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Future challenges and chances in the diagnosis and management of invasive mould infections in cancer patients

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Abstract

Diagnosis, treatment, and management of invasive mould infections (IMI) are challenged by several risk factors, including local epidemiological characteristics, the emergence of fungal resistance and the innate resistance of emerging pathogens, the use of new immunosuppressants, as well as off-target effects of new oncological drugs. The presence of specific host genetic variants and the patient's immune system status may also influence the establishment of an IMI and the outcome of its therapy. Immunological components can thus be expected to play a pivotal role not only in the risk assessment and diagnosis, but also in the treatment of IMI. Cytokines could improve the reliability of an invasive aspergillosis diagnosis by serving as biomarkers as do serological and molecular assays, since they can be easily measured, and the turnaround time is short. The use of immunological markers in the assessment of treatment response could be helpful to reduce overtreatment in high risk patients and allow prompt escalation of antifungal treatment. Mould-active prophylaxis could be better targeted to individual host needs, leading to a targeted prophylaxis in patients with known immunological profiles associated with high susceptibility for IMI, in particular invasive aspergillosis. The alteration of cellular antifungal immune response through oncological drugs and immunosuppressants heavily influences the outcome and may be even more important than the choice of the antifungal treatment. There is a need for the development of new antifungal strategies, including individualized approaches for prevention and treatment of IMI that consider genetic traits of the patients.

Lay Abstract

Anticancer and immunosuppressive drugs may alter the ability of the immune system to fight invasive mould infections and may be more important than the choice of the antifungal treatment. Individualized approaches for prevention and treatment of invasive mold infections are needed.

Key words: invasive pulmonary aspergillosis, immunological status, hematology, hemato-oncological malignancies, mucormycosis.

Introduction

Managing invasive mould infections (IMI) has proven to be a daunting task: diagnosis and treatment are, at times, difficult, and their management also often interferes with the therapy of the underlying disease. For instance, the often severe and long-lasting neutropenia as well as genetic host factors, comorbidities, and exposure to an elevated fungal spore burden are known risk factors for IMI acquisition in hemato-oncological patients.[1] In addition, immunological factors,[2] the emergence of resistant fungal strains,[3–5] and the widespread use of novel therapeutic agents such as tyrosine kinase inhibitors,[6] have complicated matters further. In solid organ transplant (SOT) recipients, immunosuppression is often linked to the occurrence of IMI, and toxicity and interactions of antifungals may lead to graft loss, morbidity, and death.[7]

Several guidelines define the diagnostic workup and the treatment to be used when IMI are suspected.[8–11] Some authors have addressed more specifically diagnosis and treatment of mucormycoses,[12–15] for which a specific guideline has recently been published.[16] Recent work has also discussed the use of (pro)inflammatory parameters for the diagnosis and evaluation of treatment outcome in IMI,[15,17-19] underlining the need for a multifactorial approach that must include a set of diagnostically relevant markers[20] in addition to the patient's own clinical characteristics.[17]

Presently, IMI management is further challenged by new risk factors, the emergence of fungal resistance in *Aspergillus* and other moulds and yeasts, as well as the innate resistance of selected emerging pathogens.[21–23] Breakthrough mould infections after prophylaxis, new immunosuppressants, as well as potential off-target effects of new anti-cancer drugs that may increase the risk for IMI in patients previously not considered at risk are additional challenges. On the other hand, new immune-based diagnostic tools as well as the possibility of determining the host's genetic risk factors, potentially leading to personalized treatment approaches, are opportunities that will facilitate individual management of IMI.

Invasive aspergillosis (IA) is still the main cause of IMI and is associated with high mortality rates in hematological/oncological patients and SOT recipients alike.[11] This review addresses the challenges and chances in the diagnosis and management of IMI, mainly IA and to a lesser extent mucormycoses, in cancer patients.

Risk assessment

Risk factors for IMI in hemato-oncological patients and solid organ transplant recipients have been summarized,[24] but the list is continuously increasing. An emerging risk factor for IMI acquisition is the widespread use of new immunosuppressants, particularly in older and therefore more comorbid patients. There is also a lack of well performed epidemiological studies with sufficient sample size, high quality data, and state-of-the-art statistical analysis to allow weighting and balancing the various, often strongly interconnected risk factors such as age and comorbidities against each other. The changing epidemiology of IMI and the occurrence of resistance in opportunistic pathogens are factors that heavily influence the diagnostic and therapeutic workup in patients suspected of being infected by opportunistic fungal pathogens.

