

## Mini-Symposium: Fungi and The Paediatric Lung

*Aspergillus* and the paediatric lungElpis Hatziagorou<sup>1</sup>, Thomas J. Walsh<sup>2</sup>, John N. Tsanakas<sup>1</sup>, Emmanuel Roilides<sup>1,2,\*</sup><sup>1</sup> 3rd Department of Paediatrics, Aristotle University, Hippokraton Hospital, Konstantinoupoleos 49, GR-54642 Thessaloniki, Greece<sup>2</sup> Immunocompromised Host Section, Paediatric Oncology Branch, National Cancer Institute, Bethesda MD, USA

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## SUMMARY

*Aspergillus* spp produce a wide range of saprophytic and invasive syndromes in the lungs, including allergic bronchopulmonary aspergillosis (ABPA), aspergilloma and invasive pulmonary aspergillosis (IPA). ABPA results from hypersensitivity to the fungus, and mainly affects patients with asthma or cystic fibrosis (CF). The treatment of choice consists of systemic corticosteroids and itraconazole. Aspergilloma is managed by observation or surgery. IPA is predominantly seen in patients with haematological malignancies, chronic granulomatous disease or immunosuppressive treatment. With the use of aggressive therapies for end-stage CF, such as heart-lung transplantation, the potential for a patient to convert from colonization or ABPA to IPA has increased. Suggestive clinical and radiological findings, supplemented with mycological data using serology and molecular biology, have enhanced the capacity to diagnose IPA in paediatric patients. While voriconazole is considered the first-line therapy in IPA, several other antifungal agents may be appropriate alternatives.

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## INTRODUCTION

*Aspergillus* spp are ubiquitous fungi in nature and are commonly found as saprophytes in soil, plants and decomposing organic matter.<sup>1</sup> *Aspergillus* spp are typical opportunistic organisms producing a wide range of saprophytic and invasive syndromes in the lungs. Pulmonary infections due to *Aspergillus* spp are usually acquired through inhalation of conidia.<sup>2</sup> These forms measure 2–3.5 µm and are therefore able to reach the terminal airways and alveoli where they can grow to hyphae at human body temperature.

The innate host defence system against *Aspergillus* includes dedicated phagocytic cells [peripheral blood monocytes, monocyte-derived macrophages, pulmonary alveolar macrophages, neutrophils, myeloid dendritic cells (DC) and natural killer (NK) cells], cytokines, chemokines, Toll-like receptors and antimicrobial peptides.<sup>3</sup> These arms of the innate host response are part of a precisely regulated and complex network. Factors contributing to development of invasive aspergillosis (IA) include neutropenia, disorders in phagocytosis, deficiencies in T-cell number or function, immunosuppressive therapy and use of invasive devices, together with fixation methods like arm boards and adhesive tape, especially in children.<sup>4</sup>

Inhalation of *Aspergillus* conidia or hyphal fragments may result in colonization of the airways. Colonization may result in

innocuous non-pathogenic saprophytic growth in healthy individuals or in disease. Aspergillomas, which may be quiescent or cause symptoms, especially recurrent haemoptysis, may develop in pre-existing cavities. In contrast, invasive disease in the lungs and elsewhere may develop in patients with compromised local or systemic antifungal defence mechanisms. In addition to its ability to colonize and invade the human respiratory tract, *Aspergillus* has a significant potential to act as a powerful allergen, resulting in asthma and allergic bronchopulmonary aspergillosis (ABPA).<sup>2</sup> In individuals with a predilection for allergic reactions, as in patients with ABPA, *Aspergillus* conidia and hyphal fragments in the bronchial tree induce an allergic state.

The most common species causing invasive pulmonary aspergillosis (IPA) is *Aspergillus fumigatus* (up to 90% in some series) followed by *A. flavus*, *A. niger*, *A. terreus* and *A. nidulans*. On the other hand, the most common allergens are *A. fumigatus* and *A. clavatus*. *Aspergillus* spp are the most frequent opportunistic organisms. Depending on the host's reaction, *Aspergillus* spp produce both invasive and allergic disease in humans.<sup>2</sup> The various presentations of pulmonary aspergillosis in children caused by *Aspergillus* spp, allergic disease, aspergilloma or invasive disease are reviewed here, focusing on the clinical aspects rather than the basic science.

## COLONIZATION AND SACROPHYTIC INVOLVEMENT OF THE LOWER RESPIRATORY TRACT

*Aspergillus* spp may colonize the human respiratory tract without causing tissue damage. Saprophytic involvement also is a

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common finding in otherwise healthy individuals with damaged airways. If asymptomatic, patients are seldom treated. However, in patients with increased susceptibility to invasive fungal infections, such as recipients of stem cell or organ transplants and those receiving antineoplastic chemotherapy for cancer, antifungal prophylaxis is often warranted.

Saprophytic involvement of the lower respiratory tract is found with increased incidence in patients with underlying pulmonary diseases, such as advanced stages of chronic obstructive pulmonary disease (COPD), asthma requiring corticosteroid therapy and primary ciliary dyskinesia syndrome. In patients with cystic fibrosis (CF), colonization of the airways is a common finding.

## ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

### Epidemiology (underlying lung disease)

ABPA is a recognized complication of asthma and CF. It most often affects teenagers with CF and young to middle-aged adults with asthma; however, it has also been diagnosed in infants with CF and is extremely rare in paediatric asthma. While *A. fumigatus* is usually the causative organism, *A. niger* and occasionally other fungi are less commonly implicated.

ABPA is reported in 1–25% of adult asthmatic patients and in 6–25% of patients with CF.<sup>5,6</sup> North American statistics indicate its presence in 7–14% of corticosteroid-dependent asthmatics and in about 7% of patients with CF.<sup>7</sup> Data from European multicentre studies involving patients with CF showed an ABPA prevalence of 7.8% (2.1–13.6%).<sup>8</sup> The variation in ABPA prevalence is probably related to the lack of uniform criteria for the diagnosis of the disease, variation in laboratory techniques, as well as lack of clinical recognition. The prevalence of ABPA increases with patient age.<sup>9</sup>

Isolation of *Aspergillus* spp from respiratory secretions in patients with CF is relatively frequent (9–57%). The wide variability in the prevalence of bronchial colonization by the fungus is related to the degree of exposure to spores. Patients living in rural areas show relatively high rates of colonization, as do those living in inadequately ventilated houses or houses containing moulds.<sup>9</sup> However, the simple presence of the fungus is not associated with a worsening of lung function.

### Pathophysiology

The factors underlying the development of ABPA remain unclear. The roles of genetic factors, mucus quality, pre-activation of epithelial cells and the extent to which this activation facilitates the germination of *Aspergillus* conidia into hyphae, the bronchial penetration of fungi, the immune response, and bronchial/bronchiolar inflammation and destruction are not yet fully understood. Hence, the mechanisms involved in ABPA development are complex<sup>10</sup> (Fig. 1).

Certain HLA antigens, especially HLA-DR2/DR5 and possibly DR4/DR7, predispose some individuals to ABPA, while HLA-DQ2 seems to have a protective role. Genetic variability may therefore protect or enhance susceptibility to ABPA.<sup>11</sup> It is hypothesized that a key role is played by antigen-presenting (dendritic) cells that express HLA-DR2/DR5 and have increased IL-10 synthesis along with increased sensitivity to IL-4. In addition to genetic factors, patients with CF and asthma may be more susceptible to ABPA due to the presence of excessive mucus in their airways. This mucus makes the efficient clearance of conidia from the airways difficult. The conidia subsequently germinate and release antigenic proteins. This provokes a host response that is predominantly mediated by Th2 lymphocytes. The Th2 cells attracted to the airways may be especially susceptible to cytokines such as IL-4 and IL-10, and respond by enhancing both the synthesis of IgE by B cells and the attraction of eosinophils into the tissue.<sup>12</sup> The Th2 response also results in increased mucus secretion into the airway, episodic eosinophil-rich pulmonary infiltrations and remodeling of the airway.

Patients with ABPA have markedly elevated total serum IgE levels as well as increased IgE antibodies to *A. fumigatus*. Furthermore, specific IgG and IgA levels may also be upregulated. In patients with CF, prolonged colonization with *Pseudomonas aeruginosa* or *Stenotrophomonas maltophilia* may increase the risk of developing ABPA.<sup>13</sup>

### Diagnosis

ABPA can lead to extensive bronchiectasis and fibrosis if not diagnosed and treated early. This makes a timely diagnosis critical. The classical clinical and laboratory manifestations include episodes of wheezing, transient pulmonary infiltrates, a positive

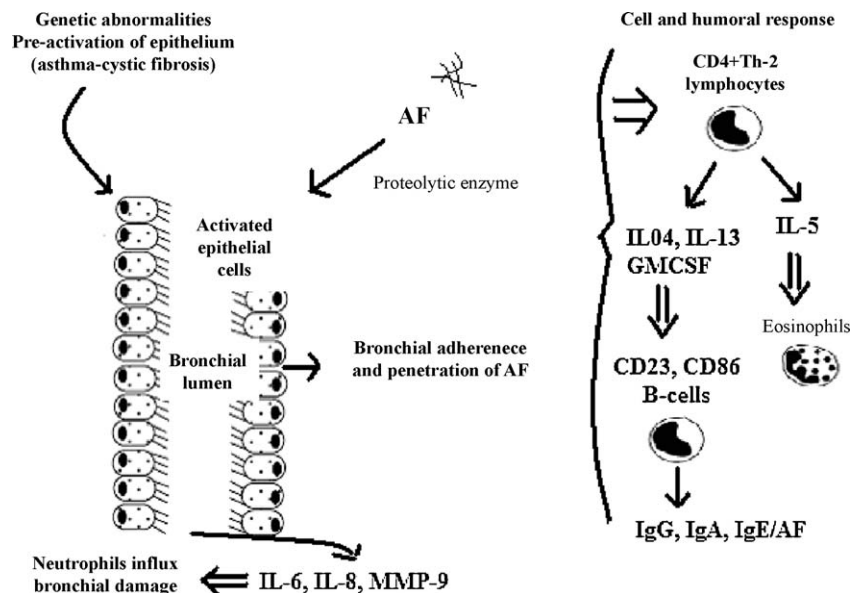


Fig. 1. Pathophysiology of allergic bronchopulmonary aspergillosis: from *Aspergillus* adherence and penetration of the bronchial mucosa to the B- and T-cell response.

