

Diagnostic and therapeutic approach to persistent or recurrent fevers of unknown origin in adult stem cell transplantation and haematological malignancy

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Abstract

Persistent or recurrent fevers of unknown origin (PFUO) in neutropenic patients on broad-spectrum antibiotics have traditionally been treated with empirical antifungal therapy (EAFT). The lack of survival benefit seen with the use of amphotericin B deoxycholate (AmB-D) as EAFT has been attributed to its toxicities. More recently, newer, less toxic and more expensive antifungal agents such as the lipid formulations of AmB, the newer azoles (fluconazole, itraconazole and voriconazole) and caspofungin have been analysed in a number of EAFT trials. Compared with AmB-D the newer agents have superior safety but are of equivalent efficacy. This lack of survival advantage is related to the fact that the trigger for commencement of EAFT is late and non-specific. Thus, alternative approaches are required. New sensitive serological and molecular tests for the detection of *Aspergillus* antigens and genomic DNA have been developed and evaluated in accuracy studies. These tests have been incorporated into management strategies (i.e. pre-emptive strategies) to direct antifungal therapy. The pre-emptive approach has been shown to be safe and feasible but its impact on clinically important patient outcomes such as survival is less clear. Other advances include the introduction of effective, non-toxic mould-active antifungal prophylaxis and patient risk-group stratification. In this paper we provide new evidence-based algorithms for the diagnosis and treatment of PFUO in adult patients undergoing stem cell transplantation and chemotherapy for haematological malignancy which incorporate these newer diagnostic tests and are directed by the risk category of the patient and type of antifungal prophylaxis the patient is receiving.

Historical rationale

In the 1980s it was recognized that invasive fungal infections (IFI) were increasing in incidence, early treatment of IFI was associated with improved outcomes, patients

treated with amphotericin B deoxycholate (AmB-D) for persistent or recurrent fevers of unknown origin (PFUO) had a lower mortality rate compared with historical controls, and IFIs were difficult to diagnose using traditional biopsy and culture.^{1–3}

Based on these observations, two prospective, randomised controlled-trials (RCT) were performed to compare the efficacy of AmB-D versus continuing antibiotics alone and/or discontinuing all antimicrobial therapy for the treatment of PFUO.^{4,5} In both studies, those randomized to AmB-D had a non-significant reduction in the incidence of IFI (6/32 vs. 1/18, $P > 0.1$ and 6/64 vs. 1/68, $P = 0.1$, respectively) and IFI-related mortality (6% vs. 0%, $P = 0.05$), but did not have an increase in overall survival (21% vs. 16%, $P > 0.05$). Importantly, neither study was blinded nor statistically powered to detect differences in the primary end-point or subgroup analysis.⁶ Nevertheless, the results of these early trials established and justified the strategy of administering AmB-D empirically to treat possible IFI in patients who develop PFUO while receiving broad-spectrum antibiotics, as the standard of care.

Empirical antifungal therapy trials and newer antifungal agents

AmB-D was the first agent used as empirical antifungal therapy (EAFT). Many experts attribute the lack of overall survival advantage observed with AmB-D, when used as EAFT, to its well-known toxicities. Several RCTs have since explored the efficacy and safety of newer but more expensive agents such as liposomal amphotericin-B (L-AMB), fluconazole, amphotericin B lipid complex (ABLCL), itraconazole, voriconazole and caspofungin using the composite end-point.⁷⁻¹² A table detailing the pertinent results of these trials is available in Appendix II, Table A1 (see electronic appendix). These trials used the composite end-point to compare overall success rates and while the trials did not demonstrate that any of the newer agents have superior efficacy they did show that these newer agents have superior tolerability. These results, and the failure of EAFT to improve overall survival, are probably due, in part, to the fact that the 'trigger' for commencing EAFT – persistent fever – is an insensitive, non-specific and late sign of IFI.

The concept of the composite end-point and its impact on the choice of empirical antifungal agent

The composite end-point (specifically developed for the EAFT efficacy trials discussed above⁷⁻¹²) is comprised of five components: (i) successful treatment of any baseline fungal infection, (ii) absence of breakthrough fungal infection, (iii) resolution of fever at a designated time point, (iv) survival at a designated time point and (v) no premature drug discontinuation due to lack of efficacy or

toxicity. All five components must be fulfilled for a case to contribute to the overall success rate of an antifungal agent under investigation. While the composite end-point enabled the EAFT efficacy trials to be completed in a timely fashion, components of the end-point, such as fever, exerted a disproportionate influence on the overall success rate of the trials, such that the true efficacy of the antifungal agents under investigation is likely to have been underestimated.¹³ As such, clinicians should not rely on the results of these trials alone when choosing an empirical antifungal agent.

Alternatives to EAFT: the role of a pre-emptive treatment strategy

Given that EAFT has never actually increased overall survival, an alternative antifungal strategy – one that diagnoses IFI both accurately and early; guides subsequent management, including the prompt institution of antifungal therapy where appropriate; and reduces morbidity and mortality – would be welcomed. This type of approach is commonly referred to in the literature as a pre-emptive treatment strategy as it relies on the fact that early diagnosis of an IFI and prompt institution of therapy appear to increase survival.¹⁴

As part of this approach, many researchers have focused on developing diagnostic tests that are rapid, sensitive and specific. Methods such as high resolution computed tomographic scan (HRCT) scans of the thorax, antigen detection assays and nucleic acid amplification assays are now available. Initial studies indicate that these tests may fulfil the above criteria and may be clinically useful if incorporated into a pre-emptive treatment strategy.

New diagnostic tests for IFI

HRCT scan of the thorax

A halo sign (i.e. a mass-like pulmonary infiltrate surrounded by a hazy edge of low attenuation) on a HRCT during neutropenia has a sensitivity of 89% for the diagnosis of IFI.¹⁵ In two retrospective studies, systematic HRCT scanning of the thorax (i.e. weekly during neutropenia or in the setting of febrile neutropenia, once an infiltrate is detected on a chest x-ray), led to a diagnosis of invasive aspergillosis (IA), on average 5 days earlier, with a 66% relative reduction in IA-related mortality.^{16,17} Some HRCT findings, however, are non-specific (e.g. diffuse infiltrates) and often require further evaluation with invasive procedures such as bronchoscopy. This, along with the fact that urgent HRCT scanning is not

