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Defining the diagnosis of invasive aspergillosis

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Invasive aspergillosis (IA) is a leading cause of death in severely immunocompromised patients. Delayed diagnosis remains a major impediment to successful treatment of IA. Conventional diagnostic methods based on use of histology and culture remain the cornerstone of diagnosis of IA. However, they have a low yield, and performance of them is frequently precluded in patients with cytopenia and/or underlying comorbidities. In recent years, efforts have been directed toward identifying non-culture-based markers for early, reliable diagnosis of IA by detection of *Aspergillus* components such as galactomannan (GM), 1,3-β-D-glucan, and DNA. Studies of profoundly neutropenic hematopoietic stem cell transplant recipients on fluconazole prophylaxis have shown high sensitivity (67–100%) and specificity (86–99%) rates for the GM assay. However, the performance of the GM assay in other settings, including pediatric patients, patients receiving mould-active antifungal prophylaxis, patients with GvHD and recipients of solid organ transplants, is suboptimal. Other factors, such as the pretest probability of infection, sequestration of *Aspergillus* lesions, the patient's immune status, the presence of anti-GM antibodies, antibacterial therapy, and diet, may also affect both the performance and interpretation of the GM assay. A colorimetric assay for the detection of 1,3-β-D-glucan, an integral cell-wall component of most pathogenic fungi, has shown promising sensitivity (55–100%) and specificity (52–100%) in limited studies. Polymerase chain reaction (PCR) analysis of *Aspergillus* DNA is also a promising method for early detection of IA and other opportunistic fungal infections. The sensitivity of PCR is excellent, but its low level of specificity for invasive infections can be problematic. Multiple unresolved issues accompany the use of PCR for diagnosis of IA, including the sample type, amplification strategy, protocol, and primer selection, and account for the lack of a standardized, commercially available assay. Comparative prospective evaluation of non-culture-based assays would facilitate their incorporation into pre-emptive strategies for the optimal management of IA. Combinations of non-culture-based assays could enhance their performance and broaden the spectrum of detectable fungi. Clearly, there is a need for innovation in this area. Preclinical studies suggest that detection of secondary metabolites for *Aspergillus* and use of novel immunolabeling approaches to positron emission tomography are potentially new avenues for the development of novel diagnostics. In view of the evolving

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epidemiology of opportunistic mould infections, developing diagnostic strategies to distinguish between pulmonary aspergillosis and other lung mycoses is another important future research direction.

Keywords *Aspergillus*, computed tomography, diagnosis, galactomannan, gliotoxin, 1,3-β-D-glucan, PCR

Introduction

Invasive aspergillosis (IA) is a leading cause of death in severely immunocompromised patients, with mortality rates ranging from 70–90% in patients with leukemia and recipients of hematopoietic stem cell transplants (HSCTs) [1]. The incidence of *Aspergillus* infections has significantly increased in this patient population over the past two decades, and the spectrum of patients at risk for IA is expanding [1–3].

A concerning trend in the epidemiology of IA is the increasing incidence of infections caused by non-*A. fumigatus* spp. that are resistant to antifungals [4–6]. In addition, several cancer centers have recently reported a substantial increase in the incidence of infections caused by uncommon, difficult-to-treat opportunistic moulds such as the *Zygomycetes*, *Fusarium* spp., and *Scedosporium* spp. [7,8]. These emerging mycoses clinically manifest similarly to IA and typically occur in patients receiving *Aspergillus*-active antifungal agents [8]. Hence, early differentiation of aspergillosis from other opportunistic moulds is important.

Substantial delay in establishing an early diagnosis remains a major impediment to the successful treatment of IA [1]. Although there might be signs and symptoms suggestive of invasive pulmonary aspergillosis (IPA) [9], timely diagnosis of IA is challenging because of the lack of specific clinical manifestations of the infection [1]. Furthermore, differentiating IPA from other pulmonary mycoses is difficult [10]. Despite significant advances associated with the recent introduction of standardized diagnostic criteria for IA, which combine clinical/radiological, microbiological, and serological parameters, over one third of *Aspergillus* infections still remain undiagnosed ante-mortem [12,13]. Novel approaches to early detection of subclinical infections and prompt initiation of pre-emptive antifungal-based treatment could improve outcomes in patients with IA.

