Original

False positive galactomannan results in adult hematological patients treated with piperacillin-tazobactam

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Summary

In this prospective study including 78 adult patients with hematological malignancy (90 episodes) we performed galactomannan (GM) (Platelia *Aspergillus*) screening twice weekly for the diagnosis of invasive aspergillosis. There were five proven and four probable invasive aspergillosis cases. The sensitivity, specificity and positive and negative predictive values were 100, 88, 47 and 100%, respectively. There were eight patients with false positive GM (10.2%). In six patients the false GM reactivity was due to the administration of piperacillin-tazobactam (P-T). A significant association was found between false positive GM ($\geqslant$ 0.5) and the administration of P-T ($p < 0.01$). Two other patients with no invasive aspergillosis (2.5%) and false GM reactivity had graft versus host disease (GVHD) and one of them had also mucositis grade IV. The kinetic patterns of false positive GM due to P-T is discussed.

Key words Galactomannan, Piperacillin-tazobactam, False-positives, Aspergillosis, Diagnosis.

Resultados falsos positivos de galactomanano en pacientes hematológicos adultos tratados con piperacilina-tazobactam

Resumen

Se han estudiado prospectivamente dos veces por semana los niveles séricos de galactomanano (GM) (Platelia *Aspergillus*) en 78 pacientes con cáncer hematológico (90 episodios) para el diagnóstico de aspergilosis invasora (AI). Hubo cinco casos de AI probada y cuatro de AI probable. La sensibilidad, especificidad y valor predictivo positivo y negativo fueron de 100, 88, 47 y 100% respectivamente. Hubo ocho pacientes con GM falsos positivos (10,2%). En seis enfermos la falsa reactividad de GM fue debida a la administración de piperacilina-tazobactam (P-T), encontrándose una asociación significativa entre galactomananos falsos positivos y la administración de P-T ($p < 0.01$). Otros dos pacientes sin AI y GM falsos positivos (2,5%) tuvieron como posible causa de falsa positividad la enfermedad injerto contra huésped y uno de ellos además tenía mucositis grado IV. En el trabajo se han analizado los patrones cinéticos con falsa reactividad de GM en relación a P-T.

Palabras clave Galactomanano, Piperacilina-tazobactam, Falsos positivos, Aspergilosis, Diagnóstico

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The commercial sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia *Aspergillus*, Bio-Rad, Marnes La Coquette, France) is widely used worldwide for the diagnosis of invasive aspergillosis (IA) in adult immunocompromised patients [9,20,28,32,42]. Galactomamman (GM) is a polysaccharide from the cell wall of *Aspergillus* spp. usually secreted to the blood in patients with IA. Currently, it is detected by the Platelia *Aspergillus* ELISA test by means of the monoclonal antibody EB-A2 which acts both as a captor and detector [42]. The monoclonal antibody EB-A2 is an immunoglobulin M (IgM) that recognizes the 1-5-β-D galactofuranoside side chains of the *Aspergillus* GM molecule but cross-reaction with several fungal exoantigens from other genera has also been reported [8,24,42].

It is out of doubt the value of screening prospectively for circulating *Aspergillus* GM in adult hematological cancer patients using a stratification scheme defined by Prentice et al. [38] for the diagnosis of IA.

This marker provides an early diagnosis of IA and the implementation of pre-emptive therapeutic strategies [18]. The sensitivity and specificity of GM ELISA appears to be adequate and timely since a positive GM appears before the onset of clinical symptoms or radiological abnormalities detected by high resolution computed tomography scanning (HRCT) [9,18,20,24,32,43]. However, a pitfall in the indirect diagnosis of IA is the occurrence of false positive GM ELISA results which widely varies from 5% to 15% in adults [20,24,28,32,42] to as much as 83% in neonates [43].

Herein we report the occurrence of false positive GM ELISA results associated with the use of piperacillin-tazobactam (P-T) treatment and other possible factors in patients with hematological malignancies using a risk stratification scheme. In order to validate the proposal of Prentice et al. [38], our group has studied prospectively a cohort of 78 adult neutropenic patients (90 episodes) exploring the incidence of IFI and IA with the use of GM screening as a diagnostic tool [27]. The incidence of IFI and IA correlated directly and significantly with risk stratification, with the highest incidence (31%) in the high risk group (n = 16), followed by the intermediate high risk (12% incidence) (n = 17) and intermediate low risk group (8% incidence) (n = 37).

