treatment for *Neoscytalidium* infection of the nails.

7.9 Prevention

In recent years, several non-dermatophyte moulds (including *Fusarium* species and *S. brevicaulis*) that cause nail infection have emerged as occasional causes of the invasive fungal disease in immunocompromised individuals (see Chapter 23). Usually, these infections have followed inhalation. In some cases, however, the source of the invasive infection has been identified as a pre-existing toenail infection. Because of the risk of life-threatening invasive fungal disease, it is essential that patients who require immunosuppressive or cytotoxic treatment be evaluated by a dermatologist before commencing immunosuppression. All sites of tissue breakdown should be identified and suspicious skin and nail lesions cultured.

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CHAPTER 8

Keratomycosis

8.1 Definition

Keratomycosis or fungal keratitis is a rare but severe cause of corneal infection. This condition is difficult to treat and can cause severe visual impairment or blindness.

8.2 Geographical distribution

The condition is worldwide in distribution, but is more common in developing countries and regions with tropical or sub-tropical climates.

8.3 The causal organisms and their habitat

More than 70 different fungi have been reported to cause mycotic keratitis. Moulds are the principal aetiological agents of this infection worldwide, but especially in tropical and sub-tropical regions. Members of the genera *Fusarium* (in particular *Fusarium solani*) and *Aspergillus* (in particular *Aspergillus fumigatus* and *Aspergillus flavus*) are the most common causes of keratomycosis in the tropics and sub-tropics, accounting for 36% and 30% of isolates in a recent report from India. Other hyaline moulds that have been reported include *Acremonium* species, *Paecilomyces* species, *Penicillium* species and *Scedosporium* species. Dematiaceous moulds (in particular the genera *Curvularia*, *Bipolaris* and *Exserohilum*) are the third most common group of aetiological agents of mycotic keratitis, accounting for 16% of cases in India.

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Members of the genus *Candida*, particularly *Candida albicans*, *Candida guilliermondii and Candida parapsilosis*, have also been reported to cause corneal infection. These human commensal organisms are an infrequent cause of corneal disease in the tropics. However, in the United States and other countries where traumatic keratitis is uncommon, but where other predisposing factors are important, *Candida* species are the most frequent cause of keratomycosis.

Cases of mixed bacterial and fungal keratitis are increasingly being reported. *Fusarium* species, *Candida* species and *Staphylococcus epidermidis* are the most frequent aetiological agents.

8.4 Epidemiology

The aetiological agents of keratomycosis are widespread in the environment, being commonly found in indoor and outdoor air, in the soil and dust, and on decomposing plant matter. Human infection usually follows the traumatic implantation of spores into the corneal epithelium. Much less commonly, it results from their inadvertent introduction during surgical procedures, such as corneal transplantation.

In developing countries, keratomycosis is most common in men, particularly those engaged in agricultural work or with another outdoor occupation. The principal, and sometimes only, predisposing factor is minor trauma. Often this trauma is so slight as not to attract the patient's attention. The most common traumatizing agents are plant materials or objects that have come into contact with soil or vegetation. The traumatizing agent itself may harbour fungal spores that are thus implanted into the cornea, or the injuring agent may cause a superficial abrasion that exposes the cornea to exogenous infection. Seasonal variations in the incidence of the disease have been noted, with infections being more common during the harvesting season in India, and during the hot, humid summer months in the southern United States.

In North America, 11–44% of cases of fungal keratitis have followed ocular trauma. More commonly reported risk factors have included chronic ocular surface disease, contact lens wear and use of topical antibiotics or corticosteroids.

Contact lens wear is considered to be one of the most significant risk factors for the development of microbial keratitis. In most cases, this is caused by bacterial pathogens. Fungal keratitis among contact lens wearers is rare, even in regions where non-contact-lens-associated fungal keratitis is more common, comprising less than 5% of all cases of microbial keratitis among contact lens wearers. In the past few years, however, changes

in the content and formulation of several soft contact lens solutions intended to improve patient compliance have resulted in an increase in the incidence of infections caused by atypical organisms. In 2005 and 2006, a large outbreak of *Fusarium* keratitis in the United States and a number of other countries was associated with a particular brand of contact lens storage solution. The outbreak was attributed to failure of the solution to disinfect after point-of-use contamination, rather than intrinsic contamination with *Fusarium*. After this solution was removed from the market, there was a dramatic decline in the number of cases of contact lens-related *Fusarium* keratitis.

Information on the incidence and prevalence of mycotic keratitis is limited, but published reports suggest that it accounts for 40–50% of all cases of ulcerative keratitis in developing countries, such as India and Thailand. In developed countries, fewer than 10% of all cases of microbial keratitis are fungus-related. In the United States, keratomycosis has been reported most frequently from the warmer, more humid southern states. In south Florida, fungal infections account for up to 35% of cases of microbial keratitis compared with 1% in New York.

8.5 Clinical manifestations

The clinical manifestations of keratomycosis are similar regardless of the organism involved. The infection is often insidious in onset, with mild symptoms having been present for some time. Often the patient has been treated with antibiotics and the correct diagnosis is not suspected until the condition fails to respond. The most common symptoms include increasing ocular pain, ocular redness, photophobia, diminished vision, tearing and discharge. On examination, the eye appears injected, and the cornea may have a noticeable haze, or an area of opacification. A mucopurulent discharge may be present.

Examination of the affected eye should be carried out using a slit lamp. The usual clinical finding is a raised corneal ulcer with a white, ragged border. Around and beneath the ulcer is a dense infiltrate extending deep into the corneal stroma. Although the lesion has a radiating margin, it is well defined. Discrete satellite lesions often develop. In the more severe cases, involvement of the anterior chamber occurs, leading to formation of a sterile hypopyon. If left untreated, the infection will spread into the anterior chamber and result in corneal perforation and loss of the eye. Enucleation is usually required because of pain.

Although the clinical manifestations of mycotic keratitis are similar, regardless of the mould involved, the rate of progression of the infection

shows marked differences. In cases due to *Fusarium* species, the infection is usually severe, tends to progress rapidly and may end in deep lesions, perforation or malignant glaucoma. In keratitis due to *Aspergillus* species, the infection is usually less severe, does not progress as rapidly and is more amenable to treatment than *Fusarium* infection. In keratitis due to *Curvularia* species, a low-grade keratitis lasting for several weeks with minimal inflammation or structural alteration is usually observed. Macroscopic pigmentation is seen only in about 30% of cases.

In contrast to mould infections, mycotic keratitis due to *Candida* species presents as a small, well-demarcated area of epithelial ulceration and expanding, but discrete, stromal infiltration. The infection may be indistinguishable from Gram-positive bacterial keratitis.

Confocal microscopy is a non-invasive procedure for imaging the cornea that may be useful for the presumptive identification of corneal pathogens at an early stage of infection. Moulds, such as *Aspergillus* and *Fusarium*, are seen as hyper-reflective, thin, branching filaments of variable length.

8.6 Essential investigations and their interpretation

Although the clinical picture is distinctive, the diagnosis of keratomycosis requires the demonstration of fungal elements on microscopic examination of smears prepared from corneal scrapings, together with the isolation and identification of the aetiological agent and the elimination of other causes for the disease.

Because the aetiological agents of keratomycosis are common contaminants of the corneal surface, isolation alone is inadequate for making a diagnosis. Neither is a superficial corneal surface specimen adequate. The organisms are often difficult to find, being located deep inside the corneal stroma, rather than on the surface.

Specimens should be taken using a sterile platinum spatula to scrape corneal fragments from the margins and base of the ulcer. Swabs are not appropriate. Because the amount of material that can be obtained will be small, it is best transferred to a clean glass slide for microscopic examination and to an agar plate for culture at the bedside. The scrapings should be examined with a potassium hydroxide wet mount, Calcofluor white staining, or a Gram stain. Some material should also be inoculated on to plates or slopes of glucose peptone (Sabouraud's dextrose) agar, blood agar or chocolate agar supplemented with an antibacterial antibiotic, such as chloramphenicol. These should be incubated at 25–30°C (rather than 37°C) for at least 2 weeks before being discarded as negative.

The colonies of most aetiological agents of keratomycosis appear within 3–5 days. Isolation of a fungus is more convincing if multiple colonies are obtained on a plate or if the same organism is recovered on more than one occasion.

If corneal scrapings are negative, but a diagnosis of keratomycosis is still suspected because the patient is not responding to antibacterial treatment, it is essential for the lesion to be biopsied. This should involve partial thickness trephination, with half the specimen being sent for culture and the remainder for histopathological examination. Corneal material may also be collected while performing a penetrating keratoplasty.

Anterior chamber paracentesis is not recommended because it is not without risk and the hypopyon, if present, is usually sterile.

8.7 Management

The management of keratomycosis entails removal of infected tissue, discontinuation of topical corticosteroids and topical or oral treatment with an antifungal drug. Prolonged treatment with careful follow-up is essential.

Topical treatment with natamycin 5% solution is often recommended for the initial management of corneal mould infection. Topical amphotericin B remains the preferred treatment for *Candida* infection. This drug can be toxic, but a 0.15% solution is well tolerated and shows reasonable ocular penetration. The imidazole compounds clotrimazole, econazole and miconazole are active against many moulds and topical treatment with a 1% solution is well tolerated. Miconazole achieves good concentrations in damaged corneal tissue and the aqueous following topical or sub-conjunctival administration. Although topical amphotericin B has sometimes proved unsuccessful against the dematiaceous moulds, topical miconazole has proved effective against these organisms. A number of recent clinical case reports indicate that topical treatment with 1% voriconazole solution is effective in keratitis caused by *Aspergillus*, *Fusarium* and *Candida* species.

Topical treatment should be applied at hourly intervals for the first week. Thereafter, it should be applied at similar intervals when the patient is awake. Treatment should be continued for at least 6 weeks for a *Candida* infection and for up to 12 weeks for a mould infection. Signs of improvement include lessening of pain, disappearance of satellite lesions, decreasing size of the infiltrate and rounding out of the ulcer margin. Negative scrapings during treatment are not significant and should not be regarded as an indication of response to treatment.

Oral treatment should be considered in patients with severe or worsening lesions. Fluconazole is not effective in mould infections, but shows good corneal penetration. It can be used to treat *Candida* infection at a dose of 200–800 mg/day. Ketoconazole (400 mg/day) has benefited some patients with mould infection, but itraconazole (200 mg twice daily) has proved more successful. There are several reports of successful salvage treatment of *Fusarium* keratitis with intravenous, oral and intravitreal voriconazole.

Surgical intervention is indicated in cases of medical treatment failure. Superficial debridement will improve the penetration of topical antifungal drugs. Superficial or lamellar keratectomy may be effective if the lesion is small and localized and, particularly, if it is located in the peripheral cornea. If, despite appropriate antifungal treatment, the inflammatory reaction or infection leads to corneal necrosis with actual or impending perforation, a penetrating keratoplasty (corneal transplant) may be required. However, about 95% of grafts will fail, usually within 4 weeks. Nonetheless, repeat corneal transplant can be successfully performed several months after resolution of infection.

Fusarium infections often result in rapid corneal sloughing and marked visual loss, and are difficult to treat. *Aspergillus* infections are less difficult to manage with antifungal agents, but the larger the ulcer and the deeper the hypopyon, the greater the likelihood of loss of vision. Even with intensive antifungal treatment, progression to corneal perforation, scleral suppuration or anterior chamber infection can occur. Corneal scarring with consequent reduction in vision is a frequent complication, even with successful treatment.

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CHAPTER 9

Otomycosis

9.1 Definition

Otomycosis or fungal otitis externa is a superficial fungal infection of the external ear canal that sometimes leads to complications involving the middle ear.

9.2 Geographical distribution

The condition is worldwide in distribution, but is more common in humid tropical and sub-tropical regions.

9.3 The causal organisms and their habitat

Otomycosis is most commonly caused by *Aspergillus* species, particularly *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus nidulans* and *Aspergillus flavus*, and *Candida* species, particularly *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis*. In temperate regions, there is a slight preponderance of infections with *Candida* species while in tropical and sub-tropical regions, *A. niger* is the most common cause of infection. Other moulds that have been implicated include *Penicillium* and *Rhizopus* species. Mixed bacterial and fungal infections are common and account for more than 50% of all cultures in otomycosis.

9.4 Epidemiology

The aetiological agents of otomycosis are commonly found in indoor and outdoor air, in the soil and dust, and on decomposing plant matter. Their

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prevalence varies with climatic conditions, but warm humid environments support their growth, and the human ear canal is ideal for their proliferation. In the tropics, otomycosis accounts for up to 30% of patients with symptomatic ear disease, but the condition is less prevalent in temperate climates, accounting for 9% of cases of otitis externa in one report from the United Kingdom. In temperate regions, it is most frequently seen during the summer months. Otomycosis occurs in men and women of all ages; children are less commonly affected. It is not a contagious condition.

Otomycosis often develops in individuals with pre-existing dermatological conditions of the ear, such as sebhorrhoeic dermatitis or psoriasis. Bacterial infection is common in such cases, and the prolonged use of topical antibiotics and steroids is a risk factor for fungal superinfection. The incidence of otomycosis in children has increased in recent years, as a result of the extensive use of topical fluoroquinolone antibiotics for the treatment of acute otitis media and acute otitis externa.

9.5 Clinical manifestations

The initial symptoms of fungal and bacterial otitis externa are often indistinguishable. Pain and discharge are the most common symptoms of otomycosis, followed by hearing loss, aural fullness and puritus. Tinnitus is an occasional complaint. In more than 90% of cases, only one ear is affected.

Otoscopic examination often reveals the presence of debris and an oedematous and erythematous ear canal. If *A. niger* is the causative agent, a mat of fungus, often covered with black sporing heads, can be seen. This mass lining the meatus, which it can obstruct, is often described as resembling greyish blotting paper. In chronic infections, eczematoid changes and lichenification of the canal can become marked. Complications are rare, but include serious otitis media, tympanic membrane perforation and external auditory canal osteitis. Most perforations will heal spontaneously usually within one month.

In most cases, children with presumed bacterial otorrhoea will have symptom resolution following empirical treatment directed at common bacterial pathogens. However, should the discharge persist despite repeated antibacterial treatment, fungal cultures should be performed to rule out the diagnosis of a fungal superinfection.

In immunosuppressed patients with haematological malignancies or persons with the end stage of the acquired immunodeficiency syndrome, fungal infection of the external ear can progress to a necrotizing

(malignant) otitis externa. There is erythema and superficial ulceration, together with bleeding and discharge. Pain is common. The infection can spread to the middle ear and mastoid. The tympanic membrane is thickened and frequently necrotic. This is most commonly caused by *A. fumigatus*, but *Scedosporium apiospermum* has also been implicated.

9.6 Differential diagnosis

The clinical diagnosis of otomycosis is difficult, although a lack of response to topical antibiotics and steroids, and the onset of hearing loss are suggestive of fungal infection. The diagnosis can be established with confidence only by mycological investigation.

9.7 Essential investigations and their interpretation

Debris and secretions should be obtained from the auditory canal. Direct microscopic examination using a 10–20% potassium hydroxide preparation will reveal branching hyphae, budding yeast cells or both. In cases of *Aspergillus* infection, the typical sporing heads can sometimes be seen.

Isolation of the aetiological agent in culture will enable the species of the fungus involved to be identified.

9.8 Management

The treatment of otomycosis consists of removal of debris from the ear canal and thorough cleaning, together with the local application of an antifungal agent. Most patients will respond to a single application of ketoconazole cream, often in less than 2 weeks; a second application should be used for persistent infection. Other topical imidazoles that have given good results include clotrimazole (which has the added advantage of having an antibacterial effect), bifonazole and miconazole. Other options include aluminium acetate and cresylate drops, although the latter should be avoided in patients with tympanic membrane perforation because of the risk of hearing loss. In developing countries, or when these agents are not readily available, mercurochrome, gentian violet and boric acid have been used with success.

Most patients with otomycosis will respond to treatment. However, recurrence is not uncommon and eradication of disease can be difficult in patients who have undergone a mastoidectomy. Careful follow-up is therefore important.

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CHAPTER 10

Aspergillosis

10.1 Definition

The term aspergillosis is used to refer to infections caused by moulds belonging to the genus *Aspergillus*. In immunocompromised individuals, inhalation of spores can give rise to life-threatening invasive infection of the lungs or sinuses and dissemination to other organs often follows. This condition is termed invasive aspergillosis. In non-immunocompromised persons, these moulds can cause localized infection of the lungs, sinuses, and other sites. Human disease can also result from non-infectious mechanisms: inhalation of spores of these ubiquitous organisms can exacerbate allergic symptoms in both atopic and non-atopic individuals. Several *Aspergillus* species produce mycotoxins that are harmful to humans and animals when ingested.

10.2 Geographical distribution

These conditions are worldwide in distribution.

10.3 The causal organisms and their habitat

Moulds of the anamorphic genus *Aspergillus* are widespread in the environment, growing in the soil, on plants and on decomposing organic matter. These moulds are often found in the outdoor and indoor air, in water, on food items and in dust. Although there are several hundred species of *Aspergillus*, fewer than 40 cause diseases in humans or animals, and some of these have been reported only once. *Aspergillus fumigatus* still

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accounts for most human cases of aspergillosis, with *Aspergillus flavus* the second most frequent pathogen. *Aspergillus terreus* is resistant to amphotericin B and is increasingly being reported as a cause of invasive disease in immunocompromised patients in some hospitals. *A. fumigatus* is also important as a human allergen, while *Aspergillus clavatus* and *A. fumigatus* are among the occupational causes of extrinsic allergic bronchoalveolitis.

10.4 Epidemiology

Inhalation of *Aspergillus* spores is the usual mode of infection in humans. The incubation period is unknown. Less frequently, infection follows the traumatic implantation of spores as in corneal infection, or direct inoculation from contaminated dressings. Inhalation of contaminated water aerosols during patient showering has been suggested as an additional potential source of infection.

The aetiological agents of aspergillosis are ubiquitous in the environment and the likelihood that infection will occur following inhalation or implantation of spores largely depends on host factors. Invasive aspergillosis has emerged as a major problem in several groups of immunocompromised patients. Those at greatest risk include persons with haematological malignancies, particularly acute leukaemia, haematopoietic stem cell transplant (HSCT) recipients, solid organ transplant (SOT) recipients, individuals receiving high-dose corticosteroid treatment and persons with neutrophil deficiencies or dysfunction, such as children with chronic granulomatous disease (CGD). Emerging at-risk populations include persons with the acquired immunodeficiency syndrome (AIDS), patients receiving intensive care, patients with chronic obstructive pulmonary disease and patients receiving tumour necrosis factor (TNF) blockers. The likelihood of aspergillosis developing in these individuals depends on a number of host factors, the most important of which is the level of immunosuppression, whether this manifests as profound or prolonged neutropenia, as graft-versus-host disease (GVHD) in HSCT recipients or as rejection in SOT recipients. With new developments in the management of the underlying disorders, the patient groups who are at highest risk of developing invasive aspergillosis are changing, and there is an ongoing need to reassess the factors that can affect different groups of susceptible hosts.

The occurrence rate of invasive aspergillosis in neutropenic patients with haematological malignancies is variable and is dependent on the underlying disorder, the treatment regimen, and other supportive measures used. Most at risk are those patients who remain neutropenic for 2 weeks

or longer. The use of corticosteroids in an antineoplastic regimen increases the risk for aspergillosis. Patients who have gone through several periods of profound neutropenia (during remission induction, consolidation or relapse treatment) are at increasing risk of developing aspergillosis, and a history of infection is often predictive of relapse during a subsequent period of immunosuppression. However, some high-risk individuals do not develop the disease, suggesting an unidentified protection mechanism.

Allogeneic HSCT recipients, particularly HLA-mismatched and unrelated donor recipients, are at high risk for invasive aspergillosis for a prolonged period of time because of disruption of mucosal barriers, delayed engraftment, GVHD, and the use of corticosteroids. Active acute GVHD has been identified as a major risk factor for the early development of *Aspergillus* infection, while extensive chronic grade III–IV GVHD is a major risk factor for late infection. The risk of aspergillosis is further increased by concomitant cytomegalovirus (CMV) infection.

Among SOT recipients, those with renal allografts are least at risk. In contrast, lung transplantation carries a high risk of invasive aspergillosis. Among the risk factors that have been identified are graft rejection, obliterative bronchiolitis, CMV infection and increased immunosuppression. Liver transplant recipients are another high-risk group. Many of those who develop aspergillosis have signs of poor allograft function and/or renal dysfunction that required dialysis.

It has long been recognized that few patients with haematological malignancies and invasive aspergillosis will survive unless their neutrophil count recovers. Earlier diagnosis and optimal treatment have also been associated with a better prognosis. Risk factors associated with mortality in invasive aspergillosis include progression of an underlying malignancy, receipt of allogeneic HSCT, pre-existing respiratory function impairment, renal impairment, diffuse lung involvement or disseminated infection, and prior corticosteroid treatment. Among the factors that have been associated with a poor outcome in allogeneic HSCT recipients with aspergillosis are active acute GVHD of grade II or more, extensive chronic GVHD at the time of diagnosis, and a high cumulative dose of prednisolone.

The incidence of invasive aspergillosis in high-risk patient groups is difficult to determine for a number of reasons. In the past, these included the lack of a consistent case definition that made it difficult to compare the incidence rates reported in different studies. This situation has improved with the adoption of an agreed set of consensus definitions for proven and probable invasive fungal infection. However, because these definitions require the use of aggressive diagnostic procedures that may not be

performed in all cases, it is likely that incidence figures will underestimate the true burden of disease.

There is a widespread perception that there has been a marked increase in the prevalence of invasive aspergillosis over the past several decades. Although this belief may be correct, the evidence most frequently cited in its support has been derived from longitudinal studies conducted among HSCT recipients in individual hospitals and may not be representative of the situation in other patient groups, institutions or countries. However, there are other studies that support the perception that aspergillosis is increasing in incidence. Most of these have relied on retrospective analysis of large databases, such as hospital discharge and death records. Although helpful for investigating trends, these databases tend to underestimate the incidence of fungal infections, such as aspergillosis. Nonetheless, these data indicate that the incidence of aspergillosis as a hospital discharge diagnosis in the United States increased about eightfold between 1976 and 1996, from 4.8 to 38 cases per million population.

The incidence of invasive aspergillosis differs from one high-risk patient group to another. Among 8000 HSCT and SOT recipients at 19 hospitals in the United States during 2001 and 2002, the cumulative 12-month incidence of aspergillosis was 0.5% after autologous HSCT and ranged from 2.3% after allogeneic HSCT from an HLA-matched related donor to 3.9% after transplantation from an unrelated donor. Among SOT recipients, the incidence was 2.4% after lung transplantation, 0.8% after heart transplantation, 0.3% after liver transplantation and 0.1% after kidney transplantation. These figures are lower than those published in earlier reports. Many factors could account for this, including changes in transplantation practices, including the use of non-myeloablative conditioning regimens and receipt of peripheral blood stem cells; improved diagnostic procedures, including the use of galactomannan antigen testing; and improvements in supportive care.

The case fatality rate from invasive aspergillosis remains high, ranging from 50–100% in almost all groups of immunocompromised patients. In a large review of cases reported up to 1995, case-fatality rates for cerebral, pulmonary and sinus infections were 99%, 86% and 66%, respectively. Some improvement has been achieved since 1995. Among 642 patients who developed invasive aspergillosis at 23 hospitals in the United States between 2001 and 2005, 58% of HSCT recipients died within 12 weeks of transplantation, compared with 34% of SOT recipients. Other reports have also described an improvement in outcome over time: in a recent report from a group of Italian hospitals, the attributable mortality rate

among patients with acute leukaemia fell from 60% in 1987–1988 to 32% in 2002–2003.

The most important extrinsic risk factor for invasive aspergillosis is the presence of *Aspergillus* spores in the hospital environment. Healthcare-associated outbreaks of the disease have become a well-recognized complication of construction or renovation work in or near units in which high-risk patients are housed. These reports have served to highlight the fact that the hospital environment is often contaminated with *Aspergillus* spores and have contributed to the widespread perception that most cases of aspergillosis in immunocompromised persons are hospital acquired. However, despite the fact that most hospital outbreaks of aspergillosis go unpublished, it is clear that these events in general are uncommon.

Most cases of invasive aspergillosis are sporadic in nature, and it is much more difficult to determine whether these infections are acquired inside or outside the hospital setting. It seems probable that some individuals are colonized before their admission to hospital and develop invasive disease when rendered neutropenic. Indeed, it has been estimated that up to 70% of cases of invasive aspergillosis diagnosed over a 2-year period of surveillance during construction in one North American hospital were acquired outside the hospital setting.

There is other evidence that a significant number of sporadic cases of invasive aspergillosis are now being acquired outside the hospital environment. In the 1970s and 1980s, most cases of *Aspergillus* infection among leukaemic patients occurred during the first few weeks after transplantation, before engraftment had occurred, or while individuals were undergoing intensive remission induction treatment. However, recent reports have indicated that many HSCT recipients now develop aspergillosis some months after transplantation, usually in association with GVHD and its treatment.

10.5 Clinical manifestations

Inhalation of *Aspergillus* spores can give rise to a number of different clinical forms of aspergillosis, depending on the immunological status of the host. In immunocompromised patients, there is widespread growth of the fungus in the lungs or sinuses and dissemination to other organs often follows. It must, however, be emphasized that with early diagnosis and treatment, a significant number of patients can now be cured. In non-compromised individuals, *Aspergillus* can cause localized infection of the lungs, sinuses and other sites, or act as a potent allergen. A wide range of sub-acute or chronic forms of aspergillosis are now recognized, which are

distinguished from each other and from acute invasive aspergillosis by their radiological appearance, rate of clinical progression and histopathological findings. However, there is considerable overlap between these forms of disease.

10.5.1 Acute invasive pulmonary aspergillosis

Acute invasive pulmonary aspergillosis occurs in immunocompromised individuals. It can be classified as focal or diffuse, the former having the better prognosis. Haematogenous dissemination to distant organs is a frequent complication in neutropenic cancer patients and transplant recipients. The central nervous system (CNS) is the most common secondary site of invasive disease, being involved in 10–20% of cases. In individuals with CGD or AIDS, a more indolent course is typical, but local spread of infection into the ribs or spine can occur.

The hallmark of invasive *Aspergillus* infection in the neutropenic patient is angioinvasion. Once this occurs, thrombosis, infarction and necrosis of the surrounding lung tissue follows. Erosion of the vessel wall can occur, leading to catastrophic haemorrhage. In non-neutropenic individuals, including HSCT and SOT recipients, persons with severe GVHD, persons with AIDS and those exposed to corticosteroids, the underlying pathology consists of a pyogranulomatous inflammatory reaction without angioinvasion. Cavitation is frequently observed, probably as a result of tissue necrosis. Individuals with CGD present with multifocal nodular, micronodular or military infiltrates with scattered non-caseating, non-confluent granulomas with variable amounts of necrosis.

The clinical presentation of invasive pulmonary aspergillosis is varied and diagnosis is difficult. The commonest initial presentation in the neutropenic patient is an unremitting fever (greater than 38°C), without any respiratory tract symptoms, that fails to respond to broad-spectrum antibacterial treatment. Corticosteroid-treated patients often present without fever, and low-grade chest pain, which can be pleuritic, is a common finding. Non-productive cough is another common presenting symptom, but haemoptysis is unusual. The presentation of invasive pulmonary aspergillosis in SOT recipients and persons receiving high-dose corticosteroids may be less fulminant than that seen in HSCT recipients or persons with haematological malignancies.

Initial investigations should include a chest radiograph and blood cultures, although the latter are usually negative in patients with invasive *Aspergillus* infection. Wedge-shaped, pleural-based densities and cavities (both late findings) are highly suggestive of invasive pulmonary aspergillosis. However, the disease can also present as diffuse infiltrates.

High-resolution computed tomographic (CT) scans are an essential tool in managing patients with suspected acute invasive pulmonary aspergillosis. Chest CT scans often reveal lesions in the lungs of immunocompromised patients at an early stage of infection. In neutropenic patients, the most distinctive signs of focal *Aspergillus* infection are small nodules of 1 cm or more in diameter and/or small pleural-based lesions. At least one nodule is present in about 90% of patients with invasive pulmonary aspergillosis. Often there is a characteristic region of lower attenuation (termed a halo sign) around the nodules. The halo sign is highly transitory and is related to the presence of haemorrhage around a necrotic nodule. Over time, the nodules may cavitate (often as the neutrophil count recovers), resulting in the characteristic crescent sign, a thin air crescent near the edge of a lung nodule, caused by contraction of infarcted tissue. The air-crescent sign is of limited usefulness in diagnosis in that it is a late sign, appearing during convalescence. Although the halo sign and air-crescent sign are characteristic of invasive pulmonary aspergillosis in HSCT recipients and neutropenic patients with haematological malignancies, other angioinvasive fungi, including the Mucorales (see Chapter 13) and *Fusarium* species (see Chapter 23), can produce similar radiological findings. The radiographic signs of *Aspergillus* infection of the lungs of non-neutropenic patients are much less distinctive and other investigations are required to confirm the diagnosis.

If focal disease is identified on a CT scan or radiograph, percutaneous lung biopsies should be obtained for microbiological and histopathogical investigation. Bronchoscopic examination is seldom helpful. However, if diffuse disease is seen on a CT scan or radiograph, bronchoscopic examination is essential. In neutropenic cancer patients and HSCT recipients, microscopic examination and culture of bronchoalveolar lavage (BAL) specimens is the most useful method for confirming the diagnosis of invasive pulmonary aspergillosis.

10.5.2 Tracheobronchitis and obstructing bronchial aspergillosis

Lung and heart-lung transplant recipients are at high risk for the development of invasive aspergillosis at the site of the anastomosis between the recipient trachea and the donor trachea, or at the site of the junction of the main bronchus. *Aspergillus* tracheobronchitis has also been described in HSCT recipients, in patients with lymphoma and in persons with AIDS. In lung transplant recipients, the disease usually occurs during the first month after transplantation. Patients are often asymptomatic or have symptoms attributable to the transplant, with chest radiographs

unchanged from baseline. However, symptoms become more pronounced as the infection develops. The most frequent are dyspnoea and wheezing, but some patients develop a cough and fever. Bronchoscopic examination will reveal multiple ulcers at the site of anastomosis and will permit the diagnosis to be established. In some cases, patients develop acute invasive pulmonary aspergillosis, while others progress to severe bronchial stenosis, anastomotic dehiscence and bronchial necrosis with bronchoarterial fistula formation. Some patients develop extensive pseudomembranes and die as a result of occlusion of the trachea or a bronchus.

Obstructing bronchial aspergillosis is a non-invasive condition that has been described in persons with AIDS. The presenting symptoms include cough, fever and wheezing. Large mucus plugs full of *Aspergillus* mycelium are expectorated or are encountered during bronchoscopic examination. These plugs form in the bronchi, leading to segmental or lobar atelectasis. Chest radiographs reveal bilateral lower lobe or generalized infiltrates. If left untreated, the disease can become invasive and spread upwards to produce tracheobronchitis. The diagnosis is established by bronchoscopic examination.

10.5.3 Acute invasive *Aspergillus* rhinosinusitis

This is a rapidly progressive disease that is most commonly seen in neutropenic cancer patients, HSCT recipients and other severely immunocompromised individuals. Acute invasive *Aspergillus* rhinosinusitis is much less common than invasive pulmonary disease in these host groups. *A. flavus* and *A. fumigatus* are the commonest aetiological agents. The clinical presentation is similar to that of rhinocerebral mucormycosis (see Chapter 13). The presenting symptoms include fever, unilateral facial swelling, unilateral headache, nasal or sinus congestion or pain, and a serosanguinous nasal discharge. Necrotic black lesions on the hard palate or nasal turbinate are a characteristic diagnostic sign. As the infection spreads into the orbit, periorbital or perinasal swelling occurs and progresses to disfiguring destruction of facial tissue. Ptosis, proptosis, ophthalmoplegia, and loss of vision can occur. In 25% of cases, the infection spreads into the brain and causes death.

CT scans can be used to determine the extent of bone destruction and pattern of sinus and orbital involvement. The commonest findings include involvement of several sinuses (in particular ethmoid and sphenoid), but with a clear unilateral predilection; no air-fluid levels; thickening of sinus linings and destruction of surrounding bone. Magnetic resonance imaging (MRI) scans are not clearly superior to CT, but are more accurate in providing information about cavernous sinus and cerebral involvement.

Tissue biopsy with histopathological examination and culture will confirm the diagnosis, but carries the risk of haemorrhage in patients with thrombocytopenia and may require general anaesthesia.

10.5.4 CNS aspergillosis

It is much more common for aspergillosis of the CNS to occur following haematogenous dissemination of infection from the lungs or heart than for it to result from direct spread from the sinuses. The CNS is involved in 10–40% of cases of disseminated aspergillosis, but the infection is seldom diagnosed during life. There is a spectrum of CNS disease and frequently more than one pathological process may be present at the same time. In immunocompromised individuals, the spectrum of disease includes cerebrovascular aspergillosis, cerebral abscess and meningitis. In immunocompetent individuals and those with CGD, solid intracerebral lesions are sometimes observed.

Cerebrovascular aspergillosis is most commonly seen in individuals with significant immunological impairment, particularly HSCT and SOT recipients. Less frequently, it is encountered in persons with AIDS, injection drug users, patients with native or prosthetic valve endocarditis and persons receiving corticosteroid treatment. Vascular invasion and thrombosis leads to infarction and necrosis and/or haemorrhage without suppuration. The clinical presentation is non-specific and typically includes an acute stroke-like illness with focal neurological signs, fits and a progressive loss of consciousness. The illness is rapidly progressive.

Aspergillus cerebral abscess formation has been reported in SOT recipients, persons with CGD, persons with AIDS, patients with endocarditis, injection drug users and patients receiving corticosteroid treatment. There are no specific symptoms or clinical signs, but the diagnosis should be considered in those patients with neurological signs and symptoms with consistent neuro-radiological abnormalities. The appearance of a cerebral abscess in the early period following SOT is characteristic of *Aspergillus* infection.

Focal *Aspergillus* meningitis is rare. More commonly, meningeal involvement represents a complication of cerebrovascular aspergillosis or cerebral abscess. There are no specific symptoms or clinical signs. Irrespective of the initial form of CNS infection, examination of the CSF is seldom helpful. The protein concentration may be moderately increased, the glucose concentration may be low or normal and a neutrophilic pleocytosis may be present.

The term cerebral granuloma is used to describe a less aggressive form of CNS aspergillosis that is characterized by solid intracerebral lesions

with florid granulomatous inflammation, but without suppuration. This form of infection has been described in individuals with CGD, as well as in immunocompetent patients from North Africa, the Middle East and the Indian sub-continent. It may arise following haematogenous dissemination or following extension from the sinuses (see Section 10.5.9).

Because early recognition and treatment may limit neurological damage, aggressive diagnostic and therapeutic intervention is important in patients with documented invasive pulmonary aspergillosis who develop signs of neurological deficits or unexplained abnormalities on CT or MRI scans. In cerebrovascular aspergillosis, CT scans may reveal one or more non-enhancing, hypodense lesions with little associated mass effect, but the findings are non-specific. MRI will often reveal additional lesions in the brain. In patients with a cerebral abscess, CT scans will reveal single or multiple space-occupying lesions showing ring enhancement, mass effect and associated cerebral oedema. In cerebral granuloma, CT scan will reveal an enhancing space-occupying lesion.

Biopsy or aspiration of material from a suspicious lesion is seldom possible, but if feasible, will often permit the aetiological agent to be detected on microscopic examination or isolated in culture. *Aspergillus* galactomannan antigen may sometimes be detected in CSF, thereby avoiding the need for an invasive neurological procedure for histopathological diagnosis.

10.5.5 Cutaneous aspergillosis

Two forms of cutaneous *Aspergillus* infection have been described in immunocompromised patients. In primary cutaneous aspergillosis, the lesion arises at or near an intravenous catheter insertion site in patients who have had contaminated dressings or splints applied to their skin. The lesion, which may act as the source of a subsequent disseminated infection, begins as an erythematous to violaceous, indurated plaque and evolves into a necrotic ulcer covered with a black eschar.

In secondary cutaneous aspergillosis, the lesions result either from haematogenous spread of infection from the lungs, or from extension to the skin from underlying infected tissue. This form of aspergillosis is seen in about 5% of patients with invasive disease. The lesions may be single or multiple, erythematous macules or papules. They evolve into necrotic ulcers with distinct borders covered with a black eschar. The lesions enlarge and may become confluent.

It is essential to biopsy cutaneous lesions to confirm the diagnosis, as similar necrotic lesions often arise in *Fusarium* infections and mucormycosis (see Chapters 13 and 23).

10.5.6 Simple pulmonary aspergilloma

An aspergilloma (or fungal ball) is a dense amorphous mass of fungal mycelium that is sometimes found in a lung cavity that forms following chronic diseases such as tuberculosis, sarcoidosis, bronchiectasis, pneumoconiosis or ankylosing spondylitis. Haemoptysis is the only serious complication. Fungal balls are usually located in the upper lobes. Less frequently they occur in the apical segments of the lower lobes. Spontaneous lysis has been reported to occur in up to 10% of cases. If there are multiple cavities, or if the single cavity with the aspergilloma is enlarging, the patient is considered to have chronic pulmonary aspergillosis (see Section 10.5.7).

Patients are often asymptomatic, but may present with chronic cough, malaise and weight loss. Haemoptysis is the most common symptom, occurring in 50–90% of cases. Most patients have infrequent episodes of small amounts of bleeding, but up to 25% suffer massive life-threatening haemoptysis.

Chest radiographs will reveal a characteristic solid oval or round mass, with a radiolucent halo or crescent of air over the superior aspect. If peripheral, localized pleural thickening overlying a cavity is characteristic. The mass can often be shown to move as the patient changes position. CT scans will help to delineate the lesion.

10.5.7 Chronic pulmonary aspergillosis

In addition to acute invasive disease and aspergilloma, a number of chronic forms of *Aspergillus* lung infection have been described. In the past, the distinction between these different clinical forms was not well-defined. A new nomenclature, based on clinical, radiological and histopathological findings, has now been introduced, separating patients with chronic pulmonary aspergillosis into three groups: those with chronic necrotizing pulmonary aspergillosis (CNPA), chronic cavitary pulmonary aspergillosis (CCPA) and chronic fibrosing pulmonary aspergillosis (CFPA). Further refinements to this classification have been suggested, but it should be recognized that the boundaries between some forms of chronic pulmonary aspergillosis and sub-acute invasive aspergillosis remain indistinct.

CCPA is characterized by the formation and expansion of multiple cavities, some of which may contain fungal balls. In some cases, the infection progresses to marked and extensive pulmonary fibrosis, and then is termed CFPA. In some cases, there is local extension into the pleura, either as a result of direct invasion or as fibrosis. In CNPA, there is

progressive enlargement of a single cavity, usually with a thin wall, in some cases to substantial dimensions, occurring slowly over months or rapidly in weeks. CCPA and CFPA typically affect patients without obvious immunological defects, but CNPA has been described in individuals with some degree of immunocompromise, including persons with AIDS, diabetes mellitus and alcoholism, and those who have received low-dose corticosteroid treatment.

Chronic pulmonary aspergillosis is an indolent, progressive condition that tends to occur in middle-aged or older persons with an underlying lung disease, such as inactive tuberculosis, bronchiectasis, sarcoidosis or pneumoconiosis. More men than women are affected. Patients with a simple aspergilloma of their lungs have a similar clinical profile and it is sometimes difficult to distinguish between the two disorders, because CCPA is often complicated by fungal ball formation. Usually, there is less pleural thickening in CCPA than in aspergilloma, but more pulmonary fibrosis or an enlarging cavity is seen. Many cavities in CCPA do not have fungus balls in them.

The most frequent presenting symptoms of CCPA include chronic productive cough and weight loss, with mild haemoptysis, dyspnoea, malaise and fatigue. Fever is absent and chest pain is uncommon. The earliest radiological changes are ill-defined areas of consolidation, typically within the upper lobes, that progress to form multiple, well-defined cavities that may or may not contain fungal balls, debris or fluids. In some cases, the cavities have thick walls and are adjacent to thickened pleura, but more commonly they are thin-walled without pleural thickening.

10.5.8 Chronic invasive *Aspergillus* rhinosinusitis

Aspergillus species are a frequent cause of chronic invasive rhinosinusitis (see also Chapter 25). This is a slowly progressive condition, analogous to chronic pulmonary aspergillosis that most commonly affects the ethmoid and sphenoid sinuses, but may involve any paranasal sinus. It has a worldwide distribution, but the largest number of cases has been reported from North America. It can occur in normal individuals, but is more often seen in subtly immunocompromised individuals, such as persons with AIDS, patients with diabetes mellitus, and those who have received corticosteroids. Affected individuals often complain of chronic nasal discharge and blockage, loss of smell and persistent headache. Thick nasal polyposis and thick purulent mucus are common. If left untreated, the infection can spread from the ethmoid sinuses into the orbit, leading to impaired vision and restricted ocular movement. Proptosis can also occur. Posterior

erosion from the ethmoid sinus can result in cavernous venous thrombosis. *A. fumigatus* is the most common cause of this condition.

Imaging studies can assist in determining the presence and the anatomical extent of sinus disease; however, plain radiographs are insensitive and do not distinguish fungal from bacterial infection. CT scanning is more discriminating. In patients with chronic invasive rhinosinusitis, noncontrast CT scans will reveal a hyperdense mass within the involved sinus with erosion of the sinus walls.

10.5.9 Chronic granulomatous *Aspergillus* sinusitis

This condition, which has also been termed paranasal granuloma, has been reported most commonly from North Africa, the Middle East and the Indian sub-continent. This is another slowly progressive invasive disease that occurs in immunocompetent individuals who often have had chronic sinusitis. The ethmoid sinuses are the most commonly involved site, with pansinusitis developing in many patients. Affected individuals present with long-standing symptoms of nasal obstruction and unilateral facial discomfort, or with a silent unilateral proptosis, but are otherwise normal. There is profuse fungal growth with localized tissue invasion. The granulomatous response is often intense enough to cause pressure necrosis of sinus walls and proptosis if the orbit is breached. Unless removed, the fungal mass can spread into the orbit and brain. *A. flavus* is the almost exclusive cause of this condition.

The most common radiographic findings are opacifications of the ethmoid, maxillary or all sinuses, together with bone erosions.

In chronic invasive rhinosinusitis, the fungus invades contiguous structures, whereas in granulomatous sinusitis, tissue destruction results from expansion of the mass. In chronic invasive disease, the sinus mass is composed of friable, necrotic or purulent material, but in chronic granulomatous sinusitis, the mass is firm and difficult to remove.

10.5.10 Paranasal sinus fungal ball

A paranasal sinus fungal ball is a dense mass of fungal mycelium that is sometimes found in the maxillary or sphenoid sinus cavities of patients undergoing investigation for chronic sinusitis, nasal obstruction, facial pain or other conditions. There is no fungal invasion of the mucosa, associated blood vessels or bone. Older individuals appear to be more susceptible; the condition is more common in women than men. The symptoms are often similar to those of chronic or recurrent bacterial rhinosinusitis, but many patients are asymptomatic. Affected persons often present with long-standing nasal obstruction, purulent nasal discharge, cacosmia

(foetid smell) or facial pain. The symptoms are often unilateral. *A. fumigatus* is the predominant cause.

CT scans will reveal partial or total opacification of the involved sinus, often associated with calcification. The maxillary sinus is the most common site affected, but the sphenoid or frontal sinus may occasionally be involved. Air-fluid levels, which are characteristic of acute bacterial sinusitis, are uncommon.

10.5.11 Endocarditis, myocarditis and pericarditis

Aspergillus endocarditis may present as a valvular or mural endocardial infection. Valvular vegetations most commonly develop on prosthetic valves, but can occur on natural valves, particularly in parenteral drug abusers. The aortic and mitral valves are the most frequent sites of infection. Valvular vegetations and, occasionally, mural vegetations may be large and friable, with a high risk of embolic complications. Mural endocarditis may be the result of disseminated *Aspergillus* infection.

The symptoms and clinical signs are similar to those of bacterial endocarditis. The illness may be abrupt in onset or insidious. Fever, weight loss, fatigue and loss of appetite are common, but non-specific symptoms. Heart murmurs can be detected in 50–90% of patients, and an enlarged spleen in 30%. More specific diagnostic signs include large friable vegetations. Emboli that obstruct major arteries, particularly those of the brain, occur in about 80% of cases.

Aspergillus infection of vascular prosthetic grafts has been reported in a few immunocompetent patients. In most instances, the grafts appear to have been contaminated at the time of insertion, and most cases have involved the thoracic or abdominal aorta. Some patients have presented with adjacent vertebral osteomyelitis.

Aspergillus myocarditis is common in patients with disseminated disease, being found in up to 25% of cases, but is seldom diagnosed antemortem. *Aspergillus* pericarditis has been reported in around 15% of cases of cardiac aspergillosis and is nearly always fatal. It usually arises as a result of extension of infection from a myocardial, mediastinal or pulmonary lesion, or intraoperative contamination.

10.5.12 Osteomyelitis and arthritis

Aspergillus osteomyelitis is an uncommon condition, but children with CGD seem to be at particular risk. In these individuals, spread from an adjacent pulmonary lesion is usual and the ribs and spine are the most common sites of *Aspergillus* infection. In immunocompromised adult patients, the spine is also the commonest site of infection, but

haematogenous dissemination of the organism may be more common. *Aspergillus* osteomyelitis can also result from the inadvertent inoculation of organisms during surgical procedures.

The clinical and radiological findings in vertebral aspergillosis are similar to those of tuberculosis. Most patients complain of fever and of pain and tenderness at the affected site. Many patients with *Aspergillus* osteomyelitis also have surrounding soft-tissue involvement, with pleural disease and paraspinal abscesses. Diagnostic imaging with CT and MRI scans is essential for staging the disease and for guiding orthopedic and/or neurosurgical intervention.

Aspergillus arthritis is rare, but it may develop from haematogenous dissemination in immunocompromised patients and parenteral drug abusers, or by direct traumatic inoculation in immunocompetent individuals. In many cases, it arises by extension from an adjacent focus of *Aspergillus* osteomyelitis.

10.5.13 Endophthalmitis

Aspergillus endophthalmitis is an uncommon condition, but it has been described in drug abusers, in patients with endocarditis and in organ transplant recipients. It is a devastating infection that may result in irreparable loss of vision and can arise following ocular trauma or haematogenous spread of the fungus. The latter is more usual in immunocompromised patients and parenteral drug abusers. The symptoms include ocular pain and impaired vision. Diagnosis requires direct ophthalmoscopic examination and culture of vitreous humour or aqueous humour specimens. On examination, most patients have an iridocyclitis or vitritis in association with yellow-white retinal lesions. Retinal haemorrhage or abscess can occur and hypopyon has also been described. *A. fumigatus* is the usual aetiological agent, but *A. flavus*, *Aspergillus niger* and *A. terreus* have also been implicated.

10.5.14 Allergic bronchopulmonary aspergillosis

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity disease of the lungs, most often seen in atopic individuals who develop episodic bronchial obstruction (asthma) and peripheral blood eosinophilia following inhalation of *Aspergillus* spores. Antigens released from the fungus colonizing the bronchi cause a vigorous humoral response that leads to eosinophilia and the release of immune mediators. Mucus secretion becomes pronounced and results in the formation of plugs that obstruct the bronchi; chronic obstruction leads to bronchiectasis. The illness can be mild, but it often progresses through a series of remissions

and exacerbations from acute corticosteroid-responsive asthma to corticosteroid-dependent asthma and then to fibrotic end-stage lung disease that is associated with a poor long-term prognosis

ABPA is seen in 1–3% of adults with asthma and 2–15% of those with cystic fibrosis, but can present in childhood. The most frequent symptoms include fever, intractable asthma, productive cough, malaise and weight loss. The diagnostic criteria include episodic bronchial obstruction (asthma), peripheral blood eosinophilia, an immediate prick test reaction to *Aspergillus* antigen, precipitating antibodies to *Aspergillus* antigen, elevated serum IgE concentration, history of pulmonary infiltrates (transient or fixed) and central bronchiectasis on chest CT. Other findings include repeated detection of *Aspergillus* in sputum, expectoration of brown eosinophilic mucus plugs, elevated specific IgE against *Aspergillus* antigen, Arthus reaction to prick test with *Aspergillus* antigen and characteristic defects on intrabronchial challenge with *Aspergillus*.

The radiographic findings range from small, fleeting, unilateral or bilateral infiltrates with ill-defined margins (often in the upper lobes) and hilar or paratracheal lymph node enlargement, to chronic consolidation and lobar contractions. The typical transient opacifications are usually due to bronchial obstruction with mucus plugs and disappear when these are expectorated. Chest infiltrates may be detectable on CT when plain radiographs are unremarkable. Some patients have concurrent ABPA and chronic pulmonary aspergillosis with overlapping radiological presentations.

10.5.15 Allergic rhinosinusitis

Aspergillus species are a frequent cause of allergic fungal sinusitis (see also Chapter 25). This condition occurs in young immunocompetent adults with chronic relapsing rhinosinusitis, unresponsive to antibiotics, antihistamines or corticosteroids.

Patients often present with unilateral nasal polyposis and thick yellow-green nasal or sinus mucus. The nasal polyposis may form an expansive mass that causes bone necrosis of the thin walls of the sinus. Should the ethmoid bone be traversed, allergic mucin can enter the orbit and cause proptosis. Polypoid material can also cause nasal septal deviation. Sinus radiographs show mucosal thickening. CT scans often reveal a characteristic serpigious sinus opacification of more than one sinus, mucosal thickening and erosion of bone, but this does not represent tissue invasion.

The diagnosis of allergic sinusitis requires the microscopic examination of the characteristic eosinophilic mucin (either at the time of surgical debridement for chronic sinusitis or endoscopic examination for drainage)

to determine the presence of eosinophils and fungal hyphae, and of sinus tissue to rule out invasion. The laboratory findings are identical to those seen in ABPA, including eosinophilia, elevated serum IgE concentration, elevated specific IgE against *Aspergillus* antigen, and an immediate prick test reaction to *Aspergillus* antigen.

10.6 Essential investigations and their interpretation

Establishing the diagnosis of invasive aspergillosis in patients with haematological malignancies and transplant recipients is often difficult to accomplish. In most cases, the diagnosis is based on a combination of clinical, radiological, microbiological and histopathological findings. These might include a positive culture result for a specimen obtained from a normally sterile and clinically or radiologically abnormal site or histopathological or cytopathological examination showing hyphae consistent with *Aspergillus* in a biopsy specimen or aspirate. Unfortunately, those patients at highest risk of invasive infection are also at highest risk for complications from the invasive procedures needed to make a definitive diagnosis. Therefore, in many clinical settings, the diagnosis is presumptive, and based upon chest CT findings and clinical signs in a patient with recognized risk factors. Several non-culture-based methods of diagnosis have been the subject of extensive evaluation in recent years. These include tests for detection of *Aspergillus* antigens in blood and other body fluids, as well as molecular methods to detect circulating *Aspergillus* DNA.

10.6.1 Microscopy

Among the most reliable methods for the diagnosis of invasive aspergillosis is the histopathological examination of stained biopsy sections. The detection of non-pigmented, septate hyphae that show repeated dichotomous branching is characteristic of *Aspergillus* infection. However, other less common hyaline moulds, such as *Fusarium* and *Scedosporium* species, can appear similar (see Chapter 23), and isolation of the aetiological agent in culture is essential to confirm the diagnosis. More precise identification can sometimes be achieved with immunohistochemical staining methods.

Direct microscopic examination of sputum preparations is seldom helpful in patients with suspected invasive aspergillosis, but examination of bronchoscopy-obtained specimens is often rewarding. Typical branching, septate hyphae may also be detected in 10–20% potassium hydroxide preparations of necrotic material from cutaneous or sinus lesions.

Microscopic examination of sputum is often helpful in the diagnosis of ABPA, because abundant branching, septate hyphae with characteristic dichotomous branching are usually seen.

10.6.2 Culture

In general, culture is not a sensitive method for the diagnosis of invasive forms of aspergillosis, but it may be the earliest clue to the diagnosis in less common risk groups. Moreover, because *Aspergillus* species are commonly found in the air, their isolation must be interpreted with caution. These moulds are rarely recovered from blood or CSF specimens, although cultures of the former have been positive in occasional patients with endocarditis. More often, however, their isolation is due to laboratory contamination.

Isolation of an *Aspergillus* species from a respiratory tract specimen obtained from an immunocompromised patient with a pulmonary infiltrate is usually indicative of infection, but the success rate does depend on the patient group and the pattern of disease. In neutropenic cancer patients and HSCT recipients with diffuse infection, culture of BAL fluid is positive in up to 60% of cases. The success rate is lower in organ transplant patients, with the exception of lung transplant recipients. Culture of BAL fluid is seldom helpful in patients with focal lung disease.

A. fumigatus can be recovered from sputum specimens from up to 60% of patients with ABPA, if multiple samples are tested.

10.6.3 Skin tests

Skin tests with *Aspergillus* antigens are only useful in the diagnosis of allergic aspergillosis. Patients with uncomplicated asthma due to *Aspergillus* give an immediate type I reaction. Those with ABPA give an immediate type I reaction and 70% also give a delayed type III reaction. Reagents from different manufacturers may give differing results.

10.6.4 Serological tests

Tests for *Aspergillus* antibodies are often helpful in the diagnosis of the different forms of aspergillosis that occur in the non-compromised patient. The available tests include immunodiffusion (ID), indirect haemagglutination and ELISA. The ID test is simple to perform and precipitins can be detected in up to 70% of patients with ABPA and over 90% of those with pulmonary aspergilloma or chronic pulmonary aspergillosis. The ID test is also useful for diagnosing other invasive forms of *Aspergillus* infection, such as endocarditis, provided the patient is not immunosuppressed.

Tests for *Aspergillus* antibodies have been evaluated for the diagnosis of invasive aspergillosis, but their role remains uncertain. The detection of precipitins in a neutropenic patient with unresponsive fever or a pulmonary infiltrate is often sufficient to prompt the initiation of antifungal treatment, but it must be stressed that a positive ID test result is not proof of infection. Nor does a negative test result preclude the diagnosis of aspergillosis in an immunosuppressed patient, because such individuals are often incapable of mounting a detectable antibody response. However, antibodies may appear following successful treatment. There is limited evidence that the detection of *Aspergillus* antibodies in SOT recipients, critically ill patients and other groups of relatively immunocompetent individuals at risk of invasive aspergillosis may be useful as a means of establishing the diagnosis, but antigen detection tests are a more appropriate diagnostic approach for those who remain immunocompromised.

10.6.5 Antigen detection tests

Tests for the detection of *Aspergillus* antigen in blood and other biological fluids offer a rapid means of diagnosing aspergillosis in the immunocompromised patient. Numerous clinical evaluations have demonstrated that low concentrations of galactomannan, a major cell wall component of *Aspergillus*, can be detected in serum, urine and BAL fluid specimens from HSCT recipients and neutropenic patients with haematological malignancies with invasive aspergillosis. Less information is available regarding the usefulness of antigen testing in other populations, such as SOT recipients and critical care patients.

Two tests to detect circulating *Aspergillus* galactomannan are commercially available. A latex particle agglutination (LPA) test was the first to be developed (Pastorex *Aspergillus*, BioRad Laboratories, Paris, France), but despite its ease of use, it is relatively insensitive. More recently, a sandwich ELISA (Platelia *Aspergillus*, BioRadLaboratories) has been marketed for the detection of *Aspergillus* galactomannan. This test is included within the revised consensus definitions for diagnosing invasive fungal disease, and it is now considered part of the expected standard of care for haematological patients in some countries.

As clinical experience has increased, it has become clear that results obtained with the Platelia *Aspergillus* ELISA depend on a number of factors, including the stage of the disease at the time of testing, and the use of mould-active antifungal drugs as treatment or prophylaxis. The latter factor has been shown to significantly reduce levels of circulating galactomannan. Published results have also depended on several test

parameters, including the cutoff value used, and whether two consecutive positive test results were required for significance.

The Platelia *Aspergillus* ELISA results are reported as a ratio between the optical density of the patient's sample and that of a control with a low but detectable amount of galactomannan (1 ng/mL). The optimal cutoff value to maximize test sensitivity and specificity has been the subject of debate since this test first became available. The manufacturer initially considered a ratio of >1.5 to be positive. This cutoff value was used in many early studies, but has been progressively revised downwards. The US Food and Drug Administration (FDA) approved a value of 0.5, and this is now accepted worldwide. This greatly increases the sensitivity of the test, albeit at some loss of specificity.

The diagnostic potential of the Platelia *Aspergillus* ELISA has been confirmed in numerous large-scale evaluations. In some studies, positive results were only obtained during the later stages of infection, although early detection of galactomannan in serum has been noted in other reports. However, direct comparisons between the results of these studies are difficult due to differences in the populations studied and the test parameters selected. The positive predictive value of the test depends on the prevalence of the disease in the population studied. In a meta-analysis of 27 different studies, this value increased from 31% in a population with a prevalence of 5%, to 69% when the prevalence was 20%.

False-positive results have been reported with the Platelia *Aspergillus* ELISA in patients receiving piperacillin-tazobactam and other beta-lactam antibiotics, including ampicillin, amoxicillin and amoxicillin-clavulanic acid. Piperacillin and amoxicillin are semi-synthetic drugs derived from natural compounds produced by moulds of the genus *Penicillium* that contain galactomannan in the cell wall. Other causes of false-positive results have included neonatal gastrointestinal colonization with *Bifidobacterium*, and enteral feeding with a liquid nutrient that contained soybean protein.

The FDA has recently licenced the Platelia *Aspergillus* ELISA for testing the BAL fluid samples. Several recent reports suggest a cutoff value of 1.0 is optimal for these samples. In SOT recipients, galactomannan detection in BAL appears to be more sensitive than detection of antigen in serum or isolation of *Aspergillus* in culture.

At present, it appears that maximum cost-benefit can be obtained from the Platelia *Aspergillus* ELISA if it is used as a screening test in those patient groups that are at highest risk to develop the disease. These might include allogeneic HSCT recipients, acute myeloid leukaemia patients and patients undergoing aggressive chemotherapeutic regimens for relapsed disease. Serial samples should be obtained from these patients and

should be tested prospectively. If an individual is at high risk of having aspergillosis, obtaining two consecutive positive results appears to offer strong support for the diagnosis, provided the patient is not being treated with piperacillin or amoxicillin.

10.6.6 β-D-glucan detection

In addition to antigen detection, a number of other approaches have been developed for the rapid diagnosis of invasive aspergillosis. These include tests to detect the presence in serum of (1,3)-β-D-glucan, a cell wall component of many fungi. The Fungitell assay (Associates of Cape Cod, Falmouth, Massachusetts, USA) is approved by the FDA for the presumptive diagnosis of invasive fungal disease, including aspergillosis, candidosis and trichosporonosis. The negative predictive value of the test appears to be high. However, false-positive results have been reported in patients with bacteraemia, in those who have recently received albumin or immunoglobulin products, or undergone haemodialysis with cellulose membranes, and among those exposed to other sources of glucan, such as certain parenteral antibiotics.

10.6.7 Molecular diagnostics

Numerous nucleic acid amplification methods have been developed for the detection of *Aspergillus* DNA in blood, serum, BAL fluid, and other clinical specimens. Several regions within the *Aspergillus* genome have been evaluated as potential targets for detection, but most effort has focused on the ribosomal DNA genes (18S and 28S) or the internal transcribed spacer regions. Other performance variables include amplification platforms and detection of amplicons. Molecular diagnostics appear promising for the rapid detection of *Aspergillus* infection directly from tissue or body fluid specimens, but until ongoing efforts to reach some form of international consensus are concluded, use of these tests in the diagnosis of invasive aspergillosis will remain investigational. Standardized PCR assays are now becoming commercially available in some countries (MycAssay *Aspergillus* PCR, Myconostica Ltd., Cambridge, UK).

10.7 Management

10.7.1 Acute invasive pulmonary aspergillosis

The successful management of acute invasive pulmonary aspergillosis in the immunocompromised patient depends on the early initiation of effective antifungal treatment, correction of the underlying immunological defect that precipitated the infection and surgical intervention as

appropriate. Early initiation of antifungal treatment in patients with suspected invasive aspergillosis is justified while a diagnostic evaluation is conducted. Although the CT halo sign is not specific for invasive pulmonary aspergillosis, initiation of antifungal treatment on the basis of this sign alone is associated with an improved prognosis.

Voriconazole is now the drug of choice for the initial management of invasive pulmonary aspergillosis in most patients. In a large clinical trial, 53% of patients randomized to voriconazole had a successful outcome, as compared with 32% of those treated with conventional amphotericin B. Moreover, survival was better among those treated with voriconazole, and fewer adverse events occurred among this group.