In addition, while the risk ranking so far proposed[24] considers implicitly the patient's immune status, the complex interactions between the host's immune system and the fungal pathogen should receive more attention. Cellular response, with the innate immune system being probably the most important structure involved,[25–27] is key in the host defense to fungal infections, but interactions between other components of the immune system and the fungal pathogens are also important and more complex than so far assumed.

Different receptors play a relevant role in the cellular antifungal immune response and their malfunction can lead to a higher susceptibility to IMI. For example, the C-type lectin receptor dectin-1 is present on myelomonocytic cells and mediates ß-glucan recognition and cytokine production, for example, interleukin (IL)-17 triggering Th-17 differentiation. Mutations in this receptor, for example, by Y238X early stop codon polymorphism, favor IA onset, as it has been shown for patients after allogeneic hematopoietic stem cell transplantation (HSCT).[28] The ß-glucan receptor CR3 (CD11b/CD18b) is known to contribute to the production of polymorphonuclear neutrophils (PMN) reactive oxygen species (ROS) and formation of neutrophil extracellular trap (NET).[29] It also plays a role in executing PMN

phagocytosis towards fungal pathogens[30] and could thus exert a negative impact on antifungal defense.

After receptor activation, different signaling pathways are involved in antifungal immune response. Innate immune cells such as the natural killer cells,[31] dendritic cells,[32] and innate lymphoid cells,[33] have been shown to influence host response to fungal infections as well. The adaptive immune system (mainly $CD4^+$ T cells subsets and B cells) contributes also substantially to antifungal defence.[34] In particular, type 2 (Th2) and type 17 (Th17) T-helper cells play a relevant role in coordinating and enhancing the cellular antifungal defence.[34]

The signaling pathways mentioned above may also be altered by immunomodulating drugs, for example, calcineurin/NFAT inhibitors[35] such as cyclosporine A and tacrolimus, new anticancer drugs,[36] or possibly the antifungals themselves,[37–40] leading to impaired effector functions. For example, calcineurin/nuclear factor of activated T cell (NFAT) signaling negatively regulates myeloid lineage development [41] and may influence macrophage effector functions through the TLR9-BTK signaling pathway as described in SOT-related IA.[42–44] Calcineurin has also been shown to influence pentraxin-3 (PTX3) expression, resulting in an impaired antifungal-defense of CD11-expressing PMN cells and increased susceptibility to *Aspergillus fumigatus* infections.[45] PTX3 acts as an opsonin against conidia, facilitating their phagocytosis and activating the complement system.[46] Mutations in PTX3 genes induce an increased susceptibility to IMI in knockout mice and in stem cell transplant recipients if these mutation are present in donor-derived immune cells.[47]

Small molecule kinase inhibitors (SMI) such as BTK, JAK, and PI3K inhibitors are increasingly used in hematological cancer therapy and have been shown to cause immunological off-target effects that can lead to IMI.[36] IMI have been described with a number of SMI,[6,48] in particular ibrutinib.[6,36,49,50] IMI during ibrutinib therapy are caused by several species, *Aspergillus* spp. being prominent (80%), and are frequently associated with dissemination, brain infections, and poor prognosis for the patients involved.[49,51] It is not clear whether second generation BTK-inhibitors currently under development (e.g., acalabrutinib)[52–54] will be more selective and associated with a lower IFI incidence.

Overall, the incidence of IMI is poorly investigated, and a comprehensive and effective prophylactic or therapeutic approach has not yet been defined. Selected patients at risk, however, might benefit from an antifungal prophylaxis, but the known interactions of SMI with some triazoles[55] in a population composed mainly of outpatients, sometimes only seen by general practitioners and only at longer intervals by the hematologist or oncologist, render it problematic. In addition, the long half-life of some SMIs and the consequent potentially permanent cell damage need to be taken also into consideration, because stopping the SMI treatment to fight the underlying IMI may not preclude the possibility of interactions. Finally, the risk of relapse of the underlying disease when the SMI treatment is interrupted implies the need for close monitoring. Reevaluation of existing phase III trials is thus essential to identify patients at special risk, to select patients who might profit from prophylaxis, and to define second-line risk factors.