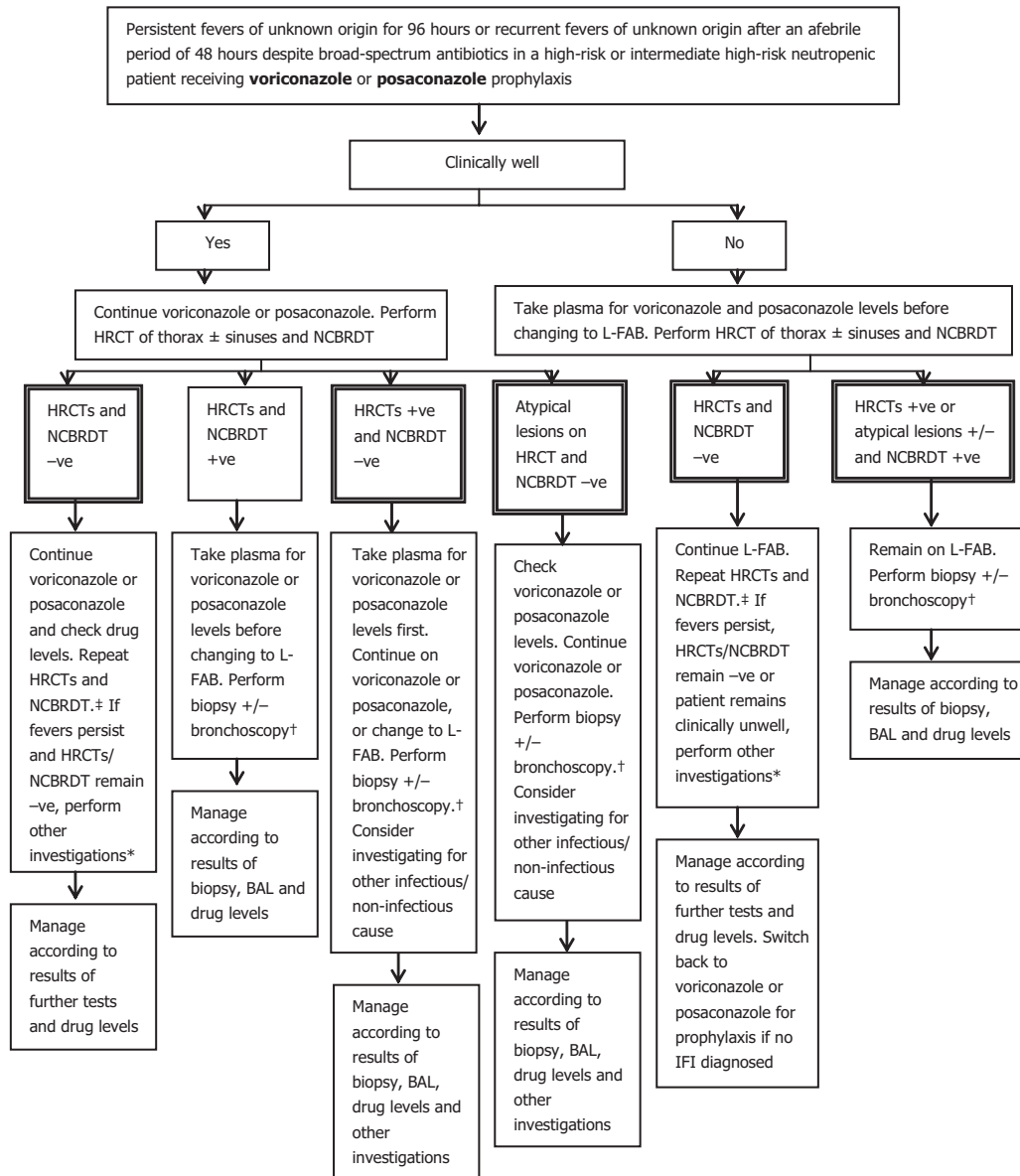


Figure 1 Algorithm for management of high-risk or intermediate high-risk neutropenic patients receiving voriconazole or posaconazole prophylaxis. Please refer to Table 1 of these guidelines for IFI risk groups and to Ascioğlu *et al.*³⁹ for probability-based IFI definitions ('probable', 'possible', 'proven'). If NCBRDT are not available within an adequate timeframe (48-h turnaround time), proceed down the relevant arm of the algorithm according to the result of the HRCT scans only (see double-lined boxes). Note: NCBRDT includes *Aspergillus* GM-ELISA and *Aspergillus* PCR assay. These are available in some Australasian specialist reference laboratories as part of the ASPID clinical trial (see main text for detail) or on a non-trial basis. Positive NCBRDT result = positive results from *Aspergillus* GM-ELISA and/or *Aspergillus* PCR assay, i.e. optical density index of GM ≥ 0.5 and/or a band of 236 bp detected on gel electrophoresis or exponential increase in fluorescence during the first 30 cycles of amplification. Positive HRCT result = presence of lesions meeting criteria for IFI, e.g. halo sign, nodule, cavitation in area of consolidation. Atypical lesions refer to changes on HRCT not meeting criteria for IFI, e.g. diffuse infiltrates, non-cavitary consolidation. *Example: repeat blood cultures, serum/plasma/urine for PCR viral testing, faecal sampling for *C. difficile*, ova, cysts, parasites and adenovirus, urine culture, colonic biopsy for CMV or GVHD and other tests as clinically relevant. †If HRCT lesions are focal or consistent with pulmonary IFI, e.g. halo sign, biopsy is the diagnostic test of choice; if HRCT shows atypical or non-focal pulmonary lesion, e.g. diffuse infiltrates, bronchoscopy is appropriate. All lesions in the sinuses should be biopsied. ‡Repeat HRCT scans of thorax \pm sinuses in the following 7–10 days and NCBRDT a minimum of twice in the following week to capture any evolving breakthrough IFI. AmB-D, conventional amphotericin B; L-FAB, lipid formulation of amphotericin B; L-AMB, liposomal amphotericin B; BAL, bronchoalveolar lavage; IA, invasive aspergillosis; IFI, invasive fungal infection; HRCT, high-resolution computed tomography; NCBRDT, non-culture-based rapid diagnostic tests; PCR, polymerase chain reaction.

Table 1 Invasive fungal infection risk groups**Low risk**

Peripheral blood stem cell autologous bone marrow transplant
 Childhood acute lymphoblastic leukaemia except for *Pneumocystis jiroveci* (*P. carinii*) pneumonia
 Lymphoma

Intermediate risk

Low

Moderate neutropenia $0.1\text{--}0.5 \times 10^9/\text{L}$ for <3 weeks, lymphocytes $<0.5 \times 10^9/\text{L}$ + antibiotics, e.g. trimethoprim and sulfamethoxazole (Septrin®)
 Older age
 Central venous catheter

High

Colonized >1 site or heavy at one site, neutropenia <0.5 to $>0.1 \times 10^9/\text{L}$ for >3 to <5 weeks
 Acute myeloid leukaemia
 Total body irradiation
 Allogeneic matched sibling donor bone marrow transplant

High risk

Neutrophils $<0.1 \times 10^9/\text{L}$ for >3 weeks
 Colonized by *Candida tropicalis*, allogeneic unrelated or mismatched donor bone marrow transplant
 Graft-versus-host disease
 Neutropenia $<0.5 \times 10^9/\text{L}$ for >5 weeks
 Prednisolone >1 mg/kg and neutrophils $<1 \times 10^9/\text{L}$ for >1 week
 Prednisolone >2 mg/kg for >2 weeks
 High-dose cytosine arabinoside (Ara-C)
 Fludarabine (uncertain)

Adapted from Prentice *et al.*⁴⁸

always available at all centres and at-risk patients must leave their protected environment in order to undergo scanning, can limit the usefulness of HRCT scanning. Current evidence suggests that HRCT scanning is most useful when combined with serological and molecular diagnostic tests to support a pre-emptive approach.¹⁸

Serological assays**Galactomannan**

Galactomannan (GM), an antigen component of the *Aspergillus* cell wall, can be detected in serum and other bodily fluids of patients with IA. A commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (PlateLIA® *Aspergillus* ELISA kit, Bio-Rad, Marne-la-Coquette, France) has a lower limit of detection of 0.5–1.0 ng/mL of GM in serum and is the most widely used assay.¹⁹ A recent meta-analysis calculated its pooled sensitivity and specificity for proven and probable IA as 0.61 (95% CI 0.59–0.63) and 0.93 (95% CI 0.92–0.94), respectively.²⁰ The test's sensitivity is affected by the cut-off value used to determine positivity (i.e. optical density index), the

concurrent use of mould-active antifungal therapy, the frequency of testing, the duration of testing in relation to the at-risk period and whether the patient group is at a high or low risk of developing an IFI.^{21,22} Its specificity is compromised by the concurrent administration of some antibiotics, e.g. piperacillin-tazobactam.²³

A number of clinically important issues around GM testing still require resolution: the optimal frequency of testing, the behaviour of the test over time, whether the test should be performed in patients receiving mould-active antifungal prophylaxis, the optimal cut-off for positivity, whether this cut-off should be different for patients with acute leukaemia versus stem cell transplant recipients, and a better understanding of the conditions associated with false-positivity and false-negativity.²⁴ Current opinion suggests that the true clinical value of this assay will only become apparent when it is incorporated into a pre-emptive treatment strategy to guide antifungal therapy.

