Journal of Microbiology, Immunology and Infection (2018) 51, 802—809

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Clinical characteristics, radiologic findings, risk factors and outcomes of serum galactomannan-negative invasive pulmonary aspergillosis

Jiwon Jung [a,b], Mi Young Kim [c,**], Yong Pil Chong [a], Sang-Oh Lee [a], Sang-Ho Choi [a], Yang Soo Kim [a], Jun Hee Woo [a], Sung-Han Kim [a,*]

[a] *Department of Infectious Diseases, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea*
[b] *Division of Infectious Diseases, Department of Internal Medicine, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Republic of Korea*
[c] *Department of Radiology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea*

Received 14 November 2016; received in revised form 14 May 2017; accepted 17 May 2017
Available online 29 June 2017

KEYWORDS
Galactomannan;
Invasive pulmonary aspergillosis;
Neutropenia

Abstract *Background:* The sensitivity of galactomannan (GM) assay is suboptimal for detecting invasive pulmonary aspergillosis (IPA) in serum samples. However, the clinical characteristics, radiologic findings, and outcomes in patients with GM-negative IPA have not been fully elucidated.
Methods: Over a 7-year period, adult patients with proven or probable IPA by the EORTC/MSG definition were retrospectively enrolled. Patients with negative GM results and positive *Aspergillus* spp. cultures from sputum or bronchoalveolar lavage were classified into GM-negative IPA group. GM-positive and culture-negative IPA cases were selected at a 1:2 ratio.
Results: Thirty-four patients with GM-negative IPA were compared to 68 randomly selected patients from 158 patients with GM-positive and culture-negative IPA. Patients with diabetes mellitus, chronic kidney disease, and steroid use were more common but those with hematologic malignancy, prior receipt of mold-active antifungal drugs, and neutropenia were less common in GM-negative IPA than in GM-positive IPA. Regarding radiologic findings, angioinvasive aspergillosis was less common in GM-negative IPA than in GM-positive IPA. The median number of days

* Corresponding author. Department of Infectious Diseases, Asan Medical Center, University of Ulsan College of Medicine, 86 Asanbyeongwon-Gil, Songpa-Gu, Seoul, 138-736, Republic of Korea. Fax: +82 2 3010 6970.
** Corresponding author. Department of Radiology and Research Institute of Radiology, Asan Medical Center, University of Ulsan College of Medicine, 86 Asanbyeongwon-Gil, Songpa-Gu, Seoul 138-736, Republic of Korea. Fax: +82 2 3010 0090.
E-mail addresses: mimowdr@gmail.com (M.Y. Kim), kimsunghanmd@hotmail.com (S.-H. Kim).

http://dx.doi.org/10.1016/j.jmii.2017.05.007

from diagnosis to appropriate antifungal therapy was higher in GM-negative IPA than in GM-positive IPA. Multivariate analysis indicated that neutropenia (adjusted odds ratio [aOR], 0.10) and prior receipt of mold-active antifungal drugs (aOR, 0.12) were inversely associated with GM-negative IPA. The 30-day and 90-day mortality were similar between the two groups.
Conclusion: Neutropenia and prior receipt of mold-active antifungal drugs before GM assay were independently associated with GM positivity among patients with proven/probable IPA. Angioinvasive aspergillosis was less common in GM-negative IPA than in GM-positive IPA.

Introduction

As invasive pulmonary aspergillosis (IPA) is a life-threatening infection, prompt diagnosis and treatment is crucial to improve outcomes. Because early diagnosis based on cultures and/or microscopy of histopathologic specimens is generally difficult, clinicians rely heavily on chest computed tomography (CT) and serum assays for galactomannan (GM). GM assays are noninvasive and their results are useful markers for early diagnosis of IPA. However, their sensitivity is still suboptimal (approximately 70%),[1] and clinicians often encounter GM-negative invasive pulmonary mold infection. Several studies have suggested that non-neutropenia and concurrent anti-mold therapy are associated with the low sensitivity of the GM assay.[1,2] However, clinical characteristics, radiologic findings, and outcomes in patients with GM-negative IPA have not yet been elucidated. We investigated the clinical risk factors, radiologic findings, and outcomes of patients with GM-negative IPA compared to those with GM-positive IPA.

Methods

Study population

This study was performed at Asan Medical Center, a 2700-bed tertiary-care teaching hospital in Seoul, South Korea. The medical records of adult patients (aged $\geq$16 years) who met the criteria for proven or probable IPA by the revised EORTC/MSG definitions from January 2008 to December 2014 were retrospectively reviewed.

Serum GM was routinely measured twice per week in neutropenic cancer patients, and once per week in hematopoietic stem cell transplant (HCT) recipients and liver transplant recipients; otherwise, the assay was performed whenever IPA was suspected. Bronchoalveolar lavage (BAL) GM was routinely measured in patients with suspected IPA who received bronchoscopy at the discretion of each attending physician.[3] Empirical antifungal therapy such as caspofungin or (liposomal) amphotericin-B was given in neutropenic cancer patients who retained fever regardless of broad spectrum antibiotic use. Preemptive antifungal therapy was not adopted in neutropenic cancer patients during the study period. The routine antifungal prophylaxis included micafungin in HCT recipients, posaconazole, itraconazole, or fluconazole in acute myeloid leukemia or myelodysplastic syndrome patients, voriconazole in lung transplant recipients, and liposomal amphotericin-B in liver transplant recipients. No routine antifungal prophylaxis was used in kidney, pancreas, or heart transplant recipients.

Definitions

Patients were assigned with a proven or probable diagnosis of IPA according to the modification of previous studies.[4–7] Proven IPA was defined by histological evidence of tissue invasion consisting of septated, acutely branching filamentous fungi plus recovery of *Aspergillus* species in cultures of pulmonary tissue, or positivity in immunohistochemical staining with anti-*Aspergillus* monoclonal antibody (LSBio, Seattle, WA).[7] Probable IPA was defined as the presence of host factors together with one or more clinical indications such as dense, well-circumscribed lesions with or without a halo sign, and an air-crescent sign or cavity in CT scans, and mycological evidence of fungal infection (by culture or cytological analysis of BAL