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APPLIED MICROBIAL AND CELL PHYSIOLOGY

Clinical validity of bis(methylthio)gliotoxin for the diagnosis of invasive aspergillosis

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Abstract Early and accurate diagnosis of invasive aspergillosis (IA) is one of the most critical steps needed to efficiently treat the infection and reduce the high mortality rates that can occur. We have previously found that the *Aspergillus* spp. secondary metabolite, bis(methylthio)gliotoxin (bmGT), can be detected in the serum from patients with possible/probable IA. Thus, it could be used as a diagnosis marker of the infection. However, there is no data available concerning the sensitivity, specificity and performance of bmGT to detect the infection. Here, we have performed a prospective study comparing bmGT detection with galactomannan (GM), the most frequently used and adopted approach for IA diagnosis, in 357 sera from 90 episodes of patients at risk of IA. Our results, involving 79 patients that finally met inclusion criteria, suggest that bmGT presents higher sensitivity and positive predictive value (PPV) than GM and similar specificity and negative predictive value (NPV). Importantly, the combination of GM and bmGT increased the PPV (100 %) and NPV (97.5 %) of the individual biomarkers, demonstrating its potential utility in empirical antifungal treatment guidance and withdrawal. These results indicate that bmGT could be a good biomarker candidate for IA diagnosis and, in combination with GM, could result in highly specific diagnosis of IA and management of patients at risk of infection.

Keywords Bis(methylthio)gliotoxin · Invasive aspergillosis · Diagnostic accuracy · Galactomannan · Gliotoxin

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Introduction

Invasive aspergillosis (IA) is a severe opportunistic infection with high morbidity and mortality rates caused by fungi of the genus *Aspergillus*, which mainly affects neutropenic patients (Kousha et al. 2011). The high mortality rates are in part due to deficiencies associated with early and specific diagnosis. IA diagnosis has evolved during the last years thanks to the introduction of galactomannan (GM) detection (Maertens et al. 1999, 2001, 2002). In 2008, this biomarker was included by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) as defining criteria of probable IA (De Pauw et al. 2008). Nevertheless, there is increasing concern about its reliability due to false positives primarily caused by beta-lactam antibiotics or intravenous solutions (Sulahian et al. 2003; Martín-Rabadán et al. 2012) and false negatives due to antimould prophylaxis (Marr et al. 2005). Although other non-culture-based diagnostic tools have been developed, its routine use in clinical diagnosis is unequal due to several reasons: 1,3-beta-D-glucan is not specific of *Aspergillus* species since it is a cell wall component of many fungi; PCR is a promising tool but

the lack of standardization has precluded its widespread clinical adoption (White et al. 2010; Arvanitis et al. 2014). The clinical utility of the first commercially available PCR assay MycAssay *Aspergillus* (Myconostica, Ltd) has to be properly evaluated in large prospective studies (White et al. 2011; Danylo et al. 2014). The same applies to the recently commercialized lateral flow device which detects an extracellular glycoprotein (White et al. 2013). Finally, it has been described that certain volatile organic compounds such as 2-pentylfuran can be detected in the breath of patients infected with *Aspergillus* spp. as they tend to produce them (Chambers et al. 2009). However, large studies are needed to warrant its clinical utility.

