Outcomes for invasive fungal infections have greatly improved in the past decade, and several new antifungal drugs have been or will be licensed in the next few years. Early accurate diagnosis and appropriate treatment have major impact on survival. In a 1995 survey of laboratory practice in the UK for mycology, major disparities were seen, with many laboratories not undertaking even simple diagnostic procedures. Delays in processing and inadequate procedures for handling samples, incomplete or delayed reporting of results, or a combination of these, compromise the care of patients. In randomised trials of antifungal chemotherapy, optimum treatments and good alternatives for others have been defined for some infections. High-quality care requires a multidisciplinary approach to diagnosis and management. In this review, we propose microbiology, histopathology, radiology, and clinical auditing standards, with the evidence base for each reviewed. The standards are absolutes, and, therefore, provide a straightforward basis for improving services to patients if they are all implemented.

The incidence of invasive fungal infections in all developed nations has increased notably in the past two decades. All over the world the rate of invasive fungal infections increased in patients with HIV infection until the advent of combination antiretroviral chemotherapy reversed this trend. Diagnosis of some invasive fungal infections is straightforward, such as in cryptococcal meningitis, but others are problematic, especially invasive aspergillosis. Units with active surveillance and diagnostic programmes help to keep mortality to a minimum, whereas delayed or missed diagnosis inevitably means death. The differences can be striking, such as in Dijon, France, where mortality from invasive aspergillosis fell from about 60% to 12% over 6 years because of improved management.5

The most important improvements in mortality from cancer have come from improved supportive care, including the management of invasive fungal infection. The UK has a poor outcome from cancer compared with other developed countries, and attempts to rectify this situation in the past 5 years have been successful only in certain instances. The National Health Service Cancer Plan (http://www.doh.gov.uk/cancer/cancerplan.htm) makes no mention of infection as a major complication in cancer patients, and in particular does not address supportive care, including the diagnosis and treatment of complications of therapy (including infection). The actual practice of UK microbiology laboratories for mycology is far from ideal.6

In view of these issues, the British Society for Medical Mycology set out to define standards of care to lower deaths from invasive fungal infections to a minimum. Around £30 million is spent in the hospital sector on antifungal treatments,7 not always in an optimum way. Here, we define some absolute standards of care that can be audited, resulting from extensive consultation. Scales for the quality of evidence underpinning these standards are those adopted by the Infectious Diseases Society of America.8 In all cases a category from E to A is used (panel 1), and for therapeutic interventions a scale of 3–1 (panel 2) has been used.

We have set out the standards of care by medical specialty, rather than by disease. The standards are collected together in panels for ease of use in the clinical setting (panels 3–6).

**Microbiology standards of care**

All fungi (yeasts and moulds) obtained from sterile sites, including blood and continuous ambulatory peritoneal dialysis (CAPD) fluids, and intravenous-line tips should be identified to species level by referral to a specialist laboratory.
Aspergillus niger is intrinsically resistant to all licensed azoles; and neutropenic patients and probably in patients in intensive human gut, and is highly likely to become invasive in respiratory fluids, particularly in patients in whom the identification for all relevance to clinical care. If a reference mycology laboratory identify to species level than others, and some have little (figure 1).

Important examples of fungi that have low susceptibility to antifungal agents include: Candida krusei, which is intrinsically resistant to fluconazole and less susceptible to amphotericin B than are other Candida spp; Aspergillus terreus; Scedosporium apiospermum (Pseudallescheria boydii), Trichosporon beigelii, and Scopulariopsis spp, which are resistant to amphotericin B; Mucorales spp, which are all intrinsically resistant to all licensed azoles; and Candida glabrata, which is frequently less susceptible to fluconazole than are other Candida spp.

Several outbreaks of invasive fungal infection have been uncovered because of the unusual species implicated (recommendation B), such as Candida lusitaniae, Candida tropicalis, Candida parapsilosis, C krusei, Candida lipolytica, Pichia anomala, among others. Interruption of transmission in the hospital first requires recognition.

Certain species of fungi are more or less likely to cause disease than others (B). Examples include C tropicalis and Aspergillus niger. C tropicalis is occasionally found in the human gut, and is highly likely to become invasive in neutropenic patients and probably in patients in intensive care, but not in liver-transplant patients. Some isolates are resistant to fluconazole. By contrast, A niger is less likely than Aspergillus fumigatus to cause pulmonary disease in patients with leukaemia; therefore, if it is isolated from bronchoalveolar lavage, it could be colonising but not the cause of invasive infection, although this distinction is not absolute. Some genera of fungi are more difficult to identify to species level than others, and some have little relevance to clinical care. If a reference mycology laboratory cannot identify an isolate to species level, a judgment should be made as to whether the species is required or not.