**Table 1**

Minimal diagnostic criteria for allergic bronchopulmonary aspergillosis (ABPA) in patients with asthma.<sup>59</sup>

<p><b>ABPA central bronchiectasis</b></p> <ul style="list-style-type: none"> <li>• Asthma</li> <li>• Central bronchiectasis</li> <li>• Immediate cutaneous reactivity to <i>Aspergillus</i> species</li> <li>• Elevated total serum IgE (&gt; 417 IU/ml or &gt; 1000 ng/ml)</li> <li>• Elevated serum IgE and/or IgG to <i>A. fumigatus</i></li> </ul> <p><b>ABPA seropositive</b></p> <ul style="list-style-type: none"> <li>• Asthma</li> <li>• Immediate cutaneous reactivity to <i>Aspergillus</i> species</li> <li>• Elevated total serum IgE (&gt; 417 IU/ml or &gt; 1000 ng/ml)</li> <li>• Elevated serum IgE and/or IgG to <i>A. fumigatus</i></li> </ul>
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*A. fumigatus* skin reaction, elevated total serum IgE level, elevated *Aspergillus*-specific IgE and IgG concentrations, eosinophilia, positive precipitins and central bronchiectasis. Symptoms consist of worsening asthma, cough, expectoration of brown mucus plugs, fever, dyspnoea, wheezing and malaise. Frequently, however, patients are asymptomatic. Physical examination is often normal, with the most frequent positive findings being crackles and wheezes. In more advanced disease, clubbing, cyanosis or cor pulmonale may be present.

Chest radiographs and computerized tomography (CT) scans are the primary imaging studies in ABPA. Infiltrates seen in ABPA typically involve the middle or upper lobes and may be transient or permanent. The form of bronchiectasis associated with ABPA is central and involves the inner two-thirds of the lung. High-resolution CT scans are more sensitive than chest radiographs for detecting infiltrates and bronchiectasis.

When evaluating a patient with asthma or CF for ABPA, certain diagnostic criteria are available. Greenberger proposed two sets of minimal criteria to diagnose ABPA in asthmatic patients (Table 1).<sup>14</sup> One set is for patients with central bronchiectasis and one for those without bronchiectasis. The presence of precipitating antibodies to *A. fumigatus* supports the diagnosis of ABPA, and total IgE levels predict ABPA exacerbations. The Cystic Fibrosis Foundation recommends its own set of classic and minimal criteria for the diagnosis of ABPA in patients with CF (Table 2).<sup>15</sup>

The recommended screening test for *Aspergillus* among asthmatic patients is skin testing for *Aspergillus* reactivity. If the skin test is negative, ABPA can be excluded. Positive reactivity, however, is not specific for ABPA. The prevalence of skin reactivity to *Aspergillus* is 23–28% in patients with asthma and 29% in patients with CF.<sup>16</sup> A positive skin prick test must therefore be followed by further serological and radiological testing to determine whether minimal diagnostic criteria are met.

**Table 2**

Recommendations of Cystic Fibrosis Foundation for the diagnosis of allergic bronchopulmonary aspergillosis.<sup>5</sup>

<p><b>Classical criteria</b></p> <ul style="list-style-type: none"> <li>• Clinical deterioration</li> <li>• Immediate cutaneous reactivity to <i>Aspergillus</i> species or positive RAST</li> <li>• Elevated total serum IgE (&gt; 1000 IU/ml or &gt; 2398 ng/ml)</li> <li>• <i>A. fumigatus</i> – positive precipitins or presence of anti-<i>A. fumigatus</i> IgG</li> <li>• Altered chest X-ray</li> </ul> <p><b>Minimal diagnostic criteria</b></p> <ul style="list-style-type: none"> <li>• Clinical deterioration</li> <li>• Elevated total serum IgE (&gt;500 IU/ml)</li> <li>• Immediate cutaneous reactivity to <i>Aspergillus</i> species or positive RAST</li> </ul> <p><b>Suggestion for annual screening</b></p> <ul style="list-style-type: none"> <li>• Persistence of a clinical suspicion</li> <li>• Serum IgE &gt; 500 IU/ml, perform immediate skin test or RAST</li> <li>• Serum IgE 200–500 IU/ml, repeat if clinical suspicion was strong</li> </ul>
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In addition, several other findings are consistent with ABPA. Sputum from patients with ABPA may contain golden-brown mucus plugs, hyphae and/or large numbers of eosinophils. However, the presence of *A. fumigatus* in sputum is not specific for ABPA.

