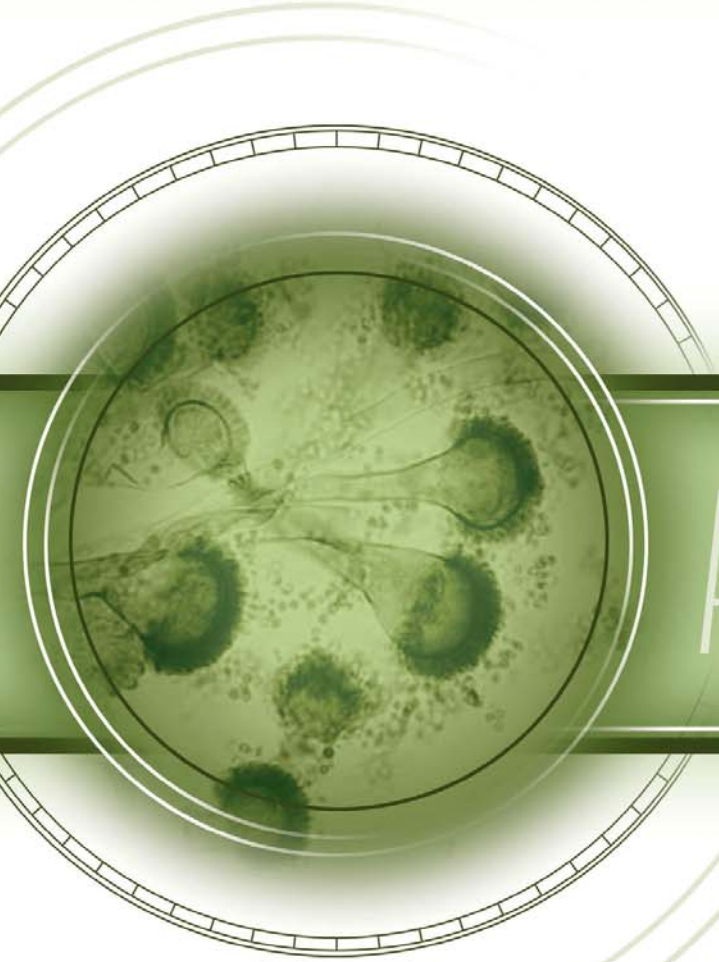

ASPERGILLOSIS

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KEY POINTS ABOUT ASPERGILLOSIS

- *Aspergillus* is a fungus that is found in every region of the world (ubiquitous) and causes a wide range of disorders, including pulmonary and nasal allergy, chronic pulmonary infection, and invasive disease
- *Aspergillus* is most commonly found in decaying vegetation but is also found in soil, water, food, and air
- Pulmonary disorders, corticosteroid use and diabetes mellitus are the top 3 conditions placing the patient at increased risk for colonization with *Aspergillus*
- The clinical importance of invasive *Aspergillus* infections has increased as the number of immunocompromised patients has risen
- The mortality rate of untreated invasive aspergillosis is nearly 100%, and invasive aspergillosis accounts for up to 7% of deaths in European teaching hospitals
- *A fumigatus*, the most pathogenic species, is responsible for about 90% of all invasive aspergillosis cases. *A terreus* is emerging as a cause of opportunistic infection and is associated with high mortality
- The usual route of infection is inhalation but infection sometimes follows local tissue invasion, through surgical wounds or contaminated intravenous catheters
- Corticosteroids increase host susceptibility to *Aspergillus* by reducing oxidative killing of the organism by pulmonary macrophages, increasing its linear growth rate by 30% to 40%, and increasing cell synthesis by >150%

- Current guidelines have established that definitive diagnosis of invasive fungal infections, including invasive aspergillosis, requires histologic and cultural evidence from tissue biopsies or resection material or positive cultures from normally sterile body fluids. Although these measures are the gold standard for invasive fungal infection diagnosis and are widely used in the research setting, they are of less use in the real-world clinical setting
- Two principal signs on radiographs or computed tomography (CT) scans may be suggestive of a diagnosis of invasive aspergillosis: the halo sign on a CT scan or the air-crescent sign on a chest x-ray or CT scan. Timing and infection stage, however, play a key role in the diagnostic utility of radiographic diagnostic methods
- Non–culture-based diagnostic methods are designed to facilitate rapid diagnosis, monitor the progression of *Aspergillus* infection, and monitor response to therapy. Methods that are being developed and studied include antigen-based assays, metabolite detection, and molecular detection of fungal DNA from body fluid samples
- Primary assays of interest for non–culture-based methods of identifying invasive aspergillosis include galactomannan, (1,3)-β-D-glucan assays, and polymerase chain reaction-based assays
- Currently, there is a shift in emphasis from waiting for definitive diagnosis to screening high-risk patients using non–culture-based methods so that physicians can administer antifungal therapy early, when it can improve patient outcome

CASE STUDY

The chest radiograph (**Figure 1**) shows a patient who underwent left lung transplantation in May 2003 for cryptogenic fibrosing alveolitis, which was diagnosed posttransplantation as sarcoidosis. The donor was cytomegalovirus negative, the recipient was cytomegalovirus-positive, and the ischemic time of the transplanted lung was 5 hours. Bronchoscopy 1 week posttransplantation revealed no evidence of airway narrowing. However, the patient developed an area of necrotic tissue, and sputum plugging partially obstructed the left main bronchus over the next couple of months; these conditions were managed bronchoscopically without evidence of infection. There was also some evidence of narrowing of the airways, which required insertion of a bronchial stent 3 months posttransplantation. Three months later, the stent was in a good position but was surrounded by a considerable amount of mucus and pus, which were removed bronchoscopically. The mucus and pus grew *Aspergillus fumigatus* on culture.

Figure 1. **Chest radiograph in a transplant patient, misdiagnosed as sarcoidosis**[1]

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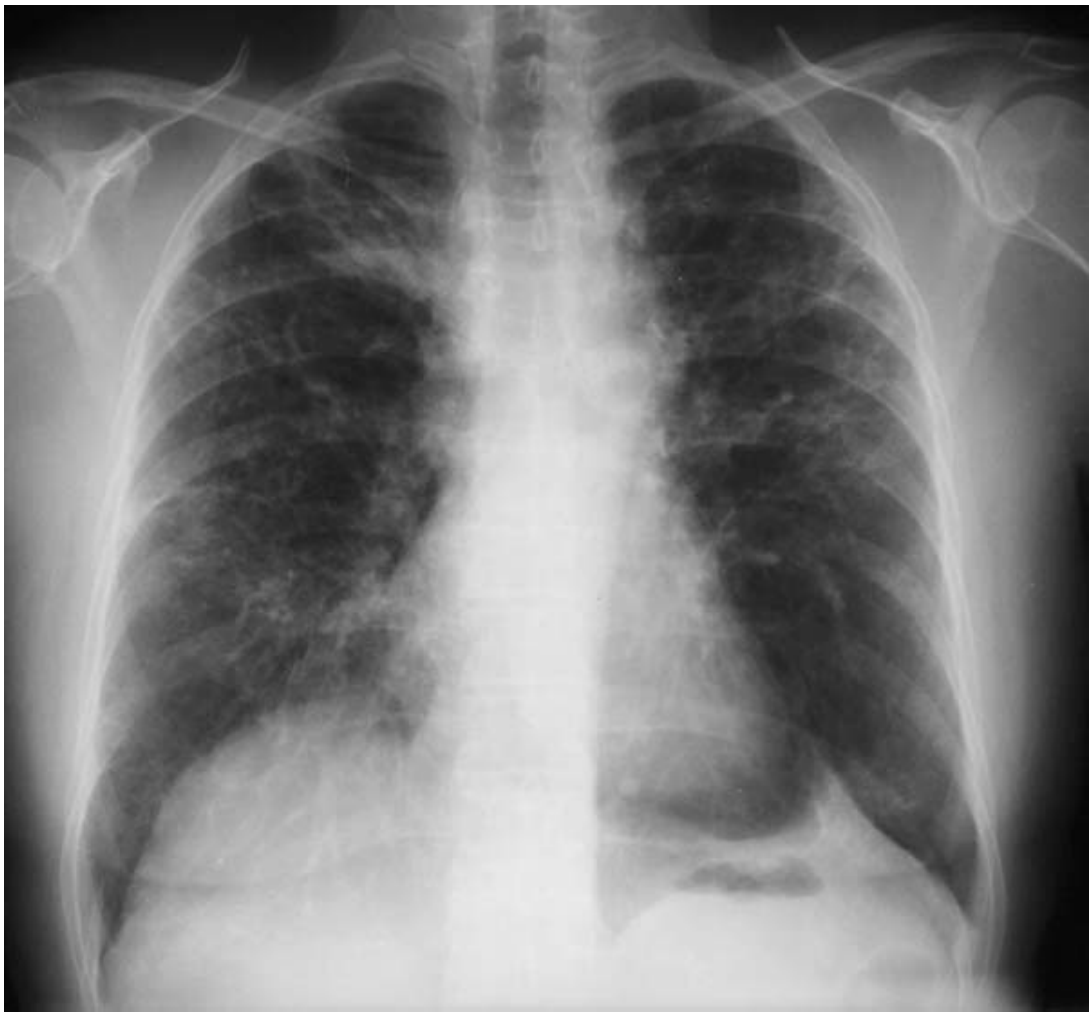


INTRODUCTION TO *ASPERGILLUS*

Taxonomic Classification

The taxonomic classification of *Aspergillus* is shown in **Table 1**.[2] About 300 *Aspergillus* species are currently accepted, and new species continue to be described.[3] Identification of the *Aspergillus* genus and common pathogenic species is seldom difficult in experienced clinical microbiology laboratories, but species-level identification of less common members can be labor-intensive.[4] Nevertheless, species identification is increasingly important due to differences in pathogenicity and antifungal drug susceptibility.[3,4]

Table 1. Taxonomic classification of *Aspergillus*[2]

Kingdom	Fungi
Phylum	Ascomycota
Order	Eurotiales
Family	Trichocomaceae
Genus	*Aspergillus*
Major species	*A fumigatus*, *A flavus*, *A terreus*, *A niger*, *A nidulans*, *A ustus*

Morphologic Characteristics

Macroscopic Features

Pathogenic *Aspergillus* species usually grow easily and relatively quickly on routine bacteriologic and mycologic media.[3] The yield is 35% better on mycologic media.[5] After incubation at 25°C (77°F) for 7 days, colonies of most species have diameters of 1 to 9

cm, whereas colonies of *A nidulans* and *A glaucus* are smaller, reaching only 0.5 to 1 cm in diameter.[2] Only pathogenic species (for example *A fumigatus*, *A flavus*, *A terreus, A niger, A nidulans*) can grow at 35° to 37°C (95-99°F), that is, at body temperature.[3]

Aspergillus colonies have downy-to-powdery textures (**Figure 2**), and the surface color varies with the species (**Table 2**).[2] Viewed from underneath (the reverse), the colonies are uncolored to pale yellow in most isolates. Spores of the major pathogenic species usually develop within 36 to 48 hours of incubation at 30° to 37°C (86-99°F), but spores of unusual species may not appear for days or weeks.[3]

Figure 2. ***Aspergillus fumigatus.*** **A 4-day culture on malt extract agar.**[1] Reprinted from http://www.aspergillus.org.uk with permission from the Fungal Research Trust.

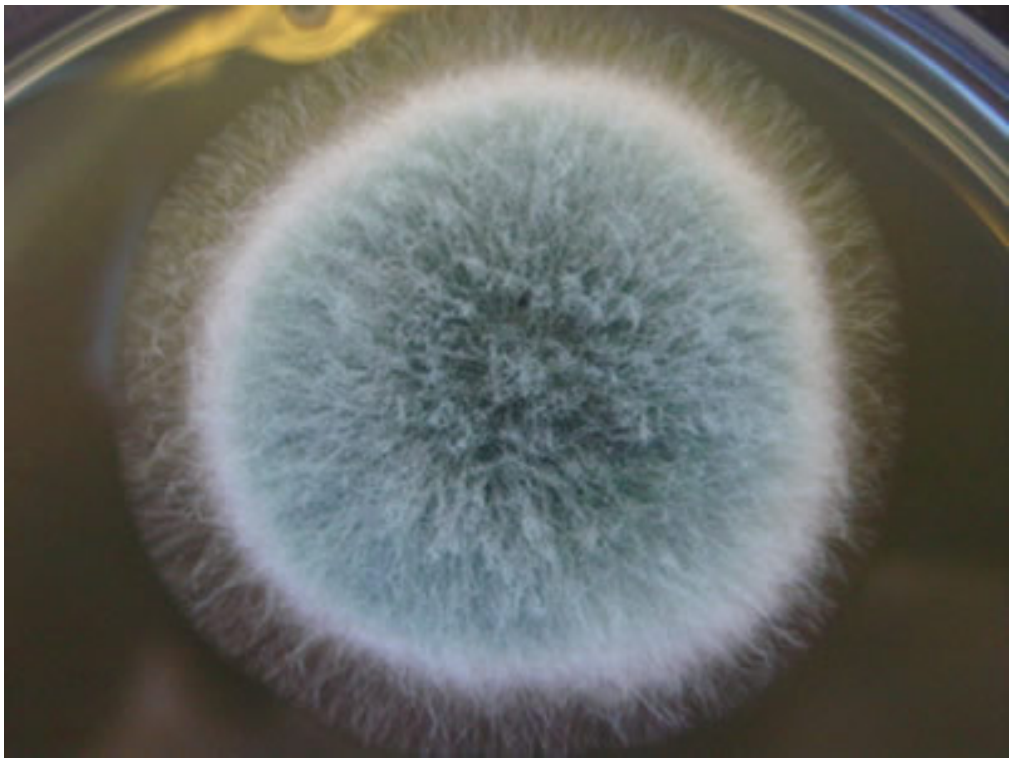


Table 2. Selected macroscopic and microscopic features of major *Aspergillus* species[2] Adapted with permission from www.doctorfungus.org © 2005.