Uncertainty surrounds the usefulness of species-level identification for all Candida spp isolates cultured from respiratory fluids, particularly in patients in whom the isolation is repeated (C). Recognition of Cryptococcus neoformans in the context of immunocompromised patients is very important, however, and if a laboratory decides not to speciate all yeasts isolated from respiratory fluids, a means of screening for C neoformans needs to be implemented (figure 1).

**Fungi cultured from urine of special-care and transplant patients**

All fungi from urine of patients in intensive care, special-care baby units, burn units, and from any transplant patients should be speciated (A). Candida spp are cultured from urine in 1–9% of patients staying in hospital. The importance of the proportion, from a treatment perspective, depends on the clinical context. In infants in special-care baby units, this finding frequently represents invasive infection, and can precede documentation of candidaemia. The isolate in the urine is generally identical to that in blood or at other sites in multiply colonised patients. In other patients, urine represents an important site of colonisation that can inform prophylactic and pre-emptive strategies. The most common species encountered are Candida albicans and C glabrata, but any pathogenic Candida spp and occasionally other fungi, such as Aspergillus spp, C neoformans, and Coccioidoides immitis (as an example of an imported infection), may be involved. The possibility of disseminated disease in patients with such infections is high and early identification of the most likely infecting species is important.

No evidence shows that higher colony counts in urine are more or less likely to indicate local or systemic disease, and isolation from urine derived from an in-dwelling bladder catheter should be viewed in the same light as that obtained from spontaneously voided urine. Up to 50% of patients with autopsy-proven invasive candidiasis have negative blood cultures, despite multiple blood cultures, but most have positive cultures from other sites, including urine. If enough blood is cultured (20 mL for aerobic culture is recommended) new blood-culture systems have a high yield, although not 100%.

**Importance of microscopy**

Microscopy is an important investigation for several reasons. First, the diagnostic yield is more than that for culture alone in infections (A). Several studies attest to the substantially higher yield with microscopy than that with culture in bronchoalveolar lavage fluid—in the order of 20% in immunocompromised patients. For example, in a study in cancer and stem-cell-transplant patients, culture from bronchoalveolar lavage fluid was positive in 40% and cytology in 64%; either or both tests were positive in 67% of patients. In another study, 22 patients with invasive aspergillosis were diagnosed by bronchoalveolar lavage, and
Invasive fungal infections

Panel 3. Microbiology auditing standards in systemic fungal disease (strength of recommendation)

1 All fungi (yeasts and moulds) obtained from sterile sites, including blood and CAPD fluids, and intravenous line tips, should be identified to species level by referral to a specialised laboratory if necessary (A, see panel 1). Bronchoscopy fluid is regarded as sterile in this context for all fungi except Candida spp.

2 All fungi from urine of patients in intensive care, special care baby and burn units, and any transplant patients should be specified (A)

3 All bronchoscopy fluids from patients suspected of infection should be examined microscopically for hyphae and cultured on specialised media (A)

4 All clinical isolates of aspergillus should be identified to species level, by referral to a specialised laboratory if necessary (A)

5 All cerebrospinal fluid (CSF) specimens from HIV seropositive patients, transplant recipients or patients with sarcoidosis, or CSF specimens showing abnormal concentrations of glucose, protein, or leucocytes without an adequate explanation should be tested for cryptococcal antigen, if the Gram stain is negative (B)

6 All CSF specimens from immunocompromised patients or those with sarcoidosis, or CSF specimens showing concentrations of glucose, protein, or leucocytes without an adequate explanation should be cultured and antigen tested for C neoformans and all bacterial plates incubated for a minimum of 5 days and fungal plates at 30°C for up to 21 days (B)

Detailed studies in which methods are compared, such as different approaches to sample concentration and the importance of different stains, have not been done. We believe that the sample should be concentrated by centrifugation (B), and the pellet cultured for fungi, mycobacteria, and bacteria and stained with Gram stain or fluorescent (for chitin) stain for rapid.38 Results should be available within 2–4 h of the sample being received by the laboratory. Since delayed diagnosis of an invasive mould infection of the lung may be fatal for the patient, rapid processing is important.