1,3-β-D-glucan

1,3-β-D-glucan is a component of the cell wall of many fungi. Fungitec-G and Fungitell assays produced by Seikagaku Kogyo Corporation (Tokyo, Japan) and Associates of Cape Cod (Falmouth, MA, USA), respectively, are the most widely available assays. Only a few published studies are available. These studies report sensitivities of 70–100% and specificities of 76–83.8%.^{25–27} These assays have been assessed in only a small number of IA cases^{25–27} and require further evaluation before being included in a pre-emptive antifungal treatment strategy.

Nucleic acid assays

Numerous polymerase chain reaction (PCR)-based assays have been developed for the detection and identification of *Aspergillus* species in clinical specimens including whole blood, bronchoalveolar lavage (BAL) fluid and tissue. However, current molecular methods are not standardized; many different extraction methods, amplification targets and amplicon-detection methods are used. Technical factors can also affect the performance of these assays. The most sensitive assays use whole blood as the test specimen, use techniques for high-efficiency extraction of *Aspergillus* DNA (e.g. red cell and white lysis buffers, lyticase, heat-alkali treatment, β-mercaptoethanol, EDTA), target multicopy genes such as the rDNA gene complex or mitochondrial DNA and use a nested PCR or PCR-ELISA format.²⁸ When such techniques are used, sensitivities of 63.6–100% and specificities of 63.5–100% are reported.^{29–38} Studies have shown

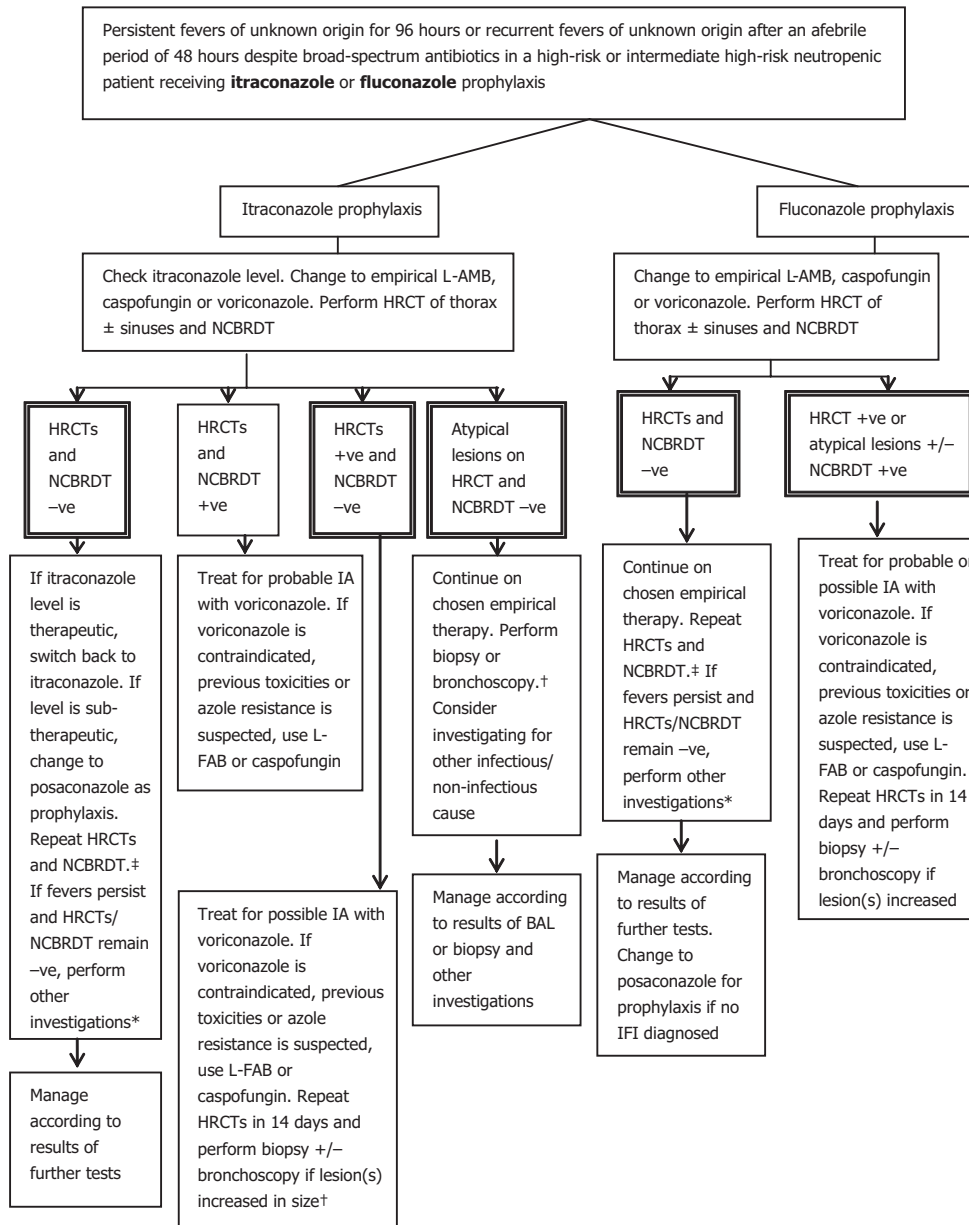


Figure 2 Algorithm for management of high-risk or intermediate high-risk neutropenic patients receiving itraconazole or fluconazole prophylaxis. Please refer to Table 1 of these guidelines for IFI risk groups and to Ascoglu *et al.*³⁹ for probability-based IFI definitions ('probable', 'possible', 'proven'). If NCBRDT are not available within an adequate timeframe (48-h turnaround time), proceed down the relevant arm of the algorithm according to the result of the HRCT scans only (see double-lined boxes). Note: NCBRDT includes *Aspergillus* GM-ELISA and/or *Aspergillus* PCR assay. These are available in some Australasian specialist reference laboratories as part of the ASPID clinical trial (see main text for detail) or on a non-trial basis. Positive NCBRDT result = positive results from *Aspergillus* GM-ELISA and *Aspergillus* PCR assay, i.e. optical density index of GM ≥ 0.5 and/or a band of 236 bp detected on gel electrophoresis or exponential increase in fluorescence during the first 30 cycles of amplification. Positive HRCT result = presence of lesions meeting criteria for IFI, e.g. halo sign, nodule, cavitation in area of consolidation. Atypical lesions refer to changes on HRCT not meeting criteria for IFI, e.g. diffuse infiltrates, non-cavitary consolidation. *Example: repeat blood cultures, serum/plasma/urine for PCR viral testing, faecal sampling for *C. difficile*, ova, cysts, parasites and adenovirus, urine culture, colonic biopsy for CMV or GVHD and other tests as clinically relevant. †If HRCT lesions are focal or consistent with pulmonary IFI, e.g. halo sign, biopsy is the diagnostic test of choice; if HRCT shows atypical or non-focal pulmonary lesion, e.g. diffuse infiltrates, bronchoscopy is appropriate. ‡Repeat HRCT scans of thorax \pm sinuses in the following 7–10 days and NCBRDT a minimum of twice in the following week to capture any evolving breakthrough IFI. Amb-D, conventional amphotericin B; L-FAB, lipid formulation of amphotericin B; L-AMB, liposomal amphotericin B; BAL, bronchoalveolar lavage; IA, invasive aspergillosis; IFI, invasive fungal infection; HRCT, high-resolution computed tomography; NCBRDT, non-culture-based rapid diagnostic tests; PCR, polymerase chain reaction.

