Diagnosing pulmonary aspergillosis is much easier than it used to be: a new diagnostic landscape

D. W. Denning1,2
Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK; Global Action Fund for Fungal Infections, Geneva, Switzerland

SUMMARY

Significant innovations in the past decade have resulted in more sensitive and faster diagnosis of allergic, chronic and invasive pulmonary aspergillosis, as well as *Aspergillus* bronchitis and *Aspergillus* nodules. This new diagnostic landscape has revealed that the incidence and prevalence of aspergillosis is substantially higher than previously understood, and is summarised in this review. Oral and intravenous antifungal treatment offers good clinical response rates for affected patients. Nevertheless, missed diagnoses mean that patients are over-treated with antibacterial agents, corticosteroids and anti-TB drugs, resulting in continuing illness and often death. The clinical introduction of several high performing diagnostic tests is helping to redefine patient management. It is well-known that *Aspergillus* antigen can be detected in 70–95% of bronchoscopy samples in patients with invasive and chronic aspergillosis in less than 1 hour. *Aspergillus* immunoglobulin G (IgG) (precipitins) is >90% sensitive and >85% specific for chronic and allergic aspergillosis. High-volume respiratory fungal culture and *Aspergillus* polymerase chain reaction have 3–5-fold higher sensitivity than routine bacterial culture. *Aspergillus* IgE (or skin prick testing) diagnoses *Aspergillus* sensitisation in asthma, cystic fibrosis, chronic obstructive pulmonary disease and post-TB, and correlates well with poorer lung function and/or exacerbations. Clinicians and laboratorians across the world need to mainstream these excellent new tools to improve clinical outcomes by delivering results in a more timely and accurate fashion.

KEY WORDS: *Aspergillus*; antibody; immunodeficiency; HIV/AIDS; leukaemia

*Aspergillus fumigatus* is responsible for remarkably diverse disease manifestations, ranging from infection in healthy and immunocompromised people to several allergic and locally invasive conditions. This diversity of different disease patterns is greater than those caused by any other microorganism, and reflects a complex fungal/host interaction, including invasive and chronic forms of pulmonary and sinus disease, dissemination to every organ, post-operative aspergillosis, trauma/burn and corneal local invasion, onychomycosis, external otitis and allergic disease. Every breath drawn includes *Aspergillus* spores, and in general, innate immune defences are able to eradicate these slowly. *Aspergillus* spores are found in normal lungs; 70% of normal lungs yielded positive culture results when sampled after death from other causes.1 The clinical and imaging manifestations of all forms of aspergillosis are subtle in their early stages and mimic other conditions. Only in the more advanced cases are the clinical features more apparent, but even then, are rarely distinctive. All forms of pulmonary and bronchial aspergillosis are ‘stealth’ conditions.

The most common comorbid conditions for invasive, chronic and allergic aspergillosis are shown in Table 1.2–35 Also documented are approximate frequencies (incidence or prevalence) in these different clinical backgrounds.

NEW DIAGNOSTIC TOOLS AND DATA ON ASPERGILLOSIS

Given the subtle nature of most forms of aspergillosis, and its clinical and radiological overlap with other pulmonary conditions, mycological tests are mandatory to confirm or rule out the diagnosis. The turnaround times (TATs) quoted below are in-laboratory ones, and do not include transport to the laboratory or delays in delivering the result, which are often considerable for send away tests.

Fungal culture

Culture testing of respiratory samples should be
performed on fungal (not bacterial) media, as the yield is 30% higher for Aspergillus (Table 2). As overall culture positivity rates in all forms of aspergillosis are under 30% with standard fungal culture techniques, these cannot be used to exclude infection. High-volume culture techniques boost culture-positive rates by at least three-fold. Bronchoalveolar lavage fluid (BAL) is inferior to

---

### Table 1: Manifestations of pulmonary aspergillosis and their approximate incidence and prevalence in different patient groups

