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When to change treatment of acute invasive aspergillosis: an expert viewpoint

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Invasive aspergillosis (IA) is an acute infection affecting patients who are immunocompromised, as a result of receiving chemotherapy for malignancy, or immunosuppressant agents for transplantation or auto-immune disease. Whilst criteria exist to define the probability of infection for clinical trials, there is little evidence in the literature or clinical guidelines on when to change antifungal treatment in patients who are receiving prophylaxis or treatment for IA. To try and address this significant gap, an advisory board of experts was convened to develop criteria for the management of IA for use in designing clinical trials, which could also be used in clinical practice. For primary treatment failure, a change in antifungal therapy should be made: (i) when mycological susceptibility testing identifies an organism from a confirmed site of infection, which is resistant to the antifungal given for primary therapy, or a resistance mutation is identified by molecular testing; (ii) at, or after, 8 days of primary antifungal treatment if there is increasing serum galacto-mannan, *or* galactomannan positivity in serum, or bronchoalveolar lavage fluid when the antigen was previously undetectable, *or* there is sudden clinical deterioration, *or* a new clearly distinct site of infection is detected; and (iii) at, or after, 15 days of primary antifungal treatment if the patient is clinically stable but with ≥2 serum galactomannan measurements persistently elevated compared with baseline or increasing, *or* if the original lesions on CT or other imaging, show progression by >25% in size in the context of no apparent change in immune status.

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Introduction

Over the last two decades, the choice of antifungal for primary therapy for invasive aspergillosis (IA) has widened alongside an expansion of diagnostic tests for detection of invasive fungal diseases (IFDs). Use of mould-active antifungal prophylaxis has also become more common over the last decade. Studies of prophylaxis and primary therapy with antifungals for preventing or managing acute IA in the haemato-oncology or transplant setting have used various iterations of the IFD consensus definitions developed by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) to advance clinical and epidemiological research.[1] These definitions were not designed for use in clinical practice, which has left a gap in terms of guidance for physicians treating IA outside a clinical trial. Moreover, there are no generally accepted criteria for defining treatment failure, either in a trial setting or in clinical practice.

Voriconazole, liposomal amphotericin B, isavuconazole, posaconazole and voriconazole plus anidulafungin have been studied for primary therapy of acute IA[2–6] whilst caspofungin, posaconazole, micafungin and caspofungin plus other antifungals have been studied as salvage therapy.[7–10] Most of these studies have been performed in patients with acute myeloid leukaemia and recipients of an allogeneic haematopoietic stem cell transplantation, although it is now clear that the pool of patients at risk of IA extends well beyond these two settings.

'Salvage' therapy implies failed treatment and a poor outcome, and this concept was imported from oncology trials, yet the studies of second-line IFD therapy also included patients who experienced adverse events on their primary therapy and therefore 'intolerance', rather than clinical failure, was a criterion for switching therapy.[7–10] Since patients showing intolerance may respond therapeutically to their first-line antifungal whilst also being intolerant, the cases enrolled in salvage trials generally involve different treatment response categories with intolerant patients potentially having better outcomes than those who are failing treatment, considered 'refractory'.

The definition of the 'stable disease' is also contentious as it implies treatment failure although it is questionable whether it is reasonable to regard 'stable disease' of a severely immunocompromised host as true treatment failure as it seems unreasonable to expect more than a stabilizing effect of antifungal therapy in any patient with limited or no immune response. Answering these questions may be dependent on whether the situation arises in a real-world clinical scenario or in a clinical trial. EORTC/MSGERC criteria for evaluating therapeutic responses in Phase III trials of IFDs state that stable response represents treatment failure, and hence requires a change in the antifungal used for therapy, even though stable disease may be a reasonable therapeutic goal until immune recovery occurs.[11,12] However, these criteria were adapted from oncology trials where visualization of reduction in tumour volume is weighted heavily towards response and may not be representative of the healing process seen in angio-invasive fungal diseases.[13] In an immunocompromised host, stable disease may represent an early sign of control of the disease process, and thus the beginning of treatment success. The European Confederation of Medical Mycology (ECMM)/MSGERC recently defined 'persistence' as disease that is unchanged since treatment initiation and needs further antifungal therapy but is distinct from refractory disease.[14]

Different imaging modalities may also show discordance, e.g. between positron emission tomography (PET)/CT and CT, with PET/CT showing response of the lesion to treatment by absence of metabolic activity and CT showing persistence of the tissue lesion.[15] PET/CT is a useful clinical diagnostic tool, which has not yet been incorporated into the EORTC/MSGERC criteria for use in clinical trials of IA, whilst CT scanning is one of the major diagnostic tools.