Breakthrough infections during prophylaxis

Breakthrough fungal infections result from a failure of prophylaxis. They are relatively rare, but they may occur and are generally associated with a poor outcome.[56] In patients with hematological malignancies, breakthrough fungal infections under triazoles, in particular posaconazole, [11,57] have been reported to be less than 5%.[57,58] In most studies, mainly dealing with patients with hematological malignancies,[56,57,59–64] fungal infections were attributable to *Aspergillus* spp., but they are quite often also caused by *Mucorales*, sometimes as mixed infections with *Aspergillus*.[59,63]

Local epidemiology probably determines the spectrum of species involved in IMI,[56–60,62–65] while risk factors such as the host's immune status and environmental exposure to moulds may be the main factors determining their incidence and prevalence.[66] Clinical presentation of IMI is often non-specific and may reflect the involved fungal pathogens. Necrotic, disseminated and/or painful skin or nail lesions, fever, and myalgia should raise suspicion of disseminated fungal infection, especially fusariosis.[67] Fever, cough, hemoptysis, and sinusitis have often been observed in cases of mucormycoses, but they can be seen in other IMI as well.[56] *Mucorales* infections are increasingly frequent in clinical settings, and in one study their incidence reached 37% of all breakthrough infections observed in patients treated prophylactically with either posaconazole or voriconazole,[21] two drugs that have variable efficacy against Mucormycota.[16] Real-life data show variable rates of breakthrough infections,[56,59,60,62–64,68,69] with opportunistic, generally saprophytic fungi such as *Hormographiella aspergillata* (*Coprinus cinereus*) also being recorded.[70]

Some moulds, for example, *A. terreus, A. ustus,* and other rare *Aspergillus* spp., are intrinsically resistant to selected antifungals,[71,72] as are some *Mucorales*, *Lomentospora prolificans* and *Fusarium* spp.[73] It cannot be excluded that intensive prophylaxis in patients at risk may cause a shift toward resistant species and strains. One hypothesis is that antifungal prophylaxis might create ecological niches for opportunistic fungi.[21,72,73] These organisms are difficult to distinguish in the microbiological routine laboratory, and clinical data are usually lacking. Based on current insight, however, the occurrence of breakthrough infections could be primarily driven by a change in the local spectrum of pathogenic opportunistic fungal species rather than the development of resistant strains in most countries; future study of the mycobiome present not only in the hospital but also at the patients' homes and surroundings may be key to understanding their insurgence.

Samples of culture-positive breakthrough infections should always be sent to reference centers for species identification and resistance testing. For many breakthrough infections with intrinsically resistant or azole-resistant moulds, polyenes are the first line of treatment, but echinocandins and combination therapy are important options for selected cases.[73] No high-level clinical evidence, however, is yet available to support the use of a combination therapy as primary treatment option as opposed to monotherapy.[11]

Emerging and innate resistance in *Aspergillus* species

The last decade has seen an abrupt increase in the isolation of azole-resistant *Aspergilli*.[4,74,75] In one study in The Netherlands, 19% of all isolated strains were azole resistant, with an excess overall mortality of 21% at day 42 and 25% at day 90 as compared to nonresistant strains.[76] The prevalence in other countries is much lower: in Germany, for instance, it reached 6.4% in acute myeloid leukemia and 3.8% in acute lymphocytic leukaemia.[77] Overall, cases have occurred in many countries with varying prevalence,[78-84] and infections are often observed in patients without prior azole exposure.[3] A low prevalence has been reported from the USA,[81] France,[85] and Germany,[77,79,86] but higher rates of resistant strains have been reported from countries (The Netherlands, Denmark, Colombia) with extensive flower cultivation.[87–89] Occurrence of resistant strains seem also to be tightly linked to the local epidemiology: in The Netherlands, a gradient has been observed that seems to be correlated with the extent of flower cultivation,[89] thus supporting the hypothesis that azole resistance in *Aspergillus* is correlated with fungicide use in agriculture.[5]

Azole resistance seems to be mainly determined by the TR_{34}/TR_{46} mutations in CYP51A,[75,90–92] but other mutations in the same gene have also been reported.[74,81] Azole resistance in *A. fumigatus* develops mainly during exposure of the fungus to azoles in the natural environment and not in the patient,[5] but resistance is also apparently associated with the use of long-term azole therapy and switching between antifungal azoles in patients with chronic pulmonary aspergillosis.[93]