Treatment with voriconazole should be started with an intravenous loading dose of 6 mg/kg at 12-hour intervals for two doses followed by 4 mg/kg at 12-hour intervals. Each dose should be infused over a 1–2-hour period. Once the patient can tolerate oral medication, the usual adult dose is 200 mg twice daily. The duration of treatment will differ from patient to patient and depend on the nature of the infection and the clinical response. The dosage of voriconazole should be reduced in patients with mild to moderate hepatic impairment, and it should be avoided in those with severe hepatic impairment. Blood levels of voriconazole are significantly reduced by co-administration with drugs that induce hepatic cytochrome P-450 enzyme activity (see Chapter 3).

Amphotericin B is the historical standard for treatment of invasive pulmonary aspergillosis, particularly for life-threatening and severe infections, despite the fact that it is not particularly effective. Moreover, use of the conventional deoxycholate formulation is limited by a range of serious side effects, particularly renal damage. There are numerous regimens for administration of this drug, but widespread agreement that in immunocompromised patients, it is important to give the maximum tolerated dose of amphotericin B (1–1.5 mg/kg per day) from the outset (see Chapter 3). Most isolates of *A. terreus* are resistant to amphotericin B, and an antifungal triazole should be used to treat infections caused by this species. Other uncommon species of *Aspergillus* that may be resistant to amphotericin B include *Aspergillus lentulus* and *Aspergillus ustus*.

The three lipid-based formulations of amphotericin B are approved as second-line agents for patients with invasive aspergillosis. Amphotericin B lipid complex (ABLC) and amphotericin B colloidal dispersion (ABCD) are approved at dosages of 5 mg/kg per day and 3–4 mg/kg per day, respectively, and liposomal amphotericin B is licenced at a dosage of 3–5 mg/kg per day for salvage treatment of aspergillosis. Although ABCD is the least expensive of the lipid-based preparations, it is seldom used

because of its significant infusion-related toxicities. The optimal dosage for treatment of invasive aspergillosis has not been defined for any of the lipid-based formulations. Liposomal amphotericin B has been safely administered at dosages as high as 15 mg/kg, but a randomized trial that compared a daily dosage of 3 mg/kg with a dosage of 10 mg/kg found the higher dose to confer no advantage for the treatment of aspergillosis. The dose–response rates for ABLC and ABCD have not been well studied.

Itraconazole (200 mg twice daily) was the first oral triazole antifungal to be approved for the treatment of invasive aspergillosis. However, its use is limited by a number of important drug interactions (see Chapter 3). In addition, poor absorption has been a serious problem with the original oral capsule formulation of this agent, particularly in neutropenic patients and HSCT recipients, and blood concentrations need be measured at regular intervals. It is now recommended that itraconazole should not be used for treatment of aspergillosis in patients with life-threatening infection.

Although posaconazole is currently the only triazole antifungal agent licenced for the prevention of invasive aspergillosis (see Section 10.8.2), it has not been approved as an initial treatment for this disease. Limited published data indicate that it might be useful for salvage treatment of invasive aspergillosis in patients whose disease has not responded to other antifungal treatment or who are intolerant of previous treatment. The recommended dosage for salvage treatment in adults is 800 mg/day given as four divided doses.

Caspofungin has been approved for the treatment of invasive aspergillosis in patients who have failed to respond to, or are intolerant of, other antifungal agents. Treatment should be started with an intravenous loading dose of 70 mg followed by 50 mg/day thereafter. Each dose should be infused over a 1-hour period. Although it is not licenced for this indication, caspofungin has been evaluated as first-line treatment for invasive aspergillosis in two small open trials in HSCT recipients: 42% of allogeneic and 33% of autologous transplant recipients had a complete or partial response at the end of treatment. Survival rates at 12 weeks were 50% and 53%, respectively. The drug was well tolerated in these high-risk populations.

Micafungin and anidulafungin are currently not approved for the treatment of aspergillosis.

The benefits of combination treatment for invasive aspergillosis have been much discussed, but have been difficult to confirm in clinical practice. In the absence of a well-controlled prospective trial, routine administration of combination treatment for the initial management of

patients is not recommended. However, in the context of salvage treatment, an additional antifungal agent might be added or a combination of antifungal drugs from different classes other than those in the initial regimen may be used.

Some clinicians recommend immediate surgical resection of one or more localized *Aspergillus* lesions in the lungs during neutropenia. Most, however, reserve surgical intervention for patients with persistent pulmonary lesions who require further intensive induction treatment or HSCT, for those with significant haemoptysis from a single focus, for those with invasion of the chest wall, or for those with focal lesions in a central location (near the mediastinum). The latter group is at higher risk of death from perforation of a bronchus, the trachea or one of the great vessels, and rapid surgical intervention is essential.

The optimum duration of antifungal treatment has not been well-defined, but it is dependent on the extent of the infection, the response to treatment, and the patient's underlying disease. In general, treatment of invasive pulmonary aspergillosis should be continued for a minimum of 12 weeks. In immunocompromised patients, treatment should be continued throughout the period of immunosuppression and until there has been complete or near complete resolution of disease. In neutropenic patients, this means continuing treatment until the neutrophil count is greater than 1×10^9/L and until relevant clinical and radiological abnormalities are resolving. AIDS patients and organ transplant recipients also require long-term treatment.

Granulocyte transfusion has been used with success as an adjunctive treatment in individual neutropenic patients with invasive aspergillosis. Effectiveness appears to depend at least in part on the dose of granulocytes transfused and the compatibility of the donor leucocytes with the recipient. Some patients experience serious transfusion reactions, including pulmonary dysfunction. There have been reports in the past of fatal pulmonary reactions following simultaneous infusion of amphotericin B and granulocytes, and these products are no longer given at the same time. Use of granulocyte transfusions may be considered in persistently neutropenic patients for whom standard treatment has failed, as well as in children with CGD.

Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have also been employed as adjunctive treatments in neutropenic patients with invasive aspergillosis. GM-CSF may be more effective than G-CSF, but is less well tolerated than G-CSF. Recovery of neutrophil counts and immune function in patients with invasive aspergillosis may be associated with an

immune reconstitution inflammatory syndrome and may result in significant clinical deterioration.

Other immune-based treatment approaches for invasive aspergillosis include administration of cytokines, such as recombinant human interferon-γ. This has been shown to prevent serious infections (primarily bacterial) in children with CGD and has been used successfully in combination with antifungal agents to treat CGD patients with aspergillosis.

10.7.2 Tracheobronchitis and obstructing bronchial aspergillosis

Early treatment of tracheobronchial aspergillosis may prevent anastomotic disruption and loss of the graft, as well as resolution of ulcerative tracheobronchial lesions in lung transplant recipients. Voriconazole is recommended as the initial treatment for this infection. Treatment should be started with an intravenous loading dose of 6 mg/kg at 12-hour intervals for two doses followed by 4 mg/kg twice daily. Each dose should be infused over a 1–2-hour period. Reduction of immunosuppression, where possible, is an important element in improving the outcome.

The infection has also been treated with itraconazole (400 mg/day for 4–6 months) or parenteral amphotericin B. Direct instillation of aerosolized amphotericin B or liposomal amphotericin B permits high concentrations of the antifungal agent to be delivered to the site of the infection, but this approach remains investigational. Surgical resection and stent placement may be required if dehiscence of the anastomosis should occur.

Patients with obstructing bronchial aspergillosis often respond to oral treatment with itraconazole (400 mg/day).

10.7.3 Acute invasive *Aspergillus* rhinosinusitis

Immunocompromised patients with acute invasive *Aspergillus* rhinosinusitis should be treated with voriconazole. Treatment should be started with an intravenous loading dose of 6 mg/kg at 12-hour intervals for two doses followed by 4 mg/kg twice daily. If the aetiological agent has not been identified or if histopathological examination is still pending, treatment with a lipid-based formulation of amphotericin B, at a dosage of 3–5 mg/kg per day, should be initiated in anticipation of possible mucormycosis (see Chapter 13).

Treatment should be continued at least until progression of disease ceases and the underlying disorder is well-controlled. Surgical debridement of infected sinus material is useful, but carries the risk of haemorrhage in thrombocytopenic patients.

10.7.4 Central nervous system aspergillosis

This infection has a dismal prognosis, and even with aggressive treatment, many patients will die. The lesions are often located deep in the brain and are difficult to remove without causing serious damage. Voriconazole is recommended for the initial management of this infection. Treatment should be started with an intravenous loading dose of 6 mg/kg at 12-hour intervals for two doses followed by 4 mg/kg twice daily. Blood levels of voriconazole are significantly reduced by co-administration with drugs that induce hepatic cytochrome P-450 enzyme activity (see Chapter 3). Itraconazole, posaconazole or a lipid-based formulation of amphotericin B are recommended for patients who are intolerant of, or fail to respond to, voriconazole. Surgical resection of lesions that would not result in worsening of neurological deficits is an important adjunct to antifungal treatment.

10.7.5 Cutaneous aspergillosis

The management of this disease depends on the extent of the infection and the underlying status of the patient. Systemic antifungal treatment with voriconazole is recommended for neutropenic individuals and most other immunocompromised patients. Treatment should be started with an intravenous loading dose of 6 mg/kg at 12-hour intervals for two doses followed by 4 mg/kg twice daily. Alternative agents include amphotericin B, posaconazole and itraconazole. Surgical debridement is essential for successful management of lesions that arise at catheter insertion sites.

10.7.6 Simple pulmonary aspergilloma

Surgical removal of the lesion is recommended. On occasion, segmental or wedge resection will suffice, but lobectomy is usually required to ensure complete eradication of the disease. Serious post-operative complications, such as haemorrhage and bronchopleural fistulae, occur in around 10% of cases.

If surgical intervention is contraindicated, endobronchial instillation or percutaneous injection of amphotericin B may be helpful. The optimum dosage has not been determined, but 10–20 mg amphotericin B in 10–20 mL distilled water instilled 2 or 3 times per week for about 6 weeks has proved successful. Larger doses (40–50 mg) have been instilled into lung cavities using percutaneous catheters.

The treatment of mild to moderate bleeding in asymptomatic patients remains controversial, but either tranexamic acid given orally or bronchial artery embolization may be at least partially effective.

10.7.7 Chronic pulmonary aspergillosis

Treatment of chronic pulmonary aspergillosis requires long-term, perhaps lifelong, administration of an oral antifungal drug, such as itraconazole (200–400 mg/day) or voriconazole (200 mg twice daily). This will often relieve the symptoms of this condition, but most patients will relapse once treatment is stopped. Resection plays a small role in the treatment of this form of aspergillosis because of underlying lung disease in many patients and a high rate of surgical complications.

10.7.8 Chronic invasive *Aspergillus* rhinosinusitis

Invasion of adjacent structures is a common complication of this infection. For this reason, extensive surgical debridement with removal of all necrotic material, combined with antifungal treatment is recommended. The optimum duration and total dose of amphotericin B that should be given have not been defined, and it is not uncommon for this condition to recur, necessitating further surgical intervention. The role of the newer triazole antifungal agents, such as voriconazole and posaconazole, is unclear but promising. There is limited evidence that long-term suppressive treatment with itraconazole can reduce the rate of recurrence following surgical resection and amphotericin B treatment.

10.7.9 Chronic granulomatous *Aspergillus* rhinosinusitis

Surgical removal of the fungal mass is recommended. Often, however, complete removal is difficult and the condition will recur, necessitating further surgical intervention. There is some evidence that post-operative treatment with itraconazole (200–400 mg/day for at least 6 weeks) reduces the rate of recurrence.

10.7.10 Paranasal sinus fungal ball

Surgical removal is recommended. Endoscopic techniques are also adequate, although recurrences have occurred with this form of treatment. Antifungal treatment is not indicated.

10.7.11 Endocarditis, myocarditis and pericarditis

Aspergillus endocarditis requires early recognition, followed by aggressive medical and surgical treatment to prevent embolic complications and valve destruction. Endocardial lesions require surgical resection: infected valves should be replaced 1–2 weeks after antifungal treatment has started, but earlier surgical intervention is indicated if there are large vegetations, signs of heart failure, or dysfunction of a prosthetic valve.

Although experience is limited, voriconazole appears to be an effective agent for management of *Aspergillus* endocarditis. Treatment should be started with an intravenous loading dose of 6 mg/kg at 12-hour intervals for two doses followed by 4 mg/kg twice daily. Amphotericin B is the historical standard for treatment of this infection, and treatment with a lipid-based formulation (3–5 mg/kg per day) is an alternative to voriconazole. The optimum length of antifungal treatment is uncertain, but it should be continued for at least 6 weeks after surgical intervention to reduce the likelihood of relapse. Because of the potential for recurrent infection following replacement of an infected prosthetic valve, lifelong treatment with an oral antifungal, such as voriconazole or posaconazole, may be justified.

Aspergillus myocarditis and pericarditis require aggressive antifungal treatment and, where indicated, surgical intervention.

10.7.12 Osteomyelitis and arthritis

Management of *Aspergillus* osteomyelitis and joint infections is difficult. Combined medical and surgical management is recommended, where feasible. Although experience is limited, voriconazole, either alone or in combination with aggressive surgical debridement of necrotic tissue, appears to be an effective agent for treatment of *Aspergillus* osteomyelitis. Treatment should be started with an intravenous loading dose of 6 mg/kg at 12-hour intervals for two doses followed by 4 mg/kg twice daily. Amphotericin B is the historical standard for management of this infection, and treatment with a lipid-based formulation (3–5 mg/kg per day) is an alternative to voriconazole. The optimum length of antifungal treatment is uncertain, but it should be continued for 6–8 weeks in immunocompetent patients. Itraconazole penetrates bone well, and long-term treatment with this drug (400 mg/day) has sometimes proved successful.

Most reported cases of *Aspergillus* arthritis have been treated with amphotericin B; experience with azoles is limited.

10.7.13 Endophthalmitis

Aspergillus endophthalmitis requires both medical and surgical treatment. Penetration of amphotericin B into the vitreous and aqueous humours following parenteral administration is inadequate to treat this infection. The drug is toxic and can cause focal retinal necrosis, but intravitreal doses of 5–10 μg in 50–100 μL can be tolerated. Injection should be carried out slowly into the central vitreous cavity and as far as possible from the retinal surface. Early vitrectomy can be sight-saving and is an essential part of the management of this infection.

Although experience is limited, combined systemic and intravitreal administration of voriconazole (at a dose of 100 μg) has proved successful in several cases of *Aspergillus* endophthalmitis.

10.7.14 Allergic bronchopulmonary aspergillosis

Management of this disease is directed at treating the acute asthmatic exacerbations and avoiding end-stage fibrosis. Mild disease may not require treatment. Increasing serum IgE concentrations, and new or worsening infiltrates on chest radiographs, are among the findings that suggest that corticosteroids should be used. Prednisone is the drug of choice because it is effective in reducing symptoms, improving chest radiographs, and abolishing positive sputum cultures. The usual dosage regimen is 1 mg/kg per day until radiographs are clear, then 0.5 mg/kg per day for 2 weeks. The same dose is then given at 48-hour intervals for another 3–6 months, and then the dose is tapered off over another 3 months. The initial regimen should be resumed if the condition recurs. Bronchodilators and postural drainage may help to prevent mucus plugging.

The role of antifungal drug treatment is unclear, but itraconazole appears useful as a corticosteroid-sparing agent. Two randomized trials showed that itraconazole, at a dose of 200 mg twice daily for 16 weeks, had a beneficial effect. Voriconazole and posaconazole have not been studied in this disease.

10.7.15 Allergic rhinosinusitis

Treatment of allergic rhinosinusitis consists of surgical debridement to remove polyps and the eosinophilic mucin containing fungal debris, which is thought to be the cause of the immune reaction in the sinus mucosa. More than one surgical procedure may be required to accomplish this goal. Adjunctive medical management is also required because it is unlikely that all fungal debris can be removed. Despite surgical debridement and post-operative systemic corticosteroid treatment, the condition recurs in up to two-thirds of patients.

There is limited evidence that oral antifungal treatment with itraconazole is of benefit in allergic fungal rhinosinusitis and may spare the use of steroids. Other azoles have not been evaluated.

10.8 Empirical treatment of suspected invasive aspergillosis

Empirical treatment with amphotericin B is recommended for high-risk neutropenic patients with haematological malignancies and those

undergoing HSCT who remain febrile despite the administration of broad-spectrum antibacterial agents in adequate dosage. Liposomal amphotericin B is as effective, but less nephrotoxic than the conventional formulation; empirical treatment with the latter should generally be limited to clinical situations where other safer antifungals are unavailable. Other options for empirical treatment include voriconazole and caspofungin; these drugs are ineffective against the mucoraceous moulds and caution is thus required. The recommended dosages are the same as for proven aspergillosis. Once begun, empirical treatment should be continued until the neutrophil counts recovers. Empirical treatment is not recommended for patients who are anticipated to have neutropenia of short duration (less than 10 days), unless other findings indicate the presence of an invasive fungal infection.

Although empirical treatment is the standard of care for neutropenic patients who remain febrile despite broad-spectrum antibacterial treatment, recent improvements in diagnosis of invasive fungal disease have brought into question the appropriateness of using persistent or recurrent fever as the sole criterion for initiation of antifungal treatment. High-resolution CT of the chest permits earlier diagnosis and treatment and thus better rates of survival from acute invasive aspergillosis. Used in combination with new screening tools, such as the Platelia ELISA for *Aspergillus* galactomannan antigen detection, it is possible to select for pre-emptive treatment those febrile neutropenic patients at greatest risk for invasive *Aspergillus* infection. This targeted approach appears to be safe and may lead to substantial reductions in use of healthcare resources. It is possible, however, that newer prophylactic agents, such as posaconazole, could reduce disease prevalence to levels where the performance of diagnostic screening tests becomes sub-optimal and these tools cease to be cost-effective.

10.9 Prevention

Because the diagnosis and treatment of invasive aspergillosis is difficult and because the mortality rate from this disease exceeds 90% in some high-risk patient groups, preventive measures have become an issue of major importance in the management of all high-risk groups. These measures can be divided into two main approaches: environmental precautions and antifungal drug prophylaxis.

10.9.1 Environmental strategies

Aspergillus infection can be acquired following a wide range of exposures inside or outside the hospital environment. Infection control

measures may be effective in reducing the occurrence of this and other mould infections among immunocompromised patients, and detailed guidelines for specific measures have been published.

Because most cases of invasive aspergillosis either start in, or are confined to, the lungs, and because *Aspergillus* spores are commonly found in the indoor and outdoor air, inhalation is thought to be the usual mode of infection in humans. Therefore, the first step in the prevention of infection in high-risk patients should consist of measures to reduce or eliminate obvious sources of environmental exposure, such as removing potted plants and flower arrangements from rooms where these individuals are being treated. Food items, such as spices, that are often contaminated with moulds should not be offered to immunocompromised patients. Thorough and regular cleaning of all surfaces should be undertaken to prevent dust accumulation.

It has been shown that HEPA filtration can reduce or even eliminate *Aspergillus* spores from the air. Caring for high-risk patients in hospital rooms supplied with HEPA-filtered air has led to reduced rates of nosocomial *Aspergillus* infection. Components of a protected hospital environment should include a well-sealed room, HEPA filtration of incoming air, directed (or laminar) airflow within the room, positive room air pressure relative to the corridor and high rates of room air exchange. Meticulous attention to maintenance and proper cleaning to ensure that dust does not build up in rooms or in ventilation systems, or become aerosolized during maintenance and cleaning procedures is essential. This approach is expensive, and while it is feasible for the highest-risk groups for limited periods, housing patients in rooms supplied with sterile air has never been cost-effective for all groups at risk. Moreover, *Aspergillus* infection can develop if patients are colonized before their admission to hospital or are moved from the protected environment to other parts of the hospital for essential procedures to be performed. It must be emphasized that improper operation or poor maintenance of even the most elaborate air filtration and ventilation systems represents a high risk for aspergillosis and can lead to nosocomial outbreaks of infection.

Water is another potential environmental source of *Aspergillus* spores and has been implicated in cases of infection acquired in hospitals. Avoidance of activities, such as showering, that can result in aerosolization of spore-contaminated water droplets may be advisable for high-risk patients. Although *Aspergillus* has been recovered from hospital showerheads and hot water taps, further work is need to confirm transmission from water and the proportion of cases it accounts for. In addition to

hospital-based investigations, it will be essential to conduct epidemiological studies to look for potential sources of water-borne infection in the home environment.

Construction work in or near hospital units in which high-risk patients are housed has been shown to be an independent risk factor for invasive aspergillosis. Immunocompromised individuals should not be treated in units with ongoing, adjacent construction work, but if this cannot be avoided, measures should be instituted to minimize the entry of dust and contaminated air. Patient care areas should be sealed off from areas of hospital construction by plastic sheeting or other suitable barriers.

Following discharge from hospital, immunosuppressed patients must continue to observe certain precautions to minimize the risk of *Aspergillus* infection. It is recommended that HSCT recipients avoid dust-laden indoor and outdoor environments, occupations involving contact with soil, food containing moulds and naturopathic medicines. Immunosuppressed individuals should not smoke tobacco or marijuana, because these have been shown to be contaminated with numerous moulds and have been implicated in cases of invasive infection.

10.9.2 Therapeutic strategies

Environmental control measures designed to protect high-risk individuals from exposure to *Aspergillus* spores in the hospital or at home are difficult. For this reason, other approaches to prevention are needed. Antifungal prophylaxis with posaconazole is now recommended for HSCT recipients with GVHD who are at high risk for invasive aspergillosis as well as for high-risk neutropenic patients undergoing induction chemotherapy for acute myelogenous leukaemia or myelodysplastic syndrome. Posaconazole is licenced for the prophylaxis of invasive fungal infections at a dose of 200 mg (5 mL) given three times daily. A potential limitation of posaconazole is erratic oral absorption, particularly in patients with intestinal GVHD. In this situation, drug levels should be monitored or an intravenous mould-active antifungal should be considered.

A large multicentre comparison of voriconazole (200 mg twice daily) with fluconazole as prophylaxis following myeloablative allogeneic HSCT has recently been published. Fungal free survival rates were similar in the two treatment groups at 6 months. Relapse-free and overall survival rates were also similar. There was a trend to fewer *Aspergillus* infections in the voriconazole group. Voriconazole inhibits several cytochrome P-450 enzymes, and there is considerable potential for the drug to increases the concentrations of other drugs metabolized by this system (see Chapter 3).

Co-administration with sirolimus, which can be used to prevent or treat GVHD, is contraindicated.

Itraconazole prophylaxis has been widely employed to prevent invasive aspergillosis among HSCT and lung transplant recipients, but few large comparative trials have been conducted and its usefulness remains controversial. According to a meta-analysis, however, itraconazole oral solution prevents invasive fungal infections, including aspergillosis, among neutropenic patients with haematological malignancies and allogeneic HSCT recipients, and reduces mortality from these infections. Itraconazole use is limited by the unpleasant taste of the cyclodextrin-containing oral solution and the erratic absorption of the oral capsule formulation. Moreover, the concomitant administration of itraconazole with cyclophosphamide, vincristine and other antineoplastic drugs metabolized by the hepatic cytochrome P-450 enzyme system may lead to harmful drug interactions and greater toxicity of the chemotherapy (see Chapter 3).

Amphotericin B, while broader in its spectrum than itraconazole, has largely been avoided as a prophylactic agent because of its nephrotoxicity. Numerous anecdotal reports of breakthrough infection occurring while patients were receiving low-dose parenteral amphotericin B (up to 0.25 mg/kg per day) indicate that this approach is unreliable as prophylaxis against aspergillosis in neutropenic cancer patients and transplant recipients. Lipid-based formulations of amphotericin B are less nephrotoxic, but more expensive, and have not been clearly shown to provide effective prophylaxis. However, many of the clinical trials that have been conducted with these agents used lower doses than are recommended for treatment of proven invasive fungal infections. Despite these disappointing results, there has been considerable interest in the use of nebulized amphotericin B, administered by inhalation, as prophylaxis to prevent *Aspergillus* infections in HSCT and lung transplant recipients. It has been reported that aerosolized ABLC is better tolerated than the conventional formulation. However, there are numerous issues to be addressed before routine use of aerosolized amphotericin B can be recommended, including optimization of dose, formulation and delivery method.

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CHAPTER 11

Invasive candidosis

11.1 Definition

The term candidosis (candidiasis) is used to refer to infections caused by yeasts belonging to the ascomycetous genus *Candida*. In addition to causing mucosal and cutaneous infection (see Chapter 5), these opportunist pathogens can cause acute or chronic invasive infection in immunocompromised or debilitated individuals. This may be confined to one organ or become widespread (disseminated candidosis).

11.2 Geographical distribution

These conditions are worldwide in distribution.

11.3 The causal organisms and their habitat

More than 200 species of *Candida* have been described, but many have not been implicated in human disease. More than 90–95% of all *Candida* bloodstream infections worldwide are caused by four species: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*. The remaining infections are caused by 15–20 other *Candida* species, such as *Candida dubliniensis*, *Candida famata*, *Candida guilliermondii*, *Candida inconspicua*, *Candida krusei*, *Candida lusitaniae*, *Candida norvegensis, Candida pelliculosa* and *Candida rugosa*. Although these species are uncommon causes of candidosis, several have been observed to occur in nosocomial clusters, or demonstrate intrinsic or acquired resistance to one or more antifungal agents.

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C. albicans remains the predominant cause of both superficial and invasive forms of candidosis worldwide, although the proportion of serious infections attributed to other species, such as *C. glabrata*, is rising. *C. albicans* is found in the mouth and gastrointestinal tract of around 30–50% of normal individuals, but much higher isolation rates have been recorded among patients receiving medical attention. Although *C. albicans* is still the most prevalent species recovered from both normal and sick individuals, it appears to be less common in the environment than many other *Candida* species. *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* have also been recovered from the digestive tract of normal individuals. In addition, *C. parapsilosis* is a colonizer of the skin and can persist on inert surfaces in hospital environments.

11.4 Epidemiology

In most cases, invasive *Candida* infection is endogenous in origin, but transmission of organisms from person to person can also occur. In the healthcare setting, the main mechanisms of cross-infection include direct patient-to-patient transmission; transmission from a colonized or infected person to a recipient via a third person, often a healthcare worker; transmission from a colonized or infected person to another individual via a medical device and more or less simultaneous transmission to two or more patients from a common source, such as contaminated intravenous infusions. In most reports of outbreaks or cross-infections due to *C. albicans* in intensive care unit (ICU) patients, the suspected or proven source has been the hands of healthcare workers. Outbreaks of *C. parapsilosis* infection among ICU patients have been linked to nosocomial acquisition via medical devices, such as vascular catheters, and/or parenteral nutrition. There are also a number of reports of *C. parapsilosis* cross-infection related to hand carriage by healthcare workers.

Invasive forms of candidosis are a significant problem in several distinct groups of hospitalized patients. These groups include neutropenic cancer patients and recipients of haematopoietic stem cell transplants (HSCT); recipients of liver transplants and patients who have been hospitalized in an ICU, particularly adult surgical units and neonatal units. More cases of invasive candidosis now occur among ICU patients than are diagnosed among neutropenic individuals. The reduction in incidence of the disease among high-risk cancer patients and HSCT recipients is due to the widespread routine use of fluconazole prophylaxis during periods of prolonged neutropenia, together with shortened neutropenia, less mucositis,

improved catheter use, and earlier initiation of empirical antifungal drug treatment in febrile patients.

Invasive candidosis is not a notifiable disease. As a result, most of the published data on the incidence of this infection have been derived from individual institutions. These studies sometimes have similar designs, but different denominators have been used, making it difficult to compare the rates of infection. Although still few in number, sentinel and population-based surveillance programs have helped to define changing trends in species distribution and have served to highlight the growing importance of different *Candida* species as compared with other groups of hospital-associated pathogens.

Longitudinal data from the US Centers for Disease Control and Prevention's National Nosocomial Infections Surveillance (NNIS) program showed that the rates of *Candida* bloodstream infection almost doubled among ICU patients in the United States during the 1980s. More recent data from the NNIS program showed that the incidence in this patient group decreased during the 1990s. This decline was mostly due to a reduction in the rate of *C. albicans* infections, from 8 cases per 10,000 catheter days in 1989 to only 2 cases per 10,000 catheter days in 1999. The incidence of *C. glabrata* infections, however, increased significantly (from 0.2 to 0.5 cases per 10,000 catheter days), while those of other *Candida* species remained unchanged.

In contrast, other data from the United States suggest that the number of patients discharged from hospital with fungal sepsis has tripled over the last decade, with *Candida* bloodstream infection being the most frequent cause. Although this infection is often associated with critical care, recent reports from the United States and Spain indicate that only one-third of patients were in an ICU when the *Candida* infection was diagnosed. Furthermore, these population-based studies found that 11–28% of patients with *Candida* bloodstream infection had disease onset outside of the hospital. Clearly, the risk factors associated with this infection are no longer limited to critical care patients.

In the United States, *Candida* species are ranked as the fourth most common cause of hospital-acquired bloodstream infection, with an annual incidence of 1.5 cases per 10,000 patient days or 8 infections per 10,000 hospital discharges. In comparable European studies, the incidence is slightly lower, at 0.5–0.7 cases per 10,000 patient days or 2–5 infections per 10,000 discharges from the hospital.

Although hospital-based epidemiological studies have provided much useful information about *Candida* bloodstream infection, the data obtained may not be as representative as those derived from active

population-based surveillance systems. Several population-based surveillance studies of *Candida* bloodstream infection have been reported from the United States, where annual incidence rates have ranged from 6 per 100,000 in the state of Iowa between 1998 and 2001, to 24 per 100,000 population in Baltimore, Maryland, between 1998 and 2000. For reasons that are still unclear, the overall incidence of *Candida* bloodstream infection in the United States is higher than in a number of European countries, including Finland (2 per 100,000 population between 1995 and 1999), Iceland (4.9 per 100,000 population between 1995 and 1999) and Spain (4.3 per 100,000 population between 2002 and 2003). These differences in rates between countries could be due, in part at least, to different distribution of risk factors in the populations studied, different age distributions, or differences in methodologies employed. For instance, the proportion of outpatient infections was lower in Spain than in the United States (11% versus 28% within 24 hours of hospital admission), possibly due to a difference in the frequency of outpatient central venous catheter use.

Sentinel and population-based surveillance programs have enabled trends in the rank order of species distribution to be studied. *C. albicans* remains the predominant cause of *Candida* bloodstream infection worldwide, but the frequency with which this and other species of *Candida* are recovered from blood samples varies according to the local, regional or global setting, and demographics of the population studied. In the United States, *C. albicans* accounted for 45–55% of *Candida* bloodstream infections during the 1990s. In recent reports from hospital-based sentinel surveillance programs in Brazil and Japan, this species, while predominant, only accounted for 40% of *Candida* bloodstream infections. In contrast, 70% of these infections in several Nordic countries during the 1990s were caused by *C. albicans*.

C. glabrata has become the second most common cause of *Candida* bloodstream infection in the United States, accounting for 20–26% of cases in some recent reports. However, for reasons that are not well understood, *C. glabrata* remains an infrequent cause of *Candida* bloodstream infection in Latin America (4–6% of cases) where *C. tropicalis* and *C. parapsilosis* are the second and third most frequent aetiologic agents. *C. glabrata* bloodstream infection is rare among infants and children, but increases significantly in incidence with increasing age. This species is responsible for more than one-third of *Candida* bloodstream infections among patients aged 60 years or older. The emergence of *C. glabrata* as an important cause of bloodstream infection is of concern because of reports that 5–10% of incident bloodstream isolates of this species are resistant to fluconazole. In addition, cross-resistance to other azole agents has been well documented.

Although increasing fluconazole usage might have contributed to the emergence of *C. glabrata*, it is likely that other host and healthcare factors have also had an effect on this trend.

C. parapsilosis is more common than *C. glabrata* as a cause of bloodstream infection in European and Latin American countries. It is the third most common cause of these infections in North America, accounting for 10–20% of cases in recent reports. *C. parapsilosis* is the most common *Candida* species found on the hands of healthcare workers, and nosocomial clusters of infection have occurred in critical care patients, likely because of its association with indwelling vascular devices and parenteral nutrition. *C. parapsilosis* is known for its ability to form biofilms on catheters and other implanted devices. In neonates, *C. albicans* has long been the leading cause of *Candida* bloodstream infection, followed by *C. parapsilosis* and *C. tropicalis*. However, recent reports have documented a decrease in the proportion of infections due to *C. albicans* and an increase in the proportion of *C. parapsilosis* infections.

C. parapsilosis isolates can be divided into three groups on the basis of molecular analysis. The *C. parapsilosis* complex is now known to be comprised of three separate species: *C. parapsilosis, Candida orthopsilosis* and *Candida metapsilosis*. The three species account for around 92%, 6% and 2% of *C. parapsilosis* complex isolates, respectively. *C. parapsilosis* is less susceptible to the echinocandins than are most other *Candida* species, and breakthrough infections have been reported in HSCT recipients receiving long-term caspofungin treatment. However, bloodstream infections caused by this species are associated with a lower mortality rate than are infections caused by other common *Candida* species. *C. orthopsilosis* and *C. metapsilosis* are susceptible to the echinocandins.

C. tropicalis has long been considered as an important cause of invasive candidosis in HSCT recipients and patients with haematological malignancies. Although it is only the third or fourth most frequent cause of *Candida* bloodstream infection in North America, accounting for about 8% of cases in recent reports, it ranks second in South America and is also more common than *C. glabrata* in the Asia-Pacific region. The incidence of fluconazole resistance among *C. tropicalis* bloodstream isolates is negligible, and this may account for the marked decrease in incidence of this species that followed the widespread use of this agent for prophylaxis in HSCT recipients and patients with leukaemia or lymphoma.

Among the remaining 20 or so *Candida* species that have been reported to cause invasive infection, there are several that merit mention because they show decreased susceptibility to one or more antifungal agents. *C. krusei* is not a common cause of *Candida* bloodstream infection in most

countries, accounting for 1–5% of cases in most population-based and sentinel surveillance programs. This species is most notable for its intrinsic resistance to fluconazole and its propensity to emerge in settings where this drug is used as prophylaxis. However, it is also less susceptible to amphotericin B and flucytosine. It should be noted that colonization and infection with *C. krusei* had become a problem in certain hospitals well before the widespread use of fluconazole. In longitudinal studies, the proportion of *C. krusei* infections has remained almost constant over time. *C. lusitaniae* most often causes bloodstream infection in patients with malignancies or other serious underlying conditions. This species is notable for developing resistance to amphotericin B during the course of treatment, and it has presented as breakthrough fungaemia in immunocompromised patients. *C. guilliermondii* and *C. rugosa* have been associated with clusters of infection in healthcare settings. Both species demonstrate decreased susceptibility to amphotericin B, fluconazole and the echinocandins.

Many risk factors for invasive candidosis have been described. These can be divided into host-related factors, such as underlying immunosuppression and low birth weight, and healthcare-related factors, such as intravascular catheterization, broad-spectrum antibiotic use, total parenteral nutrition, haemodialysis and surgical procedures. In general, crude case-fatality rates for *Candida* bloodstream infections have ranged from 20% to 60% (40% in adults and 20% in premature infants). Estimates of the mortality attributable to candidaemia and other forms of invasive candidosis have been reported from retrospective matched cohort studies conducted in single institutions as well as from population-based surveillance. Attributable mortality has ranged from 10% to 50% and has not changed over the last two decades, despite the introduction of new and effective antifungal agents. Because of the multiple host- and healthcare-related factors involved, it is difficult to compare species-attributable case-fatality rates. However, *C. parapsilosis* is consistently associated with a lower mortality, while *C. glabrata* is sometimes associated with a higher mortality, due in large part to the fact that it tends to occur in sicker, older and more debilitated individuals.

Among adult patients cared for in surgical ICUs, *Candida* bloodstream infection has a case-fatality ratio of about 40%. The major risk factors independently associated with candidosis in this setting include previous surgical procedures, acute renal failure and receipt of parenteral nutrition. In addition, triple-lumen catheters have been associated with an increased risk of infection among surgical patients. However, not all patients in the surgical ICU are at equal risk of invasive candidosis. Those who undergo neurosurgical or ear, nose and throat procedures appear to be at lowest

risk, while those who undergo abdominal procedures are at the highest risk. Invasive *Candida* infections are infrequent if adequate closure and resolution of the underlying problem is achieved. However, complications, such as anastomotic breakdown and recurrent abdominal leakage, are important predisposing factors that identify a high-risk subgroup that might benefit from antifungal prophylaxis.

Candida species are the third most common cause of neonatal hospital-associated bloodstream infection. Among premature infants cared for in neonatal ICUs, *Candida* bloodstream infection has a case-fatality rate of about 20%. The incidence of this infection among pre-term infants in neonatal ICUs has increased since the 1980s. It has been reported in 0.25% of infants weighing 2500 g or more, in 3% of very low birth weight (VLBW) infants (less than 1500 g birth weight) and in 5.5–7% of extremely low birth weight (ELBW) infants (less than 1000 g birth weight). When birth weight is adjusted for, other risk factors include gestational age of <32 weeks, 5 minute APGAR score of <5, presence of central vascular catheters, receipt of parenteral nutrition, treatment with broad-spectrum antibiotics (in particular third generation cephalosporins), H2 blocker treatment and intubation. Differences in the incidence of *Candida* bloodstream infection between white and black infants have been reported from the United States, with rates among black infants being two to three times higher than among white infants. These differences have been attributed to the higher prevalence of low birth weight among black infants.

Without chemoprophylaxis, invasive candidosis develops in about 15–20% of allogeneic HSCT recipients. In this setting, the condition has a case-fatality rate of 40–50%. With fluconazole chemoprophylaxis, the case-fatality rate has fallen to around 20%, largely due to the virtual elimination of *C. albicans* and *C. tropicalis* infections. Recent reports suggest that risk factors, such as prolonged neutropenia, patient age, receipt of corticosteroids, and presence of acute graft-versus-host disease (GVHD) are less significant predictors of *Candida* bloodstream infections in patients receiving fluconazole. Other factors, including prior colonization, bacteraemia and cytomegalovirus infection now appear to be significant risk factors for the development of *Candida* infections in HSCT recipients.

Among recipients of solid organ transplants, invasive candidosis is most prevalent in liver and pancreas transplant recipients. In earlier reports, the disease was documented in 16–30% of liver transplant recipients. More recently, many transplant units have reported an incidence of less than 10%, even without fluconazole prophylaxis. Improved methods of immunosuppression and surgical technological improvements are among the factors that are thought to have led to this reduction in

incidence of candidosis. The major risk factors independently associated with invasive *Candida* infection in this setting include renal failure, high transfusion requirement during operation, retransplantation, choledochojejunostomy and fungal colonization detected within the first 3 days after transplantation. The risk for candidosis following pancreas transplantation is similar to that following liver transplantation. Up to 10% of pancreas transplant recipients have developed intra-abdominal infection after transplantation.

In all risk groups, previous colonization is an independent risk factor for bloodstream infection with all *Candida* species except *C. parapsilosis* (perhaps because this species usually is acquired through an indwelling catheter). The risk of infection increases with the number of sites colonized, but depends on the colonizing species (*C. tropicalis* colonization is more predictive of infection than *C. albicans* colonization). The extent of colonization, expressed in terms of the number of sites colonized, has sometimes been helpful in predicting invasive *Candida* infections in surgical patients receiving intensive care.

11.5 Clinical manifestations

11.5.1 Acute disseminated candidosis and candidaemia

Candidaemia is defined as the isolation of *Candida* species from one or more blood cultures. It can lead to the development of deep-organ candidosis or follow the development of such an infection. It is a critical diagnostic sign of acute disseminated candidosis, which is defined as infection of two or more non-contiguous organs following haematogenous spread. Acute disseminated candidosis is most commonly seen in seriously ill individuals who usually have one or more indwelling vascular catheters, although these are not necessarily the source of the infection. Many cases are thought to arise from translocation of organisms across the wall of the intestinal tract. In this situation, haematogenous spread can then lead to catheter colonization.

Adults with candidaemia usually present with a persistent fever that fails to respond to broad-spectrum antibacterial treatment, but with few other symptoms or clinical signs. Macronodular cutaneous lesions (seen in about 10% of neutropenic individuals, but rare in ICU patients) and endophthalmitis (found in up to 45% of ICU patients, but seldom seen in neutropenic individuals) are useful clinical signs of acute disseminated candidosis. Other manifestations include meningitis, brain abscess, renal abscess, myositis, myocarditis and endocarditis. Late complications include osteomyelitis and septic arthritis. Occasional patients present with

septic shock, which has a poor prognosis. Others present with minimal symptoms, and the isolation of *Candida* species from blood cultures may be the only evidence of invasive candidosis. It was this group of patients who gave rise to the term 'benign transient candidaemia', a condition that was thought not to require antifungal treatment. This has now been shown to be a hazardous interpretation, given the high incidence of metastatic complications.

In neonates, invasive candidosis can present as candidaemia, as single organ candidosis or as disseminated candidosis. The disease is most common in VLBW and ELBW infants requiring prolonged neonatal intensive care. It usually presents between 2 and 5 weeks of life with non-specific signs, such as deteriorating respiratory function, apnoea, bradycardia, hypotension, acidosis, fever, feeding intolerance and abdominal distension. Septic shock has also been reported. Often the presenting signs are the result of end-organ involvement. End-organ damage in the neonate is usually the result of haematogenous dissemination of infection. Meningitis occurs more frequently (about 15% of cases) than in older patients and is sometimes associated with arthritis and osteomyelitis. Renal involvement is another common manifestation. Endophthalmitis is rare.

C. albicans is the leading cause of *Candida* bloodstream infection in preterm infants. However, several recent reports have documented an increase in the proportion of *C. parapsilosis* infections in this group. Comparisons of neonatal bloodstream infections due to *C. albicans* and *C. parapsilosis* have demonstrated no significant differences in the overall mortality rate.

11.5.2 Chronic disseminated candidosis

This is an indolent illness that occurs in around 5% of leukaemic patients who have regained an adequate neutrophil count after remission induction treatment. It has sometimes been called hepatosplenic candidosis, but lesions are found in other organs as well. It is thought that initial colonization of the gastrointestinal tract with *Candida* species, followed by liver invasion through the portal venous circulation, is the most probable route of infection. It has recently been suggested that chronic disseminated candidosis may be a form of immune reconstitution inflammatory syndrome (IRIS).

In most cases, the infection begins while the patient is neutropenic and presents as a persistent fever that fails to respond to broad-spectrum antibacterial treatment. There are no discernible lesions in any organ, nor are there signs of infection in any particular organ. The neutrophil count then returns to normal, but the fever persists and is often associated with

continuing weight loss. The patient may complain of abdominal pain, and hepatic and/or splenic enlargement may be detected. Many patients have highly elevated blood levels of alkaline phosphatase, but other liver function abnormalities may be mild or absent. The diagnosis should be suspected if computed tomographic (CT) scans reveal numerous, small radiolucent lesions in the liver or spleen.

Microscopic examination of stained sections of biopsied tissue will often confirm the diagnosis, but the organism is isolated from no more than 30% of specimens. In most cases, blood cultures are negative. *C. albicans* is the principal aetiological agent, but *C. tropicalis* has sometimes been implicated.

11.5.3 Lower urinary tract candidosis

The presence of *Candida* species in the urine (termed candiduria) is sometimes indicative of renal candidosis, but more commonly it represents lower urinary tract colonization or infection. Ascending infection is the most common route of infection for the bladder. Lower urinary tract candidosis is more common in women because of a shorter urethra and frequent vulvovestibular colonization with *Candida* species. Other risk factors for candiduria include advanced age, recent antibiotic use, indwelling urethral catheters, prior surgical procedures and diabetes mellitus. The clinical presentation is varied, but unlike those with bacterial infections, most patients with candiduria have no symptoms. In some cases, however, lower urinary tract candidosis can cause dysuria and suprapubic tenderness. Candiduria tends to persist while the catheter is in situ, whether or not antifungal treatment is given. In most cases, however, the problem resolves with the removal of the catheter.

The diagnosis of *Candida* urinary tract infection is difficult because there are no definitive tests to localize the site of infection or to determine whether the presence of the organisms is due to colonization or infection (see Section 11.7.2). Although *C. albicans* is the commonest organism, accounting for 50–70% of isolations from the urine, *C. glabrata* is the second most common species, accounting for 20–30% of isolates. *C. glabrata* is often recovered from patients with diabetes mellitus and those who have received previous treatment with fluconazole. Older adults have more frequent isolation of *C. glabrata* from the urine than younger individuals. In neonates, *C. parapsilosis* is more common than *C. glabrata*.

11.5.4 Renal candidosis

It is much more common for renal candidosis to result from the haematogenous spread of organisms than for it to occur as a result of

ascending infection. At least 80% of patients with disseminated candidosis develop renal infection. For this reason, invasive infection should be suspected in a septic patient with persistent candiduria. Renal infection often results in microabscess formation. Less commonly, it results in the formation of clumps of mycelium (fungus balls) that can obstruct the pelvis or ureters, leading to hydronephrosis or anuria.

In adults, the symptoms of renal candidosis include fever, rigors, leukocytosis, lumbar pain and abdominal pain, indistinguishable from bacterial pyelonephritis. There are no specific radiological signs, apart from fungus balls in the renal pelvis or ureters, which appear as radiolucent, irregular filling defects. Imaging procedures, such as ultrasound and CT scans, are helpful in documenting obstruction and the presence of fungus balls. Pyuria is not a consistent indicator of infection. In neonates, renal candidosis can be asymptomatic or present with oliguria, anuria, haematuria and hypertension. Up to 35% of infants with candiduria have fungus balls that obstruct the pelvis or ureters, leading to hydronephrosis. Candiduria is often the first sign of disseminated neonatal candidosis.

11.5.5 Respiratory tract candidosis

Laryngeal candidosis is usually seen in individuals with human immunodeficiency virus (HIV) infection. Less commonly, it is encountered in patients with haematological malignancies. Hoarseness and dysphagia are the principal symptoms, but some patients present with stridor. The clinical signs are white plaques on the laryngeal mucosa. The diagnosis can be established by histopathological examination and culture of biopsy specimens collected during laryngoscopy. *Candida* tracheobronchitis is an unusual form of candidosis seen in HIV-positive or other immunocompromised individuals complaining of fever, productive cough, and dyspnoea. The diagnosis generally is made by bronchoscopy.

Candida pneumonia is a rare form of candidosis that has been described in neutropenic patients as well as in those receiving intensive care. It is seldom diagnosed during life, due to difficulties in distinguishing lower respiratory tract colonization from infection. It can arise following haematogenous dissemination of organisms or, more rarely, it occurs as a result of aspiration of colonized oropharyngeal or gastric contents. The clinical and radiological presentation is non-specific. Patients present with fever, cough, tachypnoea and diffuse nodular infiltrates on chest radiographs, indistinguishable from other causes of nosocomial pneumonia. Microscopic examination and culture of sputum, endotracheal secretions or bronchoalveolar lavage specimens does not distinguish colonization from infection. The definitive diagnosis of this form of candidosis depends

on the histopathological demonstration of organisms in lung tissue with associated inflammation.

11.5.6 Osteomyelitis and arthritis

Candida osteomyelitis usually occurs as a late complication of the haematogenous dissemination of organisms in neutropenic patients and low-birth-weight infants. It can also result from direct inoculation or spread from contiguous tissue following surgical or accidental trauma. In adults, the lumbar spine is often involved; in children, the long bones are the commonest site of infection. The commonest symptom is local pain, but fever is often absent. The infection gives rise to characteristic osteolytic lesions. Osteomyelitis may present weeks or months after dissemination of organisms, and consequently, blood cultures are usually negative.

Arthritis is usually the result of haematogenous dissemination, but it can also result from local trauma, the inadvertent inoculation of organisms into the joint during aspiration, injection of corticosteroids or during insertion of a prosthetic joint replacement. Patients with underlying joint disease, such as rheumatoid arthritis or prosthetic joints, are at increased risk. The symptoms include indolent joint pain and effusion. The large joints are most commonly involved.

11.5.7 Peritonitis

Candida peritonitis is one of the most common forms of candidosis among patients in intensive care. Risk factors for peritonitis include acute pancreatitis, recent or concomitant antimicrobial treatment and multiple abdominal surgeries. Pancreatic transplantation, especially with enteric drainage, is associated with abdominal *Candida* abscess formation.

Candida species form part of the normal flora of the human digestive tract, and can often be isolated from abdominal drain fluid specimens from patients with perforation of the gastrointestinal tract or a leaking intestinal anastomosis. The clinical significance of *Candida* isolation from post-surgical peritoneal specimens is often unclear: according to one report, 61% of cases from whom *Candida* was isolated had no signs of peritonitis. The decision to begin antifungal treatment should be based on clinical signs and risk factors in each patient. The symptoms and signs of *Candida* peritonitis are similar to those of bacterial peritonitis. The commonest symptoms are fever, abdominal pain and tenderness, abdominal distension, and paralytic ileus. *C. albicans* is the commonest pathogen, but *C. tropicalis* and *C. parapsilosis* are sometimes involved.

Candida peritonitis is an uncommon complication of peritoneal dialysis. It seldom results in positive blood cultures or haematogenous

dissemination. The isolation of *Candida* species from polymicrobial peritoneal fluid cultures suggests traumatic perforation of the bowel by the Tenchoff catheter.

11.5.8 Endocarditis, myocarditis, pericarditis and other vascular infections

Candida infection is the commonest form of fungal endocarditis, now accounting for 50–70% of cases of this disease. Three groups of individuals develop this condition: patients with underlying native valve disease, patients with prosthetic heart valves and intravenous drug abusers. The aortic and mitral valves are the most frequent sites of infection, but the tricuspid valve is sometimes involved in drug abusers.

In surgical patients, endocarditis tends to occur within the first 2 months after the operation, although it can also develop much later (>12 months). The prognosis has been poor and more than 80% of patients given antifungal treatment alone have died. Earlier and improved surgical intervention combined with aggressive antifungal treatment has led to more patients surviving: mortality rates of around 45% are now typical.

The symptoms and clinical signs are similar to those of bacterial endocarditis. The illness may be abrupt in onset or insidious. Fever, weight loss, fatigue and loss of appetite are common, but non-specific, symptoms. Heart murmurs can be detected in 50–90% of patients, and an enlarged spleen in 30–50%. More specific diagnostic signs that can help to distinguish the infection from bacterial endocarditis include large vegetations, large emboli to major vessels (more than two-thirds of patients) and endophthalmitis. Blood cultures are positive in up to 80% of cases, although the disease is often diagnosed post-mortem following histopathological examination and culture of vegetations.

Myocardial infection with abscess formation is a complication of endocarditis, but it may also occur as a result of haematogenous spread of organisms in immunocompromised patients. It has been found in 50–60% of individuals dying with disseminated candidosis. The diagnosis is difficult. Non-specific echocardiogram abnormalities and shock are common.

Purulent pericarditis is an unusual complication of haematogenous dissemination of *Candida* infection. It can also arise from extension of endocarditis or a superficial myocardial abscess. The diagnostic signs are non-specific and include chest pain, pericardial friction rub and pericardial effusion.

Other vascular infections include septic thrombophlebitis of peripheral or central veins and prosthetic graft infections. *Candida* phlebitis is often

associated with subcutaneous catheters. Delay in treatment can result in extensive vascular thrombosis and suppuration.

11.5.9 Central nervous system candidosis

Candida meningitis is found in around 15% of low-birth-weight infants with disseminated candidosis. It has a high mortality rate and survivors have a significant incidence of neurological deficits. *Candida* infection of the central nervous system (CNS) often results in granuloma or abscess formation and vasculitis. Complications include obstructive hydrocephalus, calcifications, thrombosis, and encephalomalacia. The presenting signs are non-specific, and cerebrospinal fluid (CSF) findings may be normal. CSF cultures are positive in about 50% of cases.

In adults, *Candida* meningitis is often a post-neurosurgical complication (especially after CSF shunt placement). Prolonged broad-spectrum antibacterial treatment, multiple neurosurgical procedures, prior bacterial meningitis and persistent CSF leakage are significant risk factors. Infection can also occur as a result of haematogenous dissemination, direct inoculation of organisms into the subdural space or retrograde spread along the distal portion of a ventriculo-peritoneal shunt (if the distal tip has caused bowel perforation). *Candida* meningitis often follows an indolent course with minimal fever.

The diagnosis is difficult, but *Candida* meningitis should be suspected in a neurosurgical patient with bacterial meningitis who fails to respond or deteriorates despite appropriate antibacterial treatment. It should also be suspected in a patient with other signs of disseminated candidosis who develops neurological signs.

The CSF findings are indistinguishable from those of bacterial meningitis. The protein concentration may be increased, the glucose concentration may be low or normal and a neutrophilic or lymphocytic pleocytosis may be present. Isolation of a *Candida* species from the CSF confirms the diagnosis; *C. albicans* is the principal aetiological agent.

In adults, many cases are diagnosed by isolation of *Candida* species from CSF obtained through indwelling neurosurgical devices. Multiple isolations from these specimens in patients with clinical sepsis confirm the diagnosis. However, even a single positive CSF culture in a patient with symptoms consistent with meningitis should prompt the initiation of antifungal treatment.

Other forms of CNS candidosis include brain abscess and diffuse metastatic encephalitis. These manifestations are seldom diagnosed during life. Large brain abscesses may give rise to focal neurological signs. Once CNS involvement is established or suspected, imaging should be

considered. The lesions can be detected with CT or magnetic resonance imaging (MRI) scans. More often, however, haematogenous spread of organisms results in multiple small lesions that produce no obvious neurological deficits. In neonates, CT scanning with IV contrast allows for visualization of abscesses and is superior to MRI for visualizing calcifications.

11.5.10 Endophthalmitis and chorioretinitis

Haematogenous dissemination of *Candida* species to the eye can lead to chorioretinitis that can then spread to the vitreous to cause endophthalmitis. This condition has been reported to occur in up to 45% of non-neutropenic patients with disseminated candidosis, but with the more widespread use of fluconazole prophylaxis in high-risk patients, the incidence of ocular complications has declined. If left untreated, the infection can progress to retinal necrosis and visual loss, and thus early diagnosis and treatment is essential.

Patients with ocular *Candida* infection usually present with a subacute onset of blurred vision and floaters that may be associated with ocular discomfort and photophobia. Early or peripheral lesions may be asymptomatic. Fundoscopic examination will reveal the typical yellow-white retinal, chorioretinal, or vitreoretinal cotton ball-like lesions with indistinct borders. The lesions are commonly multiple, can be bilateral and can develop into a vitreous abscess. Extension into the anterior chamber may occur. The definitive diagnosis is made by histopathological demonstration or isolation of a *Candida* species from an intravitreal biopsy specimen. *C. albicans* is the most common cause, but up to 40% of cases are due to other *Candida* species.

11.5.11 Oesophagitis

Candida oesophagitis often develops in persons with the acquired immunodeficiency syndrome (AIDS) or patients undergoing treatment for cancer. Although oesophagitis can occur in individuals with oropharyngeal candidosis (see Chapter 5), in many cases the oesophagus is the only site involved. The condition tends to occur later than oropharyngeal infection and at much lower CD4 T-lymphocyte counts. Prior to the introduction of highly active antiretroviral therapy (HAART), *Candida* oesophagitis was the AIDS-defining illness in 10–15% of HIV-infected individuals in the United States. The widespread implementation of HAART in the United States and other developed countries has led to a dramatic decrease in the incidence of this infection.

In AIDS patients, *Candida* oesophagitis may be asymptomatic despite extensive oesophageal involvement. The principal symptoms include

dysphagia, odynophagia and retrosternal pain. In patients with more advanced infection, barium contrast radiographs will often reveal irregular ragged mucosal margins, ulcers, large filling defects or oedematous mucosal folds. However, endoscopic examination is required to confirm the cause of the ulceration. The characteristic finding is white plaques with intense inflammation and varying degrees of ulceration. This method has permitted a diagnosis of oesophageal candidosis to be established in up to 25% of patients with normal oesophagrams. The differential diagnosis includes radiation oesophagitis, reflux oesophagitis, and cytomegalovirus or herpes simplex virus infection. Up to 30% of AIDS patients with oesophageal candidosis have concurrent viral oesophagitis.

11.5.12 Gastrointestinal candidosis

Although gastric candidosis is common in debilitated cancer patients, this condition is often asymptomatic and is seldom diagnosed during life. Mucosal ulcerations are the most common lesions, but it is unclear to what extent these are chronic gastric ulcers superinfected with *Candida* species. Perforation can lead to disseminated infection.

Intestinal candidosis is a controversial condition. It is clear that intestinal infection does result in mucosal ulceration in some debilitated individuals with AIDS or cancer. It is much more difficult to be certain whether intestinal colonization leads to illness in less sick individuals. It has been claimed that *Candida* colonization can produce an alarming range of symptoms, including weight loss, headaches, diarrhoea and general malaise. However, these claims have not been subjected to rigorous clinical investigation.

11.5.13 Intrauterine candidosis

Although symptomatic candidosis of the lower genital tract is one of the most common infections encountered in pregnant women (see Chapter 5), foetal infection is unusual. Intrauterine candidosis is believed to result from ascending infection of the maternal genital tract. In most cases, foetal infection follows contamination of the amniotic fluid. Spontaneous abortion associated with foetal candidosis has been reported in women fitted with an intrauterine contraceptive device.

Intrauterine candidosis presents as multiple small yellow-white lesions scattered over the surface of the umbilical cord. In some cases, the fungus affects the foetus, and in live births, such infections manifest as the characteristic lesions of congenital cutaneous candidosis (see Chapter 5). Umbilical cord lesions are often associated with other lesions that are less characteristic and take the form of diffuse, generalized chorioamnionitis.

11.6 Candidosis in special hosts

11.6.1 Drug abusers

Addicts who inject heroin solutions contaminated with *C. albicans* may develop an unusual form of disseminated candidosis. This consists of a purulent follicular and nodular cutaneous infection associated with ocular and osteoarticular lesions. The symptoms include sudden onset of fever, rigors, headache and myalgia several hours after the injection of heroin. The fever lasts between 24 and 72 hours, and cutaneous lesions then appear in more than 90% of patients. Endophthalmitis develops in 40–60% of patients, occurring 1–2 weeks after the onset of fever. Osteoarticular lesions develop in 20–30% of patients, appearing 2 weeks to several months after the cutaneous lesions. Costrochondral involvement is a frequent and characteristic finding.

11.7 Essential investigations and their interpretation

Establishing the diagnosis of invasive candidosis is difficult because the clinical presentation is varied and non-specific and because the results of laboratory tests are often difficult to interpret. In most cases, the diagnosis is based on a combination of clinical, radiological, microbiological and histopathological findings. These might include a positive blood culture result, or a positive culture result for a specimen obtained from a normally sterile and clinically or radiologically abnormal site, or histopathological or cytopathological examination showing budding yeast cells or pseudohyphae consistent with *Candida* in a biopsy specimen or aspirate. The use of antigen detection and polymerase chain reaction (PCR)-based tests has been reported, but the methods are still undergoing evaluation and their routine use cannot be recommended at present.

11.7.1 Microscopy

The microscopic detection of typical budding yeast cells, pseudohyphae, and/or true hyphae of *Candida* species in tissue sections or normally sterile body fluids is indicative of invasive candidosis. Typically, *C. glabrata* produces only yeast cells, and only *C. albicans* produces true hyphae in tissues.

11.7.2 Culture

Many *Candida* species are normal commensal inhabitants of the mouth and gastrointestinal tract, and their isolation from sputum or faecal specimens cannot be considered diagnostic of infection. Prior colonization has

been identified as an independent risk factor for invasive candidosis in adult patients receiving intensive care. However, routine assessment of colonization is labour-intensive and expensive.

Isolation of *Candida* species from blood or other normally sterile body fluids, or from tissue or aspirates from other closed sites, is indicative of deep-seated infection. It is important that specimens are processed as soon as possible after collection to avoid problems of interpretation because of multiplication of organisms. It is recommended that all isolates from blood or other significant sites are speciated, because of the increasing incidence of infections caused by species other than *C. albicans* and the differing susceptibilities of individual *Candida* species to certain antifungal agents.

Blood cultures should be performed in all cases of suspected invasive candidosis. However, it is not unusual for several attempts to be required before the organism is recovered. *Candida* species can be recovered from the blood via a peripheral venous puncture, through an indwelling catheter, from an arterial puncture or from the tip of a vascular catheter that has been removed and cultured. Positive cultures from specimens of blood obtained through an intravascular catheter are more likely to contain contaminants than are positive cultures from specimens obtained by venipuncture, but it is seldom safe to ignore the isolation of a *Candida* species from a blood culture, regardless of the source. In contrast, a positive culture of a catheter tip in the absence of a positive blood culture should not be used as the basis of a decision to begin treatment. Modern, automated, continuous-monitoring blood culture systems have largely replaced the lysis centrifugation method for the detection of *Candida* species. Depending on the inoculum, amount of blood cultured, medium and detection system, growth can be detected in 24–48 hours, but species identification takes longer. Rapid identification and differentiation of *C. albicans* from *C. glabrata* directly from blood cultures can now be achieved within 3 hours using a commercial peptide nucleic acid fluorescent in situ hybridization method (PNA FISH, AdvanDx, Woburn, Massachusetts, USA).