Microscopy may help to discern whether an infection is caused by a septate or non-septate mould (figure 2). The former includes Aspergillus spp, and the latter mucorales such as Rhizomucor spp, Rhizopus spp, Mucor spp, Absidia corymbifera, Cunninghamella bertholetiata, and members of the families Sakkenaceae, Mortierellaceae, and Syncephalastraceae. Invasive aspergillosis can be treated with the azoles itraconazole, voriconazole, or posaconazole (when licensed), the mucorales can only thus far be treated with amphotericin B. Pulmonary mucormycosis responds well to surgical resection (11% mortality vs 68% with pharmacotherapy in recommendation B), if clinically possible.45 In addition to an early indication of the best treatment, pending a positive culture, most cases of pulmonary infection caused by mucorales do not yield positive culture. Mucorales are particularly susceptible to chilling in the refrigerator, and the potential yield may fall with temporary storage of the sample. They can also be damaged by tissue homogenisation and fail to grow. Thus the only means of establishing a causal diagnosis (aside from biopsy or autopsy) is microscopy.

An important differential diagnosis of pneumonia in immunocompromised patients is Pneumocystis carinii pneumonia. This infection can be diagnosed only by microscopy with use of a monoclonal antibody stain, or by molecular means, since the fungus cannot be cultured on standard media. Co-trimoxazole prophylaxis is partly protective, but resistance is emerging.44

Specialised fungal media, such as Sabouraud dextrose agar provide higher yield in recommendation A infections,45 including a greater frequency of isolation of common fungi such as Aspergillus spp, and is the only means (with the possible exception of serology) of making the diagnosis of infection with more fastidious fungi such as Histoplasma capsulatum, C immitis, Penicillium marneffei, and other rare fungi. As international travel increases, the frequency of such rarities is likely to rise, and it is important to identify which species is implicated to guide therapy.

Isolates of Aspergillus spp

All clinical isolates of Aspergillus spp should be identified to species level, by referral to a specialist laboratory if necessary. Aspergillus terreus is resistant to amphotericin B.43,44 This pathogen used to represent perhaps only 3% of all Aspergillus spp that caused invasive disease, but in a randomised study of voriconazole compared with amphotericin B for invasive aspergillosis, A terreus caused 6% of infections.46 In patients with chronic granulomatous disease, Aspergillus nidulans may be implicated and does not respond as well to amphotericin B as does A fumigatus.46 Resistance to triazoles (other than fluconazole) in Aspergillus spp has been described only in clinical isolates of A fumigatus and not other species.46 A niger is proportionately more likely to represent colonisation than infection.46

Investigation of cerebrospinal fluid

All cerebrospinal fluid (CSF) samples from HIV-seropositive patients, transplant recipients, or patients with sarcoidosis, or...
CSF samples with abnormal concentrations of glucose, protein, or leucocytes without adequate explanation should be tested for cryptococcal antigen, if the Gram stain is negative (B). Cryptococcal meningitis may be diagnosed reliably by antigen testing (A).\(^5\) Most cases of cryptococcal meningitis occur in HIV-antibody-positive patients (whether previously documented or not), transplant recipients, occasionally in other corticosteroid-treated patients, and in patients who have sarcoidosis. Antigen testing is appropriate in some patients with other immunocompromised states, such as those with hairy-cell or chronic lymphocytic leukaemia, but these groups of patients are difficult to define absolutely and, therefore, are omitted from the standard. Rarely, in the UK, cryptococcal meningitis occurs in non-immunocompromised patients, in whom the onset is gradual and commonly manifests as idiopathic hydrocephalus.\(^6\) In immunocompromised patients, the CSF antigen is positive in more than 95% of cases. Culture is occasionally negative because the sample volume was small (eg, <5 mL), was not cultured for fungus (although *C neoformans* will normally grow on conventional media), or the culture was discarded too soon.

A diagnostic dilemma arises if a CSF sample is abnormal but no diagnosis is established. Cryptococcal meningitis should be included in the differential diagnosis. In non-immunocompromised patients, culture of large volumes of CSF are sometimes necessary to establish the diagnosis, whereas the cryptococcal antigen is generally detectable, at low titres. The ELISA testing format that has been introduced may be the most sensitive method.\(^4\) Rarely, false-positive results occur, almost all in undiluted samples or 1-in-2 dilutions. Other fungal causes of meningitis include *Candida* spp, *Aspergillus* spp,\(^4\) *H capsulatum*,\(^4\) and *Sporothrix schenckii*,\(^4\) all of which are best diagnosed with specialised serology, except *Candida* spp meningitis, which is best diagnosed by culture.