<table>
<thead>
<tr>
<th>Entity</th>
<th>Comorbid condition</th>
<th>Frequency in that condition</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community-acquired Aspergillus pneumonia Chronic</td>
<td>Influenza, COPD, diabetes, mild immunosuppression, TB and NTM infection</td>
<td>Uncommon</td>
<td>Difficult to document as diagnostic approach unclear</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>COPD</td>
<td>&lt;1%</td>
<td>Co-infection and post-TB most common</td>
<td>3, 4</td>
</tr>
<tr>
<td></td>
<td>Sarcoïdosis</td>
<td>&lt;4%</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Lung cancer surgery</td>
<td>3.5%</td>
<td>Cumulative incidence after 10 years</td>
<td>6, 7</td>
</tr>
<tr>
<td></td>
<td>Asthma, ABPA Pneumothorax</td>
<td>&lt;5%</td>
<td>Uncertain</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>?</td>
<td>No follow-up study published; probably rare and linked to bullae</td>
<td>9, 10</td>
</tr>
<tr>
<td>ABPA*</td>
<td>Adult asthma</td>
<td>0.7–5%</td>
<td>More common in the Indian subcontinent</td>
<td>11, 12</td>
</tr>
<tr>
<td></td>
<td>Childhood asthma</td>
<td>?</td>
<td>Described in several case series in India</td>
<td>13, 14</td>
</tr>
<tr>
<td>Severe asthma with fungal sensitisation*</td>
<td>Cystic fibrosis</td>
<td>10–25%</td>
<td>Peak risk period is late teenage years or early adulthood</td>
<td>15, 16</td>
</tr>
<tr>
<td>Aspergillus sensitisation</td>
<td>COPD exacerbations</td>
<td>Doubles hospitalisations, increases mortality</td>
<td>Airborne fungal sensitisation and home exposure in South East Asia strongly linked to hospital admission and worse COPD</td>
<td>17, 18</td>
</tr>
<tr>
<td>Aspergillus bronchitis</td>
<td>Bronchiectasis and cystic fibrosis</td>
<td>30% in cystic fibrosis</td>
<td>Data are evolving and incomplete</td>
<td>19</td>
</tr>
<tr>
<td>Extrinsic allergic alveolitis</td>
<td>Occupational exposure</td>
<td>Industry-specific</td>
<td>Malt worker’s lung, tobacco worker’s lung; occupational asthma may be induced by fungal exposures</td>
<td>20</td>
</tr>
<tr>
<td>Invasive</td>
<td>Leukaemia and lymphoma, multiple myeloma, myelodysplasia</td>
<td>2–15%</td>
<td>Primarily linked to neutropenia, corticosteroids and ibrutinib; reduced by prophylaxis</td>
<td>21–23</td>
</tr>
<tr>
<td></td>
<td>Transplantation</td>
<td>0.5–26%</td>
<td>Airways disease most common in lung transplantation; linked to GvHD in allogeneic HSCT</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>ICU patients</td>
<td>1–12%</td>
<td>Most common in influenza (8–24%), COVID-19 (~20%) and medical patients</td>
<td>24–28</td>
</tr>
<tr>
<td></td>
<td>Lung cancer</td>
<td>2.6%</td>
<td>Many probably sub-acute invasive</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Autoimmune disorders such as SLE</td>
<td>&lt;4%</td>
<td>Especially linked with corticosteroid and other immunocompromising treatments</td>
<td>30, 31</td>
</tr>
<tr>
<td></td>
<td>COPD admissions to hospital</td>
<td>1.3–3.9%</td>
<td>Some cases linked to oral corticosteroid use</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Liver failure</td>
<td>5–14%</td>
<td>Partially linked to corticosteroids</td>
<td>21, 32</td>
</tr>
<tr>
<td></td>
<td>Severe fever with thrombocytopenia syndrome</td>
<td>20%</td>
<td>Tick-borne infection in Northeast Asia</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>HIV/AIDS</td>
<td>~4%</td>
<td>Advanced disease, corticosteroids and neutropenia</td>
<td>34, 35</td>
</tr>
<tr>
<td></td>
<td>Chronic granulomatous disease and other primary immune deficiencies†</td>
<td>~40% lifetime risk</td>
<td>Lifelong prophylaxis reduces risk</td>
<td>21</td>
</tr>
</tbody>
</table>

* Also known collectively as fungal asthma, a catch-all term for several entities linking fungal allergy/sensitisation and airway infection with worse asthma.
† Other primary immunodeficiency disorders with moderate or high risk of invasive aspergillosis include caspase recruitment domain family member 9 deficiency, hyper-immunoglobulin E syndrome, MonoMAC syndrome due to defects in GATA2 and other even rarer disorders.

COPD = chronic obstructive pulmonary disease; NTM = non-tuberculous mycobacteria; ABPA = allergic bronchopulmonary aspergillosis; GvHD = graft versus host disease; HSCT = haematopoetic stem cell transplant; ICU = intensive care unit; SLE = systemic lupus erythematosus.
brachial aspirate and sputum obtained pre- or postbronchoscopy, indicating that most *Aspergillus* resides in the larger airways, not the alveoli, unlike cytomegalovirus and *Pneumocystis jiroveci*. The more samples submitted, the higher the yield, which may go up to ~60%. Culture rates in chronic obstructive pulmonary disease (COPD) patients may be higher, as airway macrophages and epithelial cells are poor at destroying ingested spores in these patients.

There is no relationship between colony counts and severity of disease; a single colony can reflect rapidly invasive aspergillosis, whereas high colony counts may signal tracheobronchitis rather than parenchymal disease. Blood cultures are almost always negative for *Aspergillus*. Positive cultures can be tested for azole resistance. Culture TAT is usually 48–96 h.