Isolation of *Candida* from urine is often indicative of serious infection, provided the patient does not have an indwelling urinary catheter. In non-catheterized patients, care must be taken to ensure that vaginal or perineal infection does not lead to contamination of urine specimens. In infants, suprapubic aspiration is the best method of urine collection. A negative urine culture does not exclude invasive candidosis. Isolation of *C. tropicalis* from urine is more often indicative of invasive candidosis than isolation of *C. albicans*. It has been suggested that counts of $>1 \times 104$ cfu/mL in

a non-catheterized patient should be regarded as significant, but this has never been validated. High counts in a patient with an indwelling urinary catheter are seldom significant.

Isolation of *Candida* from the CSF provides reliable evidence for the diagnosis of meningitis, but often requires repeated culture of large amounts of fluid. All specimens obtained from ventricular shunts or reservoirs should be cultured for *Candida* species.

11.7.3 Serological tests

Tests for *Candida* antibodies have been extensively evaluated, but remain of limited usefulness in the diagnosis of invasive forms of candidosis. These tests are complicated by false-positive results in patients with mucosal colonization or superficial infection and by false-negative results in immunocompromised individuals. In an attempt to reduce the number of false-positive results, efforts have been made to identify antigens of *Candida* species that are associated with invasive infection rather than colonization. However, despite the numerous methods and reagents that have been developed for antibody detection, none of them is sufficiently reliable to be used as the basis of a decision to begin treatment.

11.7.4 Antigen detection tests

Antigen detection tests have also been extensively evaluated for the rapid diagnosis of invasive forms of candidosis. Several circulating antigens have been studied as potential targets, including mannan (a heat-stable cell wall component), enolase, proteinase and other immunodominant cytoplasmic antigens. Test formats that have been evaluated include latex particle agglutination (LPA), ELISA and dot immunoassay.

Mannan or mannoprotein is the immunodominant surface antigen of *C. albicans*. It is released from the *Candida* cell wall during infection, and much effort has been devoted to the development of rapid serologic tests for its detection. However, even in immunocompromised individuals, mannan usually circulates in the form of immune complexes that must first be dissociated for optimal detection of the antigen. This can be accomplished through boiling in the presence of EDTA. Mannan is rapidly cleared from the circulation by receptor-mediated endocytosis in the liver and spleen. Thus, testing of multiple serum samples is essential to optimize the sensitivity of tests for mannan detection.

Two tests for mannan detection are commercially available in a number of countries. An LPA test was the first to be developed (Pastorex Candida, Bio-Rad Laboratories, Paris, France) but, despite its ease of use,

it has been found to be relatively insensitive. The monoclonal antibody, EB-CA1, used in this test is directed against an epitope derived from *C. albicans* mannan, but it also reacts with several other species including *C. glabrata, C. parapsilosis* and *C. tropicalis*. More recently, a sandwich ELISA (Platelia Candida Antigen, Bio-Rad Laboratories) has been marketed, which employs the same monoclonal antibody, EB-CA1. Although this test has not been extensively assessed, it appears to be more sensitive than the LPA test. By combining the results of the Platelia Candida Antigen ELISA with those of a commercial ELISA for detection of antimannan antibodies (Platelia Candida Antibody, Bio-Rad Laboratories), mannan antigen and/or antimannan antibodies have been found in 69% of patients with invasive candidosis by the time the first positive blood culture was obtained. Non-neutropenic surgical patients present first with positive antibody tests, while haematologic patients present with antigenaemia.

11.7.5 β-D-glucan detection

In addition to antigen detection, a number of other approaches have been developed for the rapid diagnosis of invasive candidosis. These include tests to detect the presence in serum of (1-3)-β-D-glucan, a cell wall component of many fungi. The Fungitell assay (Associates of Cape Cod, Falmouth, Massachusetts, USA) is approved by the US Food and Drug Administration for the presumptive diagnosis of invasive fungal infections, including candidosis, trichosporonosis and aspergillosis. The absence of a positive test result has been reported to have a high negative predictive value. False-positive results have been reported in patients with bacteraemia, in those who have recently received albumin or immunoglobulin products, or undergone haemodialysis with cellulose-containing membranes, and among those exposed to other sources of glucan, such as certain parenteral antibiotics.

11.7.6 D-arabinitol detection

Another approach to the diagnosis of invasive candidosis involves the detection in serum or urine of a metabolite, D-arabinitol, which is produced by most of the medically important *Candida* species, with the exception of *C. krusei* and perhaps *C. glabrata*. Because increased levels of arabinitol are also found in human body fluids when renal function is impaired, the results are reported as the D-arabinitol:creatinine ratio. Although several large studies have demonstrated that patients with candidaemia have elevated serum D-arabinitol:creatinine ratios, this approach remains investigational.

11.7.7 Molecular diagnostics

Numerous nucleic acid amplification methods have been developed for the detection of *Candida* DNA in blood, serum, CSF and other clinical specimens. Several regions within the *Candida* genome have been evaluated as potential targets for detection, but most effort has focused on the ribosomal DNA gene complex. Both nested and pan-fungal PCR formats have been employed. More recently, quantitative real-time PCR methods have been developed. Molecular diagnostics appear promising for the rapid diagnosis of *Candida* infection directly from tissue or body fluid specimens, but until some form of international consensus is agreed and/or validated commercial systems are available, use of these tests in the diagnosis of invasive candidosis will remain investigational.

11.8 Management

11.8.1 Acute disseminated candidosis and candidaemia

All patients with candidaemia require treatment, regardless of whether or not this is associated with an intravascular catheter. Early initiation of effective antifungal treatment is critical for the successful management of candidaemia: mortality rates are higher among patients whose therapy is delayed. If there are no metastatic complications, treatment should be continued for 2 weeks after the last positive blood culture and resolution of signs and symptoms attributable to the infection. This approach is generally associated with few complications or relapses.

If feasible, all existing central vascular catheters should be removed from patients with documented candidaemia. The evidence for this recommendation is strongest for non-neutropenic patients. In neutropenic individuals, the gastrointestinal tract is a frequent source for disseminated infection, removal of catheters often creates significant intravenous access problems and the evidence for routine catheter removal is less compelling. An exception is *C. parapsilosis* bloodstream infection, which is very often catheter-related and not associated with previous colonization.

All patients with positive blood cultures should undergo at least one dilated retinal examination to exclude *Candida* endophthalmitis. If the initial assessment is negative, a repeat evaluation should be conducted two weeks later. Neutropenic patients tend not to develop visible endophthalmitis until the neutrophil count has recovered, and ophthalmological examination should be deferred until this point.

Further blood cultures should be obtained to document the resolution of the infection. An intravascular focus of infection, such as an

intravascular catheter, endocarditis or septic thrombophlebitis, should be suspected when blood cultures remain positive despite treatment. In patients with blood cultures that clear, but whose condition does not improve, an undrained focus of infection should be suspected. If drug resistance is suspected, treatment with a drug from a different class should be substituted, the isolate should be identified to species level and susceptibility testing should be performed.

The choice of antifungal treatment will depend on the clinical status of the patient, the species of infecting organism and/or the antifungal susceptibility profile of the infecting isolate, the relative toxicity of the different drugs, the presence of organ dysfunction that would affect drug clearance and the patient's prior exposure to antifungal agents. In general, bloodstream isolates of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. lusitaniae* and *C. guilliermondii* are susceptible to the azoles, the echinocandins and flucytosine, and infections with these organisms can be treated with any of these drugs. Both *C. parapsilosis* and *C. guilliermondii* are less susceptible to the echinocandins than other *Candida* species. However, in clinical trials there has been no evidence of reduced responsiveness of *C. parapsilosis* infections to these agents. Both *C. glabrata* and *C. krusei* are susceptible to the echinocandins, but both species are intrinsically less susceptible to fluconazole. *C. krusei* also shows decreased susceptibility to amphotericin B and flucytosine. *C. guilliermondii* and *C. lusitaniae* may develop resistance to amphotericin B during treatment.

11.8.1.1 Non-neutropenic patients

In non-neutropenic patients in whom the species of infecting organism has not been determined, treatment can be initiated with either fluconazole or an echinocandin. Initial treatment with an echinocandin is preferred for patients who have moderately severe or severe illness, have recently received azole treatment, or are at high risk of *C. glabrata* or *C. krusei* infection. Treatment with anidulafungin should be initiated with an intravenous loading dose of 200 mg on the first day, followed by 100 mg/day thereafter. Treatment with caspofungin should be started with a loading dose of 70 mg, followed by 50 mg/day thereafter. The recommended dosage for micafungin is 100 mg/day; a loading dose is not required. None of the echinocandins require dosage adjustment for renal impairment or haemodialysis. Caspofungin is the only echinocandin for which dosage adjustment is recommended for patients with moderate to severe hepatic dysfunction. If the response to echinocandin treatment is rapid and if the patient is stable, it is reasonable to change to oral fluconazole (400 mg/day) after 1–2 weeks.

Fluconazole is recommended for patients who are less critically ill, have not recently received azole treatment and do not belong in a group at high risk of *C. glabrata* infection. Patients with candidaemia and suspected concomitant endocardial or CNS involvement should probably not receive fluconazole as initial treatment. Fluconazole should be administered with a loading dose of 800 mg (12 mg/kg), followed by a daily dose of 400 mg (6 mg/kg). A lower dosage is indicated in patients with a creatinine clearance of less than 50 mL/min. To achieve comparable drug exposure, the fluconazole dosage needs to be doubled, from 6 to 12 mg/kg per day, in neonates and children.

In patients with proven or suspected infection due to *C. glabrata* or *C. krusei,* an echinocandin is the preferred initial treatment. Transition to fluconazole or voriconazole treatment (for *C. krusei* infection) is not recommended unless the infecting isolate has been confirmed to be susceptible to these agents. In those patients who received initial treatment with fluconazole or voriconazole, whose clinical condition has improved and whose follow-up cultures are negative, continuing an azole to completion of treatment is reasonable.

In patients with proven or suspected infection due to *C. parapsilosis*, fluconazole is the preferred initial treatment. In those patients who received initial treatment with an echinocandin, and whose clinical condition is stable, continuing an echinocandin to completion of treatment is reasonable.

Voriconazole is an effective treatment for candidaemia, but the need for twice daily administration, less predictable pharmacokinetics and more frequent adverse reactions, compared with other antifungals, makes it a less attractive choice for initial treatment of candidosis. It is, however, useful for patients with fluconazole-resistant *C. krusei* or *C. glabrata* infection who are ready for transition from parenteral to oral treatment. Adults should receive two loading doses of 400 mg of oral voriconazole 12-hour apart, followed by 200 mg twice daily. The dosage of voriconazole should be reduced in patients with mild to moderate hepatic impairment, and it should be avoided in those with severe hepatic impairment.

In resource-limited environments, or where echinocandins are unavailable, amphotericin B is an effective alternative. For most forms of invasive candidosis, the typical dosage of conventional amphotericin B is 0.3–0.5 mg/kg per day, but dosages as high as 1 mg/kg per day should be considered for infections caused by less susceptible species such as *C. krusei*. In non-neutropenic patients with more severe infections, the full dose of conventional amphotericin B (50 mg/day) can be given from the outset (see Chapter 3). Lipid-based formulations of amphotericin B have been approved for the treatment of candidosis in patients who have failed

to respond to the conventional parenteral formulation, who have developed severe side effects to conventional amphotericin B, or in whom conventional amphotericin B is contraindicated because of renal impairment. In general, if a lipid-based form is chosen, a dosage of 3–5 mg/kg per day should be given.

11.8.1.2 Neutropenic patients

In neutropenic adult patients in whom the species of infecting organism has not been determined, treatment should be initiated with an echinocandin or a lipid-based formulation of amphotericin B. Fluconazole (loading dose of 800 mg, then 400 mg daily) is a reasonable alternative for patients who are less critically ill and who have not recently received azole treatment. Voriconazole (loading dose 400 mg twice daily for 1 day, then 200 mg twice daily) may be preferred in situations where additional mould coverage is desired. Treatment should be continued for 2 weeks after the last positive blood culture, resolution of signs and symptoms of infection, and resolution of neutropenia. Patients should receive adjunctive treatment with a cytokine, such as granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor, to assist recovery of their neutrophil counts and immune function.

Among neutropenic patients with proven or suspected infection due to *C. glabrata*, an echinocandin or lipid-based form of amphotericin B is the preferred initial treatment. In those patients who received initial treatment with voriconazole or fluconazole, whose clinical condition has improved and whose follow-up cultures are negative, continuing the azole to completion of treatment is reasonable. Among neutropenic patients with proven or suspected infection due to *C. parapsilosis*, lipid-based amphotericin B or fluconazole is the preferred initial treatment. Patients with *C. krusei* infection should be treated with an echinocandin or voriconazole.

11.8.1.3 Neonatal patients

Neonatal candidosis differs from invasive disease in older patients. CNS disease should be assumed to be present in infants with positive blood cultures, because of the high incidence of this complication. In pre-term infants, central venous catheters should be removed or replaced within 24 hours of the first positive blood culture. Failure to do so places the infant at increased risk of prolonged infection and has been associated with increased mortality and long-term, irreversible neuro-developmental impairment. Infants with sterile body fluid and/or urine cultures positive for *Candida* species should undergo a lumbar puncture and a dilated retinal

examination. Imaging of the liver, spleen and genitourinary tract should be performed if sterile body fluid cultures are persistently positive.

The drug of choice for neonatal candidosis is conventional amphotericin B (1 mg/kg per day). If urinary tract involvement is excluded, lipid-based formulations of amphotericin B, at a dosage of 3–5 mg/kg daily, can be used. In contrast to adults, amphotericin B is well tolerated in full- and pre-term infants, but close monitoring of patients is still an essential component of care. Measurement of renal function and serum potassium and magnesium levels is important (see Chapter 3). Although levels of amphotericin B in the CSF are less than 5% of the simultaneous serum concentration in adults, CSF levels may reach 40–90% of blood levels in preterm infants. Treatment should be continued for 2–3 weeks after the last positive blood culture and resolution of signs and symptoms of infection. Fluconazole (12 mg/kg per day) is a reasonable alternative treatment, but echinocandins should only be used in situations where amphotericin B and fluconazole are precluded.

11.8.2 Chronic disseminated candidosis

Administration of fluconazole (400 mg or 6 mg/kg per day) for 3–6 months has proved successful in cancer patients with chronic disseminated candidosis (hepatosplenic candidosis). This is now the preferred treatment option for individuals who are stable. Conventional amphotericin B (0.5–0.7 mg/kg per day) or a lipid-based form of amphotericin B (3–5 mg/kg per day) is recommended for acutely ill patients and those who are not responding to fluconazole treatment. Another option is to give all patients an initial 1–2 week course of amphotericin B, followed by a prolonged course of fluconazole, at a dosage of 400 mg daily, provided the patient is stable or has improved. The echinocandins have been used successfully in small numbers of cases and are alternatives for initial treatment. They should be followed by oral fluconazole when clinically appropriate.

Treatment should be continued until clinical signs have resolved and calcification or resolution of the lesions has occurred. Premature discontinuation of treatment can lead to recurrent infection. Patients with chronic disseminated candidosis may continue to receive cytotoxic chemotherapy, including ablative treatment for recipients of bone marrow or stem cell transplants.

There is limited evidence that adjuvant oral corticosteroid treatment involving a prednisolone equivalent at a dosage of 0.5–1 mg/kg per day for at least 3 weeks is associated with prompt resolution of symptoms and of inflammatory response in both adults and children. These findings

support the suggestion that chronic disseminated candidosis is a form of fungus-related IRIS.

11.8.3 Lower urinary tract candidosis

Most asymptomatic individuals with persistent candiduria and an indwelling urinary catheter should have the catheter changed, but do not require antifungal drug treatment. Candiduria should be treated in symptomatic patients as well as in asymptomatic patients at high risk of developing candidaemia or in whom the presence of candiduria infers disseminated infection. These patient groups include VLBW infants, neutropenic patients, renal transplant recipients and patients undergoing urological procedures. In VLBW infants, candiduria is often indicative of renal infection with fungus ball formation. Removal of indwelling stents and catheters is effective in eradicating candiduria in as many as 40% of patients, but if complete removal is not possible, replacement with new materials can be beneficial. However, it is likely that the organisms will return within a short time period. Correcting structural problems and relieving obstruction can lead to long-term eradication of *Candida* species from the urine.

Fluconazole is the drug of choice for most *Candida* infections of the lower urinary tract. Treatment should be initiated with an oral loading dose of 400 mg, followed by 200 mg/day for 1–2 weeks. The dosage should be reduced in patients with renal impairment (see Chapter 3). Fluconazole should not be used to treat *C. krusei* infections. Other azole agents and echinocandins are not recommended for the treatment of lower urinary tract infections because they fail to achieve adequate concentrations in the urine.

In patients with *C. glabrata* infection, the first step should consist of removal of all foreign material, discontinuation of antibiotic treatment and relief of obstruction. Fluconazole, at a dose of 800 mg/day, can be tried as the initial antifungal treatment, in the hope that the organism is susceptible. Patients failing fluconazole treatment can then be treated with conventional amphotericin B (0.3–0.6 mg/kg for 1–7 days). Lipid-based formulations of this drug appear to be ineffective, because adequate concentrations in the urine are not achieved. Oral flucytosine is another option in patients with normal renal function when fluconazole cannot be given or has proved unsuccessful. The usual dosage is 25 mg/kg at 6-hour intervals for 7–10 days. Most *Candida* species, with the exception of *C. krusei*, are susceptible to flucytosine, but resistance often emerges when this drug is used alone. The likelihood of resistance developing is reduced when flucytosine is given for less than 1 week.

Local instillation of amphotericin B is sometimes helpful in individuals with persistent candiduria due to infection with fluconazole-resistant organisms, provided there are no signs of pyelonephritis or of renal or ureteric obstruction. These patients often respond following catheter change and intermittent instillation (200–300 mL of a 50 mg/L solution in sterile water at 6- to 8-hour intervals) or continuous irrigation with amphotericin B (50 mg/L in sterile water) for 5–7 days. However, the effect is often brief and candiduria often returns within 1–2 weeks. This method of treatment is no longer commonly used, given its limitations and the availability of more convenient oral treatment options.

11.8.4 Renal candidosis

There are three basic approaches to the management of renal candidosis: local irrigation of the renal pelvis with antifungal drugs, oral or parenteral treatment with antifungals and surgical removal of obstructions or resection. In practice, a combined approach is often required.

Fluconazole is excreted unchanged and in high concentrations in the urine. It should not be used to treat *C. glabrata* or *C. krusei* infections, but provided the infecting organism is susceptible, the recommended dose is 400 mg/day for 2 weeks. The dosage should be reduced in patients with impaired renal function (see Chapter 3). For fluconazole-resistant infections, particularly those caused by *C. glabrata*, amphotericin B (0.7–1 mg/kg per day), with or without flucytosine (25 mg/kg at 6-hour intervals), may be used.

11.8.5 Respiratory tract candidosis

The decision to initiate antifungal treatment of suspected *Candida* pneumonia should not be made on the basis of respiratory tract culture results alone. Most cases of *Candida* pneumonia occur as a result of disseminated infection and should be managed on that basis (see Section 11.8.1). Patients with *Candida* pneumonia arising as a result of aspiration have been successfully treated with amphotericin B (0.7–1 mg/kg per day).

Most patients with laryngeal candidosis will respond to amphotericin B (0.7–1 mg/kg per day). Oral fluconazole may be appropriate for treatment once symptoms and signs are improving.

11.8.6 Osteomyelitis and arthritis

Candida osteomyelitis is best managed with a combination of surgical debridement and antifungal treatment. Debridement of necrotic bone (with bone grafting at the same procedure) is important if extensive vertebral destruction is present. Amphotericin B (0.5–1 mg/kg per day

for 6–10 weeks) has proved successful for the treatment of *Candida* osteomyelitis, as has fluconazole (400 mg or 6 mg/kg per day for 6–12 months). Current recommendations suggest an initial course of a lipid-based formulation of amphotericin B, at a dosage of 3–5 mg/kg daily, for at least 2 weeks followed by fluconazole, at a dosage of 400 mg daily, for 6–12 months.

In patients with infected native joints, adequate and/or repeated drainage is often essential for successful management. In particular, management of *Candida* arthritis of the hip requires open drainage. Case reports have documented success with amphotericin B, fluconazole and caspofungin when given in conjunction with adequate drainage. Current recommendations suggest fluconazole (400 mg daily) for at least 6 weeks, or a lipid-based formulation of amphotericin B (3–5 mg/kg daily) for at least 2 weeks, followed by fluconazole (400 mg daily). Intra-articular injection of amphotericin B is seldom required, and its use is now discouraged.

In patients with infected prosthetic joints, treatment involves removal of all foreign material and necrotic bone tissue. Subsequent medical treatment is the same as that for native joint disease, and a new prosthesis may be inserted after successful eradication of the infection. This is defined by lack of return of symptoms following cessation of antifungal treatment. Eradication of infection is often difficult to achieve, and long-term maintenance treatment with an azole may be an alternative, particularly in older patients.

11.8.7 Peritonitis

It is not unusual for *Candida* species to be recovered from abdominal drains in a post-operative patient receiving intensive care. If the individual is stable, is asymptomatic with respect to a possible peritoneal infection or an intra-abdominal abscess and is not growing *Candida* species from other sites, antifungal treatment may not be needed. If, however, the patient is febrile despite appropriate antibacterial treatment, and is at high risk for disseminated candidosis, antifungal treatment should be administered. *Candida* peritonitis due to intra-abdominal leakage of faecal material first requires surgical repair and drainage. It should then be treated with either fluconazole (6 mg/kg per day) or amphotericin B (0.7–1 mg/kg per day). Intraperitoneal amphotericin B has been used, but most patients develop a painful chemical peritonitis. Local instillation of the drug should be avoided if possible.

In cases where *Candida* peritonitis has developed during peritoneal dialysis, the catheter should be removed as soon as possible and the patient

should be started on either fluconazole (6 mg/kg per day) or amphotericin B (1 mg/kg per day), with or without flucytosine (25 mg/kg at 6-hour intervals), and haemodialysis (if required). If the dialysate is grossly turbid, peritoneal lavage (until the returning fluid is clear) should be performed before the catheter is removed. It can be replaced once antifungal treatment has been discontinued.

Patients who are diagnosed as having *Candida* peritonitis within 2 weeks of starting dialysis can sometimes be managed without catheter removal, provided their symptoms and clinical signs are mild. Patients who cannot have their catheter removed should be treated with amphotericin B or fluconazole, depending on the organism. Intraperitoneal administration of amphotericin B is painful, but may need to be considered. The peritoneal catheter should be removed as soon as the patient can be managed with haemodialysis.

The optimum duration of treatment for all forms of *Candida* peritonitis has not been well defined and should be guided by the patient's response. In general, 2–3 weeks of treatment is required. Prophylactic treatment with fluconazole (400 mg/day) has been shown to reduce the risk of infection in surgical patients with recurrent gastrointestinal perforations or anastomotic leakage.

11.8.8 Endocarditis, myocarditis, pericarditis and other vascular infections

The management of *Candida* endocarditis is difficult and most cases require a combination of medical and surgical treatment. For native- and prosthetic-valve endocarditis, treatment with a lipid-based formulation of amphotericin B (3–5 mg/kg per day), with or without flucytosine (25 mg/kg at 6-hour intervals), is recommended and should be commenced as soon as the diagnosis is made. Other treatment regimens include conventional amphotericin B (0.6–1 mg/kg per day) with or without flucytosine, or an echinocandin. Higher than usual dosages may be required to treat *Candida* endocarditis. Both native and prosthetic valves should be replaced 1–2 weeks after treatment has started, but earlier surgical intervention is indicated if there are large vegetations, signs of heart failure or dysfunction of a prosthesis. The optimum length of treatment remains uncertain, but treatment should continue for at least 6 weeks after valve replacement to reduce the likelihood of relapse. Because of the risk of late relapse, patients require careful follow-up for several years. If valve replacement is not possible, life-long suppressive treatment with fluconazole (400–800 mg daily) is recommended.

Candida myocarditis should be treated as described for endocarditis. Septic central vein thrombosis is often curable with antifungal treatment alone. The catheter should be removed and amphotericin B given for 1–2 months; flucytosine can be added during the initial phase of treatment. Anticoagulation is desirable, but not essential for a successful outcome. *Candida* pericarditis is best managed with a lipid-based formulation of amphotericin B (3–5 mg/kg per day), an echinocandin or fluconazole (400–800 mg daily) for as long as several months, in combination with surgical debridement or resection of tissue (depending on the extent of the infection). Septic *Candida* thrombophlebitis of a peripheral vein requires catheter removal and incision and drainage or resection of the vein followed by treatment with amphotericin B, fluconazole or an echinocandin.

Candida infections of transvenous pacemakers and implantable cardiac defibrillators should be managed by removal of the entire device followed by treatment with a lipid-based formulation of amphotericin B (3–5 mg/kg per day) with or without flucytosine (25 mg/kg at 6-hour intervals) for at least 6 weeks.

11.8.9 CNS candidosis

Meningitis in neurosurgical patients and low-birth-weight infants is best treated with a lipid-based formulation of amphotericin B (3–5 mg/kg per day) with or without flucytosine (25 mg/kg at 6-hour intervals; provided the infecting organism is susceptible). The half-life of flucytosine is prolonged in small infants and the drug should be administered at 12- or 24-hour intervals. Treatment should be continued for a minimum of 4 weeks after resolution of all signs and symptoms related to the infection. Infected shunts should be removed or replaced.

Few patients with *Candida* meningitis have been treated from the outset with fluconazole, but this drug (at a dosage of 400–800 mg/day) has been employed both as follow-up treatment and as part of a longer-term suppressive regimen after the patient responds to initial treatment with amphotericin B and flucytosine.

11.8.10 Endophthalmitis and chorioretinitis

Amphotericin B (0.7–1 mg/kg per day), combined with flucytosine (25 mg/kg at 6-hour intervals), is recommended for treatment of advancing lesions or lesions threatening the macula. Fluconazole (loading dose of 12 mg/kg, then 6–12 mg/kg daily) is an alternative for patients with less severe endophthalmitis. Lipid-based forms of amphotericin B (3–5 mg/kg per day), voriconazole (6 mg/kg twice daily for two doses, then 3–4 mg/kg twice daily) or an echinocandin can be used to treat patients

who are intolerant of, or not responding to, conventional amphotericin B or fluconazole. Treatment should be continued for at least 4–6 weeks, until complete resolution of visible lesions or convincing stabilization. The echinocandins should be used with caution because of their poor ocular penetration.

Lesions extending into the vitreous usually require vitrectomy at the outset and intravitreal injection of amphotericin B. The drug is toxic and can cause focal retinal necrosis, but doses of 5–10 μg in 50–100 μL can be tolerated. Injection should be carried out slowly into the central vitreous cavity and as far as possible from the retinal surface. Although experience is limited, combined systemic and intravitreal administration of voriconazole (at a dose of 100 μg) has proved successful in a few cases of *Candida* endophthalmitis.

11.8.11 Oesophagitis

Oesophageal candidosis can be treated with oral fluconazole (200–400 mg/day) or itraconazole oral solution (200 mg/day) for 2–3 weeks. Treatment should be continued for at least 2 weeks following resolution of symptoms. Itraconazole capsules and ketoconazole are less effective because of variable absorption. Intravenous fluconazole (400 mg daily) or an echinocandin should be used for patients who cannot tolerate oral treatment. Patients who remain symptomatic after 2 weeks require further investigation for drug resistance, drug interactions and other causes of oesophagitis.

Management of oesophagitis in AIDS patients from whom fluconazole-resistant strains of *C. albicans* have been isolated is difficult. Higher doses of fluconazole (400–800 mg/day) should be tried, but the benefit is often transient. Voriconazole (200 mg twice daily) or itraconazole solution (200 mg/day) can be prescribed, as these have sometimes proved successful in patients who had failed to respond to fluconazole.

Patients with otherwise unresponsive *Candida* oesophagitis can also be managed with intravenous caspofungin (50 mg/day) or micafungin (150 mg/day); a loading dose is not required. Treatment with anidulafungin should be initiated with an intravenous loading dose of 100 mg on the first day, followed by 50 mg/day. Treatment should be given for a minimum of 2 weeks and should be continued for a minimum of 1 week after resolution of symptoms.

In persons with AIDS, treatment of the underlying HIV infection with HAART is critical for prevention and management of *Candida* oesophagitis.

11.9 Empirical treatment of suspected invasive candidosis

Because it has become clear that the earlier treatment is started the better the prognosis, it has become the standard practice of care to begin antifungal treatment without waiting for formal proof that a patient who remains febrile and profoundly neutropenic, despite the administration of broad-spectrum antibacterial agents in adequate dosage, has candidosis. Empirical antifungal treatment with a lipid-based form of amphotericin B caspofungin, or voriconazole is recommended for high-risk patients with prolonged neutropenia who remain febrile despite broad-spectrum antibiotic treatment. The recommended dosages are the same as for proven candidosis. Treatment with caspofungin has been shown to be as effective as and better tolerated than liposomal amphotericin B. It may be preferred for patients who have recently received azole treatment or who may be at high risk for *C. glabrata* or *C. krusei* infection. Empirical treatment with conventional amphotericin B should generally be limited to clinical situations where other safer antifungals are unavailable. Once begun, empirical treatment should be continued until the neutrophil counts recovers. Empirical treatment is not recommended for patients who are anticipated to have neutropenia of short duration (less than 10 days), unless other findings indicate the presence of an invasive fungal infection.

The role of empirical treatment in the management of suspected disseminated candidosis in febrile non-neutropenic patients has not been well defined. Early initiation of antifungal treatment with fluconazole or an echinocandin should be considered in critically ill patients with multiple sites of *Candida* colonization, other risk factors for invasive candidosis and no other uncorrected causes of fever. The recommended dosages are the same as for proven candidosis. An echinocandin should be preferred in unstable patients, in those previously exposed to azoles and in those known to be colonized with azole-resistant *Candida* species. Empirical treatment with fluconazole may be considered in non-critically ill patients colonized with azole-susceptible *Candida* species or who have no prior exposure to azoles.

11.10 Prevention

Although many risk factors have been identified for invasive candidosis, few of these factors are preventable or modifiable. The only prevention measures shown to be effective have been in the setting of outbreaks and have usually consisted of improved hygiene and hand washing

procedures among healthcare workers or removal of a contaminated source of infection. Hand washing should be encouraged between patient contacts and after contact with infectious materials. Hand antisepsis is recommended before the insertion of invasive devices. Gloves should be used in addition to hand washing, to provide an additional physical barrier to cross-transmission. Gloves should be changed before and after contact with patients or with infectious materials.

Good barrier precautions during the insertion of vascular catheters should provide adequate protection against *Candida* infection, and the risk of subsequent catheter contamination and catheter-related bloodstream infection will be minimized. The longer the duration of catheterization, the greater is the risk of infection. However, routine replacement of central venous catheters at specified intervals is not currently recommended.

In several large randomized trials, chemoprophylaxis with fluconazole was found to reduce the incidence of superficial and deep forms of candidosis among HSCT recipients during the period prior to engraftment. Long-term follow-up (8 years) of one of these trials showed a significant survival benefit among the fluconazole recipients. Moreover, fewer patients who received fluconazole developed acute GVHD involving the gastrointestinal tract. The use of fluconazole prophylaxis, at a dosage of 400 mg/day during the period of neutropenia, has been endorsed in published guidelines for prevention of infection in allogeneic HSCT recipients. Oral posaconazole, at a dosage of 200 mg three times daily, is an effective alternative. Oral itraconazole, at a dosage of 200 mg daily, is as effective as fluconazole in neutropenic patients with haematological malignancies, but is less well tolerated than other agents.

Fluconazole prophylaxis also appears to be effective in reducing the incidence of invasive candidosis among some high-risk non-neutropenic patient populations. These include surgical patients with recurrent gastrointestinal perforations or anastomotic leakages, and some liver, pancreas and small bowel transplant recipients. The risk of invasive candidosis after transplantation of other solid organs appears to be too low to justify routine prophylaxis. Published guidelines recommend the use of fluconazole, at a dosage of 200–400 mg/day, as post-operative prophylaxis for high-risk liver, pancreas and small bowel transplant recipients. The use of fluconazole, at a dosage of 400 mg/day, is also recommended for high-risk patients in adult ICUs with a high incidence of invasive candidosis in the immediate post-operative period. To better target prophylaxis, many of the well-established risk factors for invasive candidosis have been integrated into risk predictive models. To date, these models remain limited by suboptimal performance characteristics, derivation from small ICU

cohorts, and a lack of validation on prospective and independent ICU patient cohorts.

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CHAPTER 12

Cryptococcosis

12.1 Definition

The term cryptococcosis is used to refer to infections caused by encapsulated yeasts belonging to the basidiomycetous genus *Cryptococcus*. These ubiquitous organisms can cause disease in normal individuals, but a large proportion of human infections occur in immunocompromised persons. Infection follows inhalation, but meningitis is the most common clinical presentation, and widespread disseminated infection can also occur.

12.2 Geographical distribution

The disease is worldwide in distribution.

12.3 The causal organisms and their habitat

Although there are more than 30 species of *Cryptococcus*, almost all cases of human infection are caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. Two varieties are recognized within *C. neoformans*, namely *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii. C. gattii* used to be regarded as a third variety of *C. neoformans*. However, based on differences in a number of biochemical, ecological, epidemiological and molecular characteristics, it is now recognized as a separate species.

C. neoformans and *C. gattii* were previously classified into five serotypes of *C. neoformans*, termed A, B, C, D and AD (hybrids between serotypes A and D) on the basis of antigenic differences between their capsular polysaccharides. The two varieties of *C. neoformans*, var. *grubii* and

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var. *neoformans*, are comprised of serotypes A and D respectively, while *C. gattii* is comprised of serotypes B and C. The teleomorph species *Filobasidiella neoformans* has been found to be produced by strains of serotype A and D, while *Filobasidiella bacillispora* is produced by strains of serotype B and C.

C. neoformans and *C. gattii* differ in their geographical distribution and natural habitat. *C. neoformans* serotype A accounts for about 95% of reported human cases of cryptoccosis and is found worldwide. The remaining 5% of infections are caused by *C. neoformans* serotype D or *C. gattii* serotypes B and C. Serotype D is predominantly found in European countries. *C. gattii* is found primarily in tropical and sub-tropical regions worldwide, but has recently emerged as a human and animal pathogen in north western North America.

C. neoformans has been isolated most frequently from soil contaminated with pigeon or other bird droppings. It has also been recovered from old dried accumulations of bird droppings in buildings, but has not been found in fresh wet droppings. Despite this ecological association, the precise link between birds and *C. neoformans* remains unclear. The evidence suggests that birds are not infected, but serve as a vector, spreading the fungus from vegetation throughout the environment. There is no evidence for an association between *C. neoformans* and specific trees, but the two varieties have been isolated from decaying wood of many different species of trees.

In nature, *C. gattii* was first isolated from decaying wood in the red gum group of eucalyptus trees. These trees are indigenous to Australia, but have been planted in numerous countries with a tropical or sub-tropical climate, including the United States, parts of Central and South America, Africa, India, China and the Mediterranean region. In recent years, the outbreak of cryptococcosis in north western North America has indicated that many native species of coniferous and deciduous trees are also hosts for *C. gattii*. Unlike *C. neoformans*, *C. gattii* has not been isolated from bird droppings.

Until recently *C. gattii* was thought to be confined to tropical and subtropical regions, with the distribution of human infections in general corresponding to the distribution of the host gum trees. Most cases were reported from Australia, parts of Africa, Asia, South America and other tropical regions. The geographic range of *C. gattii* has expanded since 1999 with the recognition of an unprecedented number of cases among humans and animals living on Vancouver Island, British Columbia (BC). Environmental sampling identified *C. gattii* as being associated with a broad range of native tree species, including alder, cedar, fir and oak, as well as

with soil, debris and air around the trees. The precise means by which the fungus was first introduced to this temperate environment has not been established, although one hypothesis implicates importation of contaminated trees. The recent spread of the outbreak strain of *C. gattii* to the BC mainland, as well as to the states of Washington, Oregon, Idaho and California in the United States, has been attributed, at least in part, to human-mediated dispersal.

The emergence of *C. gattii* in a temperate climate suggests that the pathogen may have adapted to a new climatic niche, or that climatic warming might have created an environment favourable to its development. However, it is also possible that the environmental conditions supportive of *C. gattii* are broader than previously suspected. Many cases of *C. gattii* have likely not been recognized because distinguishing between *C. gattii* and *C. neoformans* requires plating on differential media and this is seldom performed in clinical laboratories.

12.4 Epidemiology

Inhalation of airborne propagules from an environmental source is the usual mode of infection with *C. neoformans* and *C. gattii* in humans and animals. However, it remains unclear whether the infectious particles are small desiccated acapsular yeast cells or basidiospores of the teleomorph stage of the fungus. It is thought that the encapsulated yeast cells are too large to penetrate the upper respiratory tract.

Outbreaks, investigations of which have helped to elucidate the sources, incubation periods and risk factors for other fungal infections, such as histoplasmosis, have not been described for *C. neoformans* infection. Thus, while bird roosting sites have been clearly implicated in outbreaks of histoplasmosis, the association of *C. neoformans* infection with exposure to pigeon droppings, the best recognized of the putative environmental risk factors for this disease, is mostly derived from information on isolation of the fungus from the environment, not from studies that attribute disease to this exposure. The incubation period for *C. neoformans* is unknown and could be weeks, months or even longer. Estimated incubation periods for outbreak cases of *C. gattii* infection among residents of Vancouver Island, BC, and elsewhere have ranged from 2 to 13 months (median 6–7 months).

It remains unclear whether cryptococcosis is caused by acute infection or reactivation of a latent infection. There is considerable evidence to suggest that reactivation may be the more common mechanism. For

instance, an investigation of children aged 2 years and older in New York showed that most of them had serological evidence of asymptomatic *C. neoformans* infection. However, acute infection can also occur when immunocompromised individuals are exposed to large numbers of cryptococcal cells.

The likelihood that an infection with *C. neoformans* will develop after inhalation depends, in major part, on host factors. Even before the advent of the acquired immunodeficiency syndrome (AIDS), infections with *C. neoformans* tended to occur in individuals with T-cell-mediated immunological defects, such as are found in cancer patients with lymphoproliferative malignancies and those receiving corticosteroid treatment. Solid organ transplant (SOT) recipients also appear to be a high-risk group for cryptococcosis; many of these infections occur several years after transplantation. Nevertheless, human immunodeficiency virus (HIV) infection remains the single most common immunocompromising illness, being associated with more than 80% of cases of *C. neoformans* infection worldwide. In HIV-infected persons, cryptococcosis is associated with severe immunological impairment, as reflected by CD4 T-lymphocyte counts of less than 100 cells per μL.

C. gattii infections occur predominantly in immunocompetent individuals. Less than 40% of cases from the outbreak in north western North America have had an identifiable immunocompromising condition and reports from sub-Saharan Africa indicate that only 10–20% of HIV-associated cases of cryptococcosis are caused by this species. The major risk factor for development of infection with *C. gattii* appears to be environmental exposure. Activities that disturb colonized soil or trees increase the likelihood of exposure and have been reported as the most significant risk factor for *C. gattii* infection in BC.

Until the 1980s, cryptococcosis was a rare disease. With the advent of the AIDS epidemic, *C. neoformans* infection became the most common cause of meningitis in hospitals in which persons with HIV infection are treated. In the United States, active population-based surveillance, conducted between 1992 and 1994, showed that cryptococcosis developed in 2–5% of HIV-infected persons per annum. The annual incidence of the disease has declined following the widespread use of fluconazole and the introduction of more effective combination antiretroviral treatments, including highly active antiretroviral therapy (HAART). In developed countries, cryptococcosis continues to occur in those with undiagnosed HIV infection and in those disadvantaged HIV-positive individuals without access to routine HIV-supportive care. The acute case fatality rate from

cryptococcosis among HIV-infected individuals in developed countries remains in the range of 5–20% despite antifungal treatment. Among HIV-negative persons in the United States, the average annual incidence of cryptococcosis has remained almost constant at about 1 case per 100,000 population; about 20% of these individuals have no obvious predisposing factors. Among HIV-negative persons with cryptococcosis, the mortality rate is about 21%.

The incidence of cryptococcosis remains high in resource-limited settings such as sub-Saharan Africa and Southeast Asia, where lack of access to antifungal treatment and HAART is a major problem. According to recent estimates from the US Centers for Disease Control and Prevention, around 958,000 cases of cryptococcal meningitis occur worldwide in persons with HIV infection each year. The region with the greatest number of cases is sub-Saharan Africa, with 720,000 cases, followed by south and South East Asia with 120,000 cases. The disease is one of the leading causes of infection-related mortality in sub-Saharan Africa, with around 500,000 deaths each year. Moreover, cryptococcosis is estimated to cause more deaths in this region than diseases such as tuberculosis, which are more common in the population.

12.5 Clinical manifestations

Most cases of cryptococcosis are not diagnosed until signs of central nervous system (CNS) disease have appeared. However, it is well established that the lungs are the usual initial site of involvement. In many patients, infection of the lungs occurs and resolves weeks to months before disseminated infection is detected.

In HIV-negative individuals, the clinical manifestations of *C. gattii* infection can be distinguished from those of *C. neoformans* by a much higher incidence of mass lesions in the brain (up to 30% of cases) and/or lungs (termed cryptococcomas), more severe neurological deficits, and a slower response to treatment. In HIV-associated cases of cryptococcosis in South Africa, the clinical presentation of *C. gattii* infection was similar to that of *C. neoformans*.

The clinical manifestations of cryptococcosis in persons with HIV infection are similar to those in other groups of immunocompromised individuals. However, infected sites often contain a much higher burden of organisms. More than 80% of persons with AIDS have meningitis or meningoencephalitis at the time of diagnosis, but the disease is often more widespread. Because the clinical presentation can be so

non-specific, it is important to consider the diagnosis in all HIV-infected patients who present with a fever and to re-evaluate them at regular intervals for cryptococcal infection, even if the initial investigations are negative.

12.5.1 Pulmonary cryptococcosis

Up to one-third of immunocompetent individuals develop no symptoms following inhalation of *Cryptococcus,* and their infection remains unrecognized until it has spread to other organs. However, the remainder present with symptoms, such as fever, productive cough, respiratory distress, chest pain and weight loss. The most frequent radiological findings are well-defined, non-calcified, single or multiple nodules. Less common findings include indistinct mass-like infiltrates, lobar infiltrates, hilar and mediastinal lymphadenitis, pleural effusions and cavities.

In immunocompromised individuals, especially those with HIV infection, pulmonary cryptococcosis is usually symptomatic and can progress to an acute respiratory distress syndrome (ARDS). The disease follows a much more rapid course and patients often present with disseminated infection. More than 90% of HIV-infected persons in whom cryptococcosis of the lungs is diagnosed have concurrent meningeal involvement at the time of diagnosis. Immunocompromised patients with pulmonary cryptococcosis commonly present with fever, malaise, chest pain, weight loss and dyspnoea. Chest radiographs will reveal diffuse interstitial or alveolar infiltrates that can mimic *Pneumocystis* pneumonia. Nodules, pleural effusions and cavities are sometimes found.

12.5.2 CNS cryptococcosis

Infection of the brain and meninges is the commonest clinical form of cryptococcosis and the most frequent cause of death. It follows dissemination of the organism from the lungs.

In HIV-negative patients, the symptoms and signs are often indolent in onset. Headache is the most common presenting symptom: the pain is dull, bilateral and diffuse. Fever is often minimal or absent until late in the course of the infection. Mental changes, such as drowsiness and confusion, also occur. Hydrocephalus is a serious complication, but focal neurological lesions are uncommon in *C. neoformans* infection. In contrast, cerebral cryptococcomas with or without hydrocephalus can occur up to 25% of apparently immunocompetent HIV-negative patients with *C. gattii* infection. These lesions are insidious in onset and present with focal signs. Computed tomographic (CT) scans will reveal single or

multiple, enhancing or non-enhancing nodules. Magnetic resonance imaging (MRI) scans are more sensitive than CT scans in detecting abnormalities. In some HIV-negative patients, CNS disease is associated with concurrent infection of other sites. In most, however, it presents without other manifestations of disease.

About 90% of HIV-negative patients have abnormal cerebrospinal fluid (CSF) findings, including increased pressure, raised protein concentration, lowered glucose concentration and a lymphocytic pleocytosis. In persons with HIV infection, the CSF may appear normal: the protein and glucose concentrations may be unchanged, and there is often only a very mild pleocytosis.

In HIV-infected persons, the burden of fungal organisms in the CNS is usually higher. These patients may have a shorter onset of symptoms and clinical signs, higher intracranial pressures, higher CSF antigen titres and slower CSF sterilization after starting antifungal treatment. Headache and fever are common, but overt meningeal symptoms and signs are unusual: fewer than 20% of patients are somnolent, confused or obtunded. Focal neurological signs are uncommon on initial presentation, occurring in about 10% of patients.

About 50% of HIV-positive patients with cryptococcal meningoencephalitis have an elevated baseline intracranial pressure (>25 cm of CSF). The underlying mechanism is unclear, but it is thought to be due, at least in part, to interference with CSF reabsorption in the arachnoid villi, caused by accumulation of fungal polysaccharide. This increased CSF pressure can be associated with a rapid deterioration in their condition and death, particularly early after starting antifungal treatment. If cryptococcal meningoencephalitis is suspected, opening pressure during the initial lumbar puncture should be measured. Imaging of the CNS is recommended before the initial lumbar puncture is performed in individuals with focal neurological signs or impaired mentation.

In retrospective reports, 10–50% of AIDS patients with cryptococcosis have developed immune reconstitution inflammatory syndrome (IRIS) after starting HAART. The timing of this complication appears to be variable, ranging from less than 2 weeks to 4 months. Manifestations of IRIS include headache, new inflammation noted on MRI scans and/or an increased intracranial pressure. These new symptoms can be wrongly construed as signs of treatment failure.

Although initiation of HAART within 30–60 days of starting treatment for cryptococcosis has been reported to be associated with a higher risk of IRIS in retrospective investigations, no association between the timing of HAART initiation and IRIS was found in a recent prospective

investigation. In earlier reports, the risk factors for IRIS in HIV-positive patients included lack of previous antiretroviral treatment, low CD4 T-lymphocyte counts, introduction of HAART during the initial stage of induction antifungal treatment, rapid initial decrease in HIV load in response to HAART and lack of CSF sterilization after two weeks of treatment. In contrast, in a recent prospective investigation, the only risk factor identified was a high baseline serum cryptococcal antigen titre. This indicates that a greater fungal burden at the onset of infection can predispose a patient to IRIS when HAART is initiated. However, regardless of the presence of IRIS, clinical outcomes are better among patients who receive HAART within 6 months of the diagnosis of cryptococcal meningitis.

Cryptococcosis-associated IRIS has also been reported in SOT recipients where it has tended to occur more frequently in those receiving more intensive immunosuppressive regimens, such as tacrolimus treatment. In renal transplant recipients, IRIS has led to graft loss.

The differentiation of IRIS from progressive cryptococcal meningitis or meningoencephalitis is important because treatment approaches and outcomes are different for the two diseases. This can be difficult because there are no specific markers that can reliably establish the diagnosis of IRIS. Cultures of CSF and lymph node aspirates are negative in patients with *Cryptococcus*-associated IRIS even though yeast cells may be present in smears. Patients with IRIS tend to have higher CSF opening pressures, glucose levels and white blood cell counts when compared with those with typical HIV-associated cryptococcal meningitis.

12.5.3 Cutaneous cryptococcosis

Haematogenous spread of *Cryptococcus* gives rise to skin lesions in 10–15% of patients with disseminated cryptococcosis. Direct extension from a bone lesion can also occur. In some cases, these lesions are the earliest clinical sign of infection. They are often located on the face and scalp, but may occur on the trunk or limbs.

Cutaneous lesions are common in HIV-infected persons with cryptococcosis. Multiple small papular or maculopapular lesions, some of which show central necrotic ulceration resembling molluscum contagiosum, are frequently reported. These lesions cannot be distinguished from those found in patients with histoplasmosis or *Penicillium marneffei* infection. Other cutaneous lesions include single or multiple nodules, which sometimes progress to ulcers or abscesses. Because there are many different manifestations that mimic other infections, microscopic examination and culture of biopsy material from a suspicious lesion is essential to permit the correct diagnosis to be established.

12.5.4 Osteomyelitis

Osteomyelitis occurs in 5–10% of patients with disseminated cryptococcosis. Most patients present with a single isolated lesion, the commonest site of involvement being the spine. Patients may complain of local pain and soft tissue tenderness at the affected site. Radiographs will reveal well-defined osteolytic lesions without marginal sclerosis or periosteal change. Joint involvement is rare. CT scans can be used to define the extent of bone involvement. Microscopic examination and culture of aspirates or biopsy material from a suspicious lesion should permit the diagnosis to be established.

12.5.5 Other forms of cryptococcosis

Ocular infection with *Cryptococcus* is not uncommon, being noted in up to 40% of patients with meningitis before the AIDS epidemic. In some cases, endophthalmitis is present before the diagnosis of meningitis is made. Prompt diagnosis and rapid treatment are essential to prevent visual loss. Catastrophic visual loss has also been reported in persons without ocular lesions. In some cases, visual loss has occurred over a period as short as 12–24 hours due to invasion of the optic nerve. Others have presented with slower visual loss related to raised intracranial pressure following CNS infection.

Asymptomatic infection of the prostate is common in HIV-infected men with cryptococcosis. Moreover, cultures of urine and prostatic secretions often remain positive following apparently successful antifungal treatment of meningitis and other disseminated forms of cryptococcosis. Before the advent of HAART, the prostate was considered an important reservoir for relapse of infection.

12.6 Essential investigations and their interpretation

Establishing the diagnosis of cryptococcosis is less difficult than diagnosis of many other fungal infections. It requires the isolation of *Cryptococcus* in culture from body fluids or tissue, or the detection of cryptococcal capsular antigen in blood, CSF or urine.

12.6.1 Microscopy

Encapsulated *Cryptococcus* cells can often be detected in specimens of CSF or other host fluids or secretions mounted in India ink or nigrosin. About 50% of HIV-negative and more than 80% of HIV-positive patients have a positive India ink examination of the CSF. However, intact lymphocytes, in particular, can be confused with the organism. In persons with AIDS,

Cryptococcus cells are usually plentiful in the CSF, although the capsules can be small, making recognition difficult. Persistently positive CSF findings in patients undergoing treatment should be considered evidence of failure or relapse only if they are confirmed by a deterioration in the patient's clinical condition or by positive cultures.

12.6.2 Culture

Cryptococcus can be isolated from the CSF in 75–90% of cases of cryptococcal meningitis. The likelihood of success is increased if multiple specimens are taken, and the centrifuged sediment of large amounts (3–5 mL) of fluid are plated out. The organism grows well on standard microbiological media. It grows best at 30–35°C and, although most isolates from untreated patients can be detected in culture after 2–3 days, it is advisable to prolong incubation of plates in suspected cases for up to 2 weeks.

Cryptococcus can also be recovered from blood, bronchoalveolar lavage (BAL) fluid, sputum, urine, prostatic fluid and other specimens. Positive blood cultures have been obtained in 35–70% of HIV-infected persons with cryptococcosis. The lysis-centrifugation method (Isolator, Wampole Laboratories, Cranbury, New Jersey, USA) is the most sensitive procedure for recovering *Cryptococcus* species from blood. Because of the greater load of organisms, microscopic examination and culture of other specimens is more often positive in untreated patients with HIV infection than in other individuals. Isolation of the organisms from sputum, BAL fluid or lung tissue should be attempted if pulmonary cryptococcosis is suspected, especially in persons with AIDS in whom the recovery rate is high. There are a number of special media, such as canavanine–glycine–bromthymol blue agar, which can be used to differentiate *C. gattii* from *C. neoformans*.

12.6.3 Serological tests

Antibodies to *Cryptococcus* can often be detected in patients with localized or past infection, but are seldom found in patients with untreated meningeal or disseminated disease in whom tests for antigen are much more helpful.

12.6.4 Antigen detection tests

Testing for *Cryptococcus* capsular antigen is one of the most reliable methods for the diagnosis of cryptococcosis. Several latex particle agglutination and ELISA tests have been marketed for the detection of antigen in serum, CSF, urine and BAL fluid specimens. These tests are sensitive and highly specific, provided that rheumatoid and other interfering factors are removed. False-negative results can occur if the organism load is low or if

the organisms are not well encapsulated. It is also important to remember that different manufacturers' products can give different titres with the same clinical specimen.

A serum antigen titre of 1:8 or greater is considered strong presumptive evidence of *Cryptococcus* infection. A negative serum antigen test result does not exclude the diagnosis of cryptococcosis, particularly if only a single specimen has been tested and the patient has symptoms consistent with the infection. Repeated negative test results for serum antigen in HIV-infected persons without neurological symptoms or signs make the diagnosis improbable, but should not preclude the clinician who still suspects cryptococcal meningitis from performing a lumbar puncture.

The CSF antigen test is positive in over 90% of patients with untreated meningeal cryptococcosis. Levels of antigen in the CSF often decline with treatment, but the test can remain positive for several weeks. This is more common in patients whose initial load of organisms is high. Positive CSF antigen test results may be obtained despite failure to recover *Cryptococcus* from the CSF. This may represent the persistent release of antigen from dead cells or slow elimination of capsular antigen from the CSF, rather than ongoing infection.

In HIV-negative individuals, high levels of serum and CSF antigen prior to treatment are often predictive of death during treatment. In persons with AIDS, abnormal mental status prior to treatment is the most important factor, but a high CSF antigen titre (greater than 1:1000) is also a sign of a poor prognosis.

In HIV-negative patients, high antigen levels at the end of treatment are often predictive of later relapse. In contrast, in persons with AIDS, changes in serum antigen levels do not correlate with response, and although an unchanged or increased titre of antigen in CSF is often associated with clinical and mycological failure to respond to treatment, its usefulness in management is uncertain.

12.7 Management

All patients with cryptococcosis, apart from a few immunocompetent individuals with asymptomatic infection of the lungs, require treatment. The choice of treatment depends on both the anatomic sites of involvement and the immunological status of the host. The choice of treatment is usually the same regardless of whether the infection is caused by *C. neoformans* or *C. gattii*. However, *C. gattii* infections are often slower to respond, largely because of the high incidence of cryptococcomas in the brain and/or lungs.

12.7.1 CNS cryptococcosis

12.7.1.1 HIV-infected patients

HIV-infected persons with CNS cryptococcosis should be treated with amphotericin B deoxycholate (0.7–1 mg/kg per day) and flucytosine (100 mg/kg per day in four divided doses) for at least 2 weeks. In cases where flucytosine cannot be used, amphotericin B alone (0.7–1 mg/kg per day) is an acceptable alternative. If the initial 2-week period of induction treatment is successful in sterilizing the CSF, consolidation treatment can then be commenced with oral fluconazole (400 mg/day or 6 mg/kg per day) for a further 8 weeks, or until CSF cultures are sterile.

Administration of one of the lipid-based forms of amphotericin B should be considered in patients in whom the conventional formulation is contraindicated. For induction, liposomal amphotericin B should be administered at a dose of 3–4 mg/kg per day and amphotericin B lipid complex (ABLC) at a dose of 5 mg/kg per day for at least 2 weeks.

Other options for induction and consolidation treatment include combination treatment with amphotericin B (0.7 mg/kg per day) and oral fluconazole (800 mg/day) for 2 weeks, followed by fluconazole (800 mg/day) for a minimum of 8 weeks; fluconazole (800–1200 mg/day) and flucytosine (100 mg/day in four divided doses) for 6 weeks; and fluconazole (1200–2000 mg/day) for 10–12 weeks.

The rate of recurrence of cryptococcal disease among persons with AIDS is high, and it has long been recommended that all patients who survive beyond the initial induction treatment should receive maintenance treatment to prevent relapse, either lifelong or until immune reconstitution occurs as a result of HAART. The drug of choice is oral fluconazole (200 mg/day), as this has proven superior to itraconazole (200 mg twice daily). The latter drug can be used in cases where the patient is intolerant of fluconazole or has failed fluconazole treatment. HAART can be initiated 2–10 weeks after beginning initial antifungal treatment.

Because of its more rapid clearance in children, fluconazole should be administered at 12-hour intervals. During consolidation treatment, a dosage of 10–12 mg/kg per day is recommended. A dosage of 6 mg/kg per day is recommended for maintenance treatment in HIV-positive children.

The risk for recurrence of cryptococcosis appears low when patients have completed an initial course of antifungal treatment, have remained asymptomatic and have had a sustained increase (for 3 months or longer) in their CD4 T-lymphocyte count to greater than 100 cells per μL during HAART. The number of individuals who have been evaluated

remains limited, but guidelines now suggest that it is reasonable to discontinue maintenance treatment after 12 months of antifungal therapy among such patients. Maintenance treatment should be restarted if the CD4 T-lymphocyte count falls below 100 cells per μL.

12.7.1.2 Organ transplant recipients

SOT recipients with CNS cryptococcosis should be treated with liposomal amphotericin B (3–4 mg/kg per day) or ABLC (5 mg/kg per day) and flucytosine (100 mg/kg per day in four divided doses) for at least 2 weeks. This should be followed by oral fluconazole (400–800 mg/day) for 8 weeks, and then by maintenance treatment with a lower dose of fluconazole (200–400 mg/day) for 6–12 months. If the induction regimen does not include flucytosine, a lipid-based form of amphotericin B should be given for at least 4–6 weeks. Liposomal amphotericin B at a dosage of 6 mg/kg per day may be used in patients with high-burden disease or relapse.

12.7.1.3 Other patients

The recommended treatment for immunocompetent patients with meningoencephalitis, but without neurological complications, is the combination of amphotericin B deoxycholate (0.7–1 mg/kg per day) and flucytosine (100 mg/kg per day in four divided doses) for at least 4 weeks. One of the lipid-based forms of amphotericin B may be substituted for the second 2-week period if renal toxicity becomes a problem. In patients with neurological complications, the period of induction treatment may need to be extended from 4 to 6 weeks, and a lipid-based form of amphotericin B may be given for the last 4 weeks of the induction period. Induction treatment should be followed by consolidation treatment with fluconazole (400 mg/day) for an additional 8 weeks.

If flucytosine is not given or treatment is interrupted, the period of induction treatment with conventional or lipid-based forms of amphotericin B should be lengthened by at least 2 weeks. Patients who respond well to induction treatment with amphotericin B and flucytosine may be changed after 2 weeks to consolidation treatment with fluconazole (800 mg/day) for a further 8 weeks. After induction and consolidation therapy, patients should receive maintenance treatment with oral fluconazole (200 mg/day) for 6–12 months.

12.7.2 Pulmonary cryptococcosis

Immunosuppressed patients with pulmonary cryptococcosis should have a lumbar puncture performed to rule out concomitant CNS infection. Patients with disseminated disease and/or severe pneumonia (ARDS)

should be treated the same as those with CNS disease (see the preceding text). Patients who present with mild or moderate pulmonary symptoms (without diffuse infiltrates) and without signs of disseminated infection may be treated with oral fluconazole (400 mg/day) for 6–12 months. It is reasonable to discontinue maintenance treatment after 12 months in HIV-infected patients who are receiving HAART and have a CD4 T-lymphocyte count of greater than 100 cells per μL.

Immunocompetent individuals with asymptomatic pulmonary infection have done well without treatment. However, careful observation is essential. Patients with severe pulmonary disease should be treated the same as those with CNS infection (see the preceding text). Those who present with mild or moderate symptoms should be treated with fluconazole (400 mg/day) for 6–12 months. If fluconazole is contraindicated, oral treatment with voriconazole (200 mg twice daily) or posaconazole (400 mg twice daily) is an alternative.

Surgical intervention should be considered in patients with persistent radiological abnormalities and symptoms that are not responding to treatment.

12.7.3 Cryptococcoma

Patients with cerebral cryptococcomas should be treated with amphotericin B deoxycholate (0.7–1 mg/kg per day), liposomal amphotericin B (3–4 mg/kg per day) or ABLC (5 mg/kg per day) plus flucytosine (100 mg/kg per day in four divided doses) for at least 6 weeks. This should be followed by consolidation and maintenance treatment with fluconazole (400–800 mg/day) for 6–18 months. Adjunctive treatment with corticosteroids should be considered in patients with cryptococcomas with significant surrounding oedema, particularly if there are neurological deficits. If corticosteroids are used, the dose should be reduced gradually to prevent a 'rebound' IRIS phenomenon. Surgical removal of accessible lesions may be required to reduce life-threatening cerebral mass effects if there is substantial surrounding oedema and to achieve cure in cases unresponsive to prolonged or repeated courses of induction treatment.

