REVIEW

Update on invasive aspergillosis: clinical and diagnostic aspects

P. Muñoz, J. Guinea and E. Bouza

Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario 'Gregorio Marañón', Madrid, Spain

ABSTRACT

Apergillus is a ubiquitious mould that can cause a wide variety of clinical syndromes ranging from mere colonisation to fulminant invasive disease. Invasive aspergillosis (IA) is the most severe presentation of aspergillosis. The lung is usually the portal of entry, from which the pathogen may disseminate to almost any organ, often the brain and skin. The diagnosis remains a significant challenge. It is usually based on a combination of compatible clinical findings in a patient with risk-factors and isolation of the microorganism, radiological data, serological detection of antibodies or antigens, or histopathological evidence of invasion. Chest radiographic findings in patients with pulmonary *Aspergillus* may initially be normal in up to 10% of cases. Computed tomography scanning is probably the most useful imaging technique for the diagnosis of IA, since it may reveal lung lesions up to 5 days earlier than would radiograph techniques simply. Currently available laboratory diagnostic methods include several techniques: histopathological evidence of invasion; isolation of the microorganism and direct microscopy from clinical samples and non-invasive procedures (serological detection of antigens or nucleic material of *Aspergillus*; detection of antibodies). The histological diagnosis of IA requires the presence of invasion by fungus of the *Aspergillus* species. The truth is that, if no other variables are considered, the positive predictive value is very low, and most of the isolates of *A. fumigatus* do not represent proven or probable infection. Several molecules could be used as markers of infection, but two of them are of special interest: *Aspergillus* galactomannan (GM) and (1 → 3)-β-glucan (BG). GM has a high specificity (above 85%) and a reported sensitivity that varies widely (between 30% and 100%). BG, a main cell wall polysaccharide component of *Aspergillus*, can be colourimetrically detected and is useful in diagnosis, with a sensitivity ranging from 50% to 87.5%. A specific *Aspergillus* PCR assay has also been used in the diagnosis of IA and has shown very good results, with a sensitivity and specificity of 100% and 89%, respectively.

Keywords Aspergillosis, *Aspergillus*, galactomannan, 1-3 β-glucan

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INTRODUCTION

Aspergillus is airborne in all environments, both inside and outside the hospital. Only a few species cause human illness, and the immunological status of the individual and the condition of the lung determine the pattern of disease.

In all hosts, the most common species of *Aspergillus* causing disease is *Aspergillus fumigatus*. Far less common are infections caused by *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus*. Mortality associated with invasive aspergillosis (IA) is nearly 100% if the disease is not treated [1]. The attributable mortality of IA varies depending on the type of infectious episode: 60% in global terms, 40% for pulmonary disease, 90% for disseminated disease and practically 100% for disseminated disease with central nervous system (CNS) involvement [2].

In this review, the epidemiology of *Aspergillus* infection and the therapeutic and prophylactic approach to this infection are examined by concentrating on the clinical and diagnostic aspects of the disease. We summarise the status of current knowledge and recommend three excellent reviews that may be of interest [3–5].

Corresponding author and reprint requests: P. Muñoz, Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario 'Gregorio Marañón', Dr Esquerdo 46, 28007 Madrid, Spain
E-mail: pmunoz@.micro.hggm.es

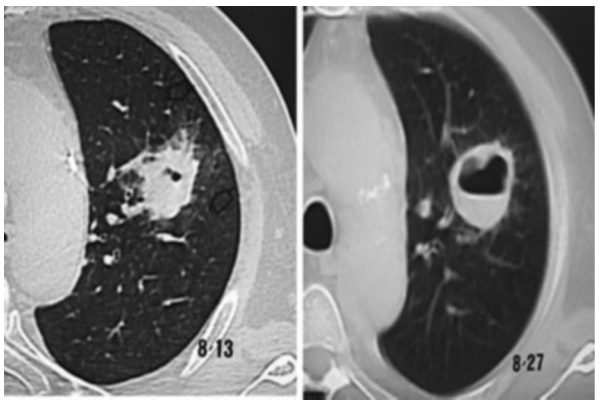

Fig. 1. Pulmonary aspergillosis in a transplant recipient.

Clinical manifestations of *Aspergillus* infection

Aspergillus can cause a wide variety of clinical syndromes, ranging from mere colonisation to fulminant invasive disease. As clinicians become more aware of the classic foms of the disease (i.e., IA in neutropenic patients) and effective preventive measures are established, the face of the disease is changing. In institutions such as ours, IA in chronic obstructive pulmonary disease patients now outnumbers cases in 'classic patients' [6]. Table 1 shows the underlying conditions of the cases of IA diagnosed in our institution over the last few years.

Furthermore, recently characterised 'border presentations' such as semi-invasive or chronic forms demonstrate that there is a certain overlap between entities. Progression from a less aggressive to a more aggressive form is not common [7].

Table 1. Underlying conditions of 260 patients with *Aspergillus fumigatus* recovered in culture (406 isolates) and 31 patients with invasive aspergillosis detected in a general hospital

Underlying conditions	No. of patients	No. of isolates	Cases of invasive aspergillosis
Solid-organ tumour	29	39	3 (10.3%)
COPD + corticoids	100	179	10 (10%)
Leukaemia	10	18	6 (60%)
Lymphoma	6	8	1 (16.7%)
Transplant patients	17	29	2 (11.8%)
HIV	27	50	4 (15%)
Other risk factors	71	91	5 (7%)
Total	260	404	31

COPD, chronic obstructive pulmonary disease.

