Epidemiology of invasive aspergillosis and risk factors in non neutropaenic patients

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Available online 15 May 2010

Summary

Introduction. — Invasive aspergillosis is a major cause of mortality in allogeneic bone marrow transplant recipients and patients treated for blood malignancies. The diagnostic tools, treatments and preventive strategies, essentially developed for neutropaenic patients, have not been assessed in populations whose immune systems are considered to be competent.

State of the art. — Beside the standard picture of chronic Aspergillus infection, the incidence of invasive aspergillosis is increasing in non neutropaenic patients, such as those with chronic lung diseases or systemic disease treated with long-term immunosuppressive drugs and solid organ transplant recipients. This study reviews the specific features of invasive aspergillosis in non neutropaenic subjects (NNS) and discusses the value of the diagnostic tools and treatment in this population.

Prospects. — A better understanding of the pathophysiology and the epidemiological characteristics of invasive aspergillosis would provide a means of adapting the staging and classification of the disease for NNS.

Conclusions. — Invasive aspergillosis is under diagnosed in NNS who may already be colonised when they receive immunosuppressive treatment; this can lead to an adverse outcome in patients who are considered to be a moderate risk population.

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KEYWORDS
Invasive aspergillosis; Aspergillus fumigatus; Non neutropaenic; Solid organ transplantation; Immunosuppressive treatment


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0761-8425/$ — see front matter © 2010 Published by Elsevier Masson SAS on behalf of SPLF.
doi:10.1016/j.rmr.2010.01.004
Introduction

The clinical spectrum of *Aspergillus* infection is particularly broad; it can present as a standard picture of colonisation (sinuses, bronchi, lungs and external ear), there are also immuno-allergic forms, mycotoxicoses, and even in some cases multivisceral invasive infections that are burdened with a very poor outcome [1].

The determinant factors in the clinical presentation of invasive aspergillosis (IA) that we are considering here are usually related to a specific underlying terrain and predisposing risk factors. There are two different clinical entities:

- **pulmonary aspergillosis** affecting subjects with severe, long-term neutropenia. This form develops rapidly, becomes invasive and then spreads. It was first described when the cytolytic treatments used mainly in haematology were introduced. Neutropaenic subjects (NS) are the group of patients that are the most exposed to invasive pulmonary aspergillosis [2,3];
- **pulmonary aspergillosis in non neutropaenic subjects (NNS).** The main risk factor compromising the immune response in NNS is corticosteroid exposure and this affects two main populations:
  - subjects whose pulmonary defence system is compromised locally, not by a medical treatment in this case but by a chronic underlying disease [6,7]. This category of patients usually suffers from chronic respiratory failure caused by emphysema, bronchial fibrosis or dilatation. Modern methods of managing these chronic disorders with early screening, rehabilitation and long-term systemic or inhaled corticosteroids have extended the patients’ life-expectancy. However, increased survival automatically increases the exposure of these subjects to fungi and airborne contaminants in the environment.
  - solid organ transplant recipients, who have to take long-term immunosuppressive corticosteroids to tolerate their graft. This population ranks second in terms of the risk of invasive pulmonary aspergillosis [4,5].

The literature concerning IA shows that most of the published articles focus on the epidemiology, diagnostic methods, management and treatment of NS with IA. In the recent past, several teams from France and abroad have demonstrated that 30 to 50% of the cases of IA are now diagnosed in NNS and the mortality rate in this category is at least as high as in haematology patients [8—11]. IA is a diagnosis that seems to be envisaged too late in these patients because they are considered as a moderate risk population and the delay in treatment can often result in a fatal outcome. This is why we feel that it is important to consider the epidemiological and clinical features of IA that are specific to NNS, since they are not always identical to those reported in NS.

**Epidemiology and pathophysiology**

Invasive aspergillosis in patients with chronic respiratory disorders

Chronic obstructive pulmonary disease (COPD), emphysema and bronchial dilatation (BD)

The specific feature of *A. fumigatus* in patients with COPD, emphysema, chronic asthma or BD [12] is that it can present as several, sometimes complex, clinical pictures/entities. These are classified according to how they develop and the type of underlying pulmonary lesion and include:

- chronic necrotising pneumonia: in general the disease develops in less than 3 months creating thick-walled de novo cavitary lesions, usually in patients with emphysema, but also in some cases in patients with pulmonary fibrosis or bronchial dilatation;
- chronic cavitary pneumonia: develops in preformed cavitary lesions on diseased lungs inside which the fungus develops slowly, finally spreading outside the walls of the lesion. These cavitary lesions are usually sequelae of tuberculosis, bronchial dystrophy caused by fibrosing pneumonia, lobectomy or pleurectomy cavities. This form usually develops at a slower pace than the chronic necrotising form;
- aspergilloma: in its standard presentation, the fungus forms a mycetoma or fungus ball that remains confined in a pre-existing cavity, although it can also develop into the cavitary form;
- *Aspergillus* bronchitis is a rare form that can develop even in patients with no case history of respiratory disease.

