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ORIGINAL ARTICLE

The limited role of serum galactomannan assay in screening for invasive pulmonary aspergillosis in allogeneic stem cell transplantation recipients on micafungin prophylaxis: a retrospective study

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Background
We evaluated the outcomes of serum galactomannan (GM) assay for the screening of invasive pulmonary aspergillosis (IPA) in allogeneic hematopoietic stem cell transplantation (alloHSCT) recipients while on primary antifungal prophylaxis (PAP).

Methods
This study included patients with hematologic disorders who underwent alloHSCT from January 2013 to November 2015. Patients received routine PAP with fluconazole before 2014 and micafungin after 2014; serum GM tests were performed and retrospectively analyzed. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of serum GM tests for detection of probable/proven IPA were evaluated. The serial change of serum GM levels was illustrated on a time series plot.

Results
A total of 136 alloHSCT recipients at Seoul National University Hospital were included in the study. Fluconazole was administered in 72 patients for PAP, while micafungin was administered in the remaining 64 patients. The overall sensitivity, specificity, and NPV of serum GM assays were 95.8% (95% confidence interval [CI] 78.9–99.9%), 93.8% (95% CI 91.7–95.5%), and 99.8% (95% CI 99.1–100.0%), respectively. However, the PPV of GM tests was relatively low at 35.4% (95% CI 23.9–48.2%). The serial change in serum GM levels differed according to the antifungal agents used. With effective PAP using micafungin, serial serum GM levels showed zero order kinetics during the neutropenic period.

Conclusion
Although the serum GM assay is a sensitive and specific test for detecting IPA in alloHSCT recipients, its role for routine surveillance in an era of effective PAP with micafungin is limited.

Key Words Hematology, Stem cell transplantation, Galactomannan, Invasive pulmonary aspergillosis, Antifungal prophylaxis

INTRODUCTION

Invasive pulmonary aspergillosis (IPA) is the most common life-threatening opportunistic invasive mycosis in immunocompromised hosts. The incidence of IPA is continuously increasing, mainly due to the growing number of patients undergoing intensive chemotherapy and hematopoietic stem cell transplantation (HSCT). Primary antifungal prophylaxis (PAP) reduces the incidence of IPA and improves clinical outcomes [1-3]; however, IPA continues to be a leading cause of morbidity and mortality in HSCT recipients.

Early diagnosis of IPA along with immediate initiation of appropriate therapy may further improve patient outcomes [4]. However, the diagnosis of IPA is challenging because

of nonspecific clinical signs and symptoms coupled with the frequent absence of characteristic lesions on chest imaging. Furthermore, tissue biopsy for definitive diagnosis is often invasive, and many patients' statuses preclude the use of such invasive technique. Instead, the detection of serum galactomannan (GM) antigen, a heteropolysaccharide component of the aspergillar cell wall [5], has proven useful in diagnosing IPA [6-8] and has therefore been widely used in alloHSCT recipients for IPA screening. GM is excreted by the Aspergillus during the growth phase, and serum GM levels have been suggested to be proportional to the tissue fungal load [9, 10].

The advent of newer antifungal agents such as micafungin, an echinocandin, has demonstrated improved PAP in high-risk hematology patients when compared with prior therapies [11-15]. Due to the increased efficacy of micafungin prophylaxis and resultant decreased prevalence of IPA, the clinical reliability of the serum GM assay remains uncertain. In this study, we evaluated the efficacy of the serum GM assay for IPA screening in hematologic alloHSCT recipients receiving micafungin prophylaxis, by comparing the diagnostic performance of the assay in patients receiving fluconazole prophylaxis. We hypothesized that the serum GM levels might follow zero order kinetics, which may potentially limit the clinical use of the test.

