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Diagnostic-driven management of invasive fungal disease in hematology in the era of prophylaxis and resistance emergence: Dutch courage?

E. A. de Kort [1,*], J. Maertens [2], P. E. Verweij [3,4], B. J. A. Rijnders [5] and N. M. A. Blijlevens [1]

[1]Department of Haematology, Radboud University Medical Center, Nijmegen, the Netherlands, [2]Department of Haematology, Universitaire Ziekenhuizen Leuven, Leuven, Belgium; Department of Microbiology and Immunology, University of Leuven, Leuven, Belgium, [3]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, the Netherlands, [4]Center of Expertise in Mycology Radboudumc/CWZ, Nijmegen, the Netherlands and [5]Department of Microbiology, Erasmus university medical center, Rotterdam, the Netherlands

*To whom correspondence should be addressed: Elizabeth de Kort, MSc, Department of Haematology, Radboud University Medical Center, Nijmegen, the Netherlands. Tel: 31-243618823; E-mail: Elizabeth.DeKort@Radboudumc.nl

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Abstract

Patients receiving intensive anti-leukemic treatment or recipients of allogeneic hematopoietic stem cell transplantation (HSCT) are prone to develop invasive fungal disease caused by both *Aspergillus* and non-*Aspergillus* moulds. Overall mortality following invasive mould disease (IMD) is high; adequate and timely antifungal treatment seems to ameliorate the outcome, yet early diagnosis in the haematological patient remains a challenge for most clinicians. Prophylaxis and the empiric addition of antifungal therapy to neutropaenic patients with fever persisting or recurring during broad-spectrum antibiotic treatment is therefore standard of care in many institutions. However, aside from the potential for overtreatment and important side effects, the emergence of resistance to medical triazoles in *Aspergillus fumigatus* poses a risk for inadequate initial treatment. Initial voriconazole therapy in patients with azole-resistant invasive aspergillosis was recently shown to be associated with a 23% increased mortality rate compared to the patients with azole-susceptible infection, despite changing to appropriate antifungal therapy once resistance was detected. Moreover, fever is not always present with IMD; therefore, cases may be missed when relying solely on this symptom for starting diagnostic procedures and antifungal treatment. At our institution, a diagnostic-driven treatment approach for IMD was implemented relying on clinical but also laboratory markers to start antifungal treatment. We describe the basis and clinical implementation of our diagnostic-driven approach in this review.

Key words: invasive fungal disease, diagnostic driven management, haematopoietic stem cell transplantation, acute leukemia, azole resistance.

Introduction

Patients receiving intensive anti-leukemic treatment or recipients of allogeneic hematopoietic stem cell transplantation (HSCT) are prone to develop invasive fungal disease.[1] Pulmonary and sinocerebral disease are the most commonly encountered manifestations of invasive mould disease (IMD) caused by both *Aspergillus* and non-*Aspergillus* moulds. Overall mortality following IMD is high, with 12-week mortality rates of 18%–26% for probable/proven aspergillosis.[2–4] Timely antifungal treatment seems to improve the outcome, yet early diagnosis in the hematological patient remains a challenge for most clinicians. Cytopenias often make diagnostic sampling of deeper tissues less attractive if not impossible. Noninvasively obtained respiratory tract cultures are neither specific nor sensitive and

are therefore not discriminatory. Inconclusive results owing to erroneous tissue sampling or previous antifungal treatment are also frequently encountered. Prophylaxis and the empiric addition of antifungal therapy to neutropenic patients with fever persisting or recurring during broad-spectrum antibiotic treatment is therefore standard of care in many institutions. However, this approach can lead to overtreatment and may delay effective antifungal therapy, as identification of the fungus causing IMD is hampered. In addition, the emergence of resistance to medical triazoles in *Aspergillus fumigatus* further increases the risk of inappropriate initial therapy. Initial voriconazole therapy in patients with azole-resistant invasive aspergillosis was recently shown to be associated with a 23% increased mortality rate compared to the patients with azole-susceptible infection, despite changing to appropriate antifungal therapy once resistance was detected.[5] Furthermore, fever is not always present with IMD; therefore cases may be missed when relying solely on this symptom for starting diagnostic procedures and antifungal treatment.[6] At the Radboudumc, we implemented a diagnostic driven treatment approach for IMD, relying on clinical but also laboratory markers to start antifungal treatment without delay. We describe the basis and implementation of this approach, as well as the outcome in this review.