12.7.4 Other forms of cryptococcosis

Patients with fungaemia or disseminated forms of disease that do not involve the lungs or CNS should be treated the same as those with CNS disease (see the preceding text). If CNS disease is not present, fungaemia is absent, infection occurs at a single site and there are no immunosuppressive risk factors, the patient can be treated with oral fluconazole (400 mg/day) for 6–12 months.

12.7.5 Management of persistent and relapsing cryptococcosis

Patients with persistent (defined as a positive culture after a minimum of 4 weeks of treatment) or relapsing infection should receive a second course of induction treatment for 4–10 weeks. If the initial dose of conventional amphotericin B was 0.7 mg/kg or less, or 3 mg/kg or less of a lipid formulation, the drug should be restarted at a higher dose (up to 1 mg/kg of the conventional formulation, or 6 mg/kg of liposomal amphotericin B). If the patient is intolerant of amphotericin B, an alternative is to give a combination of fluconazole (at least 800 mg/day) and flucytosine (100 mg/kg per day in four divided doses). If the patient is flucytosine-intolerant, an alternative regimen is to give amphotericin B deoxycholate (0.7 mg/kg per day) plus fluconazole (800 mg/day). The use of intrathecal or intraventricular amphotericin B is generally discouraged and should only be considered in cases where all other forms of treatment have failed.

Following the second course of induction treatment, patients with relapsed infection may receive salvage consolidation treatment with fluconazole (800–1200 mg/day), voriconazole (200–400 mg twice daily) or posaconazole (200 mg four times daily or 400 mg twice daily) for 10–12 weeks. If the infecting strain remains susceptible, the previous suppressive dose of fluconazole may be reinstated

12.7.6 Management of increased intracranial pressure

In all cases of cryptococcal meningitis, careful attention must be paid to the management of intracranial pressure to assure optimal clinical outcome. Elevated CSF opening pressure has been associated with a higher organism load, a higher incidence of neuropathies and decreased short-term survival. The principal intervention for reducing elevated intracranial pressure is percutaneous lumbar drainage. In cases where repeated lumbar punctures or use of a lumbar drain fail to control symptoms of elevated pressure, or when persistent or progressive neurological deficits are present, a permanent ventriculoperitoneal shunt may be indicated. Adjunctive treatment with acetazolamide and corticosteroids (unless part of IRIS treatment) should be avoided. Nor has mannitol been shown to provide any clear benefit in the management of elevated pressure resulting from cryptococcal meningitis.

12.7.7 Management of IRIS

There are no definitive treatment recommendations for individuals with mild IRIS, as this will usually resolve spontaneously within days or weeks. In patients with severe symptomatic manifestations of IRIS, such as CNS inflammation and/or elevated intracranial pressure, some experts recommend short-course corticosteroids (0.5–1 mg/kg per day of prednisone

equivalent for 2–6 weeks). It is prudent to delay the initiation of potent antiretroviral treatment until patients are stable, have completed induction treatment for cryptococcosis and have microbiological evidence of response. Long delays in initiating HAART can place individuals at risk of dying from other complications of HIV infection.

12.8 Prevention

Several randomized clinical trials in the United States and Thailand have demonstrated that primary prophylaxis with fluconazole (200 mg/day to 400 mg/week) or itraconazole (200 mg/day) can significantly reduce the incidence of cryptococcosis among adults with advanced or severe HIV infection, particularly those with CD4 T-lymphocyte counts of less than 50–100 cells per μL. Moreover, a controlled clinical trial in Thailand with fluconazole (400 mg/week) showed a survival benefit associated with prophylaxis. In developed countries, current guidelines do not recommend routine antifungal prophylaxis to prevent cryptococcosis because of the relatively low prevalence of the disease, the high cost, the lack of survival benefits associated with prophylaxis and the potential for drug interactions or development of drug resistance. This approach may, however, be more beneficial in resource-limited settings because of the higher incidence of disease and more limited availability of diagnosis and antifungal treatment. In Thailand, national guidelines recommend fluconazole (400 mg/week) for patients with CD4 counts of less than 100 cells per μL.

There is currently growing interest in integrating serum cryptococcal antigen screening into national antiretroviral treatment programs in sub-Saharan Africa. If screening detects the presence of antigen, treatment with high-dose fluconazole can be initiated, thereby preventing most, if not all, cases of cryptococcal meningitis that develop during HAART. With the advent of a simple, low-cost, rapid dipstick test for cryptococcal antigen (Immuno-Mycologics Inc., Norman, Oklahoma, USA), a pre-emptive 'screen and treat' approach is becoming a cost-effective option for resource-limited settings where mortality from cryptococcosis is high.

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CHAPTER 13

Mucormycosis

13.1 Definition

The term mucormycosis is now used to refer to infections caused by moulds belonging to the order Mucorales. Traditionally, this order was assigned to the phylum Zygomycota together with the order Entomophthorales (see Chapter 20), and the different forms of disease caused by the two groups of organisms were often referred to as zygomycosis. However, following molecular analysis, the phylum Zygomycota is no longer accepted due to its polyphyletic nature. The sub-phylum Mucoromycotina has been proposed to accommodate the Mucorales and the sub-phylum Entomophthoromycotina has been created for the Entomophthorales.

Fungi of the order Mucorales can cause rhinocerebral, pulmonary, gastrointestinal, cutaneous or disseminated disease in predisposed individuals, the different clinical forms often being associated with particular underlying disorders. Mucormycosis is the second most frequent mould infection seen in immunocompromised individuals.

13.2 Geographical distribution

These infections are worldwide in distribution.

13.3 The causal organisms and their habitat

Many different organisms have been implicated as aetiological agents of mucormycosis, but the commonest causes of human infection are *Rhizopus oryzae* (*R. arrhizus*) and *Rhizopus microsporus*. Other aetiological agents

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include *Apophysomyces elegans*, *Cokeromyces recurvatus*, *Cunninghamella bertholletiae*, *Lichtheimia* (formerly *Absidia*) *corymbifera*, *Rhizomucor pusillus*, *Saksenaea vasiformis* and *Syncephalastrum racemosum*. In tissue and in culture, these moulds form characteristic broad, non-septate or sparsely septate hyphae with right-angled branching. All these moulds cause similar diseases in humans, and the diagnostic and therapeutic approaches are similar.

The species isolated from cases of human and animal infection are thermotolerant, and many are ubiquitous in the soil and on decomposing organic matter. These moulds are found in the indoor and outdoor air, on food items and in dust.

13.4 Epidemiology

Most human infections follow inhalation of spores that have been released into the air and the lungs and nasal sinuses are the commonest initial sites of infection. Infection may also follow cutaneous or percutaneous inoculation of organisms, either through traumatic disruption of skin barriers or as a result of catheter insertion or injections. Less frequently, infection follows ingestion of contaminated food.

Many of the aetiological agents of mucormycosis are ubiquitous in the environment and the likelihood that infection will occur following inhalation, implantation, or ingestion of spores largely depends on host factors. The major risk factors predisposing individuals to the disease include prolonged or profound neutropenia, uncontrolled diabetic and metabolic acidosis, malnutrition, use of corticosteroids, and treatment with the iron-chelating agent, desferrioxamine (deferoxamine). High-risk patient groups include haematopoietic stem cell transplant (HSCT) recipients, solid organ transplant (SOT) recipients, patients with haematological malignancies, patients with type I or II diabetes mellitus, burns patients, injection drug users and individuals with no apparent immunological defects. Certain predisposing conditions seem to be more commonly associated with particular clinical forms of disease. For instance, persons with diabetic ketoacidosis often develop fulminant rhinocerebral mucormycosis, while individuals with haematological malignancies and transplant recipients often develop pulmonary disease. Injection drug users frequently develop isolated cerebral infection. A significant number of cases of cutaneous mucormycosis occur in individuals with no underlying condition at the time of infection. In many of these cases, there is a history of penetrating trauma, burns or road traffic accidents before the development of the infection.

Uncontrolled diabetes mellitus is the principal cause of acidosis in most patients with mucormycosis, but the disease has also been seen in patients with persistent metabolic acidosis due to other causes, such as renal disease. Impairment of macrophage and neutrophil function has been identified as the principal factor responsible for the increased risk of developing mucormycosis in diabetic individuals. Iron has an important role in the pathogenesis of infections caused by the Mucorales, with fungal growth being promoted in the presence of increased iron uptake. The availability of serum iron is increased in the presence of an acidic pH, and this could help to account for the development of mucormycosis in patients with diabetic ketoacidosis. Desferrioxamine is an iron chelator that mobilizes iron from the host and makes it available to the infecting fungus. The use of this drug in patients with iron overload or on dialysis has been associated with the development of more severe, disseminated, and often lethal mucormycosis.

The widespread use of voriconazole to prevent or treat aspergillosis in patients with high-risk malignancies or following HSCT has been associated with an apparent rise in cases of confirmed mucormycosis in many institutions worldwide. While this phenomenon is still not completely understood, it is clear that the use of broader-spectrum antifungal agents for prophylaxis has the potential to lead to the selection of organisms, such as the Mucorales, that are intrinsically resistant to available antifungal agents.

Mucormycosis is a rare infection, but recent reports suggest it may be increasing in incidence. In the United States, active population-based surveillance, conducted in 1992 and 1993, showed that the disease developed in 1.7 persons per million population per annum (in the same report the incidence of aspergillosis was 12.4 per million population). In France, population-based surveillance, conducted between 1997 and 2006, showed an increase in incidence from 0.7 to 1.2 cases per million population per annum. Most of the increase was accounted for by infections among HSCT recipients or persons with haematological malignancies. Among HSCT and SOT recipients, most cases of mucormycosis occur late (more than 100 days) after transplantation.

The case fatality rate for mucormycosis ranges from 35% among individuals with no underlying condition, 44% among patients with diabetes and 66% in patients with malignancies. The higher survival rate among diabetics than among those with underlying malignancies has been attributed to the relative ease with which the underlying ketoacidosis can be reversed. Case fatality rates also vary according to the site of infection: 62% among those with rhinocerebral disease, 76% in those with

pulmonary disease, 85% among those with gastrointestinal disease, and 96% in those with disseminated disease. Pulmonary mucormycosis has a high case fatality rate because it is difficult to diagnose, frequently occurs in neutropenic patients and because surgical intervention is seldom possible.

Nosocomial outbreaks of mucormycosis are not as common as healthcare-associated *Aspergillus* infections, but have sometimes been linked to construction or renovation work, as well as to contaminated ventilation systems. Nosocomial clusters of cutaneous infections with *Rhizopus rhizopodiformis* and *Rhizopus microsporus* have been traced to contaminated biomedical devices, or associated with the use of non-sterile surgical dressings, tape, adhesive bandages and splints. However, most cases of mucormycosis in hospitalized patients are sporadic in nature and it is much more difficult to determine whether these infections are acquired inside or outside the hospital setting.

13.5 Clinical manifestations

There are five major clinical forms of mucormycosis, of which rhinocerebral and pulmonary infections are by far the most common. Other manifestations include cutaneous, gastrointestinal and disseminated disease. Like the aetiological agents of aspergillosis, the causal organisms of mucormycosis have a predilection for vascular invasion causing thrombosis, infarction and necrosis of the surrounding tissue. The clinical hallmark of mucormycosis is the rapid onset of necrosis and fever. In most cases, progress is rapid and death follows unless aggressive treatment is initiated.

13.5.1 Rhinocerebral mucormycosis

The terms rhinocerebral and craniofacial mucormycosis are used to describe an infection that begins in the paranasal sinuses and then spreads to involve the orbit, face, palate and/or brain. The clinical presentation is similar to that of acute invasive *Aspergillus* rhinosinusitis (see Chapter 10). This is a rapidly progressive disease that is most commonly seen in diabetics with uncontrolled ketoacidosis, as well as in neutropenic cancer patients and HSCT recipients. Immunocompetent individuals are seldom affected. It is the commonest clinical form of mucormycosis, accounting for 39% of all cases of the disease and 66% of cases in diabetics. If left untreated, the infection is often fatal within a week of onset.

The presenting symptoms include fever, unilateral facial swelling, unilateral headache, nasal or sinus congestion or pain and a blood-tinged nasal discharge. Necrotic black ulcerations on the hard palate or nasal

turbinate are a characteristic diagnostic sign. As the infection spreads into the orbit, periorbital or perinasal swelling occurs and progresses to disfiguring destruction of facial tissue. Ptosis, proptosis, ophthalmoplegia and loss of vision can occur. Drainage of black pus from the eye is a useful diagnostic sign. From the orbit, infection may spread into the brain, leading to frontal lobe necrosis and abscess formation.

Imaging studies are helpful in assessing the extent of bone and soft-tissue involvement, but are more useful in guiding more invasive procedures to obtain biopsy specimens than in establishing a diagnosis. Computed tomographic (CT) scans often reveal involvement of several sinuses (in particular ethmoid and sphenoid), but with a clear unilateral predilection, no air-fluid levels, thickening of sinus linings and destruction of surrounding bone. Magnetic resonance imaging (MRI) is superior to CT scanning for detecting the extension of infection to the adjacent soft tissue of the orbit and brain and may be preferred for the diabetic patient in whom CT contrast agents may be contraindicated.

13.5.2 Pulmonary mucormycosis

This is the second most common clinical form of mucormycosis. It is most commonly seen in neutropenic patients with underlying haematological malignancies who are undergoing remission induction treatment. It has also been reported in allogeneic HSCT recipients, SOT recipients, and those receiving desferrioxamine treatment. Mucormycosis may develop in the lungs as a result of aspiration of infectious material, or following inhalation, or from haematogenous or lymphatic spread during dissemination. In about 50% of reported cases, the infection remains confined to the lung; in the remainder, it spreads into adjacent tissues or becomes more widely disseminated. If left untreated, the infection is usually fatal within 2–3 weeks.

The clinical presentation is non-specific. Patients often present with an unremitting fever (greater than 38°C) that fails to respond to broad-spectrum antibacterial treatment. Non-productive cough is a common presenting symptom. Haemoptysis and pleuritic chest pain are uncommon, but when present are helpful in suggesting a fungal infection. However, there are no characteristic symptoms or clinical signs to distinguish mucormycosis from aspergillosis (see Chapter 10).

The radiological signs are also non-specific, but infiltrates and nodules are more frequent than consolidation or cavitation. Pleural effusion is uncommon. As with pulmonary aspergillosis, high-resolution CT scanning is more sensitive than plain chest radiographs for early diagnosis of infection and is the best method for determining the extent of the disease.

The halo and air crescent signs, which are recognized radiological signs of aspergillosis (see Chapter 10), have also been reported in patients with mucormycosis. In patients with pulmonary mucormycosis, the presence of an air crescent sign is associated with an increased risk for massive haemoptysis. Mucormycosis has been reported to have a predilection for the upper lobes; however, any part of the lung may be involved, and bilateral disease is not uncommon.

13.5.3 Cutaneous mucormycosis

Although inhalation is the usual route of infection in patients with mucormycosis, traumatic inoculation of spores can lead to extensive necrotic cutaneous infections. This form of disease is most often seen in patients with burns and severe soft-tissue trauma. Cutaeneous mucormycosis has sometimes developed as a result of the application of contaminated surgical dressings, tape, adhesive bandages and splints. In diabetic or immunosuppressed patients, cutaneous lesions may arise at an insulin injection site or a catheter insertion site. Cutaneous mucormycosis is an aggressive disease, even in the face of surgical debridement and antifungal treatment. In 44% of cases, it leads to extensive local necrotizing fasciitis or widespread disseminated infection.

The initial signs include cutaneous erythema and subcutaneous swelling. The margins of the lesion become raised and indurated, and the central region becomes necrotic and evolves into an ulcer covered with a black eschar. The lesions are indistinguishable from those caused by *Aspergillus* species (see Chapter 10) and can resemble ecthyma gangrenosum. The lesions are painful, and the patient can be febrile. The development of severe underlying necrosis and infarction in a burn should suggest the diagnosis.

Much less commonly, cutaneous mucormycosis develops following haematogenous dissemination of the fungus in immunosuppressed patients. The lesions begin as an erythematous, indurated painful cellulitis, then evolve into ulcers covered with a black eschar.

13.5.4 Gastrointestinal mucormycosis

This is a rare condition that has usually been encountered in malnourished individuals and low-birth-weight infants, in whom it may present as necrotizing enterocolitis. All segments of the gastrointestinal tract can be involved, but lesions are most common in the stomach, colon and ileum. The disease is seldom diagnosed during life.

The symptoms are varied and depend on the site affected. Fever, abdominal pain, distention, vomiting and haematemesis are typical. Complications include gastric or intestination perforation, perirenal abscesses

and renal infarction. Intestinal mucormycosis is a fulminant illness ending in death within several weeks due to bowel infarction, sepsis or haemorrhagic shock.

13.5.5 Disseminated mucormycosis

This may follow any of the four forms of mucormycosis described so far, but it is most frequently associated with pulmonary disease. Less commonly, dissemination occurs from the gastrointestinal tract, burns or other cutaneous lesions. The commonest site of spread is the brain, but metastatic necrotic lesions have also been found in the spleen, heart and other organs. Disseminated mucormycosis is seldom diagnosed during life, but occasional patients develop metastatic cutaneous lesions that permit an earlier diagnosis.

13.5.6 Other forms of mucormycosis

Isolated mucormycotic brain lesions without rhino-orbital involvement have been reported in leukaemic patients and injection drug users. The lesions often lead to focal neurological signs. The diagnosis is difficult, but should be considered in a neutropenic patient who becomes lethargic, confused and obtunded. CT and MRI scans are useful in locating the lesions, but the findings are non-specific. Other rare forms of mucormycosis include endocarditis, osteomyelitis and pyelonephritis. Isolated peritonitis has been associated with peritoneal dialysis.

13.6 Differential diagnosis

Rhinocerebral mucormycosis is a dramatic and distinctive condition, but it can be confused with cavernous sinus thrombosis, bacterial orbital cellulitis and other forms of acute invasive fungal sinusitis, such as aspergillosis.

The clinical manifestations of pulmonary mucormycosis cannot be distinguished from Gram-negative bacterial pneumonia, or aspergillosis or hyalohyphomycosis.

13.7 Essential investigations and their interpretation

Because mucormycosis is such an aggressive infection, an early diagnosis is essential for successful management.

13.7.1 Microscopy

The microscopic demonstration of Mucorales in clinical material taken from necrotic lesions or in sputum or bronchoalveolar lavage (BAL) fluid is more significant than their isolation in culture. These organisms can be

distinguished from other moulds, such as *Aspergillus*, *Fusarium* and *Scedosporium* species, by their characteristic wide, non-septate hyphae with right-angled branching. Other moulds have thinner, septate hyphae that typically branch at acute angles.

13.7.2 Culture

Isolation in culture is required for specific identification of the Mucorales. Despite their predilection for angioinvasion and haematogenous dissemination, blood cultures in all forms of mucormycosis are always negative. Nasal, palatal and sputum cultures are seldom helpful. Because the Mucorales are common contaminants, isolation of these organisms from material obtained from a necrotic lesion, or from sputum or BAL fluid must be interpreted with caution. However, if the patient is diabetic or immunosuppressed, the isolation should not be ignored.

13.7.3 Serological and molecular tests

There are no routine serological or molecular tests for diagnosis of mucormycosis available at present.

13.8 Management

If the management of mucormycosis is to be successful, treatment should be initiated as soon as the diagnosis is suspected, the underlying metabolic or immunological disorder(s) that precipitated the infection must be corrected, infected necrotic tissue must be removed and an appropriate antifungal agent must be administered. Even if a timely diagnosis is made, management is challenging: disease progression is often rapid, and vascular invasion and thrombosis can prevent adequate concentrations of antifungal drugs from reaching the site of infection.

Immunosuppressive and myelotoxic drugs should be reduced in dose or discontinued, provided this will not harm the patient. Iron chelation treatment with desferrioxamine should also be discontinued. Shortening the duration of neutropenia with colony stimulating factors may be beneficial, but it remains unclear whether this improves outcome.

Amphotericin B remains the most potent drug available for mucormycosis. Most experts now recommend a lipid-based formulation as first-line treatment because of the toxic side effects of amphotericin B deoxycholate. However, it remains unclear as to whether the lipid-based formulations differ from conventional amphotericin B or each other in terms of clinical benefit in mucormycosis. Liposomal amphotericin B has been administered at dosages as high as 10 mg/kg per day, while amphotericin B lipid

complex has been given at dosages as high as 5 mg/kg per day. If lipid-based amphotericin B is unavailable, the conventional formulation should be given at the maximum tolerated dosage of 1.0–1.5 mg/kg per day from the outset (see Chapter 3).

Unlike other azole antifungals, posaconazole has significant activity against some of the Mucorales. Individual case reports and case series describing the use of this agent (at a dosage of 800 mg/day given in two or four doses) as salvage treatment in patients with mucormycosis have provided encouraging results. However, posaconazole is not currently licenced for this infection, and the lack of an intravenous formulation is a serious disadvantage when treating seriously ill patients. If posaconazole is used, drug levels should be monitored to ensure adequate serum concentrations are attained (see Chapter 3).

In individuals with sino-orbital infection, early and aggressive surgical debridement of the necrotic lesions and surrounding infected tissue is the single most important component of treatment. Delay of just a few hours may permit the organism to spread along blood vessels into the cavernous sinus or into the brain. Debridement may need to be repeated several times until a patient is stabilized.

If a patient with mucormycosis confined to one region of the lung does not respond to amphotericin B treatment within 48–72 hours, surgical resection should be considered. Although a wedge resection is sometimes sufficient, complete lobar or segmental resection is often required. The more widespread the infection, the less beneficial is surgical intervention.

In individuals with cutaneous mucormycosis, early and aggressive surgical debridement of the necrotic lesions and surrounding infected tissue is an essential component of treatment. Debridement may need to be repeated. Administration of amphotericin B is also advised. Treatment is more successful in cutaneous than other forms of mucormycosis. However, it is not without complications: curative surgical intervention is often disfiguring, and amputation of affected limbs is sometimes required.

The optimum duration and total dose of amphotericin B that should be given to patients with mucormycosis has not been determined. Treatment must be individualized according to the patient's clinical response and the rate of clearing of the infection. Treatment should be continued until clinical and radiological abnormalities are resolving, and reversible underlying predispositions have abated. The duration of antifungal treatment should be guided by clinical response rather than the total drug dose administered. The ultimate response of patients to treatment is largely related to host factors, such as resolution of neutropenia and the return of neutrophil function, and the correction of diabetic ketoacidosis.

Although desferrioxamine is a well-recognized risk factor for mucormycosis, the newer iron chelators, deferiprone and deferasirox, have the opposite effect. These agents have been shown to improve survival in animal models of mucormycosis, and there are human case reports of their successful use as an adjunct to salvage treatment. In other cases, however, iron chelation treatment has been unsuccessful, and its potential benefit awaits confirmation from a prospective clinical trial.

13.9 Prevention

Measures to reduce the incidence of mucormycosis are of major importance in the management of this difficult-to-diagnose infection. In the non-immunocompromised individual, preventive measures should focus on the underlying risk factors that lead to the development of mucormycosis. Adequate control of diabetes mellitus, the use of iron chelators other than desferrioxamine and limiting the use of aluminium-containing buffers in dialysis are among the best preventive measures that can be taken.

The Mucorales are among the most common moulds found in the environment. Therefore, the first steps in the prevention of infection in the immunocompromised patient should consist of measures to reduce or eliminate obvious sources of environmental exposure, such as removing plants and flower arrangements from rooms where these individuals are being treated. Food items, such as old bread and fruit, that are often contaminated with mucoraceous moulds should not be offered to immunocompromised patients. Clusters of cutaneous mucormycosis have sometimes been traced to contaminated biomedical devices or linked to the use of non-sterile surgical dressings.

Inhalation of spores is thought to be the commonest mode of infection in immunocompromised patients with mucormycosis. The most effective, and expensive, method of protecting these individuals from nosocomial infection is to confine them to hospital rooms provided with HEPA-filtered air (see Chapter 10). Although this reduces the risk of disease to an insignificant level, infection can still occur if patients are colonized before their admission to hospital or are moved from the protected environment to other parts of the hospital for essential procedures to be performed. Immunocompromised patients should not be nursed in units with ongoing, adjacent construction work, but if this cannot be avoided, measures should be instituted to minimize the entry of dust and contaminated air.

There is currently no effective antifungal prophylaxis available for prevention of mucormycosis.

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CHAPTER 14

Pneumocystosis

14.1 Definition

The term pneumocystosis is used to refer to infections caused by *Pneumocystis jirovecii*, a common opportunistic pathogen of humans that is now classified as a fungus but was once presumed to be a protozoan parasite. Following inhalation, this organism causes a life-threatening pneumonia in persons with human immunodeficiency virus (HIV) infection or other immunosuppressive conditions. The diagnosis of *Pneumocystis* pneumonia (PCP) continues to be challenging as there are no specific symptoms or clinical signs and the organism cannot be cultivated outside the mammalian lung.

14.2 Geographical distribution

The disease has a global distribution. The incidence of PCP among HIV-infected individuals in developed countries has declined, but it remains the leading opportunistic infection in resource-limited environments.

14.3 The causal organisms and their habitat

Members of the fungal genus *Pneumocystis* are ubiquitous, extracellular organisms found in the lungs of mammals. Once thought to comprise a single genus and species, *Pneumocystis carinii*, it is now accepted that distinct species of *Pneumocystis* infect different mammalian hosts. In the late 1980s, phylogenetic analysis based on the nuclear small-subunit ribosomal DNA sequence alignments showed that *Pneumocystis* was a

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unicellular yeast-like fungus rather than a protozoan. More recently, other gene sequence comparisons and cDNA and genomic sequence data emerging from the *Pneumocystis* Genome Project have led to the organism(s) being classified in the phylum Ascomycota.

Phylogenetic determinations have not only permitted the fungal nature of the genus to be clarified, but have provided the basis of species distinctions within the genus. Although it is anticipated that there are hundreds, if not thousands, of *Pneumocystis* species, only five have been formally described according to the International Botanical Code of Nomenclature: *P. carinii* and *Pneumocystis wakefieldiae* infect rats, *Pneumocystis murina* infects mice, *P. oryctologi* infects rabbits and *P. jirovecii* infects humans. The proposal for these changes in nomenclature was controversial, but the name *P. jirovecii* now seems well established.

All species of *Pneumocystis* appear to contain similar life-cycle stages, although the clusters of organisms recovered from the lungs of different species of mammal can differ in their size and presentation. The life cycle of *Pneumocystis* is divided into three distinct morphological stages: the cyst, a thick-walled, spherical or crescent-shaped form (5–8 μm in diameter); the sporozoite (2–5 μm in diameter), eight of which develop within each cyst; and the trophozoite, (2–5 μm in diameter), which often exists in clusters and is thought to be derived from excysted sporozoites. The terms cyst, sporozoite and trophozoite are based on the earlier classification of the organism as a protozoan parasite and are now being replaced by the mycological terms, ascus, ascospores (or intracystic bodies) and trophic forms, respectively. All three morphological forms of *Pneumocystis* and a number of intermediate stages reside within the alveolar spaces of the lungs and are often found in large, multi-layered, adherent clusters, making identification of each stage difficult.

Microscopic observations and molecular genetic studies suggest that *Pneumocystis* species have a biphasic life cycle consisting of an asexual phase characterized by binary fission, not budding, of the trophic forms and a sexual phase resulting in formation of an ascus (cyst) containing eight ascospores. The actual infectious propagule by which the organism is transmitted has not been identified, although there is some recent evidence that the cyst is the transmissive form.

14.4 Epidemiology

Pneumocystis infection is acquired by inhalation, but it remains unclear whether the source of infection is environmental or person-to-person spread. No environmental form or cycle of *Pneumocystis* has been found,

but animal models and nosocomial case clusters among immunosuppressed patients suggest that airborne transmission from one host to another is important. Epidemiological studies showing the clustering of *P. jirovecii*-specific genotypes with patients' place of residence are consistent with the hypothesis of airborne person-to-person transmission of the organism.

It has long been assumed that clinical disease results from reactivation of latent infection acquired during childhood, but there is growing evidence to suggest that it may be due to primary infection or reinfection. Numerous serological studies have demonstrated that a high proportion of the human population has evidence of asymptomatic *Pneumocystis* infection and that seroconversion typically occurs during childhood. In one cohort of normal, healthy infants, seroconversion began in the first few months of life, and by 20 months of age, 85% of the children had become sub-clinically infected with *P. jirovecii*.

If reactivation of latent infection is the predominant mechanism of infection, there is little rationale for instituting measures, such as isolation of hospitalized patients with PCP, to avoid possible exposure during adulthood. In recent years, however, evidence has emerged to cast doubt on reactivation as the sole mechanism of infection. For instance, numerous reports describe the presence of different genotypes in the same person during recurrent episodes of PCP. Moreover, individuals with newly diagnosed HIV infection have been found to have mutant strains associated with prior use of PCP prophylaxis, despite never having received such treatment. The most likely explanation of these results is acquisition of the organism from an individual with prior drug exposure.

Pneumocystis is usually well contained by an intact immune system, and PCP only occurs in individuals who are significantly immunosuppressed. Patients with PCP typically have abnormalities of T-lymphocyte function, although B-cell defects can also predispose to infection. The clinical course and outcome is dependent upon the degree of immunosuppression.

Pneumocystis infection was first described in the late 1940s among malnourished infants and children housed in sub-optimal conditions who were suspected to be immunosuppressed. In the 1970s, the organism began to be identified in patients with cancer and persons with congenital or drug-induced immunosuppression. However, it did not come to prominence until the early 1980s when it was recognized as the cause of lethal pneumonia in patients with AIDS. Prior to the widespread use of primary prophylaxis and highly active antiretroviral therapy (HAART), PCP was one of the most frequent AIDS-defining opportunistic infection in the United States, occurring in 70–80% of adults with AIDS. Despite

treatment, mortality rates as high as 55% for patients admitted to intensive care units (ICUs), and 80% for those needing mechanical ventilator support, were reported. About 90% of cases occurred among individuals with CD4 T-lymphocyte counts of less than 200 cells per μL. Other risk factors, independent of CD4 T-cell count, include previous episodes of AIDS-related illness, previous episodes of bacterial pneumonia, oropharyngeal candidosis, unintentional weight loss and a high viral load.

The prevalence of PCP has decreased substantially with the widespread use of prophylaxis and antiretroviral therapy. Among patients with AIDS in North America and western Europe, the incidence is now around two to three cases per 100 person-years. However, despite these advances, PCP remains a leading opportunistic pathogen among HIV-infected persons who are either not using or not responding to HAART, among those who have not yet been diagnosed as HIV-infected and among those not taking or breaking through on PCP prophylaxis. The case fatality rate associated with PCP has not changed significantly in the United States; currently, average rates of 10–20% are being reported, with rates as high as 40% among those who are not receiving ongoing HIV care and 80% among those who require aggressive interventions such as mechanical intervention.

Globally, PCP is still the most common opportunistic infection in adults and children with HIV/AIDS. The prevalence of PCP was once thought to be much lower in developing countries than in the United States or western Europe, but it is now apparent that the lower reported prevalence was due to a lack of access to diagnostic testing and death due to other causes (such as tuberculosis). The frequency of documented *Pneumocystis* infection is increasing in sub-Saharan Africa, with *Pneumocystis* organisms being found in up to 80% of HIV-positive infants dying from pneumonia.

The population of patients at risk for PCP has evolved and increased as a result of the development of new immunosuppressive therapies to treat malignancies, organ failure and autoimmune and inflammatory diseases. Although the cell-mediated immune response is thought to play the predominant role in host defence against *Pneumocystis* infection, individuals with humoral deficiencies can develop PCP: cases have been reported in individuals with agammaglobulinaemia or hypogammaglobulinaemia and in those with severe combined immunodeficiency. However, PCP is more commonly seen in solid organ transplant (SOT) recipients, patients with haematological or solid malignancies, persons with autoimmune inflammatory disorders such as Wegener granulomatosis and systemic lupus erythematosus, persons receiving long-term immunosuppression for connective-tissue disorders and individuals with severe malnutrition. Corticosteroids and cyclosporin, both of which inhibit

T-lymphocyte function, are among the agents most closely associated with *Pneumocystis* infection. Most recently, PCP has been reported in individuals receiving tumour necrosis factor (TNF)-alpha blocker therapy.

Prior to the widespread use of primary prophylaxis, PCP occurred in up to 80% of lung transplant recipients. With the current routine use of prophylaxis, PCP has become uncommon in SOT recipients. In patients with cancer and other immunosuppressive conditions, there has been little improvement in case fatality rates, and PCP carries a worse prognosis than among those with HIV infection. In these patient populations, case fatality rates of 30–50% have been documented in several large studies. It has been suggested that the higher case fatality rate is likely a result of delayed diagnosis and initiation of appropriate treatment.

The terms asymptomatic infection, subclinical infection and colonization are among those that have been used to describe the presence of *P. jirovecii* organisms or DNA in the absence of PCP. The effects of the presence of low numbers of *Pneumocystis* on the host remain to be determined, and the role of carriage in the underlying pathogenesis of asthma, chronic obstructive pulmonary disease and cystic fibrosis is currently being investigated.

14.5 Clinical manifestations

The symptoms of PCP are non-specific, but the diagnosis should be considered in any immunocompromised patient with a non-productive cough and dyspnoea. The presentation is more insidious in AIDS patients than in other immunocompromised individuals. While other groups tend to present with acute illness, several days after symptom onset, the most common manifestations among HIV-infected persons are the sub-acute onset of progressive dyspnoea, fever, non-productive cough and chest discomfort that worsens within days to weeks. In mild cases, pulmonary examination is usually normal at rest. With exertion, tachypnoea, tachycardia and diffuse dry rales may be observed. Fever is apparent in most cases and is the predominant symptom in some patients.

In other groups of immunocompromised adults and children, the onset of symptoms and hypoxia is usually more abrupt, with fever, tachypnoea, dyspnoea and cough, progressing to severe respiratory distress. Rales are usually not detected on pulmonary examination.

A third presentation of PCP has been seen in AIDS patients who develop immune reconstitution inflammatory syndrome (IRIS) shortly after starting HAART. These patients appear to respond well to treatment for PCP, but 3–6 weeks after starting therapy, they experience recurrence of pulmonary symptoms and chest radiograph abnormalities that coincide

with return of immune function. These new symptoms can be wrongly construed as signs of treatment failure. IRIS occurs more frequently in adults than children and has also been reported in stem cell transplant recipients who engraft while infected with *Pneumocystis*.

Chest radiographs typically demonstrate bilateral, diffuse, symmetrical interstitial infiltrates. However, they may be normal in patients with early disease, particularly in those with HIV infection. The earliest densities are perihilar, and progression proceeds peripherally, typically sparing the apices until last. The severity of abnormalities on the chest radiograph is considered prognostic. Less common chest radiograph presentations include asymmetrical infiltrates, lobar consolidation, cavities and nodules. In patients receiving aerosolized pentamidine for prophylaxis, there may be a predisposition for upper lobe localization. A spontaneous pneumothorax in a patient with HIV infection should be presumed to be due to PCP until proven otherwise. Cavitation, intrathoracic lymphadenopathy, and pleural effusion are uncommon in the absence of other pulmonary infections, and their presence may be indicative of concurrent infection with another pathogen. High-resolution computed tomographic (CT) scans are helpful when chest radiographs are equivocal. Chest CT scans often reveal patchy areas of ground-glass attenuation with a background of interlobar septal thickening.

Lactate dehydrogenase (LDH) levels are frequently elevated in patients with PCP. Although elevated LDH levels in immunocompromised individuals are not specific for PCP, very high levels should raise the suspicion of *Pneumocystis* infection. However, normal LDH levels should not exclude the diagnosis.

In addition to pneumonia, *Pneumocystis* infection may disseminate to the lymph nodes, spleen, bone marrow and liver. Less common sites of dissemination include the adrenal gland, gastrointestinal tract, genitourinary tract and meninges. Extrapulmonary disease has been reported in 1–3% of patients dying with PCP. It may be clinically silent or present as a non-specific debilitating illness. It occurs predominantly in patients with advanced HIV infection and in those not taking trimethoprim-sulphamethoxazole prophylaxis, as well as in those receiving aerosolized pentamidine.

14.6 Differential diagnosis

The differential diagnosis includes acute respiratory distress syndrome, cytomegalovirus infection, *Legionella* infection, lymphocytic interstitial pneumonia, *Mycoplasma* infection, *Mycobacterium avium* complex infection, pulmonary embolism, tuberculosis and viral pneumonia.

14.7 Essential investigations and their interpretation

Because the clinical and radiological presentation is non-specific and the organism cannot be isolated in culture, cytopathological or histopathological demonstration of *Pneumocystis* is required to establish a definitive diagnosis. Appropriate specimens for analysis include bronchoalveolar lavage (BAL) fluid, tracheal aspirate fluid, transbronchial lung biopsy, bronchial brushing, percutaneous transthoracic needle aspiration, pleural fluid and open lung biopsy. The latter is the most reliable clinical specimen, but BAL fluid is much easier to obtain. Induced sputum samples are helpful only if positive; the absence of *Pneumocystis* from an induced sputum sample does not exclude the diagnosis of PCP.

14.7.1 Microscopy

The microscopic demonstration of typical octonucleate cysts or trophozoites of *P. jirovecii* in tissues or body fluids is sufficient for diagnosis. In many cases, the organisms will be present as large clusters in which it may be difficult to distinguish the different life-cycle stages. Collapsed organisms are usually found intermingled with intact organisms. Unlike other pathogenic fungi, *P. jirovecii* does not bud, and this feature can be used to distinguish between this organism and others, such as small variants of *Blastomyces dermatitidis*, *Candida glabrata*, capsule-deficient *Cryptococcus* species and *Histoplasma capsulatum*.

Giemsa and Wright stains detect both the cyst and trophozoite forms, but do not stain the cyst wall. Methenamine silver, periodic acid-Schiff, Calcofluor white and toluidine blue stain the cyst wall. Trophozoites and cysts can also be detected by direct or indirect immunofluorescent staining of BAL fluid or induced sputum smears with fluorescein-conjugated monoclonal anti-*P. jirovecii* antibodies (DAKO Corp., Carpinteria, California, USA). Depending on the monoclonal selected, staining may target only the cyst form or all forms of the organism. In tissue sections stained with hematoxylin and eosin, PCP presents as foamy eosinophilic exudates within the lung alveoli; the organisms are unstained. In Papanicolaou-stained respiratory cytology specimens, the organisms blend into the mucous blue green background.

14.7.2 Serological tests

Serological tests for antibodies to *P. jirovecii* are useful for epidemiological studies, but not for the diagnosis of PCP. Most humans become seropositive early in their childhood and likely come into contact with the organism many times over their lifetimes. Recently, serological responses to certain fragments of the major surface glycoprotein of *P. jirovecii* have been

reported to be predictive for some clinical outcomes, such as recovery, and may be of use in monitoring the response to therapy.

14.7.3 β-D-glucan detection

(1-3)-β-D-glucan is a major cell wall polysaccharide component of many fungi, including *P. jirovecii*. The Fungitell assay (Associates of Cape Cod, Falmouth, Massachusetts, USA) is approved by the US Food and Drug Administration for the presumptive diagnosis of a number of invasive fungal diseases, including aspergillosis, candidosis and trichosporonosis. Levels of (1-3)-β-D-glucan have been reported to be elevated in patients with PCP, but the sensitivity and specificity of the assay for diagnosis of the infection is still undergoing evaluation.

14.7.4 Molecular diagnostics

Molecular diagnosis by PCR methods offers great promise as a rapid diagnostic method and is being introduced in some centres. Detection of *P. jirovecii* can be accomplished by targeting the large subunit of the mitochondrial ribosomal RNA gene or the major surface glycoprotein gene; both these genes are present in multiple copies per organism. Although more sensitive than conventional staining methods, the presence of *P. jirovecii* DNA has not been strictly correlated with underlying disease, and a positive result may only represent colonization. Standardized quantitative PCR assays are now commercially available in some countries (MycAssay *Pneumocystis* PCR, Myconostica Ltd., Cambridge, UK).

14.8 Management

Standard antifungal drugs targeting ergosterol and ergosterol biosynthesis are ineffective against *Pneumocystis* infection. Echinocandins inhibit synthesis of (1-3)-β-D-glucan, an essential cell wall component of many fungi, including the cyst form of *P. jirovecii*. However, clinical experience with these drugs is limited to anecdotal reports and they are not licenced for the treatment of PCP.

Sulphonamides, such as sulphamethoxazole and dapsone, are among the most important drugs for the treatment and prophylaxis of *Pneumocystis* infection. These drugs inhibit the enzyme dihydropteroate synthase (DHPS), an essential component of the folate synthesis pathway. In *P. jirovecii*, two point mutations in the *fas* gene, which encodes the DHPS enzyme, have been associated with prior exposure to sulphonamide drugs and concerns have been raised about the possible emergence of resistance. The prevalence of DHPS mutations has been reported to range from 5% to

80% in developed countries. In South Africa, more than 50% of adult patients were recently found to harbour *P. jirovecii* isolates with DHPS mutations, underscoring the importance of rational use of sulphonamides for treatment or prophylaxis of *Pneumocystis* infection.

The treatment of choice for *Pneumocystis* infection is a fixed-ratio (1:5) combination of the antifolate inhibitors, trimethoprim and sulphamethoxazole (cotrimoxazole). Trimethoprim, at a dosage of 15–20 mg/kg per day, is administered with sulphamethoxazole, at a dosage of 75–100 mg/kg per day. Adults may be treated at 6- or 8-hour intervals; children should be treated at 6-hour intervals. Treatment is usually administered intravenously, but it may be given orally if the infection is mild or moderate and no malabsorption or diarrhoea is present. Patients who develop PCP despite prophylaxis with trimethoprim-sulphamethoxazole can usually be treated effectively with standard doses of the combination. The duration of treatment is generally 3 weeks for patients with AIDS and 2 weeks for other individuals. The dose must be adjusted for abnormal renal function.

Careful monitoring during therapy is essential to evaluate the response to treatment and to detect toxicity as soon as possible. Adverse reactions are common in patients receiving trimethoprim-sulphamethoxazole, more so in adults than in children. Common side effects include rash (including Stevens-Johnson syndrome), fever, leucopenia, thrombocytopenia, azotemia, hepatitis and hyperkalaemia in patients with AIDS. These side effects are less frequent in other groups of patients. Supportive care for common adverse effects should be attempted before discontinuing trimethoprim-sulphamethoxazole. Rashes can often be managed with antihistamines, nausea can be controlled with antiemetics and fever can be managed with antipyretics.

Alternative treatment regimens for patients with moderate to severe disease who cannot tolerate or fail to respond to trimethoprim-sulphamethoxazole after 5–7 days include pentamidine isethionate (4 mg/kg per day) given intravenously as a single dose. Adverse reactions are frequent with pentamidine and include renal and hepatic dysfunction, hyperglycaemia or hypoglycaemia, cardiac dysrhythmia, pancreatitis and leucopenia. Aerosolized pentamidine should not be used for the treatment of PCP because of limited efficacy and more frequent relapse.

Atoquavone (750 mg/day) is an alternative treatment that has been used primarily in adults with mild to moderate disease. It is given twice daily with food. Atovaquone is less effective than trimethoprim-sulphamethoxazole, but has fewer side effects. These include headache, nausea, diarrhoea, rash, fever and transaminase elevations.

Other effective treatment regimens for mild to moderate disease include combinations of dapsone (100 mg orally once daily) and trimethoprim (15 mg/kg per day, orally in three divided doses), and primaquine (15–30 mg orally once daily) combined with clindamycin (600 mg intravenously at 8-hour intervals). One meta-analysis concluded that the combination of primaquine and clindamycin might be the most effective regimen for salvage treatment after failure with trimethoprim-sulphamethoxazole. The most common adverse effects of these treatment regimens include methemoglobinemia and haemolysis with dapsone or primaquine; rash and fever with dapsone; and anaemia, rash, fever, and diarrhoea with primaquine and clindamycin.

Administration of corticosteroids in addition to anti-*Pneumocystis* drugs increases the chances for survival in those with moderate to severe disease. Corticosteroids have been associated with significant benefit in terms of preventing deterioration in oxygenation during the first week of anti-*Pneumocystis* treatment. Patients should receive corticosteroids as early as possible and certainly within 72 hours after starting therapy. If steroids are started at a later time, their benefits are unclear, although most clinicians would use them in such circumstances for patients with severe disease. The recommended corticosteroid dose and regimen for adults and adolescents is oral prednisone 40 mg twice daily for 5 days, followed by 40 mg daily for 5 days and then 20 mg daily for 11 days. Children may receive oral prednisone at a dosage of 2 mg/kg for the first 7–10 days, followed by a tapering regimen for the next 10–14 days. Methylprednisolone (at 75% of the respective prednisone dose) can be used if parenteral administration is required.

The overall prognosis for patients whose degree of hypoxaemia requires ICU admission or mechanical ventilation remains poor, but up to 40% of patients requiring ventilatory support now survive. Because long-term survival is possible for patients in whom antiretroviral treatment is effective, patients with AIDS and severe PCP should be offered ICU admission or mechanical ventilation when appropriate. Owing to the potential for additive or synergistic toxicities when anti-*Pneumocystis* and antiretroviral treatment is administered together, some experts recommend postponing the latter until after the completion of anti-*Pneumocystis* therapy or until at least 2 weeks after starting anti-*Pneumocystis* treatment, despite some suggestion of benefit of early antiretroviral treatment in the management of PCP.

IRIS has been reported in HIV-infected patients with PCP. Most cases have occurred 3–6 weeks after starting HAART. However, there is currently insufficient information on which to base recommendations for the

optimal time to start antiretroviral treatment following a mild or severe case of *Pneumocystis* infection.

Clinical failure is defined as a lack of improvement, or worsening of respiratory function, documented by arterial blood gases after at least 4–8 days of anti-*Pneumocystis* treatment. Treatment failure attributable to drug toxicity occurs in up to one-third of patients. Failure attributable to lack of drug efficacy occurs in about 10% of those with mild to moderate disease. However, it is important to wait at least 4–8 days before changing therapy for lack of clinical improvement, and concurrent infections or non-infectious processes, such as congestive heart failure or pulmonary emboli, should first be excluded. In the absence of corticosteroid therapy, early and reversible deterioration within the first 3–5 days of therapy is typical, probably because of the inflammatory response caused by antibiotic-induced lysis of organisms in the lung.

To prevent recurrence of *Pneumocystis* infection, AIDS patients should receive maintenance treatment (secondary prophylaxis) with trimethoprim-sulphamethoxazole for life unless immune reconstitution occurs as a result of HAART. If patients are intolerant of trimethoprim-sulphamethoxazole, dapsone, dapsone combined with pyrimethamine, atovaquone or aerosolized pentamidine may be used. In adults and adolescents, guidelines now suggest that it is reasonable to discontinue maintenance treatment once the CD4 T-lymphocyte count has increased to greater than 200 cells per μL for longer than 3 months as a result of antiretroviral treatment. If the *Pneumocystis* infection developed while the CD4 count was greater than 200 cells per μL, it may be prudent to continue prophylaxis for life regardless of the CD4 count. However, data regarding the most appropriate approach in this setting are limited. Maintenance treatment should be restarted if the CD4 T-lymphocyte count falls below 200 cells per μL. If PCP recurs at a CD4 count of greater than 200 cells per μL, lifelong maintenance is recommended.

HIV-infected children who have a history of *Pneumocystis* infection should be administered lifelong maintenance treatment to prevent recurrence.

14.9 Prevention

PCP is effectively prevented by the use of antimicrobial prophylaxis. In adults and adolescents with HIV infection (including pregnant women and those on HAART), prophylaxis is indicated if individuals have a CD4 T-lymphocyte count of less than 200 cells per μL or a history of oropharyngeal candidosis. Persons who have a CD4 T-lymphocyte percentage of less than 14% or a history of an AIDS-defining illness, but do not

otherwise qualify should also be considered for prophylaxis. If it is not possible to monitor an individual's CD4 count at least every 3 months, initiation of prophylaxis at a CD4 count of greater than 200 but less than 250 cells per μL should also be considered.

Trimethoprim-sulphamethoxazole is the drug of choice for anti-*Pneumocystis* prophylaxis in both HIV-infected and -uninfected immunocompromised patients. One double-strength tablet per day is the preferred regimen. However, one single-strength tablet per day is also effective and may be better tolerated. One double-strength tablet three times per week is also effective. For patients who have an adverse reaction that is not life-threatening, prophylaxis with trimethoprim-sulphamethoxazole should be continued if clinically feasible; for those who have discontinued prophylaxis because of an adverse reaction, reinstitution of trimethoprim-sulphamethoxazole should be considered after the adverse event has resolved.

If trimethoprim-sulphamethoxazole cannot be tolerated, alternative prophylactic regimens include dapsone, dapsone plus pyrimethamine plus leucovorin, aerosolized pentamidine and atovaquone. Atovaquone appears to be as effective as aerosolized pentamidine or dapsone.

Current HIV treatment guidelines suggest that primary prophylaxis can be safely discontinued in adult and adolescent patients who have responded to HAART with a sustained increase in CD4 T-lymphocyte counts from less than 200 cells per μL to greater than 200 cells per μL for more than 3 months. Prophylaxis should be restarted if the CD4 count falls below 200 cells per μL. There are limited data regarding discontinuation of prophylaxis in other groups of immunocompromised patients. In these groups, prophylaxis should probably be continued as long as the individual remains immunosuppressed.

Children born to HIV-infected mothers should be administered prophylaxis with trimethoprim-sulphamethoxazole beginning at 4–6 weeks of age. Prophylaxis should be discontinued for children who are subsequently found not to be infected with HIV. HIV-infected children and children whose infection status remains unknown should continue to receive prophylaxis for the first year of life. The need for subsequent prophylaxis should be determined on the basis of age-specific CD4 T-lymphocyte count thresholds. The safety of discontinuing prophylaxis in HIV-infected children receiving HAART has not been studied.

Chemoprophylaxis should be administered to pregnant women as is done for other adults and adolescents. The recommended regimen is trimethoprim-sulphamethoxazole; dapsone is an alternative. Because of theoretical concerns regarding possible teratogenicity associated with drug exposures, prophylaxis may be withheld during the first trimester.

In such cases, aerosolized pentamidine may an alternative because of its lack of systemic absorption.

Although some experts recommend that persons with HIV infection who are at risk for *Pneumocystis* infection should not share a hospital room with a patient who has the disease, current HIV treatment guidelines do not support this recommendation as standard practice.

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CHAPTER 15

Blastomycosis

15.1 Definition

The term blastomycosis (North American blastomycosis) is used to refer to infections caused by the thermally dimorphic fungus *Blastomyces dermatitidis*. Following inhalation, this organism causes a wide spectrum of clinical manifestations ranging from asymptomatic pulmonary infection to acute or chronic disease of the lungs and other organs, in particular the skin, bones and genitourinary system. Blastomycosis most commonly affects immunocompetent persons.

15.2 Geographical distribution

Most cases of blastomycosis have been reported from the region around the Mississippi and Ohio Rivers, but the disease is endemic to many of the south central, south eastern, and mid-western states of the United States, and to the Canadian provinces that border the Great Lakes and the St. Lawrence River. Thus, there is some overlap with the endemic region for histoplasmosis (see Chapter 17). Sporadic cases have been reported from other continents, including southern Africa, India, and parts of Central and South America.

15.3 The causal organism and its habitat

B. dermatitidis is a thermally dimorphic fungus. It exists in nature as a mould. In human and animal tissue, it forms large, round budding yeast cells.

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The natural habitat of *B. dermatitidis* is the soil, although attempts to recover it have seldom proved successful. Environmental isolation of the fungus during disease outbreaks indicates that it grows best in warm, moist acidic soil in wooded areas enriched with organic material, such as animal droppings, rotting wood and decaying vegetation. Higher soil temperatures and recent rainfall facilitate growth of the fungus. Dogs are susceptible to *B. dermatitidis* and may be sentinels for high-risk localities.

15.4 Epidemiology

Inhalation of *B. dermatitidis* spores is the usual mode of infection in humans. The incubation period, which has been estimated from outbreaks in which exposure occurred over a limited time period, is 4–6 weeks. Occasional cases have followed traumatic cutaneous inoculation.

Outbreaks of blastomycosis are uncommon, but have provided us with important information on risk factors for development of the disease. Most reported point-source outbreaks have been associated with occupational and recreational activities, often along streams or rivers, and have resulted from exposures to moist soil enriched with organic matter. Apart from outbreaks, blastomycosis is more commonly seen in adults than in adolescents or children. Those at greatest risk for blastomycosis are middle-aged men with outdoor occupations, such as construction or farming, or recreational interests, such as hunting or fishing. As with outbreaks, sporadic cases often involve exposures that occur near to freshwater. In some reports, the prevalence of sporadic infection has been reported to be much higher among African Americans, although it is unclear whether this is due to genetic risk factors or increased exposure.

Although cases of blastomycosis have been reported in persons with the acquired immunodeficiency syndrome (AIDS), *B. dermatitidis* is uncommon as an opportunistic pathogen. However, it does tend to cause more aggressive disease in persons with underlying cell-mediated immunological defects, such as those with human immunodeficiency virus (HIV) infection, transplant recipients and those receiving steroids or other immunosuppressive treatments. Immunocompromised persons with blastomycosis have a higher case fatality rate (30–40%) than those who are not immunosuppressed (2–20%).

Blastomycosis is reportable in a few states in the United States where it is endemic, but information on the incidence of the disease is limited. In Wisconsin, as in several other endemic states, the average statewide annual incidence rate is about 1 case per 100,000 population. Within the endemic states, however, rates as high as 40–100 cases per 100,000

population have been reported from small hyperendemic areas, consistent with the hypothesis that specific environmental conditions are important for *B. dermatitidis*. In Ontario, Canada, the overall incidence rate between 1994 and 2003 was 0.3 cases per 100,000 population. Almost 60% of cases were diagnosed in the fall and winter months, suggesting that infection occurred in the summer and fall, when persons spent time outdoors and rainfall is high. Two-thirds of cases occurred in males, and 13% occurred in children and adolescents.

15.5 Clinical manifestations

Inhalation of *B. dermatitidis* spores leads to a wide spectrum of clinical manifestations, ranging from subclinical pulmonary infection that may not require treatment, to acute or chronic pulmonary infection, or to more widespread disseminated disease. The skin, bones and genitourinary system are the most frequent sites of extrapulmonary disease. Disseminated infection develops in an estimated 25–40% of cases of blastomycosis, particularly among immunosuppressed individuals, such as organ transplant recipients and persons with HIV infection. Most patients with bone or genitourinary system involvement also have active pulmonary or cutaneous infection at the time of presentation.

15.5.1 Pulmonary blastomycosis

At least 50% of individuals exposed to *B. dermatitidis* during point-source outbreaks remain asymptomatic. The rest develop acute symptomatic pulmonary disease, similar to that seen with histoplasmosis or coccidioidomycosis, about 4–6 weeks after exposure.

Acute pulmonary blastomycosis usually presents as a non-specific flu-like illness characterized by fever, chills, myalgia, arthralgia and pleuritic chest pain. Patients often present with a non-productive cough that becomes mucopurulent as the infection develops. The radiological findings are non-specific, but usually include alveolar infiltrates with lobar or segmental consolidation. Pleural effusion is uncommon. Hilar lymphadenopathy is uncommon and this is useful in distinguishing blastomycosis from acute pulmonary histoplasmosis (see Chapter 17). Spontaneous resolution of the infection has been documented, but appears to be uncommon. Some patients with acute pulmonary blastomycosis fail to recover and develop a chronic infection that may be pulmonary, extrapulmonary or both.

The symptoms of chronic pulmonary blastomycosis are similar to those of tuberculosis and include intermittent low-grade fever, mild, persistent

productive cough, chest pain, haemoptysis, malaise, night sweats and weight loss. The radiological findings are more dramatic than those seen in patients with acute infection and include alveolar infiltrates with or without cavitation, mass lesions and fibronodular infiltrates. Cavitation is less common than in tuberculosis, histoplasmosis or coccidioidomycosis. Although small pleural effusions may be present, large effusions are uncommon.

In immunocompromised individuals, particularly those with late-stage AIDS, pulmonary blastomycosis may present as acute respiratory distress syndrome (ARDS) with fever, miliary or diffuse infiltrates and progressive respiratory failure. The mortality rate from this complication is high (50–90%) and most deaths occur during the first few days of treatment.

15.5.2 Cutaneous blastomycosis

The skin is the most frequent site of disseminated blastomycosis, being involved in 40–80% of cases. The lesions tend to be painless and present either as raised, crusted verrucous lesions with an irregular shape and sharp borders, or as ulcers. Both types of skin lesions usually occur over abscesses located deeper in the subcutaneous tissue. Verrucous lesions, which are more common, begin as small maculopustular lesions that slowly spread to form large nodular or papulonodular lesions with heaped-up borders. These lesions appear on exposed sites, such as the face, neck and scalp, and can be mistaken for basal or squamous cell carcinoma. Besides the skin, ulcerative lesions can appear on the mucosa of the nose, mouth and throat. These lesions begin as a pustule that spreads to form a superficial ulcer or slightly raised lesion with a bed of red granulation tissue.

15.5.3 Osteoarticular blastomycosis

Osteomyelitis occurs in about 25–30% of patients with disseminated blastomycosis. The spine, pelvis, skull, ribs and long bones are the commonest sites of infection. The lesions often remain asymptomatic until the infection spreads into contiguous joints or into adjacent soft tissue causing abscess formation with draining sinuses. Infection of the spine can involve adjacent vertebral bodies with destruction of the intervening disc space. The lower thoracic and lumbar regions are most frequently affected.

The radiological findings are not specific and the well-defined osteolytic or osteoblastic lesions cannot be distinguished from those of other fungal or bacterial infections. Periosteal proliferation is unusual.

Arthritis is less common than osteomyelitis, occurring in up to 10% of patients with blastomycosis either as a result of haematogenous

dissemination from the lung or spread from a contiguous bone lesion. The commonest sites of infection are the knee, ankle, elbow and wrist. The symptoms include swelling, pain and limited movement in the affected joint.

15.5.4 Genitourinary blastomycosis

The prostate, epididymis or testis is involved in 10–30% of men with disseminated blastomycosis. The prostate may be tender and enlarged, resulting in dysuria and obstruction. Epididymitis presents as scrotal swelling and pain, with or without a draining sinus. Infection often spreads to the adjacent testis.

On occasion, venereal transmission of *B. dermatitidis* infection has resulted in self-limited genital ulceration or endometrial infection in the woman.

15.5.5 Other forms of disseminated blastomycosis

Haematogenous spread of infection to the central nervous system (CNS) is rare (5–10% of cases), except in immunocompromised individuals. Manifestations of CNS infection include meningitis and cranial, epidural or brain abscesses. Meningitis is indolent in onset and tends to occur late in the course of *B. dermatitidis* infection. It is often lethal and is indistinguishable from other forms of chronic meningitis such as tuberculosis or cryptococcosis. Most patients with CNS infection present with headache, confusion or focal neurological deficits. However, in immunocompromised individuals with disseminated blastomycosis, it is recommended that imaging studies of the brain be performed to assess the extent of CNS involvement.

Other organs, such as the adrenal glands, thyroid, liver, spleen, heart and gastrointestinal tract, are sometimes involved. Choroiditis and endophthalmitis have been reported.

Although blastomycosis has been reported in patients with impaired T-cell-mediated immunological function, it is much less common than histoplasmosis or coccidioidomycosis. However, immunocompromised individuals are predisposed to develop severe manifestations of the disease, which can relapse, and is often associated with a high mortality rate during the first few weeks after onset of symptoms. In persons with AIDS, disseminated blastomycosis is usually associated with CD4 T-lymphocyte counts of less than 200 cells per μL. Cutaneous lesions are less common in HIV-infected individuals than in non-immunocompromised persons. Up to 40% of AIDS patients with blastomycosis have CNS disease, which manifests as either meningitis or brain lesions. Others have presented with

a septic shock-like syndrome, similar to that seen with histoplasmosis (see Chapter 17).

15.6 Differential diagnosis

Misdiagnosis of blastomycosis is common, even in the endemic regions. The infection mimics many other more common diseases, both acute and chronic. Acute pulmonary blastomycosis may be mis-diagnosed as bacterial pneumonia or influenza, and chronic pulmonary infection is indistinguishable from tuberculosis, histoplasmosis and bronchogenic carcinoma. Tuberculosis of the skin, bones or genitourinary tract, coccidioidomycosis of the bones or meninges, mucosal histoplasmosis and mucocutaneous paracoccidioidomycosis are other infections that can be confused with blastomycosis. However, the endemic regions for blastomycosis have almost no overlap with those of coccidioidomycosis and paracoccidioidomycosis.

15.7 Essential investigations and their interpretation

15.7.1 Microscopy

Microscopic examination of wet preparations of pus, sputum, bronchial washings, urine or other clinical material can permit the diagnosis of blastomycosis if the characteristic large round to oval yeast cells with thick refractile walls and broad-based single buds are seen. The buds often attain the same size as the parent cells before becoming detached. Atypical, small *B. dermatitidis* yeast cells can, however, be confused with other pathogens, such as single or non-budding cells of *Paracoccidioides brasiliensis*, *Histoplasma capsulatum* and capsule-deficient cells of *Cryptococcus species*.