MATERIALS AND METHODS

Study subjects and treatment

A total of 136 hematology patients who received alloHSCT at Seoul National University Hospital (SNUH) from January 2013 to November 2015 were eligible for this study. Those with history of fungal infections before transplant were excluded. Patient demographics and laboratory tests were obtained by reviewing the medical records. The alloHSCT conditioning regimen varied according to the underlying hematologic disease and the overall patient condition. Prior to 2014, fluconazole 400 mg daily was administered for PAP from the start of conditioning chemotherapy until the absolute neutrophil count (ANC) of $>$1,000/μL for 3 consecutive days. Thereafter, our institution initiated routine PAP with micafungin 50 mg daily for alloHSCT recipients, to obtain data on micafungin [11-15]. Initiation of intravenous antifungal agents for PAP was dependent on clinical signs and symptoms of IPA, patient's general condition, persistent and unresponsive to broad-spectrum antibiotics fever for $>$72 hours, and/or laboratory test results.

Ethical considerations

The study was approved by the SNUH Institutional Review Board (IRB approval number: H-1607-035-776) and was conducted in accordance with Declaration of Helsinki provisions. Patient informed consent was waived because of the retrospective design of the study.

Detection and classification of the serum GM antigen

During the neutropenic period with ANC of $\leq$500/μL, serum GM assays were performed twice weekly as a routine surveillance in the absence of any signs or symptoms (GM_{surv}). Serum GM was tested more frequently as part of a diagnostic workup for infection (GM_{diag}) in the presence of suspicious signs and symptoms of IPA, namely, fever of $\geq$38.3°C, respiratory symptoms, and/or abnormal findings on chest imaging. Serum aspergillus GM antigen was detected by the direct double-sandwich enzyme-linked immunosorbent assay (Platelia Aspergillus enzyme immunoassay, Bio-Rad Laboratories, Madrid, Spain). The plates were read at an optical density (OD) of 450 nm with a reference filter of 620/630 nm. Patients were divided into four groups according to the antifungal agents used for PAP and the presence of signs/symptoms of IPA: fluconazole PAP with GM assay for surveillance (F-GM_{surv}), fluconazole PAP with GM assay for diagnostic workup (F-GM_{diag}), micafungin PAP with GM assay for surveillance (M-GMs_{urv}), and micafungin PAP with GM assay for diagnostic workup (M-GMd_{iag}) (Fig. 1). According to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) [7], the results of serum GM tests were classified into four certainty levels of IPA: no IPA, possible IPA, probable IPA, and proven IPA. In accordance with most clinical trials investigating the treatment of IPA, both probable and proven IPA were considered positive IPA [16, 17].

Statistical analysis

Descriptive and comparative analyses were performed using χ^2 test or Fisher's exact test for differences in proportions. The optimal predictive OD index of serum GM tests were

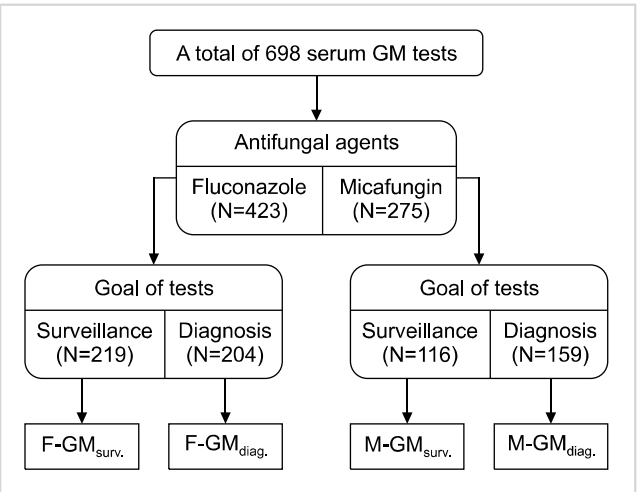

Fig. 1. Classification of a total of 698 serum galactomannan tests. A total of 423 tests were performed using fluconazole as PAP, while micafungin was administered when the remaining 275 tests were performed. Each serum GM assays were further divided according to goal of the assay: routine surveillance and diagnostic workup for infection.

obtained using the Youden index and receiver operating curve (ROC) [18], which permitted estimation of assay sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The cumulative incidence of IPA was estimated using the Kaplan–Meier method, and the Cox proportional hazard model was used to estimate the hazard ratio (HR) and its 95% confidence interval (CI). The serial change of mean ODs and their 95% CI were evaluated and illustrated on a time series plot. All reported *P*-values were two-sided, with statistical significance set at $P<0.05$. The above analysis relied on the standard software (STATA version 11; StataCor LP, College Station, Texas, USA).