CSF samples from immunocompromised patients or those who have sarcoidosis, or abnormal samples showing inexplicably abnormal concentrations of glucose, protein, or leucocytes should be cultured and antigen tested for *C neoformans*, and all bacterial plates incubated for a minimum of 5 days and fungal plates at 30°C for 21 days (B). The diagnosis of cryptococcal meningitis can be elusive; if not initially suspected on clinical grounds, the diagnosis can be established only by culture or antigen detection. As a fail-safe, and because some antigen tests are falsely negative (3–5%), culture for *C neoformans* (and other rarer fungi) on fungal media is recommended when the diagnosis is considered. The diagnosis is not, however, always considered, and *C neoformans* is sometimes cultured on bacterial media. Incubation for at least 5 days for all media plated with CSF from patients with abnormal CSF should, therefore, be routine. Fungal media should be incubated at 30°C for up to 21 days, the time limitation being drying of plates, which can be kept to a minimum with incubation in sloped or water-containing sealed containers (A).

**Histopathology standards of care**

**Use of fungal stains**

All tissues from immunocompromised (including corticosteroid-treated) patients with suspected infection should be stained with fungal stains such as periodic acid-Schiff, silver, or fluorescent stains, in parallel with regular stains. Biopsy and surgical resection of abnormal tissue are frequently definitive features in the diagnosis of invasive fungal infections. Speed is critically important in achieving an early diagnosis, and the practice of assessing haematoxylin and eosin stains of tissues before deciding whether to use specialised stains for fungi frequently introduces fatal delays for patients. Clear indication on all pathology request cards that the patient is immunocompromised is critically important (see clinical standard 1, panel 6). Hyphae and yeasts are commonly invisible on standard sections stained with haematoxylin and eosin or Gram stain alone (B).\(^6\) Often, only fragments of hyphae are present, especially on small samples such as transbronchial biopsy samples. Hyphae are best visualised by specialised stains for fungus (A), as recommended in anastomotic biopsy samples in lung-transplant recipients.\(^6\) Some histologists prefer periodic acid-Schiff staining because the morphology of the tissue adjacent to the fungi can be better visualised. Others prefer silver stains such as Gomori methenamine silver staining, since hyphae are immediately visible, but interpretation can be difficult because much besides fungal-cell walls are stained (figure 3).

Mucorales may require longer staining times, and other fungi can easily be left for too long. Therefore, inclusion of good control sections is mandatory. In one study, workers assessed different stains in experimental aspergillus keratitis and found that the Gomori methenamine silver

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**Panel 4. Histopathology auditing standards in systemic fungal disease (strength of recommendation)**

1. All tissues from immunocompromised (including corticosteroid-treated patients) with suspected infection should be stained with fungal stains such as Periodic acid-Schiff, silver, or fluorescent (for chitin) stains, in parallel with regular stains (B). Positive results should be telephoned through to clinicians immediately (A).

2. Reporting of specimens containing any fungal elements should always include the presence and absence of yeast forms, hyphae, and whether hyphae are or are not septate, if it is possible to tell, and whether there is any melanin present (A). The relative size of any fungi should be described, their cellular location, and any specialised structures or forms (A).

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**Figure 3. Histological section of stomach stained with the Grocott silver stain showing multiple hyphae, which branch irregularly and demonstrate folding, and appear as septa. The haematoxylin and eosin stain was negative for fungal elements. Cultures grew the mucorales *Absidia corymbifera*.**
stain with a haematoxylin and eosin counterstain gave the best results. The key recommendation is, therefore, that at least one specialised stain for fungi should be used for tissues from immunocompromised patients concurrently with haematoxylin and eosin and other relevant stains.

Specialised stains for cryptococcus such as the mucicarmine stain or the Masson-Fontana stain for dematiaceous fungi can be done after fungi are seen in sections. The visualisation of any fungi in sections is critically important, and the treating clinician must be informed promptly (A).

**Reporting of samples containing any fungal elements**

The reporting of samples containing any fungal elements should always include the presence and absence of yeast forms, hyphae, whether hyphae are septate or aseptate, and whether any melanin is present. The size of any fungi, their cellular location and any specialised structures or forms should be described (A).

The appearance of fungi histologically may be sufficient to partly establish the cause before culture results (A). The clinical presentation combined with the histological appearances is frequently sufficient to guide treatment appropriately until confirmatory evidence is available. For example, *Candida* spp generally manifest yeasts and hyphae in deep tissue, with the exception of *C. glabrata*, for which only medium-sized yeasts are visible without hyphae. The hyphae of the mucorales are broad, hyaline, and non-septate, and are commonly seen in necrotic tissue, whereas those of *Aspergillus* spp and other moulds are narrower and septate. Folding of hyphae can cause the false appearance of septa and rarely a septum can be seen in mucorales hyphae. Branching patterns differ as well, in that the mucorales tend to branch at right angles, at irregular intervals, whereas 45° branching is typical of *Aspergillus* spp.