The increasing incidence of triazole-resistant aspergillosis in some regions presents another reason for changing therapy. Two retrospective case series from the Netherlands and Belgium showed that mortality from proven and probable azole-resistant IA treated initially with a triazole is double that of cases with triazole-susceptible aspergillosis, if initiation of appropriate therapy is delayed until conventional resistance testing becomes available, or until clinical treatment failure is observed (median 10 days).[16,17] This strongly suggests a clear need to identify the susceptibility of *Aspergillus* species either in patients failing triazole therapy in regions with previously documented resistance or by routine resistance testing in regions where triazole resistance levels are approaching 10%,[18] in order to change therapy as soon as a resistant organism is identified or strongly suspected.

Current guidelines on when, and why, to change the initial treatment regimen of IA differ in their recommendations. ESCMID guidelines on the diagnosis and management of aspergillosis suggest assessing response after 2 weeks of treatment.[19] Neither the European Conference on Infections in Leukemia[20] nor the IDSA guidelines discuss the timelines for changing therapy. The IDSA advocates an individualized approach that takes into consideration the rapidity, severity and extent of infection, patient comorbidities, and to exclude the emergence of a new pathogen[21] whilst the salvage studies for caspofungin and posaconazole required a minimum of 7 days treatment before a change in the initial treatment could be made.[7,8]

F2G Ltd, a development-phase pharmaceutical company, convened an advisory board of international experts including haematologists, infectious disease specialists, medical mycologists and molecular microbiologists with expertise in the diagnosis and management of IFDs to discuss the aforementioned issues in the management of IA and to develop a consensus on the design of clinical trials for registration purposes. One of the goals was to develop criteria for determining when to stop prophylaxis and start therapy for breakthrough IA, and when a change of therapy should be considered in other clinical settings.

Methods

A group of clinicians and scientists with a special interest in mycology from Asia, Australia, Brazil, Europe and North America was invited to attend the advisory board. All attendees had published extensively in the field of preclinical and clinical mycology and clinical trials, were members of international and national guidelines groups, or were members of international bodies representing mycology [e.g. International Society for Human and Animal Mycology (ISHAM), ESCMID and ECMM]. One expert, J.P.D., chaired the meeting and helped design the pre- and post-meeting questionnaires sent to attendees.

A pre-meeting questionnaire was sent to the experts to review their practice in the investigation and management of IA, setting the scene for

the advisory board (Table S1, available as Supplementary data at *JAC* Online). At the meeting, the experts were divided into four workshop groups, each with a facilitator, to discuss three clinical scenarios for the management of IA based on the most likely clinical scenarios in which a change of antifungal therapy would be needed. The scenarios were: (i) breakthrough IA on triazole prophylaxis; (ii) IA failing first-line treatment with a triazole in the absence of susceptibility data or genetic markers of triazole resistance; and (iii) triazole-resistant IA confirmed by microbiological methods. Each of these topics is one where there is little published evidence to support clinical practice, hence the need for expert guidance. The questions posed to the experts for each of the three initial scenarios are shown in the Supplementary data (Table S1). The responses were tabulated and the outputs shared with the expert group prior to the meeting. Each workshop group reported their conclusions to the wider group and the outputs were discussed, particularly where opinions differed. Where there was clear agreement on the approach to take, this was used to guide the recommendations. Where there was lack of agreement, the majority view was used to generate the guidance, with the alternative approaches also provided.