Specific risk-factors and the host immune system determine which form of aspergillosis develops; for example, acutely invasive aspergillosis is usually found in deeply immunosuppressed patients.

Allergic bronchopulmonary aspergillosis

Allergic bronchopulmonary aspergillosis (ABPA), the least invasive disease caused by *Aspergillus*, is an infrequent chronic disease caused by hypersensitivity to the fungus *Aspergillus fumigatus*, which complicates the evolution of disease in 1–2% of patients with asthma and in 2–15% with cystic fibrosis [8]. More uncommon underlying conditions are hyper-immunoglobulin E syndrome or chronic granulomatous disease. ABPA must be recognised early to avoid progression to bronchiectasis and parenchymal damage.

ABPA typically presents with periods of exacerbation and others of remission. Airway colonisation by *Aspergillus* exacerbates underlying airway injury, leading to chronic inflammation and fibrosis with destruction of bronchial elements, bronchiectasis and scarring. The patients most affected are atopic individuals in their third to fifth decade, with a history of allergy and asthma that is difficult to control.

As with other diseases, e.g., rheumatic fever, no single diagnostic finding is sufficient, so a set of clinical, laboratory and radiographic criteria is needed (Table 2). Six clinical criteria make the diagnosis probable: (1) asthma; (2) peripheral eosinophilia; (3) immediate skin test reactivity to *Aspergillus* (IgE); (4) precipitating antibodies to *Aspergillus* (IgG); (5) elevated serum IgE; and (6) pulmonary infiltrates, which may be transient or fixed. The first five are required criteria for ABPA in patients with asthma.

Other possible clinical criteria include: a sputum culture positive for *Aspergillus*, or detection of compatible hyphae on smears, mucous plugs with degenerated eosinophils (Charcot–Leyden crystals) in sputum, and infiltrates on chest X-rays suggesting bronchial inflammation. In patients with cystic fibrosis, eosinophilia is not a useful diagnostic tool [9]. Lung biopsy is not usually required for the diagnosis. If it is performed, allergic mucin and fungal hyphae indicate the presence of ABPA.

Therapy is aimed at reducing inflammation and immunological activity. Management includes the

Table 2. Criteria of allergic bronchopulmonary aspergillosis for patients with asthma or cystic fibrosis

Asthmatic patients	Asthma
	Central bronchiectasis on chest tomography
	Immediate cutaneous reactivity to *Aspergillus* species (or *Aspergillus fumigatus*)
	Total serum IgE concentration greater than 417 IU/mL (1000 ng/mL)
	Elevated serum IgE and/or IgG antibody to *Aspergillus fumigatus*
	Transient infiltrates on chest radiograph
	Serum precipitins (antibodies) against *Aspergillus fumigatus*
	Peripheral blood eosinophilia
Cystic fibrosis patients	Clinical deterioration not attributable to other aetiology
	Immediate cutaneous reactivity to *Aspergillus* species or presence of IgE to *Aspergillus fumigatus*
	Total serum IgE concentration greater than 500 IU/mL (120 ng/mL)
	One of the following: precipitins to *Aspergillus fumigatus* or new or recent bronchiectasis in CT scans or chest X-ray (mucous plugging/infiltrates) that do not clear after antibiotics and physiotherapy

use of corticosteroids during exacerbations as well as antifungal drugs. A recent Cochrane meta-analysis supports the administration of itraconazole (200 mg for at least 16 weeks) [8].

Aspergillus has also been related to rare cases of allergic fungal sinusitis [10–12]. Advanced allergic sinusitis may be complicated by histological evidence of tissue invasion, and the non-invasive and invasive forms of fungal sinusitis may coexist in the same patient.

COLONISATION AND SUPERFICIAL ASPERGILLOSIS

Aspergilloma

When *Aspergillus* colonises a pre-existing lung cavity, a fungus ball formed by masses of hyphae, blood clot and cellular debris, called an aspergilloma, may appear. This typically affects apex lesions caused by previous tuberculosis (up to 17% of such patients) or other chronic lung diseases such as lung abscess, sarcoidosis, emphysema, neoplasms, or pneumocystis in AIDS.

Sometimes the patient is asymptomatic; however, most patients present with haemoptysis or productive cough, chest pain, dyspnoea, fever, weight loss, or clubbing. The diagnosis is established radiologically by computed tomography (CT) and confirmed by culture or histological identification of *Aspergillus* hyphae in sputum, bronchoalveolar lavage fluid, or trans-thoracic needle aspirates, or by serological demonstration of *Aspergillus* precipitins. Most lesions are solitary and occur in the upper lobes. Cavities average 3–5 cm in diameter. An air crescent lies above the fungus ball, which may move when the patient changes position. The wall and the adjacent pleura show variable thickening.

Therapeutic options are controversial. Approximately 10% of cases resolve spontaneously. Surgery is recommended for severe haemoptysis, but post-operative complications include bronchopleural fistula and empyema. Instillation of various antifungal agents or iodides into the cavity sometimes controls bleeding. An associated invasive disease may occur, especially in AIDS patients. The role of antifungal agents is not clear when local invasion is not established. Aspergillomas carry a 40% 5-year survival rate [13].

