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Treatment and timing in invasive mould disease

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Invasive mould disease is a growing threat for immunocompromised patients. The optimum time to use mould-active antifungal agents is much debated. Current approaches to antifungal prophylaxis, early treatment (empirical and pre-emptive therapy) and treatment of documented mould infections in onco-haematology patients are discussed.

Keywords: invasive fungal disease, aspergillosis, empirical therapy, pre-emptive therapy

Introduction

Invasive mould disease (IMD) accounts for one-half to two-thirds of all systemic mycoses in immunocompromised patients and is most frequently caused by *Aspergillus* species. Over the past two decades, the number of patients at risk has expanded due to the wider use of intensive myelosuppressive and/or immunosuppressive agents in the treatment of haematological cancers, in particular in those with acute myeloid leukaemia and myelodysplastic syndromes, the growing number of patients undergoing allogeneic haematopoietic stem cell transplantation (HSCT) and the increasing aged population.[1] While the spectrum of patients at risk is also expanding,[2,3] two-thirds of these patients still receive medical care in haematology and HSCT units. In this setting, prolonged neutropenia and graft-versus-host disease are the major risk factors for early and delayed occurrence of IMD, respectively.

Epidemiological studies suggest wide variations in fungal disease attack rates, reflecting marked differences in patient characteristics and prevention and treatment protocols, as well as environmental characteristics. Hence, national and even local surveillance programmes need to be established if one wants to tailor diagnosis and treatment more effectively to the most prevalent fungal species. Indeed, some centres have recently reported unusually high incidences of non-*Aspergillus* mould diseases, including *Fusarium* spp., *Scedosporium* spp. and Zygomycetes. These diseases are associated with very high attributable mortality rates.[4]

Current data for invasive aspergillosis[5,6] and mucormycosis[7] suggest that early initiation of antifungal therapy may improve outcome. In addition, treatment should be started with an adequate drug (an adequate dose of a drug that is active against the fungus causing the infection). The latter requires diagnostic procedures, fungal culture, species identification and possibly *in vitro* susceptibility testing. Hence, to date, fungal culture, species identification and *in vitro* susceptibility testing remain the cornerstone of fungal diagnosis.

As few data are available from paediatric patients, the present review focuses on the evidence based on studies conducted in adult patients.

Prophylaxis

Considering the high mortality rate of IMD and the difficulties in its diagnosis, mould-active primary prophylaxis has been advocated, at least in high-risk haematology patients.[8] This strategy implies the administration of broad-spectrum antifungal agents—usually at therapeutic doses—to all (or a significant subgroup of) patients who are at increased risk of mould infection, but who lack any indication of active infection at the time of administration. Recently, new data from five large prospective randomized clinical trials in onco-haematology patients have been presented: (i) in an open-label multicentre study in patients receiving chemotherapy for acute leukaemia or myelodysplastic syndrome inducing a prolonged neutropenia, posaconazole reduced the incidence of invasive aspergillosis to 1%, as opposed to 7% in those receiving standard azole prophylaxis;[9] (ii) in a double-blind multicentre study, posaconazole proved to be non-inferior to fluconazole in reducing the number of invasive

fungal infections (IFIs) in allogeneic HSCT recipients with acute or severe chronic graft-versus-host disease, and it significantly reduced the incidence of invasive aspergillosis while on treatment;[10] (iii) in a single-centre, placebo-controlled study, aerosolized liposomal amphotericin B was effective in reducing the incidence of invasive aspergillosis from 14% to 4%, and was well tolerated;[11] (iv) in a double-blind randomized study, voriconazole, when compared with fluconazole in myeloablative standard-risk allogeneic stem cell transplant recipients, tended to reduce the incidence of invasive aspergillosis ($P=0.09$), but did not significantly improve fungus-free survival;[12] (v) finally, in a randomized open study in allogeneic HSCT recipients, voriconazole proved to be superior to itraconazole, albeit due to improved tolerability only.[13]

