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SUPPLEMENT ARTICLE

Consensus guidelines for the diagnosis and management of invasive aspergillosis, 2021

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Key words

Aspergillus, invasive aspergillosis, diagnosis, antifungal therapy, haematological malignancy, stem cell transplant.

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Abstract

Invasive aspergillosis (IA) in haematology/oncology patients presents as primary infection or breakthrough infection, which can become refractory to antifungal treatment and has a high associated mortality. Other emerging patient risk groups include patients in the intensive care setting with severe respiratory viral infections, including COVID-19. These guidelines present key diagnostic and treatment recommendations in light of advances in knowledge since the previous guidelines in 2014. Culture and histological-based methods remain central to the diagnosis of IA. There is increasing evidence for the utility of non-culture methods employing fungal biomarkers in pre-emptive screening for infection, as well as for IA diagnosis when used in combination. Although azole resistance appears to be uncommon in Australia, susceptibility testing of clinical *Aspergillus fumigatus* complex isolates is recommended. Voriconazole remains the preferred first-line antifungal agent for treating primary IA, including for extrapulmonary disease. Recommendations for paediatric treatment broadly follow those for adults. For breakthrough and refractory IA, a change in class of antifungal agent is strongly recommended, and agents under clinical trial may need to be considered. Newer immunological-based imaging modalities warrant further study, while surveillance for IA and antifungal resistance remain essential to informing the relevance of current treatment recommendations.

Introduction

The expanding repertoire of treatments for various haematological and oncological malignancies (e.g. ibrutinib, chimeric antigen receptor (CAR) T-cell therapy, azacitidine) has led to longer cancer survivorship, but also new and often compounding long-term immunosuppression. This immunosuppression places patients at risk of invasive aspergillosis (IA) and other invasive fungal diseases (IFDs). Mortality from IA is high (30–60%),[1,2] and new patient risk groups have emerged. However, there have been improvements in the diagnosis of IA and new pharmacological agents have also become available for treating this challenging infection, with a multidisciplinary management approach essential for optimising patient outcomes.

The current guidelines represent an update of the 2014 invasive mould infection guidelines,[3] but focus

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only on IA in adults and children. The management of other non-*Aspergillus* invasive mould infections is covered in the accompanying guidelines by Bupha-Intr *et al.* 2021,[295] which can be found elsewhere in this supplement. Evidence-based data for children remains relatively scant, and this should be taken into consideration when implementing any recommendations. Herein, we briefly discuss changes in epidemiology and predisposing factors for IA, including patient risk groups other than haematology-oncology patients, summarise the contribution of newer diagnostic modalities and antifungal drug resistance and provide recommendations for the use of antifungal agents across various clinical contexts of IA.

It is pertinent to mention that the updated 2019 European Organisation for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) consensus definitions of IFD now incorporate: (i) *Aspergillus* polymerase chain reaction (PCR) and (ii) the molecular detection of fungal (*Aspergillus*) DNA in tissue, as diagnostic criteria for IA.[4] Further, there are newly defined specimen-specific thresholds for the *Aspergillus* galactomannan (GM) test. These changes in classifying the likelihood of IA are shown in Table 1. While these definitions are primarily designed to inform clinical research, rather than patient treatment *per se*, they do have some diagnostic relevance.

Table 1 Changes to the EORTC/MSGERC definitions of invasive aspergillosis over a decade: 2019 updated definitions (Donnelly *et al.*)[4] compared with those published in 2008 (De Pauw *et al.*)[5]

Category of IA	Host risks or diagnostic modality	Change(s) or additions made (for full definition criteria refer to Donnelly *et al.*)[4]
Possible and probable IA	Risk factors for IFD	• Addition of use of B-cell immunosuppressants (e.g. ibrutinib) • Explicit addition of solid organ transplants
	Imaging findings suggestive of pulmonary IA	• Addition of wedge-shaped and segmental or lobar consolidation
Probable IA	*Aspergillus* galactomannan	• Clear thresholds of 'positivity' established specific to the body fluid tested • Single serum or plasma BAL fluid or CSF (ODI cut-off ≥1.0) • Single serum or plasma (ODI cut-off ≥0.7) plus single BAL fluid (ODI cut-off ≥0.8)
	Aspergillus PCR	• *Aspergillus* PCR added as a mycological criterion (requiring two positive readings to qualify as a 'positive' result either from: consecutive blood samples; or duplicate samples if BAL fluid used; or a single positive from blood and a single positive from BAL fluid)
Proven IA	Molecular diagnostics (DNA sequencing)	• rRNA (ITS) gene sequencing from tissue specimens where fungal hyphae are seen on histopathology

BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; DNA, deoxyribonucleic acid; EORTC/MSGERC, European Organisation for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium; IA, invasive aspergillosis; IFD, invasive fungal disease; ITS, internal transcribed spacer; ODI, optical density index; PCR, polymerase chain reaction; rRNA, ribosomal ribonucleic acid.

Scope of guidelines

It is important to emphasise many points. First, these guidelines should be considered in conjunction with the accompanying guidelines for antifungal prophylaxis by Teh *et al.* 2021[296] and for optimising antifungal therapy and therapeutic drug monitoring (TDM) by Chau *et al.* 2021,[297] both of which can be found elsewhere in this supplement. Second, these guidelines relate only to the acute, invasive forms of aspergillosis. Guidance for the non-invasive, allergic, or other forms in the spectrum of chronic pulmonary aspergillosis, are detailed elsewhere.[6,7] The clinical context in which IA occurs is also important for informing its management. Table 2 defines the terminology that is utilised throughout these guidelines. Lastly, recommendations for the use of diagnostic tests and for choice of antifungal agent in different clinical scenarios is based on the GRADE and AGREE systems, as detailed by Chang *et al.* 2021[298] in the introductory chapter to these guidelines.[10,11]

Methodology

Questions asked

This update addresses the following questions:

1 What is new in the epidemiology of IA in haematology and oncology?
2 Do standard diagnostics for IA still have a role in 2021?

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Table 2 Clinical contexts of invasive aspergillosis – definitions

Clinical context	Definition	References
Primary IA	IA in a patient not exposed to a mould-active antifungal at presentation or within the last 7 days; first-line therapy is appropriate	8
Breakthrough IA	IA which occurs during exposure to an antifungal drug (given as either antifungal prophylaxis or treatment)	8
Refractory IA	Progression of disease, with worsening or new clinical symptoms, signs, or radiological features attributed to IA as a result of failure to respond to anti-*Aspergillus* antifungal treatment[†]	8,9

[†]As assessed by an expert physician after a clinically appropriate time interval (e.g. 2 weeks).
IA, invasive aspergillosis.

3 How has thoracic imaging and other types of imaging in IA improved?
4 How may biomarkers for aspergillosis be utilised to establish a diagnosis and to pre-emptively screen for IA?
5 How can biomarkers be used to assess treatment response in IA?
6 How prevalent is azole-resistant *Aspergillus fumigatus* and does it occur in Australia?
7 What is the role of antimicrobial susceptibility testing in managing IA?
8 How do we detect and diagnose azole resistance?
9 What recommendations should guide the first-line antifungal treatment of IA in haematology/oncology patients?
10 What is the role of combination antifungal therapy in the first-line setting?
11 How do we treat azole-resistant *A. fumigatus* in 2021 and what new anti-*Aspergillus* drugs are in the pipeline?
12 What is breakthrough IA and how should it be managed?
13 How should refractory disease be managed?
14 What is the role of TDM in managing IA?
15 Are there any adjunctive therapies available for managing IA?
16 What is new in the management of extrapulmonary and disseminated IA?

Search strategy

A literature review was performed using PubMed to identify papers published until June 2020 relating to the diagnosis and management of IA, with particular focus on new publications since January 2014. Search terms included '*Aspergillus*', 'aspergillosis', 'diagnosis', 'management', 'treatment', 'epidemiology', 'haematology', 'oncology', 'stem cell transplant', 'galactomannan', 'B-D-glucan', '*Aspergillus* polymerase chain reaction', 'panfungal polymerase chain reaction', 'imaging', 'positron emission tomography', 'susceptibility testing', 'antifungal resistance', 'invasive pulmonary aspergillosis', 'antifungal therapy', 'surgery', 'extrapulmonary aspergillosis', 'central nervous system', 'endophthalmitis', 'keratitis', 'osteomyelitis'. International guidelines relating to IA diagnosis and management were also reviewed.

Question 1: What is new in the epidemiology of IA in haematology and oncology?

Among patients with haematological malignancies, the incidence of all IFDs is highest in patients with acute myeloid leukaemia who have a proven/probable IFD incidence of up to 12%.[12,13] The risk of IFD post-allogeneic stem cell transplant is estimated at 5–15%,[14,15] while patients with acute lymphoblastic leukaemia were more recently identified as a high-risk group with an incidence of 6–10%.[16–18] Although the incidence of IFD in chronic lymphoproliferative disorders is lower at 0.5–8%, the introduction of newer treatments may change this.[12,19] *Aspergillus* species account for the largest proportion of invasive mould infections, of which the majority are caused by members of the *A. fumigatus* complex. As the predominant species varies with geography, local epidemiological data are critical.[12,14]

Traditional risk factors for IA include duration and degree of neutropenia, active malignancy, high-dose chemotherapy, previous IA and in allogeneic transplants, mismatched or unrelated donor, graft-versus-host disease (GVHD) and cytomegalovirus infection.[15,20] Multiple lines of therapy may increase risk in haematological malignancies traditionally associated with an overall lower incidence of IA. Teh *et al*. reported an increased risk of IFDs in myeloma patients receiving more than three lines of treatment, although immunomodulatory drugs or proteasome inhibitors alone did not increase risk.[21] Disease progression can induce defects in the immune system and contribute to cumulative immunosuppression and risk of opportunistic infections.[22]

Since the 2014 guidelines, small molecule targeted inhibitors and immunotherapeutic agents have revolutionised treatments in the oncology/haematology setting. Given to heavily pre-treated patients, these agents are associated with an increased risk for IA.[23,24] The increased risk of IFD associated with ibrutinib, other Bruton tyrosine kinase inhibitors, immune checkpoint inhibitors, janus kinase inhibitors and CAR modified T cells, along with the role of primary prophylaxis, are

discussed in the accompanying guidelines for antifungal prophylaxis by Teh *et al.* 2021,[296] which can be found elsewhere in this supplement. IA accompanying ibrutinib use appears to be associated with infection early after commencement of therapy and relatively high rates of CNS infection, particularly in the setting of primary cerebral lymphoma, while the risk of IA after CD19 CAR T-cell therapy is associated with prior lines of therapy, cytokine release syndrome requiring steroids or tocilizumab, and prolonged neutropenia.[25–28]

For all the above therapies, vigilance must remain high for signs and symptoms suggestive of IA, with aggressive investigation to diagnose or exclude infection (see later section on 'diagnosis'). Pharmacokinetic drug interactions with antifungal agents that are CYP3A4 inhibitors are an important consideration when using novel agents such as venetoclax and ibrutinib. Where appropriate, dose adjustments are recommended (see the accompanying optimising antifungal therapy and TDM guidelines by Chau *et al.* 2021,[297] which can be found elsewhere in this supplement).[29,30]

Other immunosuppressed patient groups, in particular solid organ transplant recipients, are at increased risk for IA. Of these, those with lung transplants are at highest risk, with kidney transplant recipients at lowest risk.[31–33] Primary immunodeficiency conditions, including chronic granulomatous disease (CGD), may also cause or present as IFD and must be considered in patients with no obvious risk factors.[34] Furthermore, patients not typically considered 'immunosuppressed' such as those in intensive care units (ICU), are also at increased risk of IA – overall incidence 0.3% to 5.8% – with high mortality.[35,36] Diagnosis is difficult in this group due to a lack of classical clinical and radiological features, plus a lack of traditional risk factors as per the EORTC/MSGERC definitions of IFD.[37] Expert bodies are in the process of formulating ICU-specific IFD definitions.[38] Finally, there appears to be an increased risk of IA in the setting of COVID-19 infection (4–33% of patients with COVID-19 admitted to ICU),[39–41] now termed COVID-19 associated pulmonary aspergillosis.[42] As reports are based on small cohort studies, incidence rates should be interpreted with caution. Please see Appendix I for incidence rates and available management guidelines in non-malignant risk groups.