Aspergillomas may also appear in the paranasal sinuses [14], kidney [15] and even brain tissue [16].

Other chronic forms of pulmonary aspergillosis

Other chronic forms of pulmonary aspergillosis that have systemic symptoms have been characterised by Denning, who proposed the following nomenclature. Chronic cavitary pulmonary aspergillosis (CCPA) is a form in which the cavities, with or without fungus balls, in the lung increase and expand; in some cases, this progresses to extensive pulmonary fibrosis, termed chronic fibrosing pulmonary aspergillosis. Pleural involvement may occur due to direct invasion or fibrosis. Fibrosis may be limited in extension, but also commonly involves the whole hemithorax. The third form is chronic necrotising pulmonary aspergillosis (CNPA), or sub-acute invasive pulmonary aspergillosis, in which slow progression of one single cavity may be detected over time (months or weeks), the cavity sometimes reaching

large dimensions. In general, expansion of a cavity is much more rapid than in patients with CCPA. Patients with CNPA usually have some degree of immunosuppression (diabetes or corticosteroid use) [13]. CNPA and aspergilloma are probably a continuum of the same pathological process. Furthermore, it is likely that what has been termed complex aspergilloma is synonymous with CCPA, although some patients with CCPA do not have aspergillomas within the cavities. Usually, there is less pleural thickening in CCPA than in aspergilloma, but more pulmonary fibrosis or an enlarging cavity is seen. The distinctions among these categories reflect the dominant clinical and radiological manifestations and their evolution over time.

Constitutional symptoms are practically always present and include weight loss (94%), malaise and fatigue (28%). Cough (78%), shortness of breath (50%), haemoptysis (58%), chest pain (17%), sputum production (11%) and fever (11%) have also been found. The most common clinical presentation involves chronic productive cough, with weight loss and shortness of breath. All patients have radiological evidence of a cavitary lesion in the lung. The proposed criteria for diagnosis of chronic pulmonary aspergillosis are shown in Table 3.

Patients with CNPA respond to systemic antifungal therapy, but this may be a life-long requirement. Itraconazole has been used as a maintenance drug, and its slight inhibition of immune response is deemed to be useful for recovery from chronic pulmonary aspergillosis. Interferon gamma was also helpful in Denning's series. Surgery should be reserved for patients with reasonable respiratory reserve and no other treatment options. It may be appropriate for patients with severe haemoptysis if embolisation fails. *Aspergillus* empyema is a complication of aspergilloma and CNPA, or of the surgery undergone during treatment of these diseases, and is slow to respond to treatment [13].

INVASIVE ASPERGILLOSIS

IA is the most severe presentation of aspergillosis. The lung is usually the portal of entry, from which the pathogen may disseminate to almost any organ [5,17], the brain and skin being the next most common targets.

Invasive pulmonary aspergillosis (IPA)

The lung is affected in up to 92% of cases of IA. Pulmonary symptoms predominate, including non-productive cough, pleuritic pain, low-grade fever, haemoptysis and dyspnoea. Occasionally, patients present with neurological manifestations indicating dissemination and CNS involvement [18].

Haemoptysis and pleuritic pain should be considered alarming symptoms that may anticipate erosion of a major vessel and fatal bleeding. In such cases, an emergency CT-scan should be performed and, if the problem is confirmed, aggressive surgery is indicated [19].

Prompt recognition of this fungal infection is essential for a successful outcome with intensive antifungal therapy. However, both clinical

Table 3. Proposed enrolment criteria for prospective clinical studies of chronic pulmonary aspergillosis (CPA)

1.	Chronic pulmonary or systemic symptoms (duration = 3 months) compatible with CPA, including at least one of the following symptoms: weight loss, productive cough, or haemoptysis
2.	Cavitary pulmonary lesion with evidence of paracavitary infiltrates, new cavity formation, or expansion of cavity size over time
3.	Either positive result of serum *Aspergillus* precipitin test or isolation of *Aspergillus* spp. from pulmonary or pleural cavity
4.	Elevated levels of inflammatory markers (C-reactive protein, plasma viscosity, or erythrocyte sedimentation rate)
5.	Exclusion of other pulmonary pathogens, by results of appropriate cultures and serological tests, that are associated with similar disease presentation, including mycobacteria and endemic fungi (especially *Coccidioides immitis* and *Histoplasma capsulatum*)
6.	No overt immunocompromising conditions (e.g., HIV infection, leukaemia, and chronic granulomatous disease)

All criteria must be met for an individual to be enrolled; for criterion 3, one of the conditions must be met.

symptoms and radiological manifestations may be non-specific at early stages of the disease.

CT-scans are preferable to conventional chest X-ray (Fig. 1). Radiological evidence of IPA includes nodular lesions, with or without cavitation, pleural-based, wedge-shaped infiltrates, alveolar infiltrates and even interstitial and ground-glass opacities [20]. Pleural effusion and pneumothorax may be found.

The diagnosis of IPA is discussed further below, and antifungal therapy is examined by other authors in this supplement. Surgery may be proposed for acute IPA in two situations: to prevent catastrophic haemoptysis due to a paravascular lesion, or for resection of sequestered mycotic deposits that could lead to generalised re-infection [21].