Material and methods

Patient selection. From September 2004 to May 2005, a cohort of 78 adult hematological cancer patients treated in the Hospital Doce de Octubre, Madrid, Spain and stratified according to the scheme of Prentice et al. [38], were prospectively analyzed (as a routine screening twice weekly) establishing the GM index (GMI) by using the commercially available sandwich ELISA (Platelia *Aspergillus)* until the risk condition for developing IFI had subsided. All patients were nursed in rooms with HEPA filtration. The clinical assessment of our patients is the standard of care in tertiary hospitals, and has been described by our group elsewhere [32].

Definition of invasive aspergillosis. IA episodes were classified on the basis of the European Organization for Research and Treatment of Cancer / Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infections Diseases Mycoses Study Group (EORTC-IFICG and NIAID-MSG) case definitions [4].

Diagnostic work-up of IFI. In cases of suspicion of IFI, or when the GMI was above 0.5, a diagnostic work-up was started; this included a pulmonary high resolution computed tomography scanning (HRCT) followed, when possible, by bronchoalveolar lavage and/or biopsy for bacterial, mycobacterial, fungal and viral cultures. Direct examination for bacteria and fungi (including *Pneumocystis jiroveci*) was performed for all patients. The presence of *Legionella* antigen in urine was tested.

GM detection. The ELISA was performed as recommended by the manufacturer. Results (GMI) were expressed as the ratio of the optical density (OD) obtained from the patient serum sample and the control (index = OD of the sample / OD of the control). A result was considered a true positive with a GMI above or equal to 0.500 (static index) [17]. The serum was retested in these cases, showing good reproducibility. An index below 0.5 was considered negative.

Mycological studies. When judged necessary, specimens from clinically infected foci were collected and processed as described by Denning et al. [10]. *Aspergillus* species were identified by their macroscopic and microscopic culture characteristics.

Table 1. Characteristics of adult oncohematological patients with evaluation of GM.

Characteristic	Total	Proven IA	Probable IA	No IA*	False positive GM	
					P-T**	Others***
Patients (n)	78	5	4	69	6	2
Age (yr)[a]	52 (16-79)	60 (36-67)	39 (33-44)	51 (16-79)	61 (27-79)	41 (19-63)
Gender (M/F)[b]	42/36	3/2	1/3	38/31	4/2	1/1
Number (%) with underlying diseses[c]						
ALL	9	1	1	7	0	0
AML	14	1	1	12	1	1
CLL	5	0	0	5	0	1
MM	14	1	1	12	2	0
MDS	5	0	0	5	0	0
NHL	24	1	1	22	3	0
HD	6	1	0	5	0	0
SAA	1	0	0	1	0	0
Number of serum samples total (range)	881 (2-55)	68 (5-28)	84 (8-36)	729 (2-55)	93 (2-33)	70 (20-50)
Number of positive samples for GM (range)	62 (1-11)	12 (2-4)	24 (2-11)	26 (1-8)	23 (1-8)	3 (1-2)

*Includes patients with no IA and patients with false positive GM results. **P-T: Piperacillin-tazobactam. ***Two patients had graft versus host disease and one of them had also grade IV mucositis.

[a] Values in parenthesis are ranges. [b] M/F: male/female. [c] ALL: acute lymphocytic leukemia; AML: acute myeloid leukemia; CLL: chronic lymphocytic leukaemia; MM: multiple myeloma; MDS: Myelodisplastic syndrome; NHL: Non-Hodgking`s lymphoma; HD: Hodgkin disease; SAA: severe aplastic anemia.