The recent molecular characterization of *A. fumigatus* antigens has improved the serological diagnosis of ABPA.<sup>17</sup> The identification and reproducibility of *A. fumigatus* antigen fractions has permitted the detection of specific antibodies against each fraction. The recombinant allergens Asp1, Asp2, Asp3, Asp4 and Asp6 have been evaluated in the diagnosis of ABPA in patients with asthma<sup>18</sup> and CF.<sup>17</sup> These studies have demonstrated that the presence of IgE antibodies against rAsp1 and rAsp3 determine a serological profile of sensitized individuals with 88% sensitivity and 100% specificity.<sup>17</sup>

### Treatment

Proper and timely treatment of ABPA can prevent its progression to severe fibrotic disease. There are several important therapeutic considerations, including environmental control, treatment of concomitant asthma or CF, treatment of ABPA itself and careful monitoring between exacerbations.

### Environmental control

While *Aspergillus* conidia are ubiquitous, avoiding areas where the fungus is present in potentially high concentrations is important. These locations include crawl spaces, moist basements, compost piles and under-carpeting laid over concrete. *A. fumigatus* has also been recovered from bird excreta, so the removal of birds from the home should be considered.<sup>6</sup>

### Systemic corticosteroids

The treatment of choice for ABPA consists of systemic corticosteroids to reduce the inflammatory response induced by the fungus. This treatment is able to control exacerbations and reduce pulmonary infiltrates but recurrences during treatment have been reported.<sup>19</sup> The use of systemic corticosteroids is problematic in patients with CF who are already predisposed to diabetes, osteopenia and growth retardation. In addition, the pharmacology of many drugs is altered in patients with CF.<sup>15</sup> Various dosage regimens have been proposed but the best scheme has yet to be defined. Initial daily dosages of 1–2 mg/kg prednisolone have been shown to be adequate and should be followed by a slow and gradual reduction according to the remission of symptoms and pulmonary infiltrates, with a minimum treatment duration of at least 3 months. Monitoring of serum total IgE levels is a useful parameter of disease control. A 30–50% reduction is expected after commencement of therapy. In patients in whom treatment cannot be discontinued without recurrence, prophylaxis for *Pneumocystis jiroveci* and maintenance treatment for bone mineralization should be considered, in addition to monitoring other adverse effects of prolonged corticosteroid therapy.<sup>20</sup> Intermittent checking of IgE is recommended as recurrences may be detected early, based on increasing IgE levels.<sup>15</sup>

### Antifungal agents

While oral corticosteroids have always been the mainstay of ABPA treatment, there is much interest in using antifungal agents as an adjunct. Itraconazole administered orally in a dosage of 200 mg twice a day has been shown beneficial in adult patients with ABPA. There are reports showing a lower frequency and adequate control of exacerbations as well as a possible reduction and even discontinuation of systemic corticosteroid therapy.<sup>7</sup> When itraconazole is used in combination with oral or inhaled

corticosteroids, adrenal suppression remains a potential concern.<sup>19</sup>

Voriconazole and posaconazole, newer antifungal triazoles, and echinocandins, namely caspofungin, micafungin and anidulafungin, are effective anti-*Aspergillus* agents; however, these newer agents have not been adequately studied in patients with ABPA. If their use is necessary, contraindications and adverse effects should be carefully respected and monitored. Among them, data only for voriconazole have been published. A retrospective single-centre study of 13 children with CF and ABPA suggested that voriconazole monotherapy is an alternative treatment strategy when oral corticosteroids are not suitable.<sup>21</sup> In a case of ABPA complicating lung transplantation for CF, a prompt clinical and physiological response was observed when nebulized amphotericin B was introduced, which allowed prednisone to be reduced and, in time, all antifungal therapy to be withdrawn.<sup>22</sup>

#### Additional therapeutic considerations

Leukotriene D4 antagonists have anti-eosinophilic activity and thus might be useful in patients with ABPA. Anti-IgE therapy could potentially be helpful, but there are insufficient data on safety and efficacy. Immunotherapy with *Aspergillus* antigens is another option in the investigational phase. Inhaled corticosteroids, commonly used in patients with CF and asthma, are actually associated with increased sensitization to *Aspergillus* and may potentially increase the risk of developing ABPA.<sup>23</sup> This is particularly true in patients with CF.

#### Patient monitoring

Monitoring patients with ABPA is an essential component of their care. Many patients may have an asymptomatic exacerbation and only through careful observation are they diagnosed and treated in a timely manner. After treatment with prednisone, total serum IgE levels should be obtained every 2 months for 1 year. A doubling of IgE level should prompt a chest radiograph even in an asymptomatic patient. Spirometry should be obtained at least annually and an unanticipated decline of  $\geq 15\%$  in functional vital capacity (FVC) might be an indication of ABPA exacerbation.<sup>15</sup>

### CHRONIC PULMONARY ASPERGILLOSIS INCLUDING ASPERGILLOMA

Chronic forms of pulmonary aspergillosis have been recognized for many years, although rarely in children, and have been variably termed pulmonary aspergillosis with cavitations, pulmonary aspergilloma or more recently chronic necrotizing aspergillosis.<sup>2</sup>

A pulmonary aspergilloma is a rounded conglomerate of hyphae, mucus and cellular debris contained within a fibrotic and thickened wall and occupying a pre-existing cavity. The origin of pre-existing cavity is most commonly an old tuberculosis lesion. However, an aspergilloma may complicate a wide range of other pulmonary diseases associated with cavitation, such as sarcoidosis, histoplasmosis, pulmonary cysts, ankylosing spondylitis, bronchiectasis, rheumatoid nodules and adenocarcinoma.<sup>24,25</sup> The fungal ball is mobile and its size may vary with time, as may the degree of adjacent pleural and cavity wall thickening.