The impact of the occurrence of azole resistant *Aspergillus* isolates on the patient outcome is not yet entirely clear, but high mortality rates, up to 2.7 times higher than in nonresistant IA, have been reported.[94] Identification of azole resistant *Aspergillus* strains at the time of diagnosis helps predict azole treatment failure,[95] and should prompt an immediate switch to an appropriate therapy. No clinical data on the best therapeutic approach are available, and there may be a need to develop new treatment strategies, considering that echinocandins might not be sufficiently effective in patients with continued immunosuppression.[96–99] The use of upfront azoles in combination with liposomal AmB (L-AmB) or an echinocandin if local resistance rates exceed 10%[100] has been suggested, but no clinical evidence exists to support this recommendation. A guideline from The Netherlands[101] recommends the use of voriconazole combined with L-AmB or an echinocandin as first line therapy until resistance has been excluded (Recommendation 12), but clinical data on efficacy and safety of these combinations are limited. Until additional data are available, azole monotherapy remains the treatment of choice, and there is no agreed threshold for local resistance rates to define an alternative. In cases of reasonable doubt, such as an increase in the local epidemiology of resistance, real-time phenotypic and polymerase chain reaction (PCR)-based detection of the most frequent CYP51A resistance associated mutation patterns TR34/L98H and TR46/T289A/Y121F (the latter directly on bronchoalveolar lavage fluid) should be performed to rule out resistance as early as possible. In such cases, existing international guidelines list liposomal amphotericin B (L-AmB) as an alternative to isavuconazole and voriconazole for treatment of IA,[10,11] thus L-AmB monotherapy is also an accepted option when triazoles cannot be used.

Studies are currently underway to define a sensible threshold when primary monotherapy with an azole is no longer acceptable and to determine an appropriate diagnostic and therapeutic scheme in the presence of high azole resistance prevalence.[102] Additional, pragmatic trials using overall and attributable mortality as endpoints are needed to help shed light on this increasingly important issue, and algorithms must be developed and evaluated to handle complexity in the context of increasing azole resistance. New drugs currently under development[103–105] may also become an option but, so far, only limited data with regard to safety and efficacy of these new compounds in patients are available.

Diagnostics

IMI diagnosis relies on the use of imaging, biomarkers (e.g., galactomannan and PCR), and culture.[106–111] The methods used for IA, in particular culture, imaging, and PCR, are applicable also to suspected mucormycoses and rare mould infections.[10,11,14,112–114] The diagnosis of *Mucorales* and other rare IMI caused by moulds remains challenging because phenotypic identification is not always possible as cultures can remain negative and their evaluation is often possible only after a comparatively long time.

The GM test has been shown to be a reliable diagnostic tool in a number of clinical trials,[106,111,115–118] although a recent study has reported a high rate of false positives in BAL samples of hematological and SOT patients using the standard cut-off value of 0.5.[119] Another problem with the use of galactomannan testing on serum is its low sensitivity, in particular in non-neutropenic patients.[120,121] PCR has the advantage to provide a reliable species identification in a relatively short time, but its sensitivity is limited when used on serum or plasma and, even

on galactomannan positive BAL fluid, the sensitivity is not optimal. After its introduction as a diagnostic test, 1-3-ß-glucan (BDG) has received considerable attention, but based on disappointing sensitivity, high workload and costs, and many false positives, it has not become a generally recommended test for IMI detection.[116,117,122]

IMI patients have been shown to have increased levels of mould-reactive *Aspergillus*- or *Mucorales*-specific $CD4^+$ cells compared to healthy controls,[123] but scant data are available on *Mucorales*-reactive T cells, with only a small patients cohort studied so far.[124–126] *Mucorales*-reactive T cells producing IL-10 and IL-4 have been detected at high rates in patients with mucormycosis[124,125] and are currently evaluated as potential surrogate diagnostic markers in the diagnosis of mucormycoses.

Immune parameters for potentially more specific diagnoses have so far been given little consideration but they are likely to provide directions about diagnosis, when a decision needs to be made regarding the use of a mould-active prophylaxis, the start of empirical antifungal treatment, early escalation, or switch to a more appropriate antifungal agent. Several cytokines may allow improving IMI diagnosis. Serum C-reactive protein (CRP) and IL-6 levels are increased at the time of diagnosis and decline in case of response to antifungal treatment.[127] IL-1β, IL-6, IL-8, IL-17A, IL-23, and tumor necrosis factor (TNF)α were significantly increased among patients with IPA, confirming that the combination of specific cytokines with other biomarkers such as GM may not only facilitate diagnosis but also improve the ability to predict the disease outcome.[128]