RESULTS

Demographics and clinical characteristics

Table 1 shows that the majority of patients were male (N=89; 65.4%) with a median age of 45 (range, 18–68) years.

Table 1. Baseline characteristics of hematology patients receiving allogeneic hematopoietic stem cell transplantation (N=136).

Characteristics	Total (N=136)	Fluconazole (N=72)	Micafungin (N=64)	*P*
Age in year, median (range)	45 (18–68)	45 (18–68)	45 (18–65)	0.698
Gender, N (%)				0.444
Male	89 (65.4)	45 (62.5)	44 (68.8)	
Female	47 (34.6)	27 (37.5)	20 (31.2)	
Hematological disease, N (%)				0.937[a]
Acute myeloid leukemia	54 (39.7)	27 (37.5)	27 (42.2)	
Acute lymphoblastic leukemia	26 (19.1)	14 (19.4)	12 (18.8)	
Chronic myeloid leukemia	4 (2.9)	1 (1.4)	3 (4.7)	
Myelodysplastic syndrome	24 (17.7)	14 (19.4)	10 (15.6)	
Aplastic anemia	7 (5.2)	4 (5.6)	3 (4.7)	
Myelofibrosis	2 (1.5)	2 (2.8)	0 (0.0)	
Multiple myeloma	6 (4.4)	3 (4.2)	3 (4.7)	
Lymphoma	11 (8.1)	6 (8.3)	5 (7.8)	
Others	2 (1.5)	1 (1.4)	1 (1.6)	
Allogeneic HSCT, N (%)				0.066
Related	78 (57.3)	36 (50.0)	42 (65.6)	
Unrelated	58 (42.7)	36 (50.0)	22 (34.4)	
Origin of stem cell, N (%)				0.601[a]
Peripheral	133 (97.8)	71 (98.6)	62 (96.9)	
Cord blood	3 (2.2)	1 (1.4)	2 (3.1)	
Duration of neutropenia in days, median (range)	10 (2–90)	11 (0–90)	9 (3–28)	0.008
N of serum GM test, median (range)	4 (1–20)	5 (1–20)	3 (1–18)	0.023
Death, N (%)	18 (18.0)	16 (22.2)	2 (7.2)	0.133[a]

[a]Fisher's exact test.
Abbreviations: HSCT, hematopoietic stem cell transplantation; GM, galactomannan; N, number of patients.

Table 2. The diagnostic performance of serum galactomannan (GM) assays. A total of 698 serum GM assays, and four subgroups classified according to antifungal agents (fluconazole vs. micafungin) and the goal of test (surveillance vs. diagnostic workup).

	Overall		Fluconazole (N=423)				Micafungin (N=275)			
			F-GM$_{surv}$		F-GM$_{diag}$		M-GM$_{surv}$		M-GM$_{diag}$	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
No/possible IPA	42	632	13	197	4	194	10	106	8	142
Probable/proven IPA	23	1	9	0	5	1	0	0	9	0
Sensitivity	95.8 (78.9–99.9)		100.0 (66.4–100.0)		83.3 (35.9–99.6)		NA		100.0 (66.4–100.0)	
Specificity	93.8 (91.7–95.5)		91.4 (86.8–94.8)		97.5 (94.2–99.2)		NA		94.7 (89.8–97.7)	
PPV	35.4 (23.9–48.2)		33.3 (16.5–54.0)		50.0 (18.7–81.3)		NA		52.9 (27.8–77.0)	
NPV	99.8 (99.1–100.0)		100.0 (98.1–100.0)		99.5 (97.2–100.0)		NA		100.0 (97.4–100.0)	
Cutoff optical index	0.8		0.8				0.9			

Abbreviations: F-GM$_{diag}$, fluconazole PAP with GM assay for diagnostic workup; F-GM$_{surv}$, fluconazole PAP with GM assay for surveillance; IPA, invasive pulmonary aspergillosis; M-GM$_{diag}$, micafungin PAP with GM assay for diagnostic workup; M-GM$_{surv}$, micafungin PAP with GM assay for surveillance; NA, not available; NPV, negative predictive value; PPV, positive predictive value.