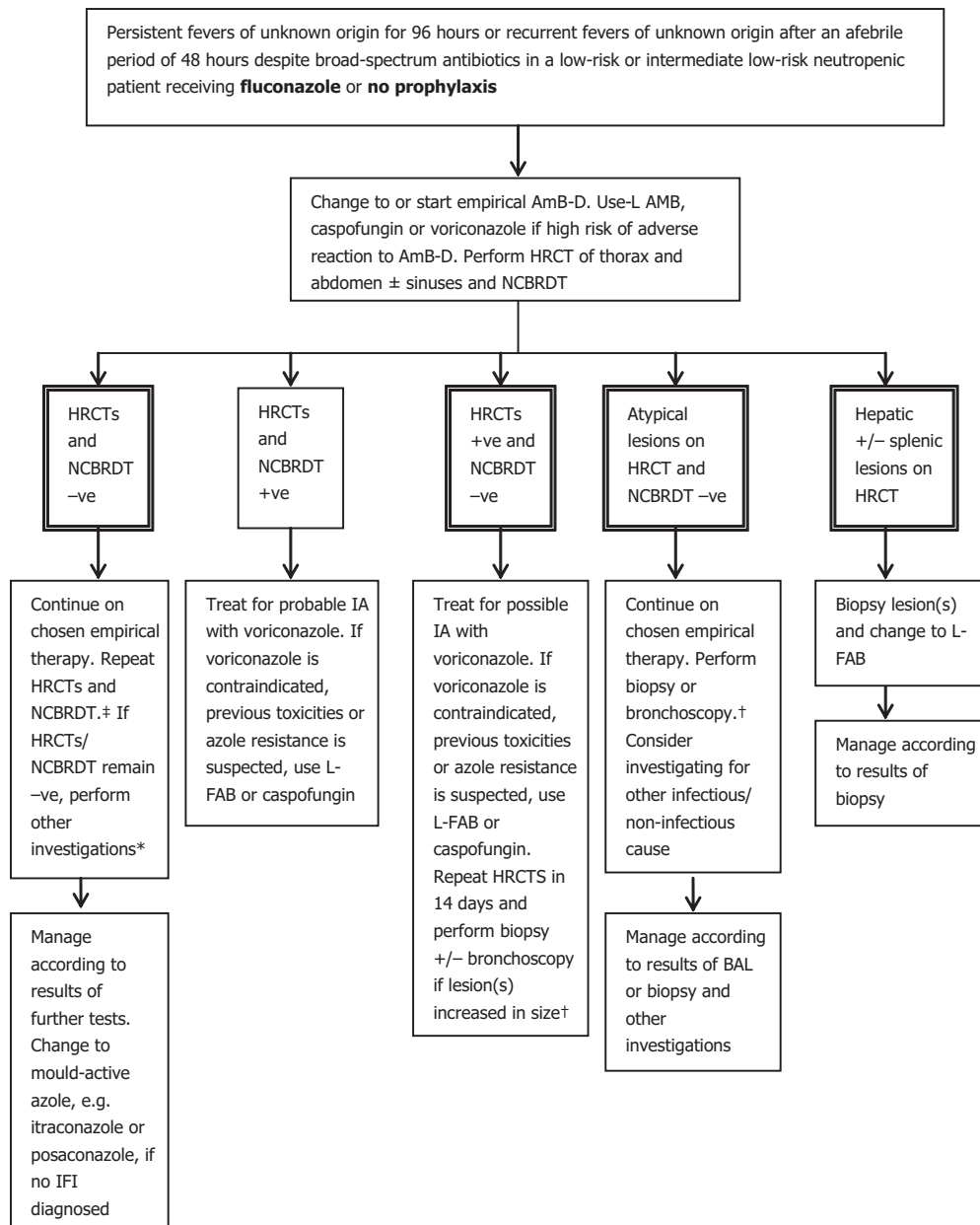


Figure 3 Algorithm for management of high-risk or intermediate high-risk neutropenic patients receiving fluconazole or no prophylaxis. Please refer to Table 1 of these guidelines for IFI risk groups and to Asciglu *et al.*³⁹ for probability-based IFI definitions ('probable', 'possible', 'proven'). If NCBRDT are not available within an adequate timeframe (48-h turnaround time), proceed down the relevant arm of the algorithm according to the result of the HRCT scans only (see double-lined boxes). Note: NCBRDT includes *Aspergillus* GM-ELISA and/or *Aspergillus* PCR assay. These are available in some Australasian specialist reference laboratories as part of the ASPID clinical trial (see main text for detail) or on a non-trial basis. Positive NCBRDT result = positive results from *Aspergillus* GM-ELISA and *Aspergillus* PCR assay, i.e. optical density index of GM ≥ 0.5 and/or a band of 236 bp detected on gel electrophoresis or exponential increase in fluorescence during the first 30 cycles of amplification. Positive HRCT result = presence of lesions meeting criteria for IFI, e.g. halo sign, nodule, cavitation in area of consolidation. Atypical lesions refer to changes on HRCT not meeting criteria for IFI, e.g. diffuse infiltrates, non-cavitary consolidation. †Example: repeat blood cultures, serum/plasma/urine for PCR viral testing, faecal sampling for *C. difficile*, ova, cysts, parasites and adenovirus, urine culture, colonic biopsy for CMV or GVHD and other tests as clinically relevant. †If HRCT lesions are focal or consistent with pulmonary IFI, e.g. halo sign, biopsy is the diagnostic test of choice; if HRCT shows atypical or non-focal pulmonary lesion, e.g. diffuse infiltrates, bronchoscopy is appropriate. All lesions in the sinuses should be biopsied. ‡Repeat HRCT scans of thorax ± sinuses in the following 7–10 days and NCBRDT a minimum of twice in the following week to capture any evolving breakthrough IFI. AmB-D, conventional amphotericin B; L-FAB, lipid formulation of amphotericin B; L-AMB, liposomal amphotericin B; BAL, bronchoalveolar lavage; IA, invasive aspergillosis; IFI, invasive fungal infection; HRCT, high-resolution computed tomography; NCBRDT, non-culture-based rapid diagnostic tests; PCR, polymerase chain reaction.

that PCR assays can detect *Aspergillus* DNA in the whole blood of patients on average 5–19.5 days before clinical signs or radiological findings are present or cultures are positive, and a median of 8–19.5 days prior to the initiation of antifungal therapy in 67% of patients.^{29,31–33,35–38} As no commercial standardized PCR-based assay is currently available, PCR is not widely used as a diagnostic modality and is not currently included in standard diagnostic criteria.³⁹ A list of other pertinent studies, which evaluate PCR-based assays in detail, is available in Appendix III (see electronic appendix).