However, primary antifungal prophylaxis has important limitations, including the potential for selection or induction of resistant fungi, drug toxicity and drug–drug interactions, therapeutic drug monitoring issues and cost. Also, mould-active prophylaxis may interfere with the accuracy of fungal-specific diagnostic assays (e.g. the galactomannan assay), thereby inducing false-negative results.[14] Finally, prophylaxis is no substitute for any of the other antifungal strategies, since breakthrough infections do occur, including infections with azole-resistant *Candida* species, *Aspergillus* species with inherited or acquired resistance, and sometimes with species for which we have limited (e.g. Zygomycetes) or no (e.g. *Scedosporium prolificans*) remaining treatment options.[15] But most importantly, identification of the appropriate patient for whom prophylaxis might be beneficial and delineation of the at-risk period are not always easy, certainly not in the absence of reliable centre-specific epidemiological data. Indeed, the perceived benefit resulting from all these prophylactic studies is largely influenced by the baseline prevalence of mould infections in the target population.[16] Lastly, while studies on antifungal prophylaxis have focused almost exclusively on patients with acute myeloid leukaemia, patients with myelodysplastic syndromes requiring intensive chemotherapy and recipients of allogeneic HSCT, sufficient supportive data are lacking for patients with acute lymphocytic leukaemia and other haematological malignancies.

Thus, the current evidence supporting mould-active prophylaxis should be weighed against gaps in knowledge as well as recent improvements in the diagnosis and outcome of fungal infections and in identifying patients who are likely to be the best candidates for prophylaxis. In the future, antifungal prophylaxis may target patients more selectively on the basis of a specific genetic profile (see the related articles in this Supplement on risk assessment and prognostic factors for mould-related diseases in immunocompromised patients[17] and the detection and investigation of IMD[18]).

Secondary prophylaxis or maintenance treatment, aiming at preventing relapse of a previous IFI during a new at-risk period, is generally recommended. The drug of choice in this particular setting should be based on the causative fungal pathogen and the prior response to antifungal therapy. Recently, an open-label prospective cohort study demonstrated the benefit of voriconazole secondary prophylaxis in allogeneic HSCT recipients with a prior history of documented fungal infection.[19]

Empirical antifungal therapy: the fever-driven approach

This approach represents another risk-based intervention and implies the commencement of antifungal therapy at the first suspicion of IFI. By definition, empirical antifungal therapy targets haematology patients that have prolonged neutropenia (e.g. acute leukaemia/myelodysplastic syndrome or myeloablative allogeneic transplantation) with persistent or relapsing fever despite the receipt of 4–7 days of adequate broad-spectrum antibiotics and in the absence of other clinical symptoms/signs, of conventional radiological and laboratory findings and of specific investigations aimed at documenting invasive fungal disease (IFD) (e.g. thoracic and abdominal CT scan, detection of circulating fungal markers). This strategy was developed in the 1980s in response to the emergence of IFIs; at that time, the tools for diagnosing mould infections were lacking and management was based on clinical features, blood cultures and conventional radiology, all of which lack sensitivity.

Empirical antifungal therapy mirrors the successful empirical antibacterial approach that was developed in the 1970s. However, the antifungal approach has been based on moderate evidence from clinical trials with small sample size and debatable methodology/design. Empirical antifungal therapy considers fungal pathogens that are not covered by the drug(s) previously used in prophylaxis. However, the low positive predictive value of persistent or relapsing fever for diagnosing fungal infection also results in significant overtreatment, toxicity and expenditure.[20] On the other hand, this fever-driven strategy may miss invasive mycoses that develop in the absence of fever, especially in patients receiving high-dose corticosteroids or other immunosuppressive drugs. Similar to primary prophylaxis, empirical therapy does not completely abolish breakthrough fungal infections, which occur in 2%–10% of cases. In addition, given the current diagnostic possibilities, one should acknowledge that all the empirical therapy studies have used a very limited diagnostic work-up (i.e. often not including CT scan and new fungal blood markers) at baseline (or later on) to confirm the presence or absence of IMD.

Pre-emptive antifungal therapy: the diagnostics-driven approach

Recent improvements in early diagnosis (for *Aspergillus* spp. in particular) have prompted a reappraisal of the diagnostics-driven use of antifungal agents.[20–23] The time period between fungal replication, invasion and appearance of signs and symptoms represents a window of opportunity for earlier treatment. However, the documentation of possible or probable fungal infection should avoid significant and deleterious delay in the initiation of antifungal therapy. In a way, such a diagnostics-driven approach resembles the pre-emptive approach developed for addressing viral infections in transplant recipients.