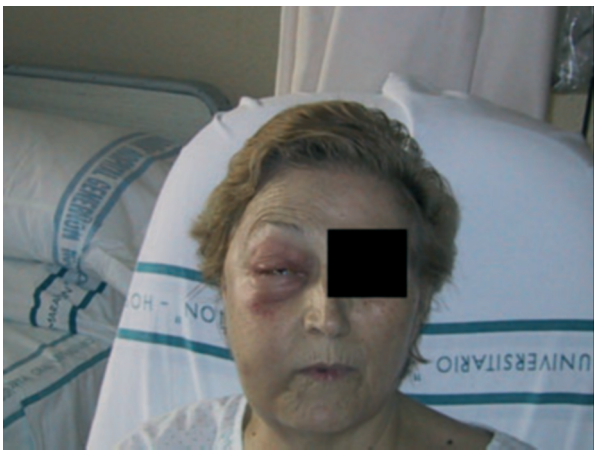

Fig. 2. *Aspergillus* sinusitis in a patient with refractory chronic lymphocytic leukaemia receiving fludarabine.

Tracheobronchitis

Aspergillus tracheobronchitis is an uncommon clinical form of IA, with fungal infection limited entirely or predominantly to the tracheobronchial tree. It has been described mainly in lung and heart–lung transplant recipients [22–24], although it may occur in other patients [25–29].

The clinical presentation includes coughing, fever, wheezing, hypoxia, haemoptysis, and sometimes dyspnoea and severe respiratory failure. Diagnosis is established by fibreoptic bronchoscopy, with fungal growth sometimes obstructing the main bronchus or nodular plaques or pseudomembranes in the trachea or bronchi. Biopsy indicates tissue necrosis and the presence of hyphal elements. Therapy requires systemic administration of antifungal agents or aerosols if dissemination is excluded.

Sinusitis

Aspergillus may invade or colonise the paranasal sinuses. Invasive *Aspergillus* sinusitis may behave as aggressively as that caused by zygomycetes. It has been described mainly in patients with haematological malignancies [30–32] or in transplant recipients [33], but cases in normal hosts have also been described [34].

The symptoms of sinusitis include persistent and significant pain followed by progressive ophthalmic signs, fever, discharge, cough, epistaxis, headache and, eventually, periorbital involvement (Fig. 2). It may, or may not, accompany lung aspergillosis. In the presence of the aforementioned symptoms, CT or magnetic resonance imaging (MRI) and biopsy of any sinonasal lesion in a high-risk patient is mandatory. Clinical features of orbital involvement or CT manifestations of extrasinus spread should signal the possibility of invasion [10,35]. The disease may extend to the palate, contiguous sinuses, orbit or brain.

CT-scans and MRI are the preferred radiological techniques [36]. Pan-fungal and fungus-specific PCR assays can be used in nasal lavages to help establish the diagnosis [37]. Endoscopy may also be useful for diagnosis and drainage [38]. Samples should be cultured in multiple media (Sabouraud dextrose agar, potato dextrose agar, and broth media). Negative specimen microscopy is common [39]. Commercially available antibodies may be useful in differentiating between *Aspergillus* and zygomycetes in histological slides [40].

The combination of systemic antifungal agents and endoscopic surgery, associated or not with Caldwell–Luc surgery, is the best therapeutic option in aggressive cases of sinusitis [41]. Long-term antifungal agents are recommended [42]. Mortality rates range from 11% to 80% and are mainly related to intracranial invasion [30,34,43].

Cerebral aspergillosis

Cerebral aspergillosis may appear as an isolated lesion or, more commonly, as part of a disseminated disease after haematogenous spread from

pulmonary aspergillosis. Neutropenia, transplantation and other forms of impaired cellular immunity are the classic underlying conditions [44,45]. Disseminated infection with CNS involvement occurred in 17% of cases of IA in solid-organ transplantation (SOT) studied in Spain. Brain abscesses are relatively uncommon (0.6%) in SOT patients, although *Aspergillus* is responsible for 78% [46] and 58% [47] of biopsied cerebral mass lesions in patients undergoing allogeneic stem-cell transplant.

Cases of cerebral aspergillosis in non-immunocompromised patients after near drowning, pregnancy or surgery have been described [48–52].

As with other forms of IA, the clinical manifestations of cerebral aspergillosis parallel the vascular tropism of the fungus, leading to infectious cerebral vasculitis, mainly involving thalamoperforating and lenticulostriate arteries, with a high frequency of thalamic or basal nuclei lesions [53]. Presentation includes fever, seizures, alteration of mental status, and CNS depression, and may even be stroke-like. CNS *Aspergillus* infections present either as mass lesions (e.g., brain abscess), or as cerebral infarcts, but rarely as meningitis.

Differential diagnosis includes tuberculosis, lymphoma, and toxoplasmosis. Radiological signs are non-specific [54], and in the case of primary lesions or in patients without a clear diagnosis, stereotactic biopsy may be necessary. The cerebrospinal fluid is almost always sterile, but detection of *Aspergillus* galactomannan in cerebrospinal fluid may contribute to the diagnosis [55]. More usually, CNS aspergillosis is associated with extraneural infection, allowing for sampling without performance of a brain biopsy.