Incorporating new diagnostic tests into a pre-emptive treatment strategy

The potential impact of a pre-emptive treatment strategy has been explored in a number of studies, including the partially completed ASPID trial (see <http://www.clinicaltrials.gov> for details of this trial: Slavin *et al.* A multicentre randomized controlled trial comparing two strategies for the diagnosis of IA in high-risk haematology patients),^{18,40–42} Please refer to Appendix II, Table A2 for a detailed summary of these studies. Collectively, these studies provide enough evidence to recommend that clinicians incorporate HRCT scans and consider, including *Aspergillus* GM and *Aspergillus* PCR assays in management algorithms for patients with PFUO (level III-2 evidence, grade B recommendation); see Figures 1–3.

Recommendations for the use of antifungal therapy in the setting of PFUO

Recommendations for the use of antifungal therapy in patients with persistent fever of unknown origin (PFUO) are summarized in Figures 1–3. These recommendations take into account current efficacy data, local fungal epidemiology, the antifungal drugs currently available and approved for use in Australasia, patient risk category, the toxicity profile of the different antifungal agents as well as the risks and consequences of developing toxicities in the various subgroups (refer to the section of the guidelines by Worth *et al.* on p. 521 for more details), the spectrum of activity of the various antifungal agents, the availability of HRCT scans and non-culture-based rapid diagnostic tests (NCBRDT), the agent previously used for antifungal prophylaxis, the degree and site of any organ dysfunction, drug–drug interactions (refer to Worth *et al.* on p. 521), and the cost of the various antifungal agents.

The clinical pathways presented in Figures 1–3 reflect the paradigm shift that has occurred following the

introduction of mould-active prophylaxis and the wider availability of better diagnostic tests, i.e. the shift away from the automatic administration of EAFT alone for PFUO to a pre-emptive treatment strategy that modifies antifungal prophylaxis according to pre-specified criteria such as clinical features, specific radiological abnormalities and/or positive NCBRT results.

As emphasized by Slavin *et al.* already in these guidelines, the choice of prophylactic agent is primarily dependent on the patient's risk of developing an IFI. In turn, the chosen prophylactic agent will determine the choice of any downstream antifungal therapy. These two factors are central to the pathways we present. The algorithms do not cover every clinical scenario and for some clinical scenarios, evidence is limited. Thus, complex, critically ill patients should be investigated and managed in consultation with your institution's Infectious Diseases Consult Service.

The rationale for the use of NCBRT in the algorithms, the choice of lipid formulation of amphotericin B (L-FAB) for empirical vs. treatment of proven or probable IFI and the recommendations for a biopsy vs. a bronchoscopy is available in Appendix I (see electronic appendix).

It is beyond the scope of this article to provide an explanation of each box in the algorithms (figures 1–3); rather below we provide summary explanatory notes as to the differential diagnoses that need to be considered, investigations that need to be performed to establish a microbiological diagnosis and antifungal agents (if any) that need to be administered for the more complex clinical scenarios.

Conclusions

The use of broad-spectrum mould-active antifungal prophylaxis and radiological, serological and molecular markers as diagnostic adjuncts has led to the development of new clinical pathways for the management of persistent or recurrent fevers in neutropenic patients. However, there is still not enough evidence to fully embrace pre-emptive strategies. The clinical pathways presented herein aim to be practical and clinically relevant. As such, they reflect the need for clinicians to (i) move away from using fever alone as a trigger to alter a patient's antifungal therapy and (ii) to proceed with caution when using NCBRT results to guide therapy. It is our intention to prospectively validate these pathways as part of our ongoing evaluation of the clinical utility of these guidelines. This clinical area is significant for the rapidity with which new data accumulate. Thus, we anticipate that the algorithms will need to be revised in the next few years.

Overview of components of algorithms (Figs 1–3)

Figure 1 Persistent or recurrent fevers of unknown origin (PFUO) in a patient receiving voriconazole or posaconazole prophylaxis and of high- to intermediate-high risk of developing an invasive fungal infection (IFI)

First, determine if the patient is clinically well or unwell (i.e. hypoxic, dyspnoeic, hypotensive, presence of markers of septicaemia, cough, haemoptysis, pleuritic chest pain, etc). PFUO in a clinically well patient receiving voriconazole or posaconazole prophylaxis are more likely to have a non-fungal aetiology, while the likelihood of breakthrough azole-resistant *Aspergillus* infection or other azole-resistant IFI is increased in those who are clinically unwell.^{43,44} We recommend continuing voriconazole or posaconazole prophylaxis in clinically well patients and changing clinically unwell patients to a lipid formulation of amphotericin B. Subsequent to this, both groups should be investigated with a HRCT scan of thorax \pm sinuses and non-culture based rapid diagnostic tests (NCBRDT) (level IV; grade C recommendation). The results of these tests will produce a number of clinical scenarios, which will determine any further downstream management.

Clinically well patients

1.1. Normal HRCT scans of thorax \pm sinuses and negative NCBRDT

While voriconazole or posaconazole may decrease the sensitivity of NCBRDT,^{21,45} the combination of negative NCBRDT with a normal HRCT scan suggests that an IFI is highly unlikely and that a non-fungal infection or non-infectious pathology is a more likely cause of the PFUO. In this clinical scenario we recommend (grade C recommendation):

1. Continuing the patient on voriconazole or posaconazole prophylaxis
2. Checking voriconazole or posaconazole levels to ensure they are therapeutic and adjusting the dose accordingly if not in the therapeutic range to minimize the risk of future breakthrough IFI or toxicity, e.g. drug fever
3. Repeating the HRCT scans of thorax \pm sinuses in the following 7–10 days and NCBRDT a minimum of twice in the following week to capture any evolving breakthrough IFI
4. Investigating for another infectious cause (e.g. viral, parasitic or nosocomial bacterial) or non-infectious cause (e.g. graft-versus-host disease (GVHD)) of PFUO.

1.2. Lesions on HRCT scans of thorax \pm sinuses consistent with an IFI and NCBRDT positive

In this scenario the differential diagnoses include:

- 1.2.1 Sub-therapeutic plasma azole levels
- 1.2.2 The presence of an *Aspergillus* isolate that has become resistant to azoles
- 1.2.3 Poor penetration of a necrotic mass of *Aspergillus* infection
- 1.2.4 Co-infection with another fungus (e.g. Zygomycetes).

To determine which is responsible, we recommend:

1. Checking voriconazole or posaconazole levels before changing to a lipid formulation of amphotericin B
2. Changing to a lipid formulation of amphotericin B while determining the cause
3. Performing a biopsy, if possible (preferable). We recommend an open lung biopsy (either thoracotomy or video-assisted), as the ability to obtain a larger volume of tissue will maximize the chance of a microbiological/histological diagnosis (see Appendix I for discussion of the choice of biopsy versus bronchoscopy).

If drug levels are sub-therapeutic, change to a higher dose of voriconazole or posaconazole. If ongoing sub-therapeutic levels are predicted (e.g. GVHD of the gastro-intestinal tract) or ongoing monitoring of levels is likely to be difficult (e.g. drug levels are performed off-site), continue on a lipid formulation of amphotericin B (5 mg/kg), i.e. L-AMB or ABLC; see Appendix I for discussion of the choice lipid formulation of amphotericin.

If an azole-resistant *Aspergillus* isolate or other resistant mould (e.g. Zygomycetes) is identified, continue L-AMB or ABLC at 5 mg/kg (see the section of the guidelines by Thursky *et al.* on p. 493 for additional treatment recommendations). If a mass of necrotic tissue is detected, resection (especially when a single lesion is present) plus L-AMB is recommended (level III-2, grade C recommendation).