**Microscopy**

The most rapid methodology for direct microscopy is the fluorescent brightener and microscope; slides can be scanned in under 3 min by an experienced technician. Alternatives include potassium hydroxide, Gram staining and silver staining (after cytocentrifugation), all of which are probably slightly less sensitive. Most samples are microscopy-negative; a positive is highly correlated with disease, often tracheobronchitis, but also invasive and chronic aspergillosis (Table 2). The TAT should be under 6 h.

**Aspergillus antigen**

Commercially available for over 25 years, the *Aspergillus* galactomannan (GM) antigen enzyme-linked immunosorbent assay (ELISA) from BioRad (Marnes-la-Coquette, France) is substantially more sensitive than culture or microscopy on BAL. Prior to antifungal therapy, sensitivity and specificity of GM ELISA is 80–90% for all forms of invasive aspergillosis and ~75% for chronic pulmonary aspergillosis (CPA). Blind tracheal aspiration in intensive care unit (ICU) patients has not been well studied. As sputum yields much higher GM values than serum or BAL, an alternative cut-off to define positivity is required. Serum samples need heating before analysis. The TAT is 24–48 h.

Recently, there have been three developments in this field, which could lead to more rapid results.

The most recent is a single sample, 1-h semi-automated ELISA launched by Vircell (Granada, Spain). Performance appears to be similar to the long-established ELISA product sold by BioRad and more recently by Dynamiker (Tianjin, China) and Era Biology (Tianjin, China). A new lateral-flow GM assay yields a result in <1 h on BAL (Immy, Norman, OK, USA). The performance appears similar to the GM ELISA assay and can be read visually or with a reader. Another lateral-flow device detects a different *Aspergillus* antigen in <1 h and has a sensitivity apparently slightly less than GM, possibly because the current comparator uses GM as a gold standard (OLM Diagnostics, Newcastle, UK).

**Aspergillus IgG and IgM antibody**

Detectable *Aspergillus* immunoglobulin (Ig) G is the cornerstone of diagnosis of CPA and is usually positive in allergic bronchopulmonary aspergillosis (ABPA) and *Aspergillus* bronchitis in asthma, cystic fibrosis and bronchiectasis patients. The value of IgM detection alone has not been fully evaluated, but it is less sensitive than IgG in CPA. Studies in CPA show a sensitivity of *Aspergillus* IgG from ~70% to >90%, depending on the assay and cut-off used. Multiple studies from many countries have evaluated the optimum cut-offs. Compared with blood donors, the specificity of *Aspergillus* IgG assays is ~98%, but this falls to ~85% if patients with respiratory disease

### Table 2 Diagnostic sensitivity of rapid tests for aspergillosis (all figures are rounded for ease of comprehension)

<table>
<thead>
<tr>
<th>Test</th>
<th>Turnaround time (hours)</th>
<th>Acute invasive (%)</th>
<th>Sub-acute invasive (%)</th>
<th>Chronic cavitary (%)</th>
<th>Aspergillus nodule (%)</th>
<th>Allergic (%)</th>
<th>Aspergillus bronchitis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture on respiratory samples</td>
<td>48–96</td>
<td>&lt;30*</td>
<td>&lt;30*</td>
<td>&lt;30</td>
<td>&lt;10</td>
<td>&lt;30</td>
<td>~80†</td>
</tr>
<tr>
<td>Microscopy on respiratory samples</td>
<td>&lt;6</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;5</td>
<td>&lt;25†</td>
<td>?</td>
<td>10–25</td>
</tr>
<tr>
<td>Antigen on BAL</td>
<td>2–24</td>
<td>70–95†</td>
<td>70–90†</td>
<td>~75†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Antigen on serum</td>
<td>2–48†</td>
<td>70–95†</td>
<td>70–90†</td>
<td>~75†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IgG antibody</td>
<td>48–96</td>
<td>&lt;25</td>
<td>70–90†</td>
<td>80–90†</td>
<td>65</td>
<td>80–90†</td>
<td>60–80†</td>
</tr>
<tr>
<td>IgE antibody</td>
<td>48–96</td>
<td>ND</td>
<td>ND</td>
<td>60–70</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Beta-D-glucan on serum</td>
<td>2–48†</td>
<td>70–95†</td>
<td>70–90†</td>
<td>~75†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Aspergillus PCR on respiratory samples</td>
<td>24–72</td>
<td>60–80†</td>
<td>ND</td>
<td>60–80†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Aspergillus PCR on serum/blood</td>
<td>24–72</td>
<td>60–80†</td>
<td>ND</td>
<td>60–80†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Yield may be higher in COPD patients with invasive aspergillosis—not carefully studied.
† Best tests to request in these clinical circumstances.
‡ Sputum plugs from ABPA usually show fungal hyphae, eosinophils and Charcot-Leyden crystals.
§ Lateral flow antigen tests are quick. Single-sample Aspergillus ELISA is <2 hours, other ELISA assays are batched.
¶ Highest sensitivity in profoundly neutropenic patients without mould-active prophylaxis.
* Single-sample assays are quicker, otherwise batch tested.
BAL = bronchial lavage; ND = no data; Ig = immunoglobulin; PCR = polymerase chain reaction; COPD = chronic obstructive pulmonary disease; ABPA = allergic bronchopulmonary aspergillosis; ELISA = enzyme-linked immunosorbent assay.
are used, probably because of some true-positives for other forms of aspergillosis among ‘controls’. The new lateral flow assay for both Aspergillus IgG and IgM is the best performing assay, but is not quantitative, unlike the other assays. Counter-immunoelectrophoresis (CIE) to detect precipitins is slower and less sensitive than ELISA and lateral flow testing. False-negative tests in CPA are linked to subtle immune defects such as mannose-binding lectin deficiency, low natural killer or plasma cell populations and poor pneumococcal antibody responses. Lateral flow assay TAT is <1 hour, 24–72 h for ELISA and automated assays as these are usually batched, and about 7 days for CIE.