A fourth clinical scenario, identified during the meeting, was explored further afterwards by means of an on-line questionnaire. This scenario was management of IA in patients receiving novel immunomodulatory and molecular targeted anti-cancer drugs where risk of drug–drug interactions might influence choice of antifungal therapy, e.g. the Bruton's tyrosine kinase inhibitor (BTKI) ibrutinib, mTOR inhibitors, Bcl-2 inhibitors (e.g. venetoclax) and other agents. This is another area where there is little published evidence to support clinical practice, despite increasing uptake of these agents.

An initial manuscript was drafted based on the outputs of the four breakout groups and the post-meeting questionnaire. Selected members of the advisory board (M.A.S., J.P.D., J.A.M. and G.R.T.III) drafted the paper and circulated it to all authors for their review, edits, comments, addition of local epidemiology data and for appropriateness of references.

Results

Breakthrough IA on mould-active prophylaxis

The experts stated that around 5%–10% of patients in their practice receiving mould-active prophylaxis (typically posaconazole but other triazoles and micafungin were considered) would develop probable or proven IA.[22,23] The proportion would be considerably higher if 'possible' infection[1] was considered a criterion for prophylaxis failure and initiating IA therapy. In clinical practice this might also include patients with non-specific radiology and mycological evidence of infection, such as persistent positive serum galactomannan (GM), who would not meet the EORTC/MSGERC criteria but, in the view of the experts, clearly qualify for antifungal treatment.[24]

Where there is clinical suspicion of IA, it was considered essential to ensure adequate drug levels prior to stopping prophylaxis; measuring posaconazole, particularly when the oral suspension is used, or voriconazole levels at 2–5 days after starting prophylaxis, depending on the drug and formulation used, and making suitable adjustments as needed. Diagnostic investigation for breakthrough infection should commence in parallel. When patients have adequate drug levels, investigations for breakthrough mould disease should commence as symptoms occurring more than 3 days after starting adequate prophylaxis would be considered more consistent with breakthrough infection, whereas the consensus was that symptoms within 3 days of starting prophylaxis would be more consistent with a pre-existing infection.

Table 1. Investigation of refractory or breakthrough infection

Investigation	Details
Serum/plasma or blood samples	GM
	β-d-glucan
	PCR
Therapeutic drug monitoring	Titrate drug dose to therapeutic levels
Fibreoptic bronchoscopy	BAL from infected lobe
	Biopsy lesion if practical
	Microscopy (using optical brighteners) and cytology
	Culture
	GM
	LFD
	PCR—positive samples can be tested further for the presence of genetic markers of resistance.
	Antifungal susceptibility on positive cultures
CT-guided biopsy or biopsy of peripheral lesion	Microscopy
	Culture
	Antifungal susceptibility on positive cultures
	Non-culture methods of identification (tissue-based molecular sequencing, immunohistochemistry, cytology)

BAL, bronchoalveolar lavage; GM, galactomannan; LFD, lateral flow device.

Where mould disease is suspected, aggressive attempts to confirm the pathogen for instance by obtaining targeted samples and testing for several biomarkers are considered essential (Table 1),[19,21] particularly as infections due to one of the agents of mucormycosis,[25] a triazole-resistant *Aspergillus* species or a rare mould with unpredictable susceptibility, would require an immediate change to a different class of antifungal agent (Table 2).

The therapeutic antifungal should be changed for every patient with adequate exposure where there is clinical or diagnostic evidence for breakthrough infection. A change to a lipid amphotericin B (e.g. AmBisome®) should be initiated when there is diagnostic evidence for breakthrough mould disease. However, if infection with a susceptible *Aspergillus* species is confirmed, isavuconazole or voriconazole may be considered an option if sub-therapeutic levels of posaconazole have been identified. Other lipid formulations of amphotericin B [e.g. amphotericin B lipid complex (ABLC), amphotericin B colloidal dispersion (ABCD)] or amphotericin B deoxycholate might be considered when there is no readily available alternative, although amphotericin B deoxycholate is not generally recommended.

A change of treatment might be futile for patients with advanced fungal disease, particularly extensive mucormycosis involving the sinuses, orbit or brain, where major surgical intervention would be the more appropriate curative approach, albeit with potentially catastrophic effects.