No symptoms can be referred to an aspergilloma other than occurrence of haemoptysis in 50–90% of patients. This is often infrequent and small in volume but occasionally may be massive and fatal. The appearance of a mobile fungal ball in a pulmonary cavity is highly suggestive of aspergilloma. The presence of precipitating antibodies to *Aspergillus* confirms the aetiology; rarely, non-*Aspergillus* fungi may be responsible.<sup>25</sup>

### INVASIVE BRONCHIAL ASPERGILLOSIS

This syndrome refers to invasive disease primarily involving the large airways. *Aspergillus* tracheobronchitis is a term reserved for cases with tracheobronchial inflammation and mucus exudate containing *Aspergillus* spp without other identifiable pathogens. The inflammation is superficial, the mucosa is intact and there is no evidence of pseudomembrane formation, deep focal ulceration or other focal endobronchial abnormalities.<sup>2,23,25</sup>

Pseudomembranous *Aspergillus* tracheobronchitis has been found in a variety of clinical settings including lung or heart-lung transplantation, post-influenza, haematological malignancy, COPD and metastatic renal cell carcinoma. Pseudomembranous tracheobronchitis may be clinically silent; progressive encroachment into the airway lumen leads to bronchial obstruction, distal atelectasis or lobar collapse, and manifests clinically as stridor, wheeze, respiratory failure and ultimately death.<sup>2,23</sup>

Ulcerative tracheobronchial aspergillosis characteristically occurs in the first 6 months following lung transplantation, and the bronchial anastomosis is the usual site of involvement. It has also been observed in a limited number of other clinical contexts including HIV/AIDS and solid cancers.<sup>2</sup>

A high index of suspicion is required to establish the diagnosis of invasive bronchial aspergillosis, since the associated symptoms and imaging abnormalities may be relatively minor and non-specific. The diagnosis can be established with bronchoscopy, which can show the nature and extent of disease and also identifies *Aspergillus* spp in the BAL specimens.

### INVASIVE PULMONARY ASPERGILLOSIS

This is the commonest manifestation of invasive aspergillosis (IA), the lungs being implicated in  $> 70\%$  of IA cases in large series.<sup>26,27</sup> Inhalation of conidia is the mode of *Aspergillus* acquisition, leading to local invasion (sinuses and lungs) in the vast majority of patients; however, inoculation through a skin lesion may also lead to growth and invasion. Dissemination from the lungs and local extension from the sinuses is frequently observed.

IA is a devastating infection that affects patients with neutropenia or neutrophil and/or macrophage dysfunction, cytotoxic chemotherapy, long-term corticosteroid therapy, bone marrow<sup>28</sup> or organ<sup>29</sup> transplantation and congenital or acquired immunodeficiency.<sup>30,31</sup> Among paediatric patients, IA is predominantly seen in patients with haematological malignancies, chronic granulomatous disease (CGD) and those on corticosteroid and other immunosuppressive therapies. Prematurely born neonates also are at risk because of their immature phagocytic capacity and the frequent use of corticosteroids.<sup>32</sup> Neutropenia is clearly the dominant risk factor for the development of IA; importantly, however, it also exerts a major influence on the specific pathology of invasive pulmonary aspergillosis (IPA). The progressive nature of this disease and its refractoriness to therapy are, in part, due to the organism's rapid growth and its tendency to invade blood vessels.

#### Pathology and pathogenesis

##### Angioinvasive invasive pulmonary aspergillosis

There are two classical pathological manifestations of IPA in which angioinvasion appears to be central to pathogenesis.<sup>33</sup> The first arises in the context of invasion of major proximal pulmonary arteries with resultant thrombosis and distal tissue infarction, leading to a wedge-shaped lesion with the base abutting the visceral pleura. The second consists of a well-circumscribed spherical nodule with a vessel in the centre of the lesion, which

is infiltrated by hyphal elements. The nodule has a pale centre consisting of an area of coagulative necrosis with extensive permeation of tissue by hyphal elements but with a relative paucity of inflammation or haemorrhage.

#### Non-angioinvasive invasive pulmonary aspergillosis

This entity is associated with a wide range of non-neutropenic hosts in whom the underlying pathology consists of a pyogranulomatous inflammatory response and inflammatory necrosis with no evidence of angioinvasion.<sup>33</sup> Cavitation is frequently observed, presumably as a result of tissue necrosis.

#### Clinical setting and clinical features

##### Angioinvasive invasive pulmonary aspergillosis

This is most frequently seen in profound and prolonged neutropenia. Furthermore, the disease is usually rapidly progressive evolving over a period of days. There are no specific clinical signs or symptoms, although fever, dyspnoea, non-productive cough, haemoptysis and chest pain are commonly cited. Pleuritic chest pain, pleural rub and haemoptysis are suggestive of haemorrhagic infarction.

