STATE-OF-THE-ART CLINICAL ARTICLE

Invasive Aspergillosis

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Since the first description of invasive aspergillosis as an opportunistic infection in 1953 [1], there has been a substantial increase in the number of cases documented at autopsy in all developed nations. The most recent figure from one major teaching hospital in Frankfurt, Germany, showed a 14-fold increase in the number of cases over the years 1978–1992; 4% of all patients autopsied were found to have invasive aspergillosis in the last year of this survey [2]. A national autopsy survey conducted in Japan from 1970 to 1995 showed that the incidence of invasive aspergillosis increased from 0.4% to 1.4% during that period [3]. The substantial worldwide use of fluconazole, which has no clinically useful activity against invasive aspergillosis, has not reduced the number of cases of this infection, which remains the most common invasive mold infection worldwide.

There has been a substantial increase in the number of patients at risk of developing invasive aspergillosis, for many reasons, including (1) the advent of AIDS; (2) the development of new intensive chemotherapy regimens for solid tumors, difficult-to-treat lymphoma, myeloma, and resistant leukemia; (3) a year-on-year worldwide increase in the number of solid organ transplant recipients, now numbering approximately .5 million annually; and (4) increased use of immunosuppressive regimens for other autoimmune diseases such as systemic lupus erythematosus. As other supportive care is improved and most bacterial infections are successfully treated, the importance of invasive aspergillosis has increased so that it is now a major direct or contributory cause of death at leukemia treatment centers and bone marrow transplantation and solid organ transplantation centers. This article reviews the current state of knowledge regarding invasive aspergillosis.

History

The first description of aspergillosis in animals was provided by Mayer in 1815 [4], who observed the infection in the air sacs and lungs of a jackdaw (table 1). The first human case of aspergillosis was described in 1842 by Bennett in Edinburgh [6]. He described what he saw microscopically in the sputum of a patient with several aspergillomas in tuberculous cavities as a cryptogamic plant.

The same year, Rayer [7] described infection of the pleura by a mold, again not identified, and Gairdner [28] reported a similar case in 1853. Virchow [29] described four cases of bronchial and pulmonary aspergillosis in 1856. Other early descriptions are summarized by Rénon in 1897 [30], by Cawley in 1947 [19], and by Hinson et al. in 1952 [21]. Rénon recognized that some cases occurred following other diseases and that *Aspergillus* could invade the lung in addition to inhabiting tuberculous cavities or other cavities [30]. Many early cases (possibly only colonization) were described in pigeon-crammers or hair combers (for wigs). Cawley also remarked on the association between aspergillosis and certain occupations such as farmers, feed-mill workers, fur cleaners who used rye flour, threshers, and those in contact with silos or grains [19].

Other landmarks in the description of aspergillosis are shown in table 1. It is of interest that among deep, invasive forms of aspergillosis, only pulmonary and renal diseases were described in the nineteenth century. Likewise, superficial aspergillosis, including otomycosis and keratitis, was described as early as 1844. As with many early descriptions, details are often sketchy, but these early observations were of great importance in the initial recognition of the pathogenic potential of *Aspergillus,* especially in immunocompetent patients. Disseminated aspergillosis was described early in the twentieth century, but the histological appearances were almost always granulomatous, and the disease occurred in apparently immunocompetent patients. It was not until the introduction of corticosteroids and cytotoxic chemotherapy in the 1950s that the first cases of invasive pulmonary aspergillosis, the opportunistic infection we now clinically recognize, began to appear.

Mycology

The first attempt to define the genus *Aspergillus* was made by Micheli in 1729 [5]. He described, named, and illustrated the fungus beautifully in his most famous work, the *Nova Geneva Plantarum,* in 1729. He noted that the pattern of the conidial head of *Aspergillus,* with its spore heads radiating

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Table 1. Landmark descriptions and reports of diseases caused by *Aspergillus* species.

Reference	Year	Author(s)	Report
[5]	1729	Micheli	First description of *Aspergillus*
[4]	1815	Mayer	Air sac and pulmonary infection in a jackdaw
[6]	1842	Bennett	Aspergilloma complicating tuberculosis with *Aspergillus* in sputum
[7]	1842	Rayer	Pleural aspergillosis
[8]	1844	Mayer	Aspergillus otomycosis
[9]	1863	Fresenius	Clear species description of *A. fumigatus* (isolated from air sacs and bronchi of a great bustard)
[10]	1879	Leber	Aspergillus keratitis following chaff entering the eye
[11]	1886	Boströem	Cutaneous aspergillosis
[12]	1887	Popoff	Allergic pulmonary disease caused by *Aspergillus*
[13]	1890	Wheaton	Aspergillus tracheobronchitis
[14]	1891	Ross	Renal aspergillosis
[15]	1891	Zarniko	Maxillary sinus aspergillosis
[16]	1897	Oppe	Sphenoid sinus aspergillosis
[17]	1931	Just	Cerebral aspergillosis
[18]	1936	Shaw and Warthen	Aspergillosis of bone
[19]	1947	Cawley	Invasive aspergillosis (probable) complicating chronic granulomatous disease (with meningitis)
[20]	1950	Zimmerman	Native valve aspergillus endocarditis
[21]	1952	Hinson, Moon, and Plummer	Allergic bronchopulmonary aspergillosis, definition of disease
[1]	1953	Rankin	Invasive pulmonary aspergillosis as an opportunistic infection (during neutropenia)
[22]	1955	Zimmerman	Invasive aspergillosis in infancy
[23]	1959	Finegold, Will, and Murray	Classification of invasive aspergillosis
[24]	1964	Newman and Cordell	Postoperative aspergillus endocarditis
[25]	1966	Milosev et al.	Paranasal aspergillus granuloma in Sudan
[26]	1970	Young et al.	Comprehensive description of pathology of invasive aspergillosis, with clinical correlation
[27]	1983	Katzenstein, Sale, and Greenberger	Allergic aspergillus sinusitis

from a central structure, resembled an aspergillum (a brush or perforated globe used for sprinkling holy water); therefore, he named the genus *Aspergillus.* He described experiments of culturing fungi from spores both in gardens and on pieces of melon. His observations were ahead of his time, and their significance was overlooked until more than a century later. It was Link [31] who first named *A. flavus* in 1809 [31]. In 1926, Thom and Church [32] first classified the genus. They accepted 69 *Aspergillus* species in 11 groups. By 1945, Thom and Raper [33] had accepted 80 species.

By 1965 this classification was outdated, and Raper and Fennel [34] detailed 151 species in 18 different groups. By then, most species with a teleomorph had been identified. Additional work, led by Samson and Pitt, has further refined the species designations with use of new technologies such as DNA hybridization and thin-layer chromatography of secondary metabolites. Approximately 150 species are now accepted, and new species continue to be described [35].

Although *Aspergillus* is a separate genus, it is closely related to *Penicillium* species in the fungal kingdom. *Aspergillus* species are ascomycetes classified in the form subdivision Deuteromycotina because most of these molds do not have a sexual reproductive phase. The taxonomy of species within the *Aspergillus* genus is gradually undergoing revision with the use of molecular taxonomic methods and is not complete. The most common species of *Aspergillus* causing invasive disease include *A. fumigatus, A. flavus, A. niger, A. terreus,* and *A. nidulans.* A number of other rarer species rarely cause disease, including *A. amstelodami, A. avenaceus, A. caesiellus, A. candidus, A. carneus, A. chevalieri, A. clavatus, A. glaucus, A. granulosus, A. oryzae, A. quadrilineatus, A. restrictus, A. sydowi, A. ustus, A. versicolor, A. wentii, Neosartorya fisheri,* and others. *A. fumigatus* accounts for ~90% of cases of invasive aspergillosis.

Pathogenic *Aspergillus* species generally grow easily and relatively quickly on routine bacteriological and mycological media in the clinical laboratory. Certain species such as *A. glaucus* require high concentrations of glucose for optimum growth. Only pathogenic species are capable of growth at 35°–37°C, and *A. fumigatus* in particular is capable of growth at ⩾50°C. Recent comparative work from a large retrospective series established that a higher yield was obtained by using

mycological media rather than standard bacteriological media in the clinical setting [36], and this procedure is recommended whenever a fungal infection, including aspergillosis, is considered diagnostically possible. Most isolates of *A. fumigatus* are capable of growth at oxygen tensions as low as 0.1% O_2 [37], and instances when isolates would grow only on anaerobic plates have been recorded. Additional work to optimize media for the growth of *Aspergillus* is warranted.

The organism grows initially as a small, fluffy white colony on the surface of agar. Spores usually develop within 36–48 hours of incubation at 30°–37°C for the major pathogenic species. For unusual species, it can take many more days, and sometimes weeks, for spores to appear, a circumstance that delays identification. Potato dextrose agar is particularly useful for inducing sporulation. Presumptive identification of *Aspergillus* at the genus level is relatively straightforward on the basis of microscopic criteria. The major pathogenic species have somewhat differently arranged spores on conidial heads in addition to different-colored colonies. Presently, formal identification may require cultures on specialized media such as Czapek-Dox and malt extract. In the future it is likely that molecular methods will be used to formally identify unusual species.

Until recently, it has not been therapeutically important to determine which species of *Aspergillus* is causing disease because no differences in outcome have been noted between infections caused by different species. Recently, however, some differences in susceptibility to itraconazole and other azoles have been noted between species (author's unpublished data; [38]). Thus, it may be more important now than previously to rapidly identify species or to test isolates for susceptibilities to make an optimal therapeutic decision.

Pathogenesis

Organism Factors

It takes ~5–12 hours for *A. fumigatus* to germinate at 37°C depending on the medium used [39]. Spores incubated in appropriate moisture conditions and temperature swell to approximately four-to-eight times their original volume. Their hydrophobic protein coat is replaced by another cell wall exterior. As hyphae appear, the logarithmic-phase growth begins. In vitro, hyphal extension and overall fungal mass increase logarithmically until ~24 hours, when the growth rate starts to plateau [39]. Branching of hyphae occurs early, and in shaking conditions in vitro, the organism takes on the appearance of multiple small balls. At the liquid-air interface in static culture, a mycelial mat is typically formed. These growth patterns probably reflect, in part, the branching characteristics of each isolate.