Brain aspergillosis has the worst prognosis among all types of IA. The mortality risk usually exceeds 90%. Data from case reports and a recent retrospective study suggest that neurosurgical interventions, e.g., abscess resections, stereotactic drainage, and intraventricular catheters, might improve the outcome in CNS aspergillosis. Voriconazole, due to good penetration into the CNS and brain tissue, has shown promising results in the treatment of this infection. In a recent retrospective study, a complete or partial response occurred in 35% of patients who were treated with voriconazole for CNS aspergillosis, and the survival rate was 31%. These data support the use of voriconazole in this clinical setting [56,57]. Intracavitary administration of amphotericin B has been used in this context, although its efficacy is controversial [58].

Cutaneous aspergillosis

Cutaneous aspergillosis may occur as a primary event [59–62] or after haematogenous dissemination. Primary cutaneous aspergillosis has been associated with many factors, including occlusive dressings [63], neonatal status [64], leukaemia [65], transplantation [66] or use of permanent intravenous catheters [59]. Surgical wounds [67] or burns [68] may also be invaded (Fig. 3).

In a recent series of patients with haematological malignancies, 15 cases of cutaneous aspergillosis were reported. The skin was involved in 4% of patients with leukaemia and documented *Aspergillus* infection. Primary cutaneous aspergillosis was diagnosed in five cases. Infection was fatal in 11 of 15 cases [69].

Clinically, cutaneous aspergillosis may appear as asymptomatic nodules or as extensive and necrotic lesions. Biopsy, which may prove to be a rapid method of establishing diagnosis, should be performed in all immunocompromised patients with skin lesions.

Other forms of focal aspergillosis

An increasing number of reports deal with unusual manifestations of invasive extrapulmonary aspergillosis, sometimes in immunocompetent

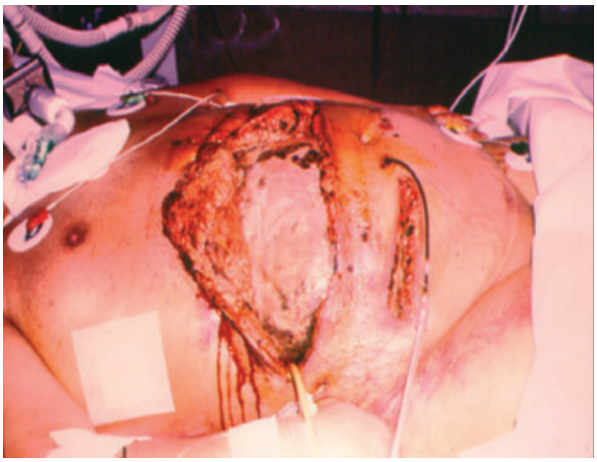

Fig. 3. Wound aspergillosis in a liver transplant recipient. Despite repeated debridements, new areas of necrosis appeared soon afterwards.

individuals. Examples include vertebral osteomyelitis, cholangitis, prosthetic vascular graft infection, endophthalmitis, pacemaker infection and infective endocarditis. Early recognition of these entities allows prompt initiation of antifungal therapies and adjunctive surgical management, if necessary, which may improve the prognosis [4,70–72].

Aspergillus accounted for 26% of cases of infective endocarditis occurring within a month of transplantation [73]. It may affect native or prosthetic valves and has been described after valvular surgery, after drug abuse and in patients requiring ventricular assistance devices [74–78]. Diagnosis is extremely difficult, since blood cultures usually remain negative [79–82]. The clinical features include fever (74%), embolic episodes (69%), a new or changing heart murmur (41%) and sudden visual loss (13%) [83]. Patients with mural endocarditis were more often immunosuppressed, especially due to SOTs, but had a lower frequency of heart murmurs and embolic episodes. Echocardiography revealed vegetations in 78% of the cases in which it was performed. Occasionally, diagnosis is established by culturing major arterial embolisms [84]. Mycotic aneurysm, invasion of the wall of major arteries and even intestinal infarction may occur [76]. The mortality rate is high and surgery is usually required.

Aspergillus osteomyelitis occurs mainly in immunosuppressed hosts, although wound and other infections in 'normal' hosts are seen. It can be induced haematogenously, contiguously or by direct inoculation. In children, contiguous spread from an adjacent pulmonary infection is most common, whereas in adults, haematogenous spread is the rule [85–91]. Cases have been described in patients with chronic granulomatous disease or intravenous drug users [92–94]. The spine is most frequently affected, and the clinical presentation is non-specific. Back pain (53.6% of cases) is the predominant symptom, while neurological deficits are present in 29.2% of patients. White blood cell counts are elevated in 12.2% of patients, and erythrocyte sedimentation rates are >40 mm/h in 39%. The overall recovery rate is 68.3%, and the mortality rate is 26.8% [88]. Radiology, CT-scan and MRI can help to establish the diagnosis, determine the extent of the disease and guide the biopsy. Diagnosis requires demonstration of characteristic hyphae in biopsy material and culture of *Aspergillus*. Although *Aspergillus* osteomyelitis is primarily treated medically, cases with involvement of vital organs and those involving neurological impairment or intolerable pain due to irreversible joint damage may require surgery [95]. Occasionally, vertebral osteomyelitis is complicated by extradural abscess [85].