Table 2. Incidence[a] of invasive fungal infection (IFI), prophylaxis and stem cell transplantation (SCT) in 78 patients (90 episodes). (Reproduced from reference [27])

Risk group	Patients	Prophylaxis		SCT		Incidence of IFI (%)	Proven IA[b] (n)	Probable IA[b] (n)	Proven Zygomycoses	Total IFI
		Fluconazole	Itraconazole	Autologous	Allogenic					
High[c]	16	9	6	5[d]	4	31	2[e]	2	1	5
Intermediate High	17	7	6	1	7	12	1[f]	1	–	2
Intermediate Low	37	25	3	20	0	8	2	1	–	3
Low	8	3	0	4	0	–	–	–	–	0

[a] Incidence of IFI, prophylaxis and risk factors were calculated in relationship with the number of patients

[b] IA: Invasive Aspergillosis

[c] All seven non SCT patients were treated with high-dose Ara-C; four of them had also neutrophils < $0.1x10^9$/l > 3 weeks

[d] One patient had neutrophils < $0.1x10^9$/l > 3 weeks, another patient was treated with high-dose Ara-C and neutrophils < $0.1x10^9$/l > 3 weeks and three patients were treated with high-dose Ara-C

[e] One patient also had Proven Invasive Candidiasis

[f] This patient also had Proven Invasive Candidiasis

n: number

Statistical analysis. Sensitivity, specificity and positive and negative predictive values were calculated as described by Kozinn et al. [15]. According to Menninkk-Kersten et al. [24] only proven and probable IA were considered truly positive and only no IA cases were considered truly negative.

Results

The baseline demographic and clinical characteristics of patients with evaluation of GM, and proven and probable IA, as well as the false positive GM results are shown in table 1. The IFI in relationship with a risk stratification scheme as defined by Prentice et al. [38], fungal prophylaxis and stem cell transplantation (SCT) is shown in table 2. These results have been published by our group elsewhere [27].

In our patients initial antibiotics for febrile neutropenia included a ß-lactam and aminoglycoside; vancomycin was added 48 hours later if fever persisted. Antimicrobial therapy could be modified on the basis of microbiological data.

There were nine patients with IA (five proven and four probable). Two patients with proven IA had also proven invasive candidiasis (IC) one of them with necropsy and biopsy of deep tissues (liver) and another patient with positive *Candida albicans* blood cultures. One other patient had also proven pulmonary zygomycosis (proven with a pulmonary biopsy) (Table 2).

Seventeen out of 78 patients (21.8%) repeatedly tested positive for GM detection (Table 1) and included 100% of patients with proven IA (five of five) and probable IA (four of four). There were no patients assessed as possible IA. Sixty nine out of 78 patients (88.4%) had no IA. However eight of them (10.2%) were positive for GM detection (false positives) and had no signs or symptoms of IA.

Eighteen patients were treated with P-T. Six of them (33.3%) showed GMI positive values despite the fact the diagnosis of IA was excluded. A significant ($p < 0.01$) association was found between the false positive GMI result and the administration of P-T. Commercial batches of P-T were not tested for the presence of GM. Two patients (2.5%) with false positive GM reactivity had graft versus host disease (GVHD) and one of them had also mucositis grade IV that required iv treatment.

The kinetics of antigenemia of some of our patients in relationship with the P-T treatment is described in figure 1. The kinetics of the decrease of GM after the cessation of P-T was analyzed for six patients; discontinuation of P-T showed an overall trend to decrease the GMI levels and became negative after five days of cessation of therapy (range 1-18 days). A representative kinetics in these patients is shown in figure 1a. The patient was first treated for ten days with P-T and a GMI of 0.508 was observed on day six of treatment. GMI antigenemia returned to baseline levels 24 h after cessation of P-T treatment. When the patient was challenged with another course of P-T two weeks later, an abrupt increase of antigenemia was observed (GMI: 0.850) on the third day of therapy, achieving a GMI of 2.237 on day 9 of treatment. The decline of GMI levels started one day after P-T was withdrawn, achieving a GMI of < 0.5, three days after the end of P-T treatment. Figure 1b shows a similar kinetics of antigenemia. The GMI one day after the start of P-T was 0.756, followed after one week of treatment with P-T, by a rapid decline of GMI one day after cessation of treatment (GMI 0.349). Figure 1c shows the kinetic of a patient treated for two weeks with P-T. On the fourth day of therapy with P-T (and also with a temporal relationship with *Escherichia coli* bacteremia) GMI was 3.194 which was maintained even nine days during the treatment course, achieving a GMI of < 0.500, 18 days after treatment cessation.