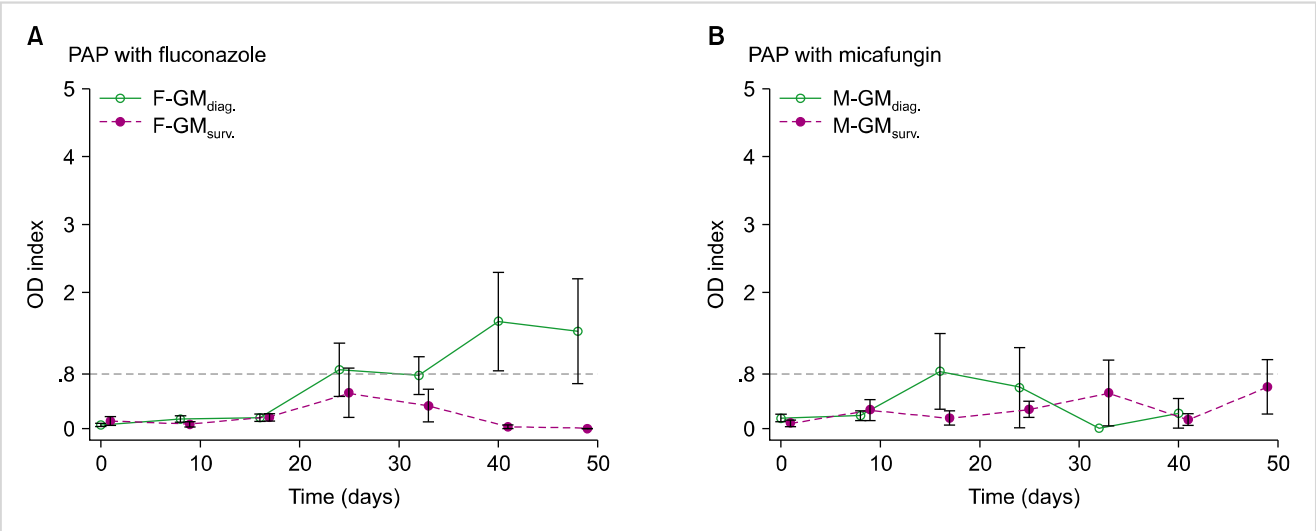

Fig. 2. Time series plots of serum galactomannan optical density (OD) index under **(A)** fluconazole prophylaxis and **(B)** micafungin prophylaxis.

The most common underlying disease that led to alloHSCT was acute myeloid leukemia (N=54, 39.7%). Overall, 78 patients (57.0%) received alloHSCT from a related donor, whereas the remaining patients (N=58, 42.7%) received stem cells from an unrelated donor. A total of 133 patients (97.8%) undergoing alloHSCT received stem cells from peripheral blood, and 3 patients (2.2%) received stem cells from cord blood. The median duration of neutropenia was 10 days (range, 2–90 days). Eighteen patients (18.0%) died during alloHSCT. Fluconazole was administered in 72 patients (52.9%) for PAP, while the remaining 64 patients (47.1%) received micafungin. Most of the clinical characteristics were comparable among all patients, except the duration of neutropenia and number of serum GM tests performed per patient.

Diagnostic performance of serum GM tests

A total of 698 serum GM assays were performed, with a median of 4 tests per patient (range, 1–20). The GM test results were classified into four certainty levels of IPA in the following proportions: no IPA (N=603; 86.4%), possible IPA (N=71; 10.2%), probable IPA (N=20; 2.9%), and proven IPA (N=4; 0.6%). Based on these data, the optimal cutoff OD indices of the serum GM level for predicting probable/proven IPA were found to be 0.8 for fluconazole prophylaxis and 0.9 for micafungin prophylaxis (Table 2). The serum GM assay with micafungin prophylaxis had an area under ROC (AUROC) of 0.750 (95% CI 0.607–0.893), which was lower than that with fluconazole prophylaxis (AUROC 0.894; 95% CI 0.819–0.968) (Supplementary Fig. 1). The overall sensitivity, specificity, and NPV of serum GM assays were 95.8% (95% CI 78.9–99.9%), 93.8% (95% CI 91.7–95.5%), and 99.8% (95% CI 99.1–100.0%), respectively. The PPV of overall serum GM assays was relatively low at 35.4% (95% CI 23.9–48.2). However, when clinically suspecting IPA, PPV was increased to 50.0% (95% CI 18.7–81.3%) in the F-GM_{diag} group and 52.9% (95% CI 27.8–77.0%) in the M-GM_{diag} group (Table 2).