Most species of *Aspergillus* are incapable of growth at 37°C, a key characteristic that distinguishes pathogenic species from nonpathogenic species [40]. There are also discernible differences in the growth rate between different species of *Aspergillus;* the most rapidly growing organism is *A. fumigatus*. Physiological and pharmacological concentrations of hydrocortisone accelerate the growth rate of *A. fumigatus* and *A. flavus* by 30%–40%. In vitro, in the presence of hydrocortisone, *A. fumigatus* has a doubling time of 48 minutes and a hyphal extension rate of 1–2 cm/h [39]. Growth rate is therefore likely to be one key determinant of the rate of progression of disease and, possibly, of pathogenicity.

In addition, *A. fumigatus* has some other characteristics that may contribute to its pathogenicity. These characteristics include very small spore size (3–5 μm), which enables the spores to penetrate deeply into the lung. Spores are capable of withstanding extraordinary atmospheric conditions, probably by virtue of the hydrophobic protein-coat layer, which may help protect them from host defenses [41–43]. Of all the pathogenic species, *A. fumigatus* in particular binds laminin [44] and fibrinogen [45] efficiently and somewhat better than other species of *Aspergillus,* presumably allowing greater adhesion in the airways before invasion.

Various putative virulence determinants have been described for *A. fumigatus,* including various proteases [46–48], ribotoxin [49–51], phospholipases [52], a hemolysin [53], gliotoxin [54–56], aflatoxin [57], phthioic acid [58], and other toxins [59, 60]. Extensive work with the alkaline protease of *A. fumigatus* with use of single or double deletants in carefully controlled animal model experiments has failed to show the importance of elastase (alkaline protease) in invasive aspergillosis [61]. However, one protease was able to induce pulmonary epithelial cell detachment as well as proinflammatory cytokine release [62]. Similarly, ribotoxin does not appear to be important in the pathogenesis of aspergillosis [51].

At least four different phospholipases are produced by *A. fumigatus* [52]. It is not yet known whether they contribute to the pathogenesis of invasive aspergillosis, but it is possible that they do. Species of *Clostridium* and *Pseudomonas* produce phospholipases, and these organisms' patterns of tissue destruction are similar to those of *A. fumigatus.* It is not yet known whether other species of *Aspergillus* produce phospholipase.

Gliotoxin has been shown to reduce macrophage and neutrophil phagocytosis [54]. Gliotoxin can also induce apoptosis [56], but whether gliotoxin contributes to pathogenesis has not yet been established. Phthioic acid is produced in small quantities by a very small number of *A. fumigatus* isolates and could conceivably contribute to granuloma formation, as it may do in tuberculosis, but it is unlikely to be a major virulence determinant.

Aspergillus produces a number of superoxide dismutases [63], at least two catalases [64–66] and mannitol [67–69]. These substances may protect the organism from damage due to singlet oxygen, hydrogen peroxide, hydroxyl radical, and other free radicals produced by phagocytes.

Host Factors

A. fumigatus is an unusual pathogen in immunocompetent patients, although many cases of *A. fumigatus* infection in such

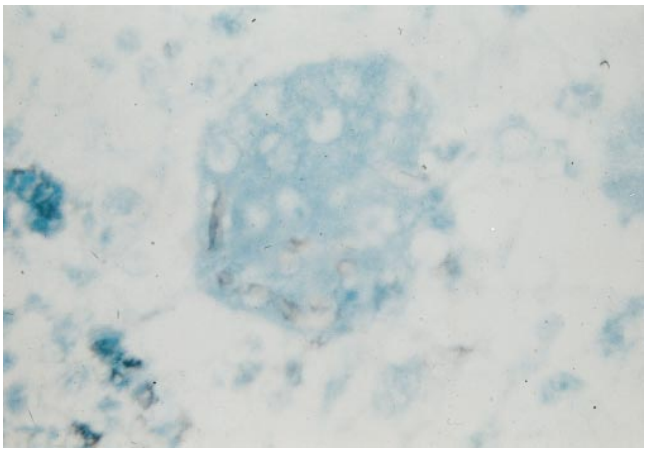

Figure 1. High-power view of a section of bone from a boy with chronic granulomatous disease who developed aspergillus osteomyelitis showing a multinucleated giant cell containing *Aspergillus* hyphae. (Courtesy of Dr. A. Jones, Hope Hospital, Manchester, UK; stain, Grocott-Gomori methenamine-silver; original magnification, ×400.)

patients have been clearly documented. Thus, *A. fumigatus* is capable of being a primary pathogen in humans. It is notable that many animals, including dogs and horses and, in particular many birds, develop *Aspergillus* infections; this finding also indicates that *A. fumigatus* is occasionally a primary pathogen. Whether the presence of an undefined immune defect is necessary to allow *A. fumigatus* to cause infection is unknown.

The first immunologic line of defense against *Aspergillus* in the lungs, and presumably the nose, are macrophages, which are capable of ingesting and killing spores [70]. Both monocyte-derived and resident macrophages contribute to spore ingestion and killing. Hyphae are primarily killed by neutrophils [70, 71], with some contribution from monocytes [72] and possibly macrophages. Swollen spores and hyphae bind complement [73]. Hyphae are too large to be ingested, and therefore, killing proceeds extracellularly; this process is unlike killing of most bacteria and *Candida albicans,* in which the organisms are first internalized. As in patients with leprosy, the histological response in patients with aspergillosis ranges from a florid granulomatous response to few organisms (figure 1) to a minimal tissue response around extensive necrosis invaded by extensive networks of hyphae (figures 2A and B), depending to a great extent on the degree of immunologic deficit. Neutropenia and dysfunctional neutrophils (which occur in patients with AIDS [74] and chronic granulomatous disease [75]) are both important risk factors for invasive aspergillosis but lead to different pathology.

This relatively simple scheme is, of course, more complex than it first appears because of the influence of various cytokines, growth factors, and corticosteroids. Corticosteroids substantially impair macrophage killing of *Aspergillus* spores and neutrophil and mononuclear cell killing of *Aspergillus* hyphae [76, 77]. These negative effects are abrogated to some extent by treatment with granulocyte colony-stimulating factor [76] and granulocyte-macrophage colony–stimulating factor (in vitro) [77]. Administration of IFN-γ also improves monocyte and neutrophil function [76, 78]. Emerging data also indicate that T cell function may be important, particularly in the more chronic forms of invasive aspergillosis [79]. An interaction between neutrophil dysfunction and T helper cells via IL-4 has been observed in experimental models of invasive aspergillosis, and this interaction could have implications for human disease [79]. In patients with invasive disease, Th 2 cell responses predominate over Th 1 cell responses, and successful therapy could necessitate reversing these T helper cell responses. There is also evidence for acquired immunity, possibly mediated in part by macrophages [80].

Epidemiology

Environmental Location

In environmental terms, *A. fumigatus* is an ubiquitous organism that has been found in every region in the world, including Antarctica. It is believed that the primary ecological niche of *A. fumigatus* is decomposing vegetable material [81], and therefore rural areas, particularly farming locations, are a major source of this organism. There is a higher incidence of colonization with *A. fumigatus* among cystic fibrosis patients in rural locations [82] than among those in urban locations. *A. fumigatus* is particularly found in and around human habitation [83]—in cellars and potted plants and in pepper and spices [84]. In addition, unfiltered marijuana smoke may yield substantial quantities of *Aspergillus* spores and patients at risk for aspergillosis should be advised of this possibility.

Various molecular typing systems for *A. fumigatus* have been developed over the last 7 years [85]. These have proven to be robust and useful in evaluating the epidemiology of invasive aspergillosis. It is clear that there is widespread dispersal of genotypes of *A. fumigatus* around the world; for example, identical genotypes cause disease in the United States and Europe [86–89]. A complete population genetic analysis of the species has not yet been conducted, although the tools are now available to do so. The majority of infections are caused by a single genotype of *A. fumigatus;* occasionally, however, two or more genotypes have been identified in patients. The latter finding is particularly true for patients with aspergillomas. There does not appear to be a group of related genotypes that are more or less pathogenic [89], but such analyses have not been performed on large numbers of isolates from minimally immunocompetent patients, in whom prominent virulence determinants are most likely to be identified.

Inoculum and Incubation Period

The development of invasive aspergillosis requires the exposure of a susceptible host to relevant inoculum. It is not known

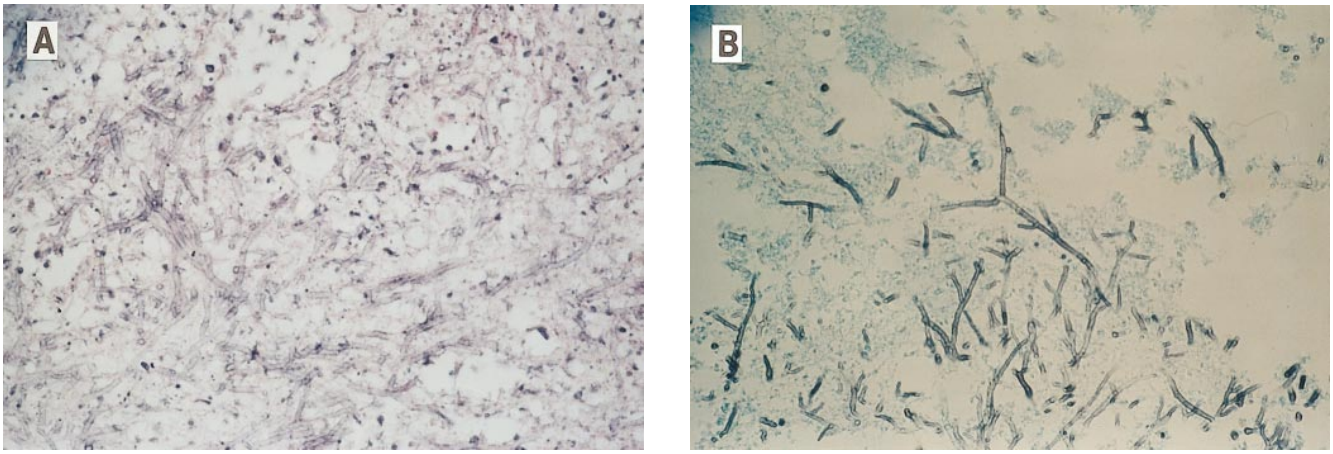

Figure 2. *A,* histological appearance under high power of an aspergillus brain abscess showing an inflammatory cell infiltrate surrounding *Aspergillus* hyphae. (Stain, hematoxylin-eosin; original magnification, ×400.) *B,* Section of the same abscess showing the characteristic radiating pattern of *Aspergillus* hyphae with septae and branching at 45° angles. (Stain, methenamine silver; original magnification, ×400.)

what the infectious inoculum for aspergillosis is, but this factor may depend upon the host.