Appearances may differ slightly after antifungal treatment. Several moulds can have hyphal forms in tissue indistinguishable from those of *Aspergillus* spp, including *Scedosporium* spp, *Penicillium* spp, *Paecilomyces* spp, *Fusarium* spp, *Alternaria* spp, or *Penicillium* spp, and others.

**Panel 5. Radiology auditing standards in systemic fungal disease (strength of recommendation)**

<table>
<thead>
<tr>
<th>1</th>
<th>All leukaemic and haematopoietic stem-cell transplant patients who are or have been profoundly neutropenic (&lt;500 neutrophils/mL) with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>a new cough, chest pain or haemoptysis</td>
</tr>
<tr>
<td>b</td>
<td>an abnormal chest radiograph</td>
</tr>
<tr>
<td>c</td>
<td>a new positive culture of an <em>Aspergillus</em> spp or other mould from any site</td>
</tr>
<tr>
<td>d</td>
<td>microscopic evidence of hyphae in any invasive sample</td>
</tr>
<tr>
<td>e</td>
<td>or unresolved temperature after 7 days of antibiotics and/or antifungals should have a high resolution (or spiral) computed tomography (CT) scan of the chest within 48 h, with immediate consultant review (A)</td>
</tr>
<tr>
<td>2</td>
<td>All transplant recipients with a new positive culture of aspergillus or other mould should have a CT scan of the chest within 48 h (B)</td>
</tr>
<tr>
<td>3</td>
<td>All immunocompromised patients with new neurological features (eg, change in mental status, seizure, stroke, persistent headache) or possible or proven meningitis should have a CT or magnetic-resonance imaging scan of the brain (A)</td>
</tr>
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</table>

**Geotrichum candidum, Acremonium spp, Scopulariopsis spp, Penicillium spp, Schizophyllum commune, and others.** Bulbous ends are typical of *Scedosporium apiospermum* (which is resistant to amphotericin B) and should be described if seen, although treatment with echinocandins may yield such structures in *Aspergillus* spp.

The finding of small intracellular yeasts is virtually diagnostic of either histoplasmosis or *P. marneffei* infection; the latter can be distinguished from the former by the appearance of one septum separating two halves of the yeast, since *P. marneffei* is a fission yeast and *H. capsulatum* a budding yeast, and *P. marneffei* stains darker. *C. neoformans* and *Blastomyces dermatitidis* appear as large yeasts, the former with narrow junctions between cells, the latter with broad-based buds. *C. neoformans* also has a capsule in most instances, best seen with the mucicarmine stain. Other rarer fungi have characteristic histological appearances including *C. immitis* (spherules) and *S. schenckii* (cigar-shaped bodies). In other diseases, such as mycetoma, grains may be seen.

**Radiology standards of care**

**Leukaemia, haematopoietic stem-cell transplant, and neutropenic patients**

All patients who have leukaemia have undergone haematopoietic stem-cell transplant, or have recently been severely neutropenic (<500 neutrophils/μL), who have also a new cough, chest pain or haemoptysis, an abnormal chest radiograph, a new positive culture of an *Aspergillus* spp or other mould from any site, microscopic evidence of hyphae in any invasive sample, or unresolved temperature after 7 days of antibiotics, antifungal, or both, should have a high-resolution spiral computed tomography (CT) scan of the chest, ideally within 48 h of changes, and immediate consultant review (A).

Imaging plays a crucial part in the diagnosis and management of pulmonary mould infections, as well as in other infections such as fungal meningitis, endocarditis, and renal and chronic disseminated candidiasis (figures 4, 5, and 6). We know from several studies that patients who have
leukaemia with invasive pulmonary aspergillosis are frequently diagnosed late because chest radiographs are falsely negative (A). In these patients, conventional or spiral CT scanning of the chest should be done initially, followed by high-resolution CT of any abnormalities identified. Thin slices (1 mm) should be taken, imaged with wide window settings (1500–2000 Hz), although thicker slices may be used for screening at a setting in which air and soft tissue can be seen to best advantage (around 500 Hz).60