Species	Macroscopic Features: Colony Color		Microscopic Features						
	Surface	Reverse	Conidiophore	Phialides	Vesicle	Sclerotia	Cleistothecia	Hulle Cells	Aleurioconidia
A clavatus	Blue-green	White, brown with age	Long, smooth	Uniseriate	Huge, clavate-shaped	–	–	–	–
A flavus	Yellow-green	Gold to red-brown	Colorless, rough	Uni- or biseriate	Round, radiate head	+ (brown in some strains)	–	–	–
A fumigatus	Blue-green to gray	White to tan	Short (<300 µm), smooth, colorless or green	Uniseriate	Round, columnar head	–	–	–	–
A glaucus group	Green with yellow areas	Yellow to brown	Variable length, smooth, colorless	Uniseriate	Round, radiate to very loosely columnar head	–	+ (yellow-orange)	–	–
A nidulans	Green, buff to yellow	Purple-red to olive	Short (<250 µm), smooth, brown	Biseriate, short	Round, columnar head	–	+ (red)	+	–
A niger	Black	White to yellow	Long, smooth, colorless or brown	Biseriate	Round, radiate head	–	–	–	–
A terreus	Cinnamon to brown	White to brown	Short (<250 µm), smooth, colorless	Biseriate	Round, compactly columnar	–	–	–	+ (solitary, round, produced directly on hyphae)
A versicolor	Initially white, then yellow, tan, pale green, or pink	White to yellow or purple-red	Long, smooth, colorless	Biseriate	Round, loosely radiate head	–	–	+ (in some strains)	–

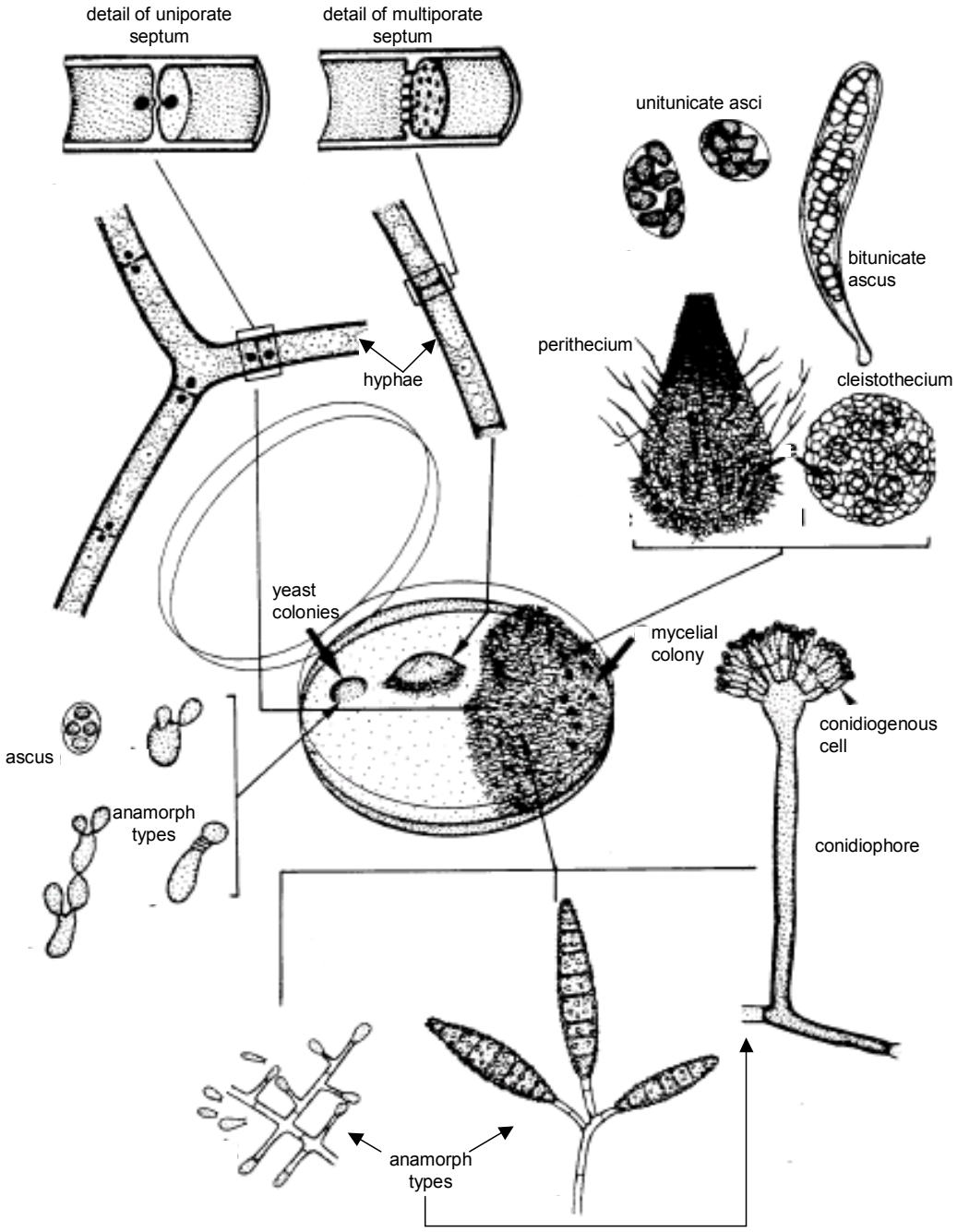
"–" = absent; "+" = present.

Sclerotia – organized masses of hyphae that remain dormant during unfavorable conditions.
Cleistothecia – enclosed, fruiting body that contains randomly dispersed asci (sac-like cells that produce sexual spores).
Aleurioconidia – special type of conidium that is produced by lysis of the cell supporting it.
Hulle cells – cells associated with *Aspergillus spp*. and have a thickened wall and small lumen.

Microscopic Features

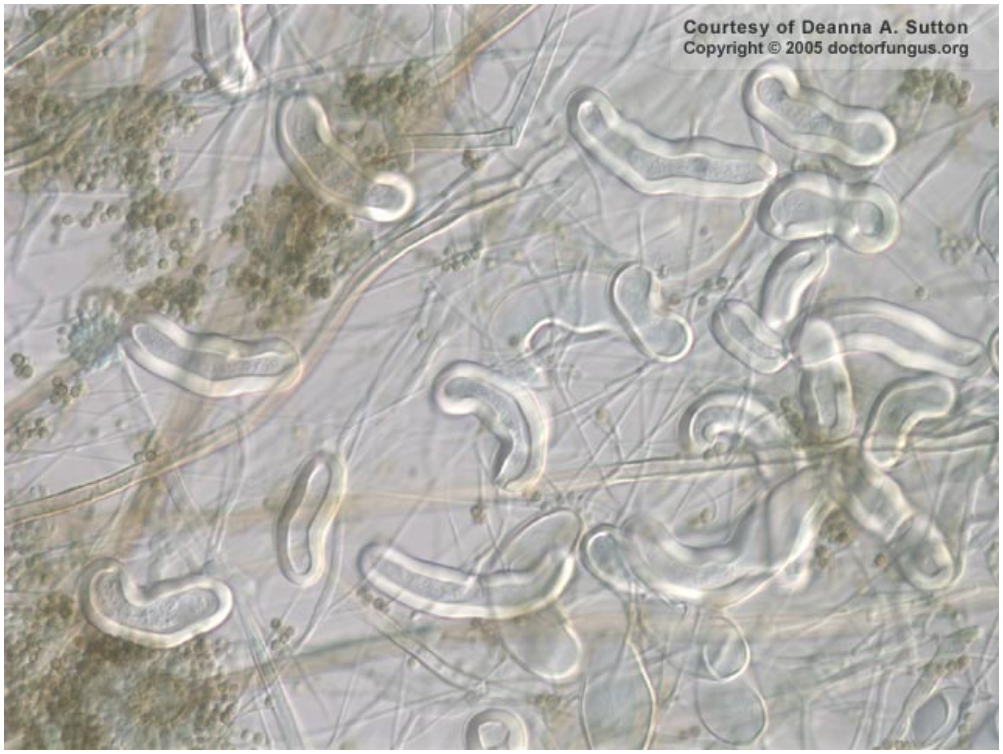
Figure 3 provides an example of some of the microscopic features of *Aspergillus* species. In all *Aspergillus* species, hyphae are septate (divided by a cross-wall) and hyaline (transparent).[2] The conidiophores originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex. The vesicle is the typical formation for the genus *Aspergillus*. The morphology and color of the conidiophore vary between species. Covering the surface of the vesicle entirely ("radiate" head) or partially only at the upper surface ("columnar" head) are the flask-shaped phialides, which are either uniseriate and attached to the vesicle directly or biseriate and attached to the vesicle via a supporting cell, the metula. Over the phialides are the round conidia (2-5 μm in diameter), which form radial chains.

Figure 3. Typical characteristics of *Aspergillus* species and other ascomycetes[6] Reprinted with permission from J. Guarro and the American Society for Microbiology.



Other microscopic structures, which appear only in some *Aspergillus* species, are sclerotia, cleistothecia, aleurioconidia, and Hulle cells **(Table 2)**.[2] The cleistothecium, which is produced during the sexual reproduction stage of some *Aspergillus* species, is a round, closed structure that encloses the asci, which carry ascospores. The asci are spread to the surrounding area when the cleistothecium bursts. The aleurioconidium is a type of conidium produced by lysis of the cell that supports it. The base of the aleurioconidium is usually truncate and carries remnants of the lysed supporting cell, which form annular frills. The Hulle cell is a large sterile cell bearing a small lumen **(Figure 4).** Like the cleistothecium, it is associated with the sexual stage of some *Aspergillus* species.

Figure 4. Thick-walled, irregularly shaped, characteristic Hulle cells of *Aspergillus ustus*. The formation of these cells may be light dependent. Image courtesy of www.doctorfungus.org © 2005.



OVERVIEW OF COMMON *ASPERGILLUS* SPP

Information about major pathogenic *Aspergillus* species causing disease in humans is presented in **Table 3**.[1,3,4,7] *A fumigatus*, the most pathogenic species, is responsible for about 90% of all invasive aspergillosis cases (**Figure 2**).[3] Several factors appear to contribute to its pathogenicity. It is the most rapidly growing species, and it is also extremely thermotolerant, capable of growing at temperatures ≥50°C (122°F).[3] It binds laminin and fibrinogen more efficiently than other species, perhaps permitting better airway adhesion before invasion.[3] Its spores have a hydrophobic protein-coat layer, which may enable them to withstand host defenses and difficult conditions, including oxygen tensions as low as 0.1% O_2. The very small size of *A fumigatus* spores (3-5 μm) enables these spores to penetrate deeply into the lung. *A fumigatus* produces a complement inhibitor, which may further increase its pathogenicity.[4]

Four other species cause most of the remaining cases of aspergillosis, namely *A terreus*, *A flavus*, *A niger*, and *A nidulans*. *A terreus* is associated with a high mortality. *A flavus* may cause sinusitis more commonly. *A nidulans* is particularly associated with infection in chronic granulomatous disease. *A niger* is a more frequent colonizing species, and is commonly associated with external otitis.[3,4]

Additional species that are less commonly seen and only occasionally cause disease are *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 versicolor*, *A wentii*, and *Neosartorya fisheri.*[3]

Table 3. Characteristics of the most common major pathogenic *Aspergillus* species

Species	Global/Regional Distribution	Pathogenicity	Typical Clinical Presentation
A fumigatus	Ubiquitous throughout world; decomposing vegetative matter is primary ecological niche; often found in and around human dwellings in rural areas; common in the home	Most pathogenic species; isolated in ~66% of clinical infections, but with decreased prevalence in recent years	Responsible for >90% of invasive aspergillosis cases; most rapidly growing species; also causes pulmonary disease, aspergilloma, allergic bronchopulmonary aspergillosis, may be resistant to amphotericin B
A flavus	Found in soil and decaying vegetation	Isolated in ~14% of clinical infections	Common isolate in sinusitis, skin, and invasive infections; produces an aflatoxin; may be amphotericin B resistant
A terreus	Found in soil; Increasingly found in water supplies	Isolated in ~5% of clinical infections	Increasingly reported in invasive infection in immunocompromised hosts; resistant to amphotericin B, more susceptible to newer azoles
A niger	Found in soil, on plants, and in food and condiments (for example pepper)	Isolated in ~5% of clinical infections	Uncommon in invasive infections; usually causes superficial infection (for example otitis externa); common colonizing isolate
A nidulans	Found in decomposing vegetative matter	Isolated in a small percentage of clinical infections	Causes diverse infections, especially in patients with chronic granulomatous disease; may be resistant to amphotericin B
A ustus	Found in decomposing vegetative matter	Isolated in a small percentage of clinical infections	Causes disseminated infection, otitis media, skin burn and cutaneous infections, and endocarditis

Data from Denning et al[3]; Patterson et al[4]; Klont et al[7]; and The Aspergillus Web site.[1]

EPIDEMIOLOGY OF ASPERGILLOSIS

Aspergillus occurs in every region in the world.[4] It is most common in decaying vegetation but is also found in soil, water, food, and air.[4]

Patient Susceptibility

Despite routine exposure, persons with normal pulmonary host defenses rarely develop infections, but individuals with altered host immunity have increased susceptibility to *Aspergillus*.[4] A study of patients at 23 medical centers in the United States and 1 center in Canada identified the clinical characteristics of 1209 patients from whom *Aspergillus* isolates were recovered **(Table 4)**.[8] The top 3 conditions placing the patient at increased risk for colonization with *Aspergillus* were pulmonary disorders, corticosteroid use, and diabetes mellitus.