It is also not clear what the incubation period between exposure to inoculum and the development of disease is. For one heart transplant recipient, it was documented that there was a 3-month time lag between the identification of *Aspergillus* in bronchoalveolar lavage fluid and the subsequent development of disseminated disease [90]. It is also notable that for neutropenic patients, invasive aspergillosis virtually never manifests before the 12th day of profound neutropenia [91], although many patients have sinus or airway colonization with *Aspergillus* on arrival at the hospital [92]. In a case in which a laparotomy wound became infected by *A. fumigatus* within 48 hours of transfer of the patient from one hospital to another, DNA typing showed that the causative organism was in the air of the intensive care unit of the receiving hospital, a finding that suggests an extremely short incubation period [93].

Prevention and Prophylaxis

In the hospital setting, patients at profound risk for aspergillosis should not be given pepper or spices that have not been sterilized [94]. Potted plants and shrubs probably pose some risk to patients, particularly if such plants are in the immediate vicinity of patients who are highly immunocompromised [84]. Local construction work is thought to be a major risk factor for highly immunocompromised patients such as bone marrow and liver transplant recipients and those with profound neutropenia [95], and some data suggest that complete separation of construction areas from clinical areas may reduce the risk to these patients [96]. The use of hepafiltration [97] and, in particular, laminar air flow [98, 99] reduces the risk of invasive aspergillosis but does not reduce it to zero, probably partly because patients are already colonized by *Aspergillus* when they arrive at the hospital, partly because of breaks in airflow (e.g., during the provision of food or during bed linen changes), and partly because patients leave these protective environments to undergo investigations.

In addition, the sudden release of large quantities of spores that occurs on cleaning or when spores are shed from clothes may be undetectable by routine spore counts but are particularly dangerous. The effects of minibursts of spores can be minimized by increasing the air change rate in protective environments [100]. However, patients at particularly high risk such as bone marrow transplant recipients and liver and lung transplant recipients, neutropenic patients, and extensively burned patients should be housed in hepafiltered environments if possible. *Aspergillus* has been found in showerheads and hot water faucets, which could represent another risk for these patients [101].

Because *Aspergillus* is ubiquitous in the air and environment, the primary means of control is by filtering the air to remove all *A. fumigatus* spores, by using pharmaceutical agents active against *Aspergillus,* and (conceptually) by administering a vaccine. Although control of air is possible within the hospital, this measure is expensive, and it does not generally include all patients at risk. For example, patients with AIDS and those receiving corticosteroids are generally not housed in hepafiltered environments, which means that prevention may be possible for the highest-risk groups for limited periods but is not currently possible for all groups at risk in hospitals. Furthermore, patients at continuing risk, such as those who have undergone solid organ transplantation, are discharged home, and the home environment is a substantial source of *Aspergillus* spores.

Several different approaches to prophylaxis for invasive aspergillosis have been attempted [94, 102]. No study has yet convincingly shown that any means of prophylaxis is com-

pletely protective. Administration of aerosolized amphotericin B may be partially protective [103], and administration of intravenous amphotericin B is possibly partially protective [104]. Recent data are consistent with a protective effect of itraconazole solution [105]. The cost-benefit equation varies with the risk and has not been well quantitated. The widespread use of effective prophylaxis, should prophylaxis become available in the future, may substantially alter the epidemiology of invasive aspergillosis. However, it is unlikely that prophylaxis will be used for all patient groups at risk, and cases of invasive aspergillosis will continue to occur.

There is presently little likelihood that a vaccine for the prevention of invasive aspergillosis will be developed, although this would be desirable. Should such a vaccine become available in the future, it could be administered before transplantation for the majority of solid organ transplant recipients, between courses of chemotherapy for many patients with leukemia, and before bone marrow transplantation. It could also be used in patients with AIDS and in other patients likely to require intermittent large doses of corticosteroids. However, there are several impediments to the development of a vaccine. One impediment is the fact that humoral immunity appears to play an insignificant part in protection against invasive aspergillosis, although patients who recover from invasive aspergillosis develop antibodies to *Aspergillus* [106]. Whether certain antibodies to *Aspergillus* act as opsonins in vivo is unclear.

More detailed work on identifying major *Aspergillus* antigens and their interactions with various parts of the immune system is warranted to try to identify protective immunodominant epitopes. Further studies of the role of T helper cells may be important. Even if a vaccine were only partially protective and slowed the rate of disease progression, allowing aspergillosis to be diagnosed and successfully treated, this effect would be of substantial importance with respect to a disease with an associated mortality that ranges from 50% to 100% despite therapy [107].

Table 2. Incidence of invasive aspergillosis according to underlying condition.

Condition	Range (%)
Heart and lung or lung transplantation	19–26*
Chronic granulomatous disease	25–40†
Acute leukemia	5–24
Allogeneic BMT	4–9
Autologous BMT without growth factors	0.5–6
AIDS	0–12
Liver transplantation	1.5–10
Heart and renal transplantation	0.5–10
Severe combined immunodeficiency	3.5
Burns	1–7
Systemic lupus erythematosus	1
Autologous BMT with growth factors	<1

NOTE. Data are from [108]. BMT = bone marrow transplantation.

* Distinguishing colonization from disease is particularly difficult in these patients.

† Lifetime incidence.

Table 3. Classification of invasive aspergillus infection.

Infection associated with tissue damage, surgery, or a foreign body
- Keratitis and/or endophthalmitis
- Cutaneous infection (e.g., burn-associated aspergillosis)
- Operative site infection (e.g., prosthetic valve endocarditis, wound infection after liver transplantation, and subdural empyema)
- Foreign body associated (e.g., Hickman catheter or other iv line or CAPD catheter)

Infection predominantly in immunocompromised hosts
- Primary cutaneous aspergillosis, especially in leukemic children
- Pulmonary aspergillosis
  - Acute invasive
  - Chronic necrotizing aspergillosis
- Airway aspergillosis
  - Obstructing bronchial aspergillosis
  - Invasive aspergillus tracheobronchitis
  - Ulcerative aspergillus tracheobronchitis
  - Pseudomembranous aspergillus tracheobronchitis
- Rhinosinusitis
- Disseminated aspergillosis, especially cerebral aspergillosis

NOTE. CAPD = continuous ambulatory peritoneal dialysis.

Clinical Features

Patterns of Disease

The incidence of invasive aspergillosis varies remarkably from center to center, and within centers, the disease tends to occur sporadically. Approximate incidence figures for different patient groups are shown in table 2.

The portals of entry for *Aspergillus* include the respiratory tract, damaged skin or other operative wounds, the cornea, and the ear. Infection arising from the gastrointestinal tract is possible but has never been clearly documented. Whichever the portal of entry is, that site is usually affected. The majority of patients (80%–90%) have pulmonary disease, but some have other manifestations of disease, including aspergillus rhinosinusitis. A clinically useful classification of invasive aspergillosis is shown in table 3. There may be local extension to the orbit from the sinuses. Local extension is distinct from disseminated disease, which refers to distant spread, presumably blood borne, from the portal of entry. Sometimes only one distant site, such as the brain, is affected.

Clinical manifestations of disease differ among patient groups. In an attempt to make these patterns of disease memorable for practicing clinicians, table 4 summarizes the typical clinical patterns in the major patient groups. In the following sections, the major body sites of disease will be described.

Pulmonary Disease

The clinical presentation of invasive pulmonary aspergillosis varies with the patient group, as does the rate at which the

Table 4. Characteristic patterns of invasive aspergillosis in commonly affected patient groups.

Underlying condition	Timing of invasive aspergillosis (% of cases)	Typical sites and features (% of cases)	Comments
Acute leukemia; multiple myeloma, stage II/III; chronic leukemia in blast crisis; aplastic anemia and immunosuppressive treatment; autologous bone marrow or PSC transplantation	During induction chemotherapy (75); during maintenance or consolidation treatment (25)	Lung (80–90); sinuses (5–10); other, e.g., brain (5–10)	High risk of fatal hemorrhage in lesions close to hilum of lung
Allogeneic bone marrow or PSC transplantation, especially if matched unrelated or mismatched donor	Early during neutropenia (20–30), late (median, 100 d), especially if grade II–III GVHD present (75)	Lung (80–90); sinuses (5–10); disseminated, including brain (5–20)	Usually bilateral diffuse disease with poor outcome
Liver transplantation	Variable, usually within 4 w of transplantation	Lung, brain, wound, disseminated	Distinguishing pulmonary shadows of aspergillosis from those of postoperative atelectasis and other infections difficult; distinguishing cerebral aspergillosis from other lesions clinically and radiologically is problematic
Lung transplantation	Variable	Airways, lung, disseminated, brain	Distinguishing colonization of airways from invasion difficult
Heart and kidney transplantation	Variable, often after treatment for rejection	Lung nodules that appear late or bilateral diffuse disease; disseminated	Good prognosis if diagnosed
HIV infection and AIDS	Late-stage AIDS, often with neutropenia and/or corticosteroid therapy	Any site, but upper-lobe thin- or thick-walled cavities and airway disease common	Subacute presentation, often mistaken for tuberculosis and other infections; poor prognosis; hemoptysis often fatal event (25% of cases)
Chronic granulomatous disease	Variable over lifetime	Lung (70); chest wall (20); bone (10); disseminated (10–15)	Slowly progressive ''cold'' abscesses
Burns	Usually within 2 mo of severe burns	Superficial, leading to disseminated disease	Progresses over 5–10 d to death, often unresponsive to medical therapy alone; amputation necessary if distal limb involved

NOTE. GVHD = graft-versus-host disease; PSC = peripheral stem cell.

disease progresses [109–129]. The most immunocompromised patients are those least likely to have symptoms, and progression is fast (e.g., 7–14 days from onset to death). Conversely, the least immunocompromised patients, such as diabetics or immunocompetent patients, usually have indolent symptomatic presentations that progress slowly (e.g., 2–3 months from onset to diagnosis).

