Diagnosis and Management of Invasive Aspergillosis

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Aspergillus was first recognized as an organism by Micheli in 1729. The first description of disease due to *Aspergillus* was in the air sacs and lungs of a jackdaw in 1815, and the first human case of aspergillosis was described in 1842 in Edinburgh, Scotland (1). This was almost certainly an example of an aspergilloma in a tuberculous cavity. Various superficial forms of aspergillosis were described in the late 1800s, and from 1890 to 1897 *Aspergillus* tracheobroncitis, renal aspergillosis, and both maxillary and sphenoid sinus aspergillosis were described. The first case of invasive pulmonary aspergillosis as an opportunistic infection was described in a patient with aplastic anemia in Britain in 1953 (2). Attempts to treat invasive aspergillosis began with amphotericin B in 1959. The first comprehensive description of the pathology of invasive aspergillosis was published in 1970 from the National Institutes of Health (3). The first multicentre trial of therapy for invasive aspergillosis was commenced in 1989 and published in 1994 by the NIAID Mycoses Study Group (4).

There are a number of different diseases produced by *Aspergillus* and these can be classified as shown in Table 14.1. The incidence of invasive aspergillosis is rising, whereas that of aspergilloma is falling in the developed world. *Aspergillus* is regarded as an opportunistic pathogen. However, there are many reports on record of invasive aspergillosis in nonimmunocompromised patients, and it is a primary pathogen of a large number of different mammals and birds and the honey bee. Thus, it is a rare primary pathogen and an increasingly common opportunistic pathogen in humans.

Aspergillus fumigatus causes approximately 85% of all forms of aspergillosis, followed by *Aspergillus flavus* (5% to 10%), *Aspergillus niger* (2% to 3%), and *Aspergillus terreus* (2% to 3%). Certain forms of aspergillosis are more common due to particular species, for example otitis externa due to *A. niger*, sinusitis due to *A. flavus*, and joint disease due to *A. glaucus*. Many other rare species of *Aspergillus* have caused disease.

Table 14.1. Classification of *Aspergillus* Infection

I. Disease in the normal host
  A. Allergic diseases
    1. Allergic bronchopulmonary aspergillosis
    2. Allergic *Aspergillus* sinusitis
  B. Superficial infection
    1. Otomycosis, onychomycosis
  C. Invasive infection
    1. Pulmonary aspergillosis, invasive sinusitis
II. Saprophytic disease
  A. Aspergilloma
  B. *Aspergillus* sinusitis
III. Infection associated with tissue damage, surgery, or foreign body
  A. Superficial infection, e.g. keratitis and/or endophthalmitis
    1. Burn wound aspergillosis
  B. Operative site infection
    1. Prosthetic valve endocarditis, empyema and pleural aspergillosis, osteomyelitis
  C. Foreign body associated
    1. Hickman or other intravenous line, chronic ambulatory peritoneal dialysis catheter
IV. Infection in the immunocompromised host
  A. Primary cutaneous aspergillosis
  B. Pulmonary aspergillosis
    1. Acute invasive
    2. Chronic necrotising aspergillosis
  C. Airways aspergillosis
    1. Invasive *Aspergillus* tracheobronchitis
    2. Obstructing bronchial aspergillosis
  D. Rhinosinusitis
  E. Disseminated
    1. Cerebral, renal, cutaneous aspergillosis

PATHOGENESIS

Aspergillus almost certainly has a substantial number of different pathogenicity factors. The small size of the conidia of *A. fumigatus* are probably one reason why it causes pulmonary infection. The ability of the pathogenic aspergilli to grow at 37°C is clearly another factor that is important for human disease. *A. fumigatus* can bind to various molecules, including laminin, fibrinogen, complement, and lactoferrin. This binding may be important in allowing adherence to epithelial surfaces and possibly endothelial surfaces.

As a eucaryotic organism that thrives on dead organic matter, *Aspergillus* spp. produce a large number of extracellular products. Various proteolytic factors have been described, in particular, elastase, which are of dubious importance as a pathogenicity factors. Other proteins, which may be toxic to the mammalian cells include restrictocin, fumigatoxin, and various cellular lytic enzymes, may

or may not contribute to pathogenicity. A poorly characterized lipid component produced by *A. fumigatus* inhibits the alternative pathways of complement, but its role in pathogenicity is not yet fully evaluated. A number of other products may also be important, such as gliotoxin, which causes apoptosis and impairs macrophage and neutrophil function, and mannitol, which acts as a hydroxyl radical scavenger. Much work remains to be done to establish which, if any, of these factors are or are not relevant to disease. There are probably other factors of importance yet to be identified.

INCIDENCE OF INVASIVE ASPERGILLOSIS

The incidence of invasive aspergillosis is increasing substantially in the Western world. A multicentre autopsy study of cancer patients from 12 centers in North America and Japan from 1980 to 1988 demonstrated that 30% of all fungal infections were due to *Aspergillus* (5). A large study of autopsies in two Frankfurt hospitals from 1978 to 1992 showed an increase of all mycoses at autopsy from 1.5% to 6% and a proportional increase due to *Aspergillus* from 17% to 60% (6).

The range of incidence of invasive aspergillosis in different host groups is substantial as shown in Table 14.2.

RISK FACTORS

Asthma and cystic fibrosis appear to be the only risk factors for allergic bronchopulmonary aspergillosis. Only cavitary lung disease is important for

Table 14.2. Incidence of Invasive Aspergillosis in Different Host Groups

	Range (%)
Allogeneic/autologous BMT	0.5–9
Acute leukemia	5–24
AIDS	0–12
Liver transplantation	1.5–10
Heart and renal transplantation	0.5–10
Heart and lung or lung transplantation	19–26[a]
Chronic granulomatous disease	25–40[b]

[a] Both colonization and disease.
[b] Lifetime incidence.
SOURCE: Denning DW. *Aspergillus*, aspergilloma and invasive aspergillosis. In: Mitchell TG, Cutler JE, Deepe GS, Hazen KC. Principles of Medical Mycology, 1st Edition. American Society for Microbiology, Washington DC 1996. In press.

pulmonary aspergilloma, and local anatomic features appear to be most important for saprophytic *Aspergillus* sinusitis.