Table 4. Underlying conditions in 1209 patients who were colonized with *Aspergillus*[8]

Condition	Patients, n (%)
Pulmonary disorder	477 (40)
Corticosteroid use	381 (32)
Diabetes mellitus	151 (12)
Human immunodeficiency virus infection	138 (11)
Cystic fibrosis	127 (11)
Surgery	126 (10)
Solid-organ cancer	124 (10)
Hematologic cancer	106 (9)
Solid-organ transplant	100 (8)
Malnutrition	99 (8)
Neutropenia*	61 (5)
Allogeneic transplant	39 (3)
Graft-versus-host disease	34 (3)
Trauma	24 (2)
Connective tissue disease	28 (2)
Autologous transplant	14 (1)
Chronic granulomatous disease	2 (<1)
None	92 (8)

Data are for individual cases in which an *Aspergillus* isolate was recovered from ≥1 culture. The data include patients considered to be colonized or infected or whose cultures were contaminated, without evidence of clinical significance. Patients may be included in ≥1 category. Data from 23 medical centers in the United States and 1 center in Canada.
*Reduction in neutrophil (type of white blood cell) count to below 500 cells/mm^3

Defense Mechanisms Against Aspergillosis

Normal pulmonary defense mechanisms can usually contain the spread of *Aspergillus* in hosts with intact pulmonary defenses. The first line of defense is ciliary clearance of the organism from the airways and limited access to the alveoli due to conidial size. When conidia reach the alveoli, the major line of defense becomes the pulmonary macrophage, which can ingest and kill *Aspergillus* conidia. Once the cells germinate, polymorphonuclear leukocytes kill the swollen conidia and hyphae.[4]

TYPES OF *ASPERGILLUS* INFECTIONS

Asymptomatic Colonization

A study that included 1209 patients at 23 medical centers in the United States and 1 Canadian center has provided valuable information about *Aspergillus* colonization.[8] Approximately 50% (735 of 1477) *Aspergillus*-positive cultures represented colonization. Of the colonizing species, 63% were *A fumigatus*, 14% were *A niger*, 9% were *A flavus*, 1% were *A nidulans*, 1% were *A terreus*, 4% were other species, and 8% were not identified. Colonization may be persistent or transient, and is more common in, but not restricted to, those with chronic chest complaints.

Superficial or Colonizing Syndromes

Superficial *Aspergillus* infections generally occur in the auditory canal (otomycosis), on the nailbeds (onychomycosis), or on the cornea (keratitis). Otomycosis is a condition of superficial colonization typically due to *A niger*.[4] The external auditory canal usually contains desquamated epithelial debris and the mould growing on cerumen. *A niger* forms a black tuft while infections due to *A fumigatus* appear greenish. *Aspergillus*

onychomycosis is a rare superficial condition that can become chronic and treatment resistant. In patients with nonresponsive onychomycosis, a nail culture can be useful in establishing the specific fungal etiology. *Aspergillus* keratitis is most likely to occur after trauma or corneal surgery. The diagnosis can be established with smears demonstrating hyphae.[4]

Allergic Responses

Allergic Bronchopulmonary Aspergillosis

Allergic bronchopulmonary aspergillosis is a hypersensitivity disease of the lungs that is almost always caused by *A fumigatus.*[9] Abnormal inflammatory host responses to *Aspergillus* underlie the pathogenesis of allergic bronchopulmonary aspergillosis .[4] This disorder is a complication of asthma and cystic fibrosis with a predominant Th2 $CD4^+$ cell immune response to multiple *Aspergillus* antigens. The immunopathogenesis of allergic bronchopulmonary aspergillosis is initiated after *Aspergillus* conidia, which are continuously inhaled into the bronchi and germinate in situ, releasing antigens. These mycelial cells release up to 75 different protein allergens that are processed by antigen-presenting cells bearing human leukocyte antigen-DR2 or -DR5 and are then presented to T cells within the bronchoalveolar lymphoid tissue. The T-cell response to these allergens favors a T helper cell 2 response, with release of cytokines interleukin-4, -5, and -13. The inflammatory response in the bronchial submucosa leads to excessive mucin production, extravasation of eosinophils into the bronchial mucin, intermittent bronchial obstruction with atelectasis, and, sometimes, to bronchiectasis.

The estimated prevalence of allergic bronchopulmonary aspergillosis in the United States is 7% to 14% in corticosteroid-dependent patients with asthma and 7% in persons with cystic fibrosis.[10] The disease may also occur in individuals with allergic fungal sinusitis, hyper–immunoglobulin E syndrome, and chronic granulomatous disease. Five stages of allergic bronchopulmonary aspergillosis have been proposed (**Table 5**).[10] The disease is often diagnosed in stage III, but diagnosis is sometimes delayed until stage V, when many patients have extensive bronchiectasis that resembles end-stage cystic fibrosis.

Table 5. Proposed stages of allergic bronchopulmonary aspergillosis[10] Reprinted with permission from the American Academy of Allergy, Asthma, and Immunology.

Stage	Description	Radiographic Infiltrates	Total Serum IgE
I	Acute	Upper lobes or middle lobe	Sharply elevated
II	Remission	No infiltrate, patient off prednisone for >6 months	Elevated or normal
III	Exacerbation	Upper lobes or middle lobe	Sharply elevated
IV	Corticosteroid-dependent asthma	Often without infiltrates, but infiltrates might occur	Elevated or normal
V	End stage	Fibrotic, bullous, or cavitary lesions	Might be normal

Severe Asthma With Fungal Sensitization

There are increasing data linking exposure and sensitization with worse asthma in adults. Multiple fungi may be involved, including *Aspergillus* species. Diagnosis currently relies on skin testing and specific fungal immunoglobulin E results and exclusion of allergic bronchopulmonary aspergillosis. Such patients have a greater need for corticosteroid therapy, are admitted to the hospital more often, and have poorly controlled asthma.

Fungal Sinusitis

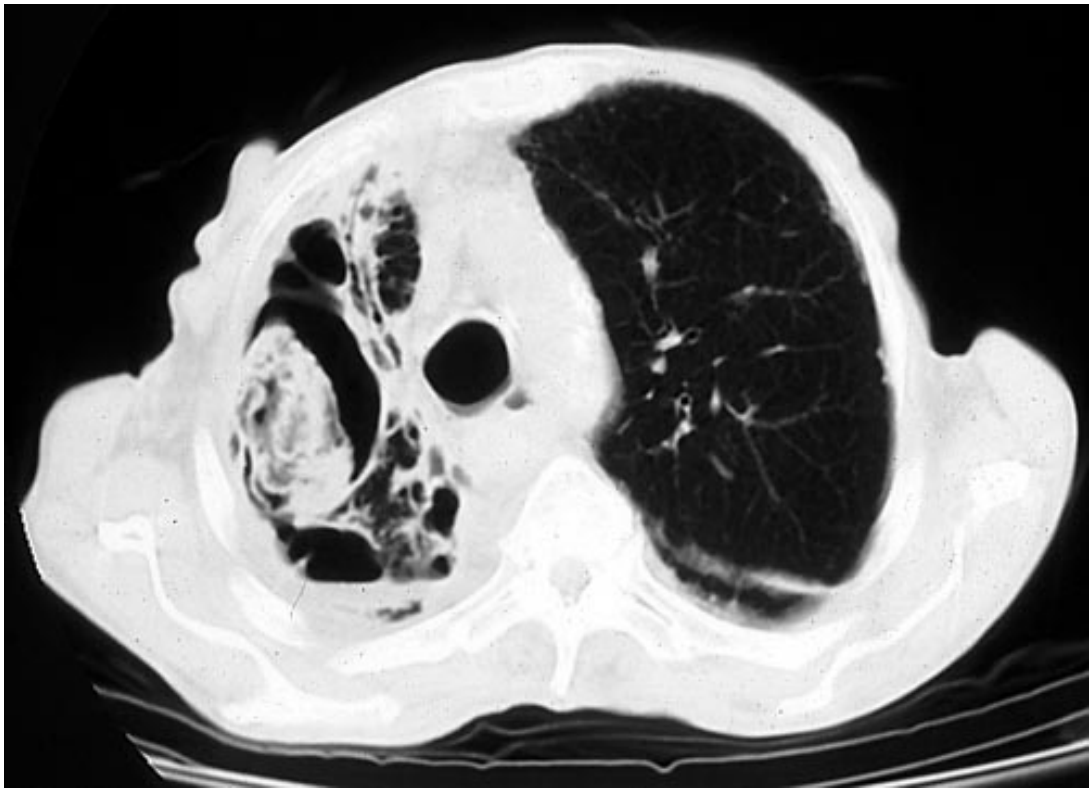
Allergic fungal sinusitis usually occurs in immunocompetent atopic young adults with a history of allergic rhinitis, nasal congestion, headache, nasal polyposis, asthma, and/or recurrent sinusitis.[9] Bone destruction occurs in 30% to 50% of cases.

Aspergilloma

An aspergilloma is a conglomeration, within a pulmonary cavity or ectatic bronchus, of intertwined *Aspergillus* hyphal matter, fibrin, mucus, and cellular debris (**Figure 5**).[9] Most patients have underlying pulmonary disease, such as fibrocystic sarcoidosis, cavitary tuberculosis or histoplasmosis, bullous emphysema, or fibrotic lung disease. Aspergillomas are sometimes considered benign colonizations of the lung, but invasive pulmonary aspergillosis may (rarely) develop from an aspergilloma. A chronic necrotizing form of *Aspergillus* infection is characterized by systemic symptoms (for example, fever and weight loss), aspergilloma, and evidence of tissue invasion as revealed by lung biopsy. Hemoptysis, a common aspergilloma symptom, causes fatal asphyxiation in about 26% of patients.[9] Risk factors associated with poor

prognosis include severity of the underlying lung disease, increasing size or number of aspergillomas on chest radiograph, immunosuppression, increasing *Aspergillus*-specific immunoglobulin G titers, sarcoidosis, and human immunodeficiency virus infection.

Figure 5. **Computed tomography scan of the thorax showing a large apical aspergilloma in the largest of several cavities in the right lung.** Reprinted from http://www.aspergillus.org.uk with permission from the Fungal Research Trust.



Chronic Pulmonary Aspergillosis

Chronic pulmonary aspergillosis usually affects middle-aged men who are immunocompetent or mildly immunosuppressed and have a history of pulmonary disease.[11] It has an indolent progressive course that lasts for years.[11] The usual presenting symptoms are chronic productive cough and weight loss, with mild hemoptysis, dyspnea, and fatigue. Pleural fibrosis and *Aspergillus* empyema complicate some cases. Three subcategories of chronic pulmonary aspergillosis have recently been defined and are discussed in the *Diagnosis* section.

Aspergillosis in the Intensive Care Unit

Patients who are admitted to intensive care units may be at high risk for aspergillosis due to underlying immunocompromising conditions or the presence of medical devices (for example intravascular catheters) that encourage the development of infection.[12] However, more recent data suggest that patients who develop aspergillosis while in the intensive care unit may not have traditional risk factors such as neutropenia.[13] In a retrospective analysis of intensive care unit admissions in a large teaching institution in Belgium during a 3-year period between 2000 and 2003, 6.9% (127 of 1850) of patients had microbiologic or histopathologic evidence of *Aspergillus* during their intensive care unit stay.[13] Excluding the patients with hematologic malignancy or cancer, the rate of proven or probable invasive aspergillosis was 3.7% and the mortality in these patients was 91%. Approximately 50% (33 out of 67) of proven or probable cases of aspergillosis had chronic obstructive pulmonary disease. Cutaneous aspergillosis most commonly occurs in neutropenic patients at or around intravenous catheter insertion sites.[3] *Aspergillus*

may also invade burns, causing a rapidly progressive necrotic lesion that is usually refractory to amphotericin B treatment, or surgical wounds.[3] Finally, liver transplant recipients may be at especially high risk for infection, perhaps because of the long duration of surgery, large operative site, and immunosuppression.

Invasive Aspergillosis

Among immunocompromised patients, the incidence of invasive aspergillosis varies between subgroups (**Table 6**).[3,13] Approximately 80% to 90% of patients have pulmonary disease (**Figure 6**), but sinus disease is also common (**Figure 7**).[3,9] Disseminated disease occurs either by hematogenous spread to distant sites or by contiguous extension from the lung.[4] The central nervous system is the most common secondary site of invasive disease and is involved in 10% to 20% of all invasive aspergillosis cases (**Figure 8**),[7,9] but the thyroid, liver, spleen, kidney, bone, heart, and skin may also be affected.[4] The rapid growth of *Aspergillus* and its tendency to invade blood vessels contribute to the progressive nature of invasive aspergillosis and its resistance to therapy.[9]

Table 6. Incidence of invasive aspergillosis according to underlying condition

Condition	Range, %
Denning 1998[3]	
Heart and lung or lung transplantation	19–26*
Chronic granulomatous disease	25–40†
Acute leukemia	5–24
Allogeneic bone marrow transplantation	4–9
Autologous bone marrow transplantation	
Without growth factors	0.5–6
With growth factors	<1
Acquired immunodeficiency syndrome	0–12
Liver transplantation	1.5–10
Heart and renal transplantation	0.5–10
Severe combined immunodeficiency	3.5
Burns	1–7
Medical intensive care unit patients (not hematology)	4
Systemic lupus erythematosus	1
Meersseman 2005[13]	
Patients with Proven or Probable Invasive Aspergillosis	
Chronic obstructive pulmonary disease	49
Systemic disease	21
Liver cirrhosis	4
Solid-organ transplantation	13
Other conditions	12

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

†Lifetime incidence.

Figure 6. Severe bilateral diffuse and nodular invasive pulmonary aspergillosis in an allogeneic bone marrow transplant recipient whose symptoms included dyspnea, hypoxia, and dry cough. Nodules and areas of atelectasis are visible at both bases. The patient later died. Reprinted from http://www.aspergillus.org.uk with permission from the Fungal Research Trust.