Acute invasive pulmonary aspergillosis. Approximately 25%–33% of patients initially have no symptoms attributable to invasive pulmonary aspergillosis. As the disease progresses, symptoms appear. Early symptoms are cough (usually dry) and fever. Corticosteroid-treated patients often do not have fever, and low-grade chest pain, which may be pleuritic but is more commonly dull and nonspecific, is a frequent finding. A pleural rub is sometimes heard. Hemoptysis can occur, although it is rarely a presenting feature. Dyspnea is more common in patients with diffuse disease. The presentation in some patients is akin to that of pulmonary embolism. In neutropenic patients, pneumothorax is an occasional presenting feature, and sharp chest pain with dyspnea is typical. The symptoms and signs of pulmonary zygomycosis are indistinguishable from those of invasive aspergillosis.

The prognosis of focal disease (and, in particular, nodular disease) is much more favorable than that of diffuse and bilateral disease, as focal disease tends to progress more slowly [111, 118, 130–131]. Resection is one therapeutic option that is not available to patients with bilateral diffuse or extensive disease. The only major problem for patients with focal disease is hemoptysis, which is often life-threatening and may occur without warning [112, 121, 124, 131].

Hypoxemia is usual in patients with diffuse disease, and hypocapnea is often present. WBC counts are usually normal, as is plasma chemistry. Elevated levels of bilirubin and lactate dehydrogenase are occasionally seen but are nonspecific findings. Coagulopathy may be present. The serum fibrinogen level is typically elevated in neutropenic patients with acute leukemia

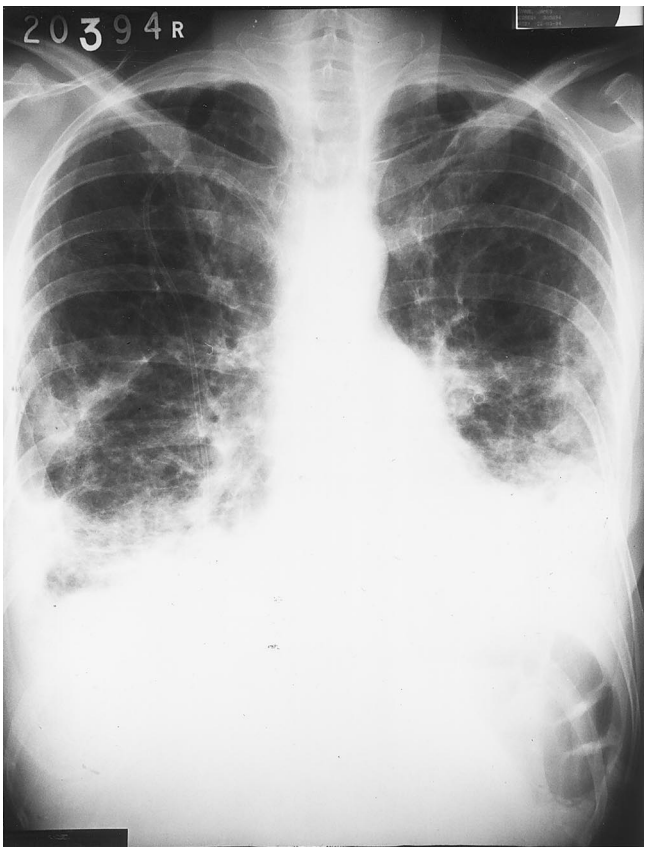

Figure 3. Chest radiograph of a patient with late-stage AIDS and probable invasive pulmonary aspergillosis, illustrating diffuse bilateral disease. The patient had cytomegalovirus retinitis and was receiving chemotherapy for Kaposi's sarcoma; he was symptomatic with a high fever and cough, and cultures of both sputum and bronchoalveolar lavage fluid yielded *Aspergillus fumigatus.*

[127]. The C-reactive protein level is often elevated, but its specificity as a marker for either fungal infection or invasive aspergillosis is in doubt.

The appearances of invasive aspergillosis on plain chest radiographs are extremely heterogenous [109, 111–117, 121–123, 129, 131–137]. Cavitation and pleural-based, wedge-shaped lesions are the most distinctive appearances of invasive aspergillosis. Nodular shadows, with and without cavitation, thick- or thin-walled cavities (in patients with AIDS and chronic necrotizing pulmonary aspergillosis), and alveolar consolidation that coalesces over time to form small nodules are typical. Diffuse, usually lower-lobe, fine shadowing is also seen (figure 3). Pleural effusions are rare. In the context of neutropenia, spontaneous pneumothorax is also highly suggestive of invasive aspergillosis or zygomycosis. For solid organ transplant recipients, the major differential diagnoses of nodular disease are nocardiasis or lymphoma. The differential diagnosis of diffuse disease is not distinctive, and the performance of further diagnostic studies (e.g., bronchoalveolar lavage) is essential.

Early in the course of rapidly progressive invasive pulmonary aspergillosis, plain radiographs are often (falsely) negative, and thus high-quality CT scans of the chest can play a major role in early diagnosis [118, 127, 133, 134, 136, 138–142]. In neutropenic patients the most distinctive early lesions are small nodules and/or small pleural-based lesions with straight edges and surrounding low attenuation (the halo sign) (figure 4). There may be only one lesion, but often there are several. As the disease progresses, the nodules may cavitate (often as the neutrophil count recovers), resulting in the air crescent sign. Both the halo and air crescent signs are highly distinctive for invasive fungal disease of the lung, usually due to *Aspergillus* but also occasionally to a Mucorales, *Trichosporon, Blastoschizomyces,* or *Fusarium.* Pathoradiological correlative studies have conclusively shown that these lesions represent infarcted lung tissue full of hyphae that ramify beyond the area of infarction.

Chronic invasive pulmonary aspergillosis. Chronic invasive pulmonary aspergillosis is less common than acute disease. Underlying conditions include AIDS [121, 143], chronic granulomatous disease [144], diabetes mellitus [145], alcoholism [145], and corticosteroid therapy for chronic pulmonary disease such as sarcoidosis [146–148]; however, many patients do not have any immunocompromising factors [149–151]. In relatively immunocompetent patients with chronic invasive pulmonary aspergillosis, symptoms are more prominent, extend over weeks or months, and are similar to those of an aspergilloma. Chronic, productive cough is usual, often with mild or moderate hemoptysis. Fever is occasionally present but is usually low grade. Malaise and weight loss are usual. Local extension of

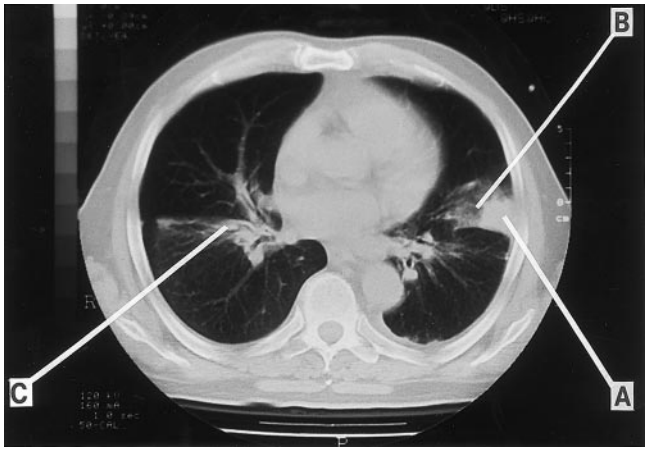

Figure 4. Thoracic CT scan of a neutropenic patient receiving treatment for acute myeloid leukemia who presented with low grade fever and chest-wall pain. A culture of sputum yielded *Aspergillus fumigatus.* An area of consolidation is apparent on the left side abutting the pleura and demarcated by an oblique fissure (*A*). Proximally the lesion has a low attenuation signal, described as the halo sign (*B*). There are also some areas of atelectasis on the right side, with features less characteristic of invasive aspergillosis but probably representing an additional site of disease (*C*).

disease into the chest wall, brachial plexus, or vertebral column is occasionally seen.

Cavitation that expands over time with surrounding consolidation in an area of lung that either did not have a cavity or previously had a small one is seen on radiographs. It can sometimes be difficult to distinguish an aspergilloma from chronic invasive pulmonary aspergillosis, particularly if a previous chest radiograph is not available. A cavitating lung tumor can have similar appearances.

Definitive diagnosis requires demonstration of *Aspergillus* hyphae in lung tissue and a culture-positive respiratory specimen. However, hyphae often are scant, and only granulomas are found, with cultures yielding *Aspergillus.* Different histological patterns have been described, including angioinvasive necrotizing granulomatous pneumonia, a granulomatous bronchiectatic cavity, and bronchocentric granulomatosis [152]. These patients are usually strongly positive for antibodies to *Aspergillus,* and this finding is helpful in inferring the diagnosis when biopsies are negative or contraindicated.

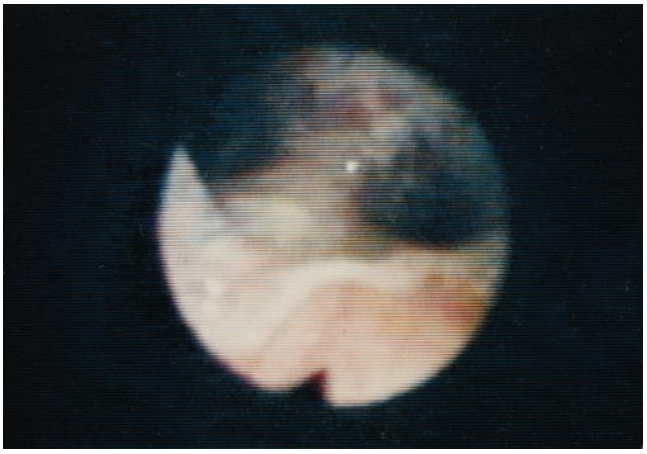

Figure 5. Bronchoscopic appearance of aspergillus tracheobronchitis in a lung transplant recipient; the mucosa is covered in a white mucus layer. (Courtesy of Dr. M. Kramer, Stanford University Medical Center, Stanford, CA.)