Table 2. Reasons for changing first-line antifungal treatment

Days since initiation of therapy	Clinical and diagnostic findings compared with baseline
At any time	Identification of a pathogen resistant to primary antifungal therapy
8 to 14	On the basis of changes in GM:
	(i) Serum: The serum GM index has not fallen by either 1 unit or to <0.5 units based on measurements taken at least 7 days apart
	(ii) BAL: Positive GM from BAL in a patient with a previous BAL test that did not meet the definition of positive (too low or entirely negative) without regard for the interval of time between samples. Note that there is not a definition for rising GM index values from BAL as these values are subject to sampling error
	Or
	Clinical deterioration consistent with persisting or progressive invasive fungal disease with no other identifiable aetiology
	Or
	New distinct site of infection detected clinically or radiologically
≥15	Any of the above criteria
	Or
	Progression of original lesions on CT (or other imaging) based on >25% growth of initial lesions in the context of no change in immune status

GM, galactomannan.
Please note that equal weighting applies to each factor.

IA failing treatment with a triazole: 'refractory disease'

The experts estimated that, in their practice, 10%–15% of patients receiving a triazole for primary therapy of acute IA might require a change of therapy due to a lack of a response or an inadequate response. Another group of patients might require a change of therapy as a result of intolerance, but this group was not considered further as this does not represent true failure of therapy.

It was agreed that results for any diagnostic test used to demonstrate triazole treatment failure should be objectively verifiable, so that others not involved in the patient's clinical care could view the data and reach the same conclusion. This is particularly important in a clinical trial setting where a data review board may need to view the clinical data, with minimal knowledge of the patient's clinical presentation.

Defining the need for second-line therapy for 'refractory disease' can be categorized according to the number of days since initiation of primary antifungal therapy (Table 2). The group agreed that primary therapy, with confirmed therapeutic drug levels where appropriate, should be given for at least 8 days to show an effect. Rising serum GM or a radiological increase in size of the initial lesion should not be considered as a reason to change

therapy until after ≥8 or ≥15 days of therapy, respectively. A lesion arising in a new site that is detected clinically or radiologically after 8 days of therapy should also be considered a criterion for change after having performed appropriate diagnostic investigations.

Other than culture of a resistant organism, or a new lesion on radiology, no single diagnostic test can be used in isolation to determine the need to change therapy. Rather, an approach that integrates clinical, radiological and mycological tests is required. In particular, an increase in the size of a lesion on CT occurring in the first week of treatment or coinciding with recovery of neutropenia should be interpreted with caution as it is more likely to represent the effects of immune reconstitution than triazole treatment failure.[20] If primary triazole therapy is thought to have failed, an aggressive approach is required to confirm the pathogen and ensure appropriately directed therapy (Table 1).

In the setting of persistent neutropenia or significant immunocompromise, there should be an attempt to exclude other causes of infection or other non-infectious pathologies. Non-specific markers of inflammation, such as C-reactive protein (CRP),[26,27] could be helpful in demonstrating a lack of response, but they need to be interpreted in the overall clinical context of the patient.

The expert panel generally considered that treatment should be changed from a triazole to the liposomal amphotericin B, AmBisome® (LAmB) or another lipid formulation of amphotericin B (e.g. ABLC), or although in some cases, the addition of an echinocandin or a switch to another triazole might be considered if inadequate drug exposure was thought to be the reason for therapeutic failure. Amphotericin B deoxycholate might be considered when there is no readily available alternative although amphotericin B deoxycholate is not generally recommended.[19,21]

When asked to consider criteria to determine futility, the experts felt that this was primarily dependent on the prognosis of the underlying disease for which the patient was receiving chemotherapy and possibly life-limiting co-morbidities. In a setting where the patient has an aggressive, poorly responsive or progressive underlying condition (e.g. haematological malignancy not responding to chemotherapy), it was considered unlikely that a change in antifungal therapy would improve the patient's life expectancy. Another potentially futile setting would be where a patient with refractory disease is likely to require extracorporeal membrane oxygenation (ECMO) or prolonged ventilation in the ICU.