15.7.2 Culture

The definitive diagnosis of blastomycosis depends on isolation of the fungus in culture from sputum, pus or biopsy material. Identifiable mould colonies, which are white to brown in colour, can be obtained after incubation at 25–30°C for 1–3 weeks on glucose peptone (Sabouraud's dextrose) agar supplemented with cycloheximide (actidione), but cultures should be retained for 4 weeks before being discarded. Unequivocal identification of an isolate as *B. dermatitidis* generally requires conversion to the yeast form. Sub-culture at 37°C on a rich medium, such as brain heart infusion agar with blood, usually results in the production of the yeast form within 2–3 days. If more rapid identification is required, molecular testing of the mould culture with the AccuProbe test (GeneProbe Inc., San Diego,

California, USA) for identification of *B. dermatitidis* can be completed within a few hours. The AccuProbe test produces a positive result with *P. brasiliensis*, so care must be taken to distinguish these organisms. Slow growing, non-sporulating isolates are more characteristic of *P. brasiliensis* than *B. dermatitidis*.

15.7.3 Serological tests

The most useful serological test is immunodiffusion (ID), but complement fixation (CF) and ELISA procedures have also been developed and evaluated. Substantial improvements in the performance of these tests have been achieved by the use of a purified surface antigen of *B. dermatitidis*, termed the A or WI-1 antigen. A positive reaction in an ID test with the A antigen of *B. dermatitidis* is specific and diagnostic for blastomycosis. However, negative ID reactions have been obtained in 10% of patients with disseminated disease and over 60% with localized infection. CF tests have been used, but lack specificity because of cross-reactions with antigens of other fungi, particularly *H. capsulatum* and *Coccidioides* species.

15.7.4 Antigen detection tests

An enzyme immunoassay for the detection of *B. dermatitidis* antigen is available from MiraVista Diagnostics (Indianapolis, Indiana, USA). Sensitivity is greatest with urine, but antigen has also been detected in serum, cerebrospinal fluid (CSF) and bronchoalveolar lavage fluid specimens. Antigen levels decline with treatment and increase with treatment failure or relapse. However, the test has been reported to give false-positive reactions with 96% of urine samples from patients with histoplasmosis, the disease from which blastomycosis is in most need of being differentiated, and 100% of urine samples from patients with paracoccidioidomycosis.

15.8 Management

All patients with chronic pulmonary or disseminated blastomycosis and all immunocompromised patients with the disease require antifungal treatment. The site(s) of infection and the severity of illness influence the choice of antifungal agent and the duration of treatment. Of note, there have been no randomized clinical trials comparing the currently available agents for the treatment of blastomycosis.

15.8.1 Pulmonary blastomycosis

Although spontaneous resolution has been reported in some immunocompetent individuals with acute pulmonary infection, most experts now

recommend that patients who have resolution of clinical and radiological signs before the diagnosis is established should be treated with itraconazole to prevent development of disseminated disease. If a decision is made to withhold treatment, patients must be followed up for long periods to detect signs of reactivation or progression of the disease.

Patients with mild to moderate pulmonary disease should be treated with oral itraconazole at a dosage of 200 mg three times daily for 3 days and then 200 mg once or twice per day for a minimum of 6–12 months. In general, treatment should be continued for several months after the resolution of symptoms and radiological signs. Patients with moderately severe to severe disease should be treated with a lipid formulation of amphotericin B at a dosage of 3–5 mg/kg per day, or conventional amphotericin B at a dosage of 0.7–1.0 mg/kg per day for 1–2 weeks, or until improvement is noted. This should be followed by oral itraconazole at a dosage of 200 mg three times daily for 3 days and then 200 mg twice per day for a total of 6–12 months. Serum levels of itraconazole should be measured after the patient has received the drug for at least 2 weeks, to ensure adequate concentrations (see Chapter 3).

If patients are unable to tolerate itraconazole, or fail to absorb the drug, or are unable to take this agent because of drug interactions, there are several alternative treatment regimens. Oral ketoconazole (400–800 mg/day) is almost as effective, but less well tolerated than itraconazole. Fluconazole (400–800 mg/day) is a less effective option than itraconazole or ketoconazole. Posaconazole and voriconazole are not licenced for the treatment of blastomycosis, but they may be effective agents.

No studies have been conducted to determine the most appropriate treatment for overwhelming pulmonary blastomycosis with ARDS. Most of these patients have been treated with the conventional formulation of amphotericin B at a dosage of 0.7–1.0 mg/kg per day, but there may be a role for higher dosages that can be given with the lipid-based formulations. Several recent case reports have suggested that patients with blastomycosis-related ARDS might benefit from treatment with corticosteroids.

15.8.2 Disseminated blastomycosis

All patients with disseminated disease require treatment. The presence or absence of CNS infection is a critical factor in determining which antifungal agent should be used.

Patients with CNS infection should be treated with a lipid-based formulation of amphotericin B at a dosage of 5 mg/kg per day for 4–6 weeks. This should be followed by treatment with an oral azole. Options include

fluconazole (800 mg/day), itraconazole (200 mg twice or three times daily), or voriconazole (200–400 mg twice daily) for at least 12 months and until resolution of CSF abnormalities. Voriconazole has excellent penetration into the CSF and brain tissue, but clinical experience with this agent in patients with blastomycosis is limited.

Patients with mild to moderate disseminated disease that does not involve the CNS should be treated with oral itraconazole at a dosage of 200 mg three times daily for 3 days and then 200 mg once or twice per day for 6–12 months. Patients with moderately severe to severe disseminated blastomycosis should be treated with a lipid formulation of amphotericin B at a dosage of 3–5 mg/kg per day or conventional amphotericin B at a dosage of 0.7–1.0 mg/kg per day for 1–2 weeks or until improvement is noted. This should be followed by oral itraconazole at a dosage of 200 mg three times daily for 3 days and then 200 mg twice per day for a total of at least 12 months. Serum levels of itraconazole should be measured after the patient has received the drug for at least 2 weeks, to ensure adequate concentrations. In general, treatment should be continued for several months after the resolution of symptoms and skin or other focal lesions.

Ketoconazole is less effective and more toxic than itraconazole and is now seldom used. Fluconazole (400–800 mg/day) is less effective than itraconazole, but can be used in patients who do not tolerate or cannot absorb that drug. The role of posaconazole and voriconazole is not clear, but these may also be effective agents. For patients whose disease progresses during azole treatment or who are unable to tolerate an azole, treatment with an amphotericin B formulation is recommended.

Antifungal treatment alone is effective in most cases of blastomycosis. In some cases, however, drainage of large abscesses may be needed for optimal management. Surgical drainage of brain or epidural abscesses may also be important when these lesions cause neurological deficits. Debridement of bone lesions is needed only when osteomyelitis fails to respond to medical management. Those with osteoarticular disease, which is more difficult to treat and more likely to relapse, should be treated with an azole for at least 12 months.

15.8.3 Immunocompromised patients with blastomycosis

Immunocompromised patients are more likely than immunocompetent persons to develop severe pulmonary infection and disseminated blastomycosis and to die of the infection. Patients who are immunocompromised should be treated with a lipid-based formulation of amphotericin B at a dosage of 3–5 mg/kg per day or conventional amphotericin B at a dosage of 0.7–1.0 mg/kg per day for 1–2 weeks or until improvement

is noted. Itraconazole, at a dosage of 200 mg three times daily for 3 days and then 200 mg twice per day, is recommended once the patient has responded to amphotericin B and should be given to complete a total of at least 12 months of treatment. Serum levels of itraconazole should be measured after the patient has received the drug for at least 2 weeks to ensure adequate concentrations.

Lifelong suppressive treatment with itraconazole (200 mg/day) may be required in immunocompromised individuals if the underlying immunosuppression cannot be reversed. Fluconazole is not as effective as itraconazole and should not be used. Experience with posaconazole and voriconazole is limited, and neither is recommended for immunocompromised patients at this time. Experience with AIDS-associated histoplasmosis has shown that it is reasonable to discontinue itraconazole maintenance once patients have completed at least 12 months of itraconazole treatment and have attained CD4 T-lymphocyte counts of more than 150 cells per μL for at least 6 months while receiving highly active antiretroviral therapy (HAART). Guidelines suggest that it is reasonable to adopt a similar approach to persons with AIDS and blastomycosis.

15.9 Prevention

It is unusual for sites that are contaminated with *B. dermatitidis* to be identified. For this reason, such sites can neither be avoided nor decontaminated. Avoidance of outdoor activities in endemic regions might decrease the risk of infection, but it is neither practical nor justified.

The prevalence of blastomycosis in immunocompromised patients is too low to justify routine antifungal prophylaxis. The endemic regions of blastomycosis overlap those of histoplasmosis. Therefore, the recommendation that itraconazole prophylaxis is considered in HIV-infected persons who live, or have lived, in areas endemic for histoplasmosis if their CD4 T-lymphocyte counts are less than 150 cells per μL should also provide protection against blastomycosis. If used, prophylaxis can be discontinued once CD4 counts exceed 150 cells per μL for 6 months in patients on HAART.

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CHAPTER 16

Coccidioidomycosis

16.1 Definition

The term coccidioidomycosis is used to refer to infections caused by two thermally dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*. Following inhalation, these organisms cause a wide spectrum of clinical manifestations, ranging from asymptomatic pulmonary infection, to acute or chronic disease of the lungs, or to more widespread disseminated disease. Although many cases are self-limited and inconsequential, progression or dissemination of the infection can result in severe effects and sometimes death, even in immunocompetent persons.

16.2 Geographical distribution

Most cases of coccidioidomycosis occur in the southwestern United States, but the disease is also endemic in parts of Central and South America. In the United States, the disease is most prevalent in south central Arizona, but the endemic region includes much of central and southern California, southern New Mexico and western Texas, as well as parts of Nevada and Utah. The endemic region extends southwards into northern and southwestern Mexico, Guatemala, Honduras, Nicaragua, Colombia, Venezuela, Brazil, Paraguay and Argentina. Outbreaks and sporadic cases of the disease have occurred among visitors to the endemic regions.

16.3 The causal organisms and their habitat

Two species of *Coccidioides* are now recognized: *C. immitis* and *C. posadasii*. The former taxon consists of strains found in California and the latter

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consists of strains from other parts of North, Central and South America. Both species are found in Arizona. Although the phylogenetic evidence is convincing, the two species are indistinguishable on clinical and biological grounds.

C. immitis and *C. posadasii* are thermally dimorphic soil fungi. They exist in nature as moulds that fragment into small, thick-walled spores, termed arthroconidia, that can better survive adverse environmental conditions. Within the lungs, the arthroconidia enlarge to form characteristic large, round, thick-walled cells, termed spherules, that undergo internal division to produce hundreds of endospores. These are released when the mature spherules rupture, spread through the host and then mature to form more spherules. Hyphae are seldom produced in tissue, but they are sometimes found in chronic lung lesions.

The natural habitat of *Coccidioides* is the soil of certain hot desert regions of North, Central and South America that correspond to the lower Sonoran life zone. An arid climate, alkaline soil, hot summers, mild winters and annual rainfall of 5–20 inches are characteristic of these regions.

16.4 Epidemiology

Infection is thought to occur when *Coccidioides* arthroconidia from disrupted soil are inhaled. The incubation period, which has been estimated from point source outbreaks in which exposure occurred over a limited time period, ranges from 1 to 3 weeks. In contrast to histoplasmosis, once individuals have recovered from *Coccidioides* infection, they are usually immune to reinfection. However, later reactivation of quiescent lesions can occur in persons who become immunocompromised. Coccidioidomycosis is not contagious but person-to-person spread has occurred by transmission from an organ donor to a recipient.

The major risk factor for infection is environmental exposure. The risk of infection depends on a number of factors, including the nature of the environmental site, the activities performed, and the duration and degree of dust or soil exposure. Longer and more intense exposures may lead to a shorter incubation time and usually result in more severe pulmonary disease. Human infection has been associated with ground-disturbing activities, such as building construction, landscaping, farming, archaeological excavation and numerous recreational pursuits. Natural events that result in the generation of dust clouds, such as earthquakes and windstorms, have been associated with an increased risk of infection and have resulted in large outbreaks.

Certain persons are at increased risk for some forms of coccidioidomycosis. In non-outbreak settings, the risk of developing severe pulmonary disease is greater in older age groups, among smokers, and in those with diabetes mellitus. Those at greatest risk for disseminated disease include members of several racial or ethnic groups in the United States, in particular those of black, Asian or Filipino race, as well as pregnant women in the third trimester. Individuals with underlying cell-mediated immunological defects, including those with the acquired immunodeficiency syndrome (AIDS), solid organ and stem cell transplant recipients, persons with haematological malignancies and those receiving high-dose corticosteroids or other immunosuppressive treatments, such as tumour necrosis factor (TNF)-alpha blockers, are at higher risk of developing aggressive pulmonary disease, as well as disseminated forms of coccidioidomycosis.

In the 1980s, coccidioidomycosis was recognized as an important opportunistic infection among human immunodeficiency virus (HIV)-infected persons living in the endemic regions. The principal risk factor for developing active coccidioidomycosis in these individuals was a CD4 T-lymphocyte count of less than 250 cells per μL. The incidence of AIDS-associated coccidioidomycosis in the endemic regions of the United States has declined since the introduction of more effective combination antiretroviral treatments, including highly active antiretroviral therapy (HAART), and improvements in HIV healthcare in general. However, the disease remains a significant opportunistic infection in resource-poor settings in Central and South America.

Coccidioidomycosis is relatively uncommon in transplant recipients, even in the endemic regions. Most reported cases have occurred among solid organ transplant (SOT) recipients, usually within the first year after transplantation, and there have been few cases among stem cell transplant recipients. Among transplant recipients, coccidioidomycosis may occur as a primary infection, as a reactivation of a latent focus of infection or as a donor-transmitted infection.

It has been estimated that upwards of 150,000 new human infections with *Coccidioides* species occur each year in the endemic areas of the southwestern United States, but whether this figure is correct is difficult to determine. The disease has been recognized as an important public health problem in several states within the endemic region. However, most cases of the disease are not reported, resulting in an underestimated number of cases. In California, the annual incidence coccidioidomycosis more than tripled between 2000 and 2006, increasing from 2.4 to 8 cases per 100,000 population. Within the state, rates as high as 150 cases per 100,000 population were reported from counties where the disease is endemic.

In Arizona, the state with the highest rate of coccidioidomycosis, the annual incidence peaked at 89 cases per 100,000 population in 2006 and then decreased to 73 cases per 100,000 population in 2008. Massive population growth in southern Arizona and central California has led to increasing numbers of previously unexposed individuals being exposed to *Coccidioides* species in the environment. Consistent with earlier reports, the latest data indicate that recent relocation to Arizona from a non-disease-endemic area is a risk factor for developing the disease.

16.5 Clinical manifestations

Inhalation of *Coccidioides* arthroconidia leads to a wide spectrum of clinical manifestations, ranging from an asymptomatic or a self-limited pulmonary illness that resolves without treatment, to chronic pulmonary infection, or to more widespread disseminated disease. *C. immitis* and *C. posadasii* cause a similar spectrum of human disease.

16.5.1 Acute pulmonary coccidioidomycosis

Among immunocompetent persons, about 40% of those newly infected with *Coccidioides* develop an acute or sub-acute pneumonia that becomes evident about 1–3 weeks after exposure. Higher rates of symptomatic respiratory infection (50–100%) have sometimes been reported during outbreaks, particularly after high-level exposure.

Acute pulmonary coccidioidomycosis usually presents as a non-specific, flu-like illness, characterized by fever, headache, pleuritic chest pain, non-productive cough, dyspnoea, myalgia, arthralgia, weight loss and malaise. Up to 50% of patients develop a transient, mild, diffuse erythematous or maculopapular rash, covering the trunk and limbs, within the first few days of the onset of symptoms. More dramatic and persistent is the rash of erythema nodosum or erythema multiforme that occur in about 5% of infected persons, and they are more common in women. These signs occur up to 3 weeks after symptoms first appear and resolve over several weeks. Both are often accompanied by non-infectious arthralgia in one or more joints. Pulmonary infiltrates can take multiple forms, are commonly associated with hilar lymphadenopathy and may also occur with pleural effusions. Many otherwise healthy persons will recover without seeking medical attention, their symptoms disappearing within 6–8 weeks.

HIV-infected persons often have a fulminant presentation, particularly when the CD4 T-lymphocyte count is less than 100 cells per μL. The symptoms are non-specific and include fever, chills, cough and weight loss.

Chest radiographs will reveal bilateral, reticulonodular or miliary infiltrates. These are seldom seen in normal persons unless there has been high-level exposure to *Coccidioides* arthroconidia. This diffuse pneumonia may result in respiratory failure. Focal pneumonia is the most common presentation of pulmonary coccidioidomycosis in HIV-infected individuals with CD4 counts greater than 250 cells per μL.

16.5.2 Chronic pulmonary coccidioidomycosis

About 5–10% of infected individuals develop chronic pulmonary lesions, which may include nodules, thin-walled cavities, abscesses or infiltrates. In 5% of cases, nodules are formed that may persist, resolve slowly, or undergo necrosis to form cavities. More commonly, peripheral thin-walled cavities develop. These may slowly resolve or may rupture into the pleural space, causing a bronchopleural fistula and empyema. Haemoptysis or rarely severe pulmonary haemorrhage may also occur. Dissemination is unusual in patients with cavitary disease.

16.5.3 Disseminated coccidioidomycosis

About 1–5% of infected individuals develop disseminated coccidioidomycosis. This is a progressive disease that usually develops within 3–12 months of the primary infection, although it can occur much later following reactivation of a quiescent infection in an immunosuppressed individual. In most cases, disseminated disease develops in individuals who have had a symptomatic pulmonary infection. Early recognition is essential for prompt initiation of treatment; delays in diagnosis are associated with increased morbidity and mortality.

The clinical manifestations of disseminated coccidioidomycosis range from a fulminant illness that is fatal within a few weeks if left untreated to an indolent chronic disease that persists for months or years. One or more sites may be involved, but the skin, soft tissue, bones, joints, and meninges are most commonly affected. Many patients with disseminated disease are symptomatic at presentation: fever, cough, night sweats and chills are the most common complaints. Patients presenting with any form of disseminated coccidioidomycosis should be evaluated with a chest radiograph, a skin examination and a bone scan. Many have normal chest radiographs; dissemination occurs week to months after the primary infection, by which time the initial pulmonary disease has often resolved. Computed tomographic (CT) scans or magnetic resonance imaging (MRI) scans of the head and lumbar puncture should be performed if there is headache or other signs of central nervous system involvement.

Cutaneous and subcutaneous lesions are among the most common manifestations of disseminated coccidioidomycosis. Cutaneous lesions may be single or multiple and can persist for long periods. Their appearance is varied: verrucous papules, erythematous plaques and nodules have been described. The nasolabial fold and the sternoclavicular area are often affected, but lesions may occur at many other sites. Underlying bone or joint lesions may be found.

Bone and/or joint disease occurs in about 20–40% of patients with disseminated coccidioidomycosis. The spine, ribs, cranial bones and ends of the long bones are the most common sites of infection. Patients often remain asymptomatic, but persistent dull pain may occur. Irregular lytic or sclerotic lesions are seen on radiographs. CT or MRI scans are useful in establishing the extent of disease and should be performed in all cases. The infection may spread into contiguous joints causing synovitis with effusion, or into adjacent soft tissue, causing subcutaneous abscess formation. Draining sinuses often appear. The ankles and knees are the most common sites of infection, but any joint may be affected.

Meningitis is the most serious manifestation of coccidioidomycosis. It occurs in 30–50% of patients with disseminated disease, and has a case fatality rate of greater than 90% at 12 months if left untreated. The symptoms are non-specific and insidious in onset. Persistent headache is the commonest and often the earliest symptom. Mental changes, such as drowsiness and confusion, also occur. Other symptoms include vomiting, loss of appetite and weight loss. Fever is minimal or absent in indolent cases. Hydrocephalus is a common and serious complication of coccidioidal meningitis. It may be rapid in onset and should be considered in any patients who experiences worsening or recurrent headache or sudden neurological deterioration. CT and MRI brain scans are abnormal in most patients, the commonest findings being basilar meningitis, cerebral infarction and hydrocephalus.

Most patients with symptomatic meningitis have abnormal cerebrospinal fluid (CSF) findings, including a high opening pressure, an elevated protein concentration, a lowered glucose concentration and a lymphocytic pleocytosis.

In persons with AIDS, coccidioidomycosis may follow a more aggressive course, depending on the CD4 T-lymphocyte cell count. In those with a CD4 count of less than 100 cells per μL, the disease tends to be severe, overwhelming and often lethal. The symptoms and signs are similar to those seen in HIV-negative individuals, except that a diffuse pneumonia is often present. Other manifestations include skin, bone and joint disease, meningitis, and liver, spleen or lymph node involvement.

16.6 Differential diagnosis

The clinical presentation of acute pulmonary coccidioidomycosis is similar to that of bacterial pneumonia or influenza, as well as other endemic fungal diseases, such as blastomycosis and histoplasmosis. The diffuse lung infiltrates that are found in some AIDS patients with coccidioidomycosis may be mis-diagnosed as *Pneumocystis jirovecii* infection. The radiological presentation in patients with residual lung cavities due to *Coccidioides* infection is similar to that of a number of other infectious and non-infectious conditions, including cryptococcosis, tuberculosis, and bacterial lung abscess. In most of these other conditions, the cavities have a thicker wall or more extensive surrounding infiltrate.

The differential diagnosis of cutaneous coccidioidomycosis includes histoplasmosis, blastomycosis, cryptococcosis, tuberculosis, sarcoidosis, squamous cell or basal cell carcinoma, verruca vulgaris and acne rosacea or vulgaris. The large, cold abscesses that develop in soft tissue adjacent to *Coccidioides* bone lesions can be mistaken for tuberculosis. The symptoms and clinical signs of chronic meningeal coccidioidomycosis are similar to those of cryptococcosis.

16.7 Essential investigations and their interpretation

With increased travel and tourism to the endemic regions, inadvertent exposure of laboratory staff to *Coccidioides* is not an uncommon cause of infection. It is essential to inform the laboratory if a diagnosis of coccidioidomycosis is suspected, to ensure that proper precautions in handling of specimens and cultures are observed.

16.7.1 Microscopy

Microscopic examination of stained tissue sections can permit the diagnosis of coccidioidomycosis if the characteristic large, thick-walled endospore-containing spherules are seen. Direct microscopic examination of wet preparations of sputum, bronchoalveolar lavage (BAL) fluid, pus or other samples is also helpful, but less sensitive. Mature spherules may be confused with *Rhinosporidium seeberi*, while immature spherules and liberated endospores may be confused with non-budding cells of *Blastomyces dermatitidis* or *Cryptococcus* species.

16.7.2 Culture

Coccidioides species are fast growing and identifiable mould colonies, which are white to gray or buff in colour, can usually be obtained after

incubation at 25–30°C for 2–7 days on glucose peptone (Sabouraud's dextrose) agar supplemented with cycloheximide (actidione). Large numbers of mature arthroconidia are usually present by 7–10 days of culture. In culture, *Coccidioides* species must be distinguished from other moulds that produce arthroconidia, such as *Malbranchea* and *Chrysosporium* species, as well as some dermatophytes. For confirmation, molecular testing of cultures with the AccuProbe test (Gen-Probe Inc., San Diego, California, USA) can be completed within a few hours. This test is sensitive and specific, although pre-treatment of isolates with formaldehyde leads to false-negative results. The AccuProbe test does not distinguish between the two *Coccidioides* species, and it is also useful for identifying atypical isolates that fail to form arthroconidia.

Cultures must be set up in secure containers (slopes rather than plates) and handled with great care because of the danger of infection from the large concentrations of easily dispersed and highly infectious arthroconidia. All procedures involving the manipulation of sporulating cultures of *Coccidioides* should be performed inside a biological cabinet under conditions of biosafety level (BSL) 3 containment. In some countries, clinical specimens suspected of containing the organisms must also be handled under BSL 3 containment. Recommendations for responding to inadvertent exposure of laboratory staff to *Coccidioides* species have been published.

In addition to lower respiratory tract samples, *Coccidioides* can be isolated from suppurative cutaneous and soft tissue lesions. Blood cultures are usually positive in patients with diffuse pneumonia. Organisms can be recovered only infrequently from CSF, and usually only after culture of large volumes (10–20 mL).

16.7.3 Skin tests

A positive coccidioidin (or spherulin) skin test result does not distinguish present from past infection. However, conversion from a negative to a positive result is a sign of recent infection, because it occurs within 4 weeks of the onset of symptoms in 90–95% of cases. False-negative results are common in anergic patients with disseminated coccidioidomycosis. Unlike the histoplasmin skin test, coccidioidin does not interfere with the results of subsequent serological tests. The coccidioidin skin testing reagent is not currently available in the United States.

16.7.4 Serological tests

Serological tests for antibodies are of proven usefulness in the diagnosis and management of coccidioidomycosis. The immunodiffusion (ID)

and complement fixation (CF) tests remain the most reliable methods. The principal antigen used in these tests is a mycelial-phase culture filtrate (termed coccidioidin).

The simultaneous use of heat-treated and unheated coccidioidin antigens permits the ID test to detect either IgM or IgG antibodies on a single plate. With heated coccidioidin as antigen, the test detects IgM antibodies against *Coccidioides* and is most useful for diagnosing recent infections. These antibodies can be found in 75% of patients within 1 week after the onset of symptoms, and 90% are positive within 3 weeks. IgM antibodies disappear within a few months in persons with acute pulmonary disease. With unheated coccidioidin as antigen, the ID test detects IgG antibodies and gives results comparable to the CF test (explained later). IgG antibodies can usually be detected within 2–6 weeks after onset of symptoms. The sensitivity of the ID test can be improved by first concentrating the serum. The ID test is useful for initial screening of specimens and can be followed by other tests if positive.

The CF test is a sensitive quantitative method in which unheated coccidioidin is used to measures IgG antibodies against *Coccidioides*. These antibodies do not appear until 4–12 weeks after infection, but may persist for long periods in individuals with chronic pulmonary or disseminated disease. Testing of serial samples to detect rising or falling titres can reveal the progression or regression of illness and the response to antifungal treatment. The failure of the CF titre to fall during treatment of disseminated disease is an ominous sign. Titres of 1:2 or 1:4 are usually indicative or early, residual or meningeal infection, but may sometimes be found in individuals without coccidioidomycosis. Titres of CF antibodies of greater than 1:16 are consistent with spread of *Coccidioides* infection beyond the respiratory tract. More than 60% of patients with disseminated coccidioidomycosis have CF titres of 1:32 or greater. However, false-negative results can occur in immunocompromised individuals, and CF titres alone should not be used as the basis for diagnosis of dissemination. Patients with clinical presentations consistent with coccidioidomycosis, but with negative or low CF titres, should be retested at 3–4 week intervals.

The CF test more sensitive but less specific than the ID test. In cases where the CF test is positive, but the ID test is negative, patients should be investigated for microbiological or histopathological evidence of histoplasmosis or blastomycosis. In addition to serum, the CF test can be performed with CSF, pleural or joint fluid samples. The detection of IgG antibodies in CSF is usually diagnostic for *Coccidioides* meningitis and remains the most useful test for diagnosis of that infection. As with serum, CF titres diminish with successful treatment.

A qualitative latex particle agglutination (LPA) test, with heat-treated coccidioidin as antigen, is available from several commercial sources (ImmunoMycologics Inc., Norman, Oklahoma, USA; Meridian Bioscience, Cincinnati, Ohio, USA) for the detection of IgM antibodies. This test is simpler and faster to perform than the ID test and is more sensitive in detecting early infection. However, it has a 5–10% false-positive rate, and positive results must be confirmed by the ID and/or CF methods. It is not recommended for testing CSF samples because false-positive reactions may occur.

A qualitative enzyme immunoassay (EIA) for the detection of IgM and IgG antibodies in serum and CSF is available from Meridian Bioscience. The antigen used contains purified heat-treated and unheated coccidioidin. False-positive reactions have been obtained with serum samples from patients with blastomycosis and a positive EIA result should be confirmed by the ID method.

16.7.5 Antigen detection tests

A quantitative EIA for the detection of *Coccidioides* antigen is available from MiraVista Diagnostics (Indianapolis, Indiana, USA). Sensitivity is greatest with urine (about 70% in moderately severe to severe disease), but antigen has also been detected in serum, CSF and BAL fluid specimens. Antigen levels decline with treatment. False-positive reactions have been obtained with samples from about 10% of patients with other endemic fungal infections and positive antigen test result should be verified by other methods.

16.8 Management

Although disease will resolve without specific antifungal treatment in most individuals who present with acute pulmonary coccidioidomycosis, patients should be followed up for 1–2 years, either to document resolution or to detect signs of pulmonary or extra-pulmonary complications. Patients who develop progressive pulmonary disease or disseminated disease require antifungal treatment, often for long periods of time.

16.8.1 Acute pulmonary coccidioidomycosis

Up to 95% of newly infected individuals have an uncomplicated, self-limited illness and recover without antifungal treatment. No clinical trials have been completed to determine whether early antifungal treatment shortens the course of the illness or reduces the likelihood of later complications. Therefore, it is reasonable to refrain from treatment if the illness

appears to be following a benign course. However, patients should undergo periodic reassessment of symptoms and radiological signs to assure resolution of their self-limited process.

Fewer than 5% of all newly infected patients require treatment. Those who should be treated to prevent progression or dissemination of infection include infants, pregnant women in the third trimester, individuals with HIV infection, organ transplant recipients, individuals receiving high doses of corticosteroids or TNF blockers, members of high-risk racial groups and patients with diabetes mellitus. Antifungal treatment is also indicated in non-immunocompromised patients with severe primary infections as adjudged by one or more of the following findings: persistent symptoms lasting for more than 2 months, weight loss of greater than 10%, intense night sweats persisting for more than 3 weeks, extensive or progressive infiltrates involving more than one half of one lung or portions of both lungs, prominent or persistent hilar adenopathy and rising or elevated CF titres (>1:16) of IgG antibodies.

Although fluconazole and itraconazole are not licenced for the treatment of coccidioidomycosis, these drugs have largely replaced amphotericin B for management of primary pulmonary disease. The usual dose of fluconazole is 400 mg once daily and this should be continued for 3–6 months. As an alternative, itraconazole (200 mg twice daily) may be prescribed. Longer courses of treatment may be needed in patients with diabetes mellitus, in members of high-risk racial groups and in immunocompromised individuals. Serum levels of itraconazole should be measured after the patient has received the drug for at least 2 weeks, to ensure adequate concentrations (see Chapter 3). Neither posaconazole nor voriconazole is currently licenced for the treatment of coccidioidomycosis, but both may be useful in cases that fail to respond to fluconazole or itraconazole. Pregnant women should be treated with amphotericin B.

Patients with diffuse pneumonia may be treated either with amphotericin B or high-dose fluconazole (800–1200 mg daily). Amphotericin B is usually preferred if significant hypoxia is present or if deterioration is rapid. Either a lipid-based formulation of amphotericin B (3–5 mg/kg per day) or the conventional formulation (1 mg/kg per day) may be used. Once the patient has improved, usually within a few weeks, treatment can be changed to oral fluconazole (400 mg/day) or itraconazole (200 mg twice daily). The length of treatment depends on the underlying condition, but should be at least 12 months. In immunocompromised individuals, long-term azole maintenance treatment should be continued.

HIV-infected patients with focal pneumonia who have responded to antifungal treatment appear to be at low risk for recurrence of

coccidioidomycosis as long as their CD4 T-lymphocyte count remains higher than 250 cells per μL and they are receiving potent antiretroviral treatment. It is reasonable to discontinue maintenance treatment after 12 months, provided periodic reassessment of radiological signs and CF titres is done to assure continued resolution of their disease. Patients who have been treated for diffuse pneumonia require lifelong maintenance treatment, even if their CD4 T-cell count exceeds 250 cells per μL.

16.8.2 Chronic pulmonary coccidioidomycosis

Individuals with solitary asymptomatic nodules, diagnosed by non-invasive means, do not require surgical resection or antifungal treatment. Nor is treatment recommended if the lesion is completely resected and the diagnosis determined from the excised tissue. Should repeated chest radiographs suggest the nodule is enlarging, re-evaluation with sputum cultures and CF testing should be done to determine if the infection is active and requires resection.

Those with small, asymptomatic cavities seldom require intervention, but should be observed until spontaneous resolution occurs. Those with cavities that are still detectable after 2 years and those with enlarging cavities often require surgical resection. This is recommended if the cavities are near the pleural surface or if serious and persistent haemoptysis or bacterial superinfection is a problem. If surgical resection is not possible, oral treatment with fluconazole or itraconazole will often lead to improvement, although symptoms can recur after the antifungal is discontinued. In young, otherwise healthy individuals, rupture of a cavity into the pleural space requires surgical intervention, with closure by lobectomy and decortication along with pre- and post-operative antifungal treatment. For patients in whom the diagnosis is delayed by a week or more or for patients with co-existing disease, management includes amphotericin B or azole treatment before surgery or chest tube drainage without surgery.

Patients with chronic fibrocavitary pneumonia should receive treatment with oral fluconazole (400 mg/day) or itraconazole (200 mg twice daily). If the patient's condition improves sufficiently, treatment should be continued for at least 12 months. If the patient does not respond, the dose of fluconazole can be increased or the treatment can be changed to amphotericin B (0.5–0.7 mg/kg per day). Surgical resection is an option if the lesions are well localized or where significant haemoptysis has occurred.

16.8.3 Disseminated coccidioidomycosis

All patients who develop disseminated disease require treatment. Infections of the skin, soft tissue, joints and bones that are not life-threatening

can be treated with an oral azole. The drug of choice is fluconazole (400 mg/day). Itraconazole (200 mg twice daily) is also effective, but less well tolerated. Treatment may need to be continued for 12 months or longer. Higher dosages of fluconazole (up to 2000 mg/day) or itraconazole (up to 800 mg/day) may be required in some patients. Amphotericin B should be substituted if the lesions worsen and are in critical locations, such as the spinal column. The dosage is similar to that for diffuse pneumonia, although the duration of treatment may be longer. Posaconazole and voriconazole have been used successfully as salvage treatment in small numbers of patients.

Surgical debridement or stabilization of infected tissue is occasionally important in the management of osteomyelitis and in the diagnosis and drainage of soft tissue lesions. Factors that favour surgical intervention are large size of abscesses, progressive enlargement of abscesses or destructive lesions, presence of bony sequestrations, instability of the spine or impingement on critical organs or tissues.

In HIV-infected persons with non-meningeal forms of disseminated coccidioidomycosis, treatment should be continued as long as the CD4 T-lymphocyte count is less than 250 cells per μL. However, it may be reasonable to discontinue maintenance treatment in those patients with higher CD4 counts if there is clinical evidence of control of the coccidioidal infection.

16.8.4 Meningitis

Because oral fluconazole is so much more benign than intrathecal amphotericin B, it is now the drug of choice for coccidioidal meningitis. The dosage of fluconazole used in clinical trials was 400 mg/day, and this led to clinical improvement in up to 80% of patients. However, some clinicians prefer to begin treatment with higher doses of oral fluconazole (800 or 1000 mg/day). Oral itraconazole, at dosages of 400–800 mg/day (in divided doses), has also been reported to be effective. Neither fluconazole nor itraconazole eradicate meningeal infection with *Coccidioides* and patients who respond to these azoles should continue this treatment for life to prevent relapse. Individuals who do not respond to fluconazole or itraconazole should be considered for intrathecal amphotericin B, with or without continuation of azole treatment. Voriconazole may be useful in cases that fail to respond to amphotericin B or other azoles. Posaconazole has poor CSF penetration, but it has been used successfully in a few patients.

Until the advent of oral treatment with fluconazole or itraconazole, the management of coccidioidal meningitis consisted of prolonged courses

of intrathecal treatment with amphotericin B. This was associated with a high incidence of side effects and complications, including chemical arachnoiditis, but an estimated 50–75% of patients achieved a sustained remission. The intrathecal dose of amphotericin B ranges from 0.1 to 1.5 mg. It is administered at intervals ranging from daily to weekly, beginning at a low dose and increasing until patient intolerance develops.

Up to 80% of HIV-infected patients with coccidioidal meningitis in whom triazoles have been discontinued have relapsed. For this reason, maintenance treatment should be lifelong, even if their CD4 T-cell count exceeds 250 cells per μL.

Hydrocephalus remains a common complication of coccidioidal meningitis. In most cases, it requires a ventriculoperitoneal shunt to reduce elevated intracranial pressure. This complication can develop regardless of the antifungal treatment being used, and changing to another drug is not required.

16.9 Prevention

With an ageing population and an increase in the number of immunosuppressed persons, serious forms of coccidioidomycosis are becoming an important public health problem in the southwestern United States. Prevention of sporadic exposure to environmental sources of *Coccidioides* arthroconidia in this and other endemic regions is difficult. Nevertheless, individuals should be advised to avoid activities associated with increased risk, such as extensive exposure to disturbed soil at construction sites or exposure to dust storms. If a contaminated site must be disturbed, workers should be provided with personal protective equipment, such as masks and respirators. Dust-control measures, such as wetting soils before disturbing the earth, should be used to decrease the amount of dust generated. Individuals should be warned to avoid transporting soil and other potential contaminated fomites (e.g. geologic specimens) from endemic regions.

Although several clinical trials have documented the effectiveness of prophylaxis with fluconazole (200 mg/day) and itraconazole (200 mg/day) in preventing histoplasmosis and cryptococcosis in HIV-infected persons, low enrolment rates led to the discontinuation of a similar trial for coccidioidomycosis. However, a case–control study, conducted to evaluate risk factors for coccidioidomycosis in persons living with AIDS, showed that having received an azole drug was associated with a reduced risk of *Coccidioides* infection. Routine prophylaxis with fluconazole is not recommended, because of concerns about the high costs of

such treatment, the lack of data on survival benefits and the potential for drug interactions and resistance. Nonetheless, given the substantial risk of disseminated coccidioidomycosis in highly immunosuppressed persons, chemoprophylaxis with fluconazole (400 mg/day) or itraconazole (200 mg twice daily) may be considered for HIV-infected individuals who live or have lived in endemic disease areas if their CD4 T-lymphocyte counts are less than 250 cells per μL, especially those who are at high-risk because of occupational exposure. If used, prophylaxis can be discontinued once CD4 T-cell counts exceed 250 cells per μL for 6 months. Prophylaxis should be restarted if the count is less than 250 cells per μL. SOT recipients with a positive serological test result for coccidioidomycosis prior to transplantation or a history of the disease may also benefit from prophylaxis with fluconazole.

In most cases, natural infection with *Coccidioides* confers lifelong immunity to reinfection. Because of this, a formalin-killed spherule vaccine was developed and tested in a human trial in the 1980s. However, the results were disappointing. New vaccines are currently being developed, and they may offer the best way to prevent coccidioidomycosis in high-risk groups. Among those who might benefit from vaccination are persons at increased risk for severe pulmonary and disseminated disease.

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CHAPTER 17

Histoplasmosis

17.1 Definition

The term histoplasmosis is used to refer to infections caused by the thermally dimorphic fungus *Histoplasma capsulatum*. Following inhalation, this organism causes a wide spectrum of clinical manifestations, ranging from asymptomatic pulmonary infection, to acute or chronic disease of the lungs, or to more widespread disseminated disease. The infection is usually asymptomatic in otherwise healthy individuals after low-level exposure. Symptomatic infection develops after high-level exposure or in persons with an underlying immunosuppressive condition.

17.2 Geographical distribution

Histoplasmosis is the most common endemic mycosis in North America, but it is also found throughout Central and South America. In the United States, the disease is most prevalent in the region around the Mississippi and Ohio Rivers, but histoplasmosis is found in localized foci throughout the eastern half of the continent. Thus, there is some overlap with the endemic region for blastomycosis (see Chapter 15). In Central and South America, the disease is found in many countries, including Mexico, Guatemala, Nicaragua, Panama, Venezuela, Colombia, Brazil, and Argentina. Other endemic regions include the Caribbean islands, parts of Africa, Australia and eastern Asia, in particular India and Malaysia. Sporadic cases have also been diagnosed in other countries among individuals who had earlier resided in, or visited, an endemic region.

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17.3 The causal organism and its habitat

H. capsulatum is a thermally dimorphic fungus. It exists in nature as a mould. In human and animal tissue, it forms small round budding yeast cells. Two varieties of *H. capsulatum* are recognized: var. *capsulatum* and var. *duboisii*. The latter is the cause of African histoplasmosis, which is found in parts of central and western Africa. The two varieties are indistinguishable in their mould forms, but differ in their parasitic forms. The cells of the tissue form of var. *duboisii* are much larger and have thicker walls than those of var. *capsulatum*.

The natural habitat of *H. capsulatum* is the soil. The fungus has been recovered most frequently from soil enriched with bird or bat droppings, particularly that found under bird roosts or in bat caves. Unlike bats, birds do not become infected with *H. capsulatum* and their droppings are primarily a nutrient source for *H. capsulatum* already present in the soil. Soil samples from sites where birds have roosted have been found to remain contaminated for many years after the roost has been cleared. Bats are capable of depositing *H. capsulatum* with their droppings and can distribute the fungus far from an existing natural focus.

17.4 Epidemiology

Inhalation of *H. capsulatum* spores is the usual mode of infection in humans. The incubation period, which has been estimated from point-source outbreaks in which exposure occurred over a limited time period, is 1–3 weeks. In cases of reinfection, the incubation period appears to be shorter (4–7 days after exposure). Histoplasmosis is not contagious but person-to-person spread has occurred by transmission from an organ donor to a recipient.

The major risk factor for development of infection with *H. capsulatum* is environmental exposure. The risk of infection depends on a number of factors, including the nature of the environmental site, the activities performed, and the duration and degree of dust or soil exposure. Longer and more intense exposures usually result in more severe pulmonary disease. Most reported outbreaks have been associated with exposures to sites contaminated with *H. capsulatum* or have followed activities that disturbed accumulations of bird or bat droppings. These include building construction, cleaning, renovation and demolition, soil excavation, spelunking and clearing sites harbouring the fungus. In many cases, however, infections with *H. capsulatum* do not involve major disturbance of infected soil. Mild dust exposure can also result in outbreaks or sporadic cases of histoplasmosis.

Certain persons are at increased risk for some forms of histoplasmosis. Infants less than 12 months of age and adults over 54 years of age are at higher risk for disseminated disease; both age groups are associated with alterations in host immunological response. Older male smokers are predisposed to develop chronic pulmonary histoplasmosis. Racial differences in predisposition to infection or disease progression do not appear to be important for histoplasmosis.

Progressive disseminated histoplasmosis is the most common endemic mycosis among individuals with underlying cell-mediated immunological defects, such as persons with human immunodeficiency virus (HIV) infection, solid organ and stem cell transplant recipients, persons with haematological malignancies and those receiving corticosteroids or other immunosuppressive treatments, such as tumour necrosis factor (TNF)-alpha blockers. Immunocompromised persons with histoplasmosis have a higher case fatality rate than those who are not immunosuppressed, but in many cases, this might be because the diagnosis is not established until late in the course of the infection.

With the advent of the acquired immunodeficiency syndrome (AIDS), histoplasmosis emerged as an important opportunistic infection among HIV-infected persons living in endemic disease areas. Early in the AIDS epidemic, histoplasmosis occurred in about 5% of persons with AIDS living in areas of endemic disease in the United States. Many cases of histoplasmosis have also been reported from cities outside the endemic disease areas, such as New York. In most cases, these patients were Hispanic men who had previously lived in countries in Central or South America with endemic histoplasmosis, suggesting that the disease was the result of reactivation of an old latent focus of infection. In contrast, the marked increase in the incidence of disseminated histoplasmosis among AIDS patients in Indianapolis during several large outbreaks of the disease in the 1980s suggests that the disease was due to direct exposure to the organism during the outbreak. The prevalence of AIDS-associated histoplasmosis in the United States has declined following the introduction of more effective combination antiretroviral treatments, including highly active antiretroviral therapy (HAART), and improvements in HIV health care in general. However, the disease remains a significant opportunistic infection in resource-poor settings in Central and South America where access to diagnostic tests and effective treatment can be difficult if not impossible.

Disseminated histoplasmosis is relatively uncommon in transplant recipients, even in the endemic regions. Most reported cases have occurred among heart or lung transplant recipients, and there have been few cases

among stem cell transplant recipients. Among transplant recipients, disseminated histoplasmosis may occur as a primary infection, as a reactivation of latent focus of infection, or as a donor-transmitted infection. The latter situation has been encountered most frequently in lung transplant recipients.

Disseminated histoplasmosis is increasingly being reported in patients treated with TNF blockers for rheumatoid arthritis, inflammatory bowel disease and dermatological conditions. It has become the most frequent life-threatening fungal infection in this group, resulting in a case fatality rate of 20%. The prevalence of histoplasmosis is higher in patients who are receiving anti-TNF monoclonal antibodies than in those who are receiving soluble-TNF receptor. Most cases have been reported from areas in which *H. capsulatum* is endemic.

It has been estimated that upwards of 500,000 new infections with *H. capsulatum* occur each year in the endemic regions of the United States, but whether this figure is correct is difficult to determine. Nonetheless, histoplasmosis has been recognized as an important public health problem. It is a notifiable condition in 13 states, but the extent of reporting of cases of the disease has been limited. As a result, the incidence of histoplasmosis in the United States is unknown. Information on the prevalence of the disease has mostly come from skin test surveys performed in the 1960s, which defined the areas within each state where reactors to histoplasmin resided. These studies demonstrated that more than 80% of young adults from the states bordering the Mississippi and Ohio Rivers had previously been infected with *H. capsulatum*. No large-scale prevalence studies have been conducted since the 1960s.

17.5 Clinical manifestations

The lungs are the usual initial site of infection, but haematogenous dissemination to other sites occurs in most patients during the first few weeks after exposure. In most cases, the primary infection is subclinical and resolves without overt symptoms or clinical signs. However, *H. capsulatum* may remain latent within the infected host and give rise to symptomatic infection months or years after the initial exposure. The immunological response of the host determines the course of the disease, with subclinical infection predominating in immunocompetent individuals after low-level exposure. Symptomatic infection develops in immunocompromised individuals, as well as in otherwise normal persons who experience high-level exposure.

17.5.1 Acute pulmonary histoplasmosis

Fewer than 5% of individuals exposed to *H. capsulatum* spores develop symptomatic disease in an endemic setting. However, during outbreaks, when higher levels of exposure occur, 50–100% of patients develop acute symptomatic and often severe infection after an incubation period of 1–3 weeks.

Acute pulmonary histoplasmosis usually presents as a non-specific flu-like illness characterized by high-grade fever, chills, headache, non-productive cough, myalgia, pleuritic chest pain, loss of appetite and fatigue. About 5–10% of patients present with arthritis or arthralgia and erythema nodosum. Most otherwise healthy persons will recover without treatment, their symptoms disappearing within 3 weeks. However, fatigue and weakness can persist for several months.

Chest radiographs will often reveal small, scattered, bilateral, nodular infiltrates. Enlarged hilar and mediastinal lymph nodes are often evident and pleural effusion may be found. The infiltrates tend to heal over several months, leaving scattered calcifications throughout both lung fields. Healing of a localized infiltrate may result in the development of a residual round nodule, often termed a histoplasmoma, which enlarges as fibrous material is deposited around the lesion. These nodules cause no symptoms and are usually identified as incidental findings on chest radiographs or computed tomographic (CT) scans. If calcification is absent, these benign lesions cannot be distinguished from a neoplasm. The most frequent symptoms of mediastinal lymphadenopathy are cough, chest pain and/or atelectasis due to bronchial compression, or dysphagia resulting from oesophageal compression. In most cases, the symptoms are mild and resolve over a few months. Obstruction is more common in children than in adults.

In immunosuppressed individuals and normal persons with high-level exposure to *H. capsulatum*, the onset of illness is abrupt, and the patients present with fever, chills, malaise, dyspnoea, cough, chest pain and weight loss. Hilar and mediastinal lymphadenopathy may or may not be present. Hepatomegaly and/or splenomegaly may be found. Chest radiographs show diffuse interstitial infiltrates that usually are described as miliary or reticulonodular. In most cases, the illness is sufficiently severe to require hospitalization, with some individuals experiencing acute respiratory distress syndrome (ARDS).

Unlike coccidioidomycosis, exposure to *H. capsulatum* does not confer immunity to reinfection. Individuals reinfected with *H. capsulatum* develop a similar illness, but this occurs after a much shorter incubation period (less than 1 week). These patients present with abrupt onset of

malaise, headache, chills, fever and cough, but the symptoms may be less severe and of shorter duration. The radiological signs are different from those seen in newly infected individuals. Multiple, small, interstitial miliary nodules are present, but there is no mediastinal lymph node enlargement and pleural effusions are not seen. Late calcification does not occur. The illness tends to resolve without treatment.

Following inhalation of *H. capsulatum*, the organism gains access to the alveolar and interstitial lung tissue and then spreads through the lymphatics to the lymph nodes. In most patients, this process results in enlargement of a few nodes that later recede and become calcified. In some, however, the process leads to massive enlargement of multiple mediastinal lymph nodes that coalesce together and undergo caseation necrosis. This is termed granulomatous mediastinitis or mediastinal granuloma. Many patients remain asymptomatic. Others develop symptoms related to tracheal, bronchial or oesophageal compression, including cough, chest pain, dyspnoea and haemoptysis. Although symptoms are often mild and usually resolve spontaneously over a few months, they may be more severe and protracted. CT scans of the chest are helpful to define the extent of nodal enlargement, the presence of necrosis and encroachment on adjacent structures. Granulomatous mediastinitis is not thought to progress to mediastinal fibrosis.

Mediastinal fibrosis, or fibrosing mediastinitis, is an uncommon, late complication of pulmonary histoplasmosis arising from the mediastinal or hilar lymph nodes and leading to invasion and occlusion of central blood vessels or the bronchi. It is seldom seen in children. CT scans will reveal the extent of invasion into the mediastinal structures. Symptoms include increasing dyspnoea, cough, haemoptysis and chest pain. Patients often report symptoms of several years duration at the time of diagnosis. The course is slowly progressive and often fatal.

Pericarditis occurs in 5–10% of individuals with acute histoplasmosis and appears to be a complication of inflammation in adjacent mediastinal lymph nodes rather than an infection of the pericardium. Symptoms include sub-sternal chest pain and dyspnoea. The disease is self-limited in most patients, but the course may be prolonged and recurrence is common.

17.5.2 Chronic pulmonary histoplasmosis

This slowly progressive illness usually occurs in middle-aged individuals with underlying lung damage, usually chronic obstructive pulmonary disease. It first manifests itself as a transient, segmental pneumonia that sometimes heals without treatment, but often progresses to fibrosis and

cavitation with destruction of significant amounts of lung tissue. If left untreated, death can result from progressive lung failure.

In patients with pneumonia, the symptoms often include productive cough, fever, chills, weight loss, malaise, night sweats and pleuritic chest pain. The typical radiological findings in these patients are interstitial infiltrates in the apical segments of the upper lobes of the lung.

The most prominent symptoms in patients with chronic fibrosis and cavitation are cough and sputum production. Other symptoms include fever, chest pain, fatigue and weight loss. Haemoptysis develops in more than 30% of these individuals. Chest radiographs will reveal progressively enlarging cavities and fibrosis. Cavities with walls thicker than 3 mm in diameter are associated with established and continuing infection. Lesions are more common in the right upper lobe than in the left, but bilateral lesions develop in about 25% of patients and, in time, the infection will spread to the lower lobes. Pleural effusion is uncommon, but pleural thickening adjacent to apical cavitary lesions is found in 50% of patients.

17.5.3 Disseminated histoplasmosis

Asymptomatic haematogenous dissemination from the lungs to other tissues occurs in most, if not all, individuals during the first 2 weeks after infection with *H. capsulatum*, before a specific immunological response has developed. In most cases, however, the infection is non-progressive and leads only to the development of calcified granulomata in the liver and spleen. Disseminated histoplasmosis has been estimated to occur in one in 2000 exposed individuals, in particular those with underlying T-cell-mediated immunological defects or those at the extremes of age.

The clinical manifestations of disseminated histoplasmosis range from an acute illness that is fatal within a few weeks if left untreated (often seen in infants and immunosuppressed patients) to an indolent, chronic illness that can affect a wide range of sites. Treatment is essential for all patients with disseminated histoplasmosis.

In non-immunosuppressed individuals, disseminated histoplasmosis follows a slowly progressive course, evolving over a period of months. This form of the disease is fatal if not treated and is usually seen in older adults with no obvious immunosuppression. Often chest radiographs are normal. Hepatic infection is common, but enlargement of the liver and spleen is not as pronounced as in patients with fulminant infection. Adrenal gland destruction is a common problem, and CT scans will reveal calcification with central necrosis. Mucosal ulcers are found in over

60% of patients with chronic progressive infection. The mouth and throat are often affected, but lesions also occur on the lip, nose, glans penis and other sites. Most patients have a single lesion, painless at first, with a characteristic, distinct heaped-up margin. Cutaneous lesions are uncommon, occurring in less than 10% of patients.

Central nervous system (CNS) disease occurs in 5–20% of patients with indolent disseminated histoplasmosis, presenting as sub-acute or chronic meningitis, focal brain or spinal lesions and encephalitis. Chronic meningitis is the most common manifestation. Headache is the most common presenting symptom in individuals with meningitis. Other symptoms include fever, headache, confusion and focal neurological deficits. Meningitis is a common manifestation of disseminated histoplasmosis in infants, but the sub-acute meningitis and focal lesions characteristic of CNS infection in adults are seldom seen in children. CT scans or magnetic resonance imaging (MRI) will reveal single or multiple ring-enhancing lesions. Most patients have abnormal cerebrospinal fluid (CSF) findings, including an elevated protein concentration, a lowered glucose concentration and a mild lymphocytic pleocytosis.

Other manifestations of chronic disseminated histoplasmosis include endocarditis (often with large vegetations) and lesions in the genitourinary tract and gastrointestinal tract. Occasional cases of bone and joint infection have occurred in infants and children.

In infants and immunosuppressed patients, acute disseminated histoplasmosis often presents as high fever, chills, prostration, malaise, loss of appetite and weight loss. The liver and spleen are enlarged and liver function tests are abnormal. Anaemia is common. Mucosal lesions can occur, but are much less common than in patients with indolent progression of the illness.

In AIDS patients, disseminated histoplasmosis is usually associated with advanced immunosuppression. Most patients have CD4 T-lymphocyte counts of less than 150 cells per μL and present with non-specific symptoms, such as fever, fatigue and weight loss, that are often gradual in onset. Up to 25% of patients have an enlarged liver and spleen and a similar proportion have anaemia, leucopenia and thrombocytopenia. Cough, chest pain and dyspnoea occur in about 50% of AIDS patients with histoplasmosis. Mucosal lesions are uncommon, but 15–20% of patients have an erythematous, maculopapular non-pruritic skin rash. About 30% of AIDS patients with histoplasmosis have normal chest radiographs on admission, but 50% have bilateral reticulonodular infiltrates. CNS involvement occurs in 10–20% of cases: the manifestations include meningitis and focal brain lesions.

Ten to 20% of AIDS patients with disseminated histoplasmosis have presented with a severe, septic shock-like syndrome, consisting of high fever, hypotension, hepatic and renal failure, ARDS and disseminated intravascular coagulation. This acute septic presentation appears to be a late manifestation of histoplasmosis in patients in whom the diagnosis has been missed. The case fatality rate in these cases is high.

17.5.4 African histoplasmosis

Although the lungs are the initial site of infection in *H. capsulatum* var. *duboisii* infection, pulmonary disease is rare. The illness is indolent in onset and the predominant sites affected are the skin, bones and soft tissue. Those with more widespread infection involving the liver, spleen and other organs have a febrile wasting illness that is fatal within weeks or months if left untreated.

Cutaneous lesions are common in patients with African histoplasmosis. Multiple papular lesions often develop on the face and trunk. Nodular lesions are less common. Both nodules and papules often enlarge and ulcerate. Osteomyelitis occurs in about 30% of patients with African histoplasmosis. The spine, ribs, cranial bones, sternum and long bones are the most common sites of infection and multiple lesions are often found. The lesions are often painless. The infection may spread into contiguous joints, causing arthritis, or into adjacent soft tissue, causing a purulent subcutaneous abscess. Draining sinus tracts often appear.

Reports of African histoplasmosis in persons with HIV infection are rare, but these individuals often present with fever, weight loss and multiorgan involvement.

17.6 Differential diagnosis

The clinical presentation of acute pulmonary histoplasmosis is similar to that of many other illnesses, including blastomycosis and atypical pneumonias, such as those due to *Mycoplasma*, *Chlamydia* and *Legionella*. The clinical and radiological presentation of chronic pulmonary histoplasmosis is similar to that of tuberculosis, sarcoidosis, blastomycosis and coccidioidomycosis. The mucocutaneous lesions of chronic disseminated histoplasmosis can be confused with a number of other infectious and non-infectious conditions, including tuberculosis, syphilis, paracoccidioidomycosis and lichen planus. The clinical manifestations of African histoplasmosis may mimic cancer or tuberculosis.

17.7 Essential investigations and their interpretation

17.7.1 Microscopy

Microscopic examination of wet preparations of clinical material, such as sputum, bronchoalveolar lavage (BAL) fluid or pus, is not a suitable method for the diagnosis of histoplasmosis. All material should be examined as stained smears.

If microscopic examination of Wright-stained peripheral blood or bone marrow smears, or stained tissue sections of lung, liver, lymph node, or other specimens from individuals who have resided in, or visited, endemic areas reveals small oval yeast cells with narrow-based buds (often clustered within monocytes and macrophages), the diagnosis of histoplasmosis should be suspected. *H. capsulatum* var. *capsulatum* cells can, however, be confused with other pathogens, such as *Candida glabrata* and *Penicillium marneffei*, as well as with atypical small yeast cells of *Blastomyces dermatitidis* and small, capsule-deficient cells of *Cryptococcus* species.

If tissue sections contain large, thick-walled yeast cells with narrow-based buds, and the patient has resided in or visited the African continent, *H. capsulatum* var. *duboisii* should be suspected as the pathogen.

Organisms tend to be much more abundant in peripheral blood smears and bronchial washings from persons with AIDS.

17.7.2 Culture

The definitive diagnosis of histoplasmosis depends on isolation of the fungus in culture. Identifiable mould colonies, which are white to brown in colour, can usually be obtained after incubation at 25–30°C for 4–6 weeks on glucose peptone (Sabouraud's dextrose) agar supplemented with cycloheximide (actidione). It is often difficult to distinguish the mycelial colonies of *H. capsulatum* from those of *B. dermatitidis* and species of *Chrysosporium* and *Sepedonium*. Unequivocal identification of a mycelial isolate as *H. capsulatum* generally requires conversion to the yeast form. Sub-culture at 37°C on a rich medium, such as brain heart infusion agar with blood, usually results in production of the yeast form within 7–10 days. If more rapid identification is required, molecular testing of the mould culture with the AccuProbe test (Gen-Probe Inc., San Diego, California, USA) can be completed within a few hours. Isolates of *H. capsulatum* var. *duboisii* also give positive results in the AccuProbe test.

H. capsulatum has been isolated from the sputum or BAL fluid specimens in 60–85% of cases of chronic pulmonary histoplasmosis if multiple specimens are tested. In disseminated disease, useful specimens for

culture include blood, urine, lymph node and bone marrow samples. The Isolator lysis–centrifugation system (Wampole Laboratories, Princeton, New Jersey, USA) is the most sensitive method for recovering *H. capsulatum* from blood. Bone marrow samples are positive in over 75% of cases. CSF samples are positive in only 25–50% of cases of *Histoplasma* meningitis.

17.7.3 Skin tests

The histoplasmin skin test is not recommended for diagnosis of histoplasmosis, because a positive result does not distinguish present from past infection. Nor does a negative result rule out recent infection. Moreover, it can induce the formation of antibodies, making the results of subsequent serological tests difficult to interpret. The histoplasmin skin testing reagent is not currently available in the United States.

17.7.4 Serological tests

Serological tests for antibodies are most useful in the diagnosis of sub-acute pulmonary histoplasmosis, chronic pulmonary histoplasmosis, granulomatous mediastinitis and pericarditis. False-negative reactions may occur during the first two months after acute exposure, as well as in immunosuppressed patients with disseminated disease.