Aspergillus polymerase chain reaction

The challenges to providing reliable, uncontaminated, sensitive polymerase chain reaction (PCR) to detect Aspergillus spp. commercially were first overcome in 2011 with the launch of the MycAssay kit (Myconostica, Camberley, UK). Since then, several companies have brought Aspergillus PCR tests to the market with slight variations in design. With respiratory samples, as with culture, Aspergillus PCR cannot distinguish colonisation from infection, and consequently will always require clinical interpretation, supported by other diagnostic tests and imaging appearances. PCR is more sensitive than culture, depending on the DNA extraction, PCR and culture methods used. Different samples can yield different answers because fungal hyphae and DNA are not evenly distributed in respiratory samples. BAL is less sensitive than bronchial and tracheal samples, probably because of dilution. Although the actual laboratory processes for DNA extraction and PCR only take a few hours, in practice, it takes 24–48 h to yield a result after the receipt of a sample in Cat 3 laboratory. The performance of PCR assays developed in-house may or may not adequate, including generic PCR when using targets such as internal transcribed spacer or 18S ribosomal RNA targets, with or without subsequent sequencing.

PCR on blood or serum samples is really only useful in profoundly immunocompromised patients. As blood tubes containing Aspergillus DNA may occasionally yield a false-positive result, confirmation on a second sample is helpful. However, as Aspergillus DNAemia may not be continuous, clinical correlation is required with all available data.

Respiratory sample acquisition

Expectorated or induced sputum can be submitted for microscopy, culture and PCR. Physiotherapy-assisted cough can yield good specimens, as does sputum induction. Bronchoscopy is useful for inspecting the airways if tracheobronchitis is considered, and BAL testing for Aspergillus antigen the most sensitive test. But the bronchial wash specimen is superior to BAL for culture and PCR, probably because more Aspergillus is present in the airways than in the alveoli. Bronchial biopsy is useful for suspicious lesions, especially in tracheobronchitis, and aspirated mucus should be submitted for microscopy or cytology, culture and histopathology.

MANIFESTATIONS OF ASPERGILLOSIS (INCLUDING RADIOLOGY)

Brief summaries of each major entity are provided below. The severity of clinical features varies substantially. Early in the disease, there are no symptoms and imaging findings are minor, or easily mistaken for other conditions. Physical examination of the chest may be abnormal, but any signs heard are rarely specific to any form of aspergillosis, except for a monophonic wheeze, which is a rare but characteristic sign of invasive Aspergillus tracheobronchitis.

Community-acquired Aspergillus pneumonia

This uncommon disorder is linked to massive airborne exposures and sometimes, influenza or a recent course of corticosteroids. The exposure history includes bark chippings, waste disposal, extensive cleaning of damp properties, composting and gardening. Patients have occasionally had influenza. Fever, anorexia, cough and breathlessness are typical, with no improvement with antibacterial agents. Patients usually present acutely with ‘pneumonia’ (usually consolidation which cavitates) or a bilateral miliary or nodular pattern which is very unusual. Undetected, severe disease is rapidly fatal; sub-acute community-acquired Aspergillus pneumonia may resolve or develop into CPA.

Invasive pulmonary aspergillosis

Early clinical features are mostly absent, but patients may have fever, dry cough, chest discomfort and malaise. Occasional patients produce sputum or have haemoptysis. Other patients present with non-pulmonary features such as stroke or rhinosinusitis. Patients with sub-acute invasive pulmonary aspergillosis have clinical and radiological features for 4–12 weeks, and are almost always symptomatic with the above features plus weight loss, anorexia and fatigue. Inflammatory markers may or may not be raised.