##### Non-angioinvasive invasive pulmonary aspergillosis

This is observed most commonly in those exposed to corticosteroids, non-neutropenic haematopoietic stem cell transplant (HSCT) recipients, solid organ transplant recipients, severe graft-versus-host disease (GVHD) and HIV/AIDS, although a multitude of other non-neutropenic contexts are described. The disease may be insidious in onset and exhibits a slower clinical course than angioinvasive IPA. The term 'subacute IPA' has been coined to denote the more slowly progressive forms of IPA; however, this term has not been rigorously defined.<sup>34</sup>

#### Epidemiology

Paediatric IA is an increasing problem, yet there are few specific data on it. The underlying diseases and treatments differ between children and adults. The incidence of IA has significantly increased in recent decades with a parallel increase in the number of immunocompromised paediatric patients.<sup>35</sup>

There have been few published reviews of paediatric IA. One reported 66 cases of proven IA from approximately 9500 children treated in Memphis between 1962 and 1996.<sup>27</sup> The median age was 11.2 (range 1.3–21.6) years. The absolute neutrophil count (ANC) for these patients was < 500 cells/ $\mu$ l for a median of 14 (1–402) days, and the interval between onset of underlying disease and IA was a median of 16 (0–180) months. Patient survival was 58% at 1 month, 25% after 2 months and 15% after 10 months. Another study reported 39 paediatric IA cases from 1979 to 1988 in Toronto.<sup>36</sup> Of those cases, 24 were proven and 15 were probable. The median age was 10 years (range 22 days–18 years), and 74% had a haematological malignancy or had received a haematopoietic stem cell transplant (HSCT). Thirty-one of 36 patients had an ANC < 500 cells/ $\mu$ l with a mean duration of ANC < 1000 cells/ $\mu$ l of 20 days. All except one patient were immunocompromised. The overall survival rate was only 23.1% (9/39), similar to the 31.8% in the age group  $\leq$  20 years from a large case review,<sup>37</sup> and generally lower than that found in adult studies.

The species distribution of *Aspergillus* isolates for paediatric and adult patients has differed in some studies. However, in two recent studies, the paediatric epidemiology paralleled previous adult studies. In the paediatric voriconazole compassionate release study, the species distribution was predominantly *A. fumigatus* (26/42), followed by *A. flavus* (6/42) and *A. nidulans* (3/42).<sup>38</sup> In another paediatric study of amphotericin B lipid complex (ABLC),

the most common isolates were *A. fumigatus* (11/23) and *A. flavus* (6/23).<sup>39</sup>

#### Specific clinical settings

##### Haematological malignancies

The most frequent underlying disease of IPA is acute leukaemia. The lungs are the primary site of IA in 78% of cases.<sup>34</sup> Among paediatric patients with IA, 74% suffered from haematological malignancies or were HSCT recipients.<sup>36</sup> *A. fungemia* seems to be a rare infection, even in high-risk patients with haematological malignancies, although neutropenia represents one of the most frequent underlying conditions.

Despite aggressive medical and surgical treatment strategies for aspergillosis, the in-hospital mortality of IA in children with HSCT is currently about 45%.<sup>35</sup> In immunocompromised children suffering from cancer, IPA has several manifestations, including pneumonia, haemoptysis and invasion of contiguous intrathoracic structures. A persistently febrile neutropenic patient with haematological malignancy, pulmonary infiltrates and haemoptysis has a high probability of having IPA.<sup>40</sup>

##### Primary immunodeficiencies

Patients with primary immunodeficiencies exhibit immune deficits that confer increased susceptibility to fungal infections. Patients with phagocytic deficiencies, the most common of which is CGD are very susceptible to IA. The frequency of IA in children with CGD is 15–40%.<sup>31</sup>

Among 322 children with CGD in the US, 21 were diagnosed with IA, reflecting an incidence of 6.5%. In another report from the US CGD registry of 368 patients, *Aspergillus* spp were the most commonly isolated organisms from patients with pneumonia (41% of 290 cases).<sup>41</sup> In this and other series, IA was the most common cause of death, accounting for over one-third of all deaths.<sup>42</sup>

The most common *Aspergillus* spp affecting CGD patients is *A. fumigatus* followed by *A. nidulans*;<sup>43,44</sup> infection with *A. flavus* also has been reported. IA usually affects CGD patients during their first two decades of life and may even be the first manifestation of this disease. The infection most commonly affects the lungs and can present as a pulmonary infiltrate in routine imaging studies of otherwise asymptomatic CGD patients. Indeed, at diagnosis, up to one-third of the patients may be asymptomatic and only around 20% may be febrile. The infection of the lungs is non-angioinvasive, consisting of pyogranulomatous lesions infiltrated by neutrophils, which are, however, non-functional due to the underlying disease. Other signs and symptoms are not specific. Leukocytosis and moderate elevation of the erythrocyte sedimentation rate may occur; however, normal values also have been observed in a significant proportion of cases.<sup>31,43,44</sup>