Question 2: Do standard diagnostics still have a role in 2021?

Recommendations

- Histopathological examination and culture remain the 'gold standard' for diagnosing IA (Strong recommendation, Level II evidence).
- It is strongly recommended that appropriate clinical specimens (e.g. tissue, bronchoalveolar lavage (BAL) fluid, sputum) be collected for fungal microscopy and culture as well as cyto-histological examination (Strong recommendation, Level II evidence).
- Growth at 50°C is a simple way to distinguish *A. fumigatus sensu stricto* from other species within the *A. fumigatus* species complex (Strong recommendation, Level II evidence).
- Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) may be used for species identification (Moderate recommendation, Level II evidence).
- Reserve DNA sequencing for isolates with atypical characteristics or for uncommon *Aspergillus* species (Strong recommendation, Level II evidence).

Timely diagnosis of IA is still a challenge and must be based on combined clinical, radiological and microbiological data. Despite significant advances in non-culture-based diagnostics, histopathological examination and culture remain the 'gold standard', with visualisation of fungal hyphae in tissue, associated with invasion, still required to classify a patient as having proven pulmonary IA and other tissue-associated IA (Fig. 1).[4] Similarly, culture of *Aspergillus* spp. from a sterile site supports a diagnosis of proven IA. The key features of these tests, along with recommendations for performing the tests, are summarised in Table 3. While the recommendations provided here apply to the haematology-oncology population, they are also broadly applicable to other patient groups. Any diagnostic test must be interpreted within the context of a patient's presentation and any prior or current antifungal

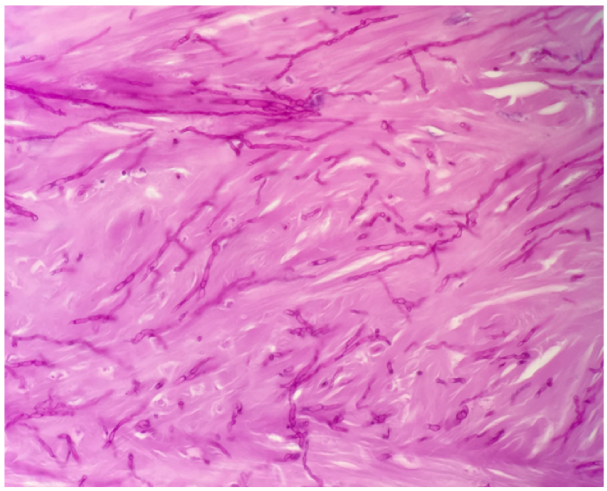

Figure 1 Typical acute dichotomous-branching septate hyphae of *Aspergillus* on direct microscopy from lung tissue with Periodic acid-Schiff staining. (Image from Dr Catriona Halliday, Westmead Laboratory, Westmead, NSW, Australia.)

Table 3 Recommendations for examination of clinical specimens by histopathology, microscopy, culture and *Aspergillus* identification from cultured isolates in haematology-oncology patients

Diagnostic approach or test	Method details	SoR	QoE	Test performance and comments	Selected references
Microscopy					
Histopathological examination of tissue sections	Gomori's methenamine silver stain, Periodic acid-Schiff stain, fluorescent dyes (e.g. calcofluor white)	A	II	Essential approach. Sensitivity depends on sampling and technical factors. Not specific for *Aspergillus* but morphology of hyphae may provide clues to fungal class. *Aspergillus* hyphae are typically septate with dichotomous acute angle (45°) branching	43,44
Immuno-histochemistry	*In situ* hybridisation with *Aspergillus*-specific monoclonal antibodies	B	II	Has potential to provide genus and species data Not widely available and requires expertise	45–49
Microscopy on fresh clinical specimens (e.g. BAL)	Fluorescent dyes (e.g. calcofluor white)	A	II	Essential approach. Not specific for *Aspergillus* but rapid. Broad applicability. Morphology may provide clues to fungal class (see above)	50–52
Culture and *Aspergillus* species identification					
Primary isolation from deep sites and sterile samples (e.g. biopsies, CSF)	Culture on standard mycological media (e.g. SDA) for up to 3 weeks at 30°C	A	II	Essential approach. May need enriched media or media containing antibiotics to recover isolates	53,54
Primary isolation from non-sterile samples (e.g. sputum, respiratory aspirates)	Culture on standard mycological media (e.g. SDA) for up to 3 weeks at 30°C	A	II	Essential approach. May need enriched media or media containing antibiotics to recover isolates	53,54
Identification of species complex and species identification of *A. fumigatus*	Macroscopic and microscopic examination from primary cultures	A	II	Colony colour, conidium size, shape, colour; conidiophore characteristics; presence of septation. Thermotolerance test (growth at 50°C for species confirmation of *A. fumigatus sensu stricto*)	53,54
	MALDI-TOF MS	B	II	In-house databases are often used to improve species identification (all species)	55–57
	Sequencing of ITS regions and β-tubulin genes	A	II	Not necessary if organism has typical growth characteristics. Important in epidemiology studies	58,59

BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; ITS, internal transcribed spacer; MALDI TOF MS, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; QoE, quality of evidence; SDA, Sabouraud dextrose agar; SoR, strength of recommendation.

administration. It is strongly recommended that appropriate clinical specimens (e.g. tissue, BAL fluid, sputum) be collected for fungal microscopy and culture as well as cyto-histological examination.

Culture of *Aspergillus* spp., particularly from non-sterile sites, should remain a focus for clinicians, as it can 'upgrade' the diagnostic likelihood from possible IA to a case of probable IA in the setting of relevant host, radiographic and clinical features.[4] It can also provide an isolate for species identification and for drug susceptibility testing (see Table 3 for common identification methods). Growth at 50°C is a simple way to distinguish *A. fumigatus sensu stricto* from other species within the *A. fumigatus* species complex.[43] Species identification by MALDI-TOF MS is increasingly used, with DNA sequencing (e.g. targeting the internal transcribed spacer region and/or the β-tubulin locus) usually reserved for isolates with atypical characteristics or for uncommon *Aspergillus* species.[43]

Question 3: How has thoracic imaging and other types of imaging in IA improved?

Recommendations

- When pulmonary IA is suspected, multislice non-contrast enhanced thoracic computed tomography (CT) is strongly recommended (Strong recommendation, Level II evidence).
- In adults, imaging of other sites including brain and sinuses should be based on signs and symptoms (Strong recommendation, Level II evidence).
- In children, brain imaging may be considered in the absence of clinical signs and symptoms of central nervous system (CNS) disease (Marginal recommendation, Level II evidence).

Multislice, multidetector CT imaging is a cornerstone for diagnosing lung infection in invasive pulmonary aspergillosis (IPA). Typical signs include single or multiple pulmonary nodules, which may have surrounding ground-glass opacities (i.e. the halo sign), cavitation or an air crescent sign.[60] However, these typical signs are often transient,[60,61] and atypical radiographic findings may be present in patients at varying levels of immunocompromise, in the presence or absence of antifungal prophylaxis,[62] and in children.[63] Atypical appearances include patchy consolidation or ground-glass change without nodules.[62] The propensity for IPA to present with a variety of radiographic signs, rather than only those classically attributed to IA, is recognised in the 2019 updated EORTC/MSGERC IFD definitions.[4]

As such, alternative imaging modalities have been assessed with the aim of improving the sensitivity and specificity for IPA. Pulmonary CT angiography may improve sensitivity by demonstrating angioinvasion,[64] but at the expense of reduced specificity. 18F-FDG-positron emission tomography (PET)/CT offers numerous advantages, including identification of lesions with high avidity, which are more suggestive of invasive disease,[65] as well as non-CNS sites of dissemination.[66] Importantly, it also provides the ability to assess responsiveness to therapy with sequential imaging.[66–68] However, the data to support the utility of 18F-FDG-PET/CT for the diagnosis of IA stem from retrospective and non-randomised prospective studies, with results from larger prospective studies still pending (*Moderate recommendation, Level II evidence*). Furthermore, access to PET/CT may be limited and is not currently funded in Australia for any infection indications.[69]

Imaging of other sites such as sinuses and brain is recommended on the basis of suggestive symptoms and signs, although routine imaging of the brain in paediatric cases may be considered (see also later section on extrapulmonary aspergillosis). Serum biomarkers of infection (see later discussion) may be evident in invasive sinusitis and CNS infection, so the absence of pathology on chest imaging in the setting of 'positive' biomarkers should prompt imaging of the CNS and sinuses as indicated. The optimal timing of follow-up imaging to assess response to therapy is uncertain. It is generally accepted that imaging at or beyond 14 days of treatment commencement is prudent, as imaging prior to this time may falsely suggest failure of therapy (given the natural history of pulmonary IA to worsen radiologically within the first 14 days of treatment).[9,60] Earlier imaging may be warranted in the setting of significant clinical deterioration if there are concerns regarding an alternative diagnosis or secondary complication such as major vessel invasion or dissemination.

Question 4: How may biomarkers for aspergillosis be utilised to establish diagnosis and to pre-emptively screen for IA?