Conventional methods of IA diagnosis

Although the use of clinical scorecards has been proposed in the past for diagnosis of IA [9], this approach seems outdated in view of the complex and evolving epidemiology of invasive mould infections (IMIs) in immunocompromised patients [7]. For example, over 40% of all IMIs in tertiary care oncology centers are now caused by non-*Aspergillus* moulds, such as *Zygomycetes* and *Fusarium* spp., which clinically manifest similarly to IA [7,8].

Conventional methods based on histology and cultures remain the cornerstone for establishing a definitive diagnosis of IA [1]. In addition, because cultures also allow for speciation and susceptibility testing of *Aspergillus* spp., they may provide useful information for the management of IA. Hence, accurate speciation of *Aspergillus* spp. is particularly important because of the emergence of non-*fumigatus Aspergillus* spp. that are less susceptible to antifungals when compared with *A. fumigatus* such as *A. terreus* [4–6]. Furthermore, *in vitro* susceptibility testing may provide clues for 'cryptic' *Aspergillus* spp. morphologically indistinguishable from *A. fumigatus* that exhibit unusual patterns of resistance to antifungals [5]. Respiratory cultures of *Aspergillus* spp. from expectorated sputum, bronchial washings, or bronchoalveolar lavage (BAL) specimens have a high positive predictive value (PPV; $>60\%$) for diagnosing IA in heavily immunocompromised patients. However, their sensitivity ($<30\%$) is unacceptably low, and they usually become positive for IA at a late stage of the infection [14]. The lack of a standardized protocol for bronchoscopic diagnosis of IA, including the method of BAL processing, lavage technique, timing, and volume of fluid obtained, may partially account for the suboptimal performance of this method. Blood cultures have little diagnostic utility for IA but may reflect true disseminated infection in cases of *Aspergillus terreus* fungemia [15].

The vast majority of current fungal culture protocols use incubation at room temperature (25–30°C) in ambient air. Whether these conditions are optimal for *Aspergillus* recovery from infected tissue is unclear. For example, Tarrand and colleagues hypothesized that growth of *Aspergillus* hyphae is adapted to the hypoxic *in vivo* environment of infected tissue and demonstrated that recovery of *Aspergillus in vitro* is

significantly enhanced at 37°C under hypoxia-modeling conditions [16].

Ultimately, invasive procedures such as biopsy may be required to definitively diagnose IA, but they may not be feasible until late in the course of the infection or recovery of pancytopenia [1]. In addition, even the use of histopathology does not provide a specific diagnosis of IA when culture of the tissue specimen remains negative. Hence, infections by other, more resistant hyalohyphomycetes such as *Fusarium*, *Scedosporium*, and *Acremonium* spp. have histopathological features indistinguishable from those of IA [17]. The ability to differentiate between these moulds before culture results are available using molecular identification methods could have significant implications on therapeutic decision-making. Hayden and colleagues reported that *in situ* hybridization directed against ribosomal 18S RNA sequences rapidly and accurately distinguished *Fusarium*, *Aspergillus*, and *Scedosporium* spp. from histopathological specimens [17]. However, further standardization of these promising molecular methods is needed.

The systemic use of high-resolution (HR) computed tomography (CT) has been a major advance in the early diagnosis and management of invasive pulmonary aspergillosis (IPA) [1]. Hence, in a recent study of febrile neutropenic patients, use of early and routine CT scanning coupled with early intensive antifungal-based treatment and surgery resulted in increased survival rates when compared with therapy initiated at the first sign of a pulmonary fungal infection [18]. Computer tomography also plays an important role in influencing various diagnostic approaches (BAL, percutaneous needle biopsy, and open lung biopsy) in patients with suspected IPA.

High-resolution CT scans of the chest may reveal small wedge-shaped subpleural lesions or nodules typically surrounded by intermediate attenuation (halo sign) in the early stages of IA [19–21]. Importantly, the halo sign has been associated with earlier diagnosis and improved outcome in patients with IA [20]. The halo sign is highly specific for acute IA in patients with severe neutropenia; however, its sensitivity is low (33–60%), and it is transient [19–21]. In fact, to be useful for diagnosis of IA, CT must be performed within 5 days of the onset of the infection, because more than 75% of initial halo signs disappear within a week [19]. With neutrophil recovery, these lesions coalesce and cavitate, forming the 'air crescent' sign, a classic sign of late filamentous invasive infection. However, the air crescent sign typically does not become evident until the third week of the infection, and appearance of it may be delayed too long to be helpful in the diagnosis of IA [19]. Furthermore, no one has systematically evaluated the role of HR CT in early diagnosis of IA in nonneutropenic patients. Importantly, CT imaging cannot discriminate reliably between IA and other opportunistic mould infections of the lung [1,21].