Due to the above-mentioned problems, investigation of new biomarkers is required (Arvanitis and Mylonakis 2015). Gliotoxin (GT) has been proposed as a diagnostic biomarker of IA (Lewis et al. 2005a; Puri et al. 2010; Cerqueira et al. 2014). GT belongs to a class of toxins with a common epipolythiodioxopiperazine structure, characterized by an internal disulphide bridge which is responsible of its biological activity (Müllbacher et al. 1986). It is well known that GT mediates immunosuppressive and proapoptotic effects, acting as virulence factor during hyphal growth (Waring et al. 1988; Sutton et al. 1996; Tsunawaki et al. 2004; Sugui et al. 2007). The most important GT producing opportunistic human pathogen is *Aspergillus fumigatus*, responsible of the majority of the IA cases (Latgé 1999). Other non-*A. fumigatus Aspergillus* species (*A. flavus*, *A. terreus*, *A. niger*) commonly causing IA can also produce GT (Lewis et al. 2005b). *Penicillium* spp., *Gliocladium* spp., *Trichoderma virens*, *Colletotrichum gloeoporoides* or *Pseudallescheria sp.* MFB165 are also able to produce GT (Gardiner et al. 2005; Li et al. 2006; Guimarães et al. 2010; Dolan et al. 2014), but they are not common opportunistic human pathogens. The genus *Candida*, the most frequent cause of invasive fungal infection, lacks to produce GT (Kupfahl et al. 2007). All these characteristics make GT suitable as a specific IA biomarker. However, GT is a very reactive compound that it is rapidly took up by tissues and circulating cells and hard to detect in body fluids (Amitani et al. 1995; Bernardo et al. 2003; Domingo et al. 2012). This could be due to the high reactivity of GT since its disulphide bridge is very unstable, and it is rapidly cell associated and removed from body fluids (Waring et al. 1988). Conversely, bis(methylthio)gliotoxin (bmGT) is a more stable inactive metabolite produced by the blockage of the disulphide bond present in GT by methylation (Müllbacher et al. 1986). GT methylation seems to be used by the fungus to regulate GT synthesis. Thus, active GT activates a fungal methyltransferase that induces the synthesis of bmGT (Dolan et al. 2014). This methyltransferase is called gliotoxin bis-thiomethyltransferase (GtmA), and it is encoded by the gene *gtmA*. It has been described that all species belonging to the filamentous ascomycetes contain a GtmA homolog and thus, the potential ability of producing bmGT. Accordingly, bmGT has been identified in several of the GT-producing fungal species

like *A. fumigatus*, *Penicillium* spp., *T. virens*, *Gliocladium* spp., *C. gloeosporoides* or *Pseudallescheria sp.* MFB165 (Kirby et al. 1980; Guimarães et al. 2010; Domingo et al. 2012; Sun et al. 2012). Studies describing the distribution of bmGT production within the *Aspergillus* genus are lacking. We have been able to identify bmGT production in cell cultures from *A. fumigatus*, *A. flavus*, *A. niger* and *A. nidulans* but not from *A. terreus* (manuscript in preparation). Our group demonstrated that bmGT is produced by a higher percentage of isolates of *A. fumigatus* than GT. BmGT is also recovered from serum of a higher amount of patients at risk of IA than GT (Domingo et al. 2012). These results suggest that bmGT is a more reliable indicator of infection than GT. Thus, bmGT has the potential to be a suitable biomarker for IA diagnosis. However, results on the clinical validity of bmGT to accurately diagnose IA are not yet available. In this study, we have assessed the diagnostic validity of bmGT compared to GM for IA diagnosis. As a secondary outcome, we have analysed its correlation with mortality and antifungal treatment.

Material and methods

Patients and study design

Patients hospitalized in a tertiary care center to whom GM detection was requested from May to December 2013 were included in the study. They were prospectively and consecutively recruited if they met the inclusion criteria: underlying disease (hematologic or oncologic malignancy and solid organ transplant recipients) or host factors according to the EORTC/MSG criteria (neutropenia for more than 10 days, allogenic stem cell transplant, prolonged use of corticosteroids, treatment with T cell immunosuppressants) (De Pauw et al. 2008). GM was requested as a screening tool of IA regardless of clinical symptoms. Clinicians were unaware of GT and bmGT results. The study followed a double-blind protocol where investigators involved in the analysis of GT and bmGT were different from those involved in medical record revision. Data collection was retrospective; if the medical record was not available, the patients were excluded from the study. The study was approved by the local ethics committee CEICA (PI12/0041 and PI15/0197). Standards for reporting of diagnostic accuracy (STARD) were followed (Altman and Bossuyt 2005).

GM, bmGT and GT assays

GM detection was performed in serum samples with the commercially available Platelia™ *Aspergillus* Ag Kit (BioRad, Hercules, CA, USA) twice weekly, according to the manufacturer's recommendations. A result was considered positive for optical reading index ≥ 0.5. A second sample was requested for confirmation of low indexes (<0.7).