Hyper-IgE syndrome (Job syndrome) is characterized by extremely elevated serum IgE levels, eosinophilia, recurrent staphylococcal infections of the skin, lungs and other organs resulting in abscess or pneumatocele formation, chronic eczematous dermatitis, skeletal abnormalities and coarse facial features. The usual pathogenetic mechanism is colonization of pre-existing pneumatoceles, which are the consequences of previous suppurative infections, with *Aspergillus* conidia leading eventually to formation of a fungus ball or aspergilloma. The usual presentation includes non-productive cough or haemoptysis. Most cases have been managed with a combination of antifungal therapy and surgical resection.<sup>43,45</sup>

##### HIV infection

In a large review of 473 HIV-infected children, seven (1.5%) developed IA.<sup>30</sup> These patients were class C3 according to CDC AIDS classification and the absolute CD4 count obtained within 3

months of IA ranged from 0 to 338 cells/ $\mu\text{l}$  (median, 2 cells/ $\mu\text{l}$ ). There were five cases of IPA, all resulting in death. Sustained neutropenia (>7 days) occurred in six (0.3%) of them.

### Neonates

While invasive candidiasis is the most common fungal infection in this age group, neonatal aspergillosis has some unique characteristics that separate it from adult and even paediatric IA. Neonatal risk factors include corticosteroid use, prolonged hospitalization and the more neonate-specific risk factors such as immature phagocytes as well as skin trauma from adhesive tapes. The pathophysiology of neonatal IA differs from that of adult IA. In one review of 44 IA cases in the first 90 days of life, 31.8% were disseminated IA, 25% cutaneous aspergillosis and 22.7% IPA cases.<sup>32</sup> Prematurity is a clear risk factor for neonatal IA, given that 43.2% of the 44 patients reviewed were premature infants. However, different from adult patients, only 2.3% of the neonatal patients had prior neutropenia. *A. fumigatus* was the most common isolate obtained (41%) with *A. flavus* the second most common (13.6%), similar to adult epidemiology.<sup>32</sup>

### Diagnosis

Effective management of IA requires early and accurate diagnosis. Despite recent advances in non-invasive surrogate markers of many diseases, radiological evaluation remains an important component of diagnosing IPA. In adult series, approximately 50% of IPA cases show cavitation and 40% air crescent. Chest CT may also be used to identify the 'halo' sign, a macronodule surrounded by a perimeter of ground-glass opacity, an early sign of IPA. In a large study of 235 adults with IPA, most patients (94%) had  $\geq 1$  macronodule, and many (61%) also had 'halo' signs. Initiation of antifungal treatment on the basis of a 'halo' sign was associated with a significantly better response to treatment and improved survival.<sup>46</sup> In a 10-year review of 27 consecutive paediatric patients, there was central cavitation of small nodules in 25% and no evidence of air crescent formation within any area of consolidation.<sup>47</sup> In other paediatric reports, there was a 22% (6/27) rate of cavitation on chest radiography or a 43% (6/14) rate of cavitation on CT.<sup>48</sup> Perhaps cavitation and air crescent formation occur more frequently in older children and adults than in younger children.

While microscopy and culture of appropriate specimens remain the gold standard of mycological diagnosis, diagnosis of paediatric IA with new antigen tests, such as galactomannan (GM) assay, is difficult because studies have shown repeated differences in paediatric and adult values.<sup>49</sup> In a recent prospective study, 826 serum samples from 64 paediatric patients were analysed. Twenty of 811 samples tested positive on repeat testing (specificity, 97.5%; 95% CI, 96.2–98.4%) including samples from eight of 63 patients without clinical evidence of IA according to study criteria (specificity, 87.3%; 95% CI, 76.9–93.4%). This study suggested that GM assay provides early, non-invasive diagnosis of IA in high-risk children and false-positive results were not common or unexplainable.<sup>50</sup>

Beta-D glucan assay has been studied in the blood of adult patients with fungal infections, including IA. The high negative predictive value of the assay allows it to be used to exclude IA; no specific data, however, exist for children. Polymerase chain reaction (PCR) in blood may be a powerful tool for early diagnosis of IPA but has not been standardized for routine use yet. No studies have addressed the issue in neonates, whereas in children PCR is probably as good as in adults.

Bronchoalveolar lavage (BAL) is widely used for the evaluation of patients with suspected IPA. BAL has been studied by several investigators and found to yield variable results with sensitivities

ranging from 25% to 75% when analysed in tissue-proven infection. Although an *A. fumigatus* culture-positive BAL fluid is indicative of IPA in febrile neutropenic children with new pulmonary infiltrates, the absence of hyphal elements or negative culture does not exclude the diagnosis.<sup>51</sup> Earlier diagnosis may be facilitated by assays that can detect *Aspergillus* GM antigen or DNA in BAL fluid. GM assay and quantitative real-time PCR seem to have greater sensitivity than cultures for detection of *A. fumigatus* in BAL fluid in experimental IPA.<sup>52</sup> Use of these methods in conjunction with culture-based diagnostic methods applied to BAL fluid could facilitate accurate diagnosis and more timely initiation of specific therapy.