However, there is as yet no consensus definition of pre-emptive antifungal therapy.[23] Such therapy should not be triggered by fever as a sole criterion, but should rest on: (i) a clear identification of those patients who are at risk of fungal disease (see the article in this Supplement on risk assessment

and prognostic factors for mould-related diseases in immunocompromised patients[17]); and (ii) utilization of sensitive techniques that facilitate rapid and early diagnosis of invasive mould infections, e.g. galactomannan, β-D-glucan or PCR testing as well as computerized radiological imaging techniques (see the article on the detection and investigation of IMD in this Supplement[18]). Importantly, in line with the revised European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) IFD definitions, positive mycological test results do not define disease when considered alone—although the predictive value may be high—but only in the presence of attributable clinical/radiological signs and symptoms, which make diagnosis of IMD more probable.[24] Therefore, these mycological tools should be used in conjunction with modern imaging techniques, especially since the systematic use of pulmonary CT scan has been shown to improve earlier diagnosis and survival in haematology patients.[6]

Empirical versus diagnostics-driven approaches: the jury is still out

The excellent negative predictive value of the antigen-based assays when used as screening tools on blood samples may persuade clinicians to withhold or modify antifungal therapy in neutropenic patients with persistent or recurring fever without other clinical, radiological and/or microbiological evidence of fungal infection. Of note, in patients receiving mould-active prophylaxis, a reduced positive predictive value of the galactomannan assay has been reported, likely due to a lower pre-test probability as a result of effective prophylaxis, reducing the incidence of IFD. Also, mould-active prophylaxis may reduce the amount of circulating galactomannan, resulting in a lower sensitivity of the test. Conversely, in a patient population in which a positive assay correlates with a high positive predictive value (i.e. in patients with a high prevalence), a positive assay should trigger a diagnostic work-up, potentially leading to early therapy.[25]

In haematology patients presenting with accessible pulmonary nodules or lesions, we strongly recommend further investigation via bronchoscopy, with lavage of the affected segment for fungal culture, microscopy and galactomannan detection. Indeed, positive galactomannan detection on bronchoalveolar lavage fluid samples taken from the radiologically affected pulmonary area supports the diagnosis of invasive pulmonary aspergillosis. A negative test result makes the diagnosis unlikely, although one should remember that the technique of sampling of bronchoalveolar lavage fluid lacks standardization and that the optimal cut-off for positivity on these samples is still a subject of ongoing clinical research.[26,27] However, a highly suggestive CT scan (e.g. nodules during neutropenia) and negative antigen-based tests should trigger a diagnostic exploration for infection caused by one of the Zygomycetes, other rare moulds or non-fungal pathogens.[28,29]

In a non-comparative pilot study, Maertens *et al*. prospectively evaluated a therapeutic algorithm incorporating CT scan and galactomannan detection (with an optical density cut-off ≥0.5) in 136 neutropenic episodes in adult haematology patients.[30] The study was designed to explore the feasibility of starting antifungal therapy based on diagnostic information, in an attempt to reduce the exposure to antifungal agents. While a purely fever-driven approach would have resulted in antifungal treatment in at least 41 of 136 episodes, a pre-emptive algorithm led to the initiation of antifungal therapy in less than one-quarter of these episodes, but identified 10 episodes of fungal infection without fever or with the presence of confounding febrile conditions. No undetected cases of invasive aspergillosis were identified. However, one case of disseminated mucormycosis was missed. The frequencies of IFI per episode and per patient were high: 15% and 24%, respectively. The overall mortality rate of 18% was acceptable for a neutropenic population with probable IFI. Importantly, all patients received fluconazole prophylaxis to prevent *Candida* infections. No patient received mould-active prophylaxis, perhaps improving the sensitivity of the assay and favouring the effectiveness of the pre-emptive approach.