BmGT and GT detection was performed by high-performance thin layer chromatography (HPTLC) as described by Domingo et al. (2012). Briefly, GT and bmGT were extracted with dichloromethane from serum samples and applied onto HPTLC plates. Separation and quantification were performed using the mixture tetrahydrofuran/n-heptane/acetonitrile (40:58:2 (v/v/v)) for 25 min in a horizontal development chamber. Peak area after ultraviolet scanning densitometry was measured for GT and bmGT quantification. Any result of GT and bmGT was considered positive.

Data analysis

Receiver operator characteristic (ROC) curves were constructed using EPIDAT 3.1. Sensitivity, specificity, positive and negative predictive values (PPV, NPV) were calculated. The cut-off value was calculated by the Youden index. As EORTC/MSG criteria include GM within the definition of probable IA, it introduces a bias for the direct comparison with bmGT. As previously described by Barnes et al. (2013), we resolved this problem by two different strategies: (i) modifying EORTC/MSG criteria to exclude GM from the definition and taking as probable cases those with positive microbiology to *Aspergillus* spp. and (ii) adding bmGT to EORTC/MSG criteria, giving the same value to bmGT as to GM. The association between qualitative variables was calculated by hypothesis testing chi-square; odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Comparison between categorical and quantitative variables was analyzed by Mann-Whitney U test. Two-sided P values <0.05 were considered statistically significant.

Results

Patient's baseline characteristics

From May to December 2013, sera from 105 consecutive patients were sent for GM determination to the laboratory. It was not possible to review the medical record from 18 patients. Eight patients did not meet inclusion criteria. Finally, a total of 79 patients met inclusion criteria, corresponding to 90 hospitalization events (Fig. 1). Sixty seven percent of the patients were male. The average age was 64.9 ± 16.9 years. The most frequent underlying disease was hematological (75.6 %); oncological patients were 18.9 % and solid organ transplant recipients 4.4 % (Table 1).

Incidence of IA

According to the revised definitions provided by the EORTC/MSG, there were 10 episodes (11.1 %) of possible IA, five episodes of probable IA (5.5 %) and a proven episode of IA (1.1 %). The incidence of proven/probable aspergillosis was

6.6 %. The proven case was histologically confirmed by brain biopsy. *A. fumigatus* was isolated from respiratory samples from three patients with probable disease; the fourth patient suffered a mixed infection with isolation of *A. flavus* and *A. terreus*. The fifth probable case was diagnosed by positive GM.

Diagnostic accuracy for the detection of IA

During the study period, a total of 357 sera from the 79 patients fulfilling inclusion criteria were analyzed. GT was analyzed in all the sera; none of the samples tested was positive. For this reason, henceforth, we will exclusively refer to bmGT. A summary of the analyses is shown in Table 2.
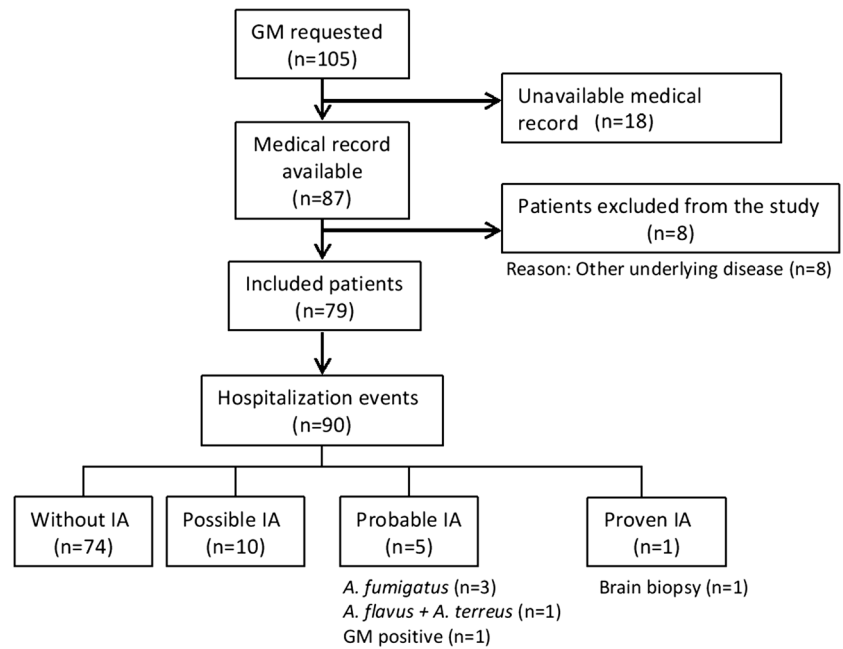
Probable/proven cases