Non-culture-based methods for diagnosis of IA

The development of tests based on identification of circulating fungal antigens, metabolites, or DNA of *Aspergillus* over the past decade has been a significant advance. To date, the two approaches that have demonstrated the greatest promise in early clinical studies are detection of antigens and measurement of *Aspergillus* nucleic acids by using polymerase chain reaction (PCR). In contrast, assays based on the detection of anti-*Aspergillus* antibodies have demonstrated very poor sensitivity for IA, especially in profoundly neutropenic patients [22]. Nevertheless, the detection of an anti-*Aspergillus* antibody could be useful in nonneutropenic patients at risk for IA, such as recipients of solid organ transplants [23] and less immunocompromised patients with semiinvasive forms of IA [24]. It is anticipated that the use of recombinant antigens instead of crude antigen extracts will further increase the reliability and performance of antibody-detection methods [25].

Galactomannan (GM) is a polysaccharide cell-wall component of *Aspergillus* spp. that is released into the circulation during fungal growth in tissues and can be detected in the serum of patients with IA [26]. A sandwich enzyme-linked immunosorbent assay capable of detecting GM at concentrations as low as 0.5 ng/ml has been recently developed. Studies evaluating the role of GM assay in the diagnosis of IA have predominantly conducted with profoundly neutropenic patients undergoing chemotherapy for leukemia or recipients of HSCTs who were on–fluconazole prophylaxis [25]. These studies have documented sensitivity rates ranging from 67–100% and specificity rates ranging from 86–99%. Furthermore, studies in animal models and humans have illustrated that GM values correlate with the *Aspergillus* fungal burden and may serve as prognostic marker for IA outcome [23]. In another study, when serially monitored, the GM assay preceded the diagnosis of IA by an average of 6–14 days [26]. However, in a recent study that evaluated the value of the GM assay in the detection of early-stage IA, the appearance of major lesions (halo sign, air crescent sign, or cavitation) on HR CT scans almost coincided

or even preceded the detection of the GM antigen in serum [27].

Furthermore, uncertainties remain regarding the performance of the GM assay in other settings, such as breakthrough IA to mould-active antifungal prophylaxis, pediatric populations, chronic granulomatous disease [28], and solid organ transplant recipients [25,29,30]. Other factors, such as the pretest probability of infection, the patient's immune status (neutropenia vs. graft-versus-host-disease), the presence of anti-GM antibodies, antifungal-based therapy, antibacterial-based therapy, and diet, may affect both the performance and interpretation of the GM assay [25]. Hence, studies have found sensitivity rates as low as 30% in nonneutropenic HSCT recipients receiving mould-active antifungal prophylaxis and lung transplant recipients [25,29,30]. In addition, false-positive results have been described in patients receiving β-lactam antibiotics, particularly piperacillin–tazobactam, as well as in those who have ingested certain cereals, pastas, nutritional supplements, and soy sauce produced with fermentation products of *Aspergillus oryzae*, a fungus commonly used in food production [25]. Furthermore, the performance of the GM assay has not been extensively studied in infections caused by non-*fumigatus Aspergillus* spp. Finally, the sampling rate, cut-off value of the GM assay, and method of statistical analysis (per test or patient) are variables that significantly affect the performance of this test [25,30].

Although the detection of GM in fluids other than serum, particularly BAL fluid, appears to be highly sensitive, specificity might be an issue [31]. For example, detection of GM in urine is feasible and obviates the need for invasive procedures, however this method is prone to contamination [31]. In addition, there is evidence that antigenemia may precede antigenuria, whereas little is known about the kinetics of GM antigenuria [31]. Of interest, small case series demonstrated that detection of GM in cerebrospinal fluid is more sensitive and becomes positive for IA earlier than cultures in patients with central nervous system IA [31]. Prospective validation of these promising findings is needed.