In patients with abnormal chest radiographs, high-resolution CT of the chest gives the best results. The appearance of a so-called halo sign (an area of ground-glass opacity) around a nodule or focal consolidation, especially seen during neutropenia, is highly characteristic of an invasive mould infection of the lung (A; figure 7). The halo sign is present in more than 60% of patients, but for only 5–7 days during severe neutropenia, and occasionally in other patients such as allogeneic haemopoietic stem-cell-transplant recipients.5,61–67 In addition, chest radiography underestimates the extent of disease (A).68

In patients with negative CT scans who have persistent or recurrent fever, reimaging should be done with CT after a short time (probably 7 days; B). Some patients with invasive pulmonary aspergillosis require surgery immediately to prevent catastrophic haemoptysis (B),68 and all those with pulmonary zygomycosis (A) if technically possible.69 Failure rates of present treatment for mould infections of the lung are high70 and alternative treatments might be required. Radiological criteria are the most important in determining therapeutic failure or lack of response,71 although apparent progression during neutropenia despite treatment can be consistent with a delayed response.68

Transplant recipients
All transplant recipients with a new positive culture of Aspergillus spp or other mould from any site should undergo CT scanning of the chest, ideally within 48 h of receipt of results (B). The positive predictive value of a positive respiratory-tract culture in solid-organ transplant recipients is substantially lower than that in neutropenic allogeneic stem-cell-transplant patients. Large multicentre series have not been done, partly because of definition difficulties, but figures quoted in the published research vary from around 10% to more than 80%.30,45,71–73 In a US series, the risk of invasive disease contemporaneously with a positive culture of Aspergillus spp was 64% for allogeneic bone-marrow-transplant recipients and 17% in those who had undergone solid-organ transplant.30

The purpose of scanning is to identify rapidly patients who have invasive disease from those who are merely colonised. Renal and heart transplant recipients have the best prognosis of all transplant recipients for invasive aspergillosis, if the diagnosis is made. New pulmonary opacities in these contexts are particularly useful. The differential diagnosis includes infection with Nocardia
spp, C neoformans, or Rhodococcus spp, community-acquired bacteria, viruses, and lymphoma. Subsequent investigations include bronchoscopy, serology (eg, for cryptococcal antigen) and needle biopsy of the lung. A sinus or nasal culture-yielding Aspergillus spp is unlikely to represent invasive disease in solid-organ-transplant recipients, and this unusual situation should be exempted from this recommendation.

**Immunocompromised patients with neurological abnormalities**

All immunocompromised patients with new neurological features (eg, change in mental status, seizure, stroke, persistent headache) or possible or proven meningitis should have a CT or magnetic resonance imaging scan of the brain (A). Dissemination of an Aspergillus spp to the brain is an occasional disastrous complication.75,76 The triazoles itraconazole and voriconazole are probably slightly better than amphotericin B for treatment of cerebral aspergillosis.77,78 Unfortunately, the clinical presentation of cerebral aspergillosis is not distinctive (with the exception of a seizure or new stroke) and can be insidious. Precise definition of precisely all the clinical features that should always prompt a scan in these complex immunocompromised patients is difficult. However, a change in mental status not associated with drugs or other infectious process, although frequently subtle, is the earliest clinical feature (A).75,77 Persistent new (but not necessarily continuous) headache is also a common early feature. A new seizure, abnormal neurological findings, or stroke are certainly indications for scanning (C).

Dependent on the nature of the immunocompromising conditions, the differential diagnosis of an abnormal brain scan may be broad.79 However in all transplant patients the most frequent cerebral infectious complication is cerebral aspergillosis (figure 8). If coagulation parameters permit, and the lesion identified is not too deep in the brain, biopsy or aspiration should be undertaken (A). Microscopy is as least as helpful in this context as analysis of bronchoalveolar lavage fluid, and the two approaches should follow similar protocols (A).

**Clinical management**

**Requests for investigations**

For sample processing requests in microbiology, histology, and radiology to be prioritised correctly, request cards must include enough clinical information to show that the patient is immunocompromised (A). Some hospitals may choose to include an immunocompromised “check box” on forms. Patients with lesser degrees of suspected immune dysfunction (such as those taking low-dose corticosteroids, antibodies to tumour necrosis factor, previous methotrexate treatment, but who are not neutropenic, etc) should be classified as immunocompromised (B). Laboratories may need to set up new ways of scanning and interpreting clinical data to take into account such changes.