There were 13 sera from six cases of probable/proven IA, 12 from probable IA and one from proven IA. Four of these six cases were positive for both biomarkers (GM and bmGT) in at least one serum (cases 1, 2, 4 and 6). Another one was negative for both biomarkers (case 3), and the last one was positive for bmGT but negative for GM (case 5) (Table 2).

Possible cases

There were 34 sera from 10 cases of possible IA without mycological evidence (culture or GM) of fungal disease but showing clinical and radiological evidences of disease. This group was considered to have no IA. From these ten cases, two patients had a GM index between 0.5 and 0.7, which was not confirmed in the following sample. Both patients (11 and 16) died between 8 and 16 days after initiating broad spectrum antimould treatment, suggesting probable disease. In contrast to GM index that was considered negative, both cases were positive for bmGT supporting a diagnosis of probable infection and the potential utility of bmGT for IA detection. From the remaining 8 cases, four were positive for bmGT; three of them required different antimould treatments and died between 33 and 55 days after (cases 8, 13 and 15). Among the patients with negative bmGT, two died in less than 25 days, one receiving voriconazole (case 14) (Table 2).

Patients not fulfilling EORTC/MSG criteria

There were 310 sera from 24 cases that did not fulfill EORTC/MSG criteria. Within this group, 20 sera corresponding to 15 cases were falsely positive in GM, and 12 sera corresponding to 9 cases were falsely positive in bmGT. The remaining sera were negative for both biomarkers.

Fig. 1 Study flowchart of patients included in the study

Diagnostic performance according to EORTC/MSG criteria:

bmGT showed higher sensitivity, PPV and NPV but slightly lower specificity than GM for probable/proven IA as shown in Table 3. ROC curve analysis showed a higher area under curve (AUC) for bmGT (0.768, 95% CI, 0.631–0.904) than for GM (0.659, 95% CI, 0.521–0.798) although the difference was not statistically significant ($p = 0.06$). The difference in

Table 1 Underlying disease and host factors of cases included in the study

	n (%)
Underlying disease	
Hematologic	68 (75.6)
Acute myeloid leukaemia	26 (38.2)
Acute lymphoblastic leukaemia	2 (2.9)
Myelodysplasia	6 (8.8)
Lymphoma	15 (22.1)
Chronic myeloid leukaemia	3 (4.4)
Chronic lymphoid leukaemia	3 (4.4)
Myeloma	8 (11.8)
Others	5 (7.4)
Oncologic	17 (18.9)
Solid organ transplant	4 (4.4)
Other underlying disease	1 (1.1)
Host factors	
Neutropenia	22 (24.4)
Stem cell transplant	12 (13.3)
Treatment with immunosuppressants	50 (55.6)
Others	6 (6.6)

AUC between GM and bmGT may be due to the fact that we have considered any value of bmGT as positive. Youden Index suggests a cut-off value of 2.17 mg/l for bmGT, with a sensitivity of 61.5 % and a specificity of 93.6 %. We looked for the correlation between quantitative bmGT and EORTC/MSG category. The Mann-Whitney U test showed statistical differences ($p < 0.01$) between the median bmGT quantity among the no IA/possible IA cases (1.31 mg/l ± 7.12 mg/l) compared to probable/proven cases (7.13 mg/l ± 10.38 mg/l).