These different clinical forms can all develop into IA and are the clinical expression of a *continuum* of pathophysiological entities that includes:

- colonisation of the airways;
- the *Aspergillus* hyphae break through the epithelial barrier, creating the first stage of invasion in the surrounding tissues, which will in turn cause pulmonary IA;
- the risk of spreading to the rest of the body.

The front line defence cells are the alveolar macrophages whose role is to kill the *Aspergillus* spore by phagocytosis, but in these subjects, they are impaired [13]. Emphysema-related epithelial impairment and an extension of the indications for corticosteroids, especially in the inhaled form, have almost certainly been instrumental in the increase of IA in the “immunocompetent” population. This aspect was recently developed by Ader et al. [7]. Recent immunological investigations have also shown the involvement of the dendritic cells and TH1/TH2 balance, but immunotolerance phenomena mediated by T lympho-
cyte regulation are now considered to be pivotal factors in the susceptibility or resistance to infection [14].

Cystic fibrosis

During the course of their disease almost half of the patients with cystic fibrosis (CF) will be colonised or have a respiratory disorder related to *A. fumigatus* [15]. The simplest clinical form is standard colonisation of the bronchi due to impairment of the bronchial epithelium and its mucus lining.

Airway colonisation by *A. fumigatus* contributes to the process that slowly deteriorates CF patients’ respiratory function during the natural history of the disease [16]. *A. fumigatus* also causes an abnormally frequent form of aspergillosis in these patients: allergic bronchopulmonary aspergillosis (ABPA) is a form that occurs in 5 to 15% of the cases according to the studies, with a prevalence that is higher than in the general population [17—19]. The diagnostic criteria for ABPA are difficult to apply in CF because of the bronchial anomalies the patients have already have; they were the subject of a recent review [17]. Some rare cases of IA reported in the literature mention that the patients had taken corticosteroid treatment [20]. However, it is surprising that a pathogen, which is so frequently encountered in CF has only been the subject of 300 published articles. No study has ever assessed the diagnostic methods or the value of antifungal treatments in eradicating *A. fumigatus* from the airways of CF patients.

- In chronic pulmonary disorders, *A. fumigatus* can present with several clinical pictures: aspergilloma, chronic necrotising pneumonia, chronic cavitary pneumonia or *Aspergillus* bronchitis.
- In CF, the spectrum of clinical presentations ranges between standard colonisation and ABPA, with *Aspergillus* bronchitis and asthma in the intermediate stages.
- All these infectious disorders can develop into an invasive form if the subject has a major risk factor.
- T-lymphocyte mediated immunotolerance phenomena are now considered to be pivotal in the susceptibility or resistance to infection.

Invasive aspergillosis in solid organ transplant recipients

Lung transplantation

**Incidence and extent of the colonisation phenomenon**

The lung is the organ that has the largest surface area in contact with airborne contaminants. Twenty-two to 85% of organ transplant recipients are colonised by *Aspergillus* spores at some point when they are transplanted [21—27]. The post-transplantation colonisation rate is the same for patients who were colonised before they were transplanted as for uncolonised patients, including those who have CF [23,24,28]. The incidence of IA in lung transplant recipients (LTR) is assessed to be between 5 and 10%, but values as high as 26% have also been published (Table 1) [4,24,26].

Aspergillus infections in LTR have a better outcome than those diagnosed in other solid organ transplantations [4]. Colonisation after transplantation is a risk factor for developing IA, but patients can develop IA without having been colonised beforehand [26]. The risk of IA peaks during the first 6 months after transplantation, but around one third of the cases occur later [26,29].