Non-culture-based diagnosis of IA

The major non culture-based biomarker tests for IA comprise GM, *Aspergillus* lateral flow antigen, *Aspergillus*-specific PCR, 1,3-β-D-glucan (BDG) and detection of *Aspergillus* DNA by broad range or panfungal PCR assays. The performance and recommendations for use of these assays in the clinical contexts of (i) 'screening' for infection; or (ii) for diagnostic purposes, are summarised in Table 4. One or more biomarkers may be detectable in blood (or blood fractions), BAL fluid and cerebrospinal fluid (CSF) with PCR assay also performed on tissue and other body fluids such as vitreous material from the eye. In general, the performance of each of these biomarker assays is optimal when used in combination.[70,108,109]

Overall, GM detection in body fluids is more sensitive than culture for the diagnosis of IA, and GM as measured by the Platelia *Aspergillus* assay (Bio-Rad, Richmond, CA, USA) is endorsed as a microbiological criterion for the diagnosis of IA in both adults and children.[4,63,110,111] GM is reported as an optical density index (ODI). In serum samples, an ODI cut-off of 0.5 results in high sensitivity and negative predictive value (NPV) in haematology patients who are not receiving mould-active prophylaxis (Table 4). Twice-weekly serial screening is recommended in patients with prolonged

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Table 4 Major non-culture-based tests for invasive aspergillosis: clinical context, performance and recommendations for use

Test approach	Clinical context	Test performance	SoR	QoE	Comments	References
Galactomannan (GM) (Platelia *Aspergillus* (Bio-Rad, California, USA))						
GM in blood	Prospective screening for IA in absence of mould-active prophylaxis	Highest test accuracy requiring a 'positive' result from two consecutive samples (ODI ≥0.5 or retesting the same sample) Pooled sensitivity 78–79%; pooled specificity 85–86%	A	I	Prospective monitoring for IA should be combined with clinical evaluation, HRCT and other biomarkers as appropriate	70–75
GM in blood	Prospective screening for IA in presence of mould-active prophylaxis	Low PPV	D	II	Low prevalence of IA in this setting	70,76,77
GM in blood	Diagnosis of IA	Overall low sensitivity at ODI cut-off of 0.5	B	II	Sensitivity highest in neutropenic patients	72,78
GM on BAL fluid	Diagnosis of pulmonary IA	At ODI cut-off of 0.5: pooled sensitivity 61–92%; pooled specificity 81–98% At ODI cut-off of 1.0: pooled specificity 94–95% with only small loss of sensitivity	A	II	For routine clinical care: ODI cut-off ≥0.5, in the context of risk factors and appropriate clinical/radiological features For probable pulmonary IA by EORTC/MSGERC criteria: a single BAL ≥1.0 or BAL ≥0.8 AND serum/plasma ≥0.7 Sensitivity is lower in patients exposed to mould-active antifungals	79–81
Aspergillus LFA						
LFA applied on BAL samples	To diagnose IPA	Sensitivity for probable IPA 100%; specificity 81%; PPV 71%; NPV 100%	B	II	$n = 37$; haematological patients and solid organ transplant patients	82
LFA on BAL samples	To diagnose IA	For proven or probable IA: pooled sensitivity 86%; specificity 83%; DOR 65.94	B	II	Meta-analysis of seven studies published 2008–2015	83
LFA on serum samples	To diagnose IA	For proven or probable IA: pooled sensitivity 68%; pooled specificity 87%; DOR 11.90	B	II	Meta-analysis of seven studies published 2008–2015	83
LFA on serum samples	Screening for IA	One positive result: sensitivity 40%; specificity 86.8%; DOR 3.03 Two positive results: sensitivity 20%; specificity 97.8%; DOR 11.13	B	II	Prospective screening of 101 allogeneic HSCT patients; compared with GM (comparable results)	84
1,3-β-D-Glucan (Fungitell kit, Associates of Cape Cod, Falmouth, Massachusetts, USA)						
Serum BDG assay	To diagnose IFD	Overall sensitivity 50–70%; specificity 91–99%	C	II	Adult haematological malignancy and HSCT	85–91
Serum BDG assay	To diagnose IA	Overall sensitivity 78–85%; specificity 91–99%; NPV 85–92%	C	II	Mixed population in ICU including haematology patients	92,93
Serum BDG assay	Screening assay for IFD	Two or more consecutive 'positive' results: sensitivity 65%; specificity 93%	C	II	Performance varies with assay and cut-off value; mixed population	88,94

Table 4 *Continued*

Test approach	Clinical context	Test performance	SoR	QoE	Comments	References
Serum BDG assay	Screening assay for IA	Overall sensitivity 46%; specificity 97%	C	II	Adult haematological malignancy and HSCT	87,88
Aspergillus PCR in conjunction with serum GM						
PCR on whole blood, serum or plasma	To screen for IA (in absence of mould-active prophylaxis)	PPV 50–80%; NPV 80–95%	A	I	Haematological malignancy and HSCT, used in conjunction with GM for greater accuracy: PPV 50–80%, NPV 80–90%	70,95
Aspergillus PCR as the sole biomarker						
PCR on whole blood, serum or plasma	To screen for IA	Single positive result: sensitivity 88%; specificity 78% Two consecutive positive tests: sensitivity 75%; specificity 87%	B	II	Haematological malignancy and HSCT, used as sole biomarker; meta-analysis, 16 studies, performed on blood	96
PCR on whole blood, serum or plasma	To screen for IA	Sensitivities for serum and whole blood were 80% and 55% respectively Specificity for serum and whole blood were 69% and 96% respectively	B	II	HSCT, used as sole biomarker, performed on whole blood and/or serum; combination of serum and whole blood superior	75,97–99
PCR on BAL fluid	To diagnose IA	Varied results due to different assays used; better performance in patients not on antifungals	B	II	HSCT and haematological malignancies	100–103
Molecular diagnostics by panfungal PCR on biopsies						
Panfungal PCR on histopathology-processed biopsy specimens where fungal hyphae are visible	rRNA gene sequencing (ITS region preferred)	Sensitivity >90%; specificity 99%	A	II	–	51,104
Panfungal PCR on histopathology-processed biopsy specimens where fungal hyphae are not visible	rRNA gene sequencing (ITS region preferred)	Sensitivity 57%; specificity 96%	C	II	Use only in conjunction with other tests	51,104
Panfungal PCR (PE specimens)	rRNA gene sequencing (ITS region preferred)	Lower sensitivity than for non-PE specimens High specificity	A	II	Extraction of paraffin required	105,106
Panfungal PCR on fresh tissue samples	rRNA gene sequencing (ITS region preferred)	Sensitivity >90%; specificity 99%	A	II	Caution against placing specimen in formalin	51,104,107

BAL, bronchoalveolar lavage; BDG, 1,3-β-D-glucan; DOR, diagnostic odds ratio; EORTC/MSGERC, European Organisation for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium; GM, galactomannan; HRCT, high-resolution computed tomography; HSCT, haemopoietic stem cell transplantation; IA, invasive aspergillosis, ICU, intensive care unit; IFD, invasive fungal disease; IPA, invasive pulmonary aspergillosis; ITS, internal transcribed spacer; LFA, lateral flow assay; NPV, negative predictive value; ODI, optical density index; PCR, polymerase chain reaction; PE, paraffin-embedded; PPV, positive predictive value; QoE, quality of evidence; rRNA, ribosomal ribonucleic acid; SoR, strength of recommendation.

neutropenia in allogenic haemopoietic stem cell transplantation (HSCT) during early engraftment phase.[111] The requirement for two consecutive positive results before a test qualifies as 'positive', improves test specificity (>95%) with only a small loss in sensitivity.[112] However, serial screening is not recommended in patients receiving mould-active prophylaxis (*Not recommended, Level II evidence*).[113,114] While the 2019 EORTC/MSGERC IFD definitions define an ODI cut-off of 1.0 for clinical research to increase specificity and

positive predictive value (PPV), it must be stressed that this cut-off is *not* for routine clinical care, and the manufacturer's cut-off for a 'positive' test remains at 0.5. The performance of serum GM appears to be similar in children and adults (pooled sensitivity of 0.81 and pooled specificity of 0.88 in children with haematological conditions).[115]

GM testing in BAL fluid can be useful, as a positive test strongly supports the diagnosis of IA (ODI cut-off ≥0.5). Using a higher cut-off for a positive test (ODI cut-off ≥1.0 vs ≥0.5) improves test specificity with only a small decrease in sensitivity (78% vs 88%; $P = 0.36$).[4,116] As for serum GM testing, an ODI cut-off of 0.5 is recommended for routine clinical care.

Other than GM, novel *Aspergillus* lateral flow assay (LFA) antigen detection tests have been examined for their utilisation in the diagnosis of IA (Table 4). The IMMY sona assay (IMMY, Norman, OK, USA) and the OLM lateral flow device (OLM Diagnostics, Newcastle Upon Tyne, UK) have both been approved for this use. Tested on serum and BAL fluid, current data indicate that the LFA may be useful in centres with low sample throughput as an alternative to GM testing,[117] but large-scale data are lacking (*Moderate recommendation, Level II evidence*). These assays are not widely available in Australia as yet.

1,3-β-D-Glucan

The serum BDG assay is not routinely available in Australia, but is widely studied in various populations in the context of screening for early IA and IFD, as well as for diagnosis of IA. The sensitivity and specificity of BDG, ranging from 46% to 80% and ≥90%, respectively, has derived largely from studies using the Fungitell assay (Associates of Cape Cod, Falmouth, MA, USA).[111,112] Test performance is improved if two consecutive positive specimens are required to call a 'positive' result, or if the assay is used in combination with *Aspergillus* GM or PCR.[108,109,118] As these results are derived largely from validation studies, with comparison against a defined gold standard rather than real-world data, the reported specificity is likely over-estimated. When used alone, the body of data (Table 4) shows that the assay has a limited role for both ruling in or out the exclusive diagnosis of IA (*Marginal recommendation, Level II evidence*).[4] Australian data on the utility of serum BDG assay are sparse. The Fungitell assay performance for the diagnosis (or exclusion) of IFD in haematology patients receiving mould-active prophylaxis from a single institution, suggests suboptimal sensitivity and PPV for the diagnosis of any IFD.[119]

There are few data on yield of BDG performed on BAL fluid. A single-centre study performed on patients with pulmonary infiltrates found modest sensitivity and specificity (56.5% and 83.2% respectively) but with poor PPV (34.2%).[120] Therefore, BDG performance on BAL fluid can only be weakly recommended (Marginal recommendation, Level III evidence). Further, BDG is not recommended for screening or in the evaluation of suspected IA in immunocompromised children (Not recommended, Level III evidence).[63]

Aspergillus PCR

Table 4 also summarises the contexts in which *Aspergillus* PCR may be performed to assist diagnosis of IA. The performance of serum PCR is not significantly different from that of whole blood,[75,98,99,121] and while the utility of PCR has been established in adults with haematological malignancies,[121–123] data in children are limited.[63] Thus, within the context of a pre-emptive strategy to screen at-risk patients not receiving mould-active prophylaxis, it is strongly recommended that *Aspergillus* PCR be performed on blood, and in combination with GM (*Strong recommendation, Level I evidence*). Methodology based on that used by the Fungal PCR Initiative consortium is strongly preferred (see the FPCRI website: http://www.fpcri.eu/).[124] In antifungal drug-naïve patients, a negative PCR result is sufficient to rule out IA (NPV 98%); however, in patients receiving mould-active prophylaxis, the PPV of PCR is only around 5%.[121,123,125] Using the criteria of two positive results to define a 'PCR positive' test improves specificity and accuracy, as does the use of PCR in combination with GM; the latter is associated with earlier diagnosis.[108,111,123]

For diagnostic purposes, detection of *Aspergillus* DNA in other clinical specimens (e.g. CSF) is useful for confirming IA.[126,127] However, for BAL fluid, while PCR is sensitive and a negative result is useful to exclude disease, a positive result cannot distinguish colonisation from IA (PPV 72%).[122] Its use is moderately recommended (*Moderate recommendation, Level II evidence*). It is essential that *Aspergillus* PCR results are interpreted within the context of the clinical presentation and antifungal drug use.

Broad range or panfungal PCR on biopsies

Broad range panfungal PCR assays followed by either DNA sequencing or high-resolution melt curve analysis, are helpful in the identification of *Aspergillus* and other fungal pathogens, although *Aspergillus*-specific assays may offer superior sensitivity and specificity for the diagnosis of IA.[128] Undertaking panfungal PCR is strongly recommended (Strong recommendation, Level II evidence) for biopsy specimens which demonstrate fungal

hyphae on histology (Table 4). If no hyphae are visible, the diagnostic yield of molecular methods is reduced (*Marginal recommendation, Level II evidence*).[129]

Question 5: How can biomarkers be used to assess treatment response in IA?