The past: how empirical treatment came to be

In the early days of hemato-oncology practice, mortality due to infections during the neutropaenic phase of anti-cancer treatment was high. Occult IMD was a common cause of death found primarily at autopsy, in an age lacking adequate diagnostic tools and systemic antifungal treatment options.[7] The discovery of conventional amphotericin B (c-AmB), which was effective against a broad range of fungi, was soon followed by studies applying this agent to the hemato-oncological population. c-AmB given empirically to patients with ongoing or remitting fever during chemotherapy-induced neutropenia seemed to decrease the incidence of fungal infections, especially caused by *Candida*.[7,8] Due to the substantial toxicity associated with c-AmB, lipid-formulations were developed. In 2002, voriconazole was shown to be superior to c-AmB for the treatment of invasive aspergillosis and replaced c-AmB as first-line therapy for invasive aspergillosis and other IMDs. The echinocandins became available as primary treatment for invasive candidiasis. These newer agents were not only more effective when compared to c-AmB, but also showed improved toxicity profiles.[3,9] However, the majority of the clinical trials involving these drugs were designed to treat neutropaenic fever, not fungal infections.

More recently, the efficacy and tolerability of posaconazole and later voriconazole in reducing the incidence of fungal infections was established by several independent studies,[10–12] and numerous guidelines support the use of mould active antifungal prophylaxis in the hematological population.

The near-present: how (biomarker guided) pre-emptive treatment came to be

Although the European Organization for Research and Treatment of Cancer (EORTC) and the Mycosis Study Group (MSG) developed definitions for diagnosing invasive fungal diseases for research purposes, many clinicians routinely apply these criteria in daily practice to guide diagnosis and treatment decisions. The EORTC/MSG definitions are universally applied in fungal research to establish a diagnosis of IMD, and they were last updated in 2008 to harbor the growing body of evidence for different diagnostic tests. It is important to note that not one single diagnostic test reaches 100% negative- or positive predictive value; therefore, in the absence of proven IMD by histopathology, a combination of criteria is needed to establish a diagnosis with a particular degree of certainty. Possible IMD is determined by a host factor (patient population at risk) and a clinical factor (most often one or more specific signs on pulmonary imaging). Probable IMD requires presence of a host factor, a clinical factor, and a mycological criterion (positive culture or nonculture based test). Proven IMD requires histopathological evidence of an invasive fungal infection by demonstrating the presence of hyphae in tissue samples or the growth of a fungus from a normally sterile body site.[13,14]

Maertens and colleagues initially explored the preemptive approach to antifungal therapy in their pilot study in 2005. In this prospective noncomparative trial, adult hematology patients at risk for IMD and receiving fluconazole prophylaxis were screened for IMD by standard daily serum galactomannan (GM) testing. A diagnostic evaluation consisting of high-resolution (HR) computed tomography (CT) of the chest and bronchoalveolar lavage (BAL) was performed, if prespecified microbiological or clinical conditions were met. Only patients with two or more consecutively positive GM (GM-index >0.5) or suggestive HRCT with microbiological support received treatment with liposomal-AmB. There were 136 investigated episodes. Fever developed in 117 episodes, and treatment according to protocol was only given in 9 episodes. This represented an estimated reduction in the use of antifungals by 27.3% when compared to an approach where antifungal treatment would have been started empirically after 5days of unexplained fever.[6] Additionally, another 10 episodes of possible/probable invasive aspergillosis (IA) by EORTC/MSG criteria received preemptive treatment following GM positivity. These patients had an alternative explanation for fever if present, did not have clinical signs of IMD, one case was identified in the absence of fever. A similar approach was independently published by an Australian group. Their randomized controlled trial included 240 patients with leukaemia or allogeneic transplantation. Survival was similar in the diagnostic-driven versus empirical therapy group. Based on serum GM, PCR screening and HRCT, 18% of patients received antifungal treatment versus 36% in the empirical therapy group. Half