The key risk factors for invasive aspergillosis in decreasing order of importance are profound neutropenia ($<100 \times 10^6$/l), prolonged neutropenia (7), neutrophil function deficits (8) (usually combined with macrophage or other cellular immune deficits) as in chronic granulomatous disease and AIDS, supraphysiologic corticosteroid therapy, graft-versus-host disease (9,10), and/or rejection in transplantation (11) (which may or may not be an independent variable as it reflects additional immunosuppression), and probably CMV disease and advanced human immunodeficiency virus infection (12) (Table 14.3). Less important risk factors appear to be diabetes mellitus, influenza, alcohol excess, prematurity, and exposure to *Aspergillus* in large quantities, (e.g., marijuana smoking or living in a rural or farm environment). Ambulatory outpatients with chronic respiratory disease treated with corticosteroids are also at higher than average risk of invasive aspergillosis but probably not at great risk. Particular groups of patients colonized with *Aspergillus* are at increased risk; this particularly includes patients with nasal colonization before chemotherapy for acute leukemia (12) and colonization of the tracheobronchial tree before lung transplantation.

There are other factors that probably do not contribute to disease. These include antibiotic therapy, cyclosporin therapy (which probably reduces risk by acting as a corticosteroid sparing agent), and use of corticosteroid aerosols in patients with respiratory disease.

Corticosteroid therapy is a risk factor by virtue of its effect to reduce pulmonary macrophage killing of *Aspergillus* conidia and neutrophil killing of *Aspergillus* hyphae (13). In addition, corticosteroids directly increase the growth rate of *A. fumigatus* and *A. flavus*. Under the influence of hydrocortisone, *Aspergillus* hyphae extend at the astonishing rate of 1 to 2cm/h (14). The detrimental effect of corticosteroids on neutrophil function can be reversed in vitro by granulocyte colony stimulating factor and gamma interferon (15).

Table 14.3. Major Risk Factors for Invasive Aspergillosis*

Neutropenia, <500 and especially $<100 \times 10^6$/l
Prolonged neutropenia, e.g., >12 days
Neutrophil ± other cellular immune deficits
Corticosteroid therapy*
Graft-versus-host disease after bone marrow transplantation*
Acute rejection after solid organ transplants*
Cytomegalovirus disease after transplantation
Advanced AIDS

*These factors are probably not independent of each other.

CLINICAL FEATURES OF INVASIVE ASPERGILLOSIS

Pulmonary Aspergillosis

At least 25% to 33% of patients initially have no symptoms attributable to invasive pulmonary aspergillosis. As the disease progresses, symptoms appear but this may be close to the time of death. Early symptoms are cough, usually dry, and fever. In corticosteroid-treated patients, fever is often absent. Chest pain is common and may be pleuritic or more commonly is mild and nonspecific. 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 pulmonary embolism. In neutropenic patients, pneumothorax is an occasional presenting feature and sharp chest pain with dyspnoea is typical (16).

In chronic granulomatous disease, acquired immunodeficiency syndrome (AIDS), and other less immunocompromised patients, a more indolent course is typical and local extension of disease into the chest wall, brachial plexus, or vertebral column is occasionally seen.

Apart from a raised respiratory rate and a fever, there are usually no signs attributable to invasive aspergillosis. Auscultation of the chest is usually unrewarding. A pleural rub is sometimes heard.

In patients with diffuse disease, hypoxemia and hypocapnea is usually present. White cell counts are usually normal as is plasma biochemistry. A raised bilirubin and lactate dehydrogenase are occasionally seen but are nonspecific. If the disease is disseminated, coagulation defects are seen that include those typical of disseminated intravascular coagulation.

In the lung, the radiographic appearances of invasive aspergillosis are extremely heterogenous (17). Nodular shadows (Figures 14.1, A and B), with and without cavitation, thin-walled cavities (in AIDS and chronic necrotizing pulmonary aspergillosis) and "alveolar" consolidation that coalesces over time to form small nodules are typical. Perhaps the most distinctive appearance of invasive aspergillosis, aside from cavitation, is the pleural-based wedge-shaped lesion, but these are uncommonly seen on plain radiographs and may be late manifestations. More commonly, diffuse, usually lower lobe, fine shadowing is seen (Figure 14.2). Pleural effusions are rare. In the context of neutropenia, a spontaneous pneumothorax is also highly suggestive of invasive aspergillosis or zygomycosis.

Early in the course of progressive invasive pulmonary aspergillosis, plain chest radiographs are falsely negative, and thus high quality computed tomography (CT) scans of the chest can play a major role in early diagnosis (18). The following comments apply mostly to hematology patients. The most distinctive early lesions on CT scan are small nodules and small pleural based lesions with straight edges. There may be only one lesion, but often there are several. The "halo" sign is common (low attenuation surrounding the lesion) (Figure 14.3). As the disease progresses, the nodules may cavitate (often as the neutrophil count recovers), revealing the "air crescent" sign (19) (Figure 14.4).