Cumulative incidence of IPA and kinetics of GM OD indices

The cumulative incidence of IPA with micafungin prophylaxis tended to be low compared to that with fluconazole prophylaxis (HR 0.37; 95% CI 0.12–1.13; *P*=0.080) (Supplementary Fig. 2). With fluconazole prophylaxis, the serial change in serum GM OD index varied based on the presence of IPA signs and symptoms (Fig. 2A). As shown, the serum GM OD index of F-GM_{diag} steadily increased and exceeded the OD index of 0.8 after 3 weeks of neutropenia. In comparison, the serum GM OD index of F-GM_{surv} increased slightly but decreased shortly after 3 weeks of neutropenia. However, as we hypothesized, when micafungin prophylaxis was used, the serial pattern of OD indices followed zero order kinetics and was independent on the presence of IPA signs and symptoms (Fig. 2B).

DISCUSSION

The diagnosis of IPA has been widely determined by the detection of serum GM. This assay has a high specificity and variable sensitivity of 29–100% in hematologic HSCT recipients [19], as confirmed in the present study. The predictive value of the serum GM assay relies on several factors, such as underlying disease, duration and intensity of neutropenia [20-27], and importantly, the pretest prevalence of IPA, which is the main determinant of the assay's predictive values [28]. Micafungin is a highly effective antifungal agent for PAP when compared with fluconazole [11-15], resulting in a low pre-test prevalence of IPA. Therefore, the serum GM test has a poor predictive value when used as a screening tool in alloHSCT recipients receiving micafungin prophylaxis. As such, there have been concerns regarding the clinical utility of the serum GM assay in IPA surveillance in this setting.

Our analysis revealed that the diagnostic performance of the serum GM assay with micafungin prophylaxis is inferior to that with fluconazole prophylaxis. Remarkably, the serial change of serum GM levels varies according to the antifungal agents used for PAP. With effective antifungal prophylaxis using micafungin, the serial pattern of serum GM levels shows zero order kinetics during the entire neutropenic period.

The GM antigen is a heteropolysaccharide component of the *Aspergillus* species cell wall [5] and is proportional to the fungal tissue load. In accordance with our initial hypothesis, the serial pattern of serum GM OD indices with micafungin prophylaxis showed zero order kinetics. This pattern was independent on the presence of clinical suspicion of IPA, which is consistent with the fact that micafungin would have effectively inhibited the synthesis of 1, 3-β-D-glucan, a critical cell wall component of *Aspergillus* species, during the entire neutropenic period. This result indicates that routine surveillance of IPA during alloHSCT on micafungin PAP is less informative than that on fluconazole PAP. Therefore, the role of the serum GM assay as a routine surveillance tool is limited in this clinical setting.

The following are several limitations that must be considered regarding the findings of this study. First, the retrospective design could bias the results. Second, the cutoff OD index of the serum GM assay in our study may not be applicable to other institutions where the patient population, antifungal strategy, and prevalence of IPA may differ. Third, as previously mentioned, the predictive value of the serum GM assay depends on various factors in addition to antifungal prophylaxis. Therefore, the heterogeneity of such factors in our patient population might bias the results. Finally, incorporation bias is also a concern when the test under study is part of the reference standard [29].