Proven triazole resistance

The experts reported that azole resistance is becoming a global problem and has been identified in up to 20% of clinical isolates of *Aspergillus fumigatus* in the Netherlands and Belgium,[28–30] 5%–8% in Taiwan,[31] 1% of haemato-oncology patients in France,[32] Germany[33] and Spain,[34] and 5% of isolates in a national survey in the USA.[35] In many regions, routine surveillance data are not available, so reliable epidemiological predictions based on local findings are difficult.

Triazole-resistant IA is associated with a high mortality.[16,17,28,29] This is probably due to the overall difficulty in diagnosing IA, as well as difficulties in determining resistance leading to delays in implementing a change to appropriate therapy. As triazole-resistant IA portends a dismal outcome on continuing azole treatment,[16,17] a change of therapy is required immediately

on identifying an azole-resistant isolate, irrespective of the duration and dose of the primary azole therapy.

It can take at least 5 days to confirm resistance with conventional susceptibility testing of *A. fumigatus* as this requires culture of the fungus, thereby limiting its usefulness for real-time clinical decision making. Nonetheless, the turnaround time can be shortened using the VIPcheck™ (EWC Diagnostics, Steenwijk, the Netherlands), which is a readily available, simple screening test that can detect azole resistance in an isolate. This device is an agar-based assay that can be run in the local laboratory with results available in 48 h and has been validated to EUCAST standards.[36,37]

Another major limitation is that cultures remain negative in up to 75% of patients diagnosed with IA.[38] However, other, faster methods are available. The AsperGenius® (Pathonostics, Maastricht, the Netherlands) PCR assay has proven useful in identifying the two most common genetic markers of triazole resistance (TR34/L98H; TR46/Y121F/T289A) and can be used directly on bronchoalveolar lavage (BAL) fluid, with results available on the day of testing.[17,39] However, this test is not universally available, is relatively expensive and lacks sensitivity, particularly in less invasive samples such as blood.

The experts agreed that in the case of proven triazole resistance, a change to liposomal amphotericin B should be made immediately. In rare cases, such as severe renal impairment (glomerular filtration rate 15–29 mL/min/1.73 m^2), the experts would consider adding an echinocandin to triazole therapy as an alternative approach.[40,41]

IA in patients receiving immunomodulating and molecular targeted anti-cancer drugs

There was less consensus on managing these patients as the body of evidence is limited, although growing. Whilst all the experts were aware of the data showing the risks of IFDs in patients receiving BTKIs such as ibrutinib, or mTOR inhibitors,[42–45] not everyone had treated such cases or been consulted about them. The experts felt that the risk of IA was variable, with most experts considering the risk as being intermediate, one expert considering the risk to be very high and about one-third considering the risk as very low. It should be noted that the risks may include fungal infections other than IA,[42,44,45] which can occur alone or concomitantly with IA.[44] In the available publications, IA infections among patients on ibrutinib or mTOR inhibitors generally occur in those with other risk factors for fungal infections, such as prior or concomitant use of potent cancer therapies.[43]

A small number of the experts felt the risk in their institutions was sufficiently high to justify antifungal prophylaxis. Administering many of these agents together with a CYP3A4 inhibitor such as an azole increases exposure to the immune modulating agent, potentially leading to toxicity. More than half of the experts would treat a patient diagnosed with IA who is receiving ibrutinib or another agent that potentially interacts with triazoles (e.g. venetoclax[46]) with liposomal amphotericin B until the infection was under control or until dose-limiting toxicity required a change of therapy, subsequently switching to the most appropriate available mould-active azole. This would minimize the need for changes in doses of BTKI or mTOR inhibitors, with the aim being to optimize therapy for the underlying haematological

malignancy. Others would treat with an echinocandin initially or would opt to give isavuconazole and monitor for adverse events, potentially reducing the dose of the immunotherapeutic agent, although therapeutic drug monitoring for the immunomodulatory drugs is not routinely available. The risk of toxicity associated with concomitant use of ibrutinib and triazoles may vary depending on which azole is used.[45] More research on co-administration of antifungals with these agents is required to better understand the risks. The experts advised following the dose guidance in the label for each individual immune modulating drug for managing co-administration with CYP3A4 inhibitors.