1.3. Lesions on HRCT scans of thorax \pm sinuses consistent with an IFI and NCBRDT negative

The likely cause of this clinical scenario includes those outlined in 1.2.1–1.2.4 with false negative NCBRDT results but may also include:

- 1.3.1 A non-fungal infection
- 1.3.2 A non-infectious pathology

To determine which is responsible, we recommend:

1. Checking voriconazole or posaconazole levels. If drug levels are sub-therapeutic, change to a higher dose. If ongoing sub-therapeutic levels are predicted (e.g. GVHD

of the gastrointestinal tract) or ongoing monitoring of levels is likely to be difficult (e.g. drug levels are performed off-site), change to a lipid formulation of amphotericin B (5 mg/kg), i.e. L-AMB or ABLC; see Appendix I (electronic appendix) for discussion of the choice lipid formulation of amphotericin.

2. Continuing voriconazole or posaconazole prophylaxis or changing to a lipid formulation of amphotericin B while determining the cause. Your choice will depend on how quickly drug levels can be obtained, how quickly a biopsy can be performed, the number and size of the lesions on the HRCT scan and the presence of other factors that increase the probability of an IFI (e.g. co-existent cytomegalovirus (CMV) disease, grade 3–4 GVHD requiring treatment with multiple immunosuppressants including high-dose corticosteroids).

3. Performing a biopsy, if possible (preferable). We recommend an open lung biopsy (either thoracotomy or video-assisted), as the ability to obtain a larger volume of tissue will maximize the chance of a microbiological/histological diagnosis (see Appendix I (electronic appendix) for discussion of the choice of biopsy versus bronchoscopy). The result of the biopsy will determine further treatment, e.g. treat an azole-resistant *Aspergillus* isolate or other resistant mould (e.g. *Zygomycetes*) with L-AMB or ABLC (5 mg/kg); treat *Pseudomonas* infection with antibiotics according to sensitivity profile; treat GVHD with corticosteroids and other immunosuppressants (Level III-3 evidence; grade C recommendation).

4. Investigating for a non-fungal infectious cause (e.g. viral or nosocomial bacterial) or non-infectious cause (e.g. GVHD) as necessary.

1.4. Atypical lesions on HRCT scan and NCBRT negative

While the causes outlined in 1.2.1–1.2.4 may cause this clinical scenario, those outlined in 1.3.1–1.3.2 are more likely. To determine which is responsible, we recommend:

1. Continuing voriconazole or posaconazole
2. Checking voriconazole or posaconazole levels and adjusting the dose as required
3. Performing an open lung biopsy (if focal lesion) or bronchoscopy (if diffuse infiltrates)
4. Investigating for a non-fungal infectious cause (e.g. viral or nosocomial bacterial) or non-infectious cause (e.g. GVHD) as necessary.

Clinically unwell patients

For clinically unwell patients with PFUO receiving voriconazole or posaconazole prophylaxis, we recommend:

1. Checking voriconazole or posaconazole levels
2. Changing to L-AMB or ABLC
3. Performing a HRCT scan of thorax ± sinuses and NCBRT (level III-3, grade C recommendation).

The results of these tests will produce a number of clinical scenarios, which will determine any further downstream management (see 1.5 and 1.6 below).

1.5. Normal HRCT scans of thorax ± sinuses and negative NCBRT in a clinically unwell patient

While voriconazole or posaconazole may decrease the sensitivity of NCBRT,^{21,45} the combination of negative NCBRT with a normal HRCT scan makes an IFI highly unlikely and a non-fungal infection or a non-infectious pathology more likely. In this clinical scenario, we recommend continuing L-AMB or ABLC while determining the cause and following recommendations (3) and (4) in section 1.1 above. If no IFI is diagnosed, switch back to voriconazole or posaconazole; use the same dose if drug levels were therapeutic or adjust accordingly.

1.6. Any abnormalities on HRCT scan of thorax ± sinuses and/or NCBRT tests are positive in a clinically unwell patient

Any abnormality on NCBRT or HRCT scans needs more thorough investigation in patients who are clinically unwell. Causes include those outlined in 1.2.1–1.2.4 and 1.3.1–1.3.2. To determine which is responsible, we recommend:

1. Continuing L-AMB or ABLC while determining the cause
2. Performing an open lung biopsy (if focal lesion) or bronchoscopy (if diffuse infiltrates)

Figure 2 Persistent or recurrent fevers of unknown origin (PFUO) in a patient receiving itraconazole or fluconazole prophylaxis and of high- to intermediate-high risk of developing an invasive fungal infection (IFI)

Compared with voriconazole or posaconazole, itraconazole prophylaxis has the disadvantage of poorer bioavailability and a narrower spectrum of antifungal activity. Thus, the likelihood of breakthrough invasive aspergillosis (IA) or other IFI is increased on itraconazole prophylaxis. As a result, patients with PFUO receiving itraconazole prophylaxis should be treated empirically with L-AMB (see Appendix I (electronic appendix) for discussion of the choice lipid formulation of amphotericin), caspofungin or voriconazole while being investi-

gated with a HRCT scan of thorax \pm sinuses and NCBDRT and itraconazole levels. The results of these tests will produce a number of clinical scenarios that will determine any further downstream management.

2.1. Normal HRCT scans of thorax \pm sinuses and negative NCBDRT

While itraconazole may decrease the sensitivity of NCBDRT,^{21,45} the combination of negative NCBDRT with a normal HRCT scan makes a diagnosis of an IFI highly unlikely. If itraconazole levels are therapeutic then it is recommended to switch back to itraconazole prophylaxis. If itraconazole levels are sub-therapeutic then a change to posaconazole as prophylaxis is recommended. Further management is the same as that recommended in (3) and (4) of section 1.1.

2.2. Atypical lesions on HRCT scan and NCBDRT negative

The causes for this clinical scenario include:

- 2.2.1 Breakthrough IA
- 2.2.2 Breakthrough infection with a non-*Aspergillus* mould
- 2.2.3 Sub-therapeutic levels of itraconazole
- 2.2.4 Non-fungal infection
- 2.2.5 Non-infectious pathology

To determine which is responsible, we recommend:

1. Continuing on chosen EAFT while determining the cause
2. Performing an open lung biopsy (if focal lesion) or bronchoscopy (if diffuse infiltrates)
3. Investigating for another infectious cause (e.g. viral, parasitic or nosocomial bacterial) and for a non-infectious cause (e.g. GVHD).

Because fluconazole has no anti-mould activity, PFUO in the setting of broad-spectrum antibiotics in a high-risk or intermediate-high risk patient may be indicative of IA or other invasive mould infection. As a result a patient should be treated empirically with L-AMB (see Appendix I (electronic appendix) for discussion of the choice lipid formulation of amphotericin), caspofungin or voriconazole while being investigated with HRCT scan of thorax \pm sinuses and NCBDRT. The results of these tests will produce a number of clinical scenarios that will determine any further downstream management.

2.3. Normal HRCT scans of thorax \pm sinuses and negative NCBDRT

Fluconazole does not decrease the sensitivity of NCBDRT (level I evidence).²⁰ Thus, these results have a high nega-

tive predictive value and breakthrough IA or other IFI is very unlikely. Further management is the same as that recommended in points (3) and (4) in section 1.1 above. However, if the HRCT scan and NCBDRT results remain serially negative then consider switching the patient to itraconazole or posaconazole prophylaxis.