FIGURE 14.1A

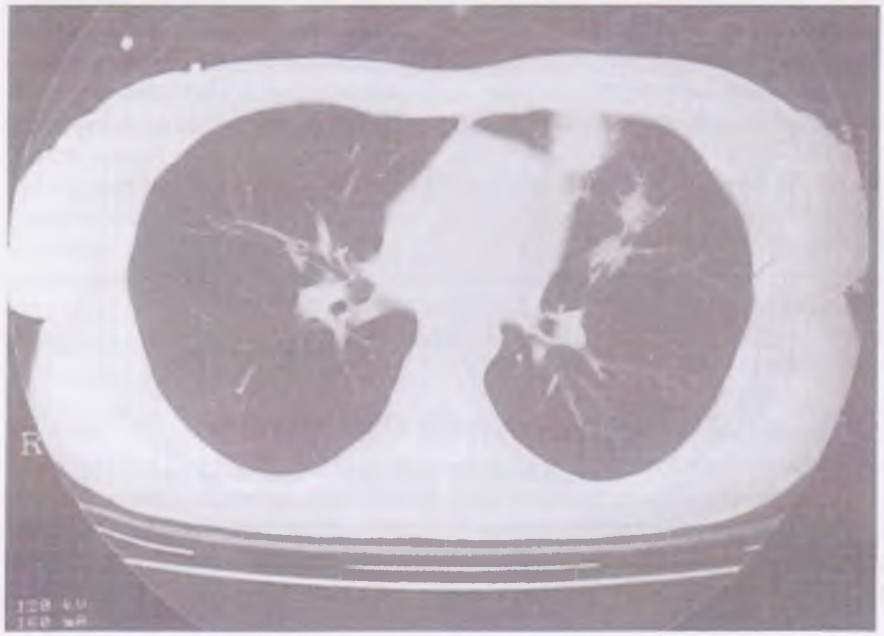

FIGURE 14.1B

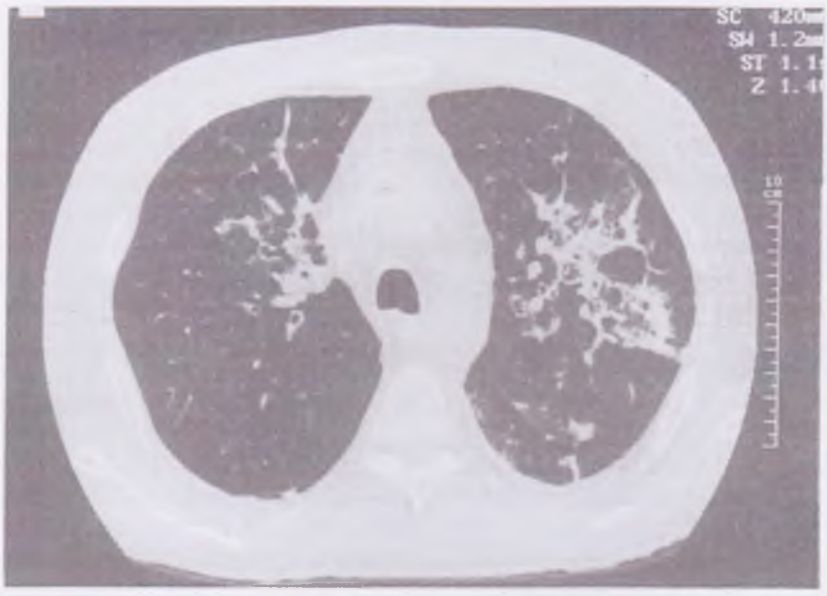

Figure 14.2. CT scan appearance of the midzones of an allogeneic BMT patient with diffuse pulmonary aspergillosis.

These lesions are highly distinctive for invasive fungal disease of the lung and are usually due to *Aspergillus* but may occasionally be due to a Mucorales, *Trichosporon*, *Blastoschizomyces*, or *Fusarium*. Pathoradiologic correlative studies have conclusively shown these lesions to represent infarcted lung tissue full of hyphae (20).

Aside from the halo and air crescent signs, consolidation is frequently seen. This is usually initially pleural based, but extensive disease can involve whole lobes. An early characteristic feature is a sharp demarcation line against the pleura, be it on the chest wall or one of the fissures. Sometimes these lesions are complicated by large or small pneumothoraces.

In solid organ transplant patients, both nodular and diffuse disease is seen. The major differential diagnoses of nodular disease in these patients are nocardial infection or lymphoma (21). There is a wider differential diagnosis for diffuse disease including *Pneumocystis carinii* pneumonia, strongyloidiasis, bacterial, or cytomegalovirus pneumonia if early after transplantation and rejec-

Figure 14.1. (A) An example of a focal lesion in the midzone of a neutropenic leukemic patient. (B) The CT scan appearance of the same patient demonstrating three lesions in the same vicinity that are superimposed on each other on the chest radiograph.

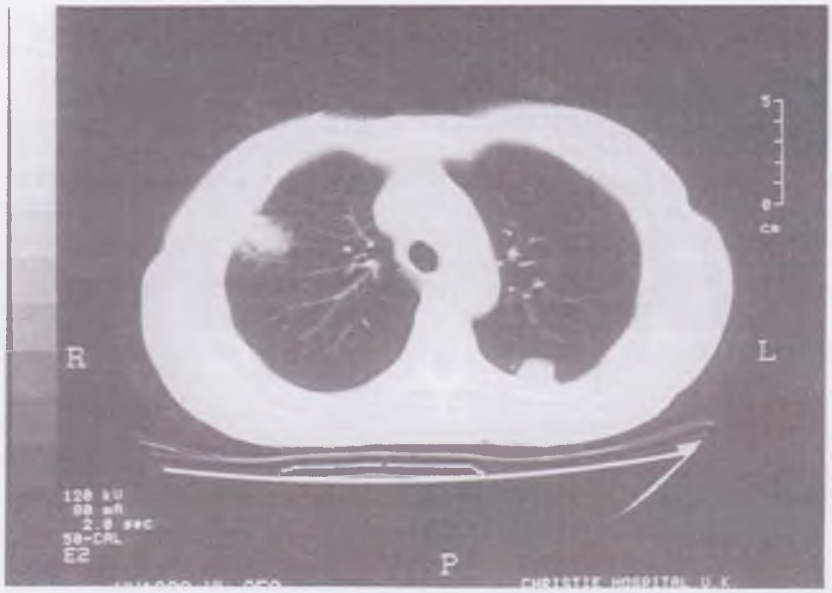

Figure 14.3. CT scan appearance of a neutropenia leukaemic patient showing a focal lesion with characteristic lower attenuation surrounding area (the "halo" sign).

tion in lung transplant recipients. The appearances of invasive aspergillosis in diffuse disease are rarely distinctive enough to obviate the need for diagnostic studies. Sometimes mixed appearances in invasive aspergillosis are seen, for example, pleural-based consolidation in one area with diffuse disease elsewhere. In the first 1 to 2 weeks after heart, lung, or liver transplantation, interpretation of radiologic abnormalities may also be confounded by postsurgical changes.

In patients with chronic granulomatous disease, AIDS, or on corticosteroid therapy, pulmonary lesions alone may be present or there may be extension into local structures such as rib. Thin- or thick-walled cavitary lesions are relatively frequent in AIDS patients with invasive aspergillosis (17,22). In addition, direct local extension from the airways into surrounding lung parenchyma, in a multifocal fashion, has also been described in AIDS (23).