**The different clinical forms of Aspergillus disease in lung transplant recipients**

These include:
- standard bronchial colonisation;
- pseudomembranous necrotising *Aspergillus* tracheobronchitis with a specific form that affects the bronchial anastomoses;
- IA, the most serious clinical form;
- ABPA, which occurs mainly as a relapse after transplantation in patients with CF.

According to a recent review of the literature, the relative frequency of the three main forms of aspergillosis in LTR is 26% for bronchial colonisation, 4% for pseudomembranous necrotising *Aspergillus* tracheobronchitis and 5% for IA with a mortality rate above 50% [29]. There are also disseminated invasive forms of the disease, *Aspergillus* endocarditis for example, that are burdened with a very poor outcome [30,31]. Pulmonary AI peaks at two time points in lung transplantation. The early phase after transplantation is the period during which the immunosuppressive treatment is the strongest and the Anastomotic areas are an ideal portal of entry for *Aspergillus* infection because they are located in the proximal part of the respiratory tree and carry a risk of ischemic lesions. The second phase, which occurs later, is when the patient develops bronchiolitis obliterans. The epithelial damage caused by chronic graft rejection can then become the seat of an *Aspergillus* infection, exacerbated by an increase in the immunosuppressive treatment [32].

When only one lung is transplanted, the native lung has also been shown to be the culprit in the onset of IA [24,33–37]. *Cytomegalovirus* (CMV) also predisposes to IA [26,27]. This factor may be directly involved in *A. fumigatus* pulmonary infection as in vitro experiments on co-infection seem to demonstrate, but it may also be due to an indirect effect of a more profoundly depressed immune response [22,23,28,33]. The role of immunosuppressive treatment as a risk factor for developing IA in lung transplantation has not been studied in detail. The dose of corticosteroids taken by the patient does not in itself seem to be a risk factor for developing IA.

Solid organ transplants other than lung

**Incidence**

The incidence is assessed to range between 0 and 15% in the transplantation of other solid organs (Table 1). However, it varies from one institution to another and also according to the organ transplanted. The incidence decreases in the following order: heart, small bowel, liver, pancreas and kidney (excluding the lungs) [5]. In a prospective survey carried out in 18 hospitals in Paris and the surrounding area between 1994 and 1999, the incidence of IA was 10.7% in bowel transplantation, 1.9% in liver transplantation, 1.3%...
in heart transplantation, 0.4% in kidney transplantation and 0% (zero out of 86) in kidney-pancreas transplantation [38]. In the same study, the incidence of post-transplantation IA observed after allogeneic bone marrow transplantation was 12.8%. At Stanford, 56 of the 844 heart transplant recipients (6.6%) operated between 1980 and 1998 presented with pulmonary or extrapulmonary IA [39]. After kidney transplantation, aspergillosis is rare, but an increased incidence has been observed when hospital maintenance work is carried out [40].

**Time of onset**

IA occurs mainly in the first 3 months after solid organ transplantation as it does in lung transplantation, when the patient’s immune system is in a state of profound immunosuppression (Table 1). The median time from transplantation to onset is usually 17 days (6–1107) in liver transplantation, 45 days (12–365) in heart transplantation and 82 days (20–801) in kidney transplantation [5,41]. An increase in the time to onset was recently observed in liver transplantation, 50% of IA now occurs over 90 days after surgery [42].

**Exacerbating factors**

The role of immunosuppressive treatment. Corticosteroids play an important role in the immunosuppressive drug regimens used in solid organ transplantation [43]. The risk is dose-dependent and increases with the duration of the treatment. Globally, the relative risk of infectious complications related to steroid treatment is 1.6 [44] but only if the daily dose of prednisone is below 10 mg or the cumulative dose is below 700 mg. In actual practice, a dose of 1 mg/kg per day or more induces a marked sensitivity to infections after a period of only a few weeks [45]. The specific role that the other immunosuppressive drugs play has not yet been established.

Role of colonisation. In a series of 25 kidney transplant recipients, it was shown that 45% of the patients with a positive culture developed IA [46]. Seventy-two per cent of liver transplant recipients with positive Aspergillus respiratory tract cultures and up to 50% of those who had positive abdominal drainage cultures developed IA [47]. In liver or kidney transplantation, the presence of more than two colonies and/or the isolation of Aspergillus in the cultures from more than one site has been associated with a positive predictive value of 70%, a sensitivity of 93% and a specificity of 75% for developing IA [48]. In heart transplantation, the positive predictive value of isolating Aspergillus ranges between 60 and 70%, 78 and 91% when the strain is A. fumigatus, and 88 to 100% when A. fumigatus is isolated from a respiratory sample other than sputum [49]. Cases of cutaneous aspergillosis [50] and aspergillosis of the surgical wound bear witness to the role of skin colonisation and prior preoperative airborne contamination and this does not only apply to immunocompromised patients.