Chest X-ray (CXR) is falsely negative in at least 10% of patients; computed tomography (CT) is a much better investigation. Contrast has often not been used to avoid the possibility of nephrotoxicity, but recent work shows that contrast is valuable because vessel occlusion can be seen. This radiological sign is useful in both neutropenic and non-neutropenic patients, but will not be discriminatory.
in Covid-19 patients, who often have intrapulmonary infarction. Bilateral findings are a strong clue to the diagnosis of invasive aspergillosis. Nodules, some with cavitation or with surrounding ground glass (halo sign), are most common, but areas of non-specific consolidation are also common (Figure 1A and B). Pleural effusions are rare, but useful if present, as they can be sampled for *Aspergillus* antigen.

**Chronic pulmonary aspergillosis**

Patients with *Aspergillus* nodules and simple aspergilloma usually have no specific symptoms for aspergillosis. Early symptoms of cavitary CPA may be minor and inconsistent with radiological appearances, which often appear more severe. Symptoms of cavitary CPA may be systemic (weight loss, marked fatigue, rarely fever and/or night sweats) or...
pulmonary (cough, haemoptysis, chest discomfort and/or breathlessness), or any combination of these. Fatigue is often profound. Symptoms typically fluctuate minimally while gradually progressing over the course of weeks to months. Multiple treatments are usually attempted with little respite. Concomitant bacterial infection is common, with slight improvement with antibiotics noted. Patients with fibrosing CPA are breathless on exertion, and usually systematically unwell.64 Inflammatory markers may or may not be raised.

Aspergillus nodules may be single or multiple (Figure 1E).64 They are increasingly being recognised during screening for lung cancer. They are most often found on a background of COPD, asthma that has required courses of oral corticosteroids or rheumatoid arthritis. They usually show low to moderate fluorodeoxyglucose uptake on positron emission tomography.

Simple aspergilloma is always unilateral and non-progressive and sometimes found incidentally. Some patients present with haemoptysis, wheeze or cough. A cavitary lesion with a fungal ball showing an air crescent on CXR or CT is typical. Fungal ball mobility is no longer a criterion for diagnosis.

In cavitary CPA, >40% of patients have bilateral abnormalities,65 and the upper lobes are involved in >85% of cases. The cardinal features of cavitary CPA are one or more cavities, which expand or coalesce over time, usually have thick walls, pleural thickening and pericavitary infiltrates (Figure 1C and D).51 On CT scan, the inside of the cavity shows irregularity of the inner cavity wall (which is fungal growth) or a fungal ball or non-descript contents, occasionally a fluid level.66 The presence of a fungal ball or aspergilloma is a late feature of CPA.65 Fat ingress adjacent to pleural thickening is common, as is associated bronchiectasis. Effective therapy reduces the thickness of cavity walls, peri-cavitary infiltrates and pleural thickening. Cavitary CPA can progress to fibrosing CPA (Figure 2),67 involving two or more lobes, usually with some persistent cavitation and often an aspergilloma.

**Allergic bronchopulmonary aspergillosis**

In patients with asthma, ABPA presents in several ways,41,68 the most common presentation being poorly controlled asthma and cough. Some patients present with typical symptoms of bronchiectasis with productive cough and green sputum. A remarkable presentation is the production of brown plugs, described variously as ‘slugs’ or ‘peas’ or ‘jelly babies’. Occasional patients present with ‘pneumonia’, having an infiltrate on their CXR, but without being as systemically ill as the CXR might suggest. Profound fatigue, faint rash or worse eczema, or a surprise finding at bronchoscopy of mucous plugging are occasional presentations. Positive sputum cultures for bacteria are common and can be misleading.

In cystic fibrosis, an exacerbation that does not remit with systemic antibiotics, sometimes with a new infiltrate is the most common presentation of ABPA.15,69 but often the diagnosis is made by screening using total IgE or Aspergillus IgE. Forced expiratory volume in 1 sec (FEV1) is usually substantially reduced in an ABPA exacerbation.

Occasional patients present with ABPA who do not have asthma or cystic fibrosis. Some are heterozygous for a cystic fibrosis transmembrane receptor (CFTR) gene abnormality or have a rare and mild CFTR defect and late-onset cystic fibrosis.70 Some patients with a very similar syndrome to ABPA are allergic to other fungi, an entity called allergic bronchopulmonary mycosis.71

Patients with ABPA may have normal CXR, but more often have bronchiectasis or an infiltrate.72 Bronchiectasis was previously thought to be more central, but may be seen anywhere, and becomes more prominent in an area previously affected by mucous plugging. A highly characteristic feature of ABPA is hyper-attenuated mucus, best seen on CT bone windows.71,72 Patients may manifest ABPA together with a form of CPA with pleural thickening and peripheral fibrosis at presentation or years later.9
Aspergillus sensitisation

Aspergillus sensitisation is defined by Aspergillus IgE, detectable in serum or on positive skin prick testing.\(^{5,17,73}\) Sensitisation to Aspergillus fumigatus is usually (but not always) accompanied by sensitisation to multiple other allergens, some airborne fungi. A. fumigatus has about 60 IgE-binding proteins, some of which cross-react with other organisms, including homologous human proteins.\(^{74,75}\)

The most common groups to manifest Aspergillus sensitisation are patients with ABPA (100% by definition), severe asthma (30–70%), COPD (35–55%), cured TB (~30%), bronchiectasis (~30%), cystic fibrosis (~40%) and CPA (~70%).\(^{3,15,17,20,68,69,71,73}\) In most cases, Aspergillus sensitisation is linked to worse lung function and/or more exacerbations. There is no radiological correlate for Aspergillus sensitisation, and it is not directly linked to airway or lung infection with Aspergillus or Aspergillus IgG positivity.