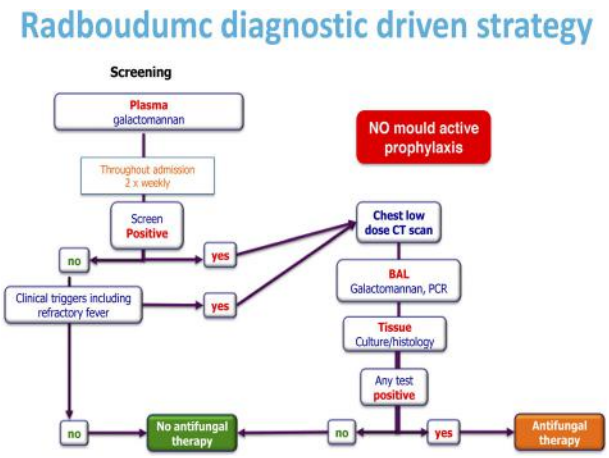

Figure 1. Radboudumc diagnostic driven strategy for treatment of invasive mould disease. This Figure is reproduced in color in the online version of *Medical Mycology*.

of the cases of probable/proven aspergillosis were established in the absence of fever by the diagnostic driven approach and thus would have been missed with empirical treatment.[15]

The present: an approach to nonempirical treatment in the real- (Dutch) world

Our diagnostic driven, nonempirical treatment approach at the Radboudumc consists of low dose HRCT prompted by clinical signs (e.g., persistent fever) or a positive serum GM found as a result of serial GM screening throughout admission. This approach is similar to the diagnostic approach in Leuven hospital and is illustrated in Fig. 1. Antifungal treatment with voriconazole is started when a case of possible IMD according to the revised EORTC/MSG criteria has been identified, while awaiting the results of a diagnostic work-up which includes bronchoscopy and BAL. In patients with nonspecific pulmonary infiltrates (i.e., no well-described nodule, no halo sign, no cavitary lesion) and a negative serum GM test, antifungal therapy is initiated only when BAL sampling confirms the presence of *Aspergillus* (by GM positivity, culture, or polymerase chain reaction [PCR]). Antifungal treatment is stopped if IMD diagnosis cannot be confirmed.

The present: adopting a diagnostic strategy

Aside from diagnosing IMD in the absence of fever, unnecessary exposure to toxic drugs, increased treatment costs and important drug-drug interactions were the most important drivers to change our clinical therapeutic strategy of fungal infections to a diagnostic driven treatment approach. Clinicians considering diagnostic-driven management of IMD, need to be familiar with the test characteristics and the conditions that need to be fulfilled before a particular diagnostic strategy can be implemented.

First, there needs to be a predetermined population at risk. In our institution, and based on previous published results, we apply this diagnostic strategy to the population expected to have a long period of neutropenia and thus to be at the highest risk of invasive fungal infection.[1,16] A formal threshold for high-risk has not been established but a prevalence of IMD > 5–8% is generally considered high risk. Our population is defined as patients with an acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) receiving intensive anti-leukemic treatment or patients who underwent an allogeneic HSCT.

Second, the screening or diagnostic tools to establish a diagnosis of IMD must be readily available, usable, and interpretable. The serum GM assay was first evaluated by Verweij and colleagues.[17] GM is a polysaccharide present in the cell wall of *Aspergillus* spp. It is released during hyphal growth and can be detected in the patient's serum. The Platelia sandwich enzyme-linked immunosorbent assay (ELISA) (Biorad, Berkeley, CA, USA) is the assay that has been used more extensively. The optimal cutoff for positivity has been set at a GM index of ≥0.5. A meta-analysis from 27 studies using GM monitoring to detect aspergillosis in the hemato-oncological patient has showed a sensitivity of 70–82% and a specificity of 86–93%. Sensitivity diminishes to 20% if systemic mould active prophylaxis is used; therefore, we limit the use of mould active antifungal prophylaxis to the patients at very high risk of IMD (i.e., allotransplant patients with grade 2 or higher GVHD).[18] Some centers screen serum from high-risk patients twice weekly for the presence of GM with results being available the same day. Other centers test for the presence of serum GM in patients with persistent fever only. Despite these differences in timing of screening, serum GM index ≥0.5 is considered suspicious and a CT scan of chest is obtained, regardless of the presence of fever. Alternatively, clinical symptoms such as persistent fever can also prompt CT evaluation, even if GM screening is negative.