Recommendation

- Serial GM measurements may be considered as a way to assess response to therapy but data are insufficient to determine the benefit of any other biomarkers (Marginal recommendation, Level II evidence).

Biomarkers may be of benefit in predicting and monitoring response to therapy. Several studies show a correlation between the level of serum GM and survival, with a recent systematic review suggesting a strong correlation between GM and survival from day 42 up to day 180.[130] There has also been a relatively consistent relationship observed between rate of GM decline and treatment response,[131–134] with a suggestion that early clearance of GM may be an early surrogate marker of response.[134] However, there has been no internal or external validation of cut-offs for clinical decision-making, and it is unclear how differing underlying conditions and prophylaxis may affect GM kinetics.[130] Thus, serial GM measurements as a way of assessing response to therapy can only be recommended with marginal strength (*Marginal recommendation, Level II evidence*).

Data are insufficient to determine any benefit of other biomarkers such as BDG or molecular measures such as quantitative *Aspergillus* PCR to assess response to therapy.[130]

Question 6: How prevalent is azole-resistant *A. fumigatus* and does it occur in Australia?

Prevalence of azole resistance

Acquired azole resistance in *A. fumigatus sensu stricto* and other *A. fumigatus* complex fungi generally develops in the setting of sustained antifungal exposure (in treatment or environment) and results mainly from point mutations in the *Aspergillus* CYP51A gene and/or an insertion of tandem repeats (TR) in the gene promotor. The most common acquired resistance mechanisms are associated with environmental origin, and comprise an insertion of a 34 or 46 base pair TR (TR_{34} or TR_{46}) in the promoter region in conjunction with a L98H or Y121F/T289A substitution(s) respectively. These mechanisms confer pan-azole (TR_{34}/L98H), or high-level voriconazole with variable itraconazole, resistance (TR_{46}/Y121F/T289A).[135,136] In contrast, drug resistance resulting from long-term azole therapy is likely to be associated with point mutations in CYP51A gene (primarily at loci G54 and M220). Non-CYP51A-mediated resistance accounts for about 10% of cases.[137,138]

The prevalence of azole-resistant *A. fumigatus* isolates varies with geographic region. A multicentre study from 19 European countries showed an overall prevalence of 3.2% (0% to 26%) with the majority of isolates containing the TR_{34}/L98H mutation.[139] Elsewhere, azole resistances rates have ranged between 2% and 12% for clinical isolates (Brazil 3.5%; China 5.8%; India 1.7%; Iran 3.2%; Japan 6.1%; Kuwait 3.2%; Pakistan 6.6%; Thailand 3.2%; and the United States 0.6–11.8%) with higher resistance rates for environmental isolates (Tanzania 13.9% and Colombia 9.3%). The variation in prevalence can be explained by the number of patient isolates included (single or multiple), underlying patient condition (haematological malignancy vs cystic fibrosis) and isolate source (invasive vs non-invasive). Azole resistance is likely under-estimated, as susceptibility testing may not be routinely performed.

Azole-resistant *A. fumigatus* in Australia

Azole resistance among clinical strains of *A. fumigatus sensu stricto* in Australia appears uncommon. Surveillance of 418 clinical isolates from 2000 to 2013 reported nine isolates with reduced susceptibility to itraconazole, voriconazole or posaconazole.[140] Two isolates harboured the TR_{34}/L98H mutation. Four additional isolates had the mutations G54R, F46Y, Y431S and S448S while three had no mutations detected[140]; the relevance of these mutations was not determined. In a follow-up study, 166 clinical (145 human, 21 veterinary) isolates and 185 environmental *A. fumigatus* isolates were screened for azole resistance using the VIP Check method (see later discussion). There were no azole-resistant environmental isolates. Only three (2.1%) human isolates had high minimum inhibitory concentrations (MICs) to one or more of itraconazole, voriconazole and posaconazole.[141] Two isolates with high MICs to itraconazole and posaconazole, but with wild-type MICs to voriconazole, contained the mutation G54R. The third isolate was pan-azole resistant and harboured the TR_{34}/L98H mutation.

Question 7: What is the role of antimicrobial susceptibility testing in managing IA?

Recommendations

- It is strongly recommended that susceptibility testing be performed in patients previously exposed to mould-

active azoles, those failing therapy, or those who have visited regions with high prevalence (>10%) of azole-resistant *Aspergillus* (Strong recommendation, Level II evidence).

- At a population level, it is strongly recommended that antifungal susceptibility testing be periodically performed (e.g. yearly) for the purpose of surveillance of azole-resistant *Aspergillus* (Strong recommendation, Level II evidence).

In an ideal setting, susceptibility testing would be performed on all clinical *Aspergillus* isolates. However, cost and time considerations limit this approach. The US guidelines suggest performing testing on those patients who have a suspected azole-resistant isolate or who are failing azole therapy, or for epidemiological purposes.[110] In contrast, the European guidelines recommend routine testing unless a patient is azole-naïve, and there are regular surveillance programmes including ≥100 isolates, which demonstrate no evidence of azole resistance[111] (likely influenced by the higher rates of azole-resistant *A. fumigatus*).

For clinical care, it is strongly recommended that susceptibility be performed for patients in the contexts stated above. At a population level, we also strongly recommend antifungal susceptibility testing be periodically performed using reference methodologies (*Strong recommendation, Level II evidence*).

Question 8: How do we detect and diagnose azole resistance?

In vitro susceptibility testing on isolates remains the gold standard and guides optimal therapy irrespective of presence of specific resistance mutations. In Australia, susceptibility testing is generally performed using the Sensititre (TREK Diagnostic Systems, West Sussex, UK) microbroth dilution assay and interpreted against Clinical and Laboratory Standards Institute clinical breakpoints, or epidemiological cut-off values (defined as the upper MIC limit of the wild-type population) in the absence of a breakpoint. Some laboratories choose to screen for azole resistance using a four-well screening system (VIPcheck agar plates, Mediaproducts BV, Groningen, The Netherlands), with an alert for potential azole resistance if the isolate grows in one or more wells containing a specific azole. This should be followed by MIC testing.

The greatest limitation of these methods is the requirement for culture. Consequently, molecular methods for the detection of both *Aspergillus* spp. and azole resistance directly from clinical samples have been developed. However, these require significant optimisation due to the small quantity of *Aspergillus* DNA present and to avoid cross-reactivity with human DNA. As a result, amplifying the whole *CYP51A* is problematic with nested PCR strategies usually required for increased sensitivity. Results require confirmation by DNA sequencing. Evaluation of two commercial assays which detect common *CYP51A* mutations in addition to *Aspergillus* identification,[142] in comparison to conventional PCR, found no difference in performance.[143] However, molecular methods are limited by target selection and false-negative results. Metagenomic approaches or whole genome sequencing of isolates are currently limited to research use.

Question 9: What recommendations should guide the first-line antifungal treatment of IA in haematology/oncology patients?

Recommendations

- Voriconazole, incorporating TDM (please refer to the accompanying optimising antifungal therapy guidelines by Chau *et al*. 2021,[297] which can be found elsewhere in this supplement), is recommended as first-line therapy for pulmonary IA in those not currently on mould-active prophylaxis (Strong recommendation, Level I evidence).
- If voriconazole cannot be used, isavuconazole is an alternative, particularly in the setting of severe and prolonged immunosuppression where coinfection by more than one fungus may be of concern (Strong recommendation, Level I evidence).
- If voriconazole cannot be used, posaconazole is also an alternative (Strong recommendation, Level I evidence). TDM is recommended (please refer to the accompanying optimising antifungal therapy guidelines by Chau *et al*. 2021,[297] which can be found elsewhere in this supplement).
- Liposomal amphotericin may be considered as an alternative treatment regimen in patients who develop IA while receiving a mould-active azole or those intolerant to voriconazole (Moderate recommendation, Level II evidence).
- Echinocandins can be considered as second-line or salvage therapy after voriconazole, isavuconazole and a lipid formulation of amphotericin B (Marginal recommendation, Level II evidence).

Antifungal treatment of IA should be initiated early in the course of disease to limit the high mortality[1,2] (*Strong recommendation, Level III evidence*) and should not be delayed while awaiting results of mycological tests.[144] Often patients are treated with empiric antifungal therapy (EAFT) on the basis of the most likely fungal pathogens. Figure 2 suggests an approach to managing a high-risk patient with suspected IA.

Overall, the choice of antifungal agent should be influenced by: (i) prior use of mould-active azole