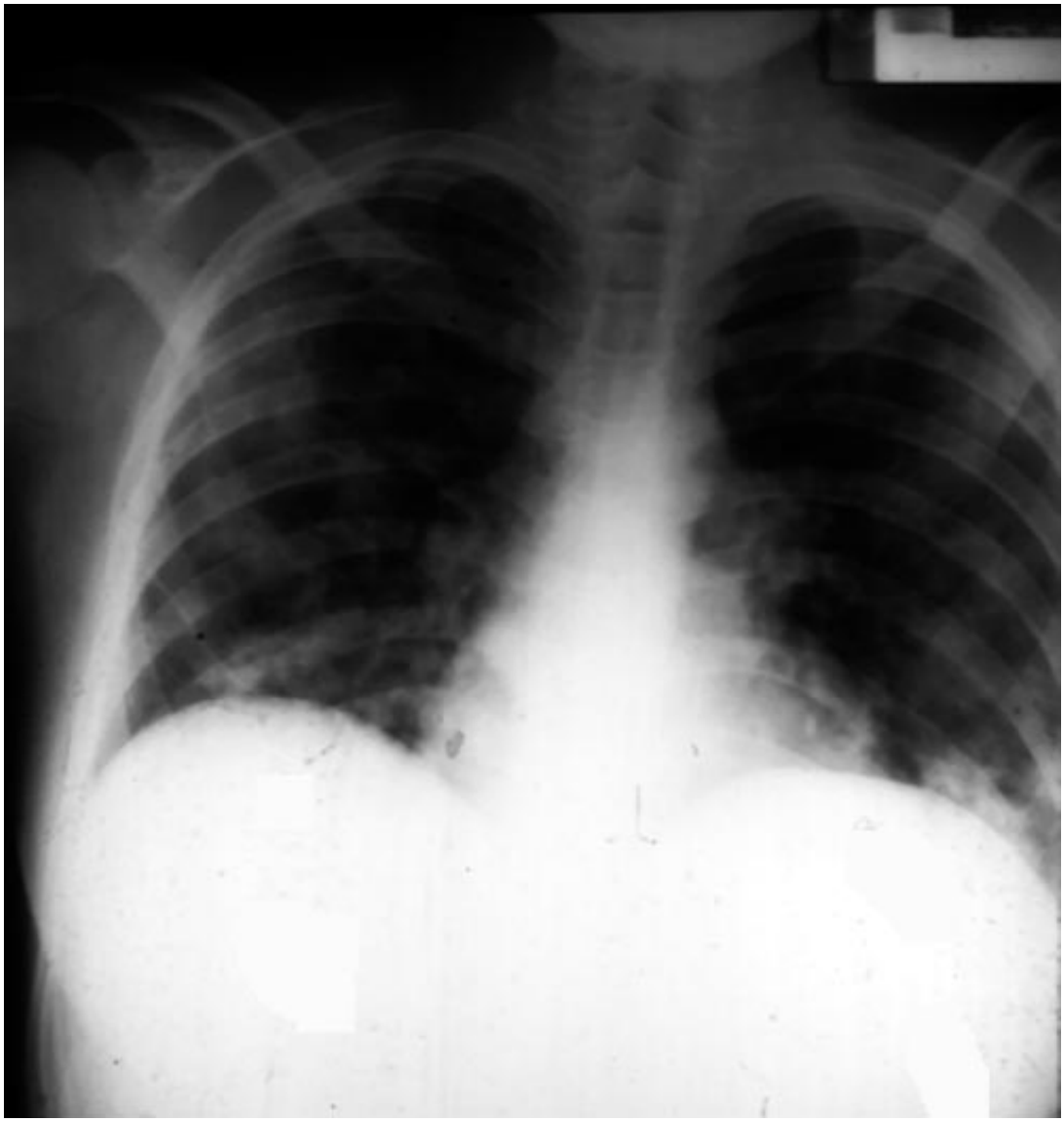


Figure 7. Magnetic resonance image showing histologically proven *Aspergillus* sinusitis in an allogeneic bone marrow transplant recipient. The maxillary sinus on the left is completely opacified. Reprinted from http://www.aspergillus.org.uk with permission from the Fungal Research Trust.

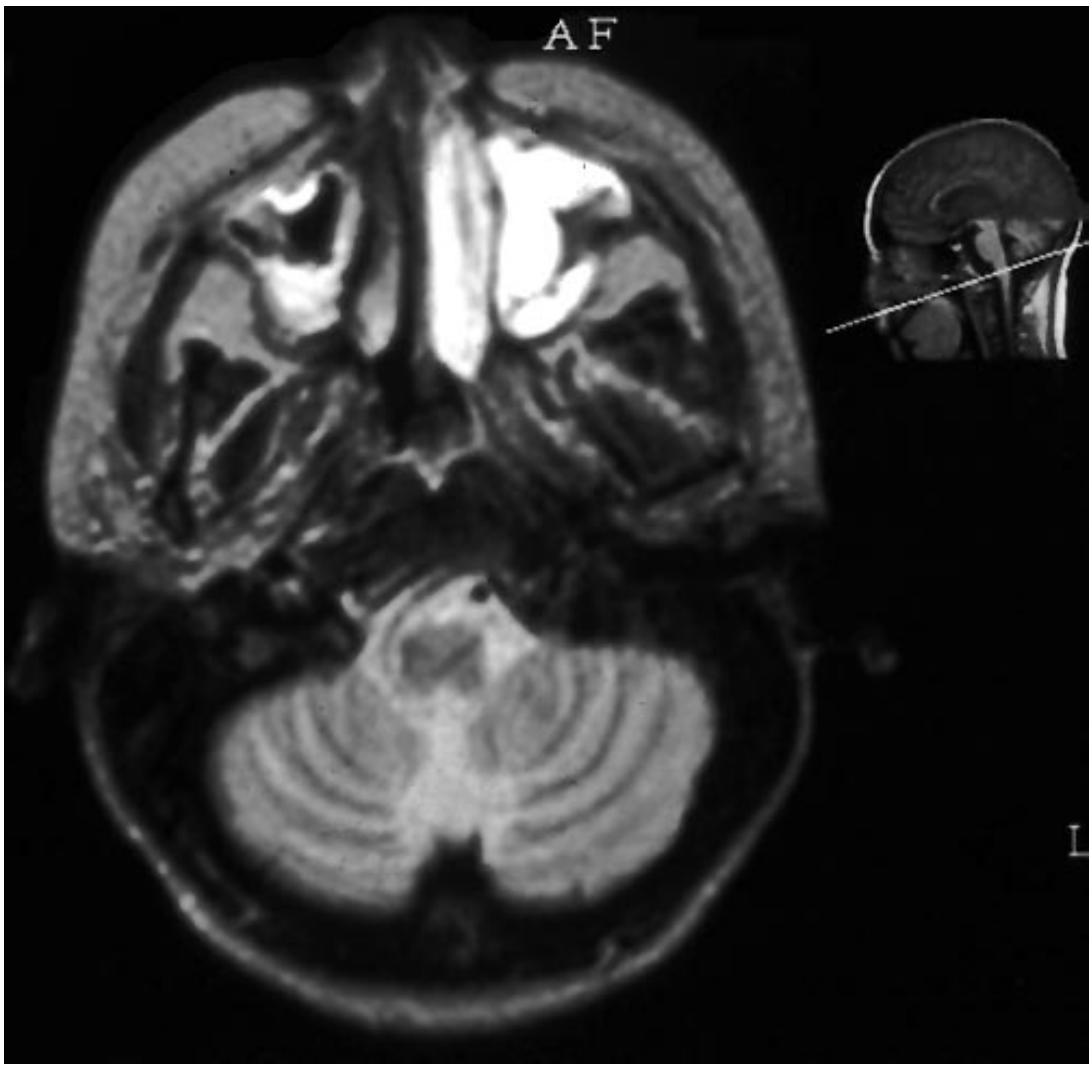
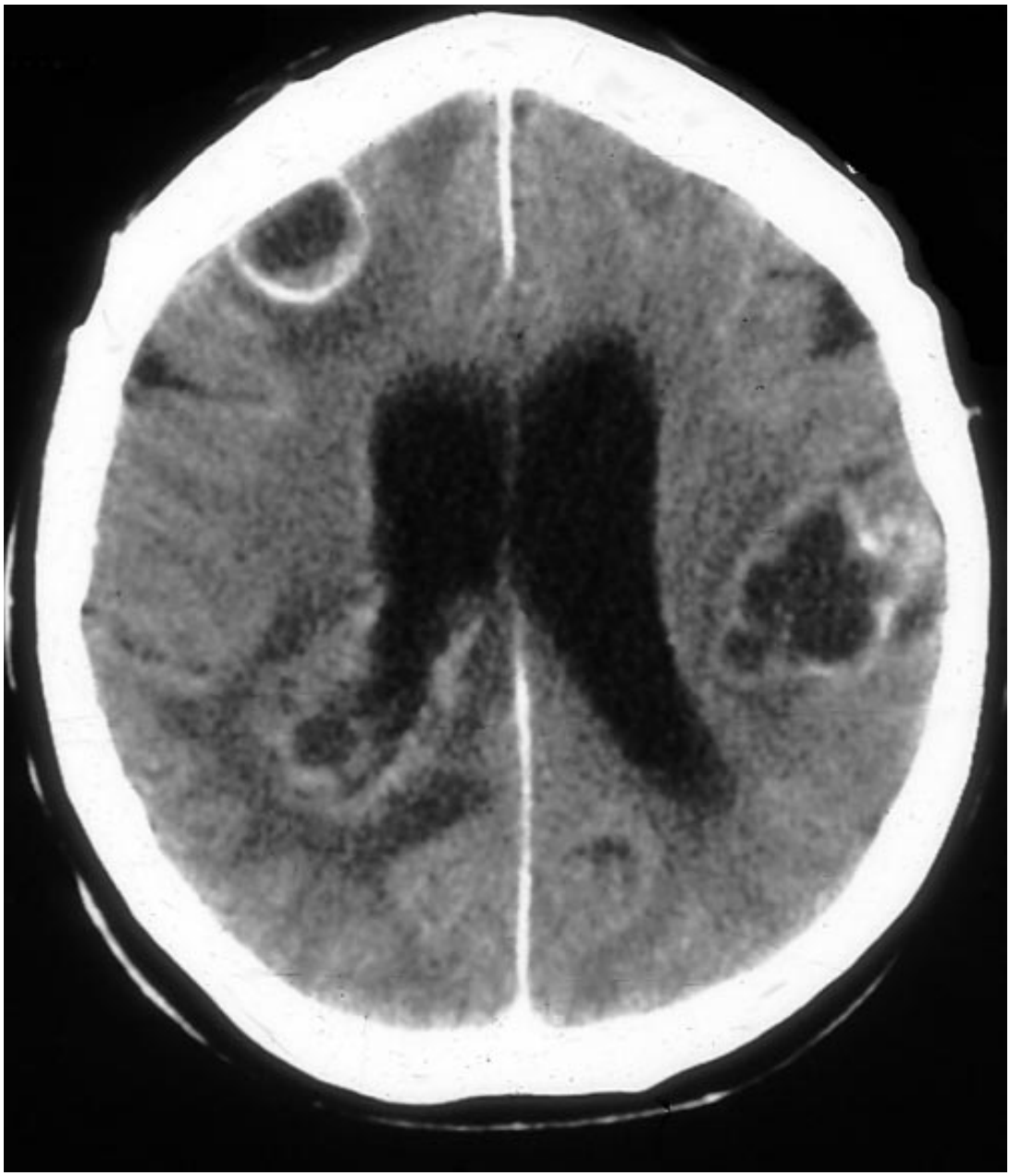
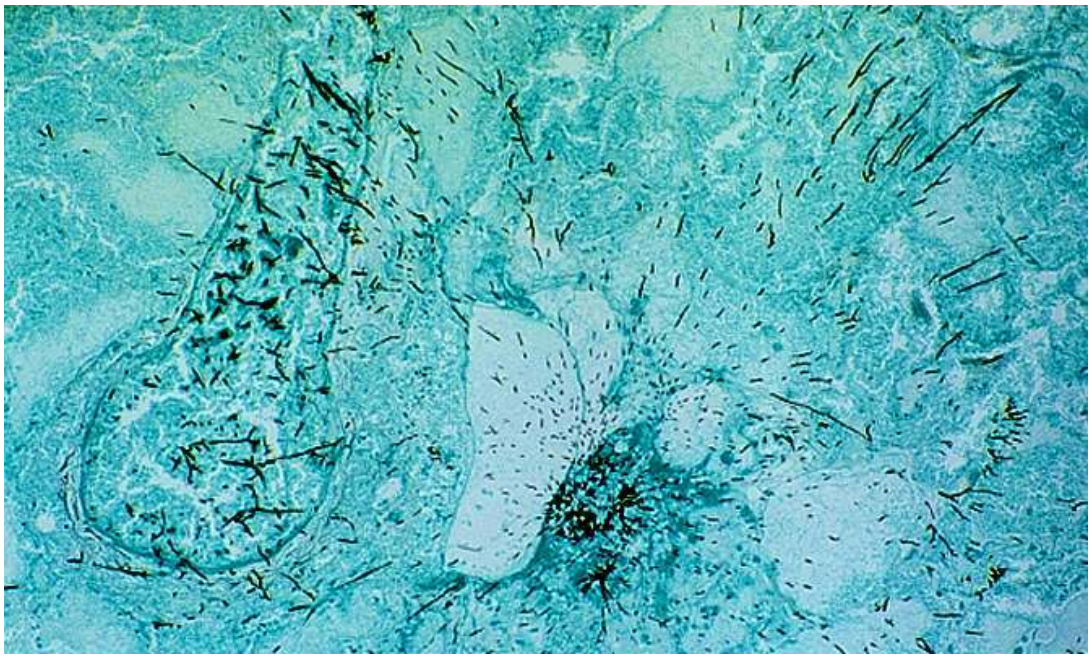


Figure 8. Cerebral aspergillosis. The patient received a renal transplant several months before developing symptoms consistent with stroke, including reduced consciousness. The enhanced computed tomography scan of her brain showed multiple ring-enhancing lesions with some surrounding edema. Biopsy confirmed invasive aspergillosis on histology and culture. Reprinted from http://www.aspergillus.org.uk with permission from the Fungal Research Trust.