In conclusion, although the serum GM assay is a sensitive and specific noninvasive test for detecting IPA in alloHSCT recipients, its use for routine surveillance is limited in an era of effective PAP with micafungin. The results of the serum GM assay should be cautiously considered in conjunction with other diagnostic procedures, such as chest imaging. Although micafungin prevents IPA effectively, IPA continues to be an important cause of life-threatening infections in immunocompromised patients and should be diagnosed in the early stages to initiate an immediate and appropriate antifungal agent. Hence, the development of a rapid and more accurate surveillance tool is of paramount importance.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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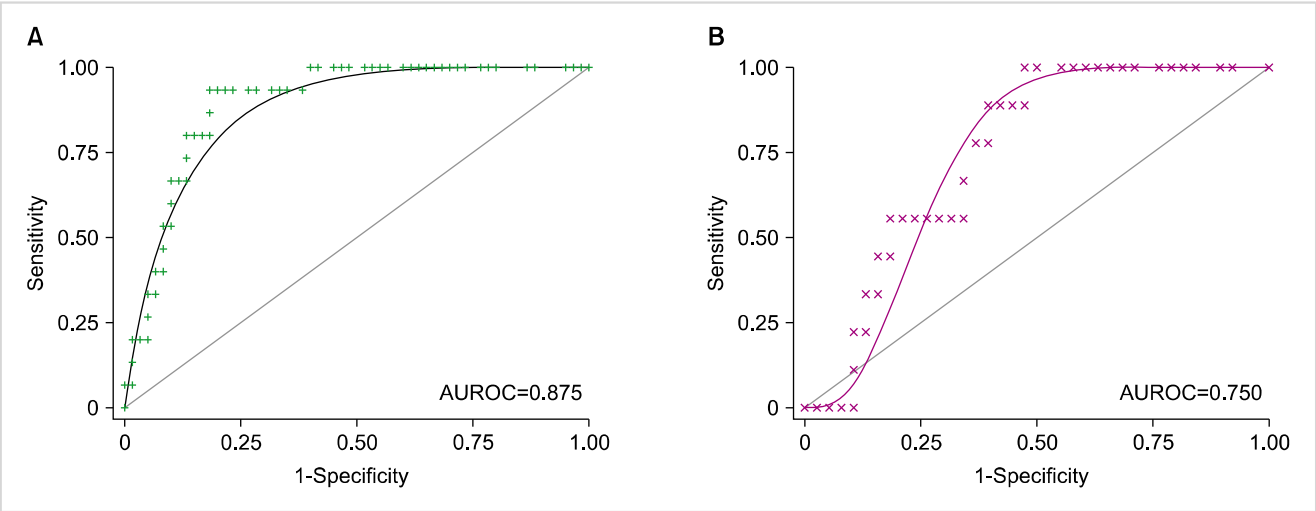
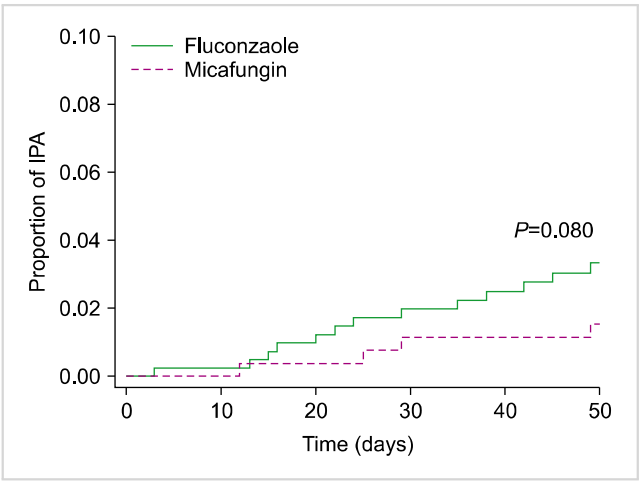


Supplementary Fig. 1. Receiver operating curves of serum galactomannan (GM) assay predicting probable or proven invasive pulmonary aspergillosis (IPA) in allogeneic hematopoietic stem cell transplant recipients receiv-ing fluconazole **(A)** and micafungin **(B)** as primary antifungal prophylaxis.
Abbreviation: AUROC, area under receiver operating curve.



Supplementary Fig. 2. Kaplan–Meier curve of cumulative incidence of invasive pulmonary aspergillosis (IPA) according to antifungal agents used as primary antifungal prophylaxis. *P*-value was estimated using the log-rank test.