### Treatment

Based upon the findings of a randomized trial demonstrating that voriconazole conferred a significant benefit of survival and overall therapeutic response, primary therapy of IPA should be initiated with voriconazole.<sup>53</sup> There has been no dedicated, prospective, large-scale investigation into treatment of paediatric IA. One large dataset of adults and children analysed separately outcomes for paediatric IA for 54 of 111 patients aged < 18 years.<sup>54</sup> The mean patient age was 9.3 years (21 days–16 years). There were 25 IA cases with a complete or partial response rate of 56%, stable response in 8% and failure in 36% of patients. The complete or partial response rate for IPA was 50% (10 cases), disseminated IA 29% (seven), sinusitis 100% (five) and single-organ extrapulmonary IA 67% (three).

In a retrospective French study of 46 paediatric patients treated with amphotericin B liquid complex (ABLC) for invasive fungal infections,<sup>39</sup> the mean age of 23 patients with IA was 9.7 years (3 months–18 years). Eighteen (78%) showed cure (52%) or improvement (26%), with 22% of them failing therapy. Three patients who initially improved later relapsed, dropping the cure or improvement rate to 65%.

An analysis of the compassionate open label use of voriconazole in children for refractory IA with clinical or radiological progression of disease after  $\geq 7$  days of systemic antifungal therapy included 42 children < 16 years with proven or probable IA.<sup>38</sup> Analysis of the response to treatment revealed a complete or partial response rate of 43%, stable disease in 7%, intolerance to therapy in 10% and 40% failing therapy.

When treating IPA, the understanding of the paediatric pharmacokinetics/pharmacodynamics of new antifungal drugs is very important. Elimination of voriconazole is linear in children after dosages of 3 and 4 mg/kg every 12 h. This linearity was based on an 11-patient, single dose, open study of children aged 2–11 years (mean age, 5.9 years) and a 28-patient multiple dose, open, multicentre study in two age cohorts (ages 2–6 and 6–12 years) (mean age, 6.4 years).<sup>55</sup> Recently, the dosage has been calculated to be 7 mg/kg twice a day for children.<sup>38</sup>

### New antifungal agents

In an initial paediatric study of caspofungin given to 39 patients (ages 2–17 years), the weight-based approach (1 mg/kg/day) resulted in suboptimal plasma concentrations in all children. The body surface area (50 mg/m<sup>2</sup>/day) regimen yielded similar plasma concentrations and increased area under the curve (AUC) to adult patients (50 mg/day).<sup>56</sup> Caspofungin pharmacokinetic studies showed that loading at 70 mg/m<sup>2</sup>/day followed by dosing at 50 mg/m<sup>2</sup>/day appears to be more appropriate in children than 1 mg/kg/day.

Posaconazole is an orally bioavailable antifungal triazole for the treatment and prophylaxis of invasive fungal infections. Krishna et al (2007)<sup>57</sup> evaluated posaconazole plasma concentration data from 12 juvenile and 194 adult patients who were intolerant of or

had invasive fungal infection refractory to standard antifungal therapies. They showed that posaconazole plasma concentrations were similar for juvenile and adult patients, suggesting that clinical outcomes are expected to be similar in adults and children with refractory invasive fungal infection.<sup>57</sup>

Combination antifungal therapy may be advantageous compared to monotherapy. For example, the combination of an echinocandin with a triazole or with a formulation of amphotericin B may be additive or synergistic in vitro and in vivo against experimental invasive aspergillosis.<sup>58</sup> However, no prospective randomized trials have been conducted to show that combinations are superior to standard monotherapy.

### PRACTICE POINTS

- ABPA should be considered in asthmatics with poorly controlled asthma or acute lung infiltrates, and patients with cystic fibrosis with exacerbation or infiltrate unresponsive to therapy.
- The current mainstay of treatment of ABPA is with systemic corticosteroids.
- Antifungal therapy may be used as an adjunct in the treatment of ABPA; it improves the clinical outcome and can reduce the requirements for systemic corticosteroids.
- Patients at risk for developing IPA are those with haematological malignancies, chronic granulomatous disease, AIDS and immunosuppressive therapy, as well as neonates.
- A persistently febrile neutropenic patient with haematological malignancy, pulmonary infiltrate and haemoptysis has a high probability of having IPA.
- The combined use of culture with galactomannan and PCR should result in earlier and more definitive diagnosis of IPA in children with clinical and/or radiological signs.
- Successful management of IPA requires an early diagnosis and prompt intervention.
- Primary therapy of IPA should be initiated with voriconazole.

### RESEARCH DIRECTIONS

- New insights into the pathophysiology of ABPA.
- Use of genetic screening to identify high-risk patients for ABPA.
- Improved treatment options for ABPA.
- Standardization of non-culture methods for earlier diagnosis of IPA in immunocompromised paediatric patients.
- Further study of antifungal agents and combination regimens in paediatric patients.
- Development of optimized antifungal therapeutic strategies for paediatric patients.

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