The sensitivity, specificity, positive and negative predictive values for GM EIA were 100, 88, 47 and 100% respectively, whereas if the 6 false positive P-T were excluded, these values would had been 100, 97, 78 and 100% respectively. Although four of the patients with false positive GMI due to P-T had clinical data that determined that they could be assigned to low and intermediate low risk group stratification as proposed by Prentice et al. [38] (see Table 3), however the administration of P-T lead to unjustified antifungal therapy (three patients), bronchoscopy (one patient) and repeated thoracic and sinus HRCT (five patients).

Discussion

Serial sampling for the diagnosis of IA in adults with hematological malignancy or stem cell transplantation with the detection of GM at a threshold of 0.5 [17] has a sensitivity that ranges from 61% to 100% and a specificity of 86% to 99% [9,17,20,24,29,31,32,34,39,42,43]. After early studies done in Europe in 2003 the test was introduced in the USA [21,45]. In patients with single serum samples tested the sensitivity was lower (around

40% or less) and consequently serial sampling should be used at least twice weekly, as long as the risk for IA persist [18,24]. An important feature in most prospective studies in adult patients is that GM could be detected at a mean of 8 days before clinical or radiological diagnosis of IA [20,24,32]. This fact enables the identification of patients that require a diagnostic work-up and leads to the implementation of pre-emptive antifungal treatment [18,24,40].

Antifungal prophylaxis or empirical therapy (excluding fluconazole) decreases the level of circulating GM and this is probably the most important factor reducing the sensitivity of the test (30-50%) [13,22,24,35].

The negative predictive value in our patients was 100%, and this value agrees with other published reports [31,34,39]. Our positive predictive value was low (47%) also in agreement with other reports [34,39]. This value was obviously influenced by the false positive GM results, namely in relationship with P-T administration, although other factors such as GVHD and grade IV mucositis were also important factors.

Our data and those of others suggest that GM assay is good at ruling out the diagnosis aspergillosis when used on a routine screening, but is less good at confirming the diagnosis [21,22,31,34,39]. Several factors influence the performance of antigen detection as discussed by Menninck-Kersten et al. [24] namely the selection of patient population, prevalence of IA [14,38] definition of an infected patient [4], cut-off [17], underlying condition and level of immunosuppression [13,38], levels of antibodies anti-*Aspergillus*, that could be as high as 36% in patients with lower immunosuppression [13], and renal clearance and hepatic metabolism [11]. At present the kinetics of GM and its detection by ELISA test are poorly understood.

In most reports the specificity of GM is greater than 85% [24,31,34,39]. False positive reactivity is due to several factors and ranges from almost 83% in newborn babies [43] to 5-15% in adult population [9,20,24,28,32,42] and is due to several factors. When mucosal barriers injury is present (newborn babies and oncohematological patients with intensive mucositis due to cytotoxic chemotherapy) translocation of fungal GM from food drink or even *Aspergillus* spp. present in the gastrointestinal tract is possible and leads to false positivity [24]. In two of our patients with false positive GM there was a well known factor: the presence of GVHD [12] and one of them had also intensive grade IV mucositis. In these cases plasma levels of ß-D-glucan (BG) (Fungitell, Associates of Cape Cod, Falmouth, MA, USA) could help to identify such false positive results, since usually they are not concordant, and the combination of GM and BG test may enable a positive predictive value of 100% [32]. Since indirect surrogate markers for the diagnosis of IA, such as GM and BG have limitations, it is possible that by using a combination of both of them could lead to overcome the inherent limitations of each individual test and improve the diagnosis and management of IA [32,37].

Another cause of false GM reactivity is high load of *Bifidobacterium* spp. in the gut of newborns babies [24,25]. Due to cross-reacting antigens other fungi such as *Penicillium* spp., *Paecilomyces lilacinus* and *Cryptococcus neoformans* may produce false positive GM results [8,24].

Early studies by Ansorg [3] showed ELISA GM reactivity of betalactam antibiotics, followed by several other European reports [1,5,26,43,46] as possible causes of false positive GM reactivity. Occasionally this fact has been questioned [33] and currently the manufacturer of P-T has not provided a satisfactory answer [48].