In invasive aspergillosis, the usual route of infection is inhalation of *Aspergillus* conidia into the lungs, but infection sometimes follows local tissue invasion, through surgical wounds or contaminated intravenous catheters.[4] After pulmonary exposure in persons with inadequate host defenses, conidia enlarge, germinate, and are transformed into hyphae (**Figure 9**). Conidial germination in vitro typically occurs in 4 to 6 hours. The incubation period for disease ranges from 2 days to 3 months. The process of hyphal growth and tissue invasion usually results in vascular invasion and pulmonary infarction—classic features of invasive pulmonary aspergillosis— in neutropenic patients. Corticosteroids increase susceptibility to *Aspergillus* by reducing oxidative killing of the organism by pulmonary macrophages, increasing its linear growth rate by 30% to 40% and increasing cell synthesis by more than 150%.[4]

Figure 9. Light microscopic appearance of conidial head of *Aspergillus fumigatus* at an air interface in pulmonary tissue (hematoxylin and eosin staining, magnification 250×).
Reprinted from http://www.aspergillus.org.uk with permission from the Fungal Research Trust.



Invasive Pulmonary Aspergillosis

The symptoms of invasive pulmonary aspergillosis are nonspecific and may lag significantly behind the disease process[4]; most patients are asymptomatic and afebrile initially. Symptoms include progressive dry cough, dyspnea, pleuritic chest pain, fever despite coverage with broad-spectrum antibiotics, and pulmonary infiltrates. Symptoms may be reduced in patients who cannot mount an inflammatory response due to profound neutropenia. Fever is common but may be absent in those receiving high doses of corticosteroids. Other clinical features include hemoptysis, pleural effusion, and pneumothorax. Due to the angioinvasive nature of *Aspergillus*, the clinical characteristics of invasive pulmonary aspergillosis may resemble those of a pulmonary embolism. Laboratory findings, which are also nonspecific, may include elevations in bilirubin, lactate dehydrogenase, C-reactive protein, and fibrinogen as well as coagulation abnormalities. In patients with extensive or progressive infection, life-threatening hypoxia may occur. Multiple diffuse nodular pulmonary infiltrates, which are readily seen on the chest radiographs of patients with extensive infection, are not diagnostic and are associated with poor prognosis.

Tracheobronchitis

Aspergillus in the airways ranges in significance from colonization and superficial bronchitis, which is common in lung transplantation, to ulcerative tracheobronchitis.[4] Tracheobronchitis typically occurs in patients undergoing lung transplantation and in persons with acquired immunodeficiency syndrome and is characterized by extensive pseudomembranous or ulcerative lesions. In patients who have undergone lung

transplantation, infection often occurs at the suture line of the transplant and can lead to dehiscence of the anastomotic site.

Symptoms of tracheobronchitis are nonspecific and include dyspnea with associated pulmonary function abnormalities, cough, chest pain, fever, or hemoptysis.[4] Symptoms may be mild and can be confused with other causes, including rejection. In more severe cases, local obstruction may cause unilateral wheeze or stridor.

Sinusitis

In immunocompromised patients, *Aspergillus* infection of the sinuses and nasal cavities manifests as acute invasive rhinosinusitis, often in association with invasive pulmonary aspergillosis (**Figure 6**).[4] Clinical manifestations include fever, cough, epistaxis, sinus discharge, and headaches.[4] Although clinical signs are nondiagnostic, the presence of an ulcerative nasal lesion with an eschar or nonsensitive area is suggestive of fungal infection.[4] In a high-risk patient, epistaxis or unexplained fever warrants endoscopy and biopsy of any nasal mucosal lesion. In patients with progressive disease, *Aspergillus* sinusitis spreads to the contiguous paranasal sinuses, palate, orbit, or brain.

Cerebral Aspergillosis

Aspergillus may invade the brain directly perioperatively, by contiguous spread from the sinuses or ear or, most commonly, by hematogenous spread as part of disseminated aspergillosis. Syndromes include single or multiple abscesses or infarctions, with or without associated local hemorrhage, meningitis, and mycotic

aneurysm. Symptoms are usually subtle initially with change in mentation, drowsiness, and focal loss of function. Seizures may occur.[3,9]

DIAGNOSIS

Allergic Bronchopulmonary Aspergillosis

Diagnostic criteria for the diagnosis of allergic bronchopulmonary aspergillosis in persons with asthma and cystic fibrosis are presented in **Table 7**.[10] Chest radiographs usually reveal transient unilateral or bilateral areas of consolidation, especially in the upper lobes, caused by bronchial obstruction with mucus plugs.[9] The mucus-filled bronchus may form a band or glove-finger shadow that disappears when the patient coughs up a plug. Chest radiographs may also show a "ring sign" or parallel shadows ("tram lines"), representing inflamed bronchi. Computed tomography with thin (1-2 mm) rather than conventional (10 mm) sections is valuable in the diagnosis of allergic bronchopulmonary aspergillosis and can identify bronchiectasis, mucus plugs or mucoid impactions, bronchial wall thickenings, atelectasis, lobar or whole-lung collapse, pulmonary fibrosis, and cavities.[10]

Table 7. Major criteria for the diagnosis of allergic bronchopulmonary aspergillosis in persons with asthma and cystic fibrosis[10] Adapted with permission from the American Academy of Allergy, Asthma, and Immunology.

Underlying Disorder	Major Diagnostic Criteria
Asthma	Allergic bronchopulmonary aspergillosis-central bronchiectasis • Asthma • Central bronchiectasis (inner two thirds of chest computed tomography field) • Immediate cutaneous reactivity to *Aspergillus* species or *A fumigatus* • Total serum immunoglobulin E concentration >417 kU/L (1000 ng/mL) • Elevated serum immunoglobulin E–*A fumigatus* and/or immunoglobulin G–*A fumigatus* Allergic bronchopulmonary aspergillosis-seropositive • Asthma • Immediate cutaneous reactivity to *Aspergillus* species or *A fumigatus* • Total serum immunoglobulin E concentration >417 kU/L (1000 ng/mL) • Elevated serum immunoglobulin E –*A fumigatus* and/or immunoglobulin G –*A fumigatus*
Cystic fibrosis	• Clinical deterioration (increased cough, wheezing, exercise intolerance, increased sputum, decreased pulmonary function) • Immediate cutaneous reactivity to *Aspergillus* or presence of serum immunoglobulin E –*A fumigatus* • Total serum immunoglobulin E concentration >1000 kU/L • Precipitating antibodies to *A fumigatus* or serum immunoglobulin G –*A fumigatus* • Abnormal chest roentgenogram (infiltrates, mucus plugging, or change from earlier films)

Useful laboratory findings include elevated total serum IgE concentrations, increased levels of immunoglobulin E – and/or immunoglobulin G –*A fumigatus*, and precipitating antibodies to *A fumigatus* or serum immunoglobulin G –*A fumigatus*. Immediate cutaneous reactivity to *Aspergillus* or the presence of serum immunoglobulin E –*A fumigatus* is another valuable finding. Extensive research is in progress to develop a sensitive, specific, and widely available test for allergic bronchopulmonary aspergillosis using selected recombinant allergens.[10]

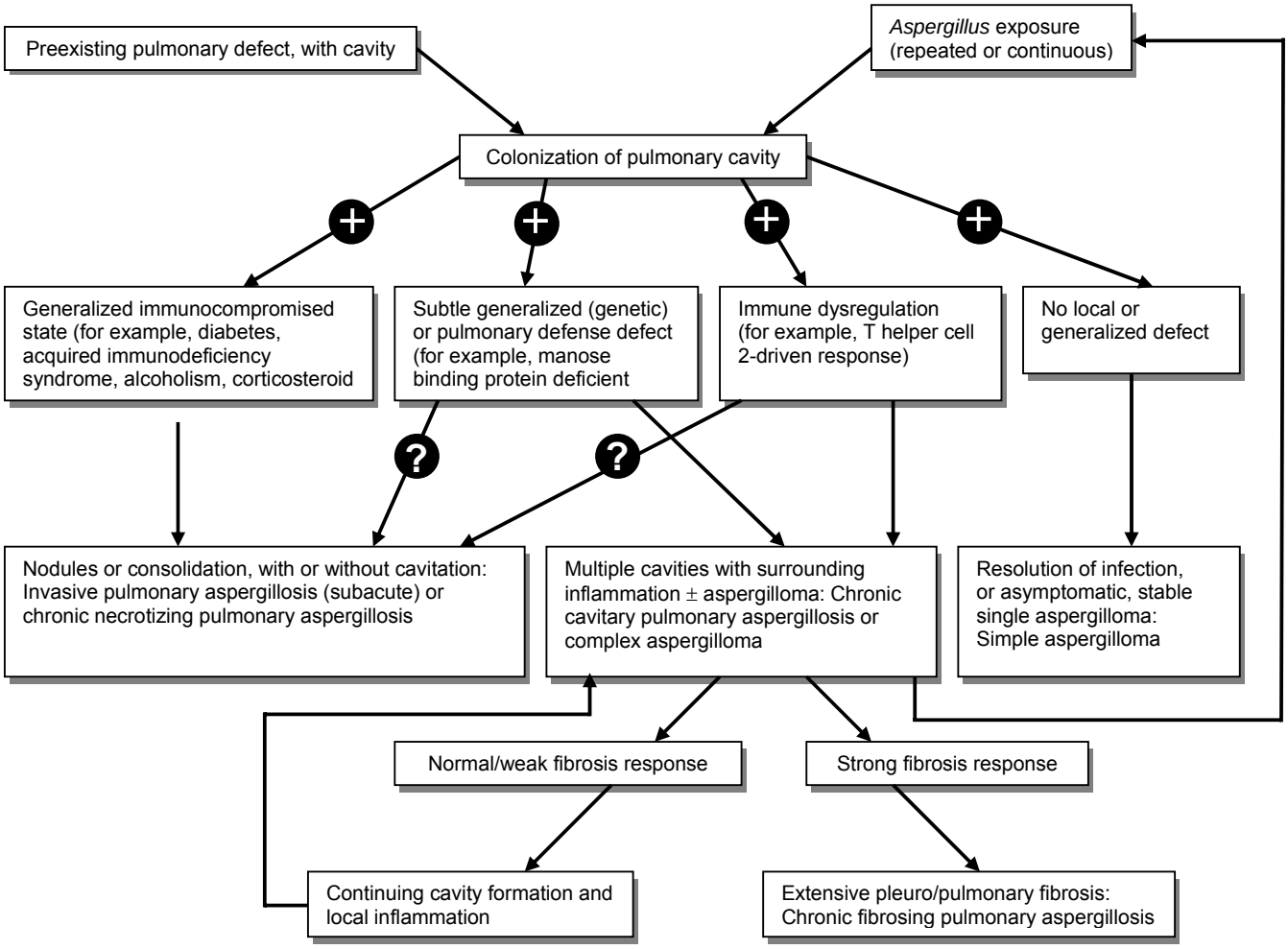
Aspergilloma

Aspergilloma is usually diagnosed by chest radiography.[9] An aspergilloma appears as a solid round mass, sometimes mobile, with the density of water. It is located in a single spherical or ovoid cavity and separated from the cavity wall by an airspace of variable size and shape. Pleural thickening occurs when the aspergilloma is peripheral. The finding of serum precipitins that are positive for an *Aspergillus* species is conclusive, because this test has >95% sensitivity for aspergilloma.[9]

Chronic Pulmonary Aspergillosis

Based on differences in clinical presentations and radiologic manifestations, 3 subcategories of chronic pulmonary aspergillosis have been proposed (**Figure 10**).[11] The distinctions among these categories, however, are not absolute.

Figure 10. Proposed classification and pathogenesis of chronic pulmonary aspergillosis. The proposed pathways (arrows) are speculative, and 1 or more may not be operative in a given patient.[11] Adapted with permission from University of Chicago Press. © 2003 by the Infectious Diseases Society of America. All rights reserved.



Chronic *cavitary* pulmonary aspergillosis is characterized by the formation and expansion of multiple cavities, some containing fungus balls.[11] Chronic *fibrosing* pulmonary aspergillosis represents a progression to marked and extensive pulmonary fibrosis.[11] Some patients with chronic fibrosing pulmonary aspergillosis have pleural involvement, either as direct invasion of the pleural cavity or as fibrosis.[11] The third category is subacute invasive pulmonary aspergillosis, which is virtually identical to chronic *necrotizing* pulmonary aspergillosis. Subacute invasive pulmonary aspergillosis is characterized by progressive enlargement of a single cavity, which may have a thick or thin wall, in some cases to substantial dimensions. This process of enlargement may take place over months or weeks. Unlike patients with chronic cavitary or fibrosing pulmonary, persons with subacute invasive pulmonary aspergillosis usually have mild to moderate immune dysfunction.

Patients with chronic cavitary pulmonary aspergillosis have radiologic evidence of a cavitary lesion in the lung.[11] Cavity expansion correlates well with continuing ill health as a radiologic marker of disease progression, and successful therapy is associated with arrest of expansion. Upon serologic testing, patients have positive results for *A fumigatus* precipitins and elevated acute-phase markers (C-reactive protein, plasma viscosity, or erythrocyte sedimentation rate).[11] Elevated levels of total immunoglobulin E and *Aspergillus*-specific immunoglobulin E are often present.[11] Histologic examination of resected lung tissue reveals localized bronchiectasis with cavity formation, chronic inflammation, and associated fibrosis in chronic cavitary pulmonary aspergillosis and a predominance of fibrosis in chronic fibrosing pulmonary aspergillosis. Culture of sputum may yield positive results for *A fumigatus*,

but positive culture results may be infrequent and occur months or years after the patient's initial presentation.

Invasive Aspergillosis

Introduction

Although diagnostic methods for some fungal infections have improved, developing accurate, quick diagnostic methods for invasive aspergillosis has proven more challenging. Invasive aspergillosis is often diagnosed late, when fungal burdens are high and the ability to fight the infection with antifungal drugs is diminished. In some cases, aspergillosis diagnosis is confirmed only at autopsy.[14,15] Traditionally recommended culture and histopathologic diagnostic methods tend to be invasive, have a low sensitivity, often yield inconclusive results, and can take a week or longer for results to become available.[3,16,17] Cultures of blood or respiratory specimens are rarely positive during the early stages of invasive aspergillosis, necessitating the use of invasive procedures to obtain tissue for histological and microbiological evaluation.[18] Many patients with invasive aspergillosis are simply too ill to undergo such invasive procedures. Consequently, there is a significant need for non–culture-based diagnostic methods.