A highly sensitive (1 pg/ml) colorimetric assay used for the detection of 1,3-β-D-glucan (BG), an integral cell-wall component in a number of pathogenic yeasts and filamentous fungi but not *Zygomycetes* or *Cryptococcus neoformans,* recently became commercially available [32–34]. Thus far, the sensitivity and specificity rates for this test in limited studies have ranged from 55–100% and 52–100%, respectively [32–36]. Authors have reported false-positive test results in patients with concomitant bacterial infections, cirrhosis, patients undergoing hemodialysis, patients following abdominal surgery, and patients receiving antibiotics [37] or chemotherapy with particular agents [32]. Furthermore, one study reported that the BG assay is less sensitive and reproducible and becomes positive later in the course of IA when compared with the GM antigen assay [35]. Nevertheless, a recent study reported that combined use of serum GM and BG assays substantially improved the sensitivity and specificity of each test in high-risk neutropenic patients with hematological malignancies [36]. Because the BG assay detects a broader spectrum of moulds than the GM assay does, it has the potential to be incorporated in pre-emptive management strategies in patients at high risk for invasive fungal infections. However, in view of the broad spectrum of fungi it detects, the diagnostic workup and the optimal pre-emptive treatment in high-risk patients with positive BG is uncertain.

Molecular diagnostic methods for IA

Polymerase chain reaction (PCR)-detection of *Aspergillus* nucleic acids is increasingly appreciated as being a method of early detection of IA. In contrast with the other non-culture-based methods, PCR has the capability of rapid detection and molecular identification of opportunistic moulds besides *Aspergillus* at the genus level [38,39]. The sensitivity of PCR is excellent, but its low level of specificity for invasive infections can be problematic, and false-positive results are common [38,39]. However, there is still a debate about whether false-positive results of PCR could represent subclinical abortive IA in patients receiving empirical antifungal-based treatment or recovering from neutropenia [38,39]. Multiple unresolved issues accompany the use of PCR for diagnosis of IA, including the sample type (serum, BAL fluid), amplification strategy (nested vs. conventional PCR), protocol (real-time quantitative vs. conventional PCR), and primer selection (*Aspergillus*-specific or pan-mould, pan-fungal primers). More importantly, the lack of standardization and absence of a commercially available system are major impediments to assessment of the role of PCR in diagnosis of IA. In addition, although circulating *Aspergillus* DNA can be detected at an early stage of infection, how and in what form the DNA is released from the site of infection and the effect of administration of antifungals on the kinetics of *Aspergillus* DNA release are unknown. Finally, because fungal necrosis is required for DNA release, there remains a question of whether PCR is indeed more sensitive for early diagnosis of IA when compared with assays detecting antigen release [40]. For example, recent *in vitro* studies of *A. fumigatus*

mycelium growth demonstrated that GM antigen was released earlier and in higher amounts as compared to both BG and DNA, whereas DNA seemed to be released only due to autolysis caused by nutrient limitation [40].

Thus far, results of PCR using whole blood appear to be sensitive enough for both early diagnosis of IA and assessment of therapeutic response, whereas assays targeting multiple copies have better detection limits; nonetheless, the use of serum in PCR could offer the advantage of simultaneous assessment of other biomarkers such as GM and BG [38,39]. Also, amplification of highly conserved multicopy sequences of 18S ribosomal subunits appears to allow for detection of a range of pathogenic fungi with a high level of sensitivity (1–10 fg/ml) [38,39]. Importantly, application of real time PCR technologies allows for rapid and effective post amplification of specific DNA sequences (e.g. ITS1 regions of 18S ribosomal subunits) for genus and species-specific identification of opportunistic fungi [38,39]. Furthermore, real-time PCR protocols, which are less prone to contamination, along with automated systems for DNA extraction appear to be a significant step towards standardization and improvement of the reliability of the existing PCR assays [39]. Because real-time PCR allows for quantification of the amount of circulating *Aspergillus* DNA, it may also be used as an indirect parameter of fungal load during monitoring of treatment with antifungal drugs.

Polymerase chain reaction using BAL to obtain material seems to be less promising for early diagnosis of IA when compared with that using serum or plasma because of the higher number of false-positive results and the fact that BAL is often performed in patients with an advanced-stage infection [38,39]. Nevertheless, as PCR with BAL specimens has proven to be more sensitive than culture [41] and has an acceptable PPV [42], it may be helpful for establishment of a specific diagnosis of IA.