(i) Consensus criteria without GM: similar results were obtained when GM was withdrawn from the criteria and only patients with a positive mycological culture were taken into account. This is likely due to the contribution of GM detection contributing to the category of probable IA only in one patient. Sensitivity, specificity, PPV and NPV were 55.6, 92.2, 15.6 and 98.8 %, respectively, for bmGT and 33.3, 94.0, 12.5 and 98.2 %, respectively, for GM. AUC for bmGT was 0.734 (95% CI, 0.563–0.904) and 0.637 for GM (95% CI, 0.468–0.805) without significant differences ($p = 0.17$).

(ii) Consensus criteria with bmGT: when bmGT was included in defining criteria, a bias was introduced as possible cases with detectable bmGT were considered as probable cases. Thus, higher sensitivity and PPV were got for this biomarker, 76 and 59.4 %, respectively. In contrast, for GM, the lowest sensitivity was found (20 %) without lowering PPV. Specificity and NPV were high (>93 %) for both biomarkers. AUC for bmGT (0.865, 95% CI, 0.778–0.952) was higher than for GM (0.566, 95% CI, 0.485–0.647), and in this case, the difference was statistically significant ($p < 0.01$). See Fig. 2.

When both biomarkers were used in combination, specificity, PPV and NPV reached encouraging high values, 100, 100 and 97.5 %, respectively, (Table 3),

Table 2 Serum GT, bmGT and GM results according to case classification

Case	EORTC/MSG	Number of serum received	Biomarker results (number of serum samples)					BmGT (mg/l)	Culture	Time to mortality (days)	Therapeutic approach	Antifungal
			GM(+) and bmGT(−)	GM and bmGT(+)	GM(−) and bmGT(+)	GM and bmGT(−)	GT(+)					
1	Proven	1	0	1	0	0	0	10.38	Neg	1	Targeted	Vor
2	Probable	1	0	1	0	0	0	13.68	*A. fumigatus*	4	Targeted	Cpf
3	Probable	1	0	0	0	1	0	nd	*A. fumigatus*	4	Targeted	Vor
4	Probable	4	0	1	2	1	0	2.17–18.68	Neg	15	Targeted	Cpf, AmB, Vor
5	Probable	4	0	0	2	2	0	2.60–2.80	*A. flavus + A. terreus*	14	Targeted	Vor
6	Probable	2	0	1	0	1	0	35.10	*A. fumigatus*	86	Targeted	Vor, Cpf
7	Possible	1	0	0	1	0	0	44.90	Neg	–	Without antimould	
8	Possible	2	0	0	1	1	0	10.45	Neg	39	Empirical	Vor, Cpf
9	Possible	1	0	0	0	1	0	nd	Neg	–	Empirical	Mic
10	Possible	2	0	0	0	2	0	nd	Neg	25	Without antimould	
11	Possible	2	0	0	1	1	0	0.86	Neg	16	Empirical	AmB, Cpf, Vor
12	Possible	7	0	0	0	7	0	nd	Neg	–	Empirical	Vor
13	Possible	2	0	0	1	1	0	9.33	Neg	33	Empirical	AmB
14	Possible	1	0	0	0	1	0	nd	Neg	7	Empirical	Vor
15	Possible	14	0	0	6	8	0	21.54–90.29	Neg	55	Empirical	AmB, Cpf, Vor
16	Possible	2	0	0	2	0	0	7.23–14.47	Neg	8	Empirical	AmB, Mic

M male, *F* female, *Neg* negative, *Vor* voriconazole, *AmB* amphotericin B, *Cpf* caspofungine, *Mic* micafungine, *nd* not detected

suggesting its potential utility in preemptive approaches for guiding antifungal treatment.