Current guidelines have established that definitive diagnosis of invasive fungal infection, including invasive aspergillosis, requires evidence—through histologic examination and culture results—from tissue biopsies or resection material or positive cultures from normally sterile body fluids.[19] The Invasive Fungal Infections Cooperative Group (IFICG) of the European Organisation for Research and

Treatment of Cancer (EORTC) and the National Institutes of Allergy and Infectious Diseases Mycoses Study Group (NIAID MSG) have published consensus guidelines for diagnosing invasive fungal infections in cancer patients and hematopoietic stem cell transplantation (HSCT) recipients.[20] These recommendations use host factors, major or minor clinical criteria, and microbiological criteria to categorize fungal infections as proven, probable, and possible (**Table 8**).[20] However, these methods may be insufficiently sensitive or specific. Although widely used in the research setting, they are of less use in the real-world clinical setting. Nevertheless, it is important to note that culture and histologic methods are the current gold standard for invasive fungal infection diagnosis.

Table 8. EORTC definitions (modified for patients with aspergillosis).[20] Reprinted with permission from Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: An international consensus. *Clin Infect Dis*. 2002;34:7-14.

Category, type of infection	Description
Proven invasive fungal infections	
Deep tissue infections	
Moulds[a]	Histopathologic or cytopathologic examination showing hyphae from needle aspiration or biopsy specimen with evidence of associated tissue damage (either microscopically or unequivocally by imaging); or positive culture result for a sample obtained by sterile procedure from normally sterile and clinically or radiologically abnormal site consistent with infection, excluding urine and mucous membranes
Fungemia	
Moulds[a]	Blood culture that yields fungi, excluding *Aspergillus* species and *Penicillium* species other than *Penicillium marneffei*, accompanied by temporally related clinical signs and symptoms compatible with relevant organism
Probable and possible invasive fungal infections[c]	
Probable invasive fungal infections	At least 1 host factor criterion; 1 microbiological criterion; and 1 major (or 2 minor) clinical criteria from abnormal site consistent with infection
Possible invasive fungal infections[b]	At least 1 host factor criterion; and 1 microbiological or 1 major (or 2 minor) clinical criteria from abnormal site consistent with infection

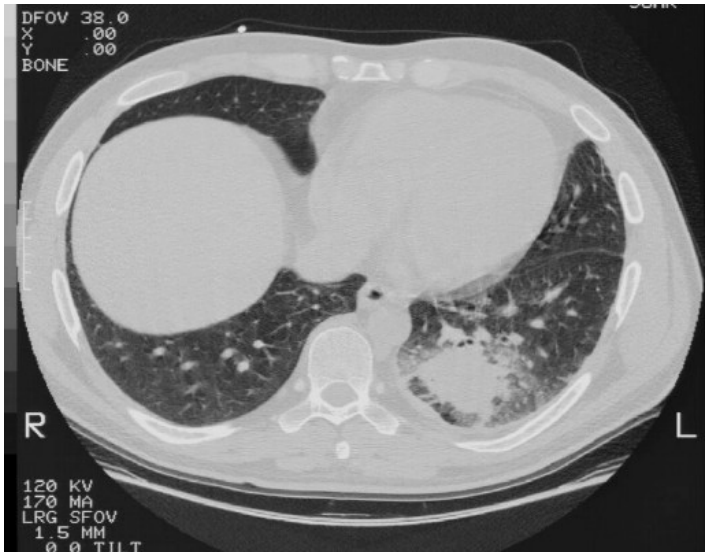
[a] Append identification at genus or species level from culture, if available.
[b] This category is not recommended for use in clinical trials of antifungal agents but might be considered for studies of empirical treatment, epidemiological studies, and studies of health economics.

Other groups, such as the Infectious Diseases Society of America, recommend that therapy be initiated promptly and aggressively upon suspicion of diagnosis, without definitive proof, because of the high mortality of invasive aspergillosis in immunocompromised patients.[9] Because traditional culture and histologic methods may delay initiation of therapy, new non–culture-based diagnostic methods may offer clinicians a method of screening patients more rapidly.

Radiologic Procedures

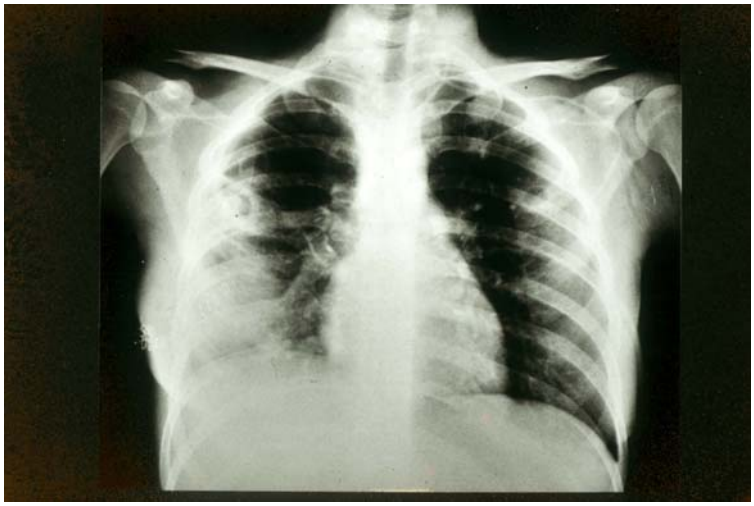
The halo sign on a computed tomography (CT scan) of the lungs and the air-crescent sign on a chest radiograph (x-ray) or CT scan may be suggestive of a diagnosis of invasive aspergillosis.[19,21] In invasive aspergillosis, fungal hyphae begin to invade and occlude small to medium-sized pulmonary arteries, leading to the formation of small nodules of necrotic (dead) tissue. On a CT scan, these nodules may be surrounded by a halo of what looks like ground glass (the halo sign), pathologically associated with hemorrhage (**Figure 11**).[21,22] Although highly suggestive of invasive aspergillosis in severely neutropenic patients, the halo sign can also be present with other fungal (eg, Zygomycetes) or bacterial (eg, *Pseudomonas* species) infections, bleeding, embolism, or leukemic or inflammatory infiltrates.[19] In addition, this finding is observed in only approximately 33% to 60% of cases, is short-lived, lasting about a week, and is neither sensitive nor specific.[23,24] Still, systematic CT scan performed in febrile neutropenic patients has allowed physicians to reduce the average time to invasive pulmonary aspergillosis diagnosis from 7 days to 1.9 days.[25] In high-risk patients, the halo sign may be taken as early evidence for pulmonary aspergillosis and warrant antifungal therapy.[26]

Figure 11. The halo sign. Reprinted with permission from Mira Vista Diagnostics.



When fragments of necrotic tissue (nodules) separate from the lung parenchyma or lining of the cavity wall, an air space may be present, and the pleura may thicken.[21] In invasive aspergillosis cases, the air-crescent sign (**Figure 12)**, if present, generally occurs 2 to 3 weeks after the initiation of treatment and only after resolution of neutropenia. This latter sign appears too late to affect the disease course.[27] Although visible on both CT and radiograph, it may be seen on a chest CT scan much earlier than on a chest radiograph and is much more indicative of another type of invasive aspergillosis, necrotizing granulomatous pneumonia.[28]

Figure 12. The air-crescent sign. Reprinted with permission from Mira Vista Diagnostics.

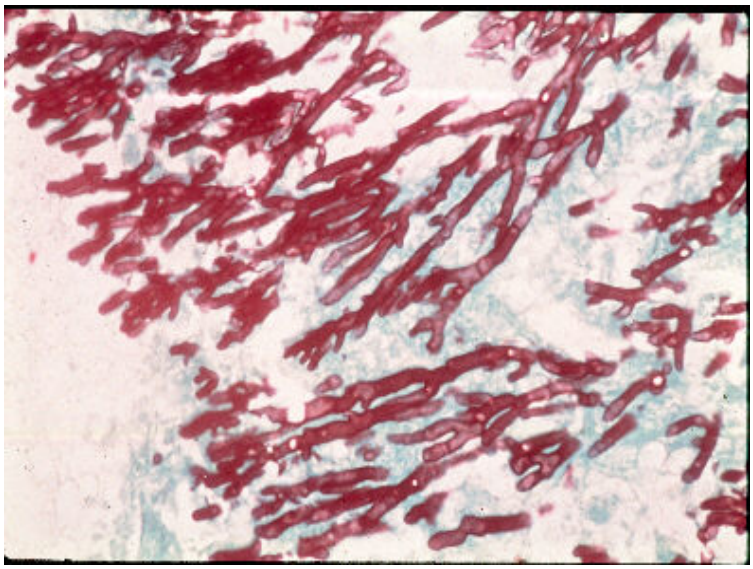


Neither the halo nor the air-crescent sign is diagnostic for invasive aspergillosis in allogeneic stem cell transplant recipients treated with steroids.[23] However, the diagnostic value of the halo sign can be much greater when applied to neutropenic patients.[23,28]

Microscopic Examination Using Special Stains

Aspergillus species can be identified only by culture and their identification requires specialized training. **Figure 13** presents a typical stain for *Aspergillus* that shows key features of its branched hyphae. However, this finding is nonspecific because several other filamentous fungi have a similar appearance under the microscope. Distinguishing *Aspergillus* spp from *Fusarium* spp or *Scedosporium* spp is important in determining antifungal susceptibility, identifying drug-resistant species, selecting appropriate antifungal therapy, and maintaining surveillance and epidemiological tracking of invasive aspergillosis.

Figure 13. A typical stain for *Aspergillus* showing key features of its branched hyphae.
Reprinted with permission from MiraVista Diagnostics.



Culture and Histopathology

Body fluids such as blood, pleural effusion, or cerebrospinal fluids are typically sterile. When a fungal infection is suspected, fluids or material obtained from a biopsy can be grown on a specific culture medium used to identify fungal elements. Samples are stained, evaluated microscopically, and cultured. However, the collection of bronchoalveolar lavage (BAL) fluid has been plagued by a lack of a uniform approach, particularly with regard to the area to lavage. Typically, BAL fluid is collected by inserting a long tube into the lungs and rinsing the area with saline solution. This solution is then extracted and the collected cells examined microscopically. Some physicians distinguish between BAL and bronchial lavage, hypothesizing that higher numbers of *Aspergillus* hyphae may be measured in the bronchi compared with the alveoli.[7]

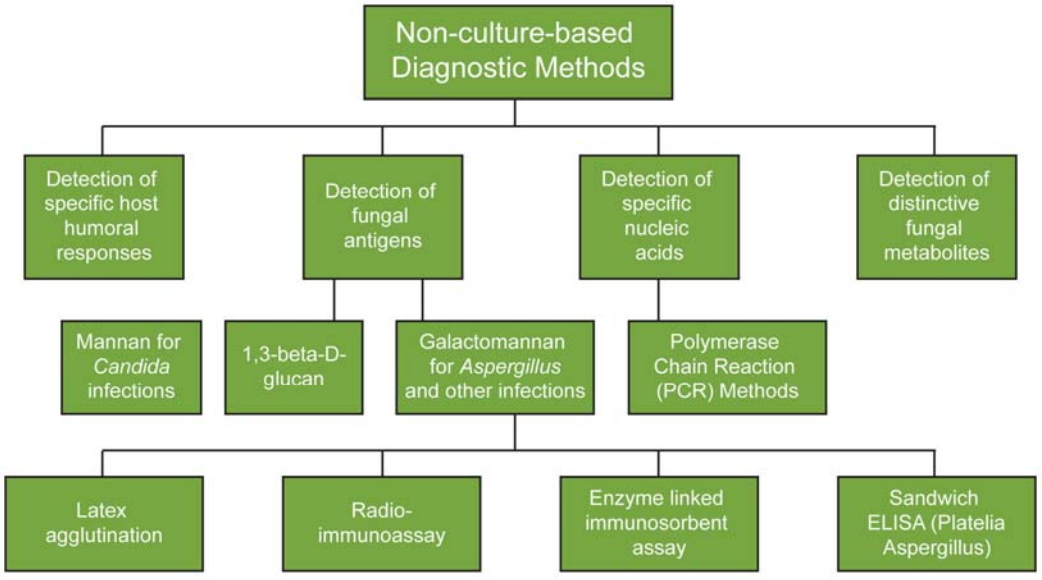
Results from blood culture are positive in less than 5% of cases of invasive aspergillosis. Most researchers have found that the significance of blood cultures positive for *Aspergillus* species is unclear because true aspergillemia (*Aspergillus* in the blood) is rare, occurs late in the course of aspergillosis, and occurs exclusively in patients with hematologic malignancies.[29] Even when present in a blood sample, *Aspergillus* tends to grow very slowly on culture medium.[3,6] Nevertheless, culture methods can be important in differential diagnosis to eliminate infection caused by *Nocardia* spp, *Legionella* spp, and the Zygomycetes, which can mimic the presentation of invasive pulmonary aspergillosis. Blood cultures are of little value except for the diagnosis of *Aspergillus terreus*,[29] but cultures of respiratory secretions can be useful in isolating and diagnosing infections caused by *Aspergillus*. Still, BAL cultures are positive in fewer than 50% of cases of invasive pulmonary aspergillosis.[6] Although cultures using BAL are the most useful for the diagnosis of aspergillosis, the sensitivity of the method depends on the timing and is low (about 30% to 40%). In the clinical setting, proven clinical diagnosis using culture methods is rarely achieved and may be achieved too late to have a positive outcome. Furthermore, the results of antifungal susceptibility testing have been compromised by diverging and imprecise definitions, parameters for estimating growth inhibition or measurement, and variable in vitro conditions.[30] A milestone in the standardized testing of filamentous fungi was the 2002 publication of the *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard* (M38-A) by the Clinical and Laboratory Standards Institute,[30] formerly the National Committee for Clinical Laboratory Standards. Despite the importance of standardized antifungal testing, this methodology has its limitations.[30] The in vitro susceptibility testing

method, for example, cannot clearly identify amphotericin B–resistant *A fumigatus* isolates but can identify azole-resistant *Aspergillus* isolates

Non–culture-based Microbiological Methods Used to Diagnose Invasive Aspergillosis (Excluding Radiological)