Recently, a non-randomized Italian study assessed the feasibility of an intensive diagnostic work-up (three consecutive galactomannan assays and chest CT scan), as opposed to routine screening, in neutropenic patients meeting standard criteria for starting empirical antifungal therapy (persistent or recurrent fever).[31] This prospective, single-centre study confirmed earlier findings in patients undergoing chemotherapy for acute leukaemia and not receiving mould-active antifungal prophylaxis: a diagnostics-based strategy appears to be feasible and safe (no undiagnosed cases of fungal infection and no excess mortality) and reduces the cost of antifungals. However, notwithstanding these results, the authors have started to administer mould-active prophylaxis to their acute myeloid leukaemia population, especially given the high incidence of invasive aspergillosis during the study period at their centre. In addition, long-term effects of delaying antifungal therapy (e.g. impact on subsequent anti-neoplastic therapy, including transplantation) were not assessed.

In 2009, Cordonnier *et al*.[32] presented a non-inferiority multicentre study comparing classical empirical and pre-emptive treatment in 293 patients with haematological malignancies with an expected duration of neutropenia of at least 10 days. The primary endpoint was overall survival. Seventeen patients developed an IFI: 4 (2.7%) in the empirical group and 13 (9%) in the pre-emptive group ($P<0.02$). However, the overall survival rates measured 2 weeks after neutrophil recovery were comparable (95% and 97%, respectively; $P=0.12$). Subanalysis revealed no difference between the two treatment approaches when the duration of neutropenia was short (<15 days); however, the longer the period of neutropenia, the greater was the risk of fungal infection within the pre-emptive therapy arm. Also, the non-inferiority of survival during remission–induction chemotherapy—the subgroup with the longest duration of neutropenia—could not be demonstrated with certainty for the pre-emptive group. Overall, the pre-emptive approach significantly reduced the use of antifungal agents (39.2% versus 61.3%, $P<0.001$) while significantly prolonging the delay between fever onset and initiation of antifungal therapy (median 13 versus 7 days, $P=0.01$). Again, long-term effects of delaying antifungal therapy, other than survival, were not assessed.

In future, prospective multicentre studies comparing pre-emptive or empirical therapy in the era of mould-active prophylaxis should further determine the impact of these more targeted approaches in terms of outcome and cost effectiveness.

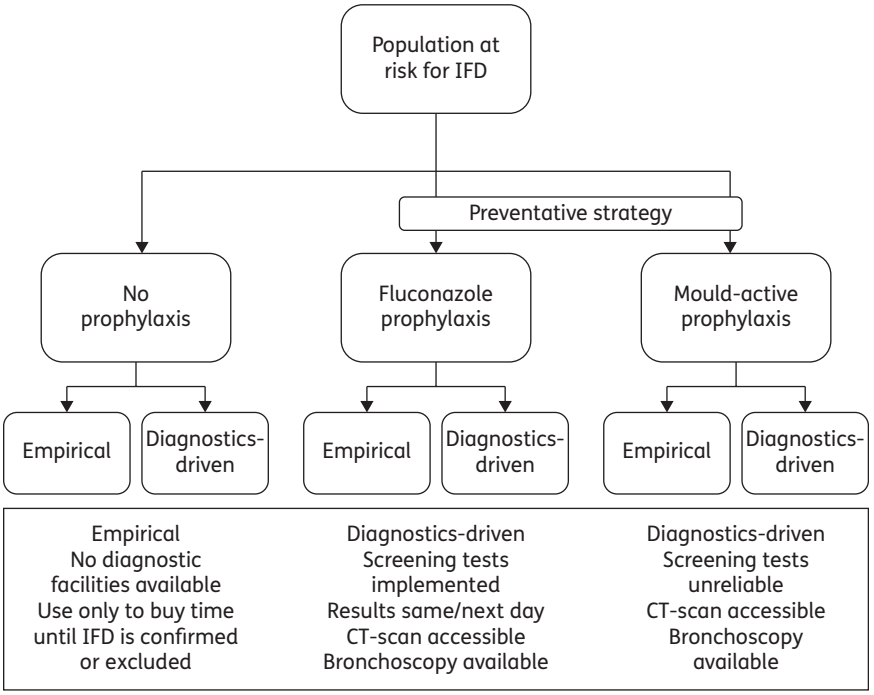

Figure 1. Antifungal strategies for patients at risk of invasive fungal disease (IFD).