Other risk factors have been reported. The risk related to lympholytic treatments (immunoglobulins anti-lymphocyte agents, OKT3) has also been acknowledged for many years [47,51]. More recently it was also demonstrated that the risk factors for IA were revision surgery or retransplantation, the indication for liver transplantation for acute liver failure, CMV infection and post-transplantation dialysis, both in liver and heart transplantation [5,49,52]. CMV infection is the only independent risk factor for late-onset aspergillosis (occurring later than 100 days after surgery) in liver transplantation [52]. This risk factor, which also applies to lung transplantation, suggests that CMV is a marker of profound immunosuppression, indicating a higher risk of Aspergillus infection. Lastly, it has also been suggested that prophylactic fluconazole treatment could also increase the subsequent risk of infection with mycelium type fungi [52,53].

Risk of dissemination. In organ transplant recipients, IA affects the lungs in 90% of the cases. It can also affect the central nervous system in 10 to 50%, although this is not as well known [51]. Cerebral abscesses are rare in organ transplantation, but are due to Aspergillus in 78% of the cases [54]. Epidural abscesses are also found [55]. Disseminated aspergillosis (involving at least two non-contiguous organs) has been reported in 9 to 36% of the cases of kidney transplantation, 20 to 35% of the cases of heart transplantation and 50 to 60% of the cases of liver transplantation [5,41,56]. The other clinical presentations include, among others: mediastinitis and osteomyelitis after heart surgery [57,58], skin infections [59,60], and infections of the surgical wound in liver transplantation, [61–64] endocarditis (26% of the cases of infectious endocarditis occur within 1 month of transplantation) [56], eye

<table>
<thead>
<tr>
<th>Type of transplant</th>
<th>Mean incidence (% and interval)</th>
<th>Mean time to onset (in days and interval)</th>
<th>% of disseminated aspergillosis</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2 (1–8)</td>
<td>17 (6–1.107)</td>
<td>50–60</td>
<td>87</td>
</tr>
<tr>
<td>Lung</td>
<td>6 (3–14)</td>
<td>120 (4–1.410)</td>
<td>15–20</td>
<td>68</td>
</tr>
<tr>
<td>Heart</td>
<td>5.2 (1–15)</td>
<td>45 (12–365)</td>
<td>20–35</td>
<td>78</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.7 (0–4)</td>
<td>82 (20–801)</td>
<td>9–36</td>
<td>77</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.1–2.9</td>
<td>NA</td>
<td>NA</td>
<td>100</td>
</tr>
<tr>
<td>Small bowel</td>
<td>2.2 (0–10)</td>
<td>289 (10–956)</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Allogeneic stem cell</td>
<td>10 (5–26)</td>
<td>78 (46–120)</td>
<td>27–30</td>
<td>78–92</td>
</tr>
<tr>
<td>Autologous stem cell</td>
<td>4.8 (2–6)</td>
<td>20 (7–456)</td>
<td>10–20</td>
<td>78–92</td>
</tr>
<tr>
<td>Non myeloablative stem cell</td>
<td>11 (8–23)</td>
<td>107 (4–282)</td>
<td>34</td>
<td>63–67</td>
</tr>
</tbody>
</table>

NA: not available.

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Table 1  Epidemiological characteristics of invasive aspergillosis in solid organ transplant recipients, adapted from Singh and Paterson [5].
Invasive aspergillosis in non-transplanted non neutropaenic patients

Systemic disease and immunosuppressive treatment
IA has also been reported in patients other than organ transplant recipients treated with immunosuppressive drugs, but the incidence is not as well known (Table 2). In these cases, the immunosuppressive treatments used almost always included corticosteroids and we now have a better understanding of their effects on the host’s immune response [13,71,72]. IA has in particular been reported in patients with lupus [73–76], vasculitis and rheumatoid arthritis [77], Horton’s disease [78], auto-immune haemolytic anaemia [79], and even in Crohn’s disease [80]. Two cases of pulmonary aspergillosis were recently reported after infliximab treatment (anti-TNFα antibodies) [81,82].