There have been no good studies examining how well PCR performs in comparison with GM detection for the early diagnosis of IA. Some studies showed that PCR is inferior to serum GM assay in animal models [43] and in some, but not all studies in humans [44,45]. In contrast, recent studies in animal models [46] and humans [47–49] using improved PCR assays have demonstrated that PCR is more sensitive than GM is, particularly in the setting of antifungal-based treatment.

In addition, because of its high negative predictive value (NPV) and ability to detect a broad spectrum of fungi (Table 1), PCR may be useful in excluding diagnosis of IA and other opportunistic fungal infections in severely immunocompromised patients. Nonetheless, in view of the multiple technical and cost issues associated with PCR technology, the use of PCR assays may be justified only in tertiary centers with high rates of IA.

Studies of combinations of surrogate markers for IA

Because none of the current non-culture-based assays are sensitive enough to select patients with IA, and because each diagnostic test could become positive at a different time point of infection [24,37], use of a combination of these tests in an effort to enhance their performance is conceptually appealing. Recently, Florent *et al.* evaluated prospectively the diagnostic utility of a serum PCR ELISA assay alone and in combination with serum GM in 167 patients with hematological malignancies receiving antifungal prophylaxis [49]. In 55 identified cases of IA, the combination of PCR and GM substantially increased their sensitivity and NPV to 83.3% and 97.6%, respectively [49]; however, the combination of these tests resulted in decreased specificity and PPV. In addition, a small retrospective study of 40 high-risk patients with hematological malignancies including 11 patients with IA, reported that the combination of GM and BG improved the specificity and PPV of both tests to 100% without affecting their sensitivity and NPV [36].

Studying combinations of different assays in other tissues could also be of value, as both *Aspergillus* GM and *Aspergillus* DNA are detectable in BAL and cerebrospinal fluid [25,38]. Nevertheless, screening of serum samples is logistically the best method for studying combinations of different assays because it allows for easy and repeated drawing of samples.

Current status of surrogate markers for diagnosis of IA

Thus far, evaluations of the diagnostic potential of most non-culture-based methods have focused primarily on patients with hematological malignancies who had profound and prolonged neutropenia. However, the spectrum of immunocompromised patients at risk for IA is continually expanding. Importantly, because angioinvasion determines the amount of circulating antigens and/or DNA of *Aspergillus*, sequestered lesions typically observed in nonneutropenic patients with IA might be associated with no detectable antigen or DNA. Therefore, the performance of non-culture-based methods in this patient setting remains uncertain.

Table 1 Comparison of non-culture-based diagnostic methods for IA.

Characteristic	GM assay	BG assay	PCR
Sensitivity (%)	29–100‡	55–100	75–100
Specificity (%)	86–99	52–100	65–92
PPV (%)	57–94	40–89	16–42
NPV (%)	95–98	73–98	98–100
Reproducibility	++	++	N/A
Standardization	++	++	–
Commercially available	++	++	–
Less laborious	+	+	–
Cost-effectiveness	?	?	?
Early diagnosis*	+/–	+/–	+/–
Studied for pre-emptive approach for IA	+	N/A	N/A
Potential for detection to *Aspergillus* sp. level	–	–	++
Potential for detection of a broad range of fungi	–#	+†	++

++, excellent; +, very good; +/–, good; –, absent; N/A, not applicable;?, questionable.
‡, In profoundly neutropenic patients with leukemia or recipients of HSCTs on fluconazole prophylaxis GM sensitivity rates ranged from 67–100%; *, Before the first CT finding and/ or empiric antifungal treatment; #, *Aspergillus* specific; †, fails to detect Zygomycetes and *Cryptococcus neoformans* spp.; ¶, requires post-amplification analysis.

In view of the evolving, complex epidemiology of opportunistic mould infections, developing clinical, laboratory, and radiological parameters that favor the differentiation of pulmonary aspergillosis from other lung mycoses is an important research direction for the future. Furthermore, because mixed infections (e.g. *Zygomycetes* plus *Aspergillus* spp.; *Fusarium* plus *Aspergillus* spp.; *Candida* plus *Aspergillus* spp.) are increasingly encountered in severely immunocompromised patients [7], relying on a single diagnostic test (e.g. GM) may lead to inadequate antifungal treatment. To that end, local fungal epidemiology could also influence both interpretation and selection of tests.