Aspergillus Tracheobronchitis

Aspergillus airway disease ranges from a relatively mild tracheobronchitis with excess mucus production and inflammation to pseudomembraneous *Aspergillus* tracheobronchitis in which a shaggy greyish lining of the whole trachea and bronchial wall is apparent at autopsy (24, 25). Intermediate forms include those with ulcers of or plaques on the bronchial lining. Some 80% of the

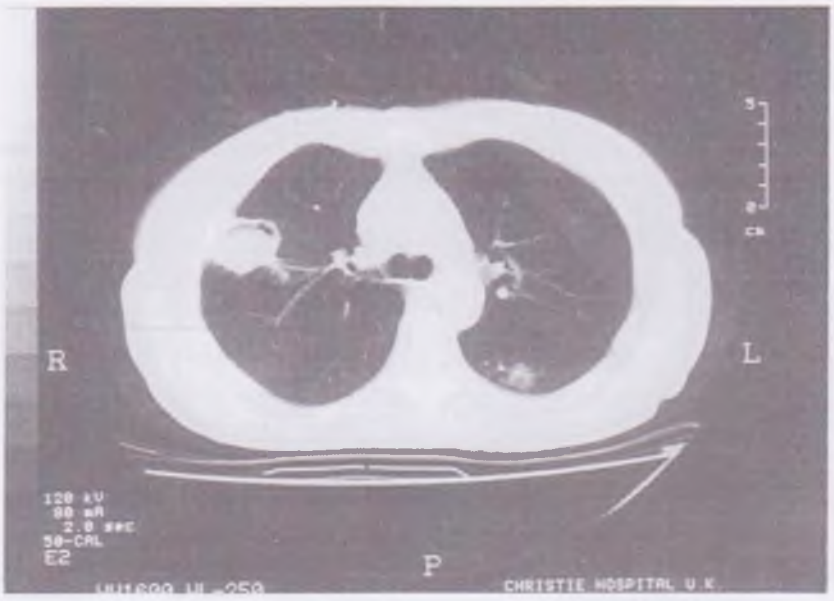

Figure 14.4. CT scan appearance of a neutropenic leukemic patient showing two lesions. The one on the right side exhibits the characteristic "air crescent" sign. It is a pleural-based lesion with at least two sharp borders, both features typical of invasive pulmonary aspergillosis. The other smaller lesion on the left posteriorly demonstrates the "halo sign".

patients are symptomatic, although symptoms may be mild. Symptoms include cough, fever and dyspnea, chest pain, and hemoptysis (25). At least 20% of patients are asymptomatic or, if symptomatic, other causes for these symptoms were identifiable (e.g., lung rejection). Almost all patients had a normal chest radiograph early in the course of disease. As the disease progresses, symptoms become more common and more severe. Those with very extensive involvement (pseudomembranous *Aspergillus* tracheobronchitis) may develop a unilateral monophonic wheeze or stridor reflecting obstruction of the lumen of the airway with necrotic material. Many patients die of respiratory insufficiency as a result of occlusion of the airway. Others develop disseminated disease in the last few days of life. Occasionally, perforation of the trachea or a bronchus occurs. About 60% of patients with tracheobronchitis have a positive respiratory culture for *Aspergillus* before death.

Bronchoscopy is essential for diagnosis (see below).

Acute Invasive *Aspergillus* Rhinosinusitis

Acute invasive *Aspergillus* rhinosinusitis occurs almost exclusively in leukemia patients after cytotoxic chemotherapy (26,27), bone marrow transplant recipi-

ents (28), in aplastic anemia, or in AIDS (9). Invasive *Aspergillus* sinusitis appears to be extremely uncommon in solid organ transplant patients.

The clinical features of acute invasive *Aspergillus* rhinosinusitis during neutropenia are variable but include fever, cough, epistaxis, headache, nasal discharge, sinus or eye pain, nasal congestion, or toothache. Early symptoms are nonspecific and easily mistaken for possible bacterial infection. Examination of the nose will show crusting of the nasal mucosa in about half the patients and a nasal or oral ulcer in about a third and either hyperemic, necrotic, or dusky nasal mucosa in 15% to 20%. About a third of the patients have sinus, facial, or nasal tenderness.

Plain radiology of the sinus is often unrevealing or merely shows opacification of the sinus. CT scanning is an essential tool to evaluate the extent of the disease and usually shows bony invasion in more advanced cases. Extension of injection from the sinuses through the roof of the mouth or into the orbit and brain is frequent.

Cerebral Aspergillosis

Cerebral aspergillosis occurs in 10% to 20% of all cases of invasive aspergillosis but is particularly common in disseminated aspergillosis. Allogeneic BMT and liver transplant recipients in particular are at high risk of dissemination (29). As with pulmonary aspergillosis, the clinical presentation and pace of progression of cerebral aspergillosis varies remarkably with the host group. Alteration in mental status is frequent (90%) as are seizures (50%) (30). Only 30% have focal neurologic deficit and less than a quarter have headache or meningeal signs. In granulocytopenic patients, focal neurologic features and seizures are more frequent. Fever may be present, but other infections are usually coexistent, including CMV disease, peritonitis, or pneumonia.

The majority of patients with cerebral aspergillosis has disease elsewhere, usually in the lungs, which implies hematogenous spread, but occasionally in the ear or sinuses.

Differential diagnoses depend on the host group. In BMT patients with a brain abscess visualized on CT scan, *Aspergillus* accounts for about half the cases and *Candida* a quarter; bacterial causes were seen in only 10% (31). In liver transplant recipients, *Aspergillus* is the most common infectious cause of brain abscess, but cerebral infarction and hemorrhage are more common (29). In other solid organ transplant recipients, cerebral aspergillosis and nocardiosis are seen with approximately equal frequency with toxoplasmosis, cryptococcosis, and lymphoma being less frequent. In AIDS patients, cerebral toxoplasmosis is a much more common cause of cerebral abscess as is lymphoma with cerebral aspergillosis being only rarely identified before death. Unfortunately, cerebrospinal fluid examination is usually abnormal but is not specific (30).