CT needs to be timely, available within 1 day of request. CT scanning of the chest has proven to be of great importance in the early detection of IMD in the lungs. Indeed, some lesions are accepted clinical criteria in the EORTC/MSG definitions of IMD.[13] The first detectable sign of IMD is usually a nodule surrounded by ground glass opacification (the halo sign). It is an early indicator of invasive aspergillosis in the neutropenic host. This lesion is transient and usually disappears within days; hence, the sensitivity of CT scan alone is low.[13,19] Evidence to support standard CT scanning early during the course of fever in high risk patients with neutropaenia was recently presented by Gerritsen et al.[20] In this study, a conventional chest x-ray and a low-dose chest CT were performed on the first day of neutropenic fever, but the result of the CT was not disclosed to the treating physician. In patients in which a diagnosis of pulmonary infection was eventually made, the low dose CT had a significantly higher sensitivity for pulmonary foci than the chest x-ray (73% vs CR 36%). Their study was underpowered to demonstrate an effect on early detection of IMD, but four out of five patients that had a possible or probable IMD had pulmonary

lesions suggestive of IMD on chest CT on the first day of neutropaenic fever.

Bronchoscopy with BAL needs to be acquired for all suspected cases, if clinically feasible. BAL fluid examination includes gross observation, cell count, bacterial, and fungal analysis. Mycological analysis of BAL-fluid includes microscopy using optical brighteners to detect fungal hyphae, culture, and detection of GM. PCR detection is another method being increasingly used to test for the presence of *Aspergillus* DNA (see below).

Third, adequate review of all determined cases should tailor future considerations. Local prevalence of IMD and epidemiology of involved pathogens should ideally be known.

Diagnostic-driven strategy and the emergence of resistance

A. fumigatus is the most frequently recovered mould in all Dutch centers. Over the past decade, resistance to medical triazoles has become an increasing concern.[21] In patients with azole-resistant invasive aspergillosis, resistance is thought to originate in the environment rather than develop during treatment. Azole fungicides are widely used for crop protection and material preservation, and azole-containing environments exist that select for azole resistance in *A. fumigatus*. As some of these azole fungicides exhibit a similar molecule structure compared with the medical triazole, azole resistance mutations also reduce the activity of the medical triazoles. A recent cohort study showed a 21% increased day-42 mortality in patients with azole-resistant invasive aspergillosis compared with azole-susceptible disease in culture-positive patients.[5] Most patients were treated with voriconazole, and switched to liposomal-AmB or voriconazole-echinocandin combination therapy when azole resistance was detected. However, despite intensive screening and MIC-testing the median time to switch was 10 days, and the day-42 mortality in patients with azole-resistant invasive aspergillosis was 23% higher than those patients with azole-susceptible infection who immediately received appropriate antifungal therapy.

Azole resistance surveillance studies show resistance rates exceeding 10% in Dutch university medical centers, and the national guideline was revised in 2017, now recommending resistance coverage with combination antifungal therapy with voriconazole and either an echinocandin or L-AmB in patients with possible, probable, or proven IA, until triazole resistance is excluded by culture or PCR.[22] Alternatively, azole monotherapy in non-critically ill hematology patients is considered warranted whilst awaiting PCR-based real-time resistance testing of BAL fluid.

The emergence of azole resistance challenges our diagnostic-driven strategy, as detection of resistance is not achieved when relying solely on GM screening.

For this purpose the Nijmegen Audit of Azole Resistant *Aspergillus* among patients at high-risk of invasive aspergillosis (NAARA) cohort study was performed and aimed to investigate the frequency of azole resistance in our haematological population at risk from 2006 to 2012. A total of 432 patients were included in the cohort and were diagnosed based on this presented strategy. Just over 16% of these patients had a possible, probable, or proven IMD according to the EORTC/MSG criteria. Approximately 8% had probable or proven IMD; 83% did not have a fungal infection. This is comparable to previous published results in other centers.[23] Overall mortality at 6 months for the entire cohort reached 50% (217 of 432), but mortality due to IMD was 2.3% (10 of 432). Mortality rate was significantly increased in the group with proven/probable IMD, 36.1% versus 17.4% in the group without IMD ($P = .01$), corresponding with an absolute difference of 18.7%. Our study was not set up to determine the implications of IMD on overall mortality; however, there are ample published results to support our findings. Slobbe et al. found a 10% absolute difference in early mortality rate (26% vs 16% at 12 weeks) in 269 patients with AML or MDS with invasive aspergillosis versus without invasive aspergillosis. Wingard et al. reported a similar outcome at 6 weeks after starting treatment for patients with probable or proven invasive aspergillosis according to the EORTC/MSG definitions.[2,24] Our findings at 6 months post transplant suggest a persistent risk for patients with IMD, possibly due to delay or even interruption of anti-leukemic treatment leading to lower complete or molecular remission rates and higher transplant related mortality as previously published.[25,26] This reiterates the importance of early and adequate diagnosis to ensure prompt, yet guided treatment in a setting with emerging azole resistance.