As recently evidenced in a clinical study evaluating PCR screening, these studies are difficult to undertake because they require the full cooperation and compliance of all parties (clinicians, microbiologists, radiologists, chest physicians, nursing team) and the strict adherence to a protocol of minimum standards of diagnosis.[33] Adequate logistic support, sound communication between all parties involved, regular monitoring and auditing of the approach, and acceptable feasibility of all related conditions (e.g. performance of diagnostic procedures over the weekend) are all prerequisites for a successful diagnostics-driven approach. Such endeavours will only be possible if healthcare providers combine their efforts and establish a consortium to support such a study using a standardized diagnostic approach that has gained wide acceptance. Thus far, although many haematology centres are using a diversity of preemptive approaches, no firm recommendation can be given; the decision must be based on the local prevalence and epidemiology of pulmonary mould infections, the availability of these mycological tests and CT scan procedures and the routine use of mould-active prophylaxis (Figure 1).

Targeted therapy of mould infections

Targeted antifungal therapy refers to the treatment of proven and probable fungal infections. However, in some prospective clinical trials, possible cases have also been included.

At present, voriconazole is recommended worldwide as the drug of choice for the first-line therapy of invasive aspergillosis based on the results of a prospective, randomized clinical trial with amphotericin B deoxycholate as comparative initial therapy in possible, probable or proven disease.[34,35] This study reported significantly better response rates and survival rates in the group of patients that started with voriconazole (53% and 71%, respectively) than in the control group, which started with conventional amphotericin B (32% and 58%, respectively).[36] Voriconazole is also considered the drug of choice for the treatment of cerebral aspergillosis.[37] However, particular clinical conditions favour the use of a non-azole-based primary treatment; these conditions include prior exposure to mould-active azoles, the concomitant use of contraindicated medication (e.g. sirolimus), the risk of severe drug interactions, moderate to severe hepatic or renal impairment (the intravenous formulation of voriconazole is discouraged in patients with a creatinine clearance of $<$50 mL/min) and the presence of mixed fungal infections (e.g. including Zygomycetes). In addition, multiazole-resistant species of *Aspergillus fumigatus* have been described and appear to be emerging.[38]

More recently, the AmBiLoad trial compared two doses of liposomal amphotericin B: a standard dose of 3 mg/kg/day versus a loading dose (for the first 14 days only) of 10 mg/kg/day. The study failed to show any advantage for the higher dose, which also proved to be more toxic and was associated with a trend towards a higher mortality rate.[39] Similar to the pivotal trial of voriconazole, the study was debated because almost 60% of the patients in the study population were enrolled based on the presence of suggestive radiological features only, without any microbiological confirmation, i.e. possible disease.[40] Thus, although the 3 mg/kg study arm reported efficacy results and survival data similar to the aforementioned voriconazole study, because of the lack of a direct comparison, liposomal amphotericin B is considered the *alternative* drug of choice for the first-line therapy of invasive aspergillosis. Of note, *Aspergillus terreus* infections display evidence of decreased activity of amphotericin B *in vitro* and *in vivo* and should be treated with a mould-active azole.[41]

Data regarding the use of echinocandins in the primary therapy of invasive aspergillosis are scarce. The activity of caspofungin has recently been assessed in a non-comparative Phase II study in two different cohorts: patients with haematological disorders ($n=61$)[42] and patients undergoing allogeneic HSCT ($n=24$).[43] Overall, the rates of favourable responses were 33% and 42%, respectively. However, a fair comparison with previously published data is not possible since all patients had mycologically documented disease and the majority had poor baseline characteristics, such as uncontrolled underlying disease, older age and low performance score. The enormous impact of baseline characteristics on outcome has also been observed by other investigators; recovery from neutropenia in particular was a major determinant of response. Nevertheless, given these results, caspofungin cannot be recommended as first-line therapy for invasive aspergillosis, but it provides an option for patients not able to receive or tolerate voriconazole or liposomal amphotericin B.