Disseminated aspergillosis

Disseminated aspergillosis is defined as the involvement of at least two non-contiguous organ sites. *Aspergillus* may disseminate from the lungs to almost any organ [5]. Overall, disseminated disease has been described in 9–36% of kidney recipients, 15–20% of lung recipients, 20–35% of heart recipients and 50–60% of liver recipients with IA [96]. Cytotoxic chemotherapy within a month of death is a risk factor associated with dissemination [97]. As mentioned, skin lesions may be the first clinical manifestation and may lead to the diagnosis [98]. Uncommon manifestations of disseminated aspergillosis include endophthalmitis [99], empyema, arthritis [100], intestinal infarction [101] and thyrotoxicosis [102].

The clinical significance of *Aspergillus* fungaemia in the setting of deep-seated aspergillosis has not been clearly established. Among 107 microbiologically documented *Aspergillus* infections in patients with haematological diseases, *Aspergillus* fungaemia was documented in nine of 89 (10.1%) patients with pulmonary aspergillosis at a median of 5 days from the onset of clinical signs of infection and in one patient with central venous catheter focal infection. The diagnostic role of *Aspergillus* fungaemia in the setting of a deep-seated infection is limited, because blood cultures become positive when a microbiological or clinical diagnosis of aspergillosis has already been made. *Aspergillus* fungaemia does not seem to be necessarily correlated with a disseminated infection or a poorer prognosis [103].

DIAGNOSIS OF INVASIVE ASPERGILLOSIS

The diagnosis of IA remains a significant challenge and is usually based on a combination of compatible clinical findings in a patient with risk factors and isolation of the microorganism, radiological data, serological detection of antibodies or antigens or histopathological evidence of

invasion. Early and accurate diagnosis of IA is essential in order to start timely antifungal therapy and has an impact on the prognosis, but unfortunately, the disease is non-specific in the early stages.

The presence of clinical signs and symptoms in a patient with recognised underlying conditions, together with the presence of a positive culture of one or more clinical samples for *Aspergillus*, is a cause for concern. However, fungus appears in clinical samples at late stages of the disease, and a positive culture for *Aspergillus* does not always indicate true infection, as is shown below. Finally, cases of IA without positive cultures would go undiagnosed. For these three reasons, other diagnostic tools are necessary. Unfortunately, none of the available diagnostic techniques are sufficiently specific and sensitive to be reliable [17].

CLINICAL DIAGNOSIS

Imaging techniques and microbiological examination should come into play in the case of a patient with underlying disease that is recognised as contributing to the development of IA, in conjunction with compatible signs and symptoms.

Imaging techniques

Chest radiographic findings in patients with pulmonary *Aspergillus* infection include nodular opacities, interstitial infiltrates, cavitary lung disease or a pulmonary embolus-type pattern; the chest X-ray may be normal initially in up to 10% of cases [104–107].

CT scanning is probably the most useful imaging technique for the diagnosis of IA, since it may reveal lung lesions up to 5 days earlier than would radiographic techniques simply. A characteristic finding is the 'halo' sign, which appears in 33–66% of patients with IA. However, it is short-lived, and approximately 75% of the initial 'halo' signs disappear within a week [108]. Therefore, to be useful for diagnosis, the CT-scan should be performed within 5 days of the onset of the disease. This lesion is not absolutely specific for IA: in SOT recipients, a pulmonary halo sign is less likely to correlate with IA, and may occur with other types of pneumonia [109].

Another manifestation revealed by imaging, the 'air crescent' sign, does not appear until the third week of the disease, which is too late for it to be useful for diagnosis [108].

Considering that, in the immunosuppressed population, mould infections affect mainly the lungs and sinuses, diagnostic approaches should concentrate on these two anatomical locations, with early and repeated CT-scans, if necessary. In patients with brain lesions, the use of CT-scans or MRI of the neuroaxis is essential for assessing the presence and nature of infectious processes.

MICROBIOLOGICAL DIAGNOSIS

Currently available laboratory diagnostic methods include three groups of techniques: (i) histopathological investigation for evidence of invasion; (ii) isolation of the microorganism and direct microscopy from clinical samples; and (iii) non-invasive procedures, i.e., serological detection of antigens or nucleic material of *Aspergillus*, or detection of antibodies.

Histopathological evidence of invasion

The histological diagnosis of IA requires the presence of invasion by fungus of the *Aspergillus* species. *Aspergillus* hyphae are 2–4 mm wide, frequently septate and branch at 45°. They are best visualised on silver stains and may be missed with routine haematoxylin–eosin stains. In rapidly progressive infections, the hyphae are of even diameter, whereas in more chronic cases, they may have bulbous, widened areas [110]. Sporulation is rarely observed in tissue, except in areas exposed to air (e.g., bronchi and cavitary lesions). In the absence of sporulation, hyphae cannot be readily distinguished from a large number of pathogenic moulds, and the mycological differential diagnosis includes *Scedosporium*, *Fusarium*, *Scopulariopsis* and many other rarer moulds. Therefore, definitive diagnosis requires a culture of the specimen positive for *Aspergillus* [17].

The condition of many susceptible patients prevents confirmatory biopsy specimens from being obtained. Furthermore, open lung biopsies have only a 50% yield, probably because of sampling error. Consequently, in practice, management should progress with the accumulation of clinical, radiological and microbiological criteria that provide different levels of certainty [107]. Any suspicious lesion, e.g., skeletal or

cutaneous, should be biopsied and cultured for fungi.