**Candidaemia**

All patients with candidaemia should have central venous catheters removed or replaced within 48 h of infection being documented, and earlier if possible (A, evidence level 2, panel 2), to lower mortality and shorten the duration of infection.80–82 Potent antifungal drugs might lessen or remove this need in the future. Although only about 10% of cases of candidaemia are thought to arise exogenously, seeding to the catheter is common, if not universal, which provides an infection focus that can remain subclinical. Peripheral venous catheters do not seem to be a focus of infection (C) and arterial lines should be changed as required (C3).

All patients with candidaemia should be treated with a systemic antifungal agent at an appropriate dose, and breakthrough fungaemia treated with an alternative agent (unless all treatment is withdrawn—eg, in palliative care; A2). Many cases of candidaemia were thought to be transient and self-limiting, but studies now show there is no means of distinguishing patients who have life-threatening disease or who will develop focal complications such as

![Figure 8. CT scan of the head showing a large contrast-enhancing abscess in the left frontoparietal region, and substantial surrounding oedema, typical of cerebral aspergillosis.](image-url)
ocular disease, endocarditis, osteomyelitis, and other foci, from those with transient candidaemia. Thus all patients with candidaemia should be treated with an appropriate antifungal drug (A2). Delays of more than 48 h in the starting of treatment worsens outcome.88 The intensity and duration of treatment may vary dependent on the disorder and immune state of the patient, and the sites and species of Candida involved.

Appropriate licensed systemic treatment includes fluconazole or amphotericin B,40–45 with or without flucytosine. The dose of fluconazole should not be less than 400 mg daily (A2) and should be adjusted downwards in patients with renal impairment (B3) and doubled in those on continuous haemofiltration (B2).86 Candidaemia caused by C krusei and C glabrata should not be treated with fluconazole (A2). A few isolates of other species of Candida (especially C albicans and C tropicalis) are fully or partly resistant to fluconazole and are best treated with an alternative drug (B2). Some data suggest that higher doses of fluconazole (800 mg daily) may be more effective than standard doses of 400 mg daily, but this approach is not recommended unless the patient is extremely ill (B2).46–48 There are concerns about use of fluconazole in profoundly neutropenic patients (C2), but these have been mostly dispelled by careful matched-pair studies.49

An alternative treatment for candidaemia is amphotericin B, but the dose required for a successful outcome is not known. Most authorities recommend at least 0·6 mg/kg daily (A1) and higher doses for very ill patients (B3) or those who have infections caused by C krusei, since this organism has poor susceptibility to amphotericin as well (B2).50–52 Lipid encapsulation probably increases the dose required for treatment based on limited animal-model data, but there are conflicting clinical data on whether doses higher than 4·0 mg/kg do or do not yield a better outcome than doses lower than 3·0 mg/kg (C3).53–55 The indications for flucytosine in candidaemia are controversial because the drug has not been well studied56 and intravenous flucytosine is not available in the USA and some other countries. Our preferences are to use it only in combination with fluconazole or amphotericin B in patients with life-threatening infection (A2) and in those in whom tissue penetration of the other agent may be poor—eg, eye, urine, and meninges (B2).57–59 The UK registered dose (200 mg/kg daily) is probably too high (A3) and is under review.

Echinocandins (caspofungin having recently been licensed) will probably have an important place in the treatment of Candida spp infections.60

**Transplant recipients and neutropenic patients**

All transplant recipients or severely neutropenic patients who have a new positive culture of an Aspergillus spp or other mould, or new pulmonary or cerebral abnormalities consistent with a fungal infection should be treated with a systemic antifungal agent at an appropriate dose active against moulds within 6 h of positive culture or documentation of the pulmonary or cerebral abnormalities (A2).

Early diagnosis and appropriate treatment of invasive mould infections are important in reducing mortality (A2). Although some cultures represent colonisation or contamination, many represent disease,61,62,77,106 with the one common exception of lung transplant recipients.72 If the radiological differential diagnosis includes fungal infection, treatment can be started (A2) and withdrawn if the culture shows non-relevant colonisation or another established diagnosis (B3). The same is true of new radiological abnormalities, although the specificity of some abnormalities is much higher than for others.

The only licensed choices for Aspergillus spp and other infections are amphotericin B, itraconazole, voriconazole, and caspofungin. Only conventional amphotericin B and voriconazole are licensed for primary treatment of invasive aspergillosis. Amphotericin B has been the broadest spectrum agent available and is active against A fumigatus, A flavus, A niger, all the mucorales, Fusarium spp, and some other moulds, but it is not active against A terreus, Scedosporium spp, Paecilomyces spp, and a few other moulds. Itraconazole is active against all Aspergillus spp, S apiospermum, and many other moulds, but is not active against the mucorales, Fusarium spp, Scedosporium prolificans, and some other rare moulds. Voriconazole has a slightly broader spectrum of activity against the moulds than does itraconazole. By contrast, caspofungin and other echinocandins have a narrow mould spectrum—probably Aspergillus spp only. Most infections are caused by A fumigatus and A flavus, which are generally susceptible to all four drugs, although treatment outcomes are not good overall.