The immunodiffusion (ID) test is a qualitative method that is performed with mycelial-phase culture filtrate antigen (termed histoplasmin). The ID test detects precipitins to the H and M glycoprotein antigens of *H. capsulatum* that are present in histoplasmin. Precipitins to M antigen are the first to appear (4–8 weeks after exposure) and can be detected in up to 75% of persons with acute histoplasmosis. However, M precipitins may persist for many months after the initial infection and may also be found in nearly all patients with chronic pulmonary histoplasmosis, as well as in those who have had a recent histoplasmin skin test. Precipitins to H antigen are specific for acute pulmonary histoplasmosis, but only occur in 10–20% of cases and disappear within 6 months of infection. H precipitins are seldom, if ever, found in the absence of precipitins to M antigen. The presence of precipitins to both H and M antigens is highly suggestive of active histoplasmosis, regardless of other serological test results.

The complement fixation (CF) test is a quantitative procedure in which two antigens are employed: histoplasmin and a suspension of intact killed *H. capsulatum* yeast cells. The CF test is more sensitive, but less specific than the ID test in histoplasmosis. It can be performed with serum and CSF specimens and is useful in the diagnosis of acute, chronic and disseminated disease. The CF test becomes positive 2–6 weeks following

infection; CF titres of 1:8 or greater with either antigen are considered presumptive evidence of histoplasmosis. Titres above 1:32 or a fourfold rise in titres between paired specimens offer stronger evidence of active infection. CF test results can be difficult to interpret because cross-reactions can occur with serum from patients with blastomycosis or coccidioidomycosis. In such instances, titres usually range between 1:8 and 1:32. However, similar titres may be obtained in tests with serum from patients with proven histoplasmosis.

Titres of CF antibodies to *H. capsulatum* decrease following resolution of infection, but increase in individuals with chronic progressive disease. In some patients, positive CF titres decline slowly and persist long after the disease has been cured. The significance of persistently elevated or fluctuating titres is unclear, as is the effect of antifungal treatment on disappearance of antibodies.

The detection of *H. capsulatum* antibodies by ID or CF in CSF specimens permits the diagnosis of *Histoplasma* meningitis, even if cultures are negative.

A semi-quantitative latex agglutination test, using histoplasmin as antigen, is available from ImmunoMycologics Inc. (Norman, Oklahoma, USA). This test detects IgM antibodies and is used primarily for the presumptive diagnosis of acute histoplasmosis.

17.7.5 Antigen detection tests

An enzyme immunoassay (EIA) for the detection of *H. capsulatum* polysaccharide antigen is available from MiraVista Diagnostics (Indianapolis, Indiana, USA). Similar tests have been developed by several other laboratories, although dissimilar results have sometimes been obtained when the tests have been compared.

The *Histoplasma* antigen test is particularly useful in immunocompromised individuals with disseminated disease and can often provide a rapid diagnosis before positive cultures can be identified. Sensitivity is greatest with urine, but antigen has also been detected in serum, CSF and BAL fluid specimens. *Histoplasma* antigen has been detected in the urine of 95–100% and in the serum of 80% of AIDS patients with disseminated histoplasmosis. Antigen detection in the urine is also useful for the rapid diagnosis of acute pulmonary histoplasmosis. In this group, antigenuria can be detected in about 75% of cases, provided samples are obtained within 2 weeks after exposure.

The amount of *Histoplasma* antigen detected in the urine can be used to monitor an individual's response to antifungal treatment. Levels decline with effective treatment, becoming undetectable in many patients.

Failure of antigen concentrations to decline during treatment is suggestive of therapeutic failure. Antigen levels decline more rapidly in serum than urine. In patients who have responded to treatment and in whom *Histoplasma* antigen levels have previously fallen, an increase in antigen is suggestive of relapse. In patients with antigenuria, levels increase in 90% of those who relapse.

The *Histoplasma* antigen test has been reported to give false-positive reactions with urine samples from patients with other fungal infections, including blastomycosis, coccidioidomycosis, paracoccidioidomycosis and penicilliosis. False-positive reactions in the urine EIA for *B. dermatitidis* antigen occur in patients with histoplasmosis. This is important because the endemic regions for the two diseases overlap.

The Platelia *Aspergillus* galactomannan EIA (see Chapter 10) has been reported to give false-positive results in patients with disseminated histoplasmosis, particularly in those who have high concentrations of circulating *Histoplasma* antigen. Patients with aspergillosis, however, do not give false-positive results in the *Histoplasma* antigen test. This difference has been attributed to the fact that patients with invasive aspergillosis have low levels of circulating antigen while those with histoplasmosis have much higher concentrations.

Patients with African histoplasmosis also give positive results in the *Histoplasma* antigen test.

17.8 Management

Treatment is indicated in severe or moderately severe acute pulmonary histoplasmosis, chronic pulmonary histoplasmosis, disseminated histoplasmosis and CNS infection. In other manifestations, the role of antifungal treatment is unclear, and efficacy has not been determined in clinical trials. The management of patients who develop disseminated histoplasmosis while receiving TNF-alpha blockers should include discontinuation of the TNF inhibitor and early initiation of antifungal treatment.

17.8.1 Acute pulmonary histoplasmosis

Antifungal treatment is not usually indicated for persons with mild symptoms caused by acute pulmonary histoplasmosis, as this form of the disease is self-limited, often resolving within a few weeks. In most cases, spontaneous improvement has begun before the condition is diagnosed. If symptoms such as fever, fatigue or weight loss persist for more than 3 weeks, this may be a sign that the patient is developing progressive disseminated disease. Treatment with oral itraconazole (200 mg once or twice

daily for 6–12 weeks) should be considered for patients who have shown no clinical improvement after 1 month of observation. However, it is unclear whether treatment hastens recovery or prevents complications.

Individuals with diffuse interstitial infiltrates following more intense exposure often develop more severe disease. They may become hypoxemic and may even require ventilatory support. Without treatment, recovery is usually slow and the outcome may be fatal. A lipid-based formulation of amphotericin B (3–5 mg/kg per day for 1–2 weeks) is the initial treatment of choice in patients with severe acute pulmonary histoplasmosis. As an alternative, the conventional formulation of amphotericin B (0.7–1.0 mg/kg per day for 1–2 weeks) can be used. Once the patient begins to show improvement, itraconazole (200 mg three times daily for 3 days and then 200 mg twice daily) should be used to complete a 12-week course of treatment. Intravenous methylprednisolone (0.5–1.0 mg/kg per day) during the first 1–2 weeks of antifungal treatment is recommended for patients who develop respiratory complications, including hypoxemia or significant respiratory distress. Antifungal treatment should not be discontinued until pulmonary infiltrates have resolved.

Arthritis, arthralgia and erythema nodosum respond to treatment with non-steroidal anti-inflammatory agents, and antifungal treatment is not required. Prednisone (0.5–1.0 mg/kg per day to a maximum of 80 mg/day) in tapering doses over 1–2 weeks is recommended for severe cases. Itraconazole (200 mg three times daily for 3 days and then 200 mg twice daily for 6–12 weeks) is recommended for patients with symptoms that require treatment with corticosteroids, to reduce the risk of progressive disseminated disease caused by corticosteroid-induced immunosuppression.

There is no evidence that antifungal treatment of asymptomatic patients with one or a few isolated lung nodules (histoplasmomas) has any beneficial effect. A few patients with mediastinal lymphadenopathy require treatment to relieve symptoms of bronchial and/or oesophageal compression. Prednisone (0.5–1.0 mg/kg per day to a maximum of 80 mg/day) in tapering doses over 1–2 weeks is recommended for severe cases with obstruction or compression of adjacent structures. Itraconazole (200 mg three times daily for 3 days and then 200 mg twice daily for 6–12 weeks) is recommended if corticosteroids are administered. Itraconazole is also recommended for patients whose symptoms last for longer than 1 month.

The treatment of choice in individuals with symptomatic granulomatous mediastinitis is itraconazole (200 mg three times daily for 3 days and then once or twice daily for 6–12 weeks). Patients who have mediastinal fibrosis do not respond to antifungal treatment and this form of

management is not recommended. Nor has corticosteroid treatment been helpful. Itraconazole (200 mg once or twice daily for 12 weeks) can be given if clinical findings cannot differentiate fibrosis from granulomatous mediastinitis. Placement of intravascular stents has been helpful in some patients with pulmonary vessel obstruction and should be considered for patients who are at highest risk because of involvement of both lungs. Surgery should be approached with great caution in patients with mediastinal fibrosis and should only be considered in those who are expected to die without intervention.

Patients with mild pericarditis respond to non-steroidal anti-inflammatory agents and do not require antifungal treatment. Prednisone (0.5–1.0 mg/kg per day to a maximum of 80 mg/day) is recommended for those with evidence of haemodynamic compromise or unremitting symptoms after several days of non-steroidal anti-inflammatory treatment. Itraconazole (200 mg three times daily for 3 days and then once or twice daily for 6–12 weeks) is recommended if corticosteroids are administered. Large pericardial effusions may require drainage.

17.8.2 Chronic pulmonary histoplasmosis

Treatment is indicated in all individuals with chronic pulmonary histoplasmosis. Itraconazole (200 mg three times daily for 3 days and then 200 mg twice daily for at least 12 months) is the drug of choice for most patients. Many experts recommend treatment for 18–24 months, with careful follow-up because of the risk of relapse. Because absorption of itraconazole can be erratic, blood levels should be measured after the patient has received the drug for at least 2 weeks, to ensure adequate concentrations (see Chapter 3).

17.8.3 Disseminated histoplasmosis

All patients with disseminated disease require treatment. Patients with moderately severe to severe disease should be treated with lipid-based formulation of amphotericin B at a dosage of 3–5 mg/kg per day for 1–2 weeks. As an alternative, the conventional formulation of amphotericin B (0.7–1.0 mg/kg per day) can be used. This should be followed by treatment with oral itraconazole (200 mg three times daily for 3 days and then 200 mg twice daily) for a total of at least 12 months. Serum levels of itraconazole should be measured after the patient has received the drug for at least 2 weeks, to ensure adequate concentrations (see Chapter 3).

In a randomized clinical trial among patients with moderately severe to severe disseminated histoplasmosis, liposomal amphotericin B (3 mg/kg per day) was more effective than conventional amphotericin B (0.7 mg/kg per day), inducing a more rapid and more complete response, lowering

mortality and reducing toxicity. Substitution of amphotericin B lipid complex (5 mg/kg per day) may be preferred in some patients because of cost or tolerability.

In patients with mild to moderate disseminated histoplasmosis, oral itraconazole (200 mg three times daily for 3 days and then 200 mg twice daily) for at least 12 months is the recommended initial treatment. Blood levels should be measured.

Lifelong suppressive treatment with oral itraconazole (200 mg/day) may be required in immunocompromised patients if the underlying immunosuppression cannot be reversed. It may also be needed in patients who relapse despite appropriate treatment. Experience has shown that it is reasonable to discontinue itraconazole maintenance once patients with AIDS-associated histoplasmosis have completed at least 12 months of itraconazole treatment, have negative blood cultures, have serum and urine antigen levels of less than 2 ng/mL and have attained CD4 T-lymphocyte counts of more than 150 cells per μL for at least 6 months while receiving HAART. Maintenance treatment should be resumed if patients discontinue antiretroviral treatment or if their CD4 cell count falls to less than 100 cells per μL.

If patients are unable to tolerate itraconazole, or fail to absorb the drug or are unable to take this agent because of drug interactions, the best alternative oral treatment is posaconazole (200 mg four times daily). This agent has been shown to be more effective than itraconazole in animal models of infection. It is currently not licenced for the treatment of histoplasmosis, but has been used successfully as salvage treatment in small numbers of patients with various forms of the disease. Ketoconazole (200–400 mg/day) is effective, but it is now rarely used because of the high rate of significant adverse events. However, it remains a useful option in resource-limited environments. Fluconazole (400–800 mg/day) is less effective than itraconazole, and relapse associated with the development of resistance has been reported in AIDS patients. The role of voriconazole is not clear, but it has been used successfully as salvage treatment in a few patients. The echinocandins are ineffective in animal models of infection and should not be used to treat histoplasmosis.

17.8.4 CNS histoplasmosis

Aggressive management of this disease is recommended because of the poor outcome. Liposomal amphotericin B (5 mg/kg per day) is now the drug of choice. It should be given to a total dose of 175 mg/kg over a 4–6 week period. Thereafter, itraconazole (200 mg twice or three times daily) should be given for at least 12 months. Blood levels of itraconazole should be measured to ensure adequate concentrations are maintained. CSF

specimens should be tested for *Histoplasma* antigen levels and CF antibody titres at regular intervals. Antifungal treatment should be continued for several months after all lesions have resolved on MRI scans.

17.9 Prevention

Prevention of sporadic exposure to sources of *H. capsulatum* spores in areas of endemic disease is difficult. Nonetheless, it is feasible to prevent large-scale exposures, such as during cleaning and construction work. If a contaminated site must be disturbed, workers should be provided with personal protective equipment, such as masks and respirators, which should be worn during activities that can lead to aerosolization of infectious material. Dust-control measures, such as wetting soils before disturbing the earth, should be used to decrease the amount of dust generated during construction, demolition or excavation.

Individuals at increased risk for severe infection, especially immunocompromised persons, should be advised to avoid sites, such as caves and bird roosts, that are often contaminated with *H. capsulatum*. These individuals should also be advised to avoid activities, such as spelunking, construction, cleaning or demolishing old buildings and soil excavation that are associated with increased risk of histoplasmosis. However, if exposure cannot be avoided, individuals should be advised to wear masks and special protective equipment.

Disinfectants have been used to eliminate or reduce foci of *H. capsulatum* infection. Of the fungicidal and chemical agents that have been used, a 3% solution of formalin is the only disinfectant that has been proved to be an effective decontaminant. Formalin is inexpensive, but its use is associated with serious health hazards and precautions must be taken during its application. Decontamination is not a feasible option for bat-infested sites, because these animals can recontaminate the environment.

Although several trials have documented the effectiveness of prophylaxis with oral itraconazole (200 mg/day) to prevent histoplasmosis in persons with advanced HIV infection, routine prophylaxis with this drug is not recommended. This is because of concerns about the high cost of such treatment, the lack of survival benefits associated with prophylaxis and the potential for the development of drug resistance. It is, however, recommended that itraconazole prophylaxis be considered for HIV-infected persons who live, or have lived, in highly endemic disease areas if their CD4 T-lymphocyte counts are less than 150 cells per μL, especially those who are at high-risk because of occupational exposure. Fluconazole is not an acceptable alternative. If used, prophylaxis can be

discontinued once CD4 counts exceed 150 cells per μL for 6 months in patients on HAART.

The usefulness of antifungal prophylaxis in other groups of immunosuppressed individuals, such as transplant recipients, with evidence of past histoplasmosis has not been studied, but the risk of reactivation appears to be low. Nonetheless, active histoplasmosis during the two-year prior period may be a basis for itraconazole prophylaxis during immunosuppression. Urine antigen levels should be measured before treatment is commenced and then at 2–3 month intervals during ongoing immunosuppression.

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CHAPTER 18

Paracoccidioidomycosis

18.1 Definition

The term paracoccidioidomycosis (South American blastomycosis) is used to refer to infections caused by the thermally dimorphic fungus *Paracoccidioides brasiliensis*. Following inhalation, this organism almost always causes sub-clinical infection. In adults, later reactivation of quiescent lesions results in chronic progressive granulomatous disease of the lungs or other organs, in particular the lymph nodes, mucous membranes and skin. In children and adolescents, acute or sub-acute disseminated disease may arise without a latent period.

18.2 Geographical distribution

Paracoccidioidomycosis is a common endemic mycosis in Latin America. The endemic region extends from Mexico to Argentina, but the disease is more prevalent in South than Central America. The greatest numbers of reported cases have come from Brazil, but the disease is also endemic in Venezuela, Colombia, Ecuador and Argentina. Imported cases of the chronic form of paracoccidioidomycosis have also been diagnosed outside Latin America among individuals who had earlier resided in an endemic region.

18.3 The causal organism and its habitat

P. brasiliensis is a thermally dimorphic fungus. It exists in nature as a mould, but in tissue it forms large oval or round cells with characteristic multiple buds encircling the mother cell.

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Although *P. brasiliensis* has on occasion been isolated from soil, its precise ecological niche remains uncertain. The lack of outbreaks and the long interval between infection and the development of disease have hindered efforts to determine the source of infection. Paracoccidioidomycosis appears to be most prevalent in rural environments with high rainfall, such as tropical or sub-tropical forests, as well as in coffee or tobacco growing regions.

18.4 Epidemiology

Inhalation of *P. brasiliensis* spores is believed to be the usual mode of infection in humans. However, in a few cases, infection may be the result of traumatic inoculation. The infection is thought usually to be acquired during the first two decades of life, but clinical disease is uncommon in this age range. The incubation period is unknown, but it is clear that the fungus can remain dormant for very long periods in the lymph nodes following asymptomatic primary infection. Among more than 70 reported cases of imported paracoccidioidomycosis diagnosed in non-endemic countries, the latent period ranged from 4 months to 60 years with an average duration of asymptomatic infection of 14 years.

Paracoccidioidomycosis is an uncommon disease in children, accounting for around 5–10% of all reported clinical cases. In contrast, skin testing of rural children living in certain endemic regions of Brazil has demonstrated that up to one-third have had sub-clinical infections with *P. brasiliensis.* Symptomatic disease is most prevalent in individuals over the age of 30 years. Although skin testing has demonstrated that the rate of infection is similar in men and women, more than 90% of cases of symptomatic disease occur in men, most of whom have or have had an agricultural occupation. Women appear to be protected from the disease, but not from infection, by oestrogen-mediated inhibition of the mould to yeast transformation.

Alcoholism and smoking, as well as malnutrition, have been identified as risk factors for paracoccidioidomycosis. In recent years, cases have been reported among several groups of immunocompromised individuals, including patients with haematological malignancies, organ transplant recipients and persons with human immunodeficiency virus (HIV) infection. In recent reports from different regions of Brazil, *P. brasiliensis* infection was estimated to occur in about 1.5% of HIV-infected persons. Thus, it is less prevalent than histoplasmosis or cryptococcosis among persons with AIDS. In contrast to HIV-negative adults with paracoccidioidomycosis, patients co-infected with HIV have been younger (age range,

20–40 years) and less involved in agricultural occupations. However, many had been extensively exposed to rural environments in the past, suggesting that disease had resulted from reactivation of latent infection.

Because paracoccidioidomycosis is not a notifiable disease in the countries in which it is endemic, information on the incidence and prevalence of the infection is limited. It has been estimated that throughout the endemic region, in which about 90 million people live, as many as 10 million individuals have been infected with *P. brasiliensis*. In Brazil, the annual incidence of symptomatic paracoccidioidomycosis has been estimated at 1–3 cases per 100,000 population. In Colombia, the annual incidence of the disease has been estimated at 0.05–0.2 cases per 100,000 population.

18.5 Clinical manifestations

The lungs are the usual initial site of infection, but the organism then spreads through the lymphatics to the regional lymph nodes. In most cases, the primary infection is sub-clinical and resolves without overt symptoms or clinical signs. However, *P. brasiliensis* may remain latent within the infected host and give rise to symptomatic infection many years after the initial exposure. The immunological response of the host determines the course of the disease, with sub-clinical infection predominating in immunocompetent individuals, and symptomatic infection appearing in those who are immunocompromised.

The most common clinical presentation of paracoccidioidomycosis is the chronic or adult form that results from reactivation of a quiescent lesion and mostly affects men older than 30 years of age. In 80% of cases, the disease involves the lungs. In many patients, the infection also involves the mucosa, skin, lymphatic system, central nervous system (CNS) and other organs. The disease is slowly progressive and may take months or even years to become established. Many patients present with significant weight loss, but fever is infrequent, unless a co-infection, such as tuberculosis, is present. Most respond to prolonged antifungal treatment, but later relapse is a common problem.

The juvenile or acute form of paracoccidioidomycosis accounts for about 5–10% of reported clinical cases of the disease. It is a serious disorder with a poor prognosis that affects children and adolescents. Among children, the proportion of affected males and females is similar, with a slight preponderance of males among adolescents. The disease is rapidly progressive following the initial infection, and within weeks to a few months, the patient's general condition is seriously impaired. The most common clinical manifestation is generalized lymphadenopathy, with

superficial and/or visceral lymph node enlargement. This may be accompanied by fever, marked weight loss, and general malaise. Single or multiple cutaneous and/or osteolytic lesions are frequent. In contrast to the chronic form, lung and mucous membrane involvement is uncommon.

In immunocompromised individuals, such as persons with AIDS, the disease usually shows a rapid progression following reactivation of a latent infection. In general, the clinical manifestations are similar to those seen in patients with the juvenile form of paracoccidioidomycosis. They include prolonged fever, weight loss, generalized lymphadenopathy, hepatomegaly and splenomegaly. In 85% of cases, the lungs are affected. Multiple cutaneous and osteolytic lesions are also common, but mucosal lesions are less frequent than in HIV-negative adults. More than 80% of cases of AIDS-associated paracoccidioidomycosis have had CD4 T-lymphocyte counts of less than 200 cells per μL.

18.5.1 Pulmonary paracoccidioidomycosis

In the chronic or adult form of disease, this illness is insidious in onset, and the patient has been ill for weeks or months before diagnosis. The most frequent symptoms include persistent cough, mucoid or purulent sputum, chest pain, dyspnoea, weight loss and malaise.

The radiological findings are characteristic, but not diagnostic. Multiple bilateral infiltrates, mostly interstitial but sometimes also alveolar, are found. Other findings include hypertrophied and calcified hilar and mediastinal lymph nodes, fibrosis, septal thickening, and pseudotumoral and fibronodular masses. High-resolution computed tomography (CT) scans of the chest will reveal interstitial abnormalities and nodules, associated with bronchiectasis and emphysema. In about 90% of cases, the abnormalities are bilateral and symmetrical and involve all parts of the lung.

In children and adolescents with the acute form of paracoccidioidomycosis, pulmonary involvement is uncommon, occurring in 10–20% of patients compared with 70–100% of adults. CT scans may reveal localized or diffuse pulmonary infiltrates, hypertrophy of the hilar and paratracheal lymph nodes, and usually unilateral pleural effusion. Many HIV-infected patients have lung lesions; sometimes these individuals present with atypical infiltrates that result from reactivation of the primary focus and from haematogenous dissemination.

18.5.2 Mucocutaneous paracoccidioidomycosis

Ulcerative mucocutaneous lesions of the face, mouth and nose are the most obvious presenting sign of chronic adult form of paracoccidioidomycosis. These occur in 60–80% of cases. In contrast, mucocutaneous lesions are seen in about 25% of children and adolescents with acute disease.

The mouth and nose are the commonest sites of mucosal infection. Lesions in the mouth usually begin as an unspecific gingivitis. Painful, infiltrated, ulcerated lesions then develop on the gums, tongue, lips and/or palate and can progress over weeks or months. Patients may present with poor nutritional status due to dysphagia and/or impairment of mastication. Perforation of the palate or nasal septum may occur. Laryngeal lesions can lead to scar formation and ulceration, resulting in hoarseness and stridor.

Cutaneous lesions often appear on the face around the mouth and nose, although patients with severe infection can have widespread scattered lesions. Small papular or nodular lesions enlarge over weeks or months into plaques with an elevated, well-defined margin. Verrucous lesions or ulcers with a rolled border may develop.

18.5.3 Other forms of disseminated paracoccidioidomycosis

Haematogenous and lymphatic spread of *P. brasiliensis* can result in widespread disseminated infection. Manifestations include nodular or ulcerative lesions of the small or large intestine, hepatic and splenic lesions, adrenal gland destruction, osteomyelitis, arthritis, focal cerebral lesions, encephalitis and meningitis, and infections of the male genitourinary tract.

Lymph node involvement is a common manifestation of paracoccidioidomycosis. In the chronic adult form of the disease, it usually represents secondary involvement of lymph nodes draining sites of mucosal infection, whereas in the juvenile acute form, it is assumed to be a primary involvement. In patients with head and neck disease, all lymph node chains may be involved, but the cervical and sub-mandibular chains are most commonly affected. Magnetic resonance imaging (MRI) is helpful in evaluating lymph nodes that are inaccessible on clinical evaluation. It is not uncommon for infection of the cervical lymph nodes to result in abscess formation and the development of draining sinuses.

About 15% of patients with the adult form of paracoccidioidomycosis develop significant adrenal gland damage; in some, it is the only clinical manifestation of the infection. In contrast, adrenal damage is not seen in children, adolescents or HIV-infected patients with acute disease.

CNS involvement has been reported in 10–25% of adults with the chronic form of paracoccidioidomycosis. It usually presents as part of a widely disseminated infection, but may occur without any other manifestation of the disease. The most common clinical signs are fits, hemiparesis, mental status changes, headaches and intracranial hypertension. Chronic meningitis or meningoencephalitis are less common presentations. CT

and MRI scans are useful for diagnosis, but are not specific. CT scans often reveal single or multiple hypodense lesions with annular or nodular enhancing, surrounded by mild oedema, after contrast injection.

18.6 Differential diagnosis

The cutaneous lesions of paracoccidioidomycosis may be mis-diagnosed as histoplasmosis, sporotrichosis, leishmaniasis, syphilis or psoriasis. Mucocutaneous leishmaniasis is endemic in the same regions as paracoccidioidomycosis and can cause similar lesions. However, nasal lesions are seldom seen in paracoccidioidomycosis. Patients with histoplasmosis may also present with oropharyngeal and laryngeal ulcers that can be differentiated from paracoccidioidomycosis by their elevated borders and the absence of the typical fine granulation surface. Laryngeal tuberculosis is difficult to distinguish from paracoccidioidomycosis. The differential diagnosis of oropharyngeal and laryngeal lesions also includes squamous cell carcinoma and lymphoma. The clinical and radiological manifestations of chronic *P. brasiliensis* infection of the lungs can be confused with tuberculosis or histoplasmosis.

18.7 Essential investigations and their interpretation

18.7.1 Microscopy

Microscopic examination of potassium hydroxide preparations of sputum, bronchoalveolar lavage (BAL) fluid, exudates, pus from draining lymph nodes and other clinical material can permit the diagnosis of paracoccidioidomycosis if the characteristic large, oval to round yeast cells with translucent walls and multiple peripheral buds are seen. Sometimes, however, yeast cells with single buds or small yeast cells may be seen that cannot be differentiated from other fungal pathogens, such as *Histoplasma capsulatum*, *Blastomyces dermatitidis*, or *Sporothrix schenckii*, or capsule-deficient *Cryptococcus* species. Histopathological examination of stained biopsy sections is usually diagnostic: *P. brasiliensis* cells will often be found enclosed within granulomas.

18.7.2 Culture

The definitive diagnosis of paracoccidioidomycosis depends on isolation of the fungus in culture. Mould colonies can be obtained after incubation at 25–30°C for 3–4 weeks on glucose peptone (Sabouraud's dextrose) agar supplemented with cycloheximide (actidione). The organism grows slowly and cultures should be retained for 4–6 weeks before

being discarded. Mycelial cultures seldom sporulate, but sub-culture of the isolate at 37°C on brain heart infusion agar, supplemented with glutamine, should result in production of the yeast form within 1–2 weeks. If more rapid identification is required (24 hours), the initial mould culture can be subjected to an exoantigen test.

There is no commercial DNA probe test for *P. brasiliensis*. The AccuProbe test for identification of *B. dermatitidis* cross-reacts with *P. brasiliensis*, so care must be taken when employing this test on isolates that may be *P. brasiliensis*. Slow growing, non-sporulating isolates are more characteristic of *P. brasiliensis* than *B. dermatitidis*.

18.7.3 Serological tests

Serological tests are useful for the rapid presumptive diagnosis of paracoccidioidomycosis, particularly in cases of untreated disease. These tests may also be used to monitor the response to treatment. The principal antigens used in these tests are derived from culture filtrates of mycelial- or yeast-phase broth cultures of *P. brasiliensis*. Antibody detection is less useful for diagnosis of paracoccidioidomycosis in persons with AIDS.

The immunodiffusion test is performed with mycelial-phase culture filtrate antigen. It is highly specific and is positive in 65–100% of patients with paracoccidioidomycosis. Although cross-reactions can occur in patients with histoplasmosis, these are uncommon. The complement fixation (CF) test, performed with yeast-phase culture-filtrate antigen, is positive in 70–100% of patients, higher titres being obtained in those with more severe disease. A CF titre of at least 1:8 is considered presumptive evidence of paracoccidioidomycosis. However, 85–95% of patients with active disease have CF titres of 1:32 or greater. The CF test is not altogether specific and cross-reactions can occur with serum from patients with other mycotic infections, particularly histoplasmosis. Non-specific CF tests tend to give titres of 1:8. Falling titres of CF antibodies may be predictive of successful treatment, and high or fluctuating titres are suggestive of a poor prognosis.

Other tests, such as indirect enzyme immunoassay (EIA) and immunoblotting, have been developed for the diagnosis of paracoccidioidomycosis. The indirect EIA measures IgG antibodies against a species-specific 43 kDa glycoprotein antigen of *P. brasiliensis*. It is simpler to perform, and more sensitive and specific than the CF test, provided that the antigen is deglycosylated. The EIA test provides a quantitative measurement of titres for use in monitoring the response to treatment. However, cross-reactions with histoplasmosis have been a problem.

A rapid dot immunobinding method for detection of *P. brasiliensis* antibodies to the 43 kDa antigen has also been developed.

18.7.4 Antigen detection tests

Tests for the detection of *P. brasiliensis* antigen in serum, urine, cerebrospinal fluid and BAL fluid appear to be useful, particularly in immunocompromised patients. They may also be helpful in evaluating the response to treatment. No validated commercial tests are currently available. Antigen tests for diagnosis of blastomycosis and histoplasmosis have been reported to give false-positive reactions with urine and other body fluid samples from patients with paracoccidioidomycosis.

18.8 Management

Without appropriate treatment, patients with the chronic form of paracoccidioidomycosis may die within a few years, while those with acute or sub-acute disease will die within a few months. Treatment is usually required for 1–2 years and is often associated with complications and relapse. Along with antifungal treatment, supportive measures such as improved diet and rest are essential to improve the general health of the patient. Agents from three different classes are currently used to treat paracoccidioidomycosis: the sulphonamides, amphotericin B and certain azoles. To date, only one randomized clinical trial has been reported and this found that sulphadiazine and the azoles (itraconazole and ketoconazole) gave similar results.

Sulphonamides were the first agents to be prescribed for the treatment of paracoccidioidomycosis. These drugs are still useful in resource-limited environments or where other drugs are unavailable. Sulphonamide treatment is effective in around 70% of cases, but needs to be continued for 3–5 years to avoid relapse, which occurs in up to 25% of cases.

Sulphadiazine is the most active sulphonamide compound and its use is indicated in non-life-threatening cases of chronic paracoccidioidomycosis, in patients with no history of previous treatment failure after sulphonamide treatment and as maintenance treatment after an initial course of amphotericin B. The usual adult dose of sulphadiazine is 100 mg/kg per day (maximum dose, 4–6 g per day), and this should be given as 1000–1500 mg orally at 6-hour intervals. Children should receive 60–100 mg/kg per day in four divided doses. Treatment must be continued without interruption for several months until clinical and mycological improvement is apparent. Then the dosage may be halved.

Cotrimoxazole is a fixed-ratio (1:5) combination of trimethoprim and sulphamethoxazole, available in oral and parenteral forms. The adult dose is 160–240 mg trimethoprim plus 800–1200 mg sulphamethoxazole at 12-hour intervals. Children should receive 8–10 mg/kg trimethoprim plus 40–50 mg/kg sulphamethoxazole at 12-hour intervals. The drugs should be administered for 12 months to patients with mild forms of paracoccidioidomycosis and for 18–24 months to those with moderate disease.

Oral itraconazole is the drug of choice for the treatment of both the adult and juvenile forms of paracoccidioidomycosis. Most experts now recommend a dosage of 200–400 mg/day in adults or 5 mg/kg per day in children, given for 12 months in mild forms of disease and for 18–24 months in moderate and severe disease. Late relapse has been uncommon (about 5%), and relapsing patients have responded to further treatment with itraconazole. Oral ketoconazole is almost as effective, but less well tolerated than itraconazole. The usual dose is 200–400 mg/day in adults or 5 mg/kg per day in children, given for 6–18 months. Treatment with ketoconazole requires regular follow-ups to prevent hepatic and gonadal dysfunction.

Although it is not licenced for the treatment of paracoccidioidomycosis, voriconazole appears to be as well tolerated and effective as itraconazole for the long-term treatment of the disease. In a recent open-label trial, patients were randomized to receive voriconazole (200 mg twice daily) or itraconazole (100 mg twice daily) for 6 months. A complete or partial response was seen in 88.6% of patients treated with voriconazole compared with 94.4% of those given itraconazole.

Amphotericin B remains a useful drug for the management of severe or refractory paracoccidioidomycosis. The usual regimen is 1.0 mg/kg per day for 4–8 weeks. Thereafter, oral treatment with an azole or a sulphonamide can be substituted. Now that other effective drugs are available, amphotericin B should be reserved for severely ill patients, for those with CNS or cardiac infection and for those unable to tolerate or unresponsive to azole treatment.

The prognosis in patients with paracoccidioidomycosis depends on the initial presentation. Cutaneous lesions will heal after a few weeks of azole treatment, but lymph node regression is slower. Radiographic improvement of pulmonary lesions does not occur until some months after the initiation of treatment. Regardless of the organs involved, paracoccidioidomycosis usually heals by fibrosis, with the formation of granulomata. These persist and constitute a source of *P. brasiliensis* that can lead to a relapse in the disease following cessation of treatment.

18.9 Prevention

It is rare for environmental sites that are contaminated with *P. brasiliensis* to be identified. For this reason, such sites can neither be avoided nor decontaminated. Avoidance of outdoor activities in endemic regions is neither practical nor justified.

The prevalence of paracoccidioidomycosis in immunocompromised patients is too low to justify routine antifungal prophylaxis. The endemic regions of this disease overlap those of histoplasmosis. Therefore, the recommendation that itraconazole prophylaxis is considered in HIV-infected persons who live, or have lived, in areas endemic for histoplasmosis, if their CD4 T-lymphocyte counts are less than 150 cells/μL, should also provide protection against paracoccidioidomycosis.

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CHAPTER 19

Chromoblastomycosis

19.1 Definition

The term chromoblastomycosis (or chromomycosis) is used to refer to a slowly progressive localized infection of the skin and subcutaneous tissue most often involving the distal limbs and characterized by slow-growing verrucous lesions. The disease is endemic worldwide, but more common in the tropics. It is caused by traumatic inoculation of the skin with a number of brown-pigmented (dematiaceous) moulds.

Chromoblastomycosis is distinguished from subcutaneous phaeohyphomycosis (see Chapter 25) and other superficial fungal infections due to dematiaceous fungi (see Chapter 6) by the finding of single or clustered, round, thick-walled, brown cells (termed muriform cells or sclerotic bodies) on microscopic examination of material from the dermis or epidermis, including the keratinized layer.

19.2 Geographical distribution

The disease has a worldwide distribution, but is most common in tropical and sub-tropical regions. It appears to be most prevalent in Central and South America, but it also occurs in southern Africa, Asia and Australia. The largest numbers of reported cases have come from Brazil, Costa Rica, Madagascar and South Africa.

19.3 The causal organisms and their habitat

Chromoblastomycosis is caused by various brown-pigmented (dematiaceous) moulds, the most common of which are *Fonsecaea pedrosoi* and

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Cladophialophora carrionii. *F. pedrosoi* is the most common aetiological agent of chromoblastomycosis worldwide, causing infections in both tropical rainforests such as the Amazon region of Brazil and northern Madagascar, as well as in temperate regions of Latin America. *C. carrionii* is the predominant agent in arid or semidesert regions such as Australia and South Africa. Other important agents are *Phialophora verrucosa*, *Fonsecaea compacta* and *Rhinocladiella acquaspersa*. However, sporadic cases of the disease have also been attributed to a number of other dematiaceous moulds, including *Exophiala jeanselmei* and *Exophiala spinifera*. It should be noted that several of these organisms (including *F. pedrosoi* and *P. verrucosa*) have also been incriminated as aetiological agents of phaeohyphomycosis (see Chapter 25). More recently, molecular analysis has indicated that *F. compacta* is a morphological variant of *F. pedrosoi*.

The aetiological agents of chromoblastomycosis are widespread in the environment of tropical and sub-tropical regions, being found as saprophytes in soil, rotting wood and decomposing plant matter.

19.4 Epidemiology

Infection follows the traumatic inoculation of the fungus into the skin or subcutaneous tissue. Minor trauma, such as abrasions or wounds due to thorns or wood splinters, is often sufficient to introduce the organism. The incubation period is unknown, but the disease is rare in children and adolescents exposed to the same environmental conditions as adults with the disease. This suggests a long latent period before signs of disease begin to appear.

Except in Japan, chromoblastomycosis is more common in men than women, reflecting the importance of occupational exposure. The disease is most prevalent among individuals over the age of 30 years with outdoor occupations, such as farmers, gardeners and agricultural labourers. As with other subcutaneous fungal infections, most affected individuals are otherwise healthy and immunocompetent, and there does not appear to be any predisposition apart from exposure. In many instances, affected persons are poor and lack adequate protective footwear and clothing.

19.5 Clinical manifestations

The clinical manifestations of chromoblastomycosis are similar, regardless of the organism causing it. The lesions are usually unilateral and occur most frequently on the exposed parts of the body, particularly the feet

and lower legs (about 50% of cases). Other less common sites include the hands, arms, buttocks, back, shoulders, neck and face. Most patients present with well-established disease.

The initial lesion is a small painless papule that develops at the site of implantation. If left untreated, this will usually enlarge only slowly to form a superficial nodule with an irregular friable surface. Established infections manifest as multiple large hyperkeratotic verrucous lesions sometimes separated from one another but clustered in the same region. Some lesions are flatter and appear plaque-like, and as they enlarge show central scarring. The lesions are seldom painful (unless bacterial superinfection occurs), but pruritus is frequent and can be severe.

Later in the disease, the lesions may become pedunculated. Autoinoculation from scratching or from superficial lymphatic spread can result in satellite lesions around the original lesion. Bacterial superinfection can occur, resulting in ulceration and a malodorous discharge. Superinfection is also responsible for the lymphadenopathy and lymphoedema that is sometimes seen in patients with longstanding chromoblastomycosis.

In rare cases, metastatic lesions develop in the lymph nodes, brain, liver, bones and other tissues. As with other proliferative epidermal processes, longstanding lesions of chromoblastomycosis can undergo carcinomatous transformation.

19.6 Differential diagnosis

Although the appearance and unilateral location of the lesions on a lower limb is often suggestive of chromoblastomycosis, the disease must be distinguished from a number of other fungal infections, including blastomycosis, paracoccidioidomycosis, phaeohyphomycosis, lobomycosis, rhinosporidiosis and sporotrichosis. It must also be differentiated from protothecosis, verrucose variants of leishmaniasis and tuberculosis, certain leprous lesions and syphilis. On the upper limbs, the erythematosquamous lesions can be confused with psoriasis or subacute or discoid lupus erythematosus.

19.7 Essential investigations and their interpretation

By definition, chromoblastomycosis is characterized by the presence of typical muriform cells in scrapings or biopsy samples. Microscopic examination of material from the lesions is therefore required to make a

definitive diagnosis, and culture should be performed to identify the aetiological agent.

19.7.1 Microscopy

Direct microscopic examination of wet preparations of scrapings from the surface of lesions can permit the diagnosis of chromoblastomycosis if clusters of the characteristic small, round, thick-walled, brown-pigmented muriform cells are seen. These cells are often divided by longitudinal and transverse septa. Muriform cells can also be seen in stained sections of biopsy specimens. In histopathological sections, the epidermis usually shows an intense reaction with pseudo-epitheliomatous hyperplasia and hyperkeratosis. The inflammatory infiltrate is primarily granulomatous with microabscesses.

19.7.2 Culture

Because the aetiological agents cannot be distinguished on the basis of microscopic examination of tissue, material from lesions should be inoculated on glucose peptone (Sabouraud's dextrose) agar and incubated at 25–30°C. Identifiable dark brown or olivaceous to black mould colonies should be formed within 1–2 weeks, but cultures should be retained for 4 weeks before being discarded. Identification of the individual causative agents can be difficult, because they have different mechanisms of sporulation. More recently, molecular methods have begun to be used for the identification of these moulds.

19.8 Management

It is rare for chromoblastomycosis to resolve without treatment: permanent scarring and disfigurement is a common problem, as is bacterial superinfection. Chromoblastomycosis is a difficult condition to treat and is associated with low cure rates and high relapse rates. Treatment choice and outcome depend on the aetiological agent, the extent of the lesions, and the presence of complications such as dermal fibrosis. In patients who present early with small lesions, the goal of treatment should be cure. In those with extensive disease, a more realistic goal is reduction of lesions and prevention of further spread, thereby minimizing the risk of complications such as bacterial superinfection and lymphatic damage.

Surgical excision should be reserved for small lesions; it carries a high risk of local dissemination and should only be attempted in conjunction

with antifungal treatment both before and after surgery. Liquid nitrogen treatment of small lesions is usually successful and sometimes requires only a single application. Cryotherapy of large lesions is usually done in stages and is more effective if combined with antifungal treatment. Thermotherapy involves the daily application of heat (42–45°C) directly onto the lesions for several hours for 2–6 months. It has been used with some success in Japan for patients with less extensive lesions.

There is no ideal antifungal treatment for chromoblastomycosis. The most effective drugs are itraconazole (200–400 mg/day) and terbinafine (500–1000 mg/day) for at least 6–12 months, preferably at the higher dosage if tolerated. Pulse itraconazole (200 mg twice daily for one week every month) has been found to be as effective as the daily regimen. Higher response rates have been reported with more prolonged treatment. Antifungal therapy should always be continued for several months after clinical cure is obtained in order to reduce the risk of relapse.

Some cases have been treated with itraconazole and terbinafine in combination. However, given the anecdotal and non-comparative nature of the studies, it is unclear whether combination treatment is more effective than either drug alone. Ketoconazole (200–400 mg/day) has been useful in some cases, but it requires regular follow-ups to prevent hepatic and gonadal dysfunction. Fluconazole appears to be ineffective. There are also a few anecdotal reports of the successful use of posaconazole (400 mg twice daily), but experience with this agent is limited due to its prohibitive cost in the major endemic regions. In the EU, posaconazole has been licenced for the salvage treatment of chromoblastomycosis.

Flucytosine (100–200 mg/kg per day given as four divided doses) for 6–12 months has proved effective in more than 50% of reported cases, but incomplete lesion resolution, relapse and development of resistance are common problems. Much better results have been obtained when flucytosine is combined with oral thiabendazole (25 mg/kg per day given as three divided doses) or amphotericin B (0.5–1.0 mg/kg per day). Both regimens appear to prevent the development of flucytosine resistance, but toxicities can be problematic.

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CHAPTER 20

Entomophthoromycosis

20.1 Introduction

The term entomophthoromycosis is now used to describe a group of fungal infections caused by moulds belonging to the order Entomophthorales. Traditionally, this order was assigned to phylum Zygomycota together with the order Mucorales, which contains the aetiological agents of mucormycosis (see Chapter 13). However, following molecular analysis, the phylum Zygomycota is no longer accepted due to its polyphyletic nature. The subphylum Mucoromycotina has been proposed to accommodate the Mucorales and the subphylum Entomophthoromycotina has been created for the Entomophthorales.

Two distinct clinical forms of entomophthoromycosis are recognized: basidiobolomycosis and conidiobolomycosis, caused by species of *Basidiobolus* and *Conidiobolus*, respectively. These diseases are usually slowly progressive subcutaneous infections that affect immunocompetent individuals and are transmitted through traumatic implantation of plant debris in tropical environments.

20.2 Basidiobolomycosis

20.2.1 Definition

The most common form of basidiobolomycosis is a chronic subcutaneous infection of the trunk and limbs caused by *Basidiobolus ranarum*. However, this mould has also caused rare cases of inflammatory disease of the gastrointestinal tract.

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20.2.2 Geographical distribution

The largest number of cases of subcutaneous disease has been reported from the tropical regions of East and West Africa and Indonesia. Sporadic cases have also been described from tropical or subtropical regions of Asia, Australia and South America. Gastrointestinal basidiobolomycosis is a rare disease that has been described in Brazil, Nigeria, and the Middle East. A small cluster of cases has been reported from the desert southwest of the United States.

20.2.3 The causal organism and its habitat

B. ranarum (formerly *Basidiobolus meristosporus* or *Basidiobolus haptosporus*) has been isolated from soil and decomposing vegetation throughout the world. It has also been recovered from the intestinal contents of several species of insectivorous bats, reptiles and amphibians.

20.2.4 Epidemiology

It is still uncertain how the disease is acquired, but subcutaneous infection is thought to follow traumatic inoculation. Minor trauma, such as a thorn prick or an insect bite, is often sufficient to introduce the organism. Basidiobolomycosis is unusual among subcutaneous fungal infections in that it is more common in children and adolescents than in adults. As with other subcutaneous fungal infections, most affected individuals are otherwise normal, and there does not appear to be any predisposition apart from exposure.

It is thought that gastrointestinal disease is acquired through ingestion of soil, animal faeces, or food contaminated by either of them. Potential risk factors include prior ranitidine use.

A case of lethal disseminated basidiobolomycosis secondary to gastrointestinal infection has been reported in an immunocompetent individual.

20.2.5 Clinical manifestations

Basidiobolomycosis typically presents as a slowly progressive subcutaneous infection of the buttocks and thighs, but the hard, movable subcutaneous swellings that characterize this condition may also be found on the trunk, arms, legs, shoulders or face. Pain and tenderness are unusual. This is a disfiguring infection, but the skin covering the lesions remains intact. The underlying bone and joints are not affected. Lymphatic obstruction may occur and can result in elephantiasis.

The presenting symptoms and signs of gastrointestinal basidiobolomycosis are non-specific. Most patients have a subacute onset of symptoms,

usually beginning with abdominal pain. Other symptoms include fever, constipation, diarrhoea, weight loss, and sometimes chills and rigors. Abdominal computed tomographic scanning and barium enema examination will reveal nodular masses and ulcerations, indistinguishable from malignancies or inflammation. It can involve the stomach, small intestine, colon or rectum.

The clinical manifestations of disseminated *Basidiobolus* infection are similar to those of mucormycosis (see Chapter 13).

20.2.6 Essential investigations and their interpretation

The clinical appearance of the subcutaneous disease is distinctive, but a firm diagnosis requires mycological and histopathological confirmation to distinguish it from other causes of chronic cellulitis. The diagnosis of gastrointestinal basidiobolomycosis requires the microscopic examination and culture of endoscopic biopsy specimens.

Microscopic examination of histopathological sections of infected tissue will reveal focal clusters of inflammation containing wide, irregular, hyphae or fragments thereof. The hyphae are usually surrounded by eosinophilic material (Splendore-Hoeppli phenomenon). Septations within the hyphae are infrequent; nonetheless, they are more prominent than those seen with the Mucorales. In contrast to the Mucorales, the hyphae of the Entomophthorales are rarely angioinvasive.

Specimens should be cultured on glucose peptone (Sabouraud's dextrose) agar at 30°C. Identifiable colonies should be obtained in less than 1 week. *B. ranarum* grows well at 30°C, but less rapidly at 37°C.

20.2.7 Management

The treatment of choice appears to be oral itraconazole (100–200 mg/day), which should be given for several months. Oral ketoconazole (400 mg/day) has sometimes been successful, but amphotericin B has seldom been helpful. Saturated potassium iodide solution is another alternative. The starting dose is 1 mL three times daily and this is increased up to 4–6 mL three times daily as tolerated. In some cases, trimethoprim-sulphamethoxazole (cotrimoxazole) has been found to be more effective than potassium iodide. The recommended dose is two tablets three times daily (each tablet contains 80 mg trimethoprim and 400 mg sulphamethoxazole). As with potassium iodide solution, treatment should be continued for 1 month after the lesions have cleared.

Should a patient have an enlarged, useless limb resistant to medical treatment, amputation should be considered, to forestall bacterial superinfection.

Patients with gastrointestinal basidiobolomycosis should undergo resection of all affected bowel and debridement of other involved tissues. This should be followed by at least 3 months of antifungal treatment with itraconazole.

20.3 Conidiobolomycosis

20.3.1 Definition

This is a chronic localized subcutaneous fungal infection caused by *Conidiobolus* species. It originates in the nasal mucosa and spreads to the adjacent subcutaneous tissue of the face, causing severe disfigurement. There have been a few reports of lethal disseminated disease in both immunocompetent and immunocompromised individuals.

20.3.2 Geographical distribution

The largest number of cases of this disease has been reported from West Africa, in particular Nigeria and Cameroon, but cases have also occurred in Madagascar, India, China and South and Central America.

20.3.3 The causal organisms and their habitat

There are more than 20 species in the genus *Conidiobolus*, but only two (*Conidiobolus coronatus* and *Conidiobolus incongruus*) have been recovered from human infections. Both are found in the soil, on decaying wood and on decomposing vegetation in tropical rain forests. *C. coronatus* is an occasional pathogen of insects. It has also has been recovered from the intestinal contents of lizards and toads.

20.3.4 Epidemiology

In most cases, the organism is introduced to the nasal mucosal by the soiled hands of the patient himself. In some instances, the infection is thought to follow spore inhalation. The disease can occur at any age, but is uncommon in children. Most cases affect men with agricultural or outdoor occupations, living in the tropical rain forests of Africa. As with other subcutaneous fungal infections, affected individuals are otherwise normal, and there does not appear to be any predisposition apart from exposure.

20.3.5 Clinical manifestations

Conidiobolomycosis may present as nasal obstruction, which is often unilateral, but it is often not diagnosed until there is noticeable swelling on the upper lip or face. Nasal discharge is a common finding, but pain, tenderness and constitutional upset are rare. If left untreated, the infection

may spread to involve the nasal bridge and upper and lower face, including the orbit. The swelling is firm and painless, and it is the resulting gross facial distortion that brings the patient for treatment.

The condition affects soft tissue, but the underlying bone is spared. The lesions have a distinct margin, but the mass is not movable over the underlying tissue. The skin is stretched, but remains intact. Invasion of local lymph nodes has been described.

The clinical manifestations of disseminated *Conidiobolus* infection are similar to those of mucormycosis (see Chapter 13).

20.3.6 Essential investigations and their interpretation

Even if, in advanced cases, the diagnosis is obvious from the typical clinical appearance, laboratory investigation is essential for its confirmation.

The diagnosis is best established by microscopic examination of smears or biopsy samples from the nasal mucosa. In stained tissue sections, the organisms appear as broad, thin-walled, irregularly branched hyphae and in most cases are surrounded by eosinophilic material (Splendore-Hoeppli phenomenon). Septations within the hyphae are infrequent; nonetheless, they are more prominent than those seen with the Mucorales. In contrast to the Mucorales, the hyphae of the Entomophthorales are rarely angioinvasive.

Culture is difficult. To optimize the recovery of fungus from clinical material, specimens must be inoculated on the largest possible number of media; they should be incubated at 25–30°C and 37°C. *Conidiobolus* grows rapidly at 37°C.

20.3.7 Management

Treatment of this condition is difficult, but patients often respond to itraconazole (200–400 mg/day), ketoconazole (200–400 mg/day) or fluconazole (200 mg/day). Treatment should be continued for at least 1 month after the lesions have cleared.

Saturated potassium iodide solution is useful for patients in resource-limited settings, because of its ease of administration and low cost. The starting dose is 1 mL three times daily, and this is increased up to 4–6 mL three times daily as tolerated. Treatment must be continued for at least 1 month after the lesions have disappeared. Allergic reactions and gastrointestinal intolerance are common complications, and relapse is common even long after successful treatment.

Surgical resection of infected tissue is seldom successful; it may hasten the spread of infection.

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CHAPTER 21

Mycetoma

21.1 Definition

The term mycetoma is used to describe a slowly progressive, suppurative disease that affects the skin, the underlying subcutaneous tissue and sometimes adjacent muscle, connective tissue and bone. The disease usually involves the feet or hands and may be caused by various species of fungi (eumycetoma) or aerobic actinomycetes (actinomycetoma) that have been inoculated into subcutaneous tissue as a result of traumatic implantation. A characteristic feature of mycetoma is the production in infected tissue of abscesses that contain large compact masses of fungal or actinomycete filaments termed 'grains'. These are discharged to the outside through sinus tracts.

21.2 Geographical distribution

Mycetomas are most common in arid tropical and sub-tropical regions between latitudes of 15°S and 30°N, the Indian subcontinent and Central and South America. The countries surrounding the Saharan and Arabian deserts (particularly Senegal, Sudan and Somalia) form the most important endemic region, but the disease is also highly endemic in Mexico, Venezuela, India and Pakistan. Occasional sporadic cases have been reported from many other parts of the world.

21.3 The causal organisms and their habitat

More than 20 species of fungi and actinomycetes have been implicated as aetiological agents of mycetoma. Many of these organisms have been

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isolated from the soil or from plants or trees, or from decomposing vegetation. About six species of fungi are common causes of eumycetoma, and five aerobic actinomycetes are common aetiological agents of actinomycetoma.

The fungi involved include *Madurella mycetomatis* (about 70% of reported cases), *Scedosporium apiospermum* (about 10% of reported cases), *Leptosphaeria senegalensis*, *Madurella grisea*, *Neotestudina rosatii* and *Pyrenochaeta romeroi*. Other fungi that have sometimes been implicated as causes of eumycetoma include *Acremonium* species and *Aspergillus nidulans*. Actinomycetomas are caused by aerobic, Gram-positive filamentous actinomycetes belonging to the genera *Actinomadura*, *Nocardia* and *Streptomyces*, including *Actinomadura madurae*, *Actinomadura pelletieri*, *Nocardia asteroides*, *Nocardia brasiliensis* (the most common organism) and *Streptomyces somaliensis*.

The predominant causes of mycetoma differ from one part of the world to another (see Table 21.1). The most important factor responsible for this variation is believed to be the climate, particularly the annual amount of rainfall. Worldwide, the prevalence rates of eumycetoma and actinomycetoma are similar. However, eumycetomas are the predominant form of the disease in Africa and southern Asia, while actinomycetomas are more common in Latin America.

Table 21.1 Aetiological agents of mycetoma

Species	*Geographical distribution*	*Colour of grain*
Eumycetomas		
Leptosphaeria senegalensis	West Africa	Black
Madurella grisea	Africa, Central and South America	Black
Madurella mycetomatis	Worldwide	Black
Neotestudina rosatii	Africa	White
Pyrenochaeta romeroi	Africa and South America	Black
Scedosporium apiospermum	North America	White
Acremonium species	Africa and Middle East	White
Aspergillus nidulans	Africa and Middle East	White
Actinomycetomas		
Actinomadura madurae	Worldwide	White/yellow
Actinomadura pelletieri	Africa	Red
Nocardia asteroides	Worldwide	White/yellow
Nocardia brasiliensis	Central America	White/yellow
Streptomyces somaliensis	North Africa and Middle East	Yellow/brown

21.4 Epidemiology

Infection results from the traumatic implantation of the pathogen into the skin or subcutaneous tissue. In some cases, the organisms are introduced on thorns or wood splinters; in others, infection is due to later contamination of the wound with soil organisms. The initial lesion appears several months after the traumatic incident, but patients most commonly present for medical attention with long-standing infection.

In some countries, men are affected more frequently than women, but there is no consistent differential sex distribution. Most patients have outdoor occupations that expose them to soil and minor penetrating wounds. Mycetoma can affect all age groups, but is most common in individuals between 20 and 40 years of age. As with other subcutaneous fungal infections, affected individuals are otherwise normal, and there does not appear to be any predisposition apart from exposure.

21.5 Clinical manifestations

The clinical features of the eumycetomas and actinomycetomas are similar, although eumycetomas tend to follow a slower and less destructive course. Although most patients present with long-standing infections, the initial lesion consists of a small, firm, painless subcutaneous nodule that can either be moved under the skin or is attached to it. With time, the nodule evolves to form an abscess with multiple sinuses that discharge a serosanguinous, viscous or purulent exudate containing the characteristic grains. These consist of clusters of actinomycotic or fungal filaments and vary in colour and size depending on the aetiological agent (Table 21.1).

Mycetomas are most common on the feet (about 70–85% of cases), particularly among those who go barefoot, followed by the hands (about 5–10% of cases) and other parts of the body that come into contact with the soil or vegetation during working, sitting or lying. Other affected sites include the back, nape of the neck and back of the head, particularly in individuals who carry wood or loads contaminated with soil.

Mycetoma lesions present as swellings covered with hypo- or hyperpigmented skin. As old sinus tracts heal, new ones appear. In time, the infection spreads to adjacent bone, leading to a destructive form of osteomyelitis. Depending on the location and size of the lesion, and also on any bone involvement, movement of the joints in the affected parts may be impaired. In spite of their unpleasant appearance, most mycetomas are

painless, even when well established. However, in about 20% of cases, pain is the main complaint that brings patients to hospital. In most cases, pain heralds the impending rupture of a sinus onto the skin surface.

In general, eumycetomas tend to remain localized, and swelling and destruction of adjacent anatomical structures is not marked until late in the course of the disease. In actinomycetoma infections, the lesions have less well-defined margins and tend to merge with the surrounding tissue. Progression is often more rapid, and involvement of bone is earlier and more extensive. Mycetomas affecting the back can spread to the spine, producing compression of the spinal cord and leading to paraplegia or other neurological manifestations depending on the site of the lesions.

Radiological examination is useful in determining the extent of bone involvement. The most common and distinctive finding is focal bone destruction with formation of cavities. These are small and abundant in cases of actinomycetoma and larger and less numerous in eumycetoma. Early evidence of bone involvement can be provided by bone scan, magnetic resonance imaging (MRI) and echoscans, although the interpretation of the latter is difficult. MRI scans are helpful in delineating the extent of the lesions.

Mycetomas often spread to adjacent tissues, but spread to the regional lymph nodes and involvement of deep organs is rare. Although lymphatic spread is found in some cases of eumycetoma, it is encountered more often in cases of actinomycetoma, where the grains are smaller. In both cases, repeated surgical interference seems to be an aggravating factor. Bacterial superinfection of the initial mycetoma lesion is common and is a frequent cause of regional lymph node enlargement and impairment of the general health of the patient.

21.6 Differential diagnosis

Mycetomas should be considered as part of the differential diagnosis of all subcutaneous swellings in patients in endemic regions. In most cases, the diagnosis of mycetoma of the foot presents no problems, but it may be difficult if other body sites are involved, particularly if no grains have been discharged at the time of examination.

The characteristic features of mycetoma are the presence of a subcutaneous mass, sinuses and discharging grains that are found to contain actinomycotic or fungal filaments. These findings distinguish mycetoma from chromoblastomycosis, botryomycosis, cutaneous tuberculosis and other conditions.

21.7 Essential investigations and their interpretation

Examination of grains is required to make a definitive diagnosis of mycetoma, and culture should be performed to identify the aetiological agent. Correct species identification is essential in order to select the most appropriate drug treatment.

21.7.1 Gross examination

The diagnosis of mycetoma depends on the identification of grains. These should, if possible, be obtained from an unruptured pustule (sinus) with a sterile needle, by puncturing the roof of the lesion and squeezing the sinus contents onto a glass slide. The grains should be picked out, rinsed in 70% alcohol and then washed in sterile saline before being cultured. Failing this, it is best to obtain material through a deep surgical biopsy. Superficial biopsies are seldom helpful.

Gross examination of the grains may afford a clue as to the aetiological agent (see Table 21.1). Black grains suggest a fungal infection; minute white grains often indicate a *Nocardia* infection and larger white grains the size of a pinhead may be of either fungal or actinomycotic origin. Small, red grains are specific to *A. pelletieri*, but yellowish-white grains may be actinomycotic or fungal in origin.

21.7.2 Microscopy

Direct microscopic examination of the grains will confirm the diagnosis of mycetoma and will also reveal whether the causal organism is a fungus or an actinomycete. Actinomycotic grains contain very fine filaments (<1 μm diameter), whereas fungal grains contain masses of short entangled hyphae (2–4 μm diameter) that are sometimes pigmented. This can be seen by direct microscopic examination of crushed grains in 20% potassium hydroxide, but is much more readily observed in stained histopathological sections. These will reveal a granulomatous inflammatory reaction with grains located within the abscess.

21.7.3 Culture

Although the identification of the causal agents of mycetoma can often be deduced from the morphological characteristics of the grains, it is also important to isolate the organism in culture. Agar plates should be inoculated with several grains (or with secretion or tissue fragments) and incubated at 25–30°C and at 37°C. The most commonly used agar medium is glucose peptone (Sabouraud's dextrose) agar, without antibiotics but with cycloheximide (actidione) for isolation of actinomycetes and with

antibiotics but without cycloheximide for fungal agents. Alternative media for isolation of actinomycetes include brain–heart infusion or blood agar. Cultures should be retained for up to 6 weeks before being discarded. Identification of actinomycetes is based on biochemical tests. More recently, molecular methods of species identification have begun to be used.

21.7.4 Serological tests

Although serological tests are sometimes available, they are seldom sufficiently informative to be helpful in diagnosis. However, these tests are helpful in monitoring the therapeutic response and can provide the earliest clue to reactivation of infection, particularly with actinomycetomas.