In highly immunocompromised patients, the CT appearances of cerebral

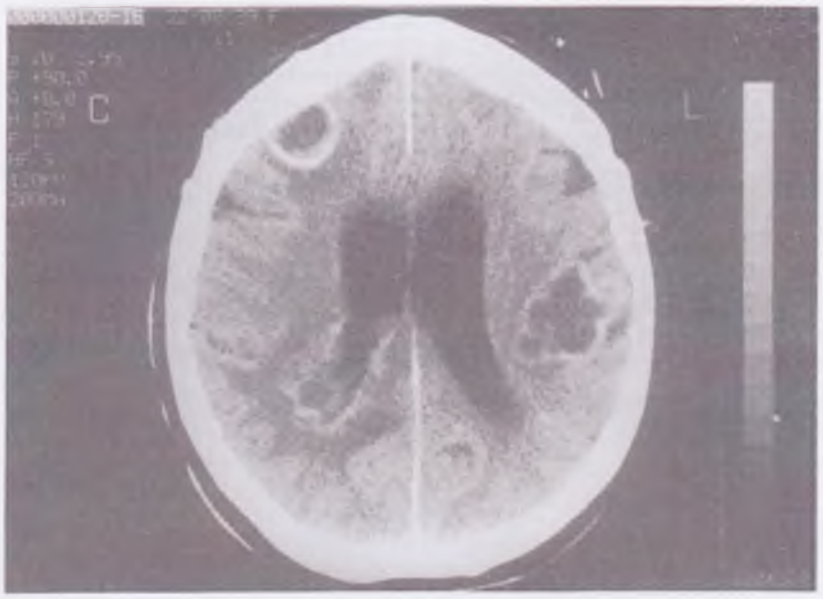

Figure 14.5. The typical appearance of multiple cerebral abscesses due to *A. fumigatus* in a renal transplant recipient. In an AIDS patient, such lesions would much more likely represent toxoplasmosis, which is also a significant differential diagnosis in the transplant population. Other filamentous fungi such as *Pseudallescheria boydii* would be a consideration in the BMT, neutropenic, and transplant patient.

aspergillosis are hypodense well-demarcated lesions (Figure 14.5). Hemorrhage and mass effect are unusual. Sometimes, clearer definition of the hypodense lesion is obtained with intravenous contrast. Magnetic resonance (MR) scans often reveal additional lesions but without distinctive features. The location of lesions are usually deep in the brain and difficult to access surgically.

DIAGNOSTIC PROCEDURES

In highly immunocompromised patients, particularly neutropenic patients, the CT scan of the chest should be the first definitive investigation when the suspicion of invasive pulmonary aspergillosis has been raised. A simple algorithm indicates the reasoning behind this (Figure 14.6). Some radiographic lesions are virtually diagnostic in this context. For less immediately diagnostic features, the optimal diagnostic (and therapeutic) approach can then be taken.

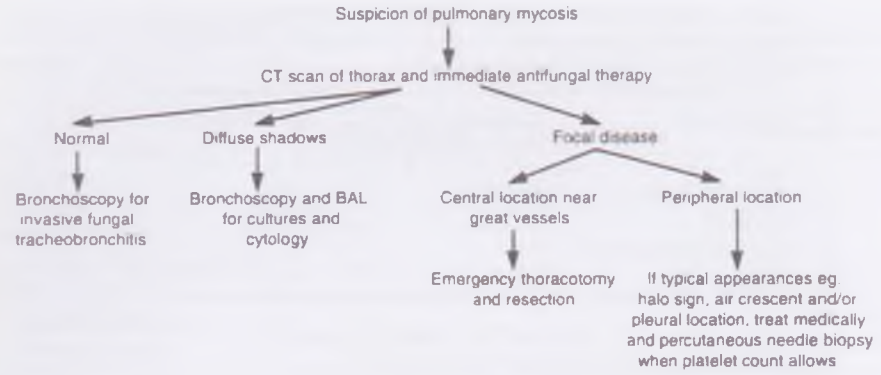

Figure 14.6. Management algorithm on suspicion of pulmonary mycosis.

Culture

The significance of *Aspergillus* cultured from sputum can be difficult to assess (32). A positive culture of *Aspergillus* from the sputum of healthy people occurs in 1% to 16%. Chronic lung disease increases the frequency of colonisation. In one British study, *Aspergillus* species were cultured from sputum, for example, 7% of 2080 hospitalized patients with various chest diseases and 57% in a group of patients with cystic fibrosis. Increased rates are also seen in those living in rural settings.

In most patients with *Aspergillus* in the sputum, no disease is present. However, a positive sputum culture in an immunocompromised patient is highly significant: all 17 leukemia patients with positive sputum cultures were subsequently shown to have invasive aspergillosis by lung biopsy. Thus, the positive predictive value for sputum culture is high, but unfortunately the negative predictive value is low in most immunocompromised patients with features suggestive of invasive aspergillosis (Table 14.4). Similar studies in other solid organ transplant patients other than liver transplant recipients are needed. In solid organ transplant patients, *Aspergillus* tracheobronchitis has been described with no symptoms and normal chest x-ray, and thus a positive *Aspergillus* culture should prompt immediate bronchoscopy. Invasive aspergillosis occurs in about 4% of patients with AIDS, but recovery of *Aspergillus* from respiratory sites in such patients may reflect colonization.

Studies examining the sensitivity and specificity of combinations of positive findings in immunocompromised patients, for example, a positive respiratory culture *and* an abnormal chest radiograph, are urgently needed.

Positive cultures of nasal swabs for *Aspergillus* are uncommon but useful in leukemia. Isolation of *Aspergillus* from the nose has a 90% correlation with invasive pulmonary aspergillosis in leukemia. A positive nasal culture before

Table 14.4. Respiratory Tract Cultures Positive for *Aspergillus* in Patients with Invasive Pulmonary Aspergillosis

Host Group	*Frequency of Positivity in IPA (%)*	*Specificity (%)*
Acute leukemia	8–34	>95
No BMT	25–38	70–95
Liver transplantation	≈80	70
AIDS	≈33%	10–20

SOURCE: Barnes AJ, Denning DW. *Aspergillus*—significance as a pathogen. Rev Med Microbiol 1993;4:176–180.

chemotherapy for leukaemia carries a high risk of invasive pulmonary aspergillosis (12). However surveillance swabs have a low yield. A positive nasal swab for *Aspergillus* in a context other than leukemia may or may not be significant unless there is overt sinus or nasal disease.

Aspergillus may be isolated in blood cultures and may represent genuine *Aspergillus* fungemia (33) or *Aspergillus* pseudofungemia. Genuine *Aspergillus* fungemia usually occurs in typical at-risk patients or patients who have received prosthetic heart valves. Many different culture systems have yielded *Aspergillus*. In a recent series of fungemia in leukemia, *Aspergillus* fungemia comprised 8% of all cases (34). *Aspergillus* pseudofungemia is more likely if the patient is not in a typical host group and/or the clinical course is not compatible. In most retrospective analyses, a laboratory source for the contamination is identified, and this has often followed a change in the usual blood culture handling procedure.