The NAARA cohort study also sought to determine the rate of recovery of azole-resistant *A. fumigatus* in our patient population. Resistance was determined by agar-dilution based resistance screening of culture-positive patients using VIPcheck™ (Mediaproducts, Groningen, the Netherlands). In case of phenotypic resistance MIC-testing and *Cyp51A*-gene sequencing was performed in order to identify the underlying resistance mutations. The rate of azole-resistant invasive aspergillosis was 1.2% (5 of 432 patients) in the NAARA cohort.[23] However, detection of resistance was hampered by the very low culture positivity rate; Only 28 *Aspergillus* cultures were positive, with five showing azole resistance (17.9%).

We performed a sub-analysis of the NAARA cohort to assess results of CT scanning, and our BAL recovery rate during febrile neutropenia episodes. A total of 113 BAL samples (cases) were analyzed for purity and recovery rate. Analysis of BAL fluid included culture, GM, microscopic assessment, and cytological assessment. In 23% of the BAL samples evidence was found for an IMD. In 86% of these 113 cases, a CT scan was performed within 1 week prior to the BAL-procedure. The halo sign was seen in 54% of our probable/proven cases with positive BAL mycology but was also identified in 22% of patients with no moulds recovered by lavage (Table 1).

Table 1. Results of CT and BAL during febrile neutropenia.

		ALL ($n = 97$)	Positive BAL* ($n = 22$)	Negative BAL ($n = 75$)
Number of micronodules (≤1 cm)	No	72 (74.2%)	15 (68.2%)	57 (76.0%)
	<10	17 (17.5%)	7 (31.8%)	10 (13.3%)
	>10	8 (8.2%)	0 (0.0%)	8 (10.7%)
Number of nodules (>1 cm)	No	66 (68.0%)	12 (54.5%)	54 (72.0%)
	<10	27 (27.8%)	8 (36.4%)	19 (25.3%)
	>10	3 (1.0%)	2 (9.1%)	1 (1.3%)
Consolidations		60 (62.5%)	11 (50.0%)	49 (66.2%)
Halo sign		29 (29.9%)	**12 (54.5%)**	**17 (22.7%)**
Cavity (within lesion)		6 (6.2%)	0 (0.0%)	6 (8.0%)
Pleural effusion		39 (40.2%)	6 (27.3%)	33 (44.0%)
Atelectasis		24 (24.7%)	4 (18.2%)	20 (26.7%)
Ground glass (other than halo sign)		39 (40.2%)	9 (40.9%)	30 (40.0%)
Tree in bud		9 (9.3%)	2 (9.1%)	7 (9.3%)
Reticular infiltrates		3 (3.1%)	1 (4.5%)	2 (2.7%)
Nonspecific		10 (10.3%)	2 (9.1%)	8 (10.7%)

*BAL was deemed positive when evidence was found via either microscopy, culture, PCR, or GM.

The near future: stepping away from empirical treatment and incorporating modern techniques

The EORTC 65091–06093 study is a prospective multicentre randomized controlled trial that ran across Europe from 2012 to July 2018. This study aims to provide a definitive answer to the pertaining questions about diagnostic-driven management, comparing overall survival in empirical (fever-driven) versus "preemptive"(diagnostic driven) antifungal therapy with caspofungin in patients with AML and MDS receiving intensive anti-leukemic treatment or allogeneic HSCT. The "diagnostic" triggers for treatment in the preemptive group were either serum GM index >0.5, *Aspergillus* culture from respiratory tract samples, and/or chest CT scan lesions consistent with IFD according to the EORTC/MSG definitions. The results of this trial have not yet been published.