Correlation with mortality

Overall 30-day mortality was 21.1 % (19/90). Among patients with possible IA, 30-day-mortality was 40 % (4/10), and among patients with probable/proven IA, it was 83.3 % (5/6). In the group without IA, mortality was 13.5 % (10/74) within 30-days. We have analyzed the correlation between the presence of individual biomarkers and mortality and found a significant correlation between bmGT and mortality (OR, 3.2, 95% CI, 1.1–9.6, $p = 0.03$) but not between GM and mortality (OR, 1.3, 95% CI, 0.4–4.2, $p = 0.62$). Moreover, there was a significant difference ($p < 0.01$) between the amount of bmGT found in patients who died (2.36 mg/l ± 4.76 mg/l) compared to those who survived (1.4 mg/l ± 7.58 mg/l).

Correlation with antifungal treatment

Antimould therapy (voriconazole, amphotericin B, echinocandins) was initiated in 42 episodes. In eight of them (19 %), antifungals were administered as prophylaxis. None of these patients developed IA, and none of them had detectable bmGT but two of them were positive in GM and were considered as false positives by clinicians. Twelve patients (28.6 %) received antimoulds as treatment: six probable/proven IA patients and six patients that did not fulfil EORTC/MSG criteria with detectable GM. The remaining 22 episodes (7 possible IA and 15 without IA), which were GM negative, required antimoulds as empirical treatment due to neutropenic febrile episodes or respiratory infection in patients at high risk of IA. We found a significant correlation between bmGT presence and requirement for antimould treatment (OR, 5.68, 95% CI, 1.61–19.99, $p = 0.01$). Requirement for increased antifungal could reflect a worse clinical situation, and thus, it could be considered as an indirect marker of illness
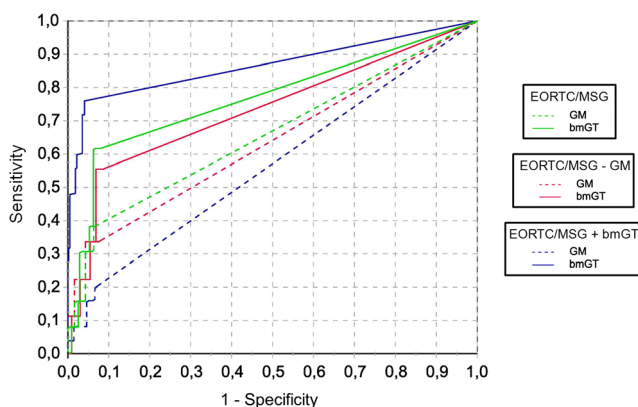

Fig. 2 ROC curve for GM and bmGT according to different criteria

Table 3 Diagnostic performance parameters for bmGT and GM

	bmGT			GM			bmGT and GM
	EORTC	EORTC minus GM	EORTC plus bmGT	EORTC	EORTC minus GM	EORTC plus bmGT	EORTC
Sens (%)	61.5	55.6	76.0	30.8	33.3	20.0	30.8
Spec (%)	93.0	92.2	96.1	94.2	94.0	93.4	100
PPV	25.0	15.6	59.4	16.7	12.5	18.5	100
NPV	98.5	98.8	98.2	97.3	98.2	93.9	97.5
AUC	0.768	0.756	0.865	0.659	0.680	0.566	0.538

Sens sensitivity, *Spec* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *AUC* area under curve

severity. Accordingly, this result suggests that bmGT might correlate with disease evolution.

Discussion

Despite recent advances, IA diagnosis is still difficult and challenging. The clinical manifestations of IA are often non-specific, and a definitive IA diagnosis depends on invasive procedures that are not always feasible due to the patient's condition. A sensitive and specific test is required to early diagnose this life-threatening complication and guide rational antifungal treatment to increase efficacy and reduce side effects and costs (Thornton 2010). Here, we compared the diagnostic accuracy of bmGT with that of GM and, in addition, the combination of both. Results are promising and suggest that bmGT and GM could be used in combination to specifically detect IA and guide antifungal therapy.

Some groups have suggested that detection of the mycotoxin GT in serum could be used for IA diagnosis (Lewis et al. 2005a; Puri et al. 2010; Cerqueira et al. 2014). In contrast to these works, we were not able to detect it in our cohort even if our technique is appropriate and shown to be sensitive (Domingo et al. 2012). This finding has been confirmed and expanded in the present work where GT was not detected in any of the analyzed sera. This is explained by the fact that we and others have shown that active GT is quickly incorporated by tissues and cells being hard to find in body fluids (Amitani et al. 1995; Bernardo et al. 2003; Domingo et al. 2012). In this sense and according to our results, bmGT seems to be a more suitable test as it was reliably detected in a large number of sera.