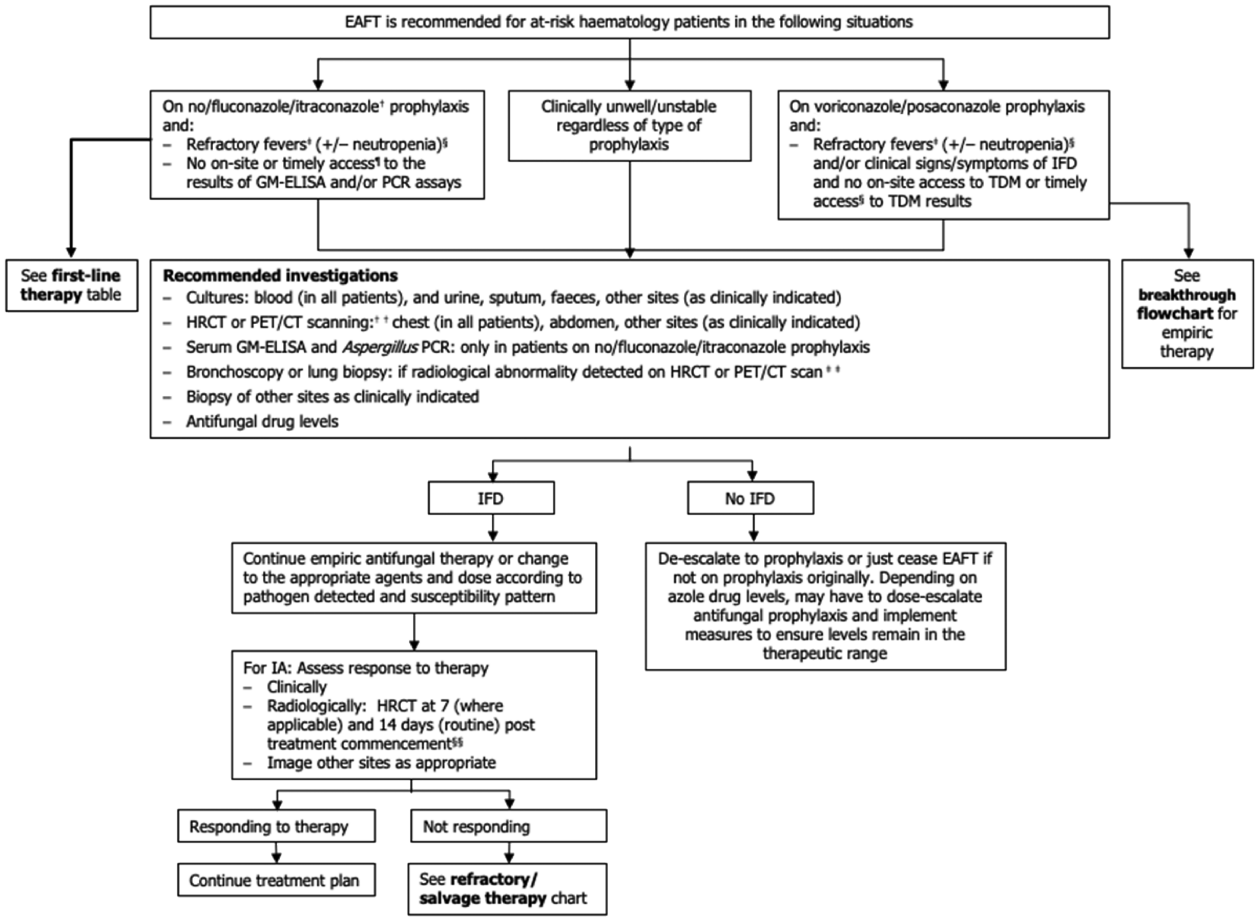

Figure 2 Approach to the diagnosis and management of suspected and confirmed invasive aspergillosis (adapted from Morrissey *et al*., 2014).[145] †Where appropriate target levels can be achieved, studies have suggested that itraconazole may be efficacious as a mould-active prophylactic agent. ‡Refractory fevers persistent (daily for 3–5 days) or recurrent (after an afebrile period of 48 h) fevers despite broad-spectrum antibiotics and negative microbial investigations. §Neutropenia (neutrophils count $<0.5 \times 10^9$/L). ¶Timely access to results; results consistently available within 3–5 days of sampling. ††FDG-PET/CT is a helpful imaging technique; however, availability may be limited in some centres. ‡‡The choice between bronchoscopy or lung biopsy is dependent on factors including location of lesion, local experience with each test, the patient's clinical status and their ability to tolerate complications of the procedure. Testing should occur within 3 days of commencing EAFT. For biopsies, ensure that a portion of the specimen is *not* placed in formalin for microbiological testing. §§Earlier radiological follow-up (e.g. at 7 days post treatment commencement) may be necessary in event of clinical deterioration; otherwise, a 14-day follow-up scan is generally recommended to assess response to therapy. CT, computed tomography; EAFT, empiric antifungal therapy; GM-ELISA, galactomannan enzyme-linked immunosorbent assay; HRCT, high-resolution computed tomography; IFD, invasive fungal disease; PCR, polymerase chain reaction; PET, positron emission tomography TDM, therapeutic drug monitoring.

prophylaxis; (ii) existing comorbidities, particularly renal impairment; (iii) the likelihood of an azole-resistant *Aspergillus* infection; (iv) likely presence of co-infection with another fungus (e.g. a mucormycete); and (v) clinical condition at the time. Tables 5 and 6 summarise the evidence and recommendations for first-line IA therapy in adults and children respectively.

The preferred first-line therapy for IA is voriconazole (*Strong recommendation, Level I evidence*). This is based on the results of the Global Comparative *Aspergillus* Study (GCAS), a randomised, unblinded, non-inferiority trial comparing voriconazole to amphotericin B.[171] Voriconazole led to more successful outcomes (53% vs 32%) and improved survival at 12 weeks (71% vs 58%) compared to standard of care therapy with amphotericin B deoxycholate. Further, voriconazole was better tolerated with fewer drug side effects. When the EORTC/MSG definitions for IFD were revised in 2008[5] to more clearly differentiate possible from probable cases of IFD, data from the GCAS were re-analysed and identified an even more favourable response for voriconazole than with amphotericin B in well-defined cases of probable and proven IA (54.7% vs 29.9%).[146]

Table 5 Recommendations for first-line therapy against invasive pulmonary aspergillosis in adults

Medication	Dosage	SoR	QoE	Notes	References
First-line					
Voriconazole	IV: 6 mg/kg twice daily on day 1, then 4 mg/kg IV twice daily Oral: 4 mg/kg twice daily	A	I	• Caution if already on triazole prophylaxis • TDM strongly recommended	146,147
Second-line or alternative options					
Isavuconazole	IV or oral: 200 mg three times daily for six doses, then 200 mg daily	A	I	• Caution if already on triazole prophylaxis	148,149
Posaconazole	IV or oral tablet: 300 mg twice daily day 1, then 300 mg daily Oral suspension: 400 mg twice daily, or 200 mg four times daily if unable to take with food	A	I	• TDM recommended	150
Liposomal amphotericin B	IV: 3 mg/kg daily	B	II	• Where there is breakthrough infection on azole therapy/ prophylaxis • In drug–drug interaction settings with azoles	151
Combination therapy: voriconazole plus anidulafungin	Voriconazole IV: 6 mg/kg twice daily on day 1, then 4 mg/kg IV twice daily Oral: 300 mg twice daily Anidulafungin IV: 200 mg on day 1, then 100 mg daily	C	I	• May have seen improved outcomes in those with positive galactomannan • May be considered in severe disease • Oral voriconazole dosing based on RCT protocol[147]	147
Caspofungin	If weight <80 kg, 70 mg IV daily on day 1, then 50 mg daily If weight >80 kg, 70 mg IV daily	C	II		152–154
Micafungin	100 mg IV daily	C	II		155–157

IV, intravenous; QoE, quality of evidence; RCT, randomised controlled trial; SoR, strength of recommendation; TDM, therapeutic drug monitoring.

Amphotericin B formulations and their dosing have also been studied for efficacy. The multinational AMBiLoad trial was a non-blinded, randomised trial comparing standard dose (3 mg/kg) to high-dose (10 mg/kg) liposomal amphotericin B for the treatment of IA in highly immunocompromised patients stratified for HSCT and duration of neutropenia.[151] The higher dose of liposomal amphotericin demonstrated no significant benefit either in response rate or survival but instead, was associated with higher rates of nephrotoxicity. The results of AMBiLoad were also recalculated in the context of the 2008 revised EORTC/MSG IFD definitions[5] and a significant number of participants with probable IA (classified based on receiving HSCT or neutropenia with radiological signs) were reclassified as having possible disease. Higher survival rates were identified at 12 weeks for possible versus probable/proven cases in the 3 mg/kg group (82% vs 58%; $P = 0.06$) compared with the 10 mg/kg group (65% vs 50%; $P = 0.15$).[172] Notably, while response rates to voriconazole and liposomal amphotericin are comparable across these trials in meta-analysis, no head to head comparison has been undertaken.[148] Based on voriconazole's more favourable toxicity profile and ease of administration as compared to liposomal amphotericin B and the above data, liposomal amphotericin is recommended with moderate support as an alternative treatment regimen in patients who develop IA while receiving a mould-active azole or those intolerant to voriconazole (*Moderate recommendation, Level II evidence*; Table 5).

The SECURE study was a phase 3, double-blind, global multicentre comparison of isavuconazole versus voriconazole standard of care, with isavuconazole providing non-inferior efficacy and a lower rate of adverse events for IA.[11] A meta-analysis of randomised controlled trials determined equivalence of isavuconazole with both liposomal amphotericin B and voriconazole.[149] These results led the Food and Drug Administration (FDA) to approve isavuconazole for the treatment of IA, with widespread adoption in the United States and Europe as a first-line agent for IA.[110,111] The appeal of isavuconazole includes bioequivalence (roughly 98%) of IV and oral formulations,[173] including in the setting of mucositis[174]; drug absorption not affected by food or drugs that alter

Table 6 Recommendations for first-line therapy against invasive pulmonary aspergillosis in children

Medication	Dosage	SoR	QoE	Notes	References
First-line					
Voriconazole (≥2 years)	Children 2 to <12 years or aged 12–14 years and weighing <50 kg IV: 8 mg/kg (day 1, 9 mg/kg) twice daily Oral: 9 mg/kg twice daily	A	I	• TDM strongly recommended • Not approved for children <2 years	Paediatric[158,159]
	Children ≥15 years or aged 12–14 years and weighing >50 kg IV: 4 mg/kg (day 1, 6 mg/kg) twice daily Oral: 200 mg twice daily				Adult[146,147]
Alternative options					
Liposomal amphotericin B	IV: 3 mg/kg daily	B	I	• First line in <2 years of age • Amphotericin B deoxycholate may be preferred in neonates	Paediatric[151,160] Adult[151]
Amphotericin B deoxycholate	IV: 1–1.5 mg/kg daily	C	II	• Used in neonates	Paediatric safety[161–164] Adult[146]
Posaconazole	Children ≥13 years IV or oral tablet: 300 mg twice daily on day 1, then 300 mg daily Oral suspension: 800 mg/day in 2–4 divided doses Children <13 years Safety and efficacy not established. Some PK data available for younger children[167]	C	II	• Awaiting studies in children • TDM recommended	Paediatric salvage[165,166]
Isavuconazole	No dosage established	D	III	• Under investigation (clinicaltrials.gov NCT03241550) • Caution against use outside clinical trials until more results are available	
Caspofungin	Children ≥1 year of age IV: 70 mg/m^2 daily on day 1, then 50 mg/m^2 daily (max 70 mg per day) Children 3–12 months IV: 50 mg/m^2 daily Infants <3 months IV: 25 mg/m^2 daily	C	II		Paediatric[168,169] Adult[152,154]
Micafungin	Children <50 kg IV: 2–4 mg/kg daily Children ≥50 kg IV: 100–200 mg daily	C	II		Paediatric safety[170] Adult[155,157]

IV, intravenous; PK, pharmacokinetic; QoE, quality of evidence; SoR, strength of recommendation; TDM, therapeutic drug monitoring.

stomach pH, so that they can be taken safely with proton pump inhibitors[175,176]; a favourable drug–drug interaction profile compared to voriconazole[177]; extensive penetration into most tissue; and a favourable safety profile, including in the setting of renal impairment due to the absence of nephrotoxic excipients to facilitate solubility.[178] Hence, we recommend that if voriconazole cannot be used, then isavuconazole is a good alternative (*Strong recommendation, Level I evidence*). Access to isavuconazole in Australia is increasing. Furthermore, due to its spectrum of activity against many non-*Aspergillus* moulds, isavuconazole is an attractive option in the setting of severe and prolonged immunosuppression where coinfection by more than one fungus may be of concern.[179] However, while real-world data support the safety and tolerability profile of isavuconazole in haematological patients for the treatment of IFD,[180] reports of IFD breakthrough and failure are increasing in the setting of prophylaxis and treatment.[181–184]

While both posaconazole oral suspension and delayed-release tablets have demonstrated efficacy for prophylaxis of IA in haematology patients, and posaconazole is licensed for salvage treatment of invasive mould disease, there are limited published data to support its first-line use to

treat IA.[185–187] However, a recently published multicentre, randomised controlled trial of posaconazole compared with voriconazole for first-line therapy of IA demonstrated non-inferiority of posaconazole to voriconazole for the outcomes of mortality and global clinical response, with improved tolerance to posaconazole compared to voriconazole.[150] Publication of pharmacokinetic data including that of TDM is awaited. Where voriconazole cannot be used, posaconazole is suitable as alternative first-line therapy (*Strong recommendation, Level I evidence*) with TDM.

Evidence for the use of echinocandins as primary therapy for IA is limited. While no head-to-head comparison has been performed, echinocandins can be considered as second-line or salvage therapy after voriconazole, isavuconazole, and a lipid formulation of amphotericin B (*Marginal recommendation, Level II evidence*; Table 5).

Switching from intravenous to oral therapy should be considered for patients who are clinically stable with reliable enteric absorption. There is no robust evidence to direct the optimal duration of therapy and this should be individualised, based on clinical response and underlying immunosuppression; however, the typical duration would be 12 weeks with potential prolongation depending on progress and state of immunosuppression.