Isolation of the microorganism and direct microscopy from clinical samples

The early diagnosis of IA in SOT recipients is difficult because fungal cultures in these patients may be negative even when infection is widely disseminated. For instance, blood cultures are notoriously insensitive in *Aspergillus* infections, even with endocarditis. Bronchoalveolar lavage fluid, bronchial or endotracheal aspirates, pleural fluid, sputum, other respiratory fluids and biopsy specimens can be cultured in the laboratory [17]. Pathogenic species of *Aspergillus* recovered from clinical samples usually grow easily and quickly on routine bacteriological and mycological media. Identification is relatively straightforward, according to microscopic criteria and colony (conidial) colour. Formal identification may require culture on specialised media, e.g., Czapek–Dox and malt extract. In the future, molecular methods will be used increasingly to identify unusual species.

Aspergillus fumigatus has been considered the most common species causing invasive aspergillosis, but several reports point to *Aspergillus terreus* as an emerging agent of IA [111–114]. Most positive *Aspergillus terreus* cultures are recovered from the lung, with a smaller proportion being recovered from bone, sinuses or skin [112].

It is increasingly admitted that cultures positive for *Aspergillus fumigatus*, in the appropriate epidemiological and clinical setting, e.g., highly immunosuppressed transplant patients, should not be disregarded, as they are strongly associated with the presence or the risk of IA [115–117]. In the case of haematological patients, the isolation of *Aspergillus fumigatus* by the microbiology laboratory, even from non-sterile samples, is generally regarded as potentially significant. In the case of heart transplant recipients in whom infection is suspected, a culture positive for *Aspergillus* has a positive predictive value (PPV) of 60–70%; the PPV is 78–91% when *Aspergillus fumigatus* is recovered, and 88–100% when *Aspergillus fumigatus* is recovered from a respiratory specimen other than sputum [118].

Unfortunately, this concept is being extrapolated to other populations, thus increasing the workload of the microbiology laboratory and leading to an overestimation of the potential clinical significance of *Aspergillus fumigatus* isolates [115]. If no other variables are considered, the PPV is very low, and most of the isolates of *Aspergillus fumigatus* do not indicate proven or probable infection [119].

We recently studied the workload created by the isolation of *Aspergillus fumigatus* and its overall clinical significance in the microbiology laboratory of a large general teaching hospital [6]. *Aspergillus fumigatus* represented 5.6% of all fungal isolates in the laboratory. It was isolated 21 times per 10 000 hospital admissions, but only four of 10 000 isolates were from patients with IA. The overall probability that a culture positive for *Aspergillus fumigatus* indicated a case of IA in an unselected hospital population was 22.3%. The PPV was 60%, but below 15% in other populations. This study provides a useful score for predicting the probability that an isolate of *Aspergillus fumigatus* indicates IA, which may be of particular interest in cases without histological confirmation. The score gives different numbers of points to the types and numbers of samples, and to the presence of leukaemia, corticosteroid therapy or neutropenia.

Another attempt to discriminate between invasive and coloniser strains resulted in the study of the pathogenicity of a specific isolate. We found a good correlation between the elastase activity index and the invasiveness of clinical isolates of *Aspergillus fumigatus*. Higher elastase activity indexes were related to IA, whereas if the elastase activity index was <1, the probability of invasive disease was very low [120].

Regarding susceptibility testing of fungal isolates, the correlation between MIC and clinical response of increased MICs for moulds has not been established using the standardised method for mould susceptibility (CLSI, formerly NCCLS, protocol M-38A). However, given the emerging development of resistance, susceptibility testing for available antifungal drugs is warranted in the case of infections caused by *Aspergillus* and other moulds.

Non-invasive procedures

Serological detection of antigens

As mentioned, traditional microbiological methods (culture of clinical samples and direct microscopy) have low sensitivity, do not help to

discriminate between infection and colonisation, and may only give positive results at late stages of the disease. Furthermore, in some cases, the underlying condition of the patient prevents the use of invasive techniques to obtain suitable clinical samples. For these reasons, methods for the detection of different circulating markers (e.g., fungal cell wall components and genomic fungal DNA) have been developed in recent years [119,121,122]. The use of serological tests of blood or other fluids (e.g., serum, urine and bronchoalveolar lavage fluid) in the diagnosis of IA is the focus of ongoing clinical investigations [117,123–125].

Several molecules could be used as markers of infection, but two of them are of special interest: *Aspergillus* galactomannan (GM) and (1 → 3)-β-glucan (BG).

GM was the first antigen detected in experimentally infected animals and in patients with IA [126–128]. It is an exo-antigen released from *Aspergillus* hyphae while they invade host tissue and can be detected in serum and other body fluids by an ELISA-based immunocapture assay. This test may help provide an early diagnosis of IA (median of 6 days before signs and symptoms of the disease become apparent) before the infection becomes too extended. The Platelia (Sanofi Diagnostic Pasteur) sandwich ELISA for the detection of GM is currently one of the methods under study. It has a high specificity (above 85%) and a reported sensitivity that varies widely (between 30% and 100%) [122,125,129,130]. A potential explanation for this variation in sensitivity may be the different cut-off values for a positive GM result in Europe and the USA (1.5 ng/mL and 0.5 ng/mL, respectively). The cut-off value is under revision, and several studies have re-evaluated it. Maertens *et al*. showed, in adult neutropenic cancer patients, that the analysis of a single clinical sample using a cut-off of 0.8 was equivalent to obtaining two consecutive positive samples using a cut-off of 0.5 ('dynamic' cut-off) [131]. The sensitivity, specificity, PPV and negative predictive value (NPV) obtained in this study are shown in Table 4. In our opinion, the use of this 'dynamic' cut-off will probably provide the best results in terms of diagnostic effectiveness, or will exclude the possibility of IA.