Figure 3 Persistent or recurrent fevers of unknown origin (PFUO) in a patient on fluconazole or no prophylaxis and of low- to intermediate-low risk of developing an invasive fungal infection (IFI)

While these patient groups are at low risk of developing a mould infection, they still have a substantial risk of developing candidaemia or invasive candidiasis (IC).⁴⁶ Thus, the empirical use of an antifungal agent while investigating the cause of PFUO is recommended (level II evidence, grade B recommendation). A patient in this category should be treated empirically with AmB-D. If they are at high risk of an adverse reaction to AmB-D, treat with L-AMB (see Appendix I (electronic appendix) for discussion of the choice lipid formulation of amphotericin), caspofungin or voriconazole while undergoing a HRCT scan of thorax \pm sinuses, CT scan of abdomen (for IC) and NCBDRT. The results of these tests will produce a number of clinical scenarios that will determine any further downstream management.

3.1. Hepatic \pm splenic lesions on HRCT scan of abdomen

The radiological appearance of hepatosplenic candidiasis is characteristic.⁴⁷ A core biopsy is recommended in order to obtain a large enough sample and maximize the chance of a microbiological/histological diagnosis (level IV, grade C recommendation).

Please refer to sections 2.1–2.3 for the differential diagnoses and recommended investigations for the remaining clinical scenarios presented in Figure 3.

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Appendix I

Rationale for the complex components of the algorithms

1. Non-culture-based rapid diagnostic test (NCBRDT) – should we include *Aspergillus* galactomannan enzyme-linked immunosorbent assay (GM ELISA) or *Aspergillus* polymerase chain reaction (PCR) assays in guidelines for the management of persistent or recurrent fevers of unknown origin (PFUO)?

There has been much discussion during the formulation of these guidelines about whether we should include non-culture-based rapid diagnostic tests (i.e. *Aspergillus* GM-ELISA and *Aspergillus* PCR assays) in the algorithms presented in Figures 1–3. Table A2 in this appendix provides level III-2 evidence (grade B recommendation) that these assays have value in the diagnosis or exclusion of invasive aspergillosis (IA) and the subsequent therapeutic decision-making process. Slavin *et al.* are currently conducting a randomized controlled trial (RCT) (Diagnosing IA in high-risk haematology patients – the ASPID trial; see <http://www.clinicaltrials.gov> for details) in Australasia to determine if stronger evidence can be provided for the utility of these assays in improving IA-related morbidity and other mortality outcomes. While we understand that recommending the use of these assays in these guidelines may slow recruitment to the trial, excluding these assays from the current guidelines would fail to recognize that many clinicians are using these assays outside the ASPID trial and that there is a need to provide expert recommendations on the interpretation of these assays. The inclusion of these assays in the algorithms (Figures 1–3) also provides an additional research avenue by which we can examine the utility of these assays while fulfilling the aim of these guidelines, i.e. to provide a general framework for best practice. Evidence is evolving at such a pace that the omission of NCBRDT from the algorithms would render them obsolete by the time of publication. Thus, a consensus decision was reached to include NCBRDT in the management algorithms we present for PFUO.

To reflect the fact that NCBRDT have not been fully evaluated, we recommend that NCBRDT results be considered in conjunction with the results of high-resolution computed tomography (HRCT) scans. For this reason, NCBRDT results alone do not drive management in any of the treatment arms presented in the algorithms (e.g. we do not include an arm for positive NCBRDT results and negative HRCT scans).

We also recognize that some centres do not have access to NCBRDT within an adequate timeframe to allow

appropriate therapeutic decision making. In these circumstances, we recommend clinicians use HRCT scans in conjunction with clinical signs and symptoms, type of antifungal prophylaxis, and a patient's risk of developing an invasive fungal infection (IFI), to determine which arm of the respective algorithm should be followed to investigate and manage PFUO. The algorithms have been developed so that the results of HRCT scans can be readily used with or without NCBRDT.

2. Which lipid formulations of amphotericin B should be used when?

When choosing a lipid formulation of amphotericin, it is important to determine the indication for use (i.e. empirical versus treatment of proven or probable IFI). The use of liposomal amphotericin B (L-AMB) (AmBisome®) as empirical therapy was as efficacious as amphotericin B deoxycholate (AmB-D) on analysis of the composite endpoint (level II evidence) (refer to Table A1 in Appendix II for further details).¹ However, comparisons of L-AMB and amphotericin B lipid complex (ABLC) (Abelcet®) as empirical therapy are primarily based on differences in toxicity rather than efficacy (level II evidence).² Given this, we recommend clinicians use L-AMB as the lipid formulation of choice for empirical therapy in the setting of PFUO when results of HRCT scans or NCBRDT are not available (grade B recommendation).

The small number of randomized trials that examine lipid formulations of amphotericin B for the treatment of proven or probable IFI (i.e. positive microbiological or positive surrogate marker of infection) indicates that L-AMB is as efficacious as ABLC; however, L-AMB has significantly less infusion-related toxicity, e.g. fevers and chills (level II evidence).^{3,4} ABLC does, however, have a cost advantage over L-AMB. Thus, we recommend using either L-AMB or ABLC as pre-emptive therapy in the setting of positive HRCT and NCBRDT results and for the treatment of proven or probable (i.e. biopsy- or culture-positive) IFI (grade B recommendation).

3. Biopsy or bronchoscopy – which procedure should be used when?

The typical radiographic abnormalities associated with IFI (e.g. halo sign) have been well described.⁵ However, it is important to remember that HRCT scans do not provide a microbiological diagnosis and that 'typical' lesions may also have a non-fungal (e.g. *M. tuberculosis*, *S. aureus*) or non-infectious (e.g. graft-versus-host-disease (GVHD), alveolar haemorrhage) aetiology.⁶ A number of clinical scenarios require a tissue sample (e.g. bronchoscopy or biopsy) in order to make a microbiological diagnosis. The

choice of procedure is dependent on a number of factors: type of lesions (nodule vs. diffuse infiltrate), location of lesions (central vs. peripheral), patient's clinical condition (e.g. hypoxia, significant thrombocytopenia), local experience with the various procedures (e.g. CT-guided percutaneous biopsy), the predicted risk of complications from the procedure and the patient's capacity to tolerate potential complications. Bronchoscopy with lavage is well tolerated and is rarely associated with serious complications; however, the diagnostic yield in patients with haematological malignancy or post haemopoietic stem cell transplantation (HSCT) is low (30–50%).^{7,8,9} CT-guided percutaneous biopsy has a low complication rate (1.1%);¹⁰ however, the diagnostic yield is quite variable (11.7–73%)^{11,12,13} as it is dependent on the timing of the biopsy in relation to the commencement of antifungal therapy, the expertise of the operator and the volume of sample obtained.^{6,13} Open lung biopsy (OLB), either thoracotomy or video-assisted, will produce a higher yield (81%) as a larger volume of tissue can generally be obtained for culture.¹⁴ However, it also has a much higher complication rate (e.g. pneumothorax, haemorrhage), of around 8–20%.¹⁵ It is important to note that OLB (thoracotomy or video-assisted) is a significantly more useful diagnostic tool when a focal lesion (e.g. nodule), rather than diffuse infiltrate, is present.¹⁶ Finally, at some centres, it is not possible to perform a biopsy without first performing a bronchoscopy. Taking all of the above into account, we recommend that, wherever possible, a biopsy (either OLB or CT-guided percutaneous biopsy) is performed for all focal lung lesions and that a bronchoscopy with lavage is performed to investigate diffuse infiltrates. An OLB (thoracotomy or video-assisted) is preferred over a CT-guided biopsy when it is essential to obtain a larger volume of tissue for culture (e.g. detection of breakthrough resistant mould infections on posaconazole prophylaxis). A bronchoscopy is recommended in those cases where a biopsy is not possible (level III-3, grade C recommendation).