Stable disease

Stable disease was not considered treatment failure in the real-world setting. If patients were consistently neutropenic or otherwise severely immunocompromised, then stable disease, particularly if there had been previous rapid progression, would be deemed a success whilst awaiting immune recovery. If patients are able to tolerate further chemotherapy for their underlying disease whilst on treatment for IA, then the experts would generally continue the primary antifungal agent. Patients with stable disease should have their IA actively managed until either a response occurs or until it is deemed futile to continue.

Discussion

Few data are available to guide clinical decision making when a change in first-line treatment for IA is required. Guidance on diagnosis and management of patients with IA and other invasive mould diseases has previously been focused on what to do in the clinical trial setting, or is based on data derived from clinical trials.[1,11,19,21] Management decisions need to be made on a daily basis and the range of patients at risk for IA extends beyond the neutropenic patient; published clinical trials predominantly refer to patients with acute myeloid leukaemia and allogeneic haematopoietic stem cell transplantation.

This viewpoint has drawn on the clinical experience of a group of international experts in the diagnosis and management of IFDs and provides criteria for the management of IA in certain real-world clinical settings, regardless of the nature of risk, when the primary antifungal therapy is not effective or not appropriate in the context of a resistant organism.

An approach that integrates clinical signs and symptoms, radiological imaging and mycological tests including microscopy and culture, PCR, and serological biomarkers such as GM and β-d-glucan, is required. Studies have shown that rising GM correlates with a poor clinical outcome,[47] whereas PCR may allow for more rapid identification of a resistant pathogen, enabling a change of antifungal therapy. While PCR on blood generally becomes negative very soon after commencing therapy, this is indicative of the low burdens in the circulation and should not be used for determining a positive response to therapy. Conversely, persisting PCR positivity when on treatment is a poor prognostic sign.[48] Relying on radiological imaging alone may lead to disease progression being incorrectly diagnosed as immune reconstitution.

An understanding of the local epidemiology is needed so that, if switching to a second-line agent in the absence of an identified pathogen, an appropriate therapeutic choice can be made, based

on local knowledge of the most likely alternative infecting organism other than triazole-susceptible *Aspergillus*.

When deciding if a patient is refractory to primary antifungal therapy, considerations include performing serial CT using the same methodology, such as high-resolution CT. Switching from one modality to another will not allow for an accurate comparison of the lesion. Ideally, the same scanner should be used for serial scans. For diagnostic purposes, GM may be detected in several different body fluids, including serum, BAL fluid and CSF. For the purposes of monitoring response to treatment, subsequent serum GM levels are useful.[47]

If the immune status of a patient remains unchanged in the context of worsening diagnostic test results (Table 2), then the patient should be considered refractory. If the neutrophil count is recovering or immunosuppression is being reduced, and a lesion is increasing in size on radiology, with GM unchanged or declining, then this is less likely to be refractory IA and could be a manifestation of immune reconstitution.

Treatment of IA is challenging, and we have provided pragmatic criteria to assist the clinician in deciding when to change therapy for breakthrough or progressive infection.

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Patricia Ingram was responsible for editing the manuscript, maintaining referencing and journal styling.

Author contributions

E.H. was responsible for devising the project and collated the outputs from all four workshop groups into an initial manuscript. E.H., R.H. and J.P.D. devised the workshop structure, the questions for the workshops and the questions for the pre-meeting questionnaire. M.A.S., J.A.M., J.P.D., E.H. and J.H.R. reviewed the initial draft, refined the definitions and timelines for refractory disease, and drafted the questions for the post-meeting on-line questionnaire addressing the fourth clinical scenario. All authors participated in discussions during the advisory board meeting, contributed to subsequent drafts of the manuscript, including literature searches, provided epidemiology data and approved the final manuscript. M.A.S. was fully involved in all discussions during and after the advisory board meeting, in the writing of the manuscript and had final responsibility for the decision to submit for publication.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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