The use of lateral-flow immunoassays has shown promising results in patients with a suspected IA,[129] and a similar immunoassay is currently under development also for *Mucorales*.[112] Compared to conventional GM testing on serum with the Platelia assay, these tests can be done on demand on patient samples and lead to results in 1–2 hours instead of the typical sampling to result time of several days for diagnostic tests that are typically pooled and performed only 2 or 3 times a week and in dedicated laboratories only. A combination of serum IL-8 levels with the BAL *Aspergillus* lateral-flow device test or BAL PCR may also allow differentiating specifically IA from non-IA pulmonary infections in hematological malignancy patients.[130,131]

The effects of genetic variants of risk-associated factors on the cytokine levels are still unknown and additional prospective studies are needed to understand the relationship between cytokine levels and the mechanisms underlying IA, including the role of immunomodulation in IA therapy.[132]

New immunological assays are under development to quickly and reliably diagnose IMI, and *Aspergillus* spp. and *Mucorales*-reactive T cells have also the potential to become interesting markers, but many confounders probably influence rare cell analysis. Published data are scant, and further work is needed to show whether these assays might be useful as alternative, noninvasive diagnostic markers, particularly for mucormycosis.

Assessment of treatment response

Predictors of treatment outcome for IA include imaging,[133], GM baseline levels and kinetics,[133–141] inflammatory parameters and pro-inflammatory cytokines.[18,127,142] PCR is apparently of limited utility as a predictor of outcome.[17]. A recent meta-analysis[12] has not provided additional information on treatment outcome. In this analysis, HSCT and *Rhizopus* infection were predictors of adverse outcome; surgery combined with antifungal therapy (mostly conventional or liposomal AmB) was associated with a reduction in overall mortality.[12]

On the other hand, changes in the levels of selected cytokines seem to provide useful information on IMI progression and resolution. High initial IL-8 and persistently high IL-6, IL-8, and CRP level have been described as predictors of adverse outcome in IA.[127] Haptoglobin, CRP, and annexin A1, three host proteins, have also been shown to have predictive values in an animal IMI model,[18] and this has been confirmed also in IA patients,[19] but the usefulness of these biomarkers in the clinical routine is not yet established.

Overall, the evaluation of response to antifungal treatment has to rely on the observation of a combination of parameters that include clinical course and the current immunological status of the patient, imaging and kinetics of biomarkers and possibly cytokines.[17]

Discussion

IMI onset is dependent on several factors, which include also local epidemiological characteristics and the increased use of new anticancer drugs targeting the immune system. The presence of specific genetic variants and the immune system status of a patient may also influence the establishment of an IMI and, together with the potential emergence of resistant strains among the pathogens, the outcome of the antifungal therapy.

Immunological components can thus be expected to play a pivotal role not only as biomarkers in the risk assessment and diagnosis but also in the treatment of IMI. Recent work, in fact, has suggested that fungus-specific T cells could be used for cellular therapeutic approaches to IMI.[143,144]

Immunological biomarkers may facilitate clinical decision making in different scenarios. They could improve the reliability of IA diagnosis by serving as biomarkers as do GM or PCR, because cytokines can be easily measured, and the turnaround time is quite short. Their use as immunological markers in the assessment of treatment response could be helpful to reduce overtreatment in high-risk patients and on the other hand allow prompt escalation of antifungal treatment, for example, in the case of persistently high IL-6 levels.[145] Mould-active prophylaxis could be better targeted to the individual host characteristics, leading to a targeted prophylaxis (as opposed to universal antifungal prophylaxis) in patients with known immunological profiles associated

with high susceptibility for IA (e.g., PTX3, TLR or dectin-1 deficiencies).

In cancer patients, the drugs used to treat the underlying and concomitant diseases may have considerable off-target effects on the immune system. In leukemia patients undergoing SMI treatment, no well-designed studies exist that investigate the complex interactions among SMIs and the immune system. Interactions of antifungals such as Amphotericin B with the immune system have also been reported[37,40] and need also to be studied in more detail. The alteration of the cellular antifungal immune response through drugs (anticancer drugs, immunosuppressants, or even antifungals) influences heavily the outcome and may be even more important than the choice of the antifungal treatment. With regard to these complex interactions, there is a need for the development of new antifungal strategies, including individualized approaches for prevention and treatment of IFI that consider also genetic traits of the patients. This means that the diagnostic and therapeutic workup must include expert consultation, in particular by infectious disease specialists.[146] Multidisciplinary teams with extensive knowledge of fungal epidemiology and antifungal treatment options will be instrumental to optimize care for patients and implement antifungal stewardship programmes.[147–149]

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