The detection of GM antigen has improved the managements of patients at risk of infection. In two large meta-analyses assessing diagnostic accuracy of GM for IA, sensitivity was around 70 % and specificity was around 90 %. These values vary depending on the used cut-off; the higher GM index was considered positive; the lower sensitivity and higher specificity were obtained (Pfeiffer et al. 2006; Leeflang et al. 2008). In a recent clinical study, GM screening was performed even in asymptomatic patients, the NPV

was 100 % and PPV was 11.8 % (Duarte et al. 2014). PPV reached 89.6 % if only patients with suspected IA were included. The low PPV is in accordance with that observed in our study, 16.7 %.

A single test with low PPV cannot be used to diagnose IA and to guide antifungal use. Therefore, screening algorithms based on high-risk populations, clinical criteria and the use of different biomarkers in combination are essential for diagnosis and management of this kind of patients and have proven to diminish empiric antifungal treatment without increasing mortality (Aguilar-Guisado et al. 2012; Barnes et al. 2013). Our results indicate that the combination of serum GM and bmGT substantially improves the PPV (100 %) in probable/proven IA keeping good specificity (100 %) and NPV (97 %). This finding suggests the potential utility of bmGT and GM in empirical antifungal treatment guidance and withdrawal. Other works have also evaluated the combination of biomarkers for IA diagnosis. In two clinical trials, the combination of PCR and GM detection lowered the empirical antifungal treatment, diagnosis was performed earlier and incidence of IA was reduced (Morrissey et al. 2013; Aguado et al. 2014). In the next steps, we will prospectively test if GM and bmGT detection renders a similar result in the management of patients at risk of IA.

Our study also suggests that bmGT could be more sensitive than GM with similar specificity and higher PPV. The significance of a positive bmGT result merits consideration as it correlates with mortality and increased antimould requirement. These two parameters are indicative of illness severity suggesting that bmGT could correlate with disease evolution. Moreover, high amounts of serum bmGT correlate with mortality and probable/proven IA according to EORTC/MSG criteria. The ROC curve analysis according to EORTC/MSG criteria rendered a cut-off value of 2.17 mg/l for bmGT. All but one probable/proven IA cases had a higher amount of bmGT than 2.17 mg/l. However, it was also detectable in high amounts in five of the ten possible cases. Unfortunately, we did not take samples of the intravenous formulations that patients were receiving, in an attempt to detect sources of false positives. More experiments will be required to show if bmGT

presence in patients with negative GM and negative mycological culture is indicative of IA, confirming a low sensitivity of those tests (Mennink-Kersten et al. 2004; Fraczek et al. 2014), or it is a false positive of the novel biomarker. If properly confirmed in large prospective studies, this finding would indicate that bmGT could be used not only as a diagnostic biomarker but also as marker of disease severity.

Retrospective data collection and the small sample size were the main limitations of our study. In addition, the use of HPTLC or high-performance liquid chromatography in clinical laboratories is not common and difficult to implement. On this regard, development of immunochemical methods like ELISA will be required, and our group is working on it. The optimal strategy for management of patients at risk of IA has still to be investigated. It depends on owns institutions particularities including IA prevalence, at-risk population, strategies for antifungal usage (prophylaxis, pre-emptive, empiric therapy), cost-economic studies and diagnostic tool availability. Due to the high morbid-mortality of IA all efforts should be made for early diagnosis of this severe disease that has a high economic impact in healthcare (Menzin et al. 2009; Ashley 2011). According to our study, bmGT shows potential usefulness as diagnostic tool of IA and in the management of patients at risk of aspergillosis. Nevertheless, additional prospective studies using a larger cohort of patients with IA will be required to confirm this conclusion and support the implementation of bmGT in hospitals.

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