It remains difficult to estimate the number of patients who are refractory to first-line therapy since an accurate assessment of the response to primary therapy is particularly difficult. The assessment uses conventionally defined global response composite endpoints that rely on non-specific signs and symptoms of infection, subjective interpretation of attributable radiological findings, and repeated culture and histopathological testing, both of which are rarely available.[44,45] In addition, often the cause of death cannot be unequivocally documented. Moreover, transient deterioration due to immune reconstitution inflammatory syndrome as a result of neutrophil recovery or tapering of immunosuppression may further obscure outcome assessment.[46] Thus, there is a pressing need for a specific and quantifiable surrogate marker for outcome evaluation. Miceli *et al.*[47] postulated that the serum galactomannan test fulfils the requirements of surrogacy for outcome evaluation in aspergillosis, and they summarized the evidence favouring serum galactomannan over conventional outcome markers. They demonstrated a strong correlation between unambiguous clinical outcome endpoints (such as survival) and the evolution of serum galactomannan. Whether serum galactomannan, or perhaps β-D-glucan, is a reliable surrogate marker of response remains to be further investigated.

As mentioned, treatment should be started with an adequate dose of the drug of choice. However, recent observations have ascribed severe drug-related toxicities, as well as therapeutic failure of mould-active azoles, to unexpectedly high or low serum concentrations due to interpatient and intrapatient variability in exposure, underscoring the potential need for therapeutic drug monitoring (see the article in this Supplement on the detection and investigation of invasive mould disease).[18] Overall, up to 50% of the patients who fail first-line therapy (as per conventional criteria) can be salvaged with second-line use of caspofungin, posaconazole or a lipid formulation of amphotericin B. Whereas the availability of new agents with different modes of action and promising preclinical studies has fuelled interest in the clinical use of combination antifungal therapy (in particular the combination of an echinocandin with a mould-active azole or a formulation of amphotericin B), this approach has only been evaluated in studies with insufficient statistical power. A large, randomized clinical trial in haematology patients is currently ongoing and will provide more insights on the general usefulness of drug combinations in first line therapy.

Recent guidelines recommend the combined use of antifungal therapy with surgical debridement, particularly in the CNS and sinus invasive mould infections, and for the prevention of severe haemorrhage when pulmonary lesions are adjacent to a large vessel.[34,35] In addition, several *in vitro* and *in vivo* data underscore the importance of restoration of the host immune defence (i.e. granulocyte transfusions, as well as the use of haematopoietic growth factors or cytokines and the preservation of organ function) for the outcome of fungal disease.

Voriconazole and lipid formulations of amphotericin B are the drugs of choice for the treatment of invasive infection caused by *Fusarium*[48] and *Scedosporium* spp.[49] Systemic antifungal treatment should, whenever possible, be combined with surgical debridement of necrotic tissue. Posaconazole can be used as salvage therapy for these infections.

Finally, a lipid-based formulation of amphotericin B is the drug of choice for the first-line therapy of invasive mucormycosis;[50] some authors have recommended higher than usual doses: 5–10 mg/kg/day for liposomal amphotericin B and 5–7.5 mg/kg/day for amphotericin B lipid complex. Posaconazole may be used for salvage or maintenance treatment. Antifungal therapy should be complemented by surgical debridement and reversal of underlying predisposing factors (such as diabetic ketoacidosis, iron overload, steroid treatment or neutropenia).

There is no firm recommendation about the duration of antifungal therapy in the treatment of invasive mould infections. Although a minimum duration of 6–12 weeks has been suggested for invasive aspergillosis in particular, we feel that the duration should be dictated by the severity and duration of the underlying immune deficits and the complete reversal of all relevant signs and symptoms of the infection.

Conclusions

IMD is mainly caused by *Aspergillus* spp. The clinical management is hampered by the difficulty of diagnosing these infections since definite diagnosis centres on histological identification of hyphae in tissue or on culture from a sterile body site. In high-risk populations, most practitioners therefore rely on antifungal prophylaxis. Recently, there has been a shift in emphasis from routine prophylaxis to screening high-risk patients so that appropriate antifungal therapy can be administered early, when it can potentially improve patient outcome. Although they do not provide fungal species identification and antifungal susceptibility results, non-culture based mycological tools are one of the key elements of this change in practice. Together with assessment of clinical signs, cultures and CT scanning, they may prove useful for starting antifungal therapy pre-emptively. In the light of changing fungal epidemiology in some (but not all) centres, future studies should focus on the combined use of 'panfungal' (PCR and β-D-glucan) and 'species-specific' (e.g. galactomannan) assays, in conjunction with sensitive imaging studies.

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