Severe asthma with fungal sensitisation

By definition (of which there are several), these patients have severe asthma, usually poorly controlled, with fungal sensitisation.\(^{76}\) Their presentation is with moderate or severe asthma symptoms, exacerbations, including hospitalisation and ICU admission and associated low mood. Many are chronically unwell and effectively disabled by their asthma. It is uncommon for these patients to have bronchiectasis – bronchial wall thickening is more common on CT scanning. Some have eosinophilic asthma, but as corticosteroid usage is often high, this may be long in the past. They do not produce brown sputum plugs.

The radiology is not distinctive: these patients do not present with infiltrates, mucous obstruction or marked bronchiectasis as ABPA does.

Aspergillus bronchitis

Patients with Aspergillus bronchitis present in two ways, primarily with productive cough and breathlessness, and almost all grow bacteria in their sputum alongside Aspergillus.\(^{19}\) First, they present with recurrent bronchitis or chest infections, which only partially clear with antibacterial therapy. Second, they present with thick tenacious sputum, violent cough and sometimes acute dyspnoea. Most, but not all, have bronchiectasis and are not immunocompromised. A similar, but often acute presentation in immunocompromised patients, is referred to as obstructing bronchial aspergillosis,\(^{77}\) which is sometimes an immune reconstitution syndrome. The CXR usually shows only the features of bronchiectasis, but occasionally, opacification due to airway obstruction. If bronchoscopy is done, multiple airways may be found to be inflamed, some with contact bleeding, and there may be multiple mucus plugs.

Aspergillus colonisation

Airway Aspergillus colonisation is relatively common, especially in COPD patients.\(^{78}\) This is distinguished from Aspergillus bronchitis by the lack of symptoms referable to the airways (i.e., bronchitis, sputum plugs), a single positive culture or PCR and lack of evidence suggesting invasive, chronic or allergic aspergillosis.\(^{19}\) The one common exception is cystic fibrosis, in which long term colonisation is relatively common and can only reliably be distinguished from Aspergillus bronchitis or ABPA by the absence of a raised Aspergillus IgG or IgE.\(^{69}\)

Occupational or exposure-related extrinsic allergic alveolitis

These patients present, as do others with extrinsic allergic alveolitis (EAA), with cough and breathlessness and sometimes, fatigue.\(^{20}\) If there is no known occupational risk, the exposure history is sometimes difficult to elicit, and is occasionally occult, as for example, with contaminated air conditioning or air intake ducts in offices or homes, undetected dampness at home or work, breathing apparatus and other examples. Imaging is highly characteristic with CT findings of extensive ground glass opacities, which are usually bilateral and symmetrical, but sometimes patchy. If linked to Aspergillus, the Aspergillus IgG antibody is raised.\(^{79}\)

Pulmonary aspergillosis in children

In children with cystic fibrosis, ABPA and Aspergillus bronchitis occur with increasing frequency in teenage years.\(^{14,15}\) Other children without cystic fibrosis may develop invasive and allergic aspergillosis, including fungal asthma, but CPA has yet to be described.

**DIAGNOSTIC APPROACH**

Diagnostic approaches have been described below by clinical context to support physicians working in different settings. Outpatients and clinic patients are addressed first, then hospitalised patients and finally, bronchoscopy. Only major clinical groups are addressed. Much guidance is published for transplant patients, who are not covered here. However, there are some common threads: 1) culture is insensitive and multiple specimens and supporting antigen or antibody data are extremely helpful; 2) most diagnoses (but not all) depend on recognised risk factors or exposures, antigen or antibody data and characteristic imaging findings; 3) to note, it is a mistake to make decisions for aspergillosis primarily on patient symptoms. The aphorism ‘Treat the patient, not the X-ray’ does aspergillosis patients a great disservice, as these disorders are so clinically quiet, symptoms
alone are a poor guide to severity or improvement in most cases. A summary diagnostic approach by disease is shown in Figure 3.

**TB clinics**

CPA may 1) mimic TB (>20% of HIV-negative pulmonary TB);80 2) be a co-infection with TB during treatment (~10%);81,82 or 3) follow TB, mostly in those left with cavities (6.5% per year, if residual cavity).3,4,83 Haemoptysis, lack of fever, radiological features of pleural thickening or thick-walled cavities and surrounding infiltrates are all clues to the diagnosis of CPA (Figure 4). Empirical dual therapy with an azole and rifampicin is ill-advised because of the profound drug interactions.