Aspergillus Tracheobronchitis

Aspergillus tracheobronchitis is proportionately more common in patients with AIDS [153] and in lung transplant recipients than in other immunocompromised patients [129, 154]; however, ~25% of patients are not apparently immunocompromised [153, 155]. Airway disease due to *Aspergillus* ranges from relatively mild tracheobronchitis with excess mucus production and inflammation to ulcerative tracheobronchitis with ulcers (often around the suture line in lung transplant recipients) and extensive pseudomembranous tracheobronchitis [154]. Distinguishing infection from colonization in lung transplant recipients can be problematic [156].

Approximately 80% of patients with aspergillus tracheobronchitis are symptomatic, although symptoms may be mild or reasonably attributable to another cause such as rejection after lung transplantation. These symptoms include cough, fever, and dyspnea (each present in <50% of patients); chest pain; and hemoptysis. As the disease progresses, symptoms become more common and more severe. Patients with pseudomembranous aspergillus tracheobronchitis may develop a unilateral monophonic wheeze or stridor, reflecting obstruction of the lumen of the airway with necrotic material and fungal material [157]. Many patients die of respiratory insufficiency secondary to occlusion of the airway; others develop disseminated disease in the last few days of life. Occasionally, perforation of the trachea or bronchi occurs.

A chest radiograph is usually normal early in the course of disease, but consolidation may occur later. CT findings include peribronchial consolidation and centrilobular nodules [158], but these findings are not specific. Performing bronchoscopy with bronchial biopsy, microscopy, and culture is the only means of making the diagnosis before death (figure 5). Biopsy of loose material in the tracheal lumen will often reveal necrotic cartilage. Patients with pseudomembraneous aspergillus tracheobronchitis have shaggy greyish linings of the whole trachea and bronchial wall. Therapy with an azole appears to be superior to that with systemic amphotericin B for this disease [154]. The role of aerolized amphotericin B has not been well evaluated but appears minor, and this agent may cause significant bronchospasm.

Invasive Aspergillus Sinusitis

The term invasive aspergillus sinusitis embraces at least three distinct disease entities. Acute rhinosinusitis is a relatively common manifestation of invasive aspergillosis in neutropenic and bone marrow transplant recipients [119, 159–162] (table 4). Invasive aspergillus sinusitis appears to be extremely uncommon in solid organ transplant recipients [163]. Chronic invasive aspergillus sinusitis occurs in healthy or only mildly immunocompromised patients [164–167], and a variant of this disease, paranasal aspergillus granuloma, is seen in the tropics [168, 169]. A. *flavus* is proportionately more common as a cause of aspergillus sinusitis [163].

Acute invasive aspergillus rhinosinusitis. Early symptoms are nonspecific and easily mistaken for possible bacterial infection [159]. Fever, cough, epistaxis, and headache are common. Other occasional symptoms include nasal discharge, sinus pain, and sore throat. Findings on examination of the anterior nares may initially be unremarkable. A careful search for insensitive areas with decreased nasal blood flow that precedes frank crusting or ulceration is particularly important. Invasive aspergillus sinusitis is frequently the only focus of aspergillus disease, although local extension to the palate [170, 171], orbit, or brain is common and relatively rapid. Aspergillus sinusitis may occur concurrently with pulmonary aspergillosis.

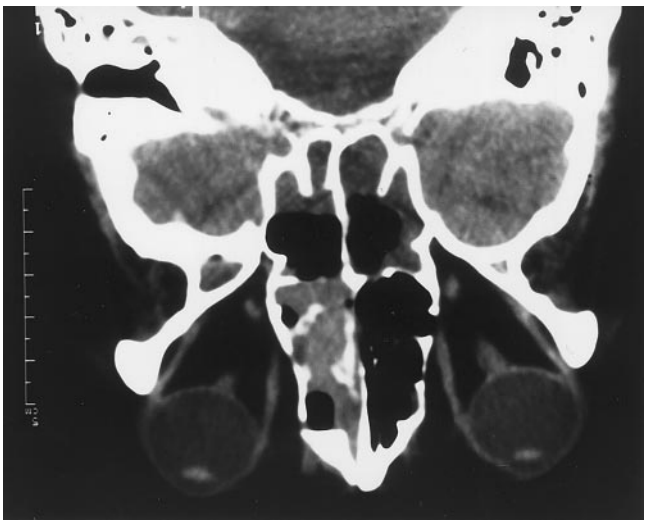

Figure 6. CT scan of the sinuses of an allogeneic bone marrow transplant recipient with diabetes showing aspergillus rhinosinusitis. The sphenoid sinus is partially opacified, and the left anterior nasal passage is completely opacified. Cultures of material from the sphenoid sinus yielded *Aspergillus fumigatus.*

Plain radiographs of the sinuses are insensitive and do not allow distinction between bacterial and fungal infection [159, 162]. CT scans show fluid opacification of the sinuses (figure 6), and any bony destruction and spread into adjacent tissues are well visualized. MRI scans also are useful. T_1-weighted images tend to appear similar to those associated with bacterial sinusitis (e.g., they may be isointense or show decreased signal intensity), but T_2-weighted images show very decreased signal intensity compared with those of bacterial sinusitis, which show increased signal intensity.

The diagnosis can be inferred if typical clinical and radiographic features are present along with a positive culture of a lesion or the finding of characteristic hyphae in tissue, and the diagnosis is confirmed if both are present.

The cornerstone of treatment is the administration of amphotericin B, as treatment with itraconazole appears to be less effective. Radical surgery may be associated with major complications [145] and appears to offer no survival advantage [172]. Response to treatment, with subsequent relapse during episodes of neutropenia or relapses of leukemia, is typical. If uncontrolled by a combination of antifungal therapy, recovery from neutropenia, and possibly surgery, local extension to the palate, facial skin, orbit, or brain, with a fatal outcome, is characteristic.

Chronic invasive aspergillus sinusitis. Most patients with chronic invasive aspergillosis have no discernible immunocompromising factors, although a substantial minority are diabetics, drink alcohol to excess, or have AIDS [121, 145, 164–167, 173]. The clinical presentation of chronic invasive sinusitis depends on how far the disease has progressed. It cannot be distinguished clinically from other fungal causes of chronic sinusitis. In addition, early cases are similar in presentation to saprophytic aspergillus sinusitis and can be distinguished only by radiographic and histopathologic examination of the sinus mucosa and/or bone. As the disease progresses, usually over months, the clinical features of local involvement become apparent. Visual symptoms are common and include diplopia, unilateral blindness, pain in the eye, and proptosis. Headaches, loss or impairment of smell, and the features of chronic sinusitis (e.g., nasal stuffiness) are also common. Fever is almost universally absent.

The radiological features of this disease are similar to those of acute disease [166, 174]. Bony destruction may occur without direct fungal invasion, apparently due either to a ''pressure'' effect or to local toxin production (speculative). However, bony invasion by hyphae may be underrecognized histopathologically because of the nature of the surgery required to remove abnormal tissue.

Invasion of mucosa and other tissue by fungal hyphae is the hallmark of disease, although in cases of chronic invasive aspergillus sinusitis, few hyphae are seen, and the diagnosis may be missed. Cultures are usually positive but may require multiple samples.

Aspergillus sphenoid sinusitis requires particular mention because of its frequent devastating complications, which include major visual disturbance, brain abscess, or stroke and/or death due to involvement of the carotid artery [175–178]. Approximately 10% of all cases of sphenoid sinusitis are due to *Aspergillus,* the commonest fungal pathogen [176]. Another intractable complication of aspergillus sinusitis is osteomyelitis of the base of the skull [179].

For both forms of chronic invasive aspergillus sinusitis, surgical debridement, followed by prolonged antifungal therapy, is required [145]. Chronicity and relapse characterize this disease.

Disseminated Aspergillosis

At autopsy, many patients who die of or with invasive aspergillosis have disseminated disease [2, 3, 109, 119, 155, 180–188]. Before death, dissemination is often suspected but rarely proven unless cerebral or cutaneous aspergillosis is diagnosed. Jaundice and disseminated intravascular coagulation are often seen in the terminal stages of disseminated aspergillosis; the precise reasons for these findings are not well delineated.

Cutaneous Aspergillosis

Cutaneous aspergillosis is most commonly seen in neutropenic patients at intravenous-catheter insertion sites [189–191] or around the catheter site as a result of adhesive dressings applied to the skin [191–195]. Aspergillosis is one cause of invasive fungal dermatitis in premature neonates [196] and may occur in children with AIDS [197]. Cutaneous aspergillosis is occasionally a manifestation of disseminated aspergillosis [119], in which case diagnosis of the systemic disease can be

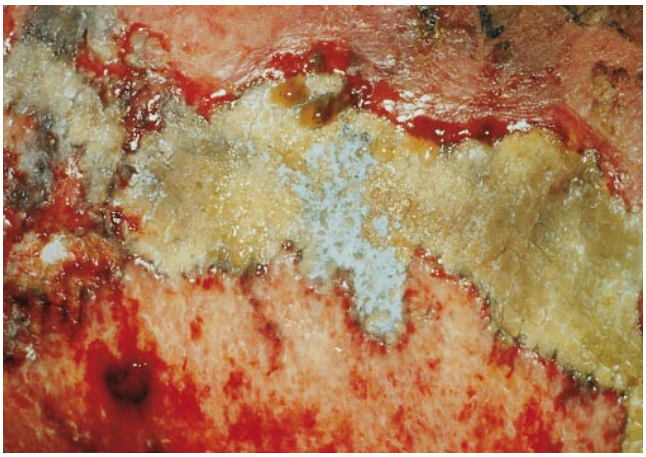

Figure 7. *Aspergillus fumigatus* growing and sporulating in a burn on a 7-year-old boy with 50% full thickness burns; he later died.

achieved rapidly with microscopic examination and culture of a skin biopsy specimen.

The clinical appearance of cutaneous aspergillosis is very similar to that of pyoderma gangrenosum, which is caused more commonly by *Pseudomonas aeruginosa.* Initially, an area of raised erythema is present that rapidly increases in size with accompanying discomfort or pain. The center of the lesion changes from red to purple and finally to black and may ulcerate. Histologic examination reveals invasion of local blood vessels and infarction of the skin, corresponding to the visual appearances. The rate of progression of the lesion usually corresponds to the patient's immune status, in that patients without circulating neutrophils tend to have rapidly progressive disease, whereas those with some neutrophils will have disease that progresses over several days.