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Appendix II

Summary evidence tables for empirical and pre-emptive therapy

Table A1 Randomized trials comparing antifungal agents as empirical antifungal therapy

Reference	Study drugs	Primary endpoint	Sample size	Efficacy outcome	Safety outcome	Level of evidence
Walsh <i>et al.</i> (1999) ⁷	AmB-D vs. L-AMB	Efficacy: Composite of survival for 7 days after starting study drug, resolution of fever during neutropenia, successful treatment of any baseline IFI, absence of breakthrough IFI during study drug treatment or within 7 days of cessation and absence of premature discontinuation of study drug because of toxicity or lack of efficacy	702	Composite: Equivalence (49% vs. 50%) Individual components: Significantly less breakthrough IFI with L-AMB (3.2% vs. 7.8%; $P = 0.009$)	Favours L-AMB: Significantly less infusion-related fevers (17% vs. 44%; $P < 0.001$) and nephrotoxicity (19% vs. 34%; $P < 0.001$)	II
Winston <i>et al.</i> (2000) ⁸	AmB-D vs. fluconazole	Efficacy: Composite of no fevers at end of therapy, absence of breakthrough IFI during therapy, no additional antifungal therapy required, no termination of study drug secondary to toxicity and alive at end of therapy	317	Composite: Equivalence (67% vs. 68%)	Favours fluconazole: Significantly less adverse events (13% vs. 81%; $P = 0.001$)	II
Wingard <i>et al.</i> (2000) ⁹	L-AMB vs. ABLC	Safety: Incidence of chills/rigors during day 1 of study drug	244		Favours L-AMB: Significantly less infusion-related chills/rigors (18.8% and 23.5% vs. 79.5%; $P < 0.001$) and nephrotoxicity (14.1% and 14.8% vs. 42.3%; $P < 0.01$)	II
Boogaerts <i>et al.</i> (2001) ¹⁰	AmB-D vs. itraconazole	Efficacy: Resolution of fevers and neutropenia	384	Equivalence (38% vs. 47%)	Favours itraconazole: Significantly less adverse events (5% vs. 54%; $P = 0.001$). Significantly less discontinuation due to toxicity (19% vs. 38%; $P = 0.001$)	II

Table A1 continued

Reference	Study drugs	Primary endpoint	Sample size	Efficacy outcome	Safety outcome	Level of evidence
Walsh <i>et al.</i> (2002) ¹¹	L-AMB vs. voriconazole	Efficacy: Composite of survival for 7 days post end of therapy, absence of breakthrough IFI, absence of premature discontinuation of study drug, resolution of fever during neutropenia and successful treatment of any baseline IFI	849	Composite: Favours L-AMB (30.6% vs. 26.0%; 95% CI: -10.6 to 1.6) Individual components: Significantly less breakthrough IFI with voriconazole (1.9% vs. 5%; $P = 0.02$)	Variable: dependent on adverse event examined	II
Walsh <i>et al.</i> (2004) ¹²	L-AMB vs. caspofungin	Efficacy: Composite of successful treatment of any baseline IFI, absence of breakthrough IFI during study drug treatment or within 7 days of end of therapy, survival for 7 days post end of therapy, resolution of fever during neutropenia and absence of premature discontinuation of study drug because of toxicity or lack of efficacy	1123	Composite: Equivalence (33.7% vs. 33.9%; 95% CI: -5.6 to 6.0) Individual components: Significantly greater successful treatment of baseline IFI with caspofungin (51.9% vs. 25.9%; $P = 0.04$)	Favours caspofungin: Significantly less discontinuation due to toxicity (10.3% vs. 14.5%; $P = 0.03$) and nephrotoxicity (2.6% vs. 11.5%; $P < 0.001$)	II

ABL, amphotericin B lipid complex; AmB-D, amphotericin B deoxycholate or conventional amphotericin B; FI, invasive fungal infection; L-AMB, liposomal amphotericin B.

Table A2 Summary of studies evaluating pre-emptive treatment strategies for invasive fungal infections (IFIs)

Reference	Study design	Population	Tests evaluated	Endpoints	Results	Limitations of study	Level of evidence
Maertens <i>et al.</i> (2005) ¹⁸	Prospective cohort	Allogeneic HSCT recipients, AML, ALL and MDS	GM-ELISA and HRCT scan of thorax	Number of patients who received PAFT vs. EAFT	Pre-emptive strategy 7.7% vs. 35% EAFT. Decreased time to initiation of antifungal agent in 10%. 1 case of <i>Zygomycetes</i> missed	An indirect estimate of diagnostic value of GM-ELISA and HRCT scan as study not randomized	III-2
Hebart <i>et al.</i> (2004) (abstract only) ⁴⁰	RCT	Allogeneic HSCT recipients	Panfungal PCR assay using <i>Aspergillus</i> -specific probes	Primary: Incidence of IFI Secondary: Survival	Pre-emptive arm Increased use of L-AMB (109 vs. 76; $P < 0.05$). Decreased mortality at day +30 (4 vs. 13; $P = 0.03$). No difference in mortality at day +100	Evaluated PCR only, study underpowered, follow-up period too short	II
Cordonnier <i>et al.</i> (2006) (abstract only) ⁴¹	RCT	Autologous HSCT recipients and haematological malignancy	GM-ELISA	Primary: Percentage of patients alive 14 days after neutrophil recovery or 60 days from randomization if severe complication	Pre-emptive arm Survival not non-inferior (95% vs. 97.3%) No difference in IFI-related mortality (2% vs. 0%). Significantly less use of antifungal agent (38.5% vs. 62.7%; $P < 0.00001$)	Evaluated GM-ELISA only, short duration of screening, high-risk patients not included	II
Barnes <i>et al.</i> (2007) (abstract only) ⁴²	Prospective cohort	Allogeneic HSCT recipients and haematological malignancy	GM-ELISA and <i>Aspergillus</i> -specific real-time PCR assay	Number of patients who received PAFT	83 patients with no evidence of IFI and antifungal therapy withheld in the majority. Multiple positive PCR results correlated strongly with IA. Decreased antifungal agent expenditure (\$250 000 USD)	An indirect estimate of diagnostic value of GM-ELISA and HRCT scan as study not randomized	III-2
Slavin <i>et al.</i> (unpubl. data) [†]	RCT	Allogeneic HSCT recipients, AML and ALL	GM-ELISA and <i>Aspergillus</i> -specific nested PCR assay	Primary: Proportion of patients who received EAFT Secondary: IA-related mortality	First safety analysis No significant difference in IA-related mortality	Not completed	II

†See <http://www.clinicaltrials.gov> for details of this trial: Slavin *et al.* A multicentre randomized controlled trial comparing two strategies for the diagnosis of IA in high-risk haematology patients. ALL, acute lymphoblastic leukaemia; L-AMB, liposomal amphotericin B; AML, acute myeloid leukaemia; EAFT, empirical antifungal therapy; GM-ELISA, galactomannan enzyme-linked immunosorbent assay; HRCT, high-resolution computed tomography; HSCT, haemopoietic stem cell transplantation; IA, invasive aspergillosis; MDS, myelodysplastic syndrome; RCT, randomized controlled trial; PAFT, pre-emptive antifungal therapy; PCR, polymerase chain reaction.

Appendix III

Pertinent references regarding nucleic acid assays for the detection of invasive fungal infections (IFIs)

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