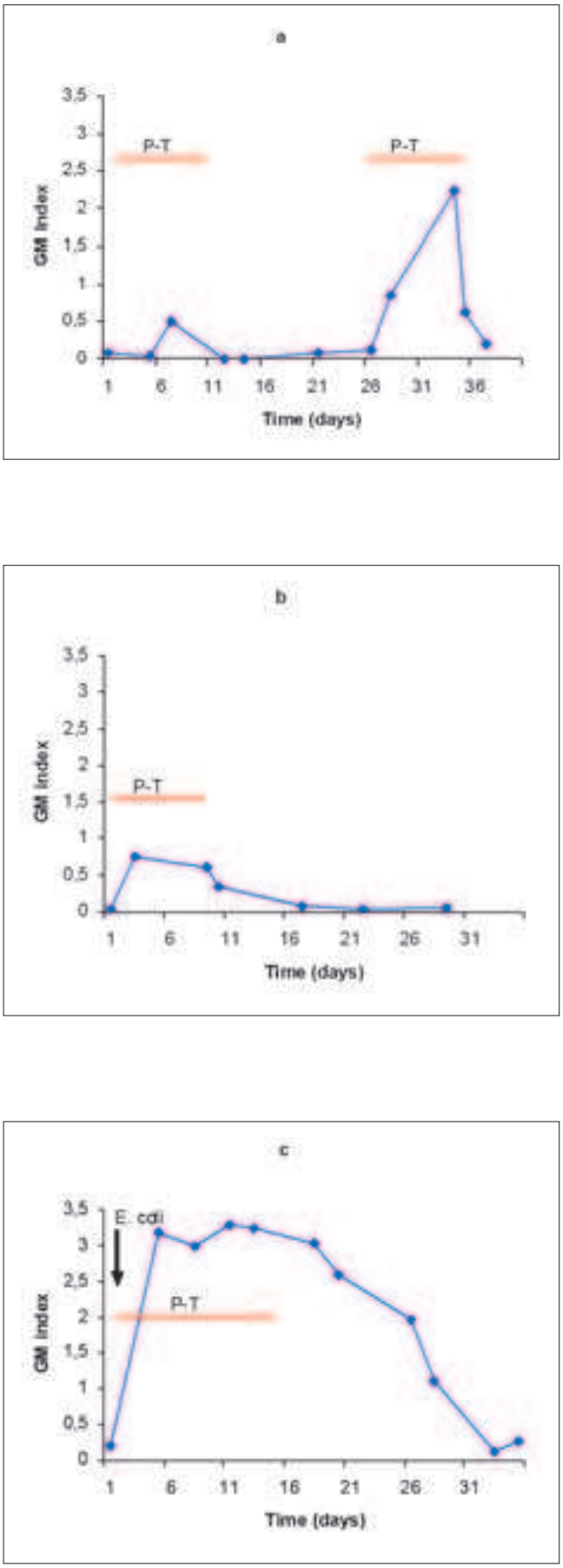

Figure 1. Three patterns of circulating GM (♦) using a Platelia *Aspergillus* in patients with false positive antigenemia due to piperacillin-tazobactam (P-T) treatment.

Table 3. Incidence of false positive galactomannan EIA results in 78 patients.

Risk group	Patients (n)	False positive GM		
		P-T	GVHD	Total
High	16	1	1	2
Intermediate High	17	1	1*	2
Intermediate Low	37	3	0	3
Low	8	1	0	1

*This patient had also grade IV mucositis.
P-T: Piperacillin-tazobactam; GVHD: graft versus host diseases.

According to Aubry et al. [5] the average half-life of elimination of GM is 2.4 days until treatment with P-T is interrupted. The kinetics of ß-lactam drugs, shows that the half-life does not exceed 1 h after intravenous infusion, therefore this would strongly suggest that the drug itself is not apparently responsible for the false positive reaction. The average time to negative antigen (GM index < 0.5) is estimated in the same report to be 5.5 days.