HIV infection
A few cases of IA have been described in AIDS [83,84]. HIV positive patients have two main predisposing factors, which are neutropaenia and corticosteroid treatment. IA is more commonly seen in subjects whose CD4 count is below 50/mm³.

Risk of IA via an unusual portal of entry
Aspergillosis can also occur in burns patients [85]. It is more common in subjects whose burns affect over 50% of their body surface, the burn wounds being the portal of entry for Aspergillus [50]. The risk of dissemination has also been reported [86]. Infections of the surgical wound have also been described after neurosurgery [87,88] or abdominal surgery [89]. Pulmonary or cerebromeningeal aspergillosis has also been reported after near-drowning [90,91]. In the literature on fungal endocarditis, the risk factors the most frequently involved are surgery, especially for valve repair and when vascular access devices are used [92,93]. Ellis reported 270 cases of fungal endocarditis, 66 of which (24%) were due to Aspergillus, and 78.5% of the patients did not have the traditional factors of immunosuppression. In another review, at least 21 patients out of 39 developed endocarditis due to a mycelium type fungus (71.8% were due to Aspergillus) and did not present the risk factors either [92,93]. With respect to Aspergillus infections on a native valve, 1/3 of the patients were not considered to be immunocompromised [94]. Cases of Aspergillus infected pacemakers have been reported in immunocompetent subjects [95]. These data suggest that surgery, implanted prosthetic devices and traumatic injuries are risk factors for aspergillosis; the infection is initially circumscribed, then spreads in a secondary stage, including in immunocompetent patients.

Absence of the standard risk factors
In a few rare cases, IA can also occur in patients with liver cirrhosis, bacterial septic shock or who are very elderly but have none of the standard risk factors [10].

Principles of diagnosis

Laboratory findings

Mycology
This involves direct microscopic examination to visualise the hyphae of the mycelium; the specimen is then cultured on specific media to show evidence of the fungal agent. In solid organ transplant recipients, sensitivity to culture may vary according to the site the specimen is harvested from and the type of transplantation; it is around 50% for different respiratory specimens [62,96]. A. fumigatus is usually present but other species of Aspergillus and sometimes other moulds (Fusarium sp., Scedosporium sp., mucorales) can also cause a similar picture and be sensitive to very different antifungal agents. The histopathology will confirm vascular invasion by the fungus, but will not confirm the species; this can only be obtained by systematic culture of the biopsy specimens. Lastly, molecular biology can also be used to detect Aspergillus sp. but is only available in specialised laboratories. It is an important part of the diagnostic work-up,
Table 2  Major risk factors for invasive aspergillosis in non neutropaenic patients.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Chronic Aspergillus disease on impaired lungs</th>
<th>Chronic obstructive pulmonary disease (COPD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chronic obstructive pulmonary disease (COPD)</td>
<td>Chronic asthma</td>
</tr>
<tr>
<td></td>
<td>Emphysema</td>
<td>Bronchial dilatation</td>
</tr>
<tr>
<td></td>
<td>Bronchial dilatation</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td></td>
<td>Cystic fibrosis</td>
<td>Pulmonary fibrosis</td>
</tr>
<tr>
<td></td>
<td>Solid organ transplantation</td>
<td>Applies to all organs</td>
</tr>
<tr>
<td></td>
<td>Applies to all organs</td>
<td>Prior colonisation of patient</td>
</tr>
<tr>
<td></td>
<td>Applies to all organs</td>
<td>Impaired immune response</td>
</tr>
<tr>
<td></td>
<td>Applies to all organs</td>
<td>Cytomegalovirus infection</td>
</tr>
<tr>
<td></td>
<td>Applies to all organs</td>
<td>Immunosuppressive treatment</td>
</tr>
<tr>
<td></td>
<td>Applies to all organs</td>
<td>Post-transplantation dialysis</td>
</tr>
<tr>
<td></td>
<td>Long-term immunosuppressive treatment</td>
<td>Corticosteroids</td>
</tr>
<tr>
<td></td>
<td>Long-term immunosuppressive treatment</td>
<td>Sirolimus</td>
</tr>
<tr>
<td></td>
<td>Long-term immunosuppressive treatment</td>
<td>Lympholytic treatment (anti-lymphocyte globulins, OKT3)</td>
</tr>
<tr>
<td></td>
<td>Long-term immunosuppressive treatment</td>
<td>Infliximab</td>
</tr>
<tr>
<td></td>
<td>Other risk factors</td>
<td>Systemic disease: lupus, vascularitis, rheumatoid arthritis, Horton’s disease, pemphigus, Crohn’s disease, auto-immune haemolytic anaemia</td>
</tr>
<tr>
<td></td>
<td>Other risk factors</td>
<td>HIV</td>
</tr>
<tr>
<td></td>
<td>Other risk factors</td>
<td>Extensive burns</td>
</tr>
<tr>
<td></td>
<td>Other risk factors</td>
<td>Infection of surgical wound</td>
</tr>
<tr>
<td></td>
<td>Other risk factors</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td></td>
<td>Other risk factors</td>
<td>Septic shock</td>
</tr>
<tr>
<td></td>
<td>Other risk factors</td>
<td>Street accident, drowning</td>
</tr>
</tbody>
</table>