Non–culture-based methods are designed to facilitate rapid diagnosis and to monitor infection progression and response to therapy. Promising methods include antigen-based assays (serology), metabolite detection, and molecular detection of fungal DNA from body fluid samples (**Figure 14)**. The ideal marker for invasive aspergillosis is present early in the infectious process, is associated with infection rather than colonization, is specific or conserved within the fungal species of interest, does not cross-react with other human or microbial antigens, is easy to perform and inexpensive, and can be standardized and validated.[18]

Figure 14. Non–culture-based methods of diagnosing invasive aspergillosis



Aspergillus *Galactomannan*

Description

Galactomannan, a water-soluble cell-wall polysaccharide released by the growing hyphae of *Aspergillus* and some other fungi (eg, *Penicillium* spp), is an antigen detectable in patients with invasive aspergillosis.[18] Galactomannan levels may be detected a median of 5 to 8 days (range 1 to 27 days) before clinical signs and symptoms are observed.[31-33] Because galactomannan levels correlate with fungal tissue burden,[34] they may be used to monitor clinical outcome and response to antifungal therapy.[18,35] Various assays have been developed, including enzyme immunoassays (EIAs), radioimmunoassays (RIAs), latex particle agglutination tests, and a sandwich enzyme-linked immunoabsorbent assay (ELISA).[7,18]

In the body, galactomannan forms immune complexes that are quickly removed from the circulation (especially in non-neutropenic hosts), potentially limiting the sensitivity of these assays.[18] More importantly, the sensitivity of the galactomannan EIA is reduced in patients receiving mould-active antifungal drugs as prophylaxis or empiric treatment on the day of testing.[36] It is unclear whether different antifungal drugs have differing effects on circulating galactomannan levels.[36]

False-positive results may theoretically occur in patients during the period of mucositis following chemotherapy because galactomannan molecules pass through the damaged gut mucosa into the bloodstream. This theory is supported by the observation that multiple positive assays and false positive results coincide with the

period within 30 days of HSCT or cytotoxic therapy.[35,37,37-39] False-positive assays are also seen in patients treated with antibiotics such as piperacillin-tazobactam or amoxicillin-clavulanate.

Types of Galactomannan Assays

Four primary galactomannan antigen detection methods have been developed, and two kits are commercially available (**Table 9**).[40-42]

Table 9. Invasive aspergillosis antigen detection methods[40-42]

Method	Manufacturer	Detection limit
Latex agglutination	Pastorex *Aspergillus*, Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France	15 ng/mL
Radioimmunoassay[40]	Not commercially available	10 ng/mL
ELISA inhibition[41]	Not commercially available	10 ng/mL
Sandwich ELISA[42]	Platelia® *Aspergillus*, Sanofi Diagnostics Pasteur	1 ng/mL

ELISA = enzyme-linked immunoabsorbent assay.

Latex Agglutination

Latex agglutination is a qualitative or semi-quantitative technique that has been used for many years. It involves attaching an antigen or antibody to tiny latex beads that agglutinate to form visible particles when mixed with a sample containing the corresponding antigen or antibody. Interpretation can be easy with strong reactions but difficult with weak or marginal reactions. Although latex agglutination is generally associated with lower individual test costs and a shorter time to results than some newer techniques, the lower limit of galactomannan detection is only 15 ng/mL—much higher than that achievable with newer methods.[18]

The Pastorex *Aspergillus* latex agglutination test (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) uses the EB-A2 rat monoclonal antibody to detect *Aspergillus* galactomannan. Use of this assay to detect galactomannan in serum samples from patients with hematologic malignancies yielded high specificity but variable sensitivity. Furthermore, galactomannan was typically detectable in serum during advanced stages of disease, negating the objective of developing a method to diagnose aspergillosis in its early stages. However, Pastorex *Aspergillus* was a good indicator of disease when BAL fluid was tested.

Radioimmunoassay

Although it is not available as a commercial kit, Talbot and colleagues validated an antigen radioimmunoassay for the detection of *A fumigatus* in 1987.[40] They reported a sensitivity of 74%, specificity of 90%, positive predictive value of 82%, and negative predictive value of 85%.

Enzyme Immunoassay (EIA)

An EIA can detect 10 ng/mL of galactomannan in serum samples.[43] In a compative study, a competitive binding inhibition EIA could potentially diagnose invasive aspergillosis from serum or urine samples with greater specificity and sensitivity than commercially available latex agglutination and sandwich enzyme immunoassay tests.[43] However, a detection kit is not commercially available.

Sandwich ELISA (Platelia® Aspergillus ELISA)

The Platelia® *Aspergillus* galactomannan enzyme-linked immunosorbent assay (ELISA) (Bio-Rad Marnes-La-Coquette, France) has demonstrated good sensitivity and specificity and can sometimes provide positive results before the development of signs and symptoms. This assay has been available for more than a decade in Europe and since 2003 in the United States. Essentially, the rat monoclonal antibody EB-A2 recognizes beta-(1,5)-linked galactofuranose.[16] It is called a sandwich ELISA because the same monoclonal antibody is used as the detecting antibody and the capture antibody.

The antibodies in this assay react with galactomannan from *Aspergillus* and a few other moulds in a 96-well plate. When galactomannan is present, a yellow color is produced, measured, and compared against a cutoff control sample. The most recent cutoff in the United States and Europe is 0.5 ng/mL. Anything above this point is considered positive, and the test is repeated (or retested) to ensure reproducibility. In one of the few comparative studies including the sandwich ELISA, real-time polymerase chain reaction (PCR), and (1,3)-beta-D-glucan assays, the sandwich ELISA method was the most sensitive at detecting invasive aspergillosis in high-risk patients with hematological disorders, using a cutoff of 0.6 ng/mL [44] Compared to the latex agglutination technique, the sandwich ELISA method allows galactomannan detection in serum at a much earlier stage, often before the onset of clinical signs and symptoms and the appearance of radiological abnormalities. Clinicians using the Platelia assay to monitor patients for invasive aspergillosis can achieve even greater sensitivity by screening at least twice weekly during the period of highest risk.[16]

Limitations associated with the use of the Platelia assay include its expense (not applicable in Europe, where the test is performed in the local laboratory), labor-intensive nature (about 4 hours per batch of assays), and susceptibility to error and contamination.[16] Its sensitivity is also affected by antifungal treatment. Results are affected by the population studied, patient age, time of test performance, with reduced specificity noted during the first few weeks after allogeneic HSCT and reduced sensitivity for late cases during immunosuppressive treatment for chronic graft-versus-host disease.

To date, no cost studies have been conducted with galactomannan assays. Despite their expense, it may be cost-effective in the long-term to diagnose invasive aspergillosis early, rather than waiting for signs and symptoms to appear.

Types of Samples Used for Galactomannan Assays

Blood serum samples can be useful in diagnosing invasive aspergillosis because a secondary mode of *Aspergillus* propagation is hematogenic. Because galactomannan is water soluble, it should also be detectable in other fluid samples, including BAL fluid, urine, CSF, and pleural fluid.[45-48]

BAL Fluid

Because *Aspergillus* organisms are primarily airborne and generally cause pulmonary infections, the microbiological yield from BAL samples is much higher (but still quite low) than that from blood samples.[49] Sandwich ELISA methods have been used to

detect galactomannan in BAL fluid. Although the correlation between serum and BAL fluid samples is excellent, serum samples were positive up to 30 days before bronchoscopy, suggesting that antigenemia may precede the emergence of clinical signs and symptoms.[50]

Urine

Galactomannan is detectable in urine samples from patients with invasive aspergillosis, indicating that at least some circulating galactomannan is cleared renally. The ability to detect galactomannan in urine samples is advantageous because urine can be collected frequently and in large volumes. Whether urine or serum samples are superior remains a matter of debate.[7] Urine samples are prone to false-positive results because of airborne fungi contamination. False reactivity has also been noted in urine samples from rats treated with cyclophosphamide and other immunosuppressive agents.[51] Sequential sampling, using higher cutoffs, or applying longer reaction times in lieu of urine concentration methods may improve specificity.[7]

Cerebrospinal Fluid (CSF)

After the lungs, the central nervous system (CNS) is the most common site of infection in invasive aspergillosis. invasive aspergillosis affects the CNS in 10% to 20% of cases.[46] Occasionally, cerebral involvement occurs without evidence of pulmonary aspergillosis.[46] Diagnosis of CNS aspergillosis, including meningitis, poses a greater diagnostic challenge for clinicians than pulmonary aspergillosis because cultures seldom yield positive results and CT scan, brain biopsy, and CSF

analysis may be inconclusive.[7,52] Galactomannan is detectable in CSF specimens from patients with CNS aspergillosis, but cutoff values may need to be lowered.[7]

Tissue

Demonstrating the presence of *Aspergillus* in tissue is the gold standard diagnosis. However, *Aspergillus* cannot be distinguished from similar types of fungi using staining and microscopic evaluation alone, necessitating the use of traditional culture techniques, with their inherent limitations. Nearly 70% of tissue specimens displaying septate hyphae yielded negative results by culture.[7]

Other Clinical Samples

In patients with invasive aspergillosis, galactomannan antigen has also been documented in cyst fluids and fluid from subphrenic abscesses.[7]

Summary of Galactomannan ELISA

Table 10 summarizes the uses, advantages, and limitations of the Platelia® galactomannan ELISA.

Table 10. Characteristics of galactomannan ELISA (Platelia®)[36,45,46,53-58]

Uses	Detection of major polysaccharide constituent of *Aspergillus* and *Penicillium* cell wall in serum
Interpretation	Two positive results on two different samples required for optimal results Results defined as ratio (index): Optical Density (OD) patient serum/OD threshold serum Cut-off for Optical Density (OD) index: 1.5 ng/L (BioRad®) 0.5 ng/L (US and Europe)
Sensitivity and Specificity	Overall from FDA data[53]: Sensitivity 80.7% Specificity 89.2% Cut-off: 1.5 / 1.0 / 0.7 Sensitivity: 57.7% / 61.5% / 73.1% Specificity: 99.4% / 98.5% / 93.9% Source: Herbrecht R et al. *J Clin Oncol*. 2002;20:1898-1906.[59] Single 0.5 / Consecutive >0.5 / Single >0.8 Sensitivity: 96.5% / 96.5% / 96.5% Specificity: 85.1% / 98.6% / 97.3% Source: Maertens J et al. *Br J Hematol*. 2004;126:852-860.[60]
Factors Affecting Performance	False-positive results • Underlying condition (false positives more common in allogeneic HSCT recipients because of increased absorption of dietary galactomannan caused by breakdown of intestinal mucosa from chemotherapy and radiation therapy) • Exposure to piperacillin-tazobactam, ampicillin-sulbactam, and amoxicillin-clavulanic acid can yield more false-positives because these are derived from *Penicillium* species which contain galactomannan [45,46,54-56] • Intake of certain foods (tea, milk, soybean) • Neonates and young children: false positives occur more frequently in children due to high galactomannan concentrations in milk and possible contamination of GI tract with organisms producing cross-reactive antigens False-negative results • Low fungal burden • Exposure to antifungal therapy[36,57] • Walled off infection Other factors[58] • Cut-off used for "positive" result used (higher with higher cutoffs) • Underlying level of immunosuppression • Pretreatment immunosuppression procedure • *Aspergillus* species causing infection • Optimal times/frequency of sample collection and intrapatient variability
Limitations [58]	• Specificity can be affected by *Aspergillus* contamination or infection or colonization with organisms containing a cross-reactive antigen • Not routinely used outside high-risk neutropenic patients • Fails to detect other pathogenic fungi (eg, *Fusarium*, Zygomycetes) • Sensitivity of single samples appears low; serial sampling is needed

HSCT = hematopoietic stem cell transplantation; GI = gastrointestinal.

(1,3)-Beta (β)-D-Glucan Assay (BDG)

Description

(1,3)-β-D-glucan (BDG) is a polysaccharide found in the cell walls of most pathogenic fungi, such as *Aspergillus*, *Candida*, and *Fusarium* species.[61] There may be species-level differences in fungal cell wall BDG concentrations.[62] As with galactomannan, the kinetics of how BDG is released from infection sites and its circulation and clearance are not well understood.[15] Currently, there are several commercially available BDG assays (**Table 11**).

Table 11. (1,3)-β-D-glucan (BDG) assays

Assay Name	Manufacturer (Location)	Positive Serum Cutoff Level
Fungitell (Glucatell)	Associates of Cape Cod (Falmouth, Massachusetts, USA	80 pg/mL
FungiTec G	Seikagaku Kogyo Corporation (Tokyo, Japan)	20 pg/mL
Wako-WB003	Wako, Wako Pure Chemical Industries (Osaka, Japan)	Unknown/Not available
B-G Star	Maruha Corporation (Tokyo, Japan)	Unknown/Not available

The Fungitell assay was approved in 2004 by the United States Food and Drug Administration for the presumptive diagnosis of fungal infection in conjunction with other diagnostic procedures,[63] and it is also widely used in Japan. In this assay, serum samples are placed into microplates, and the BDG is detected on the basis of its ability to activate factor G of the horseshoe crab coagulation cascade, which initiates a biochemical cascade reaction that ultimately produces a color. Although low levels of BDG, typically in the range of 10 to 40 picograms (pg) [10^{-12} grams]/mL, are present in normal human serum from yeasts that reside in the gastrointestinal tract, levels

above 80 pg/mL in at-risk patients suggest invasive fungal infection.[63] The assay can be completed in approximately 1 hour.

Sensitivity of BDG detection for diagnosing invasive fungal infection ranges from 50% to 63%, but can be dramatically improved by sampling twice weekly.[15] In patients with acute myelogenous leukemia or myelodysplastic syndrome receiving antifungal prophylaxis, sensitivities for detecting invasive fungal infection have varied from 60% to 100%.[64] BDG has not been systematically studied in invasive aspergillosis.[65]

BDG serum concentrations are affected by the location and encapsulation of the infection, whether the patient is receiving hemodialysis or fractionated blood products such as serum albumin and immunoglobulins, or exposure of the specimen to glucan-containing gauze.[63] No statistically significant effect upon assay sensitivity has been observed with antifungal drug treatment, but more study is required.[62,63] Potentially, this diagnostic method may be especially useful for patients receiving antifungal prophylaxis.