Aspergillus may also invade burns [97, 198–200] (table 4), causing a rapidly progressive necrotic lesion that is usually refractory to treatment with amphotericin B (figure 7). Both cutaneous and burn-associated aspergillosis are clinically indistinguishable from infection caused by the Mucorales unless *Aspergillus* sporulates, in which case direct microscopy will provide an etiologic diagnosis to the genus level. Surgical or other wounds may also be infected with *Aspergillus* acquired either perioperatively or postoperatively [93]. This occurrence appears to be relatively common among liver transplant recipients (table 4), perhaps because of the long duration of surgery [201], large operative site, and the presence of immunosuppression.

Apart from burn-associated aspergillosis, cutaneous aspergillosis responds better to therapy than do most other manifestations of invasive aspergillosis, perhaps because the diagnosis is made rapidly and with confidence [145].

Cerebral Aspergillosis

Cerebral aspergillosis occurs in 10%–20% of all cases of invasive aspergillosis, and only rarely is the brain the sole site of infection [202–216]. The clinical presentation and pace of progression of cerebral aspergillosis, like pulmonary aspergillosis, vary remarkably with the host group. The most immunocompromised patients present with nonspecific findings (i.e., alteration in mental status and seizures) shortly before death, whereas those slightly less immunocompromised are more likely to have focal features and headache. Fever sometimes occurs but may be attributed to other coexistent infections. Meningism occurs rarely.

For highly immunocompromised patients, the appearance of cerebral aspergillosis on CT scans is that of one or multiple hypodense, well-demarcated lesions (figure 8) [212, 213, 217–219]. Hemorrhage and mass effect are unusual. For patients with normal peripheral WBC counts, the findings of ring enhancement and surrounding edema are more frequent [217].

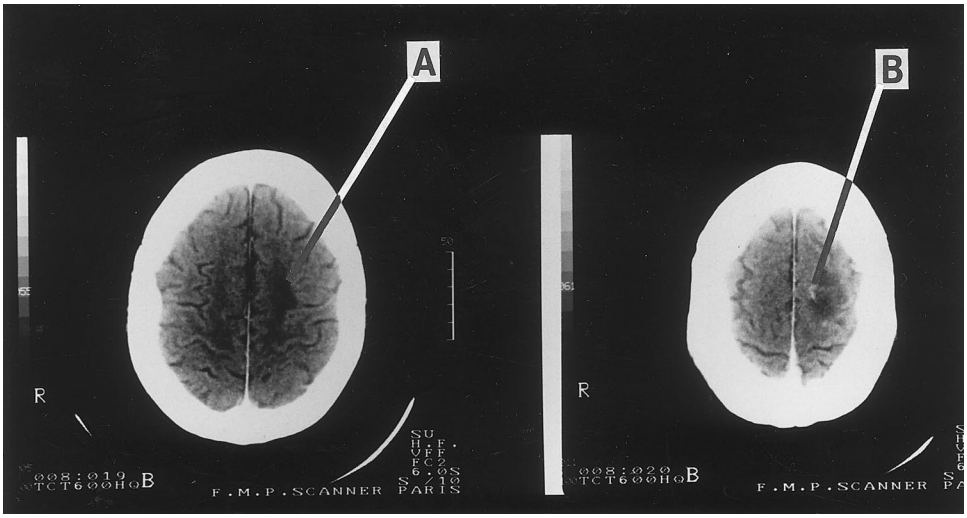

Figure 8. CT scan of the brain in a neutropenic recipient of an allogeneic bone marrow transplant showing a hypodense lesion (*A*) with slight enhancement medially (*B*), typical of invasive aspergillosis. He also had cavitating pulmonary lesions although the diagnosis of invasive aspergillosis was not proven.

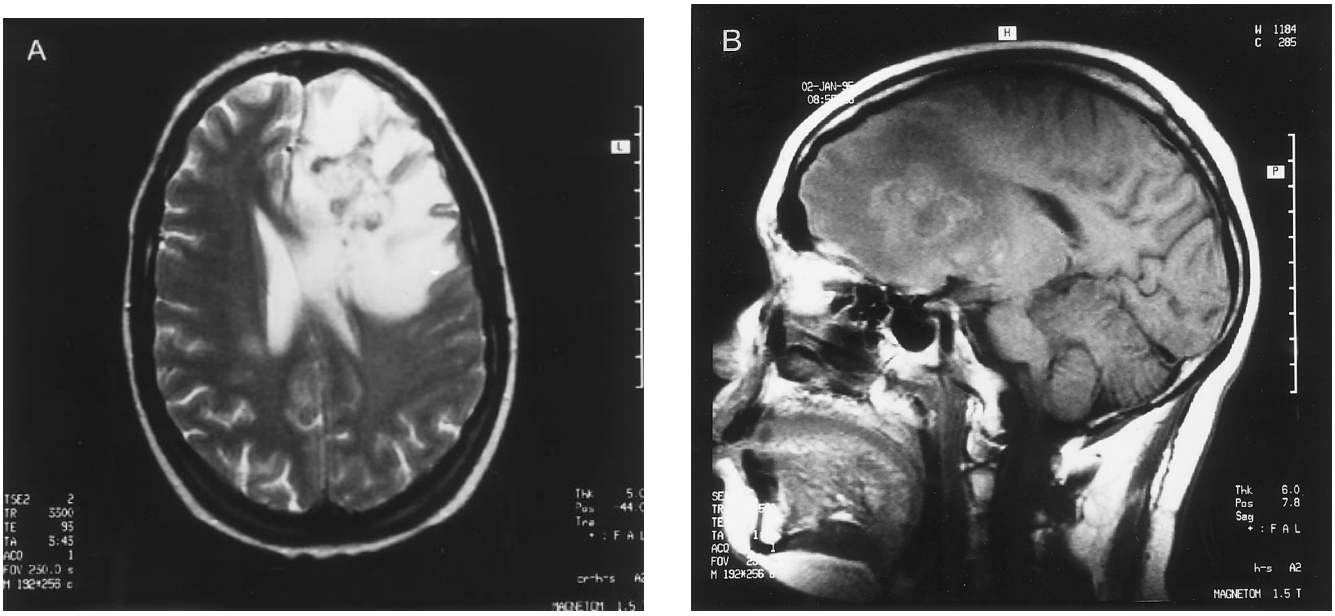

Figure 9. *A,* magnetic resonance scan (T_2-weighted) of the brain from an allogeneic bone marrow transplant recipient with graft-versus-host disease. He also had diffuse pulmonary disease and cultures of sputum and bronchoalveolar lavage fluid positive for *Aspergillus fumigatus.* The scan shows a multiloculated abscess with much surrounding edema. *B,* T_1-weighted sagittal image of same patient showing the large abscess and surrounding edema, although it is less obvious than on the T_2-weighted image.

Clearer definition of a hypodense lesion is sometimes obtained with use of intravenous contrast. MRI scans often reveal additional lesions without distinctive features (figures 9A and B) [213, 220]. These lesions are usually deep-seated and hard to access surgically. For less immunocompromised patients, a mass lesion with surrounding edema and midline shift, usually with contrast enhancement, is a typical finding [208, 217]. Many of these lesions are frontal or are in the area of the cerebellopontine angle.

Differential diagnoses depend on the host group. *Aspergillus* accounts for about half of the cases of brain abscess in bone marrow transplant recipients, and *Candida* species account for one quarter of the cases [213]. In liver transplant recipients, *Aspergillus* is the commonest infectious cause of brain abscess, but cerebral infarction and hemorrhage are more common causes of radiological abnormalities [206, 209]. In other solid organ transplant recipients, cerebral aspergillosis and nocardiosis are seen with approximately equal frequency; toxoplasmosis, cryptococcosis, and lymphoma are less frequent. In patients with AIDS, cerebral toxoplasmosis is a much more common cause of cerebral abscess, as is lymphoma. CSF findings are usually abnormal but nonspecific [205, 212]. It is important to rule out other differential diagnoses.

Definitive diagnosis requires biopsy or aspiration of a cerebral lesion, but performance of these procedures is often precluded by a patient's clinical status or by coagulation problems. An inferential diagnosis is possible if invasive aspergillosis is documented at other sites and a typical radiographic abnormality of the brain is observed.

Unusual Forms of Invasive Aspergillosis

There are many unusual or rare manifestations of invasive aspergillosis (figure 10). Some patients are immunocompromised, but most are not. Neonates, intravenous drug abusers, and patients who have undergone surgery are most commonly affected. A summary of these manifestations is provided in table 5; many of them have been reviewed previously [145, 220], but case reports that update these reviews continue to be published.

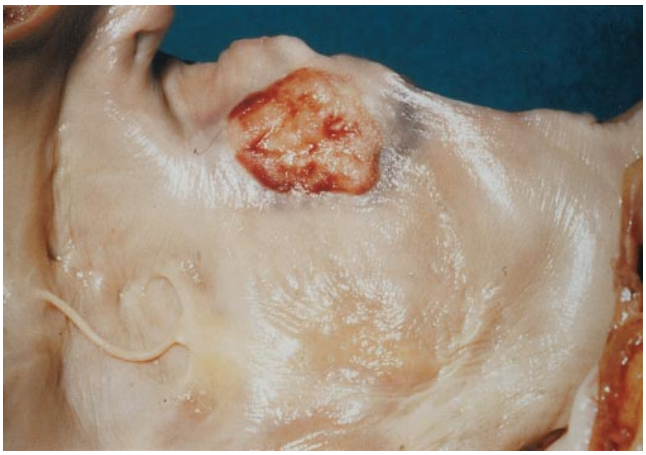

Figure 10. Macroscopic appearance of aspergillus endocarditis in an immunocompetent intensive care unit patient. Large vegetations are characteristic of this infection but not specific. (Courtesy of Dr. G. Wilson, North Manchester General Hospital.)

Table 5. Unusual forms of invasive aspergillosis.