Disease progression bias is a challenging confounder in interpretation of performance of the different non-culture-based methods in patients with IA [50]. For example, the sensitivity of a non-culture-based diagnostic test at an early stage of IA may be underestimated when compared with another test or conventional diagnostic methods performed at a more advanced stage of the infection. In view of the lack a reference diagnostic standard for IA, disease progression bias, as well as bias associated with administration of prophylactic or empirical antifungal treatment could partially account for the significant variability on the performance of non-culture-based diagnostic methods reported in the literature [50].

Because of the high NPV of current non-culture-based diagnostic assays, which is probably reflective of the low incidence of IA in some studies, development of screening strategies for excluding opportunistic mycoses in populations of high-risk patients could be an alternative diagnostic approach. Further efforts to comparatively study the performance of the different non-culture-based methods and define which of them are optimal for incorporation in pre-emptive treatment strategies in each patient setting are clearly required. However, the cost-effectiveness of testing (especially their combination) vs. empirical treatment and their impact on fungal mortality remains to be shown.

Furthermore, biomarkers could also play a significant role as adjunctive diagnostic tool for establishment of diagnosis of IA, obviating the need for more invasive diagnostic procedures. However, the value of this approach in patients with evidence of IMIs has not been systemically studied. Finally, because the true diagnostic value of these methods in patients at high risk for IA remains uncertain, autopsy validation should remain the gold standard for assessing their performance. Declining autopsy rates in tertiary care cancer centers [13] represent a vanishing gold standard in the evaluation of these new diagnostic assays in IA.

Novel directions for the diagnosis of IA

Aspergillus spp. produce a variety of enzymes and secondary metabolites during hyphal growth that could potentially be used as diagnostic markers for IA [51]. Our group recently showed that gliotoxin, a secondary metabolite of *Aspergillus* with immunomodulating properties, could be detected in the sera of patients with IA [52,53]. However, data on the prevalence and kinetics of gliotoxin in sera or other fluids in patients infected with *Aspergillus* spp. are not yet available. In addition, isolates of *A. flavus* and *A. terreus* that are important causes of IA produce less frequently gliotoxin as compared to *A. fumigatus* [53]. Further studies to better define the role of gliotoxin in the pathogenesis

of IA and screening of patient samples for mycotoxins as early diagnostic markers for IA are required.

Like other fungi, *Aspergillus* spp. are capable of releasing high amounts of specific volatile organic compounds (VOCs) [51]. Importantly, a small study recently demonstrated the feasibility of measuring pentylfuran, an *Aspergillus*-specific VOC, in patients with cystic fibrosis who had positive respiratory cultures for *A. fumigatus* [54]. Development of easily applicable noninvasive diagnostic methods for detection of *Aspergillus* VOCs deserves further evaluation.

Recently, the use of recombinant *Aspergillus* antigens was used for detection of anti-*Aspergillus* antibodies in severely immunocompromised patients [55]. The authors were able to detect *Aspergillus*-specific antibodies in 50% of patients with IA, including profoundly neutropenic patients. In addition, a correlation was found between high antibody titers and adverse infection outcome [55]. Screening for *Aspergillus*-specific antibodies could be of particular value in nonneutropenic patients with non-angioinvasive forms of IA, in whom the performance of other biomarkers such as GM assay is poor because of the distinct pathophysiology of infection. The issue of antibodies has been also discussed above in the paper.

Early invasion by *Aspergillus* hyphae in the lung parenchyma is frequently missed by even the most sensitive available radiographic methods, such as HR CT scanning of the lungs that have a low threshold (>1 mm) of detection of lesions [18]. Development of more sensitive imaging methods that exploit the pathophysiology of IA could improve their diagnostic potential. For example, identification of hypha-specific peptides may lead to new radiographic immunolabeling approaches for detection of early invasion of *Aspergillus* hyphae in the lungs [56; C. Li and D.P. Kontoyiannis, unpublished].

Because angioinvasion with subsequent thrombosis is a prominent feature of IA that occurs early in the infection course, development of imaging tools for direct detection of vessel occlusion is conceptually appealing. High-resolution CT angiography is capable of demonstrating direct vessel occlusion at the level of segmental and subsegmental arteries. A recent small series of five patients with invasive mould infections showed that HR CT angiography was more sensitive and specific than conventional HR CT in diagnosing angioinvasive IPA. Specifically, HR CT angiography was able to detect vessel occlusion caused by angioinvasive IPA before the appearance or even in the absence of the halo sign [57].