Bronchoscopy

Fibreoptic bronchoscopy is now an essential component of the diagnostic approach for pulmonary aspergillosis. It may diagnose *aspergillus* airways disease or diffuse (e.g., nonfocal) invasive pulmonary aspergillosis (Table 14.5). In addition to diagnosing aspergillosis, other important diagnoses can be made such as cytomegalovirus pneumonitis or *P. carinii* pneumonia. In the context of leukemia and bone marrow transplantation, the most useful procedure is bronchoalveolar lavage (BAL) or a bronchial wash (9,10,35–38). Samples should be processed for both culture and microscopy (or cytology). Transbronchial biopsy is simple and extremely productive if airways disease is present but has limited value for invasive pulmonary aspergillosis. Brushings contribute little to diagnosis above BAL and/or biopsy.

The positive yield for *Aspergillus* varies depending on the pattern of disease

Table 14.5. BAL for Diagnosis of Invasive Pulmonary Aspergillosis

Patients	*% Positive Result in All Those with Definitive or Probable Aspergillosis*		
	BAL culture	*BAL cytology*	*Either or both*
Leukemia	ND	ND	50
Leukemia	23	53	59
Acute leukemia	0	0	0
Leukemia, BMT, oncology	40	64	67
BMT			
Localized disease	0	0	0
Diffuse disease	100	?	100

ND, not done.
SOURCE: Reference 10,35–38.

and the host group. In leukemia and bone marrow transplantation, cytology and culture of BAL fluid will yield the diagnosis in 30% to 67% of patients (see Table 14.5). In solid organ transplant patients, the yield is probably lower, except in lung transplant patients in whom airways disease is probably more frequent (24). Diffuse disease radiologically is more likely to yield a positive result than focal disease (9). The utility of BAL for diagnosing invasive aspergillosis in AIDS is unclear.

Transbronchial biopsy is often falsely negative in cases of invasive pulmonary aspergillosis. This may be due to sampling error and in particular the depth of the biopsy. For example, in three series of AIDS patients with invasive aspergillosis that included 18 patients, transbronchial biopsy established the diagnosis in only 4; the others were confirmed by other means, usually autopsy.

Bronchoscopy with bronchial biopsy and culture is the only means of making the diagnosis of *Aspergillus* airways disease antemortem. Biopsy of loose material in the tracheal lumen, plaques, or ulcers will often reveal necrotic cartilage invaded by hyphae.

Percutaneous Lung Biopsy

Percutaneous lung biopsy or aspiration is best done with a 16–18 gauge needle; fine needle aspiration is less likely to be diagnostically useful. Percutaneous needle biopsy is indicated in patients with focal disease, particularly in the periphery of the lung, because these lesions are not accessible to the bronchoscope and BAL is often negative. The platelet count should be above 30 $\times 10^9$/l or platelet transfusions should be given. Significant bullous or emphysematous lung disease in the area of interest is a contraindication. Specimens should be processed for culture and cytology or histology. The yield with respect to *Aspergillus* has not been accurately assessed in prospective studies,

but it yielded the diagnosis in five AIDS patients with invasive aspergillosis in whom BAL was negative (22). It is also helpful in establishing the diagnosis of nocardiosis, lymphoma, *Pseudomonas*, and other rare lung mycoses. The incidence of radiologically apparent pneumothorax after percutaneous biopsy is about 10%, but only 2% of patients require a chest drain if the procedure is carefully done.

Thoracoscopy

Another approach used little to date has been thoracoscopy. This involves inserting a rigid fibreoptic instrument in the pleural cavity to visualize and biopsy any pleural or pulmonary lesions. As pleural localization is common in invasive aspergillosis, this approach is likely to be highly successful if the patient is not hypoxic or thrombocytopenic.

Open Lung Biopsy

Open lung biopsy is less frequently used to establish the diagnosis of invasive pulmonary aspergillosis, except when the diagnosis is thought to be a malignancy or other infection such as *Pneumocystis*. Studies of the usefulness of open lung biopsy for the diagnosis of invasive aspergillosis have not been done, and its role in the diagnostic cascade is presently unclear. However, when positive, it yields unequivocal evidence of invasive aspergillosis.

Serology

Aspergillus antibody is rarely detectable at the time of diagnosis of invasive aspergillosis during neutropenia but may be positive in patients after solid organ transplantation, depending on the test used. Data on the frequency of antibody positivity in other host groups, such as chronic granulomatous disease or AIDS, is lacking. The antibody response to *Aspergillus* infection has been well characterized in allergic bronchopulmonary aspergillosis and aspergilloma. More than 95% of patients with an aspergilloma have detectable precipitating immunoglobulin (Ig)G antibodies. Some have IgM antibodies as well. Successful surgical removal of an aspergilloma will result in a fall of *Aspergillus* antibody to low or undetectable levels subsequently.

Several antigen tests have been developed using galactomannan, complex protein antigens, and heat shock proteins. Only in leukemia and bone marrow transplant patients has any antigen test system been carefully examined. Reproducibility of the galactomannan latex test in serum is less than ideal, and sensitivity is around 30%. Several samples may have to be tested before positive results are obtained because antigenemia is often shortlived. Antigen positivity increases closer to time of death with progressive invasive aspergillosis. Unfortunately, many studies on antigen detection for invasive aspergillosis have not accurately described the temporal relationship of a positive antigen test and the

Table 14.6. *Aspergillus* Serology: Key Points

- Antibody is almost always detectable in cases of aspergilloma and allergic bronchopulmonary aspergillosis.
- Antibody is occasionally positive in leukemia patients with invasive aspergillosis.
- Studies of the utility of antibody for diagnosis of invasive aspergillosis in AIDS and transplant recipients have not been done.
- Antigen is detectable in about 30% of leukemic patients and or bone marrow transplant patients with invasive aspergillosis at suspicion of diagnosis.
- Multiple antigen tests necessary during neutropenia to detect positives.

early symptoms of invasive aspergillosis and initiation of treatment. The present position regarding serology for aspergillosis is shown in Table 14.6.

Sinus Aspergillosis

Invasive *Aspergillus* sinusitis requires a biopsy for diagnosis. Usually, part of the nose is affected in addition to the sinuses, and this is accessible externally. Cultures of any nasal discharge or of any crusted lesions should also be taken.

Cerebral Aspergillosis

Cerebral aspergillosis is usually confirmed at death. Aspiration of a suspicious lesion in the brain will usually yield *Aspergillus* on culture or the presence of hyphae on histologic examination. Unfortunately, histologic appearances alone may not be distinctive enough to diagnose *Aspergillus* as the hyphae are similar to those of many other pathogens, including *Pseudallescheria boydii*.