supplying information on the type of strain, although the methods are not yet standardised [97].

However, the result must always be interpreted with caution when the specimen is harvested from a non-sterile site because it is not always easy to discriminate between infection and colonisation. The presence of Aspergillus-type hyphae in the direct microscopic examination can be considered as a guide [98], especially if they are found repeatedly.

Antibody assays

Antibody assays show the host’s humoral immune response (HIR) to the fungus. This involves researching precipitating antibodies (precipitines) and is of great diagnostic value in ABPA, extrinsic alveolitis and aspergilloma. Antibody tests (total or specific IgE assays) can also be useful in the case of immune-allergic infection and also in chronic aspergillosis [5,99]. In IA, the antibody assays are more often positive when the subject is non neutropaenic than in NS, in whom the test contributes very little to the diagnosis.

The circulating galactomannan antigen of A. fumigatus

The circulating galactomannan antigen of A. fumigatus is a marker that shows that the fungus has disseminated into the blood stream. This assay was initially developed for neutropaenic patients in whom the antibody test was impossible to interpret and it has been mainly assessed in bone marrow transplant recipients with a median sensitivity ranging between 70 and 90% according to the studies [100–104]. Its sensitivity seems to peak when the Aspergillus antigenaemia test is performed systematically twice a week during the neutropaenic period, thus providing information on the kinetics of the antigen. In this same population, the specificity of a positive test result is also estimated to range between 70 and 90% [100]. There are more false positives in paediatric haematology. This can be due to various different factors: drug interactions (especially with some batches of tazocillin and amoxicillin) [105,106] or cross-reactions with bacterial epitopes such as those produced by Bifidobacterium sp. [107] and a positive response can also be related to the translocation of the galactomannan antigen from the patient’s dietary intake [108]. In NN subjects, the test can also contribute to the diagnosis, although the boundaries of interpretation are more restricted for two reasons:

- The NN population has been insufficiently assessed;
- it is difficult to interpret the result of a punctual assay because the antigen has a fast clearance rate; a negative result does not therefore eliminate the diagnosis. It would be interesting to repeat the test several times but it is more difficult to envisage systematic follow-up in these patients than in neutropaenic patients.

In view of the current data we have galactomannan antigen blood tests are disappointing in LTR because their sensitivity is only 30% whereas in liver transplantation, their
sensitivity is above 55% and the specificity is excellent [52,109,110]. Positive blood antigens seem to be a predictor of mortality and this has been reported in ICU and respiratory failure patients [10,111]. Contradictory results have been recently reported on the diagnostic input of screening for the galactomannan antigen in bronchoalveolar lavage fluid (BALF) of NNS. Meersseman et al. estimated that the sensitivity and specificity of the test were respectively 88 and 87% on a series of 100 patients, including 26 cases of IA and they consider that its input is complementary to the serum tests [111]. However, Nguyen et al. emphasize the issue of false positives, especially in cases of fungal colonisation of patients with respiratory failure [112].

Histology findings
The histology of the biopsy specimens is both sensitive and specific and therefore of paramount importance; the histology slides show the presence of hyphae inside the tissues, thus proving the invasive nature of the infection and confirming IA. However, they do not identify the fungus because the "Aspergillus-type" hyphae may very well be Aspergillus sp. mycelium, but could also be other mycelium producing fungi such as Scedosporium sp. or Fusarium sp. A CT-guided lung biopsy and/or surgery are more easily envisaged in NNS and in many cases are an effective way of obtaining a sure histological diagnosis.