Finally, the role of [F^{-18}]-fluorodeoxyglucose (FDG) positron emission tomography (PET) as a tool for early diagnosis and monitoring of antifungal-based

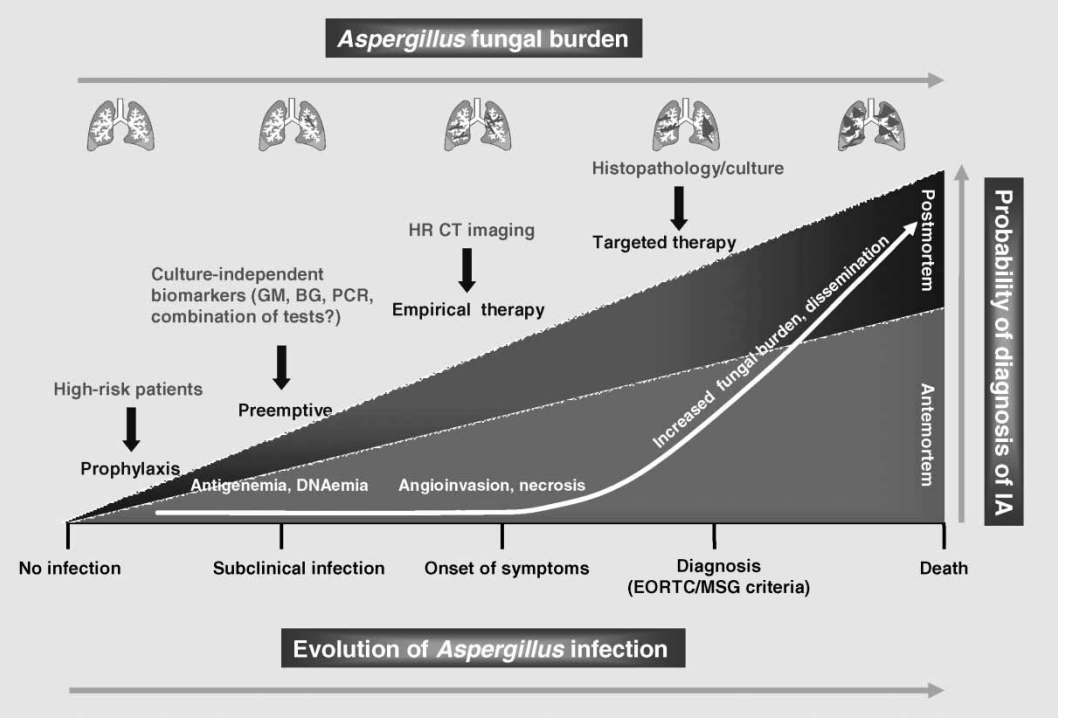

Fig. 1 The rationale for applying non-culture-based diagnostic methods for early (pre-emptive) management of invasive aspergillosis. [EORTC, European Organization for Research and Treatment of Cancer; MSG, Mycoses Study Group.]

treatment of a range of opportunistic infections, including IA is emerging [58]. In addition, anecdotal reports suggest that FDG-PET can reveal occult foci of disseminated IA and guide antifungal-based treatment [59; G. Chamilos and D.P. Kontoyiannis, unpublished]. Prospective validation of the diagnostic potential of FDG-PET for IA is needed.

Conclusions

There is no question that non-culture-based methods are the future of early IA diagnosis (Fig. 1). A recent small, phase II feasibility study examined the prospects of such pre-emptive approaches in patients with febrile neutropenia [60]. In this study, the investigators selected patients with prolonged neutropenia and/or other features suggestive of invasive fungal infections based on an algorithm that included GM performed on a daily basis and HR CT. This strategy led to administration of unnecessary treatment with antifungal agents in 78% fewer patients when compared with the standard empirical approach.

However, much more work must be done to validate surrogate markers for early diagnosis and management of IA. Defining the optimal combination or sequence of non-culture-based assays with or without diagnostic imaging tools such as chest HR CT remains a challenge in terms of cost-effectiveness and optimal performance.

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