Aspergillus IgG testing is crucial,84 and the diagnosis of CPA is supported by positive microscopy, culture and PCR from sputum or antigen from bronchoscopy.

**Asthma clinics**

In patients who are struggling, recently had an exacerbation or who have bronchiectasis, the key test is the Aspergillus IgE or skin prick test,16 supported by an Aspergillus IgG, a total IgE and respiratory fungal culture or PCR (Figure 4). The most common forms of aspergillosis in asthma are ABPA, severe asthma with fungal sensitisation and Aspergillus bronchitis.

**Cystic fibrosis**

This is very similar to asthma, except that all patients with cystic fibrosis have bronchiectasis, and Aspergillus bronchitis is as common as ABPA.14,35,69 Aspergillus colonisation is also common. Aspergillus sensitisation alone is linked to worse lung function.

**Chronic obstructive pulmonary disease**

Currently, it is not routine practice to actively seek any form of aspergillosis in COPD outpatients. Knowledge of sensitisation and whether patients are colonised adds a layer of understanding as to why individual patients are either very breathless or frequent exacerbators.5,17,18 There is no direct evidence base currently for treating such patients, unless Aspergillus bronchitis can be diagnosed, although patients with fungal asthma do respond to antifungal agents. Colonised COPD patients may be

---

**Figure 3** Summary of diagnostic tests for pulmonary aspergillosis and their overall utility, excluding imaging and histopathology.\* ✓ = <35% sensitive; ✓✓ = 35–75% sensitive; and ✓✓✓ = >75% sensitive. PCR = polymerase chain reaction; Ig = immunoglobulin.

**Figure 4** Diagnosis of CPA and fungal asthma. CT = computed tomography; SAFS = severe asthma with fungal sensitisation; ABPA = allergic bronchopulmonary aspergillosis; CPA = chronic pulmonary aspergillosis; Ig = immunoglobulin.
more at risk of developing invasive aspergillosis, as is the case with leukaemia and transplant patients, but again this is not proven.

Both invasive and chronic aspergillosis occur in patients admitted to hospital with COPD. Positive culture(s) of *Aspergillus* is found in up to 20% on admission (with two samples submitted), and 22–67% reflect invasive aspergillosis, the remainder colonisation."85,86 Clues to the diagnosis of aspergillosis include bilateral shadows, nodules, cavitation and recalcitrant wheeze (consider tracheobronchitis). Serum *Aspergillus* IgG and possibly glucan are useful in supporting a diagnosis of aspergillosis.

**Intensive care units**

Medical ICU patients are at risk of invasive aspergillosis for multiple reasons, including underlying respiratory disease, compensatory anti-inflammatory response syndrome (CARS) and its accompanying immune deficits,87 and medications inflammatory response syndrome (CARS) and its underlying respiratory disease, compensatory anti-inflammatory response syndrome (CARS) and its accompanying immune deficits,87 and medications such as corticosteroids. The baseline incidence of invasive aspergillosis in the ICU is 1–12%, but severe influenza or Covid-19 can push the incidence up to 8–25%.24–28 Cultures from bronchoscopy or tracheal aspirates are ~40% sensitive, and colonisation is as frequent as infection. BAL and *Aspergillus* antigen increases sensitivity to ~90%,38 and in some studies up to 30% of serum antigen is positive.88 Glucan may also be positive,28 but not specific for aspergillosis, as it also indicates deep *Candida* infection and *Pneumocystis* pneumonia. Tracheobronchial aspergillosis is more common than is generally realised, requiring direct inspection for diagnosis.61 Bronchoscopy in the first 48 h of admission with culture, microscopy and *Aspergillus* antigen testing, repeated after 7–10 days, is the optimal diagnostic approach. In Covid-19 and influenza patients, inspection of the airways and *Aspergillus* antigen on BAL are the most helpful diagnostic approaches.24,27,28

**Leukaemia and other haematological disorders**

Neutropenia (along with monocytopenia and thrombocytopenia), corticosteroid use and phagocyte dysfunction all contribute to the risk of invasive aspergillosis.23 Prophylaxis with mould-active antifungals such as posaconazole is commonly used in the highest-risk patients. In those not on such prophylaxis, serum *Aspergillus* antigen and serum of blood PCR have a high sensitivity, but only PCR is sensitive in those on prophylaxis.46,58 Presumptive infection is often highlighted by CT of the chest or sinuses. BAL is the best sample for diagnosis. Glucan is more useful in those without profound neutropenia.

**Lung cancer**

The optimal approach for diagnosis in these patients has not been well studied. As many cases are probably sub-acute invasive, *Aspergillus* IgG testing may alert clinicians to infection, and prompt a bronchoscopy and BAL testing for microscopy, culture and *Aspergillus* antigen and PCR. Biopsy of abnormal areas is likely to be diagnostic.