TREATMENT

Invasive aspergillosis carries a nearly 100% mortality if untreated. The only exception are patients whose immunocompromising factors are removed (e.g., permanent cessation of corticosteroids).

The pace of progression of invasive aspergillosis varies widely. In patients such as liver and bone marrow transplant recipients and patients with profound neutropenia, the course of disease from first clinical or radiologic abnormality to death is typically 10 to 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 have been missed if treatment is not started early, with fatal consequences for the patient. There is therefore room for discussion about exactly when treatment should be initiated for invasive aspergillosis.

Table 14.7 shows appropriate criteria for the initiation of therapy in the

Table 14.7. Criteria for Initiation of Therapy for Invasive Aspergillosis or Increasing the Dose of Amphotericin B to ≥1 mg/kg/d in Neutropenia (<1000 × 10^6/l) Including Aplastic Anemia

1. Isolation of *Aspergillus* from any site including nose swab, blood, BAL etc.
2. New pulmonary infiltrates on chest x-ray.
3. Infiltrates on CT scan of chest showing characteristic features, e.g.,
   a. Halo/crescent sign
   b. Pleural based sharply angulated lesion
   c. Pleural based lesion with pneumothorax
4. Persistent fevers (>7 days) with any localizing clinical features and not responding to antibiotics, e.g.,
   a. Chest pain
   b. Dry cough
   c. Facial/sinus pain
   d. Epistaxis
   e. Hoarseness
   f. New skin lesions consistent with aspergillosis
5. Any sudden intracranial event including stroke or fit with or without fever

Table 14.8. Criteria for Initiation of Therapy for Invasive Aspergillosis in Solid Organ Transplantation or Allogeneic Bone Marrow Transplantation

1. Radiologic infiltrate with isolation of *Aspergillus* from respiratory secretions, BAL, or blood.
2. Evidence of ulcers/bronchitis on bronchoscopy with hyphae visualized in BAL.
3. Histologic or cytologic evidence of hyphae from any tissue.
4. Any sudden intracranial event, including stroke or fit with or without fever.

neutropenic patient (39). In many such patients, empiric amphotericin B therapy will have been given because of persistent fever despite after 3 to 7 days of broad-spectrum antibiotics. If a dose of less than 1 mg/kg/d of conventional amphotericin B has been used for empiric therapy then the criteria given will constitute a reason to increase the dose to 1 to 1.25 mg/kg/d. If, however, empiric amphotericin B therapy is started at 1 mg/kg/d, as is more frequent these days, then a decision must be made about whether to switch therapy from this treatment to alternative therapies using the criteria stated in Table 14.7.

In solid organ or allogeneic bone marrow transplantation, the criteria for initiation of therapy are slightly different than they are during neutropenia (Table 14.8) (39). This is because there is a higher frequency of *Aspergillus* tracheobronchitis in this population and also a much wider differential diagnosis of cases of pneumonitis. If it occurs months after transplantation, the presentation is more likely to be of nodular disease that may represent *Aspergillus*,

nocardia, or development of lymphoma (21). Immediately after liver transplantation, interpretation of the chest x-ray is difficult because of changes after surgery. In addition, solid organ transplantation patients are more likely to get *Aspergillus* in operative sites (e.g., mediastinitis, wound drainage, wound infection), which are problems that do not occur in the leukemic population. After allogeneic bone marrow transplantation, invasive aspergillosis occurs during or immediately after the short period of aplasia or more often with graft-versus-host disease, weeks or months after transplantation. Progression of disease in these patients may be extremely rapid.

In the context of AIDS, invasive aspergillosis tends to be a relatively subacute disease, and there is usually time to make a positive diagnosis without having to embark upon empiric therapy (Table 14.9) (39). The major exception to this is intracranial disease. Many patients and their physicians are not willing for the patient to undergo a brain biopsy or aspiration and therefore empirical therapy may be appropriate if there is a single lesion or small number of lesions not responding to anti-*Toxoplasma* therapy.

The standard therapeutic agent for the treatment of invasive aspergillosis is conventional amphotericin B (Fungizone). The success rate of amphotericin B therapy varies substantially between different host groups. There are two major considerations in the initial choice of therapy: Can the patient take oral medication and is he/she likely to absorb it? Is the patient taking cyclosporin or is renal dysfunction already present? If the patient is able to take oral therapy and the patient is not taking drugs that induce the metabolism of P450 enzymes (e.g., rifampin, phenytoin, carbamazepine, phenobarbitone), then itraconazole would be a reasonable first choice (Table 14.10). Other factors that might reduce absorption and therefore efficacy include H2 blockers and patients with intestinal problems (such as graft-versus-host disease) and patients with AIDS. The dose of itraconazole should be 200 mg three times a day for 4 days followed by 200 mg twice daily. All doses should be taken with food or an acid drink. Itraconazole serum concentrations should be done after 5 to 10 days. Typical target concentrations of itraconazole should be at least 1 μg/mL if measured by HPLC and at least 5 μg/mL if measured by bioassay.

If the patient is not a suitable candidate for oral therapy as outlined above, intravenous therapy is necessary. Conventional amphotericin B should be used in a minimum dose of 0.8 to 1.0 mg/kg/d regardless of renal dysfunction,

Table 14.9. Criteria for Initiation of Therapy for Invasive Aspergillosis in AIDS

1. Respiratory symptoms, abnormal chest x-ray or bronchoscopy, and isolation of *Aspergillus* or visualization of hyphae in BAL.
2. Cerebral lesions unresponsive to therapy for toxoplasmosis or a single enhancing lesion consistent with aspergillosis.
3. Histologic or cytologic evidence of hyphae from any site (culture not obtained or pending).

except in neutropenic patients in whom 1 to 1.25 mg/kg/d is appropriate (see Table 14.10). If renal dysfunction is, or is likely to be, a major problem, then one of the new preparations of amphotericin B in doses of 4 to 5 mg/kg/d may be appropriate (see Table 14.10). These dosage levels should be continued for at least 2 weeks until a therapeutic response has been obtained.

The apparent severity of illness of the patient should not be a guide as to whether to give oral itraconazole or intravenous amphotericin B. Responses have been obtained in profoundly neutropenic patients with extensive disease using oral itraconazole alone, and amphotericin B therapy is often ineffective.