Walsh et al. [47] have reported that 38.5% of hospitalized patients with no evidence of IA and healthy blood bank donors receiving P-T had serum GM index values (GMI) > 0.5 compared to none of 23 subjects receiving other antibiotics. It appears consequently that among antibiotics commonly used in the setting of immnunocompromised patients, only P-T contains significant amounts of GM in vitro, and that some but not all patients receiving P-T will demonstrate circulating GM above a cut-off of 0.5, which is considered positive for IA. The data reported by us in this report agree with those of Walsh et al. [47] since from 18 patients treated with P-T, only six (33.3%) patients had GM false reactivity. In that report, Walsh et al. [47] also indicate that a significant level of GMI (> 0.5) depends upon when the blood was drawn in relation to the infusion of P-T, as well as the length of time over which the patient had been receiving P-T, suggesting that drawing the serum sample prior to the next dose of P-T may minimize but not eliminate the presence of false positive GM reactivity. This fact has also been reported by Singh et al. [41] and Machetti et al. [16]. However, in the clinical setting it could be cumbersome or difficult to comply with this timing, although what seems and important issue is that the awareness of antibiotics that the patient may be receiving is important in the interpretation of the results of a positive GM. Currently further data are needed in immunocompromised patients undergoing prolonged therapy with P-T and/or with renal or hepatic failures since the kinetics of GM is influenced by renal clearance and hepatic metabolism [11].

Our study supports evidence that false positive GM antigenemia is associated with P-T, in agreement with other published studies [1,3,5,6,16,26,41,43,46,47]. In our patients, antigenemia disappeared when P-T treatment was stopped, although it could be very rapid (one day) or even as long as 18 days in one of our patients. This patient (Fig 1c) had the longest course of P-T treatment and bacteremia caused by *E. coli,* which could perhaps explain the slower decline of GMI. Gram negative bacteremia has been shown to be associated with false positive GMI [44]. The variable concentration of GM in the batches could be a factor influencing de decline of GM after stopping treatment, although a pitfall of our study is that we did not determine GMI in the batches of P-T. This last factor is also supported by the fact that not all patients treated wit P-T have GM false reactivity, as occurred in our patients and other published reports [5,33]. The kinetics of two of our patients (Figure 1a and 1b) are similar to the patterns described by Bart-Delabesse [6].

Other antibiotics such as amoxicillin-clavulanate (A-C) may also lead to false positivity GM reactivity (cut off > 0.5) [6,19,23,26]. In our population (data not shown), nine patients (11.5%) of 78 were treated empirically with A-C, but none of them had false positive GM reactivity.

One of the difficulties for the clinician in the daily practice and management of adult patients with hematological malignancy is the interpretation of positive GM serum results, because the clinician cannot definitely rule out the possibility of IA, particularly in patients that have either high risk or even intermediate risk of developing IFI (see Table 3); an added difficulty is that in most published prospective studies in adult patients positive GM may be detected (in 40-68% of patients) at a mean of eight days before clinical signs and/or symptoms or radiologic signs appear in imaging techniques (HRCT) [18,20,24,32]. Another difficulty is that signs and symptoms of IA are not specific or may be even absent during the life of patients and the diagnosis is established post-morten. Thus, in neutropenic patients with cancer the false reactivity with GM may lead to undesirable (semi)-invasive investigations, over-treatment with expensive antifungal drugs that may produce toxicity and undesirable side-effects, as occurred in several of our patients.

For almost thirty years the standard of care of febrile neutropenic patients has been based on an empirical approach using intravenous antifungal agents [30]. The introduction of fluconazole for prophylaxis in this population virtually eliminated infections due to *Candida* spp. [36], appearing a shift to *Aspergillus* spp. [7], and currently the empirical administration of antifungals for fever refractory to broad spectrum antibiotics is the standard of care. A timely and reliable diagnosis of IA in oncohematological patients or adult patients receiving allogenic or autologous SCT is difficult, insensitive and slow resulting in late diagnosis and treatment and leading to very high mortality. But prophylaxis and empiric treatment strategies lead to high toxicities, cost and high environmental pressure which may end in the development of secondary antifungal resistance. Although the use of sensitive surrogate markers such as prospective screening of GM has limitations [2,34,39], their use may allow the shift from empirical to pre-emptive therapy when employing also imaging techniques as Maertens et al. [18] have shown in an elegant pilot study.

It should be stressed that assessment of risk is an important issue when screening GM. Since adult patients with neutropenia and hematological malignancy, stratified as either having high risk or intermediate risk, have increasing prevalence of IA [27,29,38], is in this setting were GM seems to be a useful tool for diagnosing IA, as has been shown by our group [27,29,34,38,39].

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