Radiology findings
The radiological semiology is particularly varied in Aspergillus infection. In cases of lung infection in NNS, it can range from a picture of non-specific infiltration reported during the course of an infectious lung disorder to a far more suggestive picture:
- the presence of one or more nodules is often suggestive of IA in LTR. The nodules can become/hollow and are often small and peripheral. In solid organ transplantation, larger masses or infiltrations are also highly suggestive. A high-resolution thoracic CT-scan will afford an earlier diagnosis [54]. The differential diagnoses are other infectious agents (Nocardia, Staphylococcus...) or non-infectious disorders (lymphoma, lung cancer, pulmonary embolism);
- the halo sign is non-specific [113] or may be missing in solid organ transplant recipients [114], whereas it is an early, essential sign in bone marrow transplant recipients [115]. It shows the presence of bleeding around a nodular lesion;
- the air crescent sign occurs later and shows polymuclear neutrophil detersion of the infarcted tissue on the edge of the apleastic areas.

Among the secondary sites for invasive pulmonary aspergillosis, the brain is the most frequently affected and nuclear magnetic resonance imaging (MRI) will discriminate between active lesions and sequelae (Fig. 1).

It is important to suspect possible IA in any immunocompromised or respiratory failure patient if the radiographs are suggestive and/or the patient has a lung disorder that is resistant to antibiotic treatment; a thoracic CT-scan and an Aspergillus antigen blood test must be prescribed as soon as possible or a histology sample should be taken. The diagnostic criteria published by EORTC do not apply to the diagnosis of any specific patient group; they were designed to homogenise the patient groups in clinical studies [116]. These criteria are difficult to apply to other groups of NNS. They will probably be changed in the near future in order to improve them and extend them to other immunocompromised populations such as solid organ transplant recipients.

- Diagnosis is based on the mycology findings (direct examination and isolation of Aspergillus in the cultures and on the pathology slides) confirming invasion of the blood vessels.
- Antibody serology (detection of anti-Aspergillus precipitins) yields information for the diagnosis of ABPA, extrinsic alveolitis and aspergilloma, but the only argument in favour of IA is based on detecting circulating A. fumigatus galactomannan antigen in the bloodstream; the galactomannan antigen is not very sensitive in LTR.

![Clinical case of invasive aspergillosis after cortisosteroid treatment: A. Thoracic CT-scan of the chest at the time of diagnosis. B, C. Cerebral MRI after 2 months of antifungal treatment showing active lesions. D, E, F. Sequelae lesions after recovery (8 months of treatment) on thoracic CT-scan and cerebral MRI.](image-url)
Principles of treatment

The indications and the effectiveness of antifungal treatment in IA in the course of chronic respiratory disorders has never been the subject of comparative clinical efficacy studies. In organ transplantation, the treatments for IA are no different to those used in NS. There are some specific issues related to the interaction between antifungal agents and immunosuppressive drugs. Amphotericin B desoxycholate (AmB-d) is not recommended because of the risk of renal failure related to high daily doses and length of treatment; in addition associated with immunosuppressive drugs, it is toxic for the kidneys (anticalcineurins) [117]. Lipid forms of AmB are preferable; AmB in a lipid complex appeared to be more effective than AmB-d in a retrospective study [118]. Liposomal AmB seems to be less toxic for the kidneys than AmB in a lipid complex in NS [119]. Although the usual dose is 3 mg/kg per day, the appropriate dose of liposomal AmB remains a subject of debate, a dose of 4 mg/kg per day does not seem more effective than 1 mg/kg per day [120]. Itraconazole has been associated with a complete or partial response rate of 57% [121], but substantially increases the blood concentrations of cyclosporine and, above all, of tacrolimus. More recently, voriconazole has been shown to be superior to AmB-d [122]. This study included a sub-group of 30 patients who did not have haematological disorders but had either had solid organ transplantation or were treated by steroids, with no further details. In this subgroup, voriconazole also seemed to be superior to AmB-d. The results of this study should be confirmed in a larger population of NNS who have not had haematopoietic stem cell grafts. A recent study on solid organ transplant recipients compared an association of voriconazole and caspofungin as a first line treatment (n = 40) to a historical control group treated with AmB in a lipid formulation (n = 47), although the methodology of the study is the subject of some criticism. The superiority of the association was only demonstrated in the patients who had IA caused by Aspergillus fumigatus and those with renal failure [123]. In addition, as for itraconazole, voriconazole is a powerful anticalcineurin inhibitor. The dose of cyclosporine should be divided by two in kidney transplantation [124]. The usual recommendation is also to reduce the dose of tacrolimus by two thirds. In liver transplantation, it has been demonstrated that voriconazole can increase the AUC of tacrolimus by 10 and therefore the dose should be reduced by 90% [125,126]. Posaconazole has recently been authorised in the treatment of refractory IA and also increases the AUC of tacrolimus, thereby decreasing cyclosporine clearance. However, very little data is available at present [127]. Over the past few years, a new class of antifungal agents has been available on the market: the echinocandins. The first molecule marketed for the treatment of IA in this family was caspofungine acetate. The doses of caspofungin must be reduced by half if the patient has liver failure. Caspofungin reduces the AUC of tacrolimus by 20%. Cyclosporine increases the AUC of caspofungin by around 35%. Micafungin and anidulafungin, two new echinocandins are now available in Europe. Very little clinical data assessing the value of antifungal associations is available despite the fact that the mortality rate from IA in organ transplantation is still high (Table 1) [4,38], and can reach 92% in liver transplantation [62]. In this type of organ transplantation, the mortality seems to have decreased (to around 60%) in the late-onset cases of aspergillosis [42]. Exceptionally, in heart or liver transplantation, some series have reported clearly higher survival rates rising to 70 and 83% [57,128,129]. An association of surgery and antifungal agents can contribute to improving the outcome in single or circumscribed lesions [128,130,131].