Question 10: What is the role of combination antifungal therapy in the first-line setting?

Recommendation

• Combination therapy in the first-line setting is only weakly recommended but may be considered in severe disease, in critically ill patients, or in those with suspected azole resistance (Marginal recommendation, Level I evidence).

Robust data are lacking to support combination antifungal therapy as first-line treatment of IA (Table 5) and there have been no recent publications in this area since the last iteration of these guidelines in 2014.[3] Combination therapy is usually driven by individual prescribing preferences and considered in the setting of salvage and/or severe infection.

Despite pre-clinical studies generally being supportive of combination antifungals, particularly with voriconazole or isavuconazole-echinocandin combinations,[188–192] strong supportive clinical data are lacking.[193,194] Non-randomised, single-centre, retrospective case-series have shown variable benefit but are limited by a lack of power and considerable heterogeneity in combination of agents used, duration of therapy, indications for therapy (primary or salvage) and outcome measures assessed.[155,157,195–200] A meta-analysis was not supportive of combination therapy for the primary treatment of IA.[201]

The largest combination therapy trial published to date was a multicentre, randomised, double-blind, placebo controlled trial of 459 patients comparing combination therapy with voriconazole and anidulafungin with voriconazole alone.[147] Combination therapy was administered for at least 2 weeks with a minimum total duration of 5 weeks of antifungal therapy. Combination therapy showed no benefit in 6-week mortality, (19.3% vs 27.5%; $P = 0.09$), 12-week overall mortality or aspergillosis-associated mortality. However, post hoc analysis of only serum GM-positive participants demonstrated lower mortality in the combination therapy arm (15.7% vs 27.3%; $P = 0.037$), with the hypothesis that those with high-serum GM represented a more homogeneous population diagnosed earlier in their disease course, suggesting early combination therapy may be of benefit.[147] Other international guidelines also suggest there may be a role for primary combination therapy in the setting of severe disease, but based on the strength of evidence, combination therapy for the first-line treatment of IA can only be weakly recommended (*Marginal recommendation, Level I evidence*).[110,111]

Question 11: How do we treat azole-resistant *A. fumigatus* in 2021 and what new anti-*Aspergillus* drugs are in the pipeline?

Recommendation

• Changing from voriconazole monotherapy to liposomal amphotericin B or a voriconazole/echinocandin combination is recommended for azole-resistant disease (Strong recommendation, Level III evidence).

Although best-practice treatment of infections due to azole-resistant strains remains uncertain, studies have shown that azole resistance is associated with treatment failure if an azole is used (summarised by Chowdhary *et al.*).[138] There are no controlled studies comparing azole-resistant with azole-susceptible *Aspergillus* in relation to treatment success or failure. Expert opinion strongly recommends a change from voriconazole monotherapy in documented azole-resistant disease to liposomal amphotericin B or a voriconazole/echinocandin combination (*Strong recommendation, Level III evidence*),[202] with echinocandins and polyenes typically being second-line choices for therapy in azole-susceptible disease.

Importantly, in areas with an environmental resistance rate exceeding 10%,[203] most experts favour starting

empiric first-line therapy with a voriconazole/echinocandin combination or liposomal amphotericin B.[202] Where it is suspected that CNS aspergillosis is due to an azole-resistant *Aspergillus*, expert opinion also recommends liposomal amphotericin B as core therapy, with consideration of the addition of a second agent such as 5-flucytosine.

New anti-*Aspergillus* drugs in the pipeline

The frequent toxicity and drug–drug interactions of the current antifungal armamentarium, and the emergence of resistance to the most commonly used drugs for aspergillosis, the azoles, highlights the need for new and effective antifungals for aspergillosis. Table 7 summarises the main agents of interest, their mechanisms of action, and *in vitro* and *in vivo* activities. An overview of the agents under clinical study or in development are also summarised in recent publications.[204,205] There are minimal paediatric data for these new agents, as children were not included in early clinical studies.

Details of APX001 (Fosmanogepix, Amplyx Pharmaceuticals Inc., San Diego, CA, USA) and F901318 (Olorofim; F2G, Manchester, UK) are provided in the accompanying treatment guidelines for non-*Aspergillus* moulds by Bupha-Intr *et al*. 2021,[295] which can be found elsewhere in this supplement. Briefly, APX001A (manogepix), a first-in-class small-molecule inhibitor of the conserved fungal GWT1 protein (Table 7), is administered as a prodrug. Fosmanogepix (APX001) is then converted to manogepix *in vivo*. Among *Aspergillus* spp., low MICs are found against species from the sections *Fumigati*, *Flavi*, *Terrei* and *Nigri*.[211] The anti-*Aspergillus* activity of APX001A (50% minimal effective concentration (MEC_{50}), 0.015 μg/mL; MEC_{90}, 0.03 μg/mL) is comparable in activity to anidulafungin and micafungin.[211] With regards to F901318, the compound has good *in vitro* activity against many *Aspergillus* species and is 10–100 times more active than voriconazole. In one study of 55 *A. fumigatus* isolates, the mean MIC of F901318 was 0.029 μg/mL (range 0.008–0.06) compared with a mean MIC of 0.69 μg/mL (range 0.254–16) for voriconazole.[209] In addition, the efficacy of F901318 in mouse models of *A. fumigatus* infection demonstrated superior survival compared with posaconazole treatment.[209] Importantly, F901318 has good *in vitro* activity against azole-resistant *A. fumigatus*.[212] In late 2019, Olorofim was granted Breakthrough Designation by the US FDA on the basis of preliminary clinical evidence from various clinical trial data.

CD101 (Rezafungin; Cidara Therapeutics, San Diego, CA, USA) is not included in Table 7 but is of particular interest because of its potent *in vitro* activity against *Aspergillus* species, including azole-resistant *A. fumigatus*,[205] but has not yet reached clinical trials. Although structurally resembling other echinocandins, CD101 is distinguished by its prolonged half-life in humans (approximately 130 h) and stability, which result in high plasma drug exposure and sustained drug levels, supporting less frequent dosing.[213]

Table 7 Antifungal compounds under clinical study or in development (see also Osherov and Kontoyiannis[204] and Wiederhold[205])

Compound	Mode of action	*In vitro* MIC *Aspergillus fumigatus*	*In vivo* activity in murine IA models	Human trials in *Aspergillus*	References
Agents in clinical trials					
SCY-078 (Ibrexafungerp; Synexis Inc., Jersey City, NJ, USA)	Novel glucan synthase inhibitor	MEC range 0.03–1 μg/mL compared with MEC_{90} of 8 μg/mL and 2 μg/mL for AMB and VRC	*In vivo* murine and pig models	Phase 3 combination therapy with VRC; also ODD	206,207
APX001 (Fosmanogepix)†	GPI-anchor inhibitor	0.03–0.13 μg/mL	25 mg/kg oral	Phase 2 ongoing	208
F901318 (Olorofim)	DHODH and pyrimidine biosynthesis inhibitor	<0.06 μg/mL	10 mg/kg oral	Phase 2b ongoing	209
T-2307‡	Affects mitochondrial function	0.01–1.0 μg/mL	Active 1 mg/kg subcutaneous	Phase I	210

†E1210/APX001 inhibits an early step in the glycosylphosphatidylinositol (GPI)-dependent anchoring of the fungal cell wall protein. It is highly active *in vitro* and *in vivo* against *Aspergillus* spp. and is well tolerated.[208,211]

‡An arylmidine compound that selectively targets fungal mitochondria leading to loss of membrane potential.

AMB, liposomal amphotericin B; DHODH, dihydroorotate dehydrogenase; GPI, glycosylphosphatidylinositol; HDAC, histone deacetylase; MEC, minimum effective concentration; MIC, minimum inhibitory concentration; ODD, orphan drug designation; VRC, voriconazole.

Question 12: What is breakthrough IA and how should it be managed?

Recommendations

- Verification of adherence to antifungal therapy together with TDM should be performed in suspected breakthrough IA (Strong recommendation, Level III evidence).
- If breakthrough IA occurs on triazole prophylaxis or therapy, a switch to liposomal amphotericin B is strongly recommended (Strong recommendation, Level III evidence).
- If breakthrough IA occurs on liposomal amphotericin B therapy, a switch to voriconazole or isavuconazole is strongly recommended (Strong recommendation, Level III evidence).
- Where possible, definitive treatment targeted towards the specific fungal pathogen and with an agent confirmed to be effective on antifungal susceptibility is strongly suggested (Strong recommendation, Level III evidence).

Breakthrough IA infection is defined as that which has occurred while a patient has been exposed to a mould-active antifungal agent used for primary or secondary prophylaxis (Table 2).[8,214] As many centres now use such prophylaxis in high-risk patients, breakthrough IA is not an uncommon presentation of IA. The incidence of breakthrough IA ranges between 2% and 11% in patients on voriconazole, posaconazole or isavuconazole prophylaxis[214–217] and is estimated at 1.1–7.5% in those receiving liposomal amphotericin B prophylaxis.[218–220] Early breakthrough infections are frequently seen in the setting of primary prophylaxis during newly diagnosed leukaemia or early post-allogeneic HSCT, where contributing factors include suboptimal antifungal pharmacokinetics due to poor oral absorption and drug–drug interactions.[214,221] Often, the causative *Aspergillus* organism remains susceptible to azoles.[222] In contrast, breakthrough IA which occurs late in heavily treated multiple-relapsed leukaemia with prolonged immunosuppression, or in allogeneic HSCT with chronic GVHD, may be due to azole-resistant *Aspergillus* spp. such as *A. terreus* and *A. flavus*.[139]

Breakthrough IA should be suspected if there is persistent fever and/or new cough, haemoptysis or pleuritic chest pain. Early aggressive diagnosis is strongly recommended including HRCT, bronchoscopy within 48–72 h for BAL fluid culture, GM and *Aspergillus* PCR tests. Pursuing tissue biopsy for histology and culture is recommended. In general, the majority of breakthrough IAs are diagnosed with non-culture-based testing.[223] If cultured, the causative spectrum of *Aspergillus* spp. includes species other than *A. fumigatus* complex.[139]

The principles of management are similar for adults and children. Verification of adherence to antifungal therapy together with TDM should be performed while awaiting diagnosis. The initial antifungal treatment change is dependent upon the current prophylactic agent being used (Fig. 3), but switching to a different triazole with similar or broader spectrum of activity together with the addition of liposomal amphotericin B is recommended (*Strong recommendation, Level III evidence*).[214,224] Definitive treatment should be targeted towards the specific *Aspergillus* pathogen once identified.

Question 13: How should refractory disease be managed?

Recommendations

- Switching antifungal class in refractory IA is strongly recommended (Strong recommendation, Level III evidence).
- Combination antifungal therapy and surgical management may also be considered (Moderate recommendation, Level II evidence).
- Document adequate triazole drug levels before declaring refractory IA (Strong recommendation, Level III evidence).