21.8 Management

It is essential to distinguish eumycetomas from actinomycetomas because the treatment of these conditions is different, and the drugs used to treat one are ineffective in the other.

The treatment of eumycetoma is challenging and often requires a combination of medical and surgical treatment. Medical management is justified in patients without bone disease and where regular supervision of the patient over a number of months is anticipated. Surgical management is indicated for limited and localized disease where complete removal of the lesion is possible and for patients with advanced disease unresponsive to antifungal treatment. It is essential to administer drug treatment both before and after surgical intervention.

Surgical management is more successful when the disease is limited and well defined. However, local surgical excision of eumycetoma lesions is seldom effective and relapse is not uncommon. Radical surgical removal (amputation) often becomes unavoidable in those with advanced disease. However, because the lesions are slow to spread and because it appears possible to slow the progression of the infection, the decision regarding amputation must take into account the progress and symptoms, the availability of prostheses, and the circumstances of the individual patient. If the lesions affect the back, neck, back of the head or other sites where amputation is impossible, little can be done to help the patient.

The most effective drugs for eumycetoma are itraconazole (200–400 mg/day) and terbinafine (500–1000 mg/day) given for 18–24 months or longer, preferably at the higher dosage if tolerated. Ketoconazole (400–800 mg/day) has been useful in some cases, but it requires regular follow-ups to prevent hepatic and gonadal dysfunction. Fluconazole has been found

to be ineffective. Voriconazole (400–600 mg once daily) and posaconazole (400 mg twice daily) are other options, but experience with these agents is limited due to their prohibitive cost in the major endemic regions. In the EU, posaconazole has been licenced for the salvage treatment of eumycetoma. Even with the newer triazoles, long-term treatment is still required.

In contrast to eumycetoma, medical treatment is usually effective in all but the most advanced cases of actinomycetoma. If surgical intervention is required, it often needs to be more extensive than for eumycetoma to ensure an adequate excision margin of non-diseased tissue.

The aetiological agent will determine the choice of antimicrobial agent but, in most cases of actinomycetoma, combination treatment is preferable to the administration of a single drug. Combined drug treatment will help to prevent the development of resistance and to eradicate any residual infection. Sulphonamides, such as sulphamethoxazole and dapsone, and sulphonamide combinations are among the most important drugs for the treatment of actinomycetoma. The historic standard for treatment of this disease is a fixed-ratio (1:5) combination of trimethoprim and sulphamethoxazole (cotrimoxazole), as this is more effective than sulphonamides alone. Trimethoprim-sulphamethoxazole has been used for periods of time ranging from 6 months to several years, with an average success rate of 60–70%. The addition of an aminoglycoside to trimethoprim-sulphamethoxazole has given higher success rates and allows for shorter periods of treatment. The aminoglycosides that have been used include streptomycin, amikacin, gentamicin and kanamycin. There are anecdotal reports of therapeutic success with the carbapenem, imipenem and the oxazolidinone, linezolid. However, their high cost precludes their use in resource-limited settings.

In cases of actinomycetoma due to *Nocardia* species, the combination of choice consists of trimethoprim-sulphamethoxazole (80–160 mg trimethoprim plus 400–800 mg sulphamethoxazole per day) plus dapsone (100–200 mg/day). In cases of *A. pelletieri* or *S. somaliensis* actinomycetoma, the best combination is trimethoprim-sulphamethoxazole plus streptomycin (1000 mg/day; intramuscular injection). The total dose of streptomycin should not exceed 50 g and treatment should be discontinued if ototoxic side effects develop. In cases that are resistant to trimethoprim-sulphamethoxazole, this drug can be replaced with dapsone (100–200 mg/day). Cases due to *A. madurae* are best treated with streptomycin plus dapsone.

In serious cases with difficult locations (such as the back or head), the best choice is amikacin (15 mg/kg per day). This should be administered as a single intramuscular or intravenous dose for 2–3 weeks

followed by a similar resting period. Three to five pulses are recommended. This treatment should be combined with continuous trimethoprim-sulphamethoxazole, and once the amikacin pulses have been completed, longer-term trimethoprim-sulphamethoxazole treatment should be prescribed. Amikacin treatment has proved to be one of the best treatments for *Nocardia* actinomycetomas, but is less successful in cases due to *A. madurae*. Where the actinomycete is resistant to amikacin, netilmicin (300 mg/day) may be an alternative treatment.

Treatment must be continued until the pain and swelling diminish, the discharge of secretion and grains stops, and the sinuses close. Even if these signs have disappeared, it is prudent to continue treatment for the same period of time as was required to achieve these results.

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CHAPTER 22

Sporotrichosis

22.1 Definition

The term sporotrichosis is used to refer to subacute or chronic infections caused by the thermally dimorphic fungus, *Sporothrix schenckii*. Following traumatic implantation, this organism can cause cutaneous or subcutaneous infection that commonly shows lymphatic spread. Occasionally, infection of the lungs, joints, bones or other sites occurs in predisposed persons.

22.2 Geographical distribution

Sporotrichosis is worldwide in distribution, but is most common in warm, temperate or tropical climatic regions. In recent years, the largest number of reported cases has been from Latin America and Japan. Other regions where the infection is endemic include North America, Africa, India and Australasia.

22.3 The causal organism and its habitat

S. schenckii is a thermally dimorphic fungus that is found in the soil, on decomposing vegetation and on plant materials, such as timber, hay and sphagnum moss. It exists in nature as a mould, but in human and animal tissue, it forms small budding yeast cells. Recently, molecular studies have demonstrated that *S. schenckii* is not a single species but constitutes a species complex of numerous phylogenetic species. Apart from *S. schenckii*, the species *Sporothrix brasiliensis*, *Sporothrix globosa* and *Sporothrix luriei* have also been implicated in human infections.

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22.4 Epidemiology

Infection usually follows the traumatic implantation of *S. schenckii* into the skin or subcutaneous tissue. Minor trauma, such as abrasions or wounds due to thorns or wood splinters, is often sufficient to introduce the organism. Zoonotic sporotrichosis occurs with exposure to infected animals, most commonly cats. Infection has also been transmitted from uninfected animals that transfer the organisms from soil through scratching or biting. The initial lesion usually appears 1–4 weeks after inoculation. Pulmonary and disseminated forms of infection, although uncommon, follow spore inhalation.

Infection with *S. schenckii* occurs in all age groups, but is more common in adults than children. There is no clear gender or racial predilection. Most infections are sporadic and associated with trauma sustained during the course of outdoor work or recreational activities. The disease is most prevalent among individuals whose occupation brings them into contact with soil, plants or plant materials, such as gardeners, florists, agricultural labourers and carpenters. The precise incidence of sporotrichosis has not been determined, but active population-based surveillance, conducted in the United States in 1992 and 1993, indicated that fewer than 10 persons per 100,000 developed sporotrichosis per annum.

Sporotrichosis is the most common subcutaneous mycosis in Latin America. It has been shown to be hyperendemic in a poor rural region in the south central highlands of Peru. Between 1997 and 1999, active population-based surveillance showed that the disease developed in 98 persons per 100,000 per annum. Unlike other parts of the world, the incidence was three times higher among children (156 cases per 100,000) than among adults (52 cases per 100,000). Information on risk factors in this setting is limited, but higher socioeconomic status in this region was associated with a lower incidence of infection.

Most cases of sporotrichosis are sporadic in nature. There have, however, been numerous reports of clusters of cases or outbreaks and these have led to important insights into the risk factors for acquisition of the disease. Outbreaks have occurred in different populations, including gardeners, agricultural workers and persons in contact with domestic cats. One of the earliest and largest outbreaks occurred in the South African gold mines in the 1940s when more than 3000 cases were recorded, the source of infection being contaminated timber used as pit props. In the United States, several recent outbreaks of sporotrichosis, including one that involved 84 cases in 15 states, have been associated with handling contaminated sphagnum moss. A large outbreak of zoonotic

sporotrichosis has been ongoing in the metropolitan region of Rio de Janeiro, Brazil, since 1998. Most cases have occurred among adults and children who own or care for infected domestic cats, veterinarians and others whose occupation involves cat care.

As with other subcutaneous fungal infections, most affected individuals are otherwise healthy and immunocompetent, and there does not appear to be any predisposition apart from exposure. However, individuals with human immunodeficiency virus infection or other immunosuppressive conditions, such as chronic alcoholism or diabetes mellitus, and those receiving corticosteroids or other immunosuppressive treatments, such as tumour necrosis factor-alpha blockers, may be at risk for the development of extracutaneous or disseminated forms of sporotrichosis.

22.5 Clinical manifestations

Sporotrichosis has a wide range of clinical manifestations. The most common presentation is a localized cutaneous or subcutaneous lesion. Lymphatic spread may then lead to the development of further cutaneous lesions. In contrast to adults, where infection can occur at any anatomical site, the face and upper limbs, particularly the hands and fingers, are the most common sites of infection in children. Much less commonly, the fungus may cause infection of the lungs, joints, bones, meninges and other sites. Widespread disseminated infection has been reported in diabetics and alcoholics, in drug abusers and in persons with the acquired immunodeficiency syndrome (AIDS). Extracutaneous forms of sporotrichosis are rare in children.

22.5.1 Lymphocutaneous sporotrichosis

This is the most common clinical form of sporotrichosis. It tends to affect exposed sites, such as the limbs, and in particular the hands and fingers. The right hand is affected more frequently than the left.

Following inoculation, the fungus replicates at the site of infection and then invades the regional lymphatics that drain the infected site. However, in about 25% of cases, the infection remains confined to the initial site of inoculation. Such ‘fixed’ infections are more common in children than in adults. Facial lesions also often behave in this manner.

The initial lesion is a small, firm, painless nodule that is at first movable, but later becomes attached to the neighbouring tissue. Over the ensuing weeks, the lesion slowly enlarges, and the skin and subcutaneous tissue surrounding the lesion become indurated and purplish red. The nodule becomes soft and breaks down to form a painless superficial ulcer that

discharges a serous or purulent fluid. The edge of the ulcer is often irregular and it may become oedematous and crusted.

In most cases, the infection spreads to the regional lymph nodes that drain the site of the primary infection. The resulting ascending chain of non-tender, mobile subcutaneous nodules with overlying erythematous skin is the characteristic clinical presentation of sporotrichosis. If left untreated, the nodules slowly enlarge, soften, and evolve into painless draining ulcers.

Fixed cutaneous lesions can be plaque-like or verrucous; ulceration is uncommon. Although these lesions sometimes remit, they usually return and can persist for years until treated.

In the disseminated cutaneous form of sporotrichosis, numerous small papular or nodular lesions that progress to necrotic ulcers are distributed over the trunk and limbs. This form of sporotrichosis occurs in less than 1% of patients and is usually seen in individuals with an underlying disease or predisposition. It generally follows lymphatic or haematogenous spread from an initial cutaneous or pulmonary site of infection, but may also be due to multiple cutaneous inoculations.

22.5.2 Extracutaneous sporotrichosis

Extracutaneous forms of sporotrichosis are most commonly seen in individuals with an underlying disease or predisposition, such as diabetes mellitus, alcoholism or AIDS. The most common sites of involvement are the lungs, joints and bones, but occasional cases of endophthalmitis and meningitis have been reported.

Pulmonary sporotrichosis is an uncommon, subacute or chronic infection. It is typically seen in middle-aged men with underlying risk factors of chronic obstructive pulmonary disease and alcoholism. It usually follows the inhalation of spores and is sometimes associated with enlargement of the hilar lymph nodes. In some cases, it follows hematogenous dissemination from another site of inoculation, and patients may also have cutaneous and/or osteoarticular lesions. The symptoms are non-specific and include productive cough, fever, weight loss, loss of appetite, breathlessness and haemoptysis. The latter can be massive and fatal. The most common radiological findings are unilateral or bilateral upper lobe fibronodular or cavitary lesions, similar to those seen in reactivation tuberculosis. The outcome of this condition is usually poor, often because of delay in diagnosis and severe underlying pulmonary disease.

Arthritis results from the spread of an adjacent subcutaneous infection, or from direct inoculation into the joint or from haematogenous dissemination. It is an indolent, progressive infection that tends to affect the knee

and other large weight-bearing joints, although the small joints of the hand and wrist are sometimes involved. The most common symptoms are stiffness, pain and swelling of the affected joint. It is not unusual for multiple joints to be involved. Overlying cutaneous lesions may or may not be present, and one or more joints may be involved. Tenosynovitis and bursitis are complications.

In most cases, bone disease results from the spread of infection from an adjacent subcutaneous or joint lesion. In some cases, however, it occurs as a result of haematogenous dissemination. It is a chronic and indolent infection that tends to affect the long bones. Patients often complain of focal pain, with signs of focal tenderness and minimal swelling on examination. The most common radiological finding is a lytic lesion with a periosteal reaction. Most patients have concomitant arthritis.

Endophthalmitis, although rare, may result in blindness; chorioretinitis has also been reported. Occasional cases of meningitis have also been reported; the symptoms are identical to those of tuberculous meningitis. Fever and headache are prominent symptoms, and the cerebrospinal fluid findings are those of lymphocytic meningitis with an elevated protein concentration and a lowered glucose concentration. Meningitis may be an isolated finding or a manifestation of widespread dissemination. Most of the recently reported cases of meningeal sporotrichosis have been in patients with AIDS. Disseminated sporotrichosis is very uncommon, with most cases being reported in individuals with AIDS. It may present as widespread ulcerative cutaneous lesions with or without concomitant visceral involvement.

22.6 Differential diagnosis

The diagnosis of sporotrichosis should be suspected in any patient with painless ulcerative lesions of the skin that are unresponsive to antibacterial treatment. The development of ipsilateral, ascending lymphatic nodules is also suspicious. Less typical manifestations must be differentiated from a wide range of infectious diseases, including blastomycosis, chromoblastomycosis, paracoccidioidomycosis, leishmaniasis, nocardiosis, subcutaneous abscesses of tularaemia and atypical mycobacterial infections, particularly those due to *Mycobacterium marinum*. Differentiation of the fixed cutaneous form of sporotrichosis from pyoderma gangrenosum can be difficult. Pulmonary sporotrichosis must be differentiated from tuberculosis, chronic cavitary histoplasmosis and sarcoidosis.

22.7 Essential investigations and their interpretation

22.7.1 Microscopy

Direct examination of clinical material, such as pus or tissue, is less sensitive than culture because the organisms are usually present in small numbers. However, the detection of typical round, oval or cigar-shaped cells of *S. schenckii* will help to confirm the diagnosis. The organisms are difficult to detect with hematoxylin and eosin staining, and methenamine-silver (Grocott or Gomori) and periodic acid-Schiff staining should be performed. Immunofluorescence staining has also proved to be a sensitive and specific method for detecting low numbers of *S. schenckii* cells. In some cases of sporotrichosis, a tissue reaction consisting deposits of star-like eosinophilic material, termed asteroid bodies, may be seen surrounding the yeast cells. This is also known as the Splendore Hoeppli phenomenon; it is an important feature of sporotrichosis, but can be seen in a number of other bacterial, fungal and parasitic diseases.

22.7.2 Culture

The definitive diagnosis of sporotrichosis depends on the isolation of the aetiological agent from material aspirated from a lesion or a tissue biopsy specimen. Clinical material should be inoculated onto several media, including glucose peptone (Sabouraud's dextrose) agar and brain–heart infusion agar, and incubated at 25–30°C. Identifiable mould colonies should appear in 3–5 days, but cultures should be retained for up to 4 weeks before being discarded. The colour of the colonies usually changes from cream or light brown to dark brown or black with age. Confirmation of the identification depends on the morphological characteristics of the mould form and its conversion to the yeast form on media such as blood agar or brain heart infusion agar at 37°C. Occasional isolates are difficult to convert and may require multiple subcultures and extended incubation. Transition is observed in environmental as well as clinical isolates.

22.7.3 Serological tests

At present, serological tests are not widely available for the diagnosis of sporotrichosis. Immunodiffusion and agglutination tests can be used to detect antibodies to *S. schenckii*, but are more helpful in diagnosing the unusual extracutaneous forms of sporotrichosis than in detecting cutaneous infection.

22.8 Management

Lymphocutaneous and cutaneous forms of sporotrichosis are not life-threatening, but it is unusual for them to resolve without antifungal treatment: permanent scarring and disfigurement is a common problem, as is bacterial superinfection. Although cutaneous and subcutaneous forms of sporotrichosis respond well to antifungal treatment, management of osteoarticular, other localized visceral and disseminated forms of the disease is difficult. To date, no randomized clinical trials have been reported and published treatment guidelines have been based on the results of open-label trials and anecdotal reports.

22.8.1 Lymphocutaneous sporotrichosis

Oral itraconazole is the drug of choice for patients with cutaneous and lymphocutaneous forms of sporotrichosis. Adults should be treated with a dosage of 200 mg/day or 5 mg/kg per day for 3–6 months. Children should receive a dosage of 6–10 mg/kg to a maximum of 400 mg/day. Treatment should be continued for 2–4 weeks after the lesions have cleared. Patients who fail to respond should be treated with a higher dosage of itraconazole (200 mg twice daily), or with terbinafine (500 mg twice daily) or with a saturated solution of potassium iodide (explained later). Fluconazole (400–800 mg/day) is less effective and should be regarded as a second-line treatment for patients who do not tolerate or cannot absorb itraconazole or terbinafine. Oral ketoconazole is usually ineffective in lymphocutaneous sporotrichosis.

Saturated potassium iodide solution remains a widely recommended treatment for patients in resource-limited settings who contract lymphocutaneous or cutaneous sporotrichosis, because of its ease of administration and low cost. However, no randomized trials of have been performed to assess the efficacy of this medication. Potassium iodide is inconvenient to take, and side effects, including allergic reactions and gastrointestinal intolerance, are common. The starting dose is 5 drops three times daily, and this is increased as tolerated to 40–50 drops three times daily. Children can be treated with 50 mg or 1 drop three times daily, up to a maximum of 1 drop per kg of body weight or 40–50 drops three times daily, whichever is the lowest. Treatment should be continued for 4–6 weeks after the cutaneous lesions disappear, which may take 2–4 months. If adverse reactions occur, the drug should be discontinued for several days and then re-started at a lower dose.

Thermotherapy involves the daily application of heat (42–43°C) directly onto the lesions for several hours for 2–6 months. It has been used with

some success for fixed cutaneous or lymphocutaneous sporotrichosis in patients who are intolerant to antifungal treatment. Local heat therapy is another option for patients, such as pregnant women and nursing mothers who cannot safely be treated with azoles, terbinafine or potassium iodide.

22.8.2 Extracutaneous sporotrichosis

Itraconazole (200 mg twice daily) is the treatment of choice for patients with osteoarticular forms of sporotrichosis. Treatment should be continued for at least 12 months; shorter courses have led to relapse. Amphotericin B, given as a lipid formulation at a dosage of 3–5 mg/kg per day or as the conventional formulation at a dosage of 0.7–1.0 mg/kg per day, may be indicated for patients with extensive involvement and for those in whom itraconazole is unsuccessful. After the patient has shown a favourable response, therapy may be changed to itraconazole (200 mg twice daily). Serum levels of itraconazole should be measured after the patient has received the drug for at least 2 weeks, to ensure adequate concentrations (see Chapter 3). Fluconazole is less effective and should be reserved for the occasional patient who cannot be treated with itraconazole or amphotericin B. The minimum dosage should be 800 mg/day. Terbinafine and potassium iodide are ineffective in osteoarticular sporotrichosis. Surgical debridement alone is not effective for treatment of osteoarticular sporotrichosis, but some patients may benefit from drainage of a septic joint or removal of a sequestrum. Even if osteoarticular sporotrichosis can be cured, it is rare for joint function to be fully restored.

The treatment of pulmonary sporotrichosis is difficult and relapse is a common problem. Patients who are acutely ill should be treated with a lipid formulation of amphotericin B (3–5 mg/kg per day). Conventional amphotericin B (0.7–1.0 mg/kg per day) may also be used. Itraconazole (200 mg twice daily) may be substituted once the patient's condition has improved and should be continued to a total of at least 12 months of treatment. Patients who are less severely ill may be treated with itraconazole (200 mg twice daily) from the outset. Serum levels of itraconazole should be measured after the patient has received the drug for at least 2 weeks. Fluconazole, terbinafine and potassium iodide are ineffective in pulmonary sporotrichosis. Surgical resection is an option for patients with a single pulmonary lesion that has not responded to antifungal treatment. However, many patients with pulmonary sporotrichosis have severe underlying pulmonary disease that precludes surgical resection of lesions.

Adult patients with life-threatening forms of meningeal or disseminated sporotrichosis require initial treatment with a lipid formulation of

amphotericin B (3–5 mg/kg per day) for 4–6 weeks. Conventional amphotericin B (0.7–1.0 mg/kg per day) may also be used. After the patient has shown a favourable response, therapy may be changed to itraconazole (200 gm twice daily), which should be continued to a total of at least 12 months of treatment. Children with disseminated sporotrichosis should be treated with conventional amphotericin B (0.7 mg/kg per day) followed by itraconazole (6–10 mg/kg, up to a maximum of 400 mg daily). Patients with less acute forms of disseminated infection may be treated with itraconazole (400 mg/day) from the outset. Serum levels of itraconazole should be determined after the patient has received the drug for at least 2 weeks. Fluconazole, terbinafine and potassium iodide are ineffective in meningeal and disseminated sporotrichosis.

Immunosuppressed individuals, including those with AIDS, should be given long-term suppressive therapy with itraconazole (200 mg/day) to prevent relapse. Current treatment guidelines suggest that it is reasonable to discontinue itraconazole maintenance once AIDS patients have completed at least 12 months of treatment and have attained CD4 T-lymphocyte counts of more than 200 cells per μL for at least 12 months.

Voriconazole is less active than itraconazole against *S. schenckii* in vitro and would appear to have no role in the therapy of sporotrichosis. Posaconazole is active against *S. schenckii*, but no treatment results have been published to date.

22.9 Prevention

In adults, lymphocutaneous sporotrichosis appears to be an occupational risk for those who work outdoors. The wearing of protective clothing, such as long sleeves or gloves, during work should provide some protection against the disease.

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CHAPTER 23

Hyalohyphomycosis

23.1 Introduction

The term hyalohyphomycosis is used to refer to infections caused by a diverse group of colourless (hyaline) or lightly coloured moulds that adopt a septate hyphal form in tissue. As with phaeohyphomycosis (see Chapter 25), this all-encompassing term was introduced in an attempt to stem the proliferation of new disease names each time an organism belonging to a new fungal genus was incriminated as the cause of infection. In contrast with phaeohyphomycosis, in which five major clinical forms are recognized, hyalohyphomycosis has been associated with numerous different clinical manifestations. Localized infections may occur among otherwise healthy persons, while widespread disseminated disease is usually seen in immunocompromised individuals.

The number of organisms identified as causal agents of hyalohyphomycosis is increasing with over 70 different organisms, classified in more than 30 genera of ascomycetes and basidiomycetes, being listed to date. The term hyalohyphomycosis is reserved as a general name for infections that are caused by unusual hyaline moulds that are not the cause of otherwise-named infections, such as aspergillosis. The term has also been used to designate infections caused by fungi that form brown-pigmented colonies or spores in culture, but produce hyaline or lightly coloured hyphae in tissue.

23.2 *Fusarium* infection

Fusarium species have long been recognized as a cause of nail and corneal infections in immunocompetent individuals (see Chapters 7 and 8). More

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recently, these moulds have been reported to cause locally invasive infections in both immunocompetent and immunocompromised individuals. *Fusarium* species have also emerged the second most frequent cause of life-threatening disseminated fungal infection (after aspergillosis) in severely immunocompromised individuals, particularly among neutropenic patients with haematological malignancies and haematopoietic stem cell transplant (HSCT) recipients. It should also be noted that *Fusarium* species produce numerous toxigenic metabolites that have been implicated as causes of mycotoxicoses in humans and animals following the consumption of contaminated food products.

23.2.1 Geographical distribution

These infections are worldwide in distribution. However, the largest numbers of cases of invasive *Fusarium* infection have been reported from North America and Europe.

23.2.2 The causal organisms and their habitat

Morphological identification of *Fusarium* is difficult and often confusing, even for specialists, and it is not surprising that the precise species involved has not been determined in many published reports. In fact, it has been estimated that less than one-third of clinical isolates can be identified to species level using morphological characteristics. A three-locus DNA sequence database has recently been established to facilitate molecular identification of the 69 *Fusarium* species now associated with human or animal infection. Analysis of this database has revealed that all pathogenic members of the genus *Fusarium* fall within one of eight species complexes. The two most important of these species complexes are *Fusarium solani* and *Fusarium oxysporum*. Recent work has shown that the *F. solani* species complex includes more than 45 distinct species within three major clades, but human isolates are restricted to one of these clades.

Members of the genus *Fusarium* are common soil organisms and important plant pathogens. These moulds are often found in the indoor and outdoor air, and on decomposing organic debris. *F. solani* and *F. oxysporum* have been recovered from water supply and distribution systems in a number of North American hospitals and have been found to persist for long periods of time.

23.2.3 Epidemiology

The mechanisms by which human infection is acquired are not well understood. The incubation period is unknown. There are several

suggested routes of transmission, including inhalation, implantation following trauma and acquisition via contaminated intravascular devices. In some cases, it has been found that the source of disseminated *Fusarium* infection in an immunocompromised individual was a pre-existing nail infection or a localized skin infection. The isolation of *Fusarium* species from hospital water supply and distribution systems has led some experts to suggest that inhalation of bioaerosols generated during showering could be an important source of invasive infection in HSCT recipients and other immunocompromised individuals.

Species of *Fusarium* are ubiquitous in the environment and the likelihood that infection will occur following inhalation or implantation of spores largely depends on host factors. Invasive *Fusarium* infection has emerged as a significant problem in several groups of immunocompromised individuals. Those at greatest risk include neutropenic cancer patients, particularly those with acute leukaemia, and allogeneic HSCT recipients. Less commonly, invasive *Fusarium* infection is seen in other groups of immunocompromised individuals, including burns patients and solid organ transplant (SOT) recipients. The likelihood of infection developing in these individuals depends on a number of host factors, the most important of which is the level of immunosuppression, whether this manifests as profound or prolonged neutropenia, as graft-versus-host disease (GVHD) in HSCT recipients or as rejection in organ transplant recipients.

Trauma is the major risk factor for localized *Fusarium* infection in the immunocompetent individual. Corneal infection is often the result of several factors, including traumatic inoculation and local immunosuppression due to topical corticosteroid treatment. Localized deep *Fusarium* infection is rare in immunocompetent persons, but cases of endophthalmitis, osteomyelitis and arthritis have been reported following the traumatic introduction of the organism, and peritonitis has followed contamination of peritoneal dialysis catheters.

In a retrospective analysis of 61 cases of invasive *Fusarium* infection among stem cell transplant recipients from nine hospitals in North and South America, the prevalence of disease was about 6 cases per 1000 HSCT recipients. As with aspergillosis, the prevalence was lowest among autologous transplant recipients (1.5–2 cases per 1000) and highest among recipients of mismatched unrelated-donor allogeneic transplants (20 cases per 1000). In the allogeneic HSCT population, *Fusarium* infection has a trimodal distribution with a first peak during the early neutropenic period, followed by a second peak at a median of 70 days after transplant among patients with acute GVHD receiving corticosteroids, and a third

peak more than 12 months after transplant during treatment for chronic GVHD.

The overall case fatality rate from disseminated *Fusarium* infection is high, ranging from 50% to 90% in almost all groups of immunocompromised patients and approaching 100% in patients with persistent severe immunosuppression.

23.2.4 Clinical manifestations

Fusarium species cause a broad spectrum of human disease ranging from superficial infection of the nail and cornea in immunocompetent persons (see Chapters 7 and 8) to disseminated invasive infection in severely immunocompromised patients. Localized deep infections, including cases of endophthalmitis, sinusitis, osteomyelitis, arthritis, brain abscess, thrombophlebitis and dialysis-associated peritonitis have been reported in both immunocompetent and immunocompromised individuals.

The clinical manifestations of disseminated *Fusarium* infection are similar in several respects to those of acute invasive aspergillosis (see Chapter 10). Like the aetiological agents of aspergillosis and mucormycosis, *Fusarium* species have a predilection for vascular invasion, resulting in thrombosis, infarction and tissue necrosis. The typical clinical presentation is an immunosuppressed patient with a persistent fever (greater than 38°C) that is unresponsive to antibacterial and antifungal treatment. Other findings at presentation include rhinosinusitis, cellulitis at the site of a skin or nail infection, and/or multiple painful cutaneous lesions. Patients who are persistently neutropenic often present with rapidly progressive infection.

The clinical manifestations of acute invasive *Fusarium* rhinosinusitis are indistinguishable from those of *Aspergillus* infection (see Chapter 10). Necrotic black lesions on the mucosa are a characteristic diagnostic sign. As the infection spreads into the orbit, periorbital or paranasal cellulitis occurs and may progress to disfiguring destruction of facial tissue.

Pulmonary involvement is common in patients with invasive *Fusarium* infection. The presenting signs are non-specific and include pleuritic chest pain, non-productive cough, shortness of breath and haemoptysis. The radiological presentation ranges from non-specific alveolar or interstitial infiltrates to nodules or cavitating lesions, depending on the timing of the investigation. These clinical and radiological findings are difficult to distinguish from those of invasive pulmonary aspergillosis. High-resolution computed tomographic (CT) scans are an essential tool in managing patients with suspected invasive *Fusarium* infection. Chest CT scans detect abnormalities in a much higher proportion of infected patients

than standard chest radiographs. The halo sign that is characteristic of the early stage of invasive pulmonary aspergillosis is seldom seen in neutropenic patients with *Fusarium* infection.

Unlike aspergillosis, invasive *Fusarium* infection often leads to the development of skin lesions, either as the initial site of involvement or as a metastatic site. Cutaneous involvement has been described in about 70% of reported cases of *Fusarium* infection, particularly in immunocompromised persons, compared with less than 5% of patients with aspergillosis. Immunocompromised patients with *Fusarium* infection may present with localized skin infection (cellulitis) at the site of a skin or nail lesion. More frequently, however, they present with multiple painful erythematous papules or nodules on the trunk and limbs. These lesions follow haematogenous dissemination and usually evolve over a short period of time (1–5 days), particularly in neutropenic individuals. Three types of cutaneous lesions may be observed: necrotic lesions with an ecthyma-gangrenosum-like appearance; target lesions consisting of an ecthyma-like lesion surrounded by a thin erythematous margin, 1–3 cm in diameter; and subcutaneous nodules. These presentations likely represent different stages in the evolution of the infection.

Among immunocompetent individuals, cutaneous *Fusarium* infections typically are localized and develop after skin breakdown at the site of infection. In this setting, cutaneous infections most commonly present as necrotic lesions that complicate extensive burns or trauma, as cellulitis adjacent to onychomycosis or as chronic ulcers and abscesses.

23.2.5 Essential investigations and their interpretation

Microscopic examination of histopathological sections will reveal septate hyphae with acute-angle branching that cannot be distinguished from those of other agents of hyalohyphomycosis or aspergillosis. However, *Fusarium* species may sporulate in tissue and the presence of hyphae and small, single-celled chlamydoconidia is suggestive of the diagnosis in a high-risk patient.

The definitive diagnosis of *Fusarium* infection depends on the isolation of the aetiological agent in culture from clinical specimens, such as blood or material from cutaneous lesions. In general, *Fusarium* species can be easily recovered on routine mycological media without cycloheximide (actidione). However, the choice of medium can have a profound influence on colonial appearance and sporulation. Identification of isolates to the species level is difficult and may require the use of molecular methods.

Infection with *Fusarium* has been associated with a much higher rate of isolation from blood culture (about 40–60% of cases) than has been the

case with *Aspergillus* and other mould infections. However, it has not been established whether lysis–centrifugation is any more useful than other blood culture systems. *Fusarium* can usually be isolated from biopsies of cutaneous lesions.

(1-3)-β-D-glucan, a cell wall component of many fungi, has been detected in the blood of patients with invasive *Fusarium* infection. Although clinical experience is limited, the Fungitell assay (Associates of Cape Cod, Falmouth, Massachusetts, USA) for detection of β-D-glucan has been approved for the presumptive diagnosis of invasive fungal infections, including *Fusarium*. Although a positive test result does not identify the aetiological agent, the assay can be used as a screening method, allowing the earlier initiation of antifungal treatment. A positive (1-3)-β-D-glucan test and a negative *Aspergillus* galactomannan test (see Chapter 10) in a high-risk patient is suggestive of a *Fusarium* infection.

No serological tests for the diagnosis of *Fusarium* infection are currently available.

23.2.6 Management

The most important factors influencing the outcome of *Fusarium* infection are the underlying immunological status of the patient and the extent of the infection. Most importantly, if neutropenia does not resolve, the case fatality rate approaches 100% no matter what antifungal agent is used. Because of a lack of randomized controlled clinical trials and the critical role of immunological reconstitution in the outcome, the optimum approach to management has not been established.

Isolates of *Fusarium* are often resistant to amphotericin B in vitro and breakthrough infection has been reported during empirical treatment with this agent. Nonetheless, many experts still regard high-dose amphotericin B as the regimen of choice for immunocompromised patients with disseminated *Fusarium* infection. The response rate to lipid-based formulations has been reported to be superior to that to the conventional formulation. Regardless of the formulation selected, it should be given at the maximum tolerated dosage (see Chapter 3). However, even with high-dose amphotericin B treatment, the prognosis is dismal unless the neutrophil count recovers.

Voriconazole and posaconazole are more active than amphotericin B in vitro against many species of *Fusarium*, and both these new triazoles have been used to treat *Fusarium* infection in immunocompromised patients. Voriconazole is licenced for the first-line treatment of *Fusarium* infection, while posaconazole is approved for salvage treatment. In one large case series, initial or salvage treatment with voriconazole was associated

with a 47% complete or partial response rate. In a second report, salvage treatment with posaconazole was associated with a 48% rate of complete or partial response. However, the success rate for patients who recovered from myelosuppression was 67%, compared with 20% for those with persistent neutropenia. Breakthrough *Fusarium* infection has been reported in patients with haematological malignancies receiving prophylaxis with voriconazole or posaconazole.

A limited number of cases of disseminated *Fusarium* infection have been treated with liposomal amphotericin B and voriconazole in combination. However, given the anecdotal and non-comparative nature of the studies, it remains unclear whether combination treatment is more effective than either drug alone.

In addition to antifungal treatment, immunological reconstitution is a critical factor in the treatment of *Fusarium* infection. Immunosuppressive and cytotoxic drugs should be reduced in dose or eliminated. Anecdotal reports suggest that administering colony-stimulating factors might be beneficial in treating disseminated *Fusarium* infection in persistently neutropenic individuals. Granulocyte transfusions have also been shown to be useful.

Non-immunosuppressed individuals with localized *Fusarium* infection of soft-tissue or bone usually respond to treatment. Amphotericin B remains the agent of choice for patients with osteomyelitis, but successful management often requires aggressive surgical debridement of necrotic tissue. Patients with endophthalmitis require both medical and surgical treatment. Intravitreal concentrations of amphotericin B following parenteral administration are too low to treat this infection, and intravitreal instillation is required. This will result in inflammation and retinal damage, but doses up to 10 μg can be tolerated. Surgical debridement is an essential part of the management of this infection.

23.2.7 Prevention

Measures to reduce the incidence of *Fusarium* infection should focus on protecting high-risk individuals from exposure to the organisms, and prompt diagnosis and treatment of localized skin and nail infection in patients awaiting immunosuppressive or cytotoxic treatment.

Although invasive *Fusarium* infection is an uncommon condition, it appears to be more prevalent in some hospitals than in others. Housing high-risk patients in rooms provided with HEPA-filtered air has not eliminated the problem of nosocomial *Fusarium* infection in these institutions, and it has been suggested that ingestion of contaminated tap water, or inhalation of spores released during showering, is another

potential source of *Fusarium* infection. Should hospital water be found to be contaminated with *Fusarium*, it has been recommended that immunosuppressed patients be provided with sterile (boiled) water for drinking to reduce the risk of infection. It has also been suggested that patients should use bed baths instead of showering. If individuals insist on showering during periods of intense immunosuppression, showerheads and bathroom floors and walls should be cleaned before use.

Because the skin is an important source for disseminated *Fusarium* infection, it is essential that patients who require immunosuppressive or cytotoxic therapy be evaluated by a dermatologist before commencing immunosuppression. All sites of tissue breakdown should be identified and suspicious skin and nail lesions cultured. Local debridement should be performed and topical antifungal agents, such as natamycin and amphotericin B, considered if *Fusarium* species are isolated. If possible, immunosuppressive treatment should be delayed until the lesions have healed.

23.3 *Scedosporium* infection

Scedosporium species cause a broad spectrum of human disease, ranging from transient colonization of the lungs to localized subcutaneous or deep-tissue infection and widespread disseminated infection. These moulds have emerged as a significant cause of invasive disease in immunocompromised patients, in individuals with impaired anatomical barriers (trauma, burns, etc.) and in cases of massive inoculation (near drowning in polluted water). Disseminated *Scedosporium* infection is mostly seen among immunosuppressed patients; however, cases have been reported in immunocompetent individuals.

23.3.1 Geographical distribution

These infections are worldwide in distribution.

23.3.2 The causal organisms and their habitat

Until recently, only two species of *Scedosporium* were recognized: *Scedosporium apiospermum* (the anamorph or asexual state of the ascomycete *Pseudallescheria boydii*) and *Scedosporium prolificans.* The latter species is an important cause of phaeohyphomycosis (see Chapter 25). On the basis of molecular analysis, *S. apiospermum* and *P. boydii* are now considered to be two distinct species: *S. apiospermum* (teleomorph or sexual state, *P. apiosperma*) and *S. boydii* (teleomorph, *P. boydii*). Moreover, several new phylogenetic species have recently been delineated among clinical

isolates previously identified as *S. apiospermum*, including *Scedosporium aurantiacum* and *Scedosporium dehoogii*.

Scedosporium species are ubiquitous saprophytic fungi found in the soil, in manure and sewage, and in polluted fresh water.

23.3.3 Epidemiology

Scedosporium infection is thought to follow traumatic implantation, inhalation or aspiration of contaminated water. There are also several reports of acquisition via contaminated intravascular devices. The incubation period is unknown.

Trauma is the major risk factor for localized *Scedosporium* infection in the immunocompetent individual. In addition to infection of the subcutaneous tissue and bone (mycetoma), cases of endophthalmitis, osteomyelitis and arthritis have been reported following the traumatic introduction of the organism. Unlike other opportunistic mould pathogens, *Scedosporium* species can cause pneumonia in immunocompetent individuals following aspiration of polluted water. Haematogenous dissemination to the brain is a frequent complication.

Species of *Scedosporium* are ubiquitous in the environment, and the likelihood that infection will occur following inhalation or implantation of spores largely depends on host factors. Disseminated infection has emerged as a significant problem in several groups of immunocompromised individuals. Those at greatest risk include neutropenic cancer patients, particularly those with acute leukaemia, allogeneic HSCT recipients undergoing treatment for GVHD and those receiving corticosteroid treatment. Less commonly, disseminated *Scedosporium* infection has been seen in other groups of immunocompromised individuals, including persons with the acquired immunodeficiency syndrome and SOT recipients.

The incidence of serious *Scedosporium* infection appears to have increased in recent years, particularly among neutropenic cancer patients and those undergoing allogeneic HSCT. According to one recent report from a North American cancer hospital, the incidence increased from 0.82 cases per 10,000 patient days between 1993 and 1998 to 1.33 cases per 10,000 patient days between 1999 and 2005. In a retrospective analysis of 80 cases of *Scedosporium* infection among HSCT and SOT recipients, it was found that patients who received transplants after 1999 developed the infection later (median time to onset, 6 months) than those who were transplanted in 1999 or earlier (median time to onset, 1.2 months). No fewer than 39% of infections among SOT recipients occurred more than 6 months after transplantation, compared with 25% of those among HSCT recipients.

The overall case fatality rate from *Scedosporium* infection is high, ranging from 65% to 75% in most reports. In patients with haematological malignancies, mortality is associated with dissemination and persistent neutropenia.

23.3.4 Clinical manifestations

Scedosporium is the most frequent cause of fungal mycetoma in immunocompetent individuals residing in North America and other temperate regions (see Chapter 21). This indolent disfiguring infection results from the traumatic implantation of the fungus into subcutaneous tissue. The feet and hands are the most common sites of infection. The initial lesion, which appears several months after the traumatic incident, is a small, painless, subcutaneous nodule. This enlarges and ruptures to the surface forming sinus tracts that discharge white grains, then spreads into adjacent tissue and bone, causing disfiguring swelling and tissue destruction.

The lung is the second most common site of *Scedosporium* infection in humans. The clinical manifestations range from transient local colonization to fungus ball formation and invasive infection. In the immunocompetent individual, isolation of these moulds from sputum most commonly represents transient (or more prolonged) colonization of the bronchi or lungs or both. In some cases, however, a clinical picture indistinguishable from allergic bronchopulmonary aspergillosis occurs (see Chapter 10). Despite the fact that *Scedosporium* colonization of the respiratory tract occurs in 6–10% of patients with cystic fibrosis, invasive infection is rare in these patients, even when immunosuppressed after lung transplantation. Underlying cystic or cavitating lung disease is a major factor predisposing an individual to *Scedosporium* fungus ball formation. This condition, which is radiologically indistinguishable from the more common aspergilloma, has been described in patients with residual tuberculous or bronchiectatic cavities.

Scedosporium pneumonia is rare in immunocompetent persons. However, it has been seen following the aspiration of contaminated water by previously healthy individuals. Patients tend to present within a few days to several weeks after the incident, often after a temporary improvement in their condition. Fatal dissemination to the central nervous system (CNS) is a frequent complication, and even subtle neurological symptoms should prompt CNS imaging.

In immunosuppressed patients, *Scedosporium* infection is associated with a high rate of dissemination and a poor outcome. The clinical manifestations of *Scedosporium* infection of the lungs are similar to those of invasive aspergillosis (see Chapter 10). The most common presentation

in the neutropenic patient is a persistent fever (greater than 38°C) that is unresponsive to broad-spectrum antibacterial treatment. The radiological findings range from non-specific infiltrates to areas of consolidation that can evolve to cavitation. Haematogenous dissemination to other organs is quite common.

In immunocompetent persons, *Scedosporium* infection of the CNS is most often due to spread from the paranasal sinuses, or to penetrating trauma, or to spread from the lungs following near drowning in polluted water. In immunocompromised individuals, CNS infection tends to follow haematogenous dissemination. Most CNS infections have presented as a single brain abscess or as multiple abscesses, but ventriculitis, encephalitis and meningitis have also been reported. Clinical manifestations may include fever, headache, altered mental status, seizures, hydrocephalus and brain oedema.

23.3.5 Essential investigations and their interpretation

The definitive diagnosis of *Scedosporium* infection rests on the isolation of the fungus from clinical specimens. This is because the branching septate hyphal tissue form of these moulds cannot be distinguished from that of other aetiological agents of hyalohyphomycosis or aspergillosis in wet preparations or histopathological sections. This distinction is important because *Scedosporium* species are intrinsically resistant to amphotericin B.

Scedosporium can usually be isolated from infected organs or surgical drainage of brain abscesses, but blood and CSF cultures are seldom positive. In general, these moulds can be easily recovered on routine mycological media without cycloheximide (actidione). Morphological identification of isolates to the species level is difficult and may require the use of molecular methods.

23.3.6 Management

Surgical resection remains the preferred treatment for patients with localized cavitating lesions of the lung. Other localized forms of *Scedosporium* infection, such as sinusitis, arthritis and osteomyelitis, have also been eradicated following surgical debridement of infected tissue. In some cases, cure has resulted when debridement has been combined with antifungal treatment.

S. apiospermum and related species are resistant to amphotericin B, fluconazole and ketoconazole in vitro, but susceptible to voriconazole and posaconazole. Parenteral miconazole, despite being effective in some patients with *Scedosporium* infection, is no longer available. Voriconazole is now licenced for the treatment of infections caused by these moulds.

Although no randomized controlled trials have been reported, a large case series suggests that voriconazole is effective in the management of patients with various forms of invasive *Scedosporium* infection. Overall, success was achieved in 66% of patients, with 98% of those responding receiving at least 4 weeks of treatment. The highest response rates were seen for cutaneous/subcutaneous or bone infections and the lowest for CNS or disseminated infections.

23.4 Other agents of hyalohyphomycosis

Many other colourless moulds have been incriminated as occasional aetiological agents of human hyalohyphomycosis. Isolation of these organisms alone is insufficient grounds for making a diagnosis because many are common culture contaminants. Even when the same organism is recovered on more than one occasion, the diagnosis must remain in doubt unless the fungus is demonstrated in histopathological sections of biopsy specimens.

23.4.1 *Acremonium* infection

Acremonium species are common saprophytic fungi that have long been recognized as occasional causes of nail infection, corneal infection and fungal mycetoma in immunocompetent individuals (see Chapters 7, 8 and 21). More recently, several members of this genus, including *A. kiliense*, have been implicated as causing locally invasive infections in immunocompetent persons, including sinusitis, arthritis, osteomyelitis, endocarditis, meningitis and peritonitis in dialysis patients. Disseminated infection has been reported in patients with haematological malignancies and transplant recipients. Identification of these moulds to the species level using morphological or molecular methods is very difficult, and many published reports of infection are based on unidentified species. Successful clinical outcomes have been reported after treatment with amphotericin B, voriconazole and posaconazole.

23.4.2 *Paecilomyces* infection

Paecilomyces species are common saprophytic fungi isolated from soil and decomposing plant matter. Although these moulds are uncommon pathogens, they have been reported to cause locally invasive infections in immunocompetent individuals, including sinusitis, pulmonary infection, endocarditis following heart valve replacement, oculomycosis following lens implantation, and peritonitis in dialysis patients. Disseminated infection has been described in immunocompromised individuals.

Paecilomyces lilacinus and *Paecilomyces variotii* are the species associated most frequently with human disease, but other species reported as human pathogens include *Paecilomyces marquandii* and *Paecilomyces javanicus*. *P. lilacinus* has caused outbreaks of endophthalmitis in surgical patients following its inadvertent administration in contaminated irrigation fluids. Both *P. lilacinus* and *P. variotii* are susceptible to itraconazole, posaconazole and voriconazole, but only *P. variotii* is susceptible to amphotericin B. Although clinical experience is limited, anecdotal reports suggest that voriconazole may be an effective treatment for *Paecilomyces* infections. Surgical intervention should be considered if clinically appropriate.

23.4.3 *Scopulariopsis* infection

In immunocompetent persons, *Scopulariopsis brevicaulis* has been reported to cause onychomycosis (see Chapter 7), invasive sinusitis, and prosthetic valve endocarditis. In neutropenic individuals and organ transplant recipients, this mould has caused localized invasive infection of soft tissues and lungs, as well as widespread disseminated disease. As in patients with *Fusarium* infection, cutaneous lesions adjacent to infected toe nails have been identified as the source of a subsequent disseminated infection. Invasive infections may require surgical and medical treatment and are frequently fatal.

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CHAPTER 24

Penicillium marneffei infection

24.1 Introduction

Penicillium marneffei infection has become one of the most frequent opportunistic infections encountered in human immunodeficiency virus (HIV)-infected persons who reside in East or South East Asia. Infection with this thermally dimorphic fungus is thought to follow inhalation, but widespread disseminated disease is the most common clinical presentation. The infection is associated with a high mortality rate if left untreated.

24.2 Geographical distribution

P. marneffei has a limited geographical distribution, being endemic in tropical countries in East and South East Asia, including southern China, Hong Kong, north eastern India, Indonesia, Laos, Malaysia, Myanmar, Taiwan, Thailand and Vietnam. Among the endemic countries, the greatest number of cases has been reported from northern Thailand. Imported cases have been diagnosed worldwide among individuals who had earlier resided in or visited the endemic region.

24.3 The causal organism and its habitat

P. marneffei is the only thermally dimorphic fungus in the genus *Penicillium*. It exists in nature as a mould, but in tissue it forms small round to elliptical yeast cells that divide by fission. Apart from humans, bamboo rats of the genera *Rhizomys* and *Cannomys* are the only other known natural host of *P. marneffei*.

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Although *P. marneffei* has been isolated from soil, understanding of its precise natural habitat remains limited. Further support for the hypothesis that *P. marneffei* is a soil-inhabiting fungus comes from the fact that it has been isolated from four different species of soil-digging bamboo rats, found in regions of South East Asia where human infection is prevalent. A seasonal pattern of *P. marneffei* infection has been observed in northern Thailand and Vietnam with a higher incidence during the rainy season. This suggests that heavy rainfall may provide favourable conditions for the growth and dissemination of the fungus.

24.4 Epidemiology

Infection with *P. marneffei* is thought to follow inhalation of spores that have been released from thus far unidentified environmental sources into the air. The incubation period is variable, but it is clear that the fungus can sometimes remain dormant for long periods following asymptomatic primary infection. In contrast, cases have been reported in infants and children with the acquired immunodeficiency syndrome (AIDS) in which disseminated infection occurred within a few weeks of exposure to the organism.

The likelihood that an infection with *P. marneffei* will develop after inhalation depends, in major part, on host factors. Even before the spread of AIDS to South East Asia, the disease tended to occur in individuals with T-cell mediated immunological defects, such as are found in persons with lymphoproliferative disorders or those receiving corticosteroid or cytotoxic treatment. HIV infection is now the single most common predisposing illness among patients with penicilliosis. In HIV-infected persons, *P. marneffei* infection is associated with severe immunological impairment, as reflected by CD4 T-lymphocyte counts of less than 100 cells per μL. It has been reported that the risk of symptomatic *P. marneffei* infection is greater in HIV-infected individuals with recent occupational or other exposure to soil, especially during the wet months. In contrast, exposure to or consumption of bamboo rats is not a risk factor for infection.

Until the late 1980s, human penicilliosis was uncommon with fewer than 40 natural human infections reported among individuals living in the endemic region or visitors to that region. However, following the spread of AIDS to South East Asia, penicilliosis has become much more common. It is the third most prevalent opportunistic infection (after tuberculosis and cryptococcosis) among HIV-infected individuals living in Chiang Mai province in northern Thailand, accounting for 7% of AIDS-defining illnesses reported between 1994 and 1998. In Vietnam,

P. marneffei infection accounted for 4.4% of AIDS admissions to hospital between 2004 and 2009, and 6.3% of AIDS-related hospital deaths during the same period. Despite appropriate antifungal treatment, case fatality rates remain between 10% and 28%.

24.5 Clinical manifestations

The lungs are the usual initial site of infection, but most affected individuals present with widespread disseminated infection closely resembling acute disseminated histoplasmosis (see Chapter 17). The most frequent sites of involvement are the liver, skin, gastrointestinal tract, lymph nodes and bone marrow. The illness is usually fatal if left untreated.

Penicilliosis is mostly seen in patients with late HIV infection with CD4 T-lymphocyte counts of less than 100 cells per μL; up to 80% of reported cases have had counts of less than 50 cells per μL. The most common presenting symptoms of AIDS-associated *P. marneffei* infection include fever, marked weight loss, non-productive cough and debilitation. About 60–70% of patients present with multiple papular skin lesions, some of which show a central necrotic umbilication, resembling molluscum contagiosum. These are often found on the face, neck, trunk and limbs. Molluscum-contagiosum-like lesions are also commonly present on the oral mucosa and throat.

Other presenting signs include generalized lymphadenopathy, hepatosplenomegaly, anaemia and thrombocytopenia. Chest radiographs may reveal diffuse reticulonodular infiltrates, localized alveolar infiltrates, or cavitary lesions. Pleural effusion and hilar lymph node calcification are uncommon. Other presenting signs include osteoarthritis, subcutaneous abscesses, genital ulcers and oral lesions.

Central nervous system (CNS) involvement is uncommon. However, a case series of 21 HIV-infected adults with acute altered mental state with confusion, agitation or depressed consciousness in the setting of subacute febrile illness has recently been reported from Vietnam. Symptoms of increased intracranial pressure and signs of meningeal inflammation were uncommon or absent. Cerebrospinal fluid (CSF) findings varied from a normal to mildly raised protein concentration, a normal to mildly lowered glucose concentration and an acellular to mild pleocytosis. CSF cultures were positive for *P. marneffei*. The disease course was rapidly progressive and the case fatality rate was high.

24.6 Differential diagnosis

Histoplasmosis and cryptococcosis are endemic in the same regions as *P. marneffei* infection and can cause similar necrotic skin lesions in persons

with AIDS. The radiological signs of *P. marneffei* infection can be confused with those of pulmonary tuberculosis, bacterial pneumonia and *Pneumocystis jirovecii* pneumonia. Other opportunistic infections from which penicilliosis must be distinguished include toxoplasmosis and disseminated herpes zoster infection.

24.7 Essential investigations and their interpretation

It is recommended that HIV-infected persons who have visited East or South East Asia should be investigated for signs of *P. marneffei* infection if they present with fever, cutaneous lesions, lung disease, generalized lymphadenopathy and/or hepatosplenomegaly.

24.7.1 Microscopy

Microscopic examination of Wright-stained bone marrow smears or touch smears of skin or lymph node biopsies can permit the rapid presumptive diagnosis of *P. marneffei* infection. If small, round, oval or elliptical, non-budding yeast cells, often with a prominent transverse septum are seen, the diagnosis of penicilliosis should be suspected. In histopathological sections, *P. marneffei* cells are usually found within macrophages and may appear in clusters with occasional extracellular organisms. Methenamine silver staining is useful to highlight the transverse septum that will appear to be thicker than the wall of the yeast. *P. marneffei* cells can be confused with those of *Histoplasma capsulatum*, *Candida* species, *P. jirovecii*, *Toxoplasma gondii* and *Leishmania* species due to their similar size. Distinction between them relies on the detection of the characteristic transverse septum that develops during fission of the cells.

24.7.2 Culture

The definitive diagnosis of *P. marneffei* infection depends on isolation of the fungus in culture. The organism has been isolated from skin and lymph node biopsies, blood, pus, bone marrow aspirates, sputum, bronchoalveolar lavage fluid, CSF, urine and other clinical materials. It has been recovered from almost 100% of bone marrow cultures, 90% of skin biopsy cultures and 75% of blood cultures from persons with AIDS.

Identifiable flat green mycelial colonies can be obtained on glucose peptone (Sabouraud's dextrose) agar after incubation at 25–30°C for 1 week, but cultures should be retained for up to 3 weeks before being discarded. *P. marneffei* colonies produce a distinctive red pigment that diffuses into the agar. However, some other non-pathogenic species of *Penicillium* can also produce a similar pigment. Sub-culture at 37°C on a rich medium,

such as brain–heart infusion agar, usually results in production of the yeast form within 7 days.

24.7.3 Serological tests

Various tests have been described for the detection of antibodies to *P. marneffei*, including immunodiffusion, indirect immunofluorescence and ELISA, but they are not widely available. These methods are specific, but less sensitive than culture.

Several promising methods have also been developed for the detection of *P. marneffei* infection by using monoclonal antibodies specific for *P. marneffei* to detect antigen in serum and urine. None of these tests is commercially available. The galactomannan antigen detection test for aspergillosis (Platelia *Aspergillus*, BioRad Laboratories, Paris, France; see Chapter 10) has been found to give false-positive results in HIV-infected patients with penicilliosis, and it has been suggested that this method may facilitate the early detection of *P. marneffei* infection in individuals with a compatible clinical presentation.

24.8 Management

Patients infected with *P. marneffei* have a poor prognosis without antifungal treatment. Even with treatment, the case fatality rate from disseminated infection in persons with AIDS is about 20% in northern Thailand. Furthermore, 50% of patients will relapse within 6 months of the successful completion of treatment. To date, there have been no randomized controlled trials of the acute treatment of penicilliosis.

Amphotericin B remains the drug of choice for patients with severe *P. marneffei* infection. The recommended regimen is 0.6–1.0 mg/kg per day for 2 weeks. Patients can then be changed to itraconazole (200 mg twice daily) for a further 10 weeks, provided their condition has improved on amphotericin B treatment. In milder infections, itraconazole can be used from the outset. Itraconazole should be given at a dosage of 400 mg/day for 8 weeks, followed by 200 mg/day to prevent recurrence. Serum levels of itraconazole should be measured after the patient has received the drug for at least 2 weeks, to ensure adequate absorption (see Chapter 3).

If an initial course of antifungal treatment is unsuccessful, current guidelines suggest reinitiating parenteral amphotericin B followed by another course of oral itraconazole, coupled with optimizing antiretroviral treatment, avoiding adverse drug interactions (see Chapter 3) and ensuring that adequate serum concentrations of itraconazole are attained. Voriconazole is not licenced for the treatment of *P. marneffei* infection, but a small case series reported good outcomes.

The immune reconstitution inflammatory syndrome (IRIS) has been reported in a few AIDS patients with *P. marneffei* infection. It usually occurs within a few weeks or months of starting antiretroviral treatment. This should not be withheld because of concern for the possible development of IRIS. In patients with severe symptomatic manifestations of IRIS, such as CNS inflammation and/or elevated intracranial pressure, some experts recommend short-course corticosteroids (0.5–1 mg/kg per day of prednisone equivalent for 2–6 weeks). It may be prudent to delay the initiation of potent antiretroviral treatment until patients have completed the first 2 weeks of induction treatment for penicilliosis.

All patients who successfully complete treatment for penicilliosis require long-term maintenance treatment with oral itraconazole (200 mg/day). This is safe and well tolerated, and has been shown to prevent relapse of *P. marneffei* infection in patients with AIDS. Experience in Thailand has shown that it is reasonable to discontinue itraconazole maintenance once patients have attained CD4 T-lymphocyte counts of more than 100 cells per μL for at least 6 months while receiving highly active antiretroviral therapy (HAART). Maintenance treatment should be restarted if the CD4 count falls to less than 100 cells per μL or if penicilliosis recurs at a CD4 count of more than 100 cells per μL.

24.9 Prevention

A controlled trial demonstrated that long-term prophylactic administration of itraconazole (200 mg/day) to persons with advanced HIV infection residing in northern Thailand was well tolerated and prevented the development of *P. marneffei* infection (and cryptococcosis), especially in those with CD4 T-lymphocyte counts of less than 100 cells per μL. The use of combination antiretroviral treatments, including HAART, has greatly reduced the incidence of opportunistic fungal infections among HIV-infected persons in developed countries. However, until these drugs become available in the resource-limited settings where *P. marneffei* infection is endemic, it is recommended that itraconazole prophylaxis be used in persons with CD4 counts below 100 cells per μL living in districts where there is a high burden of the disease.

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CHAPTER 25

Phaeohyphomycosis

25.1 Introduction

The term phaeohyphomycosis is used to describe infections caused by brown-pigmented (dematiaceous) moulds that appear in tissue as septate hyphae, as pseudohyphal cells, as yeast-like cells or as any combination of these forms. As with hyalohyphomycosis (see Chapter 23), the term phaeohyphomycosis was introduced in an attempt to stem the proliferation of new disease names each time an organism belonging to a new fungal genus was identified as the cause of human infection. This term was also created to segregate a heterogenous group of infections caused by dematiaceous fungi from two specific pathological conditions associated with these moulds. In chromoblastomycosis, characteristic thick-walled muriform cells are formed in subcutaneous tissue (see Chapter 18), while mycetoma is characterized by the formation of grains that consist of compact masses of fungal filaments (see Chapter 21). However, it is now clear that several organisms can cause both chromoblastomycosis and phaeohyphomycosis, and others can cause both mycetoma and phaeohyphomycosis.

Dematiaceous moulds cause a broad spectrum of disease, ranging from allergic disorders of the lungs and sinuses to localized cutaneous, subcutaneous or deep-tissue infection, and widespread disseminated infection. Localized infections usually occur among otherwise healthy persons, but life-threatening disease has been seen in both immunocompetent and immunocompromised individuals. For some conditions in immunocompetent persons, such as mycotic brain abscess and allergic fungal rhinosinusitis, dematiaceous moulds are among the most common aetiological

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agents. Infections of the skin, nails and cornea are discussed in Chapters 6–8. This chapter reviews the subcutaneous, deep tissue and disseminated forms of phaeohyphomycosis, as well as the various allergic disorders caused by dematiaceous fungi.

25.2 Geographical distribution

Phaeohyphomycosis has a worldwide distribution, but several forms of the disease are more prevalent in some regions than others. Subcutaneous infection is most often seen in regions with tropical or sub-tropical climates. Chronic invasive fungal rhinosinusitis is found worldwide, but the largest number of cases due to dematiaceous fungi has been reported from North America. The largest numbers of cases of cerebral phaeohyphomycosis have also been described from that continent.