Site, disease	Risk factor, typical host	Optimal diagnostic approach	Optimal therapeutic approach	Outcome (assuming appropriate therapy)
Epiglottis	Leukemia	Culture, with or without biopsy, with protection of the airway	iv/po therapy	Poor
Larynx	Nonimmunocompromised	Biopsy and culture	Removal of nodules, with or without po therapy	Good
Bronchial stump	Lung resection with silk sutures	Culture	Removal of sutures, with or without po therapy	Good
Empyema	Postsurgical removal of aspergilloma	Aspiration of cavity and culture	Exteriorization of cavity, with po therapy	Chronic disease
Onychomycosis	Nonimmunocompromised	Microscopy and culture of nail and subungual debris	po therapy	Good
Endophthalmitis	Disseminated aspergillosis, iv drug abuse, penetrating eye injury	Vitrectomy and intraoperative microscopy and culture of whole vitrectomy specimen on appropriate media, depending on microscopic findings	Partial or complete vitrectomy, 5–7.5 mg intravitreal amphotericin B, iv amphotericin B and flucytosine	Loss of vision likely
Meningitis	Aspergillus sinusitis, iv drug abuse, neurosurgery, mild immunocompromising conditions	CSF culture or meningeal biopsy, CSF antibody or antigen detection	iv/po therapy	Good
Endocarditis	Cardiac surgery, immunocompromised	Echocardiography (large vegetations), microscopy or culture of peripheral emboli, fungal blood culture	Valve replacement, iv amphotericin B	Fatal without surgery
Vascular graft	Nonimmunocompromised	Culture of material from graft or localized extension of infection	Removal of graft and fashioning of extra-anatomic bypass, iv/po therapy	Good if infected graft removed
Pericardium	Neutropenia	Pericardiocentesis or pericardiectomy	iv/po therapy	Poor, as diagnosis often late
Gastrointestinal tract	Immunocompromised	Biopsy of ulcer	iv/po therapy	Poor, as diagnosis often late
Peritoneum	Chronic ambulatory peritoneal dialysis	High-volume fungal culture of dialysate, (?) antigen detection	Removal of catheter, iv/po therapy	Good if diagnosis made
Liver	Leukemia	Imaging and biopsy (culture may be negative, but other foci of aspergillosis often apparent)	iv/po therapy, delay in cytotoxic chemotherapy for a short time	Reasonable, although leukemia relapse likely
Thyroid	Many	Aspiration of lesion for culture and microscopy	iv/po therapy	Poor, as usually a manifestation of disseminated disease
Renal parenchyma	Immunocompromised	Aspiration of lesion for culture and microscopy, urine culture	iv/po therapy	Poor as usually a manifestation of disseminated disease
Renal pelvis	Mildly immunocompromised (e.g., diabetes)	Culture and microscopy of urine fungus balls, culture of urine or nephrostomy drainage	Relief of hydronephrosis, nephrectomy if unilateral, iv/po therapy and local irrigation with amphotericin B if bilateral	Poor for affected kidney(s), otherwise good
Bone	Mixed, especially chronic granulomatous disease	Biopsy with microscopy and culture	Surgical debridement if localized, po therapy	Reasonable but long-term treatment necessary.

Diagnostic Approaches

The diagnostic approach to invasive aspergillosis in immunocompromised patients offers a useful paradigm for many opportunistic infections, for many reasons. First, invasive aspergillosis occurs in many different host groups, some of whom are at risk for short periods of time and some of whom are at risk for years. Second, invasive aspergillosis is relatively uncommon, and therefore, few physicians or surgeons have sufficient experience with the disease to feel entirely comfortable with either their diagnostic or therapeutic approach. Third, invasive aspergillosis has a variable and nonspecific clinical presentation, so a high index of suspicion is called for. Fourth, there is no single diagnostic test that is either universally applicable or sensitive or specific enough to establish the diagnosis. Finally, delay in the initiation of therapy is usually fatal.

Thus, several diagnostic approaches need to be taken rapidly to confirm or refute the diagnosis once an opportunistic infection is suspected. Detailed radiological evaluation is essential. This should be done with CT or MR imaging within 24 hours after the diagnosis is suspected. Occasionally, *Aspergillus* affects other organs of the body, and ultrasonography or CT may be useful in these situations. Once a lesion has been identified and characterized, the best diagnostic approach can then be decided on. For peripheral pulmonary lesions, the procedure of choice is needle biopsy or surgical resection of the lesion. For focal lesions near the hilum and the great vessels in the lung, particularly in neutropenic patients, urgent thoracotomy and resection should be considered (regardless of the presence of neutropenia or thrombocytopenia) because the risk of life-threatening hemoptysis is sufficiently high to warrant the relatively low surgical risks. For patients with bilateral alveolar or multifocal disease in whom surgical resection is impossible, the best procedures are bronchoscopy and bronchoalveolar lavage.

Microscopy and Culture

Fluid obtained by bronchoalveolar lavage, bronchial lavage, or endotracheal aspiration should be processed for microscopy or cytology, fungal culture, and any other procedures appropriate for ruling out differential diagnoses (e.g., immunofluorescence for *Pneumocystis*). Combining microscopy and culture will increase the diagnostic yield by 15%–20% [221–223] over that with culture alone (especially if the patient is receiving empirical antifungal therapy [224]), and, of course, microscopy is relatively rapid (figure 11). The use of special stains for fungi (such as calcafluor white) probably increases the sensitivity of microscopy, although this has not been systematically studied in the context of invasive aspergillosis. Microscopy is particularly useful in the context of airway and wound aspergillosis because fruiting bodies are sometimes seen, allowing direct identification of the pathogen with some confidence. Microscopy is also particularly useful for small-volume specimens obtained by aspiration of an abscess, an infected sinus, or a pulmonary lesion.

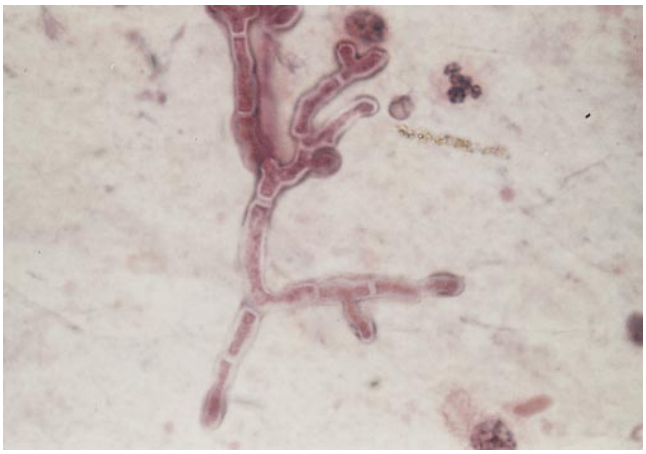

Figure 11. High-power view of *Aspergillus* hyphae found in the sputum of a patient colonized with *Aspergillus.* Microscopy or bronchoalveolar lavage and other sterile fluids are particularly useful means of detecting mold infections quickly. (Courtesy of Dr. G. Armstrong, Hope Hospital; original magnification, ×400.)

While microscopy is moderately sensitive and absolutely specific for fungal infection, it does not allow unequivocal confirmation of the diagnosis of invasive aspergillus infection because several other fungi may have similar microscopic and histological appearances. Such fungi include *Scedosporium apiospermum* (*Pseudallescheria boydii*), *Fusarium* species, *Scopulariopsis* species, and others. Immunohistochemistry is useful for distinguishing organisms seen in tissue but not grown in culture [225]. Culture of *Aspergillus* from an infected sterile site provides definitive proof of disease, allows the species to be determined, and allows the organism to be tested for susceptibility. For this reason, the performance of fungal cultures is appropriate and clinically useful. The yield from specimens is higher if specific fungal media are used [36] even though *Aspergillus* is capable of growth on blood agar and other bacterial media.

Occasionally, cultures are falsely positive in highly immunocompromised patients, and these results clearly lead to suspicion of disease [118, 126, 128, 129, 224]. In the case of respiratory specimens from patients who are clinically well but highly immunocompromised (e.g., bone marrow transplant recipients), the clinical presumption should be that such patients have acute invasive aspergillosis until proven otherwise.

Serology for *Aspergillus*

Fluids and aspirates from infected lesions may also be tested by serological methods such as specific ELISA and/or by molecular diagnostic methods, although blood is the usual sample tested. The use of serological assays has focused on detecting galactomannan. The early latex agglutination tests were highly

specific [226] but not very sensitive (~30% [227]). More recent assay procedures, such as sandwich ELISA, are considerably more sensitive (1 ng/mL of antigen can be detected, compared with 25 ng/mL with the latex test) but, unfortunately, have a false positivity rate of ~10% [227]. Such tests have been applied only to hematology patients. Routine screening, performed two-to-three times per week, provides the highest detection rate. The recently developed G-test, which detects circulating 1,3-β-D-glucan with use of a modification of the limulus assay for endotoxin, has a sensitivity of ~20 pg/mL [228, 229]. Prospective studies of the utility of the G-test have shown that it can detect 1,3-β-D-glucan in the context of confirmed invasive mycoses, including invasive aspergillosis, but it does not distinguish between species of *Candida, Aspergillus,* or other major fungi. Sera from patients with aspergillomas may also be G-test positive [228, 229], although this condition should be distinguishable on a clinical basis.

Testing for antibodies to *Aspergillus* has been performed for solid organ transplant recipients as a means of alerting clinicians to a possible diagnosis of invasive aspergillosis. Recently published early data on heart transplant recipients and more conclusive data on lung transplant recipients [230] suggest that this procedure is another valuable adjunct to assist clinicians in identifying patients who need further evaluation. Antibodies to *Aspergillus* may be detectable in other patients with invasive aspergillosis, but the sensitivity and specificity of this test are low, and in most instances the test becomes positive as a patient's condition starts to improve after initiation of therapy. However, detecting antibodies may be useful for patients with chronic invasive aspergillosis.

Molecular diagnostic approaches are in their infancy but are likely to become clinical reality over the next decade. Detection of *Aspergillus* DNA in blood would be most useful [231]. Pulmonary specimens may yield false-positive results because of the presence of extraneously introduced or colonizing spores [232–234]. In addition, information on susceptibility is not obtained.