As with galactomannan testing, false-positive results decrease the specificity of the BDG assay. Pazos and colleagues discovered that patients with false-positive results exhibit a sudden rise and fall in serum BDG levels, in contrast to a more gradual rise in BDG levels in patients with confirmed *Aspergillus* infection.[15]

According to the Fungitell package insert, sensitivity ranges from 50% to 67% and specificity ranges from 70% to 93%.[63] Others have noted higher sensitivity (90%) and specificity (84% to 100%).[65] The cutoff point used and the patient population can affect the sensitivity, specificity, and positive and negative predictive values of the assay (**Table 12**).[62,64]

Table 12. Characteristics of (1,3)-β-D-glucan (Fungitell™, Fungitec G®)[63]

Uses	Detection of polysaccharide constituent of most fungal cell walls in serum (*Aspergillus*, *Candida*, *Fusarium*) with exception of *Cryptococcus neoformans* and Zygomycetes
Interpretation	Detection limit of 1 pg/mL [65] Cut-offs:[63] Negative: <60 pg/mL Equivocal: 60-79 pg/mL Positive: 80 pg/mL (Fungitell™)
Sensitivity and Specificity	Sensitivity 50%-67%[63] Specificity 70%-93%[63] Sensitivity 90%[65] Specificity 84%-100%[65]
Factors Affecting Performance	False-positive results • Use of cellulose membranes in hemodialysis • Exposure to glucan-containing gauze • Administration of IV albumin or gamma globulin • Certain antibiotics[66] • High incidence of false-positive in ICU patients following surgery
Limitations	• Amount of (1,3)-β-D-glucan produced by certain fungi affects serum concentration of analyte • Certain healthy individuals have high circulating levels of (1,3)-β-D-glucan in equivocal zone

IV = intravenous; ICU = intensive care unit.

Nucleic Acid Detection: Polymerase Chain Reaction (PCR)

Description

Polymerase chain reaction (PCR) is a way of amplifying specific genetic sequences present in a sample so that they can be detected. PCR is potentially more sensitive than current culture-based methods, with an ability, in some assays, to detect as little as 10 fentogram (fg) [10^{-15} grams] of *Aspergillus* DNA. This corresponds to about 5

colony-forming units (CFU)/mL. These assays can test for specific genera and species.

The two basic PCR methods, panfungal PCR and species-specific PCR, differ in terms of their targets. Panfungal PCR targets specific ribosomal genes that contain conserved sequences that are common to all fungi as an initial screen for fungal infection, including the *18ss-r*RNA (ribosomal RNA), *28 r*RNA, and certain mitochondrial genes. In the second step, the DNA is hybridized with species-specific variable regions. In hematologic patients, the specificity of panfungal PCR for identifying *Aspergillus* and *Candida* species has ranged from 65% to 75%.[19] Species-specific PCR usually is described as nested or 2-step PCR. Here, the primers or targets are derived from *18sr*RNA and, in blood and BAL, have shown a specificity of 89%.

In a variation of these methods, called real-time PCR, fluorescent dye binds to the amplified DNA as it is formed within the reaction tube. The amount of fluorescence can then be measured and used to quantify the amount of DNA. Real-time PCR offers minimal contamination risk, no postamplification handling, fast turnaround time, high sensitivity and specificity using fluorescently labeled probes, and most importantly, the ability to quantify fungal burden and monitor response to treatment.

While fungal DNA has successfully been extracted and purified from many types of clinical specimens, including whole blood, serum, plasma, and BAL fluid, [67-77] the efficiency and reproducibility of the results may vary according to the type of sample

used.[18] Currently, blood and BAL fluid are the most promising specimens. The utility of PCR detection assays with CSF samples has received limited study.

Comparing the results achieved with different PCR methods is extremely difficult due to a lack of standardized methods. Because many of these assays are still in development, standards must be established for each technique. Test results are affected by the ways in which a sample is prepared and fungal DNA is handled,[18] by the amplification method used, and by patient characteristics, such as exposure to antifungal agents.

PCR appears promising because it has demonstrated high sensitivity and specificity in many studies.[19] Like other non–culture-based methods, it may also be useful in predicting the clinical course and outcome of treatment.[78] Due to lack of standardization and limited availability, PCR is currently an adjunctive test used in conjunction with Platelia ELISA, BDG, and high-resolution CT diagnostic tools.

PCR detection assays are rapid, potentially more sensitive than current culture-based methods, and may be designed to detect specific organisms.[79] PCR methods have typically shown excellent sensitivity, ranging from 88% to 100%, and have demonstrated specificities ranging from 65% to 96%.[29,79] Much of this wide variability results from the lack of standardized methodology.[79] Other sources of error include contamination, sampling frequency, inhibitors, and differences in volume sampled. **Table 13** provides a summary of PCR.

Table 13. Characteristics of polymerase chain reaction (PCR) assay[34,80]

Uses	• Detection of fungal DNA most commonly used for *Aspergillus* species in serum and other body fluids
Interpretation	• Two consecutive reproducible positive PCR results required • Detection limits 1-10 fentogram DNA[80]
Sensitivity and Specificity	• Sensitivity usually excellent (88%-100%) • Specificity (65-96%)[34] • Excellent negative predictive value
Factors Affecting Performance	• Exposure to antifungal therapy • Relationship of testing to neutrophil recovery
Limitations[79]	• Wrong choice of specimen (BAL fluid, CSF, serum or whole blood) may limit target availability and introduce false results because of contamination and PCR inhibition • Variation in quality and quantity of DNA • Use of non-specific primers may generate false-positive results • Choice of PCR platform (real-time platform preferred) • Lack of standardization leads to difficulty in comparing results

PCR = polymerase chain reaction; BAL = bronchoalveolar lavage; CSF = cerebrospinal fluid.

Combining Methods to Improve Sensitivity

(1,3)-β-D-glucan and Galactomannan

Although few studies have been conducted, the combination of the BDG and galactomannan detection assays was more effective than either test alone in diagnosing invasive aspergillosis.[15] Both markers were positive in patients with invasive aspergillosis, and the kinetics of their release into the blood were very similar.[15]

PCR and Galactomannan

The combination of quantitative PCR and sandwich ELISA galactomannan assays as part of BAL fluid analysis may improve invasive aspergillosis diagnosis.[81] In a slightly different approach, Millon and colleagues[82] used real-time PCR to process the first galactomannan-positive serum sample. They found that concomitant positive PCR and galactomannan results may justify the initiation of antifungal therapy in

neutropenic patients and that, conversely, negative PCR on the first positive galactomannan sample may justify a delay.

PCR, ELISA, and Galactomannan

Florent and colleagues[83] used a combined PCR-ELISA assay to target cases of invasive aspergillosis caused by *A fumigatus* or *A flavus* in patients with hematological malignancies. They reported sensitivity, specificity, and positive and negative predictive values for proven and probable invasive aspergillosis cases of 63.6%, 89.7%, 63.6%, and 89.7%, respectively. Additional galactomannan assays increased the sensitivity to 83.3% and the negative predictive value to 97.6%. They concluded that the success of serial screening for galactomannan antigenemia and radiological surveillance could be improved by adding PCR-ELISA.

Galactomannan and CT Scans

Maertens and colleagues[60] studied the use of a galactomannan diagnostic assay in combination with CT scans to help physicians determine whether or when to initiate antifungal therapy. This approach led to a 78% reduction in the use of empirical antifungal therapy and to the early initiation of antifungal therapy in 10 episodes that were not suspected of being invasive fungal infection on the basis of clinical features. However, no undetected cases of invasive aspergillosis were identified.

Summary: Diagnosis of Invasive Aspergillosis

Currently, there is a shift in emphasis from waiting for definitive diagnosis to screening high-risk patients so that physicians can administer antifungal therapy early, when it can potentially improve patient outcome. Non–culture-based methods are at the forefront of this paradigm shift. Commercially available and emerging methods to detect fungal antigens, metabolites, and nucleic acids may be useful when used in combination and serially over the period of highest risk. Together with assessment of clinical signs and symptoms, cultures, and radiologic imaging, they may prove useful in screening patients with hematological malignancies or those who are severely immunocompromised. Not only may they help clinicians direct early, aggressive antifungal therapy, but they also show promise as methods of monitoring the course of therapy and response to treatment. For these methods to become standard of care, ready access, appropriate training on these diagnostics, and the consistent application of these methods will be necessary to maximize the value and usefulness of these diagnostic tools. However, challenges and limitations of these methods still remain, and there is no single screening tool that can be used to diagnose all fungal infections. Therefore, the use of antifungal prophylaxis should be considered for certain patients who are at high risk for developing invasive aspergillosis.

TREATMENT GUIDELINES FOR FUNGAL INFECTIONS

Practice Guidelines for the Treatment of Fungal Infections were developed by the Mycoses Study Group of the Infectious Diseases Society of America (IDSA-MSG) and were published in 2000.[12] The guidelines reflect the increasing number of patients at risk for invasive mycotic infection and the increasing survival of these patients, as well as the ever-

improving technology available to practitioners, including intravascular catheters and immunosuppressive drugs. The guidelines also account for several important trends in the pharmaceutical industry during the 1990s: the significant increase in the number of oral and parenteral antifungal agents, the availability of more antifungal susceptibility tests, and progress in determining the application of these tests in routine clinical practice. The guidelines, which contain approximately 1000 literature references and represent the work of over 40 specialists, provide evidence-based recommendations for selecting antifungal therapy. However, since the publication of these guidelines, several agents were approved and new guidelines have been proposed by the European Conference on Infections in Leukemia (ECIL), therefore, the IDSA guidelines are not representative of current practice patterns.

The European Conference on Infections in Leukemia (ECIL), which consisted of representatives from 4 professional organizations—the European Leukemia Net (ELN), European Society for Bone Marrow Transplantation (EBMT), European Organisation for Research and Treatment of Cancer (EORTC), and the Immunocompromised Host Society (ICHS)—convened to develop guidelines on the management of infectious complications in leukemia patients. The First ECIL was held on September 30 and October 1, 2005. Guidelines for the treatment of fungal infections have been have been included in these recommendations. These guidelines, which have not yet been published, were presented at the ICHS meeting in Crans-Montana, Switzerland in July 2006.

The British Society of Medical Mycology has developed proposed standards of care for patients with invasive fungal infection.[84] This document, which was published in 2003, is targeted to improve laboratory practice in the United Kingdom for mycology, thereby reducing deaths from invasive fungal infection to a minimum. The document, which resulted from extensive consultation, defines some absolute standards of care for microbiology, histopathology, radiology and clinical practices that can be audited. Scales for the quality of evidence underlying these standards are those adopted by the Infectious Diseases Society of America.[12]

Treatment Guidelines for Diseases Caused by *Aspergillus*

Practice Guidelines for Diseases Caused by *Aspergillus* were developed by a committee of the Infectious Diseases Society of America and were published in 2000.[9] These evidence-based guidelines are intended to ensure appropriate and successful therapy for the 3 main diseases caused by *Aspergillus* species: invasive aspergillosis, allergic bronchopulmonary aspergillosis, and aspergilloma.[9] They are not intended to provide comprehensive information on the pathogenesis or diagnosis of *Aspergillus* infections. Because the guidelines were developed in the late 1990s, they are dated and do not include the newer antifungal agents.[9]

PREVENTION

Although several measures can reduce the incidence of aspergillosis in hospitalized high-risk patients (**Table 14**), it is impossible to eliminate all risk of infection.[3,4] Some patients are colonized by *Aspergillus* when they enter the hospital.[3] Patients with acquired immunodeficiency syndrome and those receiving corticosteroids are

seldom housed in high-efficiency particulate air-filtered environments. Patients with ongoing risk, such as solid-organ transplant recipients, are discharged home, and other immunocompromised patients receive care on an outpatient basis, resulting in rising numbers of community-acquired infections.[3,4]

Table 14 Measures that may reduce the incidence of aspergillosis in hospitalized high-risk patients[3,4]

- Use of high-efficiency particulate air filtration and laminar air flow
- Increasing the air-change rate in protective environments
- Complete separation of construction and clinical areas
- Attention to routine maintenance and cleaning of showers and water systems
- Preventing exposure to unsterilized pepper or spices and potted plants or shrubs

Prophylactic approaches have been partially successful.[3] Many patients are at risk for long periods of time, and *Aspergillus* is ubiquitous, making any approach other than a pharmaceutical approach untenable. There is little likelihood that an invasive aspergillosis vaccine will be developed in the near future, because humoral immunity appears to play a very small protective role.[3] Studies to identify major *Aspergillus* antigens and their interactions with various parts of the immune system are warranted, as are further investigations of the role of T helper cells. Finally, the ECIL guidelines, which are pending publication, also include recommendations based on evidence-based medicine for antifungal prophylaxis in high risk patient populations.

SUMMARY

Infections due to *Aspergillus* species are clinically important and range from acute to chronic, and localized to disseminated disease. The rate of infections due to

Aspergillus species has increased in recent years, especially in patients who are immunocompromised. The use of traditional methods for the diagnosis of invasive aspergillosis is often associated with unacceptable delays and limited sensitivity, specificity, positive predictive value, and negative predictive value. Therefore, several non–culture-based diagnostic methods are being developed and refined. These include galactomannan, (1,3)-β-D-glucan (BDG), and polymerase chain reaction (PCR)-based assays. Currently, there is a shift in emphasis from waiting for definitive diagnosis to screening high-risk patients using non–culture-based methods so that physicians can administer antifungal therapy early, when it can improve patient outcome. Despite the availability of antifungal agents with activity against *Aspergillus* species, the mortality rate due to invasive aspergillosis remains unacceptably high. Moreover, the success rates of therapies used in the treatment of IA is only about 38 to 50% (salvage) and 40% to almost 65% (primary)**.** Pathogens resistant to antifungal drugs continue to emerge, presenting challenges to physicians in the treatment of invasive aspergillosis.

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