**Outpatient bronchoscopy**

Bronchoscopy sometimes yields surprise findings, and all forms of aspergillosis can be diagnosed using bronchoscopy. Where it is possible, biopsy with fungal tissue stains are diagnostic of invasive and tracheobronchial disease. Thick mucus should be submitted for histopathology or cytology, as well as direct microscopy and culture, and additional samples sent for *Aspergillus* culture, antigen and PCR.

**FUTURE PERSPECTIVES**

Aspergillosis, like most fungal infections, is an unusual infection, but extremely important for the patient’s survival and well-being. A high level of awareness is critical, especially as ‘routine microbiology’ is so often negative, and imaging is only occasionally specific. For life-threatening, invasive aspergillosis, speed is of the essence. Time to result should be less than 48 h, and preferably shorter. Send away tests for this disease should all be ‘stat’. This urgency is not as important for chronic and allergic aspergillosis, but again ‘routine microbiology’ is of little help.

In future, better risk assessment tools may support early empirical therapy and confirmatory diagnosis. Such tools could include genetic assays in, for example, well-recognised, high-risk groups such as acute myeloid leukaemia, haematopoetic stem cell transplant recipients,89 TB and severe asthma. Recent major advances in diagnostic test performance and speed need to fully leveraged by clinicians to improve the currently poor outcomes for too many patients globally.

**Acknowledgements**

I am indebted to several colleagues who commented on the manuscript, including D Perlin, C MacDonald, D Emery, E Hammond, A Alastruey-Izquierdo and J L Rodriguez-Tudela.

This work was funded in part by the Medical Research Council (MRC) Newton Grant (MR/P017622/1) and the National Institute for Health Research Manchester Biomedical Research Centre, Manchester, UK. The publication of this article was supported by United Kingdom–Indonesia Joint Partnership on Infectious Diseases (MRC, Newton Fund, Risetekdikt; MRC grant number MR/S019989/1) and a Risetekdikt Grant (no NKB-282/UN2.RST/ HKP.05.00/2020).

Potential conflict of interest: DWD and family members hold Founder shares in F2G Ltd, a University of Manchester spin-out antifungal discovery company. DWD acts or has recently acted as a consultant to Pulmatrix (Lexington, MA, USA), Pulmocide (London, UK), Zambon (Bresso, Italy), iCo Therapeutics (Vancouver, BC, Canada), Mayne Pharma (Raleigh, NC, USA), Biosergen (Trondheim, Norway), Bright Angel Therapeutics (Toronto, ON,
Canada) and Cipla (Mumbai, India) and Pfizer (Groton, CT, USA). He sits on the Data and Safety Monitoring Board for a SARS CoV2 vaccine trial. In the last 3 years, he has been paid for talks on behalf of Dynamiker (Tianjin, China), Hikma (Amman, Jordan), Gilead (Foster City, CA, USA), Merck (Kenilworth, NJ, USA), Mylan (now Viatris) (Mumbai, India) and Pfizer. He is a longstanding member of the Infectious Disease Society of America Aspergillosis Guidelines Group, the European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines Group.

References


Des innovations significatives au cours de la dernière décennie ont abouti à un diagnostic plus sensible et plus rapide de l'aspergillose pulmonaire, allergique, chronique et invasive ainsi que de la bronchite et des nodules à Aspergillus. La nouvelle cartographie des diagnostics a révélé que l'incidence et la prévalence de l'aspergillose étaient plus élevées qu'on ne le pensait auparavant. Le traitement antifongique oral et intraveineux offre des taux de réponse clinique élevés aux patients affectés. L'absence de diagnostic laisse les patients surtraités par des agents antibactériens, des corticoïdes et des anti-tuberculeux aboutissant à une poursuite de la maladie et souvent au décès. L'introduction de plusieurs tests de diagnostic très performants contribue à redéfinir la prise en charge du patient. L'antigène d'Aspergillus peut être détecté sur 70–95% des échantillons de bronchoscopie chez les patients ayant une aspergillose invasive et chronique en moins d'une heure. La recherche d’immunoglobuline G (IgG) Aspergillus (précipitines) a une sensibilité de ≥90% et une spécificité de 98% pour l’aspergillose chronique et allergique. Une culture fongique respiratoire de grand volume et la réaction de polymérisation en chaîne à Aspergillus ont une sensibilité 3–5 fois plus élevée que la culture bactérienne de routine. Aspergillus IgE (ou le test cutané) diagnostique la sensibilisation à Aspergillus dans l’asthme, la mucoviscidose, les bronchopneumopathie chronique obstructive et après traitement de la TB et il est bien corrélé à une altération de la fonction pulmonaire et/ou à des exacerbations. Dans le monde, les cliniciens et les techniciens de laboratoire doivent intégrer ces excellents nouveaux outils pour améliorer les résultats cliniques en rendant les résultats d’une manière plus rapide et exacte.