Thus, the diagnosis of invasive aspergillosis is based ideally on histological documentation of typical hyphae and a positive culture for *Aspergillus*. In clinical practice such a diagnosis is infrequently achieved, and clinical management has to proceed pragmatically with the accumulation of symptomatic, radiological, and microbiological criteria that offer different levels of certainty of the diagnosis. Waiting for definitive proof of a diagnosis of invasive aspergillosis before therapy is initiated in patients with this disease places such patients in mortal danger from progression of the disease. However, for patients with chronic invasive aspergillosis, diagnostic procedures should proceed therapy. Anticipating the pace of the disease is critically important in determining optimal management.

Treatment

The mortality associated with invasive aspergillosis is nearly 100% if the disease is not treated [107]. The only untreated survivors are patients whose immunocompromising factors are removed (e.g., corticosteroid therapy is permanently stopped). The rate of progression of invasive aspergillosis varies widely. In patients such as liver and bone marrow transplant recipients and those with profound neutropenia, the course of disease from the appearance of the first clinical or radiological abnormality to death is typically 10–14 days. The diagnosis is often difficult to establish, and several days usually elapse between consideration of the diagnosis and partial or complete confirmation. A critical window of opportunity may be missed if treatment is not started early, with fatal consequences for patients. A culture positive for *Aspergillus,* microscopy showing the presence of hyphae, or a combination of appropriate radiological features are all sufficient to warrant starting therapy in patients at risk for this infection.

Antifungal Agents

There are currently only two antifungal agents with activity against *Aspergillus*—amphotericin B and itraconazole (table 6). The overall success rate with amphotericin B therapy is 34% (table 7) but varies substantially between different host groups. Similar response rates are seen with itraconazole. The lipid-associated formulations of amphotericins B appear to be equally efficacious but not more so.

Therefore, if a patient is able to take oral therapy, has good intestinal function, and is eating and not taking drugs that induce the metabolism of cytochrome P-450 (e.g., rifampin, phenytoin, phenobarbital, or carbamazepine), then itraconazole would be a reasonable first choice. Patients with intestinal problems (such as graft-versus-host disease) and patients with AIDS absorb itraconazole poorly. For adults, the dose of itraconazole should be 200 mg t.i.d. for 4 days, followed by 200 mg twice daily [235]; all doses of capsules should be taken with food, but the liquid formulation should be taken without food. Higher doses may be helpful for patients with cerebral aspergillosis [236] (table 7). Serum concentrations of itraconazole should be measured 5–10 days after treatment is begun. Interactions with other drugs are problematic, including that with cyclosporine (halve dose and measure levels), digoxin (monitor levels), antihistamines (stop), and protease inhibitors (observe patients carefully).

If a patient is not a suitable candidate for oral therapy as outlined above, then intravenous therapy is necessary. The dose of conventional amphotericin B should be 0.8–1.0 mg/(kg·d), regardless of the presence of renal dysfunction; neutropenic patients, for whom 1–1.25 mg/(kg·d) is appropriate, are the exception. The degree of renal dysfunction can be reduced by administering saline, and the degree of hypokalemia can be reduced by administering amiloride. If renal dysfunction is or is likely to be a major problem, as in cyclosporine-treated patients or patients intolerant of amphotericin B or the fungal infection progresses despite treatment with an adequate dose of conventional amphotericin B, then one of the lipid-associated preparations of amphotericin B in doses of 4–5 mg/(kg·d) (or

Table 6. First- and second-line therapy for invasive aspergillosis.

Agent	Initial dose	Comments
Amphotericin B deoxycholate	0.8–1.25 mg/(kg · d), iv	Considered first-line therapy but high failure rate; significant interaction with cyclosporine
Itraconazole	200 mg t.i.d. for 4 d, then 200 mg b.i.d., po*	Useful if a patient is eating and not receiving cytochrome P-450 inducers; significant interaction with cyclosporine; levels should be measured to ensure adequate absorption
Amphotericin B colloidal dispersion	4–6 mg/(kg · d), iv	Less nephrotoxic than amphotericin B deoxycholate
Liposomal amphotericin B	1–5 mg/(kg · d), iv†	Less nephrotoxic than amphotericin B deoxycholate
Amphotericin B lipid complex	5 mg/(kg · d), iv	Less nephrotoxic than amphotericin B deoxycholate

NOTE. None of these agents or regimens have been compared in controlled trials, but all have been shown to be partially effective.

* For cerebral aspergillosis, consider 400 mg b.i.d.

† In neutropenic patients with invasive pulmonary aspergillosis, 1 mg/(kg · d) is probably equivalent to 4 mg/(kg · d), but whether this is true for other patients is not clear.

itraconazole) is appropriate [237–243]. Recent data on patients with invasive pulmonary aspergillosis whose neutropenia resolved are consistent with the suggestion that 1 mg/(kg · d) of AmBisome (Nexstar, San Dimas, CA) is equivalent to 4 mg/(kg · d), although the failure rates with both regimens were considerable [244]. Recommended doses of Amphocil (Amphotec; Sequus Pharmaceuticals, Menlo Park, CA) and Abelcet (Liposome Co., Princeton, NJ) are 4 mg/(kg · d) and 5 mg/(kg · d), respectively. These dosages are my preferred option, at least until the progression of disease has been arrested. These dosages should be continued for at least 2 weeks, until a therapeutic response has been obtained.

Unfortunately, first-line therapy fails in at least 50% of patients. Deciding when therapy has failed can be difficult. For patients with rapidly progressive disease, failure of the first regimen means a fatal outcome. However, for patients with more slowly progressive disease, the evaluation of response after 10–20 days of therapy can usually be made on the basis of clinical and radiological criteria. The less immunocompromised the patient, the longer it takes to evaluate response. Many patients will respond to alternative therapy if first-line therapy fails.

Table 7. Response to treatment of invasive aspergillosis among immunocompromised patients (or survival among untreated patients).

	No. of patients who responded or survived/total no. (%)			
Site of infection	Untreated	Treated for 1–13 d*	Treated for ⩾14 d*	All treated*
Lung	0/101	1/65 (1)	90/179 (50)	91/244 (37)
Sinuses	. . .	0/11	17/35 (49)	17/46 (37)
Brain	0/49	0/8	0/7	0/15 (0)
Total	0/150	1/94 (1)	107/221 (48)	108/315 (34)

NOTE. Data are from [107].

* All patients were treated with amphotericin B deoxycholate except 75 patients with AIDS who were treated with either amphotericin B or itraconazole or both (25% response rate).

There is no a priori total dose of amphotericin B that should be given. The key to success is giving a high enough dose in the first 2 weeks of therapy and switching therapy if it is failing. Some clinicians give 2 g or 2.5 g of amphotericin B, but there is no scientific basis for this practice. Consolidation therapy with itraconazole is often appropriate, often for long periods. Relapse occurs even after months of therapy if patients remain immunocompromised. Bone marrow transplantation or further cancer chemotherapy may be possible for patients with invasive aspergillosis during remission induction therapy if secondary prophylaxis is administered [245–249]. Caution should be exercised in using azoles for this indication as they may potentiate chemotherapy toxicity by interfering with the metabolism of some cytotoxic agents [250].

Several novel agents are under investigation, including azoles and echinocandins. The most advanced azole is the broad-spectrum triazole voriconazole (UK-109,496; Pfizer, New York), which has shown good clinical efficacy and tolerability among immunocompromised patients with invasive aspergillosis [251]. Randomized trials are ongoing.

Resistance and Susceptibility Testing

In vitro resistance of *A. fumigatus* to itraconazole (confirmed in an animal model) has just been described [252, 253], although the frequency of this resistance is probably low. Different degrees of susceptibility to itraconazole have been found for different *Aspergillus* species: *A. flavus* and *A. terreus* are

generally more susceptible in vitro than are other species [38]. Furthermore, ''in vivo resistance'' of *A. fumigatus* to amphotericin B that cannot yet be detected by in vitro susceptibility testing has been shown in an animal model [254]. Given the development of resistance, more aggressive performance of cultures to detect *Aspergillus* for species-specific identification and susceptibility testing is appropriate.

Surgical Excision

Surgery has value in the treatment of focal invasive pulmonary aspergillosis, for persisting lung shadows before bone marrow transplantation or for more aggressive chemotherapy, for significant hemoptysis, and for lesions impinging on the great vessels or major airways [118, 127, 145, 246, 247, 255]. Extensive surgical debridement is often useful for invasive sinus aspergillosis but should not be done until recovery from neutropenia as hemorrhage and other operative complications are frequent in neutropenic patients [145, 172]. Surgery, other than diagnostic aspiration or biopsy, has no role for patients with cerebral aspergillosis. Other surgical approaches are described in table 5.

Cytokine Therapy

Therapy with cytokines, as an adjunct to antifungal therapy, is possibly of value. Increasing the numbers of circulating granulocytes and monocytes in patients who have been neutropenic may be critical, but the temporal impact is small. Granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and γ-IFN also act by increasing phagocytosis of and damage to *Aspergillus* hyphae in vitro [72, 76–78]. Whether the magnitude of these effects is clinically important, in combination with the effect of antifungals, has yet to be clinically demonstrated; it was not in phase 1/2 studies of macrophage colony-stimulating factor [256].

Outcome

Factors contributing to a poor outcome for patients with invasive aspergillosis are shown in table 8. There are substantial variations from patient group to patient group and within each patient group. For patients with leukemia, achieving complete remission is critical to survival [257]. Avoidance of hemoptysis by performing surgery, even during episodes of profound neutropenia, is critical to the survival of patients with proximal lung disease. Cerebral aspergillosis is still associated with a mortality of >95% among immunocompromised patients. Advances in therapy for invasive aspergillosis will follow from a greater understanding of pathogenesis, better and more sensitive diagnostics, education of clinicians, development of superior antifungal agents, and, possibly, adjunctive immunotherapy.

Table 8. Factors predicting a poor response to therapy, other than host group and site of disease, in patients with invasive aspergillosis.

- Leukemic relapse
- Persistent neutropenia
- No reduction in immunosuppression
- Diffuse pulmonary disease
- Major hemoptysis
- Delayed therapy
- Low doses of amphotericin B, especially during neutropenia
- Undetectable or very low serum itraconazole concentrations
- Lack of secondary prophylaxis during another episode of neutropenia
- Histological evidence of angioinvasion

NOTE. Data are from [227].

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