The false-positive results with GM are a major drawback of this test, and their nature remains difficult to determine. Some drugs, e.g., cyclophosphamide [132] and piperazillin–tazobactam [133], are potential inducers, and certain foods have been proposed as a source of components that lead to false-positive results [134].

Table 4. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different cut-offs with Platelia in a population of adult neutropenic cancer patients (131)

	One single positive sample (cut-off 0.8)	**Two correlative samples (cut-off 0.5)**
Sensitivity	96.5	96.5
Specificity	97.3	98.6
PPV	93.3	98.6
NPV	98.6	98.4
Clinical efficacy	97	98

BG is one of the main cell-wall polysaccharide components of *Aspergillus* [135]; it can be colourimetrically detected and is useful in diagnosis. This component is specific for fungi except for zygomycetes and cryptococci [136]. It is detectable in blood during invasive fungal infections (IFI) caused by *Aspergillus*, *Fusarium* and *Acremonium* [137]. The recently marketed Glucatell assay (Associates of Cape Cod, Falmouth, MA, USA) involves the colourimetric detection of BG in clinical samples. The current cut-off for considering an assay to be positive is a level of BG ≥120 pg/mL in at least one serum sample [138]. The sensitivity of the test ranges from 50% to 87.5% [119,121,138,139].

One of the problems with BG detection is the presence of false-positive results that decrease the specificity of the test. Haemodialysis with cellulose membranes is a well-known explanation for false-positive results with BG, but other reasons remain unexplained [138,140].

Some authors have proposed that a reasonable approach to the clinical application of these assays would be serial screening (weekly or bi-weekly) of patients at high risk of IFI. A recent study published by Pazos *et al*. compared Platelia and Glucatell in adult haematological malignancy patients who were prospectively analysed twice-weekly. The sensitivity, specificity, PPVs and NPVs are shown in Table 5. The results of this comparison are interesting: both tests were positive in the same patients with IA, and the kinetics of both markers were very similar. BG tended to become positive earlier than GM. The authors found discrepancies in patients with false-positive

Table 5. Values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in a comparison of Platelia and Glucatell in adult haematological cancer patients who were prospectively analysed twice-weekly [138]

	Galactomannan	(1 → 3)-β-glucan	Combination
Sensitivity (%)	87.5	87.5	87.5
Specificity (%)	89.6	89.6	100
PPV (%)	70	70	100
NPV (%)	96.3	96.3	96.3

results: one of the two tests was negative in these patients. The combined analysis of both markers was important to identify the patients with false-positive results, since IA could be confirmed only when both markers were positive. One possible solution to the problem of false-positive results from each test would be confirmation by other tests.

The diagnosis of IA is the most important feature of the serological detection of GM and BG, as the detection of antigenaemia dictates the start of therapy [130]. Another advantage is that the detection of these markers may correlate the concentration in serum with treatment efficacy. It has been demonstrated that, for GM, the levels decline when the patient is responding to antifungal treatment and rise with treatment failure [141,142]. Furthermore, monitoring of BG in serum has recently been shown to be a useful tool for predicting the therapeutic outcome of patients with IA [138].

Detection of nuclear material of Aspergillus

A specific *Aspergillus* PCR assay has also been studied in the diagnosis of IA. Some studies have shown very good results, with a sensitivity and specificity of 100% and 89%, respectively [143]. Lung specimens show much higher sensitivity values than blood specimens [144].

Recent protocols call for universal fungal PCR primers, which enable the detection of a broad range of fungi [145–147] with a sensitivity of 1–10 fg of fungal DNA. In one study, the sensitivity of the PCR with whole blood was 100% in patients with documented IFI when two or more samples were analysed [146].

Quantitative PCR can contribute to monitoring of the fungal burden in response to treatment, as has been shown for PCR monitoring of cytomegalovirus disease [148]. The quantitative PCR for diagnosis of IA with bronchoalveolar lavage fluid has shown sensitivity and specificity values of 67% and 100%, respectively [145]. In this study, the authors conclude that the detection of GM in bronchoalveolar lavage fluid and a quantitative PCR assay may enhance bronchoscopic identification of *Aspergillus* species as being the cause of pulmonary disease in haematopoietic stem-cell transplant recipients, and may facilitate diagnoses based on bronchoscopy in high-risk patients.

The previous studies reported that PCR has reasonable sensitivity (ranging from 50% to 100%) and specificity when used to test samples from patients at high risk of IA, but the clinical value of these assays remains unclear, despite the fact that Skladny *et al*. demonstrated a high correlation between positive histology, culture or chest CT findings and nested-PCR results [143]. Another recent study reported the sensitivity of PCR as being lower (63.5%) [149]. The authors attribute this low sensitivity to the small number of episodes of IA; however, in our opinion, it might be the actual situation in most institutions that care for patients susceptible to *Aspergillus* infections.

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