Refractory disease refers to an event where the IFD is progressing or failing to show improvement on clinical, mycological and/or radiological grounds while on treatment.[8] Treatment response should be assessed at an interval as deemed appropriate by clinical response (e.g. 2 weeks post-commencement) and reviewed by an expert in IA management if refractory disease is considered likely. Immune reconstitution needs to be excluded as a cause for apparently worsening disease.[8,225] In this setting, immune reconstitution inflammatory syndrome is defined as the onset of clinical or radiological deterioration consistent with worsening of IA and temporally related to neutrophil recovery, despite no change to antifungal therapy and an apparent treatment response prior to this time.[225]

The literature regarding management of refractory disease is difficult to interpret because: (i) studies typically lack statistical power; (ii) there is heterogeneity in when and how response to therapy has been assessed; and (iii) first-line therapy has changed since many of the original salvage therapy studies were performed (i.e. liposomal amphotericin B was historically first-line, rather than voriconazole). Furthermore, many patients may have been prescribed 'salvage therapy' when they

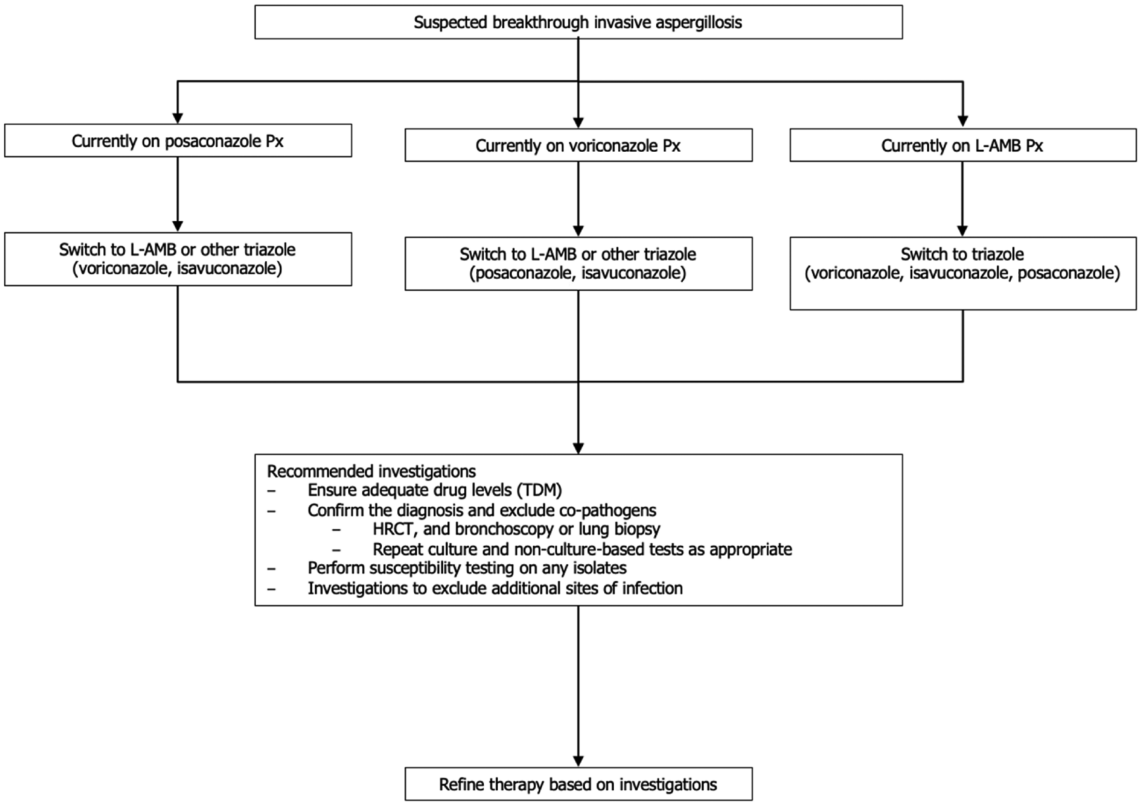

Figure 3 Suggested approaches for suspected breakthrough invasive aspergillosis treatment change. HRCT, high-resolution computed tomography; L-AMB, liposomal amphotericin B; Px, prognosis; TDM, therapeutic drug monitoring.

were intolerant to, rather than clinically failing, first-line therapy (a common issue). Finally, the assumption that first-line therapy has failed needs to take into account factors that may have led to suboptimal therapy, such as inadequate drug levels, progression of underlying disease, and the potential for misdiagnosis and/or new concomitant infection. Checking for factors that may have contributed to failure of therapy (e.g. poor vascular supply, drug compliance, subtherapeutic drug levels, sanctuary sites) and ensuring the correct diagnosis has been made, along with performing susceptibility testing, is advised.

The therapeutic approach to refractory IA suggested in Table 8 and Figure 4 is based on limited non-randomised data, with even less data in children. Generally, it is prudent to switch the class of antifungal used. Liposomal amphotericin B and voriconazole have good evidence in the salvage setting.[226,227] There is also mounting evidence to support posaconazole salvage therapy,[229,231–233] including some evidence for the efficacy of posaconazole salvage therapy following voriconazole failure.[229,230] Isavuconazole has not been reported in the salvage setting, but given its good performance as initial therapy in the SECURE study,[149] it is a reasonable option in those failing due to poor tolerance or difficulty in reaching therapeutic levels of voriconazole.

Echinocandins have been trialled for salvage therapy, with caspofungin receiving the most attention. A review of registry and cohort studies by Heinz *et al.*[234] show response rates of 28–71%. Caution is advised, however, as several of the studies used an amphotericin B formulation, fluconazole, or 'unspecified' therapy as first-line, rather than voriconazole.

Combination antifungals as first-line therapy in IA have been discussed. In the salvage setting, there was improved 12-week overall survival and a higher success rate compared to monotherapy in a systematic review and meta-analysis.[201] However, there were low numbers of cases (127 patients) and all included studies were observational. Another single-arm, observational study of combination triazole and echinocandin therapy showed good overall response rates (71% in IA); however, without a comparison to monotherapy, it is difficult to assess the benefit of such a combination.[236] No firm recommendations can be made for combination antifungal therapy in children.

In the setting of failure of multiple lines of therapy, enrolling the patient in a clinical trial of a novel agent

Table 8 Salvage therapy for invasive pulmonary aspergillosis (see Fig. 4 for drug selection based on prior therapy)

Antifungal agent	Dosage and formulation	SoR	QoE	Notes	References
Voriconazole	IV: 6 mg/kg twice daily for 1 day, then 4 mg/kg twice daily Oral: 4 mg/kg twice daily	A	II	TDM strongly recommended	226,227
Liposomal amphotericin B	IV: 3–5 mg/kg	B	II	If non-*Aspergillus* moulds are suspected	228
Posaconazole	IV or tablet: 300 mg twice daily on day 1, then 300 mg daily	C	II	Some observational data of success in voriconazole failure[229,230]	229,231–233
Isavuconazole	IV or oral: 200 mg three times daily for six doses, then 200 mg daily	C	III	No data in salvage setting specifically; consider if issues with voriconazole tolerance or poor drug levels	149
Caspofungin	IV: 70 mg daily on day 1, then 50 mg daily (if weight <80 kg)	C	II	Limited data in setting of triazole failure	234,235
Combination therapy	Voriconazole plus caspofungin, liposomal amphotericin B plus caspofungin	B	II	Observational data only in meta-analysis showed some survival benefit	201

IV, intravenous; QoE, quality of evidence; SoR, strength of recommendation; TDM, therapeutic drug monitoring.

may be an option. See also later discussion on adjunctive measures.

Question 14: What is the role of TDM in managing IA?

Recommendation

• Antifungal TDM in the management of IA is recommended during treatment with voriconazole or posaconazole (Strong recommendation, Level II evidence).

The overarching principles of antifungal TDM are outlined in the accompanying guidelines on optimising antifungal drug therapy by Chau *et al*. 2021,[297] which can be found elsewhere in this supplement. Antifungal TDM in the management of IA is recommended during treatment with voriconazole or posaconazole (*Strong recommendation, Level II evidence*). Blood drug levels are appropriate for ensuring adequate drug exposure, as subtherapeutic levels have been associated with poor outcomes, particularly for voriconazole.[237] Similarly, a prospective trial demonstrated that TDM could circumvent toxicity.[237] In patients with deteriorating clinical status, additional monitoring is warranted.[238] TDM is also able to exclude non-compliance or inadequate concentrations as the cause for poor clinical response.

Question 15: Are there any adjunctive therapies available for managing IA?

Recommendations

• Where feasible, immunosuppressive agents should be reduced (Strong recommendation, Level III evidence).
• Colony-stimulating factors may be used in neutropenic patients with IA (Marginal recommendation, Level III evidence).
• Interferon gamma is not recommended (Not recommended, Level III evidence).
• Surgical resection may be of benefit for localised and surgically accessible pulmonary disease in patients who are refractory to antifungal therapy or who have localised complications (Moderate recommendation, Level III evidence).

In the immunocompromised host with IA, reconstitution of the immune system is of paramount importance in terms of survival and treatment response. Where feasible, immunosuppressive agents should be reduced (*Strong recommendation, Level III evidence*).

In haematological malignancy, there is good evidence to support the use of granulocyte colony-stimulating factor (G-CSF) together with cytotoxic or other immune suppressing agents to reduce the duration and severity of neutropenia.[111] Pegylated G-CSF formulations have long been used during antifungal prophylaxis in the setting of non-myeloid malignancy. The use of colony-stimulating factors in other settings is less clear with a dearth of controlled studies. *In vitro* studies have alluded to the potential of both G-CSF and GM-CSF in enhancing antifungal host defence.[239] On balance, colony-stimulating factors may be used in neutropenic patients with IA (*Marginal recommendation, Level III evidence*) but there are insufficient data to recommend their use in non-neutropenic patients.

Granulocyte transfusions are seldom administered but have historically been used as an adjunct in patients with neutropenia and severe infection. Their potential benefit is balanced by the risk of acute lung injury, particularly in patients who have received amphotericin B