No antifungal agent has been assessed as a prophylactic treatment in a randomised, controlled study in lung, kidney, liver or heart transplantation. Strategies differ according to the institution, the type of transplantation (for which the incidence can vary) and when outbreaks occur. Chemoprophylaxis for IA has not been specifically recommended in solid organ transplantation and remains a subject of debate [132,133], whereas recent data stress the value of posaconazole as a prophylactic measure in NS [134–135] and itraconazole has also been evaluated in haematology [136–138]. Itraconazole prophylaxis might play a significant protective role against the onset of aspergillosis and have a positive impact on the 1-year follow-up in heart transplantation [139]. In liver transplantation, the lipid formulation of Amphotericin B seems to reduce the risk of IA in high-risk (dialysis) subjects [140].

The treatments for IA in NNS are no different to those used in neutropaenic patients.
- It has been established that voriconazole is superior to amphotericin B.
- When polyenes are used in transplant recipients, the lipid forms of AmB-d should be preferred.
- There are specific issues related to drug interactions between the antifungal agents and immunosuppressive drugs used in solid organ transplantation.
- The response rate with itraconazole is 57% but this agent increases the blood concentrations of cyclosporine and tacrolimus in particular.
- Caspofungin reduces the AUC of tacrolimus by 20% and cyclosporine increases the AUC of caspofungin by around 35%.
- The mortality rate for IA is still high in transplant recipients.
- Chemoprophylactic strategies remain a subject of debate in non neutropaenic patients.

Conclusion

Thus, the epidemiological data and clinical findings for IA in NNS are not identical to those reported in NS. It is essential
to be aware of these differences if we are to improve the management of IA in the future because it is often treated too late and can lead to a fatal outcome in subjects that are considered to be a moderate risk population. The strategies used to diagnose, treat and prevent IA need further evaluation analysing larger series of patients in the same way as in the neutropaenic population. This means that to start with specific definitions and practices must be set out for non neutropaenic patients, making a special effort to standardise them.

Learning points
- We know very little about the methods to diagnose, treat and prevent IA in subjects with a moderately compromised immune response.
- The diagnosis of IA is often made too late in NNS that are considered as a moderate risk group.
- The incidence of IA is increasing in non neutropaenic patients.
- The main risk factor compromising the immune response of NNS is corticosteroid treatment.
- IA can occur in NNS who have chronic bronchopulmonary disease, in organ transplant recipients (especially if they have CMV infection), in subjects receiving immunosuppressive treatment, those with systemic diseases and post-transplant dialysis.
- There are no clinical efficacy studies comparing the different antifungal agents in curative or preventive treatment in the non neutropaenic population.
- IA still often has a fatal outcome in NNS.

References

Invasive aspergillosis in non-neutropenic subjects


Invasive aspergillosis in non neutropaenic subjects