25.3 The causal organisms and their habitat

The dematiaceous fungi are a large and heterogenous group of organisms that have in common the presence of brown melanin or melanin-like pigments in their cell wall. More than 100 different species, classified in 60 different genera, have been incriminated as causes of human phaeohyphomycosis, but many of these are relatively rare as aetiological agents. Often these fungi have been given different names at different times, and there is therefore a great deal of confusion in the nomenclature used in different reports. The situation is due in large part to the fact that some of these organisms can produce spores by more than one mechanism, while others adopt different morphological forms, depending on the conditions under which they are grown in culture. Molecular methods are proving to be very useful in identifying dematiaceous fungi and in redefining their phylogenetic relationships.

The more important aetiological agents of phaeohyphomycosis are *Alternaria* species, *Bipolaris* species, *Cladophialophora bantiana*, *Curvularia* species, *Exophiala* species, *Exserohilum* species, *Ochroconis gallopava*, *Phaeoacremonium* species, *Phialophora* species, *Rhinocladiella* (*Ramichloridium*) *mackenziei* and *Scedosporium prolificans*. Some of these organisms are found worldwide, but others have a restricted geographic distribution. For instance, *R. mackenziei* has only been seen in patients from Middle Eastern countries, Afghanistan, Pakistan and India, or among immigrants from that region.

These organisms are widespread in the environment: many of them are found in soil, wood, polluted water and decomposing plant

matter, and others are plant pathogens. Most dematiaceous fungi are considered to be opportunistic pathogens, although some have a marked neurotropism and may function as true pathogens. Those fungi known to be neurotropic include *Bipolaris* species, *C. bantiana*, *Exophiala* species and *R. mackenziei*.

25.4 Epidemiology

Subcutaneous infection is the most frequently reported form of phaeohyphomycosis. It is thought to result from the traumatic implantation of the causative fungus into the subcutaneous tissue. Minor trauma, such as abrasions or wounds due to thorns or wood splinters is often sufficient to introduce the organism. Less commonly, it may occur as a result of haematogenous dissemination of invasive disease in an immunocompromised individual. The incubation period is unknown. The principal aetiological agents include *Bipolaris* species, *Exophiala jeanselmei*, *Exophiala* (*Wangiella*) *dermatitidis* and *Phialophora* species, but many less common moulds have also been reported to cause this condition. Most cases occur sporadically in otherwise healthy individuals, often in older men with outdoor occupations, but infections have also been reported in immunocompromised individuals, such as solid organ transplant (SOT) recipients. These patients may be at increased risk of subsequent dissemination.

Dematiaceous moulds are important causes of chronic invasive rhinosinusitis and allergic fungal rhinosinusitis. In both diseases, the disorder is thought to follow inhalation. The organisms most commonly implicated in invasive rhinosinusitis include *Alternaria* species, *Bipolaris hawaiiensis*, *Bipolaris spicifera*, *Curvularia lunata* and *Exserohilum rostratum*. Chronic invasive rhinosinusitis may occur in normal individuals, but it is more commonly seen in persons who have received corticosteroids or have diabetes mellitus. Invasive sinonasal infection has been reported in severely immunocompromised patients with cancer. Of note, more than 40% of immunocompromised individuals with sinonasal phaeohyphomycosis in a recent report had previously been diagnosed with chronic allergic sinusitis, and it is possible that immunosuppression caused an unrecognized non-invasive fungal sinusitis to become invasive.

Allergic fungal rhinosinusitis is a non-invasive disorder, usually seen in young immunocompetent adults, which is increasingly being recognized as a cause of chronic relapsing rhinosinusitis. Although patients do not have underlying immunodeficiencies, 50–70% are atopic. There is no male or female predominance. The reported prevalence of this disorder ranges from 5% to 10% of patients with chronic rhinosinusitis in

some reports to a much higher proportion in others. In earlier reports, *Aspergillus* species were believed to be the principal aetiological agents. However, most recent studies suggest that dematiaceous moulds, including *Alternaria* species, *Bipolaris* species and *Curvularia* species, are the predominant cause of allergic fungal rhinosinusitis.

Pulmonary infection is usually seen in immunocompromised individuals or those with underlying lung disease. It may be caused by a wide range of dematiaceous moulds including *Alternaria* species, *C. bantiana*, *Curvularia* species, *Exophiala* species, *Fonsecaea pedrosoi*, *O. gallopava* and *S. prolificans*.

Cerebral phaeohyphomycosis is a rare but frequently fatal disease. It is the most commonly reported systemic infection caused by dematiaceous fungi. Infection is thought to follow inhalation and haematogenous dissemination from the lungs; however, it may also result from direct spread from the paranasal sinuses. The incubation period is unknown. More cases have been described in men than women, but a particular occupational predisposition has not been noted. The age range of reported cases is wide, but most patients are young adults. Although cases of cerebral phaeohyphomycosis have been reported in patients with haematological malignancies, as well as haematopoietic stem cell transplant (HSCT) and SOT recipients, more than one-half of cases of the disease are encountered in otherwise healthy individuals with no obvious risk factors. The case fatality rate is high, and nearly identical rates have been reported for immunocompetent (74%) and immunocompromised (71%) individuals.

The most common cause of central nervous system (CNS) phaeohyphomycosis is *C. bantiana*, particularly in immunocompetent individuals. In a review of 101 published cases of CNS phaeohyphomycosis, this mould accounted for 48% of infections that, in almost all cases, consisted of a brain abscess. *C. bantiana* is found worldwide; however, infections with this organism appear to be more common in sub-tropical and humid climates. *E. dermatitidis* also occurs worldwide, but most cases of CNS infection have been encountered among immunocompetent Asians. Other aetiological agents include *B. spicifera*, *B. hawaiiensis*, *Fonsecaea monomorpha*, *O. gallopava* and *R. mackenziei*.

Disseminated phaeohyphomycosis is the most uncommon form of the disease, but recent reports suggest that its incidence may be increasing, particularly among immunocompromised individuals. According to one recent report from a North American cancer hospital, the incidence of invasive phaeohyphomycosis increased from 1.0 case per 100,000 patient days between 1993 and 1996 to 3.1 cases per 100,000 patient days between 2005 and 2008. In this retrospective analysis of 39 cases, most (82%) had

haematological malignancies, while 64% were neutropenic at the time of diagnosis and 26% had undergone an allogeneic HSCT. Of note, 36% of cases had developed the infection while receiving prophylactic or empirical antifungal treatment. One-third of patients had a widespread disseminated infection, and one-third died within 12 weeks after diagnosis. Recovery from neutropenia was the only factor that was independently associated with an improved survival rate.

In an earlier review of 72 published cases of disseminated phaeohyphomycosis, most patients were immunocompromised; a few cases occurred in immunocompetent individuals with no obvious risk factors. The most common aetiological agent was *S. prolificans,* which accounted for 42% of cases. The next most common species were *B. spicifera* and *E. dermatitidis*. The group at greatest risk for disseminated infection was neutropenic cancer patients, particularly those with acute leukaemia, but a few cases also occurred among HSCT and SOT recipients and persons with the acquired immunodeficiency syndrome (AIDS). The overall case fatality rate was 84% in immunocompromised patients, compared with 65% in immunocompetent individuals.

S. prolificans was initially reported to cause bone and joint infections and locally invasive disease in immunocompetent individuals. More recently, however, cases of disseminated infection have become more common, particularly in immunocompromised patients. In a recent retrospective analysis of 162 cases of *S. prolificans* infection, 44% of patients had disseminated disease. Almost 80% of these individuals had haematological malignancies and most were neutropenic at the time of diagnosis. The case fatality rate among patients with disseminated *S. prolificans* infection was 87.5%.

Several nosocomial outbreaks of phaeohyphomycosis have been reported. An outbreak of *E. jeanselmei* fungaemia in a Brazilian hospital was associated with contamination of hospital water used to prepare antiseptic solutions that were used for intravascular catheter site care. More recently, five cases of *E. dermatitidis* infection in four hospitals in North Carolina were traced to contaminated injectable steroids from the same source.

25.5 Clinical manifestations

25.5.1 Subcutaneous phaeohyphomycosis

Subcutaneous phaeohyphomycosis usually presents as a single asymptomatic mass or nodule at the site of prior trauma. The most common sites of infection are the feet, legs hands, arms and head. If left untreated, the nodule will slowly increase in size to form a painless cystic abscess.

Multiple lesions are rare, but sometimes can arise following incomplete excision of the original nodule. In most cases, the lesions remain localized and the overlying skin remains intact. On occasion, the infection may spread to involve joints or bone.

In immunocompromised individuals, the clinical presentation is variable. Patients may present with subcutaneous nodules, macules, papules, pustules, ulcers or eschars on the limbs. Most patients have only one or two skin lesions. Draining sinuses sometimes develop in immunocompromised patients with subcutaneous phaeohyphomycosis.

25.5.2 Rhinosinusitis

In immunocompetent individuals, chronic invasive rhinosinusitis is a slowly progressive, destructive condition that may remain confined to the sinuses or spread to the orbit and brain (see also Chapter 10). It usually affects the ethmoid and sphenoid sinuses, but may involve any paranasal sinus. In occasional cases, a fungus ball may develop. Affected individuals usually complain of long-standing symptoms of unilateral nasal discharge and blockage, loss of smell and persistent headache. Thick nasal polyposis and thick mucopurulent mucus are common. If left untreated, the infection can spread from the ethmoid sinuses into the orbit, leading to impaired vision and restricted ocular movement. Proptosis can also occur. Chronic invasive rhinosinusitis is often advanced by the time of diagnosis, with posterior erosion out of the ethmoid sinus, resulting in cavernous venous thrombosis. Non-contrast computed tomographic (CT) scans will reveal a hyperdense mass within the involved sinus with associated erosion of the sinus walls.

In immunocompromised patients, invasive rhinosinusitis most commonly affects the maxillary and ethmoid sinuses. In some patients, the disease is limited to the nasal septum, with no signs of sinus involvement on physical examination or imaging, but in others, the disease may spread to adjacent tissue, resulting in a brain abscess. The most common presenting symptom is sinus pain although this was reported in only 40% of cases in a recent review. The most frequent finding on physical examination is an eschar on the nasal septum. CT scans will reveal signs of sinusitis in most patients, but sinus wall erosion is an uncommon finding.

Allergic fungal rhinosinusitis is usually non-invasive and follows a benign albeit uncomfortable course (see also Chapter 10). Many individuals with allergic fungal rhinosinusitis have a history of chronic rhinosinusitis and have undergone multiple operations prior to diagnosis. Patients often present with unilateral nasal polyposis and thick yellow-green nasal

or sinus mucus. The nasal polyposis may form an expansive mass that causes bone necrosis of the thin walls of the sinus. Erosion of bone with or without extension of disease into adjacent anatomic spaces occurs in about 20% of cases of allergic fungal rhinosinusitis. Should the ethmoid bone be traversed, allergic mucin can enter the orbit and cause proptosis. Polypoid material can also push the nasal septum into the contralateral airway. Sinus radiographs show mucosal thickening, while CT scans often reveal a characteristic serpiginous sinus opacification of more than one sinus, mucosal thickening, and erosion of bone. This does not represent tissue invasion.

The criteria for diagnosis of this form of rhinosinusitis have undergone numerous revisions; however, a recent consensus group conference agreed on the following: the presence of chronic rhinosinusitis (confirmed by CT scan) in an otherwise immunocompetent individual; the presence of the characteristic eosinophilic mucin (mucus containing clusters of eosinophils and their degraded products) either at the time of surgical debridement for chronic sinusitis or endoscopic examination for drainage; the presence of non-invasive fungal elements within that mucin detectable on staining or culture; histopathological examination of sinus tissue to rule out invasion; radiological studies to assess the extent of disease; laboratory testing for eosinophilia, total serum immunoglobulin (Ig)E and specific IgE against fungal antigens; and a positive skin prick test to cultured fungi.

25.5.3 Pulmonary phaeohyphomycosis

In immunocompromised individuals, the clinical presentation of pulmonary phaeohyphomycosis is similar to that of acute invasive pulmonary aspergillosis (see Chapter 10). The radiographic findings range from non-specific infiltrates to nodular lesions that can evolve to cavitation. The halo sign is sometimes seen. Pleural effusion is uncommon. Disseminated infection is a frequent complication.

In patients with asthma, *Bipolaris* and *Curvularia* species may cause a clinical picture indistinguishable from allergic bronchopulmonary aspergillosis (see Chapter 10). All reported cases have had either eosinophilia or elevated IgE levels.

25.5.4 CNS phaeohyphomycosis

Brain abscess is the most common presentation of CNS phaeohyphomycosis, accounting for almost 90% of reported cases. In 70% of patients, there is a single lesion, the frontal lobes of the brain being the most common location. However, multiple lesions may occur and other sites of

involvement have been reported. Other manifestations include meningitis, encephalitis, myelitis and arachnoiditis.

The symptoms of cerebral phaeohyphomycosis are gradual in onset. Persistent headache is the most common presenting symptom. Clinical findings include neurological deficits, fever, seizures and altered mental status. Of note, however, the classic triad of fever, headache and neurological deficits described in patients with brain abscess is seldom observed. CT scans often reveal a unilateral, well-circumscribed mass with a contrast-enhancing margin. The cerebrospinal fluid (CSF) findings are usually abnormal. The opening pressure may be raised, the protein concentration may be increased, the glucose concentration may be reduced and pleocytosis may be present. CSF cultures are seldom positive, and it is unusual for the diagnosis to be established until neurologic resection is performed.

Meningitis has also been described, usually in immunocompromised patients. It may occur alone or in association with a brain abscess. Headache is again a prominent symptom, but the clinical course is more protracted.

25.5.5 Disseminated phaeohyphomycosis

In immunosuppressed individuals, the clinical manifestations of disseminated phaeohyphomycosis are similar to those of invasive aspergillosis (see Chapter 10). Fever is the commonest symptom, but was reported in only 76% of 72 cases in a recent review. Cutaneous lesions were seen in 33% of patients. The most frequent sites of involvement have included the lungs, heart and brain. The liver, spleen, lymph nodes, bones and joints have been less frequently reported as sites of infection.

Disseminated *S. prolificans* infection may be associated with septic shock.

25.5.6 Other forms of phaeohyphomycosis

Dematiaceous moulds have been reported to cause endocarditis after valve insertion or replacement and peritonitis in patients on continuous peritoneal dialysis. Post-traumatic osteomyelitis and arthritis have also been reported. Affected individuals are often immunocompromised.

25.6 Differential diagnosis

The lesions of subcutaneous phaeohyphomycosis can be confused with the small initial lesions of chromoblastomycosis, sporotrichosis, blastomycosis, coccidioidomycosis and paracoccidioidomycosis, as well as with cutaneous leishmaniasis. Lymphangitic spread of sporotrichosis and the

development of verrucous lesions in the other conditions makes the distinction easier.

In immunocompetent individuals, the clinical presentation of chronic invasive phaeohyphomycotic rhinosinusitis is indistinguishable from that of *Aspergillus* infection (see Chapter 10). In immunosuppressed patients, *Aspergillus* rhinosinusitis is a fulminant, often lethal, condition unlike phaeohyphomycosis. However, both groups of organisms have caused black necrotic lesions of the nasal septum in patients with leukaemia or AIDS.

The presenting symptoms of cerebral phaeohyphomycosis are similar to those of an untreated bacterial brain abscess, but the fungal infection is often more indolent in onset, with slowly evolving neurological signs. In immunocompromised patients, the diagnosis of cryptococcosis, histoplasmosis, coccidioidomycosis, sporotrichosis, listeriosis, nocardiosis or toxoplasmosis, among others, must be excluded.

25.7 Essential investigations and their interpretation

The aetiological agents of phaeohyphomycosis are frequently isolated from respiratory tract specimens, such as sputum and bronchoalveolar lavage fluid, and even in immunocompromised patients, their presence may represent contamination or colonization, rather than infection. Thus, the diagnosis of phaeohyphomycosis depends on a combination of histopathological examination of affected tissue and culture.

25.7.1 Microscopy

One common factor among these fungi is their melanin formation in the cell wall in culture and, in most cases, in human tissue. Microscopic examination of stained histopathological sections or wet preparations of clinical material, such as pus or skin scrapings, can permit the diagnosis of phaeohyphomycosis if brown-pigmented, septate hyphae with occasional branching are seen. The hyphae tend to be thin, but are irregularly swollen with prominent septa that show constrictions. They may show terminal or intercalated vesicular swellings with thick walls that resemble chlamydoconidia. Pigmented yeast-like cells may also be seen, and these may show septation and budding. Grains and muriform cells are not found. In cases where pigmentation is not evident with hematoxylin and eosin staining, Fontana Masson staining may be needed to detect the melanin pigment.

25.7.2 Culture

Identification of the aetiological agent is essential for correct management, and this depends on its isolation in culture. Clinical material should be

inoculated on glucose peptone (Sabouraud's dextrose) agar and incubated at 25–30°C. Identifiable dark brown or olivaceous to black mould colonies should be formed within 1–2 weeks, but cultures should be retained for at least 4 weeks before being discarded.

The identification of many of the aetiological agents of phaeohyphomycosis is difficult due to the pleomorphic nature of these fungi and their similar colonial and microscopic characteristics. Traditional identification is based on a combination of morphological and physiological tests; however, molecular identification is assuming a greater importance. In many instances, species identification may be achieved by sequencing of the internal transcribed spacer (ITS) regions of the ribosomal DNA gene complex. However, in some cases, ITS sequencing does not provide sufficient resolution, so single-copy targets, such as the β-tubulin and other genes, are used for identification.

Overall, blood cultures have been positive in around 50% of patients with disseminated phaeohyphomycosis. *S. prolificans* infection has been associated with positive blood cultures in 70–80% of cases. In most instances, however, blood cultures become positive late in the course of the infection, thus limiting their diagnostic usefulness.

25.8 Management

Management of patients with infections caused by dematiaceous fungi is based on clinical experience, much of which has been published in the form of individual case reports or small case series. Amphotericin B generally has good activity in vitro against most clinically important dematiaceous fungi. However, *S. prolificans* has been consistently resistant in vitro, as are some isolates of *Exophiala* species and *R. mackenziei*. Itraconazole has shown good activity against numerous dematiaceous moulds, and the newer triazoles, voriconazole and posaconazole also have a broad spectrum of activity. Terbinafine has shown broad activity against dematiaceous fungi, and interest in its use is increasing. However, the data for each of these agents must be interpreted with caution because there are no established correlations between in vitro test results and in vivo outcome.

25.8.1 Subcutaneous phaeohyphomycosis

Complete surgical excision of the entire lesion is usually curative and antifungal treatment is not required. Incomplete removal of involved tissues, or incision and drainage procedures will result in recurrence. Lesions that cannot be resected can be treated with an azole or terbinafine, but later relapse after discontinuation of treatment has been common, particularly

if the patient remains immunosuppressed. Combination treatment with itraconazole and terbinafine has been found to produce a good response in some cases. The optimum duration of antifungal treatment that should be given has not been defined, but several months' treatment is usually required, and close follow-up is recommended.

25.8.2 Rhinosinusitis

Invasion of adjacent structures is a common complication of chronic invasive fungal rhinosinusitis. For this reason, extensive surgical debridement with removal of all necrotic material, combined with amphotericin B treatment is recommended. The optimum duration and total dose of amphotericin B that should be given have not been defined, and it is not uncommon for this condition to recur, necessitating further surgical intervention. There is limited evidence that long-term suppressive treatment with itraconazole may reduce the rate of recurrence following surgical resection and amphotericin B treatment. Although only a small number of cases have been reported, it appears that long-term treatment with itraconazole can lead to improvement or remission in some patients who had failed to respond to amphotericin B treatment. The role of the newer triazole antifungal agents, such as voriconazole and posaconazole, is unclear but promising.

In patients with allergic fungal rhinosinusitis, treatment consists of surgical debridement to remove polyps and the eosinophilic mucin containing fungal debris, which is thought to be the cause of the immune reaction in the sinus mucosa. More than one surgical procedure may be required to accomplish this goal. Adjunctive medical management is also required because it is unlikely that all fungal debris can be removed. In small case series, use of post-operative oral corticosteroids reduced recurrence of disease.

Antifungal treatment is not usually thought to be of benefit in allergic fungal rhinosinusitis. However, there is limited evidence that post-operative treatment with itraconazole may reduce the need for re-operation. Post-operative endoscopic follow-up is recommended, because there is a poor correlation between subjective improvement and presence of objective regression of disease. Despite surgical debridement and post-operative corticosteroid treatment, the condition recurs in up to two-thirds of patients.

25.8.3 Pulmonary phaeohyphomycosis

In immunocompetent individuals, surgical resection of solitary pulmonary nodules is usually successful. Management of immunocompromised patients with pulmonary phaeohyphomycosis has usually

consisted of treatment with amphotericin B or itraconazole. The role of the newer triazole antifungal agents, such as voriconazole and posaconazole, is unclear but promising.

25.8.4 CNS phaeohyphomycosis

The clinical outcome of CNS phaeohyphomycosis is dismal, with long-term survival being reported only when radical surgical resection of solitary cerebral lesions is possible. Lesions that have not been completely removed have usually proved fatal. The classical medical treatment of amphotericin B alone or in combination with itraconazole has generally proved ineffective in patients with *C. bantiana* infection. There is now at least one anecdotal report of the successful treatment of a *C. bantiana* brain abscess with voriconazole. Failures have also been described, though these have tended to occur in immunocompromised individuals.

The clinical outcomes for patients with *R. mackenziei* infection have also been dismal, and several have died despite treatment with liposomal amphotericin B, voriconazole and/or posaconazole. There is, however, one case report describing the successful use of posaconazole for salvage treatment of a renal transplant recipient with a cerebral abscess due to *R. mackenziei*. This represents the first reported survival of infection due to this mould.

25.8.5 Disseminated phaeohyphomycosis

Too few patients have been treated to make firm recommendations. However, some dematiaceous moulds are resistant to amphotericin B in vitro and the response to this agent in patients with disseminated phaeohyphomycosis has been partial at best. According to a recent review, only 23% of 62 patients treated with this agent survived.

S. prolificans is the most frequent cause of disseminated phaeohyphomycosis. This mould appears to be resistant to most available antifungal agents and the prognosis for neutropenic patients with disseminated infection is dismal, unless the neutrophil count recovers. Newer agents such as voriconazole have shown variable results; however, there are several anecdotal reports of successful treatment of disseminated *S. prolificans* infection with a combination of itraconazole or voriconazole and terbinafine.

25.8.6 Other forms of phaeohyphomycosis

Localized forms of phaeohyphomycosis, such as endocarditis and osteomyelitis, usually require combined medical and surgical management. *S. prolificans* is the most common cause of bone and joint infection.

Several reported cases have received the combination of voriconazole and terbinafine with success.

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CHAPTER 26

Other invasive yeast infections

26.1 Introduction

In recent years, a number of yeast species that had previously been thought to represent contamination or harmless colonization when isolated from humans have been recognized as significant pathogens in compromised patients. The genera most likely to cause invasive infection include *Malassezia* and *Trichosporon*, but *Blastoschizomyces*, *Saccharomyces* and *Rhodotorula* have also been reported to cause fungaemia with or without organ invasion. Establishing the diagnosis is difficult and depends on the detection of the organism in tissue sections or smears of clinical material as well as isolation in culture.

26.2 Systemic *Malassezia* infection

Malassezia is a genus of lipophilic basidiomycetous yeasts responsible for several mild, but recurrent cutaneous diseases of humans, including pityriasis versicolor, folliculitis and seborrhoeic dermatitis (see Chapter 6). Less frequently, these organisms have caused life-threatening systemic infection in critically ill low-birth-weight infants and other immunocompromised and debilitated individuals, all of whom had received lipid-supplemented parenteral nutrition through an indwelling vascular catheter.

26.2.1 Geographical distribution

These infections are worldwide in distribution.

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26.2.2 The causal organisms and their habitat

Until recently, only three *Malassezia* species were recognized: these were two lipid-dependent species, *Malassezia furfur* and *Malassezia sympodialis*, and one non-obligate lipophile, *Malassezia pachydermatis*. On the basis of molecular analysis, the *M. furfur* species complex has been progressively enlarged to include *M. sympodialis*, and seven new taxa named *Malassezia globosa, Malassezia obtusa, Malassezia restricta*, *Malassezia slooffiae*, *Malassezia dermatis*, *Malassezia japonica* and *Malassezia yamatoensis*. Some additional members of the complex have been reported only from animals. *M. pachydermatis*, which causes ear infections and seborrhoeic dermatitis in animals, is not considered a member of the *M. furfur* complex. It is not lipid-dependent and is easily distinguished from *M. furfur* complex members by its ability to grow on routine mycological media.

26.2.3 Epidemiology

Malassezia species form part of the normal microbial flora of the skin of humans and other warm-blooded animals, and most infections are endogenous in origin. The prevalence of skin colonization with these organisms is dependent upon age, anatomical site and, to a lesser degree, race. Although *Malassezia* species are not found on the skin of healthy newborn infants, up to 30–60% of preterm infants who reside in neonatal intensive care units become colonized by their second week of life. High temperatures and high humidity appeared to be the most important exogenous risk factors affecting the colonization status of infants.

Exposure to lipid-rich intravenous infusions through a central venous catheter is the single most important risk factor for systemic *Malassezia* infection in both infants and older patients. Among neonates, other risk factors include low birth weight, early gestational age and length of hospitalization. An investigation of one outbreak of *M. furfur* infection among low-birth-weight infants, most of whom received intravenous lipids, identified the duration of antimicrobial treatment as an additional risk factor for disease. Among older children and adults, the infection has occurred in conjunction with a range of underlying illnesses, including haematological malignancies and stem cell and solid organ transplantation.

Although most infections appear to be sporadic, nosocomial outbreaks of systemic *Malassezia* infection have been reported. An investigation of one outbreak of *M. furfur* infection among low-birth-weight infants provided evidence that the organism can be transmitted from an infected or colonized infant to other infants via the hands of healthcare workers.

Nosocomial outbreaks of *M. pachydermatis* infection have also been reported. In one outbreak, the organism was introduced into the unit on healthcare workers' hands after being colonized from pet dogs at home. The organism persisted in the unit through patient-to-patient transmission, but the outbreak was interrupted by improving hand-washing practices. Other outbreak investigations have shown that *Malassezia* species can persist for prolonged periods of time on incubator surfaces, providing another source for continued transmission.

26.2.4 Clinical manifestations

In infants, *Malassezia* fungaemia usually presents as fever and/or respiratory distress. The latter may result in interstitial pneumonia. Leucocytosis or leucopenia and thrombocytopenia are common findings in this patient group. Less frequent symptoms and signs include poor feeding, hepatomegaly and splenomegaly. No signs of infection have been noted at catheter insertion sites, nor has a skin rash been evident in infants with systemic *Malassezia* infection.

Catheter-associated *Malassezia* fungaemia is a sporadic illness in compromised children and adults, and the clinical manifestations are not as well described as in premature infants. Fever is a consistent presenting symptom. Other symptoms and clinical signs include chills, rigors, myalgia, nausea, vomiting, respiratory distress with or without apnoea, pneumonia, leucopenia, thrombocytosis and leucocytosis.

Catheter-associated *Malassezia* fungaemia may result in embolic-metastatic infection of the heart and lungs and less frequently, dissemination to other organs, such as the skin, kidneys, liver, spleen and brain. Unusual manifestations include reports of thrombophlebitis, meningitis, septic arthritis, soft-tissue abscesses, and catheter-associated peritonitis in peritoneal dialysis patients. The predominant pathological changes have included mycotic thrombi around the tips of indwelling catheters, endocardial vegetations, vasculitis of vessels and inflammation of alveoli, bronchi and bronchioles.

26.2.5 Essential investigations and their interpretation

The diagnosis of *Malassezia* fungaemia should be considered in any febrile patient (particularly if there is clinical and radiological evidence of pneumonia) who is receiving lipid-containing parenteral nutrition through an indwelling vascular catheter, especially if routine blood cultures are sterile. In these patients, use of lipid-supplemented media may be justified if cultures appear sterile on routine media and yeasts have been observed on microscopy.

Malassezia fungaemia has sometimes been diagnosed following detection of the organism in stained smears prepared from catheter blood specimens. However, the diagnosis is most often based on isolation of the organism from blood taken through the catheter. In instances where the catheter can be removed, the tip of the catheter should be inoculated onto glucose peptone (Sabouraud's dextrose) agar overlaid with sterile olive oil or another lipid-enriched medium. The lipid concentration of conventional broth and agar media is usually insufficient to support the growth of *Malassezia* species (with the exception of the non-lipid-dependent species, *M. pachydermatis*), but it would appear that the blood of patients receiving parenteral nutrition often contains sufficient lipids to support initial growth of these organisms in culture. Subculture of broth onto an agar medium containing, or overlaid with, a lipid source should ensure isolation of the organism. Identifiable colonies can be obtained after incubation for 1–4 days at 35–37°C.

26.2.6 Management

The most important factor in the successful management of *Malassezia* fungaemia in preterm infants is the prompt removal of the infected vascular catheter, whether or not antifungal treatment is given. Lipid supplements should be discontinued in almost all patients, and an antifungal azole, such as parenteral fluconazole (5 mg/kg per day), should be administered. More stable patients may be able to receive fluconazole or itraconazole by mouth. *Malassezia* species are usually susceptible to voriconazole, and this agent may be another option for treatment of life-threatening infection. The duration of treatment depends on persistence of fungaemia, presence of metastatic foci of infection and removal of the catheter. It is reasonable to discontinue treatment 2 weeks after the last positive blood culture.

26.3 Trichosporonosis

In addition to causing white piedra, an uncommon asymptomatic infection of the scalp, facial and pubic hair (see Chapter 6), *Trichosporon* species can also cause a systemic infection known as trichosporonosis in immunocompromised individuals. *Trichosporon* infections in immunocompromised individuals are usually disseminated, involve major organs, and many have been fatal.

26.3.1 Geographical distribution

These infections are worldwide in distribution.

26.3.2 The causal organisms and their habitat

The disease is caused by members of the basidiomycetous yeast genus *Trichosporon*. On the basis of molecular analysis, the taxonomy of the genus has been progressively modified to the point where more than 30 species are now recognized. Four of these newly delineated taxa (*Trichosporon asahii* and less frequently *Trichosporon mucoides, Trichosporon inkin* and *Trichosporon louberi*) are currently recognized as the species associated with systemic infections in humans. In older reports, invasive isolates are identified using the now-obsolete terms, *Trichosporon beigelii* or *Trichosporon cutaneum*.

Trichosporon species have a widespread natural distribution, being found in soil and water and on plants. They are also common commensal inhabitants of the skin and gastrointestinal tract of humans.

26.3.3 Epidemiology

Most cases of trichosporonosis appear to be endogenous in origin. Like candidosis, trichosporonosis is usually preceded by gastrointestinal or respiratory tract colonization. Infection has arisen as a result of invasion from the gastrointestinal mucosa, through aspiration of gastrointestinal tract contents and via indwelling vascular catheters.

The likelihood that trichosporonosis will develop in a colonized individual depends, in major part, on host factors. Those at greatest risk include neutropenic cancer patients receiving cytotoxic treatment. Less commonly, disseminated infection has been seen in solid organ transplant recipients, burns patients, low-birth-weight infants, patients receiving corticosteroids and persons with the acquired immunodeficiency syndrome. Factors that enhance mucosal colonization and subsequent invasive infection, include broad-spectrum antibiotic treatment, central venous catheters and other breaks in anatomical barriers, such as peritoneal dialysis catheters.

The overall incidence of *Trichosporon* infections is low, even among patients with acute leukaemia, but the prevalence appears to be higher in some clinical centres than in others. The case fatality rate is high, ranging from 60% to 80% in earlier reports.

26.3.4 Clinical manifestations

The clinical manifestations of disseminated trichosporonosis are similar in most respects to those of invasive candidosis (see Chapter 11). Like candidosis, trichosporonosis may take the form of an acute or a chronic infection. The former often presents as a persistent fever that fails to respond to broad-spectrum antibacterial treatment. Multiple cutaneous lesions,

similar to those seen in disseminated candidosis, occur in up to two-thirds of patients. These evolve from maculopapules to necrotizing ulcers. Chorioretinitis has also been described. The liver, spleen, brain and heart are among the other organs that may be involved, but the lungs are the most common site of end-organ disease. In patients with *Trichosporon* pneumonia, chest radiographs will reveal reticulonodular or diffuse infiltrates.

Chronic hepatosplenic trichosporonosis has been described in leukaemic patients who recover from neutropenia. Like chronic disseminated candidosis (see Chapter 11), this infection often begins while the patient is neutropenic, but does not become apparent until after the neutrophil count has recovered. The patient remains febrile and unwell, developing an enlarged liver and spleen.

26.3.5 Essential investigations and their interpretation

The diagnosis of trichosporonosis is seldom suspected until the yeast is isolated from blood, urine or cutaneous lesions. Often, however, patients have died and the infection has remained unrecognized until material obtained post-mortem has been investigated.

Microscopic examination of smears and histopathological sections of cutaneous lesions will reveal branching hyphae with numerous rectangular arthrospores and budding blastospores. If arthrospores are sparse in tissue, *Trichosporon* species can resemble *Candida albicans*. However, *Trichosporon* sometimes produces more true hyphae and fewer pseudohyphae than *C. albicans*.

In culture, members of the genus *Trichosporon* form white to cream, heaped colonies that consist of hyaline hyphae with arthrospores and blastospores. In neutropenic patients, blood cultures are often positive and the organisms can also be recovered from biopsies of cutaneous lesions. Isolation from these specimens provides more reliable evidence of deep-seated infection than does isolation from bronchoalveolar lavage fluid, sputum or urine.

(1-3)-β-D-glucan, a cell wall component of many fungi, has been detected in the blood of patients with invasive *Trichosporon* infection. Although clinical experience is limited, the Fungitell assay (Associates of Cape Cod, Falmouth, Massachusetts, USA) for detection of β-D-glucan has been approved for the presumptive diagnosis of invasive fungal infections, including trichosporonosis. Although a positive test result does not identify the aetiological agent, the assay can be used as a screening method, allowing the earlier initiation of antifungal treatment.

Trichosporon species produce a heat-stable antigen that shares antigenic determinants with the capsular antigen of *Cryptococcus neoformans*.

Several reports have appeared, indicating that with serum the latex particle agglutination (LPA) test for *Cryptococcus* antigen is positive in patients with disseminated trichosporonosis and could be useful in making the diagnosis. However, negative results have also been obtained in LPA tests with serum and urine specimens from patients dying with disseminated trichosporonosis.

26.3.6 Management

The optimum therapy for systemic *Trichosporon* infection has not been determined. Breakthrough trichosporonosis has occurred during treatment with most antifungal agents, regardless of the dosage or duration. The intrinsic resistance of these organisms to echinocandin drugs is underscored by reports of breakthrough infections among patients with haematological malignancies and stem cell transplant recipients receiving micafungin or caspofungin.

In general, isolates of *T. asahii* appear to be less susceptible than isolates of other species to amphotericin B, fluconazole or itraconazole in vitro, while the new triazoles, voriconazole and posaconazole, are active against both *T. asahii* and the other species. Anecdotal case reports describe the successful use of voriconazole to treat neutropenic patients with disseminated *T. asahii* infection. Treatment with posaconazole led to clinical improvement in a liver transplant recipient with *T. mucoides* fungaemia, but the patient died of an unrelated infection.

26.4 Other yeast infections

Other unusual yeasts that have been reported to cause invasive infections in profoundly immunocompromised patients include *Blastoschizomyces capitatus*, *Rhodotorula* species and *Saccharomyces cerevisiae*. These organisms may present with a wide range of clinical manifestations, ranging from fungaemia to localized deep or disseminated infection. The approach to successful management is necessarily individual, but includes antifungal treatment and removal of potentially colonized vascular catheters.

26.4.1 *Blastoschizomyces* infection

Blastoschizomyces capitatus (formerly *Geotrichum capitatum*) is an ascomycetous yeast that is commonly found in the environment and can be recovered from the skin, gastrointestinal tract and respiratory tract of healthy individuals. Invasive disease is quite rare and has mostly been reported in neutropenic individuals with acute leukaemia who had received cytotoxic treatment. The mortality rate ranges from 60% to 80%.

In neutropenic patients, *B. capitatus* produces a pattern of infection similar to that of candidosis and trichosporonosis. More than 70% of patients have fungaemia, but 60–80% develops deep organ involvement, compared with 10–20% of patients with invasive candidosis. As with candidosis and trichosporonosis, chronic disseminated infection can occur in patients who recover from neutropenia. The diagnosis of *B. capitatus* infection is usually made by culture of blood.

The optimum approach to treatment remains to be defined, but *B. capitatus* is usually susceptible to amphotericin B, fluconazole, itraconazole, posaconazole and voriconazole. However, there are several reports of breakthrough infections occurring during treatment or prophylaxis with amphotericin B or fluconazole. The echinocandins are ineffective against this organism.

26.4.2 *Rhodotorula* infection

Rhodotorula is a genus of basidiomycetous yeasts that is commonly found in the environment and can also be recovered from the skin of healthy individuals. Invasive disease is quite rare: it usually presents as fungaemia and has mostly been reported in immunocompromised patients, particularly those with central venous catheters or other indwelling devices. Other risk factors include neutropenia, parenteral nutrition and broad-spectrum antibacterial treatment. The importance of central venous catheters and prophylaxis with fluconazole as risk factors was highlighted in a report of a nosocomial outbreak of *Rhodotorula mucilaginosa* fungaemia in a neonatal intensive care unit. Other forms of infection include endocarditis, meningitis and peritonitis.

The optimum approach to treatment of *Rhodotorula* infection appears to be amphotericin B coupled with catheter removal. These organisms possess intrinsic resistance to the triazoles and echinocandins.

26.4.3 *Saccharomyces* infection

S. cerevisiae is an ascomycetous yeast that is widespread in nature and can be found on plants and fruit, as well as in soil. This yeast is included in some diet or health foods and *S. cerevisiae* subtype *boulardii* is also used in probiotic preparations for the prevention and treatment of various diarrhoeal disorders, such as those associated with *Clostridium difficile* infection.

S. cerevisiae can be recovered from the mucosal surfaces, gastrointestinal tract and female genital tract of healthy individuals. Enteral translocation of ingested organisms and contamination of central venous catheter hub or insertion sites are the main portals into the bloodstream. Risk

factors for *Saccharomyces* fungaemia are similar to those for invasive candidosis, except for treatment with a probiotic containing *S. cerevisiae* subtype *boulardii*.

Invasive disease is quite rare and has mostly been seen in immunosuppressed and critically ill patients. *Saccharomyces* produces a pattern of infection similar to that of invasive candidosis. Fungaemia is common and chorioretinitis has been reported. Amphotericin B and fluconazole are effective therapeutic options, although some isolates are resistant to the latter drug. Voriconazole has been used successfully for salvage treatment of sepsis due to subtype *boulardii* following failure with fluconazole. Removal of central venous catheters and withdrawal of the probiotic regimen, if one was being given, are also advised.

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CHAPTER 27

Unusual fungal and pseudofungal infections

27.1 Introduction

This chapter describes a number of unusual or uncommon infections, including several that have been considered to be fungal based on the appearance of their characteristic structures in tissue, but have been difficult to categorize because the aetiological agents cannot be isolated in culture. In two of the four diseases described, recent molecular evidence has indicated that the causal organism, previously considered to be a fungus, is in fact a protistan parasite. *Pythium insidiosum* and *Rhinosporidium seeberi*, the aetiological agents of pythiosis and rhinosporidiosis, are now placed in the kingdoms Stramenopila (formerly Chromista) and Protozoa, respectively.

27.2 Adiaspiromycosis

27.2.1 Definition

The term adiaspiromycosis (or haplomycosis) is used to describe an uncommon infection caused by three thermally dimorphic fungi of the genus *Emmonsia* that affect humans and animals. Following inhalation, these organisms cause a wide spectrum of clinical manifestations, ranging from localized pulmonary infection to more widespread disease. Although many cases are self-limited and inconsequential, progressive pulmonary or disseminated infection can lead to respiratory failure and death.

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27.2.2 Geographical distribution

Human disease is rare, but sporadic cases have been reported from North, Central and South America, as well as from several European and Asian countries.

27.2.3 The causal organisms and their habitat

The genus *Emmonsia* currently includes three species: *Emmonsia crescens, Emmonsia parva* and *Emmonsia pasteuriana*. The last has only been recovered from a single case of cutaneous disseminated infection in a patient with the acquired immunodeficiency syndrome (AIDS). Genetic sequencing of these fungi has indicated that *E. crescens* and *E. parva* are closely related to the genus *Blastomyces*, but distinct from *E. pasteuriana*. The natural habitat of *Emmonsia* species is the soil. Infection has occurred in numerous species of burrowing mammals.

In the environment and in culture at room temperature, *Emmonsia* species exist as a mould that produces small, single-celled spores. Inside the host or upon incubation at 40°C, the conidia of *E. crescens* and *E. parva* fail to germinate, instead increasing in size to become large spherical structures termed adiaspores, which resemble the spherules of *Coccidioides* species. Although adiaspores enlarge to become enormous, thick-walled structures that induce a granulomatous response, no endospores are produced and the spores eventually die. This lack of replication in tissue is unique among pathogenic fungi.

27.2.4 Epidemiology

Inhalation is believed to be the usual mode of infection in humans and animals. The precise incubation period is unknown, but is thought to be several weeks. Adiaspiromycosis is more commonly reported in men than women, reflecting the importance of occupational exposure to soil or dust. Most affected individuals are otherwise normal, and there does not appear to be any predisposition apart from exposure. An unusual outbreak of ocular adiaspiromycosis occurred in northern Brazil in 2006. A few cases of adiaspiromycosis have been described in immunocompromised transplant recipients and persons with human immunodeficiency virus (HIV) infection.

27.2.5 Clinical manifestations

In most cases, adiaspiromycosis is a self-limited, localized pulmonary infection with few or no symptoms. Because the adiaspores enlarge but do not germinate or reproduce in host tissues, the severity of symptoms and clinical signs appears to depend upon the number of conidia inhaled. If

only a few conidia are inhaled, the disease may be limited to a few nodules within a segment or lobe of a lung; if numerous conidia are inhaled, there may be bilateral pulmonary disease. In patients with localized disease, the infection may be asymptomatic. In those with more widespread pulmonary involvement, non-productive cough, fever, chest pain, dyspnoea and weight loss may be seen. Chest radiographs may reveal bilateral reticulonodular infiltrates. Extension to the local pulmonary lymph nodes may occur. The outcome of pulmonary disease may be fatal.

Extrapulmonary dissemination is rare, but several cases of disseminated infection with *E. crescens* or *E. parva*, with cutaneous lesions, or bone involvement have been described in immunocompromised individuals. The one reported case of *E. pasteuriana* infection occurred in an AIDS patient who developed disseminated cutaneous lesions.

27.2.6 Differential diagnosis

The typical radiological appearance of pulmonary adiaspiromycosis must be distinguished from cryptococcosis, sarcoidosis, miliary tuberculosis and carcinoma. The histopathological appearance must be distinguished from coccidioidomycosis and rhinosporidiosis.

27.2.7 Essential investigations and their interpretation

The diagnosis is usually established by histopathological demonstration of the characteristic adiaspores, surrounded by granulomatous inflammation in lung tissue. Necrosis and caseation are typically not observed, but have been reported. Of note, all of the granulomas are at a similar stage of development, reflecting a single exposure without subsequent replication within the lung. Unlike *E. parva* and *E. crescens*, *E. pasteuriana* produces small yeast-like budding cells, larger thick-walled cells and pseudohyphae in tissue. Histopathological sections stained with methenamine silver (Gomori or Grocott) or periodic acid-Schiff is most helpful. Adiaspores must be distinguished from the tissue forms of *Coccidioides* species or *R. seeberi*, two other organisms that produce large spherules in tissue. In contrast to *Coccidioides* species, the adiaspores are much larger, have a thicker wall and do not contain endospores. The sporangia of *R. seeberi* are distinguished from those of *Emmonsia* species by the zonation of the internal sporangiospores and by the presence of distinctive eosinophilic globules within the mature sporangiospores.

Culture of the organism is difficult, and sputum and bronchoalveolar lavage fluid specimens are seldom positive in patients with disease. At 25°C, *Emmonsia* species grow as mould colonies. *E. crescens* produces adiaspores in vitro when cultivated at 35–37°C. *E. parva* requires culture at

40°C to produce adiaspores, while *E. pasteuriana* produces yeast-like cells on brain heart infusion agar at 37°C.

27.2.8 Management

Many cases of pulmonary adiaspiromycosis are asymptomatic or self-limited, and specific antifungal treatment is usually not required. Surgical intervention with limited resection may be needed if the condition is progressive. There are a few anecdotal reports of successful treatment with fluconazole, ketoconazole, voriconazole, thiabendazole or amphotericin B. However, because the organism does not replicate in tissue, it is unclear whether antifungal treatment accelerated clinical improvement. Some patients have benefitted from corticosteroid treatment, and this intervention may be indicated in immunocompetent individuals.

27.3 Lacaziosis

27.3.1 Definition

The term lacaziosis (or lobomycosis) is used to describe an uncommon chronic granulomatous infection of the skin and subcutaneous tissue caused by *Lacazia (Loboa) loboi*, a fungal pathogen that affects humans and dolphins. There is no systemic involvement and patients remain in general good health.

27.3.2 Geographical distribution

Most cases have been reported from the countries in northern South America and Central America. The disease is usually found in tropical and sub-tropical regions with a humid climate. Isolated cases have been seen in Europe, North America and South Africa, usually in persons with a history of travel to Latin America or contact with dolphins. Within Latin America, the greatest numbers of cases have been reported in the Amazon rain forest region.

27.3.3 The causal organism and its habitat

L. loboi has never been isolated in culture. In tissue it appears as round or elliptical yeast-like cells that reproduce by sequential budding, leading to the production of chains of cells. Genetic sequencing has indicated that *L. loboi* is closely related to *Paracoccidioides brasiliensis*. The natural habitat of *L. loboi* is still unknown; however, it is believed to be a saprophyte in soil or on vegetation and that water has an important role in human infection. Lacaziosis has also been diagnosed in several species of inshore and estuarine dolphins around the coasts of northern South America and the

southern United States, supporting the hypothesis of an aqueous habitat of the aetiological agent.

27.3.4 Epidemiology

The infection is thought to be acquired through small traumatic skin lesions after contact with the pathogen in aquatic ecological niches in nature. Lacaziosis is insidious in onset and notable for its long latent period of months or years. Some patients trace their infection to some traumatic incident, such as an insect bite, cut or an abrasion. In many cases, however, the traumatic event is so minimal that it is not remembered. The disease is more common in men than in women or children. Affected individuals are otherwise normal, and there does not appear to be any predisposition apart from contact with water.

27.3.5 Clinical manifestations

Lacaziosis is characterized by slowly evolving cutaneous lesions that most commonly develop on the legs and arms, face, ears, chest, back and buttocks. The initial lesion is a papule or a small nodule that slowly proliferates to form extensive keloidal, verrucous or ulcerated lesions, all of which may be present in a single patient. The nodular, keloid-like lesion is the most frequent presentation. The lesions are located in the dermis, the normal tissue being replaced by granulomatous tissue. Autoinoculation can lead to the development of further lesions. In advanced cases, the lesions may cover an entire limb. The mucous membranes and internal organs are not involved, but the regional lymph nodes are affected in 10–25% of cases. In most instances, the disease is symptomless. As with other proliferative epidermal processes, longstanding lesions of lacaziosis can undergo carcinomatous transformation.

Recurrent lacaziosis lesions have been documented in a patient with HIV infection.

27.3.6 Differential diagnosis

Because of its great clinical variation, the vegetative lesions of lacaziosis must be distinguished from chromoblastomycosis, paracoccidioidomycosis, leishmaniasis, nodular lepromatous lesions, keloids, dermatofibrosarcoma and xanthoma.

27.3.7 Essential investigations and their interpretation

The diagnosis of lacaziosis is usually established by histopathological examination of biopsy samples of tissue from infected sites. Histopathological sections stained with methenamine silver (Gomori or Grocott)

or periodic acid-Schiff is most helpful. These will reveal large numbers of the characteristic large, round or oval, thick-walled cells of *L. loboi* (over 10 μm in diameter). These can produce multiple buds that resemble the tissue form of *P. brasiliensis*. The cells are often formed in long unbranched chains, the adjacent cells being joined together by small bridge-like tubules within the chain. The epidermis is usually atrophic but may show pseudoepitheliomatous hyperplasia or ulceration. *L. loboi* is mostly intracellular within giant cells and macrophages. The cells are surrounded by eosinophilic material (asteroid bodies or Splendore-Hoeppli phenomenon) in about 25% of biopsies of lacaziosis.

L. loboi has never been isolated in culture. This distinguishes it from *P. brasiliensis* to which it bears a marked resemblance.

27.3.8 Management

There is no effective medical treatment for lacaziosis, although oral clofazimine (300 mg/day) has given promising results in some cases. Provided the lesions are not too extensive, the treatment of choice is surgical excision with wide margins. Cryotherapy is another option. More widespread disease usually recurs when treated surgically and does not respond to antifungal therapy.

27.4 Pythiosis

27.4.1 Definition

The term pythiosis is used to describe an infection of humans and animals caused by *P. insidiosum*, an organism that belongs to the kingdom Stramenopila, phylum Oomycota. Following traumatic implantation, *P. insidiosum* can cause a wide spectrum of clinical manifestations, ranging from cutaneous and subcutaneous infection to ocular, vascular and disseminated disease.

27.4.2 Geographical distribution

The disease has been reported from tropical, sub-tropical and some temperate regions worldwide. The disease is common in Thailand, but it has also been reported from North, Central and South America, Asia and Australia. In the United States, pythiosis is more prevalent in southern states surrounding the Gulf of Mexico, such as Alabama and Florida, but sporadic cases have been reported from states as far north as Wisconsin. In Asia, the disease has been reported from a number of countries, including India, Indonesia and Japan.

27.4.3 The causal organism and its habitat

Many *Pythium* species are important plant pathogens, but only *P. insidiosum* has been found to cause disease in humans and other animals, including horses and dogs. In tissue, *P. insidiosum* appears as wide, colourless, irregularly septate hyphae with thin, non-parallel wall and irregular branches that resemble those of the Mucorales (see Chapter 13). In nature, *P. insidiosum* is found in aquatic environments where it forms biflagellate, motile zoospores that enable the organism to spread to new plant hosts.

27.4.4 Epidemiology

The infection is thought to be acquired as a result of traumatic implantation after contact with the pathogen in aquatic ecological niches in nature. The zoospores of *P. insidiosum* have an unusual tropism for both mammalian tissue and plant foliage. However, cases of pythiosis have occurred in the absence of water, suggesting that humans and animals may develop *P. insidiosum* infection after contact with resting spores from terrestrial environments.

Pythiosis is usually diagnosed in otherwise healthy individuals, and there does not appear to be any predisposition apart from contact with stagnant water or other wetland fomites, such as plant detritus. The disease is more common in adults than children, with many cases occurring in men between 20 and 60 years of age, working in agriculture. In Thailand, the disease appears to occur more frequently in persons with thalassemia or similar blood disorders. The importance of thalassemia as a risk factor is unclear, as this is a very common condition in Thailand.

Human pythiosis is associated with significant morbidity and mortality. In Thailand, the overall case fatality rate is almost 30%; about 80% of patients with vascular infection have required limb amputation and 40% of these individuals have died. About 80% of patients with ocular pythiosis require enucleation or evisceration.

27.4.5 Clinical manifestations

There are several major clinical forms of pythiosis, of which the arterial (or vascular) form is by far the most serious. Other manifestations include cutaneous, subcutaneous and ocular infection. Like the aetiological agents of aspergillosis and mucormycosis, *P. insidiosum* has a predilection for vascular invasion causing thrombosis, infarction and necrosis of the surrounding tissue.

The ocular form of pythiosis presents as a keratitis that is indistinguishable from that caused by filamentous fungi (see Chapter 8). It usually begins after trauma to the cornea caused by plant debris contaminated with *P. insidiosum*. The symptoms and clinical signs include photophobia, pain, conjunctivitis and hypopyon, followed by the formation of corneal ulcers and eventual corneal perforation.

The initial clinical sign of cutaneous and subcutaneous infection is a small itching papule that rapidly progresses to form a large painful lesion that may later ulcerate or spread to the underlying subcutaneous tissue or adjacent bone, or both. The orbital form of pythiosis is rare and most often presents as periorbital cellulitis.

Arterial pythiosis is the most serious form of the disease, but has largely been limited to farmers in Thailand who also suffer from thalassemia. The infection begins with a small papule at a site of traumatic implantation, usually on the lower limbs. In some patients, the lesion evolves into a painful gangrenous ulcer. As the infection progresses, vascular invasion leads to an ascending arteritis with intermittent claudication, progressive ischaemia, necrosis and thrombosis of major arteries. In most cases, the infection is unilateral. If not treated, *P. insidiosum* may spread to reach the iliac and renal arteries, as well as the abdominal aorta. Angiographic findings include occlusion or aneurysm in the infected arteries. Fatal haemorrhage is common despite aggressive medical and surgical intervention.

27.4.6 Differential diagnosis

The cutaneous and subcutaneous forms of pythiosis must be distinguished from entomophthoromycosis, subcutaneous tuberculosis and cutaneous leishmaniasis. Orbital pythiosis resembles aspergillosis and mucormycosis. Arterial pythiosis must be differentiated from arteriosclerosis, diabetes mellitus and other vascular diseases.

27.4.7 Essential investigations and their interpretation

The diagnosis is established by microscopic examination and culture of corneal scrapings or biopsy specimens. The latter should be collected in sterile distilled water that contains antibiotics to inhibit bacterial growth and transported to the laboratory at room temperature. Storage of specimens at 4°C is not advised.

In contrast to the true fungi, the cell walls of *P. insidiosum* are composed of cellulose, rather than glucan, mannan and chitin. For this reason, the organism is very difficult to detect in hematoxylin and eosin preparations. In tissue sections stained with methenamine silver, *P. insidiosum* appears

as sparsely septate hyphae with occasional right-angled lateral branches. In many cases of subcutaneous infection, the hyphal elements are surrounded by eosinophilic material (Splendore-Hoeppli phenomenon). Immunofluorescence and immunoperoxidase staining have also been used for the specific identification of *P. insidiosum* in tissue.

Specimens should be cultured on glucose peptone (Sabouraud's dextrose) agar at 37°C for 2 or more days. In culture, *P. insidiosum* grows as white submerged colonies with short aerial hyphae. Definitive identification requires the development of the characteristic oogonia (sexual stage) in culture. To induce sporulation in vitro, requires culture on agar blocks floated in water. The organism forms sporangia that contain numerous round to oval, motile zoospores with two lateral flagella. More recently, molecular methods of species identification have begun to be used.

Serological diagnosis has been attempted, using immunodiffusion (ID), ELISA and Western blotting tests to detect antibodies to *P. insidiosum*. ID has proved to be a specific, but insensitive method, while ELISA and Western blotting are both sensitive and specific. Nonetheless, the detection of anti-*P. insidiosum* IgG antibodies should be interpreted with caution because sub-clinical infection can occur, and false positive results may be obtained in apparently healthy individuals.

27.4.8 Management

Although *P. insidiosum* lacks ergosterol in its cell membrane, standard antifungal drugs targeting ergosterol and ergosterol biosynthesis have sometimes proved effective against pythiosis. For instance, a child with deep facial infection was cured with a combination of itraconazole and terbinafine. The inconsistent results obtained with most antifungal drugs have led to interest in the use of an equine *P. insidiosum* vaccine to treat human cases.

Corneal infection is particularly refractory to medical management and can usually only be controlled by surgical enucleation or evisceration of the infected eye. In contrast, cutaneous and subcutaneous infection can often be eradicated with surgical debridement and antifungal treatment.

Given the serious nature of arterial pythiosis, treatment should be initiated as soon as the diagnosis is suspected, infected necrotic tissue must be removed, and an appropriate antifungal agent must be administered. Surgical debridement, amputation and aneurismectomy may be required to limit the extent of the infection and prevent fatal haemorrhage. Antifungal treatment with amphotericin B, itraconazole and terbinafine in sequence or in combination has been used with some success.

27.5 Rhinosporidiosis

27.5.1 Definition

The term rhinosporidiosis is used to refer to an unusual infection of the nasal and other mucosal surfaces and ocular conjunctiva. It is characterized by the development of large vegetative outgrowths. The causal agent is *R. seeberi*, an anomalous organism that has at different times been classified as a protozoan and a fungus.

27.5.2 Geographical distribution

The disease occurs in most tropical and sub-tropical regions worldwide, except Australia. It is most common in southern India and Sri Lanka, where it has been estimated to affect 1% of children, but sporadic cases have been reported from East Africa, Central and South America, the Middle East, South East Asia and other parts of the world. Rhinosporidiosis is endemic in north-eastern Brazil, in a transitional environment between the Amazonian rain forest and an arid region. In 1995, an outbreak of rhinosporidiosis was reported from Serbia.

27.5.3 The causal organism and its habitat

In tissue, *R. seeberi* forms characteristic abundant, large, thick-walled structures, termed sporangia. Large numbers of oval or spherical sporangiospores (endospores) are produced within the sporangia and, when mature, these are released through a pore in the wall. Each sporangiospore may develop to produce a new sporangium. It is unclear whether the released spores remain dormant in nature or have an intermediate host life cycle.

It has long been unclear whether this organism is a fungus, but sequencing of the 18S small subunit ribosomal DNA sequence from *R. seeberi* has led to its recent reclassification as a member of a novel group of aquatic parasites within the Mesomycetozoa.

Attempts to isolate *R. seeberi* in culture have failed and thus far it has not been recovered from an environmental source. It is, however, believed that stagnant pools of fresh water are an important source. However, because the disease also occurs in some arid countries in the Middle East, the hydrophilic nature of the organism has been questioned. Most probably, this pathogen evolved from an aquatic niche, in which it can still be found, to terrestrial environments by the development of resistant spores.

27.5.4 Epidemiology

The mechanism by which human infection is acquired is not known, but it is believed that the organism is usually acquired through contact with

aquatic environments contaminated with *R. seeberi*. The disease is most prevalent in rural districts, among persons bathing in public ponds or working in stagnant water, such as rice fields. In arid countries, most infections are ocular and dust is the suspected source. Rhinosporidiosis is seen in all age groups, but is most common in children and adolescents. Ocular infection is more prevalent in women, while nasal and nasopharyngeal infection more commonly affects men. Individuals with rhinosporidiosis are otherwise normal, and there does not appear to be any predisposition apart from contact with freshwater.

27.5.5 Clinical manifestations

The nose is the most common site of rhinosporidiosis, being affected in more than 70% of cases. The fungus causes the production of large, painless, sessile or pedunculated lesions that affect one or both nostrils. The infection is insidious in onset and the patient remains unaware of its existence until symptoms of obstruction develop. Rhinoscopic examination will reveal papular or nodular, smooth-surfaced lesions that become pedunculated and acquire a papillomatous or proliferative appearance. The lesions are pink, red or purple in colour. If located low in the nostril, the polyps may protrude and hang onto the upper lip. In most cases, the general health of the patient is unimpaired. Spontaneous remission is unusual and, left untreated, the polyps will continue to enlarge.

In some cases, lesions develop on the conjunctiva or the ears. Lesions may also appear on the penis and on the vulva or vagina in women. Cutaneous rhinosporidiosis is very rare and is usually due to spread from a neighbouring mucosal lesion. It presents as minute papillomas that enlarge and become pedunculated. In most cases, skin lesions are asymptomatic. Visceral dissemination has been reported, but is rare.

27.5.6 Differential diagnosis

The appearance of pedunculated or unpedunculated polyps or nodules on the nasal mucosa or the conjunctiva should suggest the diagnosis of rhinosporidiosis. The presence of small white dots on the surface of the lesions is also helpful; on microscopic examination, these are seen to be sporangia.

The condition must be distinguished from cutaneous tuberculosis, leprous lesions, leishmaniasis and from treponematoses. Lesions on the genitalia or anal region must be distinguished from warts, condylomata and haemorrhoids.

27.5.7 Essential investigations and their interpretation

The diagnosis is established by microscopic examination of biopsy specimens because the organism cannot be cultured. Microscopic examination of tissue sections or wet preparations of tissue or discharge will reveal abundant large round or oval sporangia, of different sizes and stages of development, with a thick wall and an operculum. The sporangia may contain up to 12,000 endospores.

The mature sporangia of *R. seeberi* resemble the spherules of *Coccidioides* species, but are much larger and mucicarmine positive. In addition, flattened sporogenous cells are often present at the edge of the sporangium. Mature endospores of *Coccidioides* species, however, are round regardless of their location within the spherules. The sporangia of *R. seeberi* are distinguished from those of *Emmonsia* species by the zonation of the internal sporangiospores and by the presence of distinctive eosinophilic globules within the mature sporangiospores.

27.5.8 Management

Spontaneous remission is unusual. The treatment of choice is surgical excision of lesions, with or without cauterization. No systemic drug treatment has proved effective, but local injection of amphotericin B may help to prevent recurrence. This is common (more than 20% of cases) and patients should be followed-up where possible.

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