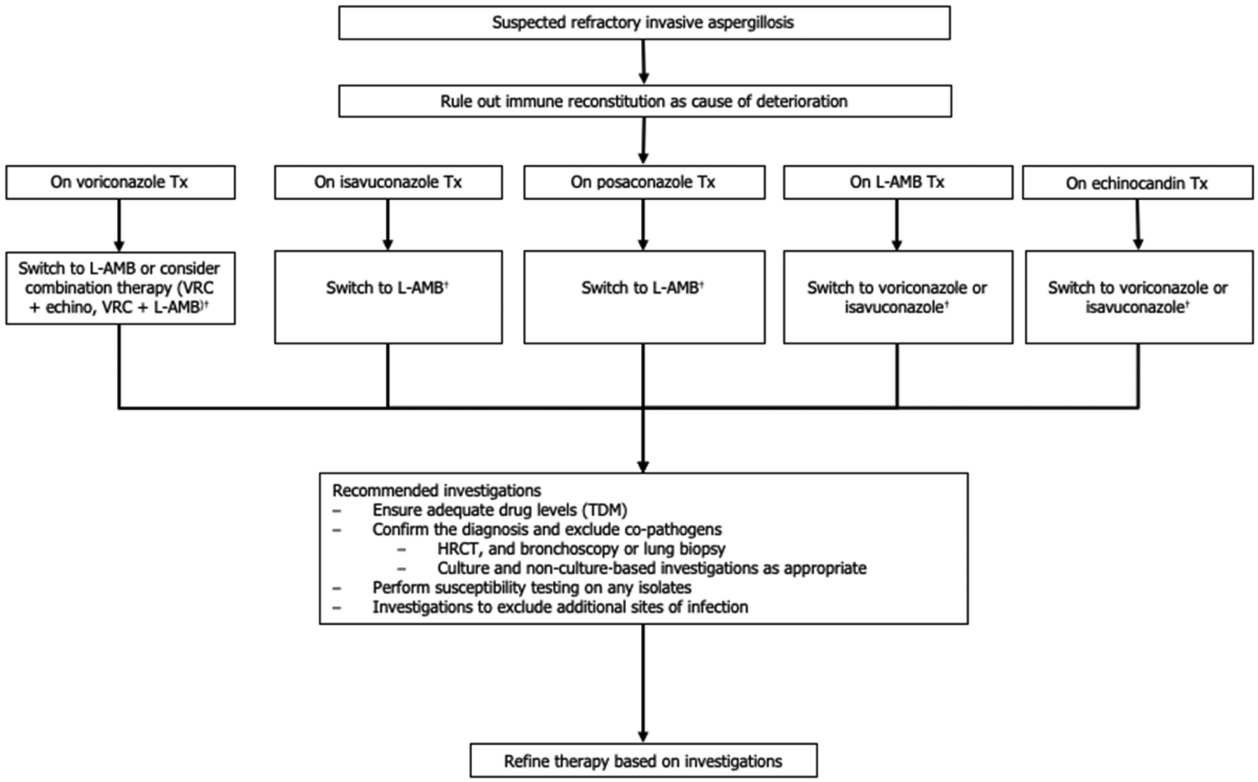

Figure 4 Approach to management of refractory invasive aspergillosis (to be used in conjunction with Table 8). †See Table 8 for dosages of antifungals and grading of recommendations. echino, echinocandin; HRCT, high-resolution computed tomography; L-AMB, liposomal amphotericin B; TDM, therapeutic drug monitoring; Tx, treatment; VRC, voriconazole.

(*Marginal recommendation, Level III evidence*).[240] Alloimmunisation following donor granulocyte infusion may also threaten stem cell or bone marrow grafts leading to graft failure.[241] A Cochrane review conducted to investigate their efficacy in neutropenic patients failed to demonstrate a mortality benefit in patients receiving granulocyte transfusions compared with those who did not.[242]

In refractory cases of IA, interferon gamma has been associated with immunomodulatory effects by augmenting macrophage and neutrophil activity.[110] Despite evidence to suggest its successful role in patients with CGD, data are very limited in haematology/oncology patients.[243] Furthermore, in allogeneic HSCT patients it may exacerbate GVHD.[244] For these reasons, interferon gamma is not recommended (*Not recommended, Level III evidence*).

Finally, surgical resection may be of benefit for localised and surgically accessible disease in patients who are refractory to antifungal therapy or who have localised complications, such as uncontrolled bleeding, neurological compromise or those at high risk of local extension.[111,245]

Question 16: What is new in the management of extrapulmonary and disseminated IA?

The management of some of the more common/important extrapulmonary forms of IA is summarised in Table 9.

Central nervous system aspergillosis

Updates in this area pertain mostly to diagnostics. There is some evidence that *Aspergillus* DNA can be detected in CSF by PCR, with moderate to high sensitivity (75–100%) and specificity (98.5%).[107,259] Likewise, utility of CSF GM has been investigated in one study, showing high sensitivity and specificity.[260] These may be options to help avoid the need for stereotactic brain biopsy. Given that paediatric patients are often asymptomatic of CNS infection, and there is a higher rate of CNS infections in this group, imaging of the CNS in children with pulmonary IA and without CNS symptoms may be considered (*Marginal recommendation, Level II evidence*).[261–264] Few data exist to guide medical therapy; in the study by

Table 9 Extrapulmonary aspergillosis management recommendations

Condition	Recommendation	Comments	SoR	QoE	References
CNS aspergillosis	Systemic voriconazole therapy		A	II	171
	Surgical resection of focal CNS lesions	Particularly if poor response to medical therapy	B	II	245–247
Acute invasive *Aspergillus* sinusitis	Urgent ENT review and surgical debridement		A	II	248,249
	Empiric liposomal amphotericin B therapy		A	II	151
	Systemic voriconazole therapy		A	II	171,250,251
Aspergillus endophthalmitis	Early vitrectomy		A	II	252,253
	Intravitreal voriconazole		A	III	252
	Systemic voriconazole therapy		A	III	254
Aspergillus keratitis	Topical natamycin		A	I	255
	Systemic voriconazole therapy	Not recommended based on RCT data	D	I	256
Aspergillus osteomyelitis	Systemic voriconazole therapy		A	II	257,258
	Surgical debridement		A	II	257,258

CNS, central nervous system; ENT, ear, nose and throat surgery; QoE, quality of evidence; RCT, randomised controlled trial; SoR, strength of recommendation.

Herbrecht *et al*., voriconazole demonstrated benefit over amphotericin B in extrapulmonary aspergillosis (*Strong recommendation, Level II evidence*) but this study included only a small number of patients with CNS infection.[171] At the time of writing, there are single-arm reports of isavuconazole's efficacy.[265] Posaconazole achieves poor and inconsistent levels in the CSF[266,267] and case reports generally show poor clinical response[233,268]; hence, its use is not recommended (*Not recommended, Level III evidence*). There are no prospective studies of surgical management of CNS IA, although surgery may have a role in combination with antifungal therapy, particularly if there is poor response to medical therapy alone (*Moderate recommendation, Level II evidence*).[245–247]

Sinus infections

There is a wide spectrum of *Aspergillus* sinus infections but these guidelines only refer to invasive *Aspergillus* sinusitis.[269] In acute invasive infection, there has been a recent review of clinical and nasendoscopy features, and surgical treatment.[270] Optimal management of this condition requires a multidisciplinary approach, with infectious diseases, ear, nose and throat surgery, ophthalmology and neurosurgery input.[269] Recent data, including a systematic review, have shown survival benefit where surgical debridement is undertaken (*Strong recommendation, Level II evidence*).[248,249] Urgent commencement of EAFT is strongly recommended, with liposomal amphotericin B recommended to cover mucormycosis and *Aspergillus* spp. (*Strong recommendation, Level II evidence*). There are few data to guide targeted medical therapy; however, as for IPA, voriconazole is the preferred agent (*Strong recommendation, Level II evidence*).[171,250,251] Recent publications have highlighted the benefit of frozen section intraoperatively,[271,272] and some reports suggest serum GM and *Aspergillus* PCR may help deliver a diagnosis with high specificity.[271,273] One particular study found that GM could aid in differentiation of invasive from non-invasive sinusitis, as well as mucormycosis versus aspergillosis. Furthermore, the kinetics of GM could show response to therapy.[274]

Eye infections

Endophthalmitis

Unlike other manifestations of IA, *Aspergillus* endophthalmitis occurs often in immunocompetent individuals following surgery or trauma.[252] In one large retrospective study, predictors of improved outcomes included better visual acuity on presentation, and vitrectomy together with intravitreal voriconazole, compared to vitrectomy with intravitreal AmB.[252] In the context of systemic and intravitreal antifungals, another retrospective study found early vitrectomy to be of likely benefit (*Strong recommendation, Level II evidence*).[253] Systemic antifungal therapy is used in some reports, varying from itraconazole, liposomal amphotericin B and voriconazole.[252,253,275,276] Despite poor quality data, given the significant consequences of failure of therapy, systemic antifungal therapy is recommended (*Strong recommendation, Level III evidence*). As voriconazole is known to achieve good intraocular concentrations[254] and

superior outcomes in other forms of IA, it is the preferred agent. The optimal duration of therapy is unclear.

Keratitis

Recent publications have described the epidemiology and outcomes of fungal keratitis cases in Australia,[277,278] with *Aspergillus* spp. the second most common cause of fungal keratitis behind *Fusarium* spp.[277] Topical natamycin remains the best therapy with a meta-analysis comparing topical voriconazole and natamycin showing inferior outcomes with voriconazole (*Strong recommendation, Level I evidence*).[255] The benefit of systemic antifungal agents remains unclear. A double-blinded, randomised placebo controlled trial (MUTT II) failed to show benefit of additional oral voriconazole to topical therapy.[256]

Osteomyelitis

Aspergillus osteomyelitis is rare but challenging to manage. Children with CGD are prone to this infection.[279] The most common bones involved are the vertebral, cranial, ribs and long bones.[257] Voriconazole is the preferred therapy, largely based on observed benefit in other forms of IA (*Strong recommendation, Level II evidence*).[257,258] Recent publications have focused on the potential benefits of surgical intervention, with a retrospective study showing significantly reduced relapse rates[257] and another showing a survival benefit of surgery,[258] although the retrospective, non-randomised nature of these data place them at risk of bias (*Strong recommendation, Level II evidence*).

Conclusion

Emerging host risk groups for IA include patients coinfected with respiratory viruses, which now also includes SARS-CoV-2. The burden of IA in these patients and implications for their management and prognosis is not defined in Australia, and it is apt to consider the need for the systematic study of such.

The expanding pipeline of *Aspergillus* biomarkers for diagnosis is welcome yet their utility in measuring response to therapy is understudied in the Australian context. Other than PET scans which are gaining acceptance, the advent of newer imaging modalities such as antibody-guided PET (immunoPET) using radiolabelled *A. fumigatus*-specific monoclonal antibodies, as well as the use of radiolabelled amphotericin B as a marker of various mould infections, warrant further study.[280,281] Azole resistance appears to be uncommon in Australia. However, large surveys on a national scale have not been performed.

Finally, treatment options for IA have not substantively changed since the last iteration of these guidelines. However, new drugs that are effective in treating IA are on the horizon. These newer agents underpin and give rise to optimism for managing this problematic infection, including cases caused by azole-resistant *A. fumigatus*. To this end, engagement of clinicians with government and the pharmaceutical industry is essential for Australia to remain on track with these developments.

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Appendix A

Table A1 Major non-haematology/oncology patient groups at risk of IA and relevant guidelines

Patient risk group	Rates of IA	Other guidelines, where available	References
Solid organ transplant recipients			
Heart	1–15%	2015 International Society of Heart and Lung Transplantation Guidelines 2019 American Society of Transplantation Guidelines	32,282,283
Lung	3–14%	2015 International Society of Heart and Lung Transplantation Guidelines 2019 American Society of Transplantation Guidelines	32,282,283
Liver	1–8%	2019 American Society of Transplantation Guidelines	32,282
Kidney	0–4%	2019 American Society of Transplantation Guidelines KHA-CARI adaptation of KDIGO Transplant Guideline	282,284
Intensive care unit patients			
Overall	0.3–5.8%	Recommendations for diagnosis of IA in ICU in development (see 'Developing definitions for invasive fungal diseases in critically ill adult patients in intensive care units. Protocol of the FUNgal infections Definitions in ICU patients (FUNDICU) project')[38]	37,38
COPD in ICU	0.4–7.4%	As above	285,286
Liver cirrhosis with respiratory failure	1.8–14%	As above	287,288
Influenza pneumonia in ICU	6.9–28.1%	As above	289–292
CAPA	4–33%	As above	39–42
Rheumatology			
TNF-alpha antagonists	0.0001%	—	293,294

AST, American Society of Transplantation; CAPA, COVID-19 associated pulmonary aspergillosis; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus diseases of 2019; EORTC/MSGERC, European Organisation for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium; ICU, intensive care unit; ISHLT, International Society of Heart and Lung Transplantation; KDIGO, Kidney Disease: Improving Global Outcomes Group.