The appropriate dose of a systemic antifungal drug with activity against moulds, requires some interpretation. The dose of amphotericin B should be 1·0 mg/kg daily (A2) or a lipid-based preparation should be used with at least three times this dose (3·0–10·0 mg/kg daily; C2). Itraconazole can be given intravenously or orally, and loading doses should be given (A2). The standard dose intravenously is 200 mg once daily, and orally is 200 mg twice daily for aspergillosis. The standard treatment dose of itraconazole is also the maintenance dose. Higher failure rates are associated with lower doses of itraconazole (B3). Very large doses of itraconazole may be appropriate for cerebral aspergillosis.77 Voriconazole, is better than amphotericin B (A1) and may provide the best alternative for invasive aspergillosis,9 but is not active against mucorales (E2). Caspofungin may be a useful alternative for invasive aspergillosis (A2).101

**Cryptococcal meningitis**

All patients with cryptococcal meningitis should be treated initially with amphotericin B deoxycholate at more than 0·7 mg/kg daily, or with lipid-based amphotericin B at more than 4·0 mg/kg daily with flucytosine 75–100 mg/kg daily (adjusted for renal function; A1).

Of all invasive fungal infections, the evidence base for the treatment of cryptococcal meningitis is the best. The first randomised study in this disease showed that the addition of flucytosine to a slightly lower dose of amphotericin B improved response rates and lowered mortality (A1).102
Search strategy and selection criteria

A consensus on the scope of the proposed standards was achieved by the authors, based on their knowledge and experience. Additional advice was sought from colleagues who are specialists in mycology. For each standard, the relevant published evidence was collected from filed reports and searches in Medline. More than 70 individual searches were done, many combining terms such as “candida” and “catheter”, “microscopy” and “fungus”, “Caflauor” and “mucorales”, etc. In subject areas for which large numbers of reports exist, we have referred to those with the best evidence in favour of or against a recommendation. Reports published in languages other than English have not been included, and books and book chapters were not accessed.

Other smaller studies comparing amphotericin B and flucytosine with intravenous amphotericin or fluconazole showed better response rates with the combination than with the azoles alone (A1). A large randomised study of cryptococcal meningitis in AIDS patients comparing amphotericin B with and without flucytosine showed a better response rate with combination treatment than with amphotericin alone, although not quite significantly so (A2), and a follow-up study in respondents done over 1 year, showed that relapse was particularly associated with a lack of flucytosine in the first 2 weeks of treatment (A1). Thus, strong data support the use of at least 2 weeks’ treatment with flucytosine combined with amphotericin B for the treatment of cryptococcal meningitis.

No good comparisons of the dose of amphotericin B have been done. However, consistently reduced mortality has been noted with higher doses of amphotericin B. A dose of 0·7mg/kg daily is, therefore, recommended as a minimum (B1), in combination with flucytosine. Higher doses of amphotericin B without flucytosine may be a reasonable alternative in patients intolerant to flucytosine (B2). If toxic effects arise, or administration of amphotericin B is impossible through a central venous catheter, a lipid-based amphotericin B formulation should be used. Poor results were reported for Abelec less than 3·0 mg/kg (D2), and good responses with AmBisome 4·0 mg/kg daily. (B2). Few data are available for Amphotil. The penetration of AmBisome into the brain is better than that with the other lipid-based amphotericin preparations and is equivalent to standard amphotericin B.

Conclusions

The absolute standards of care and proposed audit standards for patients with invasive fungal infection we describe represent the current state of the art. Developments in the next few months or years may supersede these standards, such as the routine advent of better serological and molecular diagnostic tests, better imaging methods, new antifungal drugs, combination antifungal treatments, further emergence of antifungal resistance, and so on. Nonetheless, the pace of these future developments is unlikely to change many of these standards for the next 5 years.

We could have proposed other non-absolute standards, but these can be difficult to audit and would have made the review unwieldy. Clinicians, microbiologists, histopathologists, and radiologists experienced in this specialty will, however, recognise gaps in the guidelines where the evidence base is less secure. These areas have not been ignored by the British Society for Medical Mycology and, if the evidence base improves, will be the subject of further recommendations.

Conflicts of interest

None declared.

References