There has been much discussion in case reports about the relative role of adjunctive therapy with either rifampicin or flucytosine. The use of amphotericin B with flucytosine does seem to improve clinical response rates marginally (40) and may be appropriate for sites of disease in which amphotericin B may penetrate poorly (e.g., brain, meninges, heart valve, and eye). There is a potential for antagonism in vitro, and it would be wise to have the isolate checked for this, if possible. Flucytosine serum concentrations should always be checked in patients to prevent toxicity, especially if dual therapy is prolonged, given the frequency of renal impairment with amphotericin B. The use of flucytosine for invasive aspergillosis during neutropenia has shown benefit in one or two small series but has not met with universal acceptance partly because of concern about prolonging neutropenia or thrombocytopenia (unproven).

The addition of rifampicin to amphotericin B may be synergistic in vitro (40).

Table 14.10. First- and Second-Line Therapy for Invasive Aspergillosis*

Agent	*Initial Dose*	*Comments*
Amphotericin B (Fungizone)	0.8–1.25 mg/kg/d (IV)	Considered first-line therapy but high failure rate. Significant interaction with cyclosporin.
Itraconazole (Sporonox)	200 mg tds for 4 d; then 200 mg bd (PO)	Useful if patient eating and not on P450 inducers. Significant interaction with cyclosporin. Levels should be measured to ensure adequate absorption.
Amphotericin B colloidal dispersion (Amphocil)	4 mg/kg/d (IV)	Less toxicity than Fungizone.
Liposomal amphotericin B (AmBisome)	4–5 mg/kg/d (IV)	Less toxicity than Fungizone.

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

However, it is not appropriate in the context of transplantation because rifampicin induces the metabolism of many immunosuppressants and rejection or graft-versus-host disease may result (39). In addition, because rifampicin is a powerful inducer of P450 enzymes, its use (for more than 3 days) absolutely precludes subsequent use of itraconazole, which may be useful subsequently in patients failing amphotericin B or for continued therapy after amphotericin B (39).

There has also been debate about whether combinations of amphotericin B and itraconazole are appropriate. There are theoretical reasons for considering antagonism because itraconazole inhibits the production of ergosterol and the mode of action of amphotericin B requires binding to ergosterol. There is some limited animal model work (some unpublished by the author) suggesting that antagonism may be a real phenomenon, although the magnitude of this effect is difficult to ascertain. The present recommendations are that it is better to treat with one or other drug alone. If itraconazole is the desired agent but there is concern about its absorption, then initial use of both agents until itraconazole levels have been obtained and absorption confirmed is a reasonable therapeutic strategy.

Switching Therapy

Unfortunately, a substantial proportion of patients fail the first therapy selected for them. In patients with rapidly progressive disease, failure of the first regimen means a fatal outcome, and therefore a good initial choice for these patients is critical. However, for patients with more slowly progressive disease, the evaluation of response after 10 to 20 days of therapy can be made using clinical, radiologic and other criteria to decide whether they are or are not improving and whether therapy should or should not be changed. The less immunocompromised the patient, the longer it takes to make an evaluation of response. Many patients will respond to alternative therapy if they fail the primary therapy, and therefore all patients should be offered more than one form of therapy if their response is suboptimal.

Many patients and doctors prefer to switch from initial intravenous amphotericin B as primary therapy to oral itraconazole as follow up therapy and maintenance therapy. This is reasonable strategy if the patient has no problems with absorbing itraconazole.

Duration of Therapy

Patients should be treated for as long as they are immunocompromised and until there has been either complete or near complete resolution of disease. In the neutropenic patient, this certainly means treatment until the neutrophil count is above $1000 \times 10^6/l$. If at that time there is residual evidence of disease, treatment should be continued. Eradication of disease may require surgery. In

patients whose immune status improves gradually over a long period of time (e.g., a solid organ transplant patient treated for many months), therapy should be continued until there is a complete clinical response. AIDS patients and patients who have received an allogeneic bone marrow transplant should probably receive treatment for years if they can tolerate and absorb itraconazole. If they cannot, long-term intermittent amphotericin B would be their only alternative (apart from surgical resection).

Surgical Excision

In invasive pulmonary aspergillosis, surgery has several roles (40). One group has advocated immediate surgical resection of one or more localized *Aspergillus* lesions in the lung during neutropenia. Most clinicians reserve lung resection for patients with persisting lung shadows who must undergo subsequent bone marrow transplantation or more aggressive chemotherapy, those with significant haemoptysis (19), or those with lesions impinging on the great vessels or major airways. This last point has been only recently emphasized. Erosion of invasive aspergillosis into the pulmonary vessels is not rare and is a rapidly fatal complication that can be averted by surgery.

In patients with invasive sinus aspergillosis, a biopsy of the affected area is necessary for diagnosis. Surgical debridement is useful for therapy but should not be done during neutropenia as hemorrhage and other operative complications are frequent (40).

With respect to cerebral aspergillosis, resection of the lesions is often impossible without causing untoward cerebral damage because most of the lesions are deep in the brain. For there patients, surgery has no role other than diagnostic aspiration or biopsy.

Surgery is an essential part of the management of patients with *Aspergillus* endophthalmitis, endocarditis, and probably osteomyelitis.

OUTCOME

The attributable mortality of invasive aspergillosis is from 50% to 100% in collected series (40). There are substantial variations from host group to host group and within each host group. In leukemia, achieving complete remission is critical to survival (41). Cerebral aspergillosis still has a mortality exceeding 95% in the immunocompromised patient. Many of the factors influencing the outcome of invasive aspergillosis are shown in Table 14.11.

Clearly, new agents for the treatment of invasive aspergillosis are urgently required. The role of G-CSF and other cytokines as adjunctive therapy is unclear at present. The efficacy of the primary therapeutic choice is going to be critical for improving mortality rates.

Table 14.11. Factors Influencing Outcome in Invasive Aspergillosis

Better Outcome	*Poor Outcome*
Host factors	
Resolution of neutropenia	Persistent neutropenia
Leukemia in remission	Leukemic relapse
Heart or kidney transplantation	Bone marrow transplantation
	Liver transplantation
	AIDS
Organ involvement	
Focal pulmonary disease	Diffuse pulmonary disease
	Cerebral aspergillosis
Therapeutic issues	
Early initiation of therapy	Delayed diagnosis/therapy
Switch of therapy if initial choice unsuccessful	
Appropriate surgery	

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