Adopting a diagnostic-driven approach can be quite challenging as evidence favouring one particular management strategy is still lacking. On the one hand, it is evident that the goal of every approach should be to prevent IMD-related mortality. On the other hand, how one reaches that goal is variable and depends on the incidence of IMD in a specific patient population and the availability and complications of a particular approach. In an institute with higher incidence of IMD and nonimmediate diagnostic testing capabilities, primary prophylaxis or empirical treatment might be more successful in preventing mortality than a diagnostic driven approach. In a different patient population, the number needed-to-treat to prevent one death might exceed the number needed to harm, consequently exposing too many patients to adverse reactions and complications. A clear example of the latter is the patient with acute lymphocytic leukaemia. Even though studies have shown variable IMD incidence rates in remission-induction treatment (3.3–11.7%), prophylaxis is debatable because of complicating interactions between chemotherapeutic agents and azole antifungals.[27,28] The same reasoning can be applied to empirical treatment, and therefore a diagnostic driven approach would be warranted in the management of IMD. Furthermore, the emergence of resistance increases the need to identify and characterize the fungus causing the infection. As culture-based resistance detection might not provide results early enough to prevent mortality, rapid tools such as resistance PCR have become available and might shorten the time to resistance detection. Resistance PCR could be performed in GM-positive BAL-samples and provide information on the presence of resistance mutations directly in BAL within 24 hours. A retrospective multicentre study showed that resistance mutations can be readily detected in BAL fluid of haematology patients and that the presence of resistance mutations predicted treatment failure and increased mortality.[29] However, the feasibility of such a strategy in routine practice remains to be confirmed and its usefulness will certainly depend on the resistance frequency in the local hospital or region. With respect to managing fungal diseases in AML and MDS, significant progress has been made in recent years but the jury is still out.

In the meantime, a survey conducted by Schauwvlieghe et al. on the approach to diagnosis of fungal disease in the Netherlands in the context of increasing azole-resistant *A. fumigatus*, confirmed a broad spectrum of approaches among the eight academic institutions.[30] All but one institution hold off anti-mould prophylaxis during intensive anti-leukemic treatment. All centers provide mould-active prophylaxis when steroids are given for graft-versus-host disease as well as perform a chest CT after 3 to 5 days of persistent neutropenic fever, followed by BAL if

clinically achievable. Only one center (Nijmegen) performs standard serum GM screening. *Aspergillus* and resistance PCR testing on BAL is routinely performed in three centers, culture based susceptibility testing in all. Treatment with voriconazole is started in all centres for all patients except for two centers that used L-AmB for the unstable patient or used posaconazole instead of voriconazole. This survey was performed preceding the new Dutch guidelines that recommend against azole- monotherapy in patients with possible, probable or proven IA of unknown azole resistance.[22]

Driven by mounting evidence of azole-resistance development in the Netherlands,[31] all these centers have agreed to participate in the AzorMan study which is a prospective study currently open for inclusion in the eight academic centers in the Netherlands. This study uses a single diagnostic-driven treatment approach, which will be implemented in all centers. It aims to demonstrate that PCR resistance testing on BAL fluid from haematology patients will lead to early diagnosis of triazole resistance and might improve outcome if early switch to appropriate therapy is achieved in patients with resistance markers. The study also intends to monitor the prevalence of azole-resistant *A. fumigatus* in these centres including in culture-negative individuals (https://clinicaltrials.gov/ct2/show/NCT03121235).

In addition to new microbiological tools, such as resistance PCR, important imaging techniques like CT-pulmonary angiogram also hold promise in detecting early lesions in IMD.[32] On the other hand, conventional chest radiography in the setting of neutropaenic fever will likely become obsolete and will be replaced by (early) chest CT.

We report a real-world diagnostic-driven strategy for treatment of IMD, which allows us to identify patients with low probability of IMD in whom we can safely withhold prophylaxis or empirical antifungal therapy, even if they are febrile. We have described the challenges involved in such a strategy regarding implementation and overcoming emerging risks such as antifungal resistance. We look forward to the results of the EORTC 65091–06093 trial to move us further along the line toward further improvement of a diagnostic-driven management strategy of fungal disease in the hematological patient.

Declaration of interest

J.M. has received research grants from Merck/MSD, Gilead, and Pfizer; is a consultant to Astellas, Bio-Rad, Merck/MSD, Pfizer, Schering-Plough, F2G, Gilead, Cidara, Scynexis, and Luminex; and has served at the speaker's bureau of Astellas, Gilead, Bio-Rad, Merck/MSD, Pfizer, Schering-Plough and Viropharma/Shire.PEV received grants from Merck, Pfizer, Gilead Sciences, and F2G. He has been a consultant to Basilea, Scynexis, Merck, F2G, and Siemens and received speaker's fees from Merck, Gilead Sciences, and Pfizer. B.R. received a research grant from Gilead and participated in advisory boards of Gilead and Pfizer. E.d.K. and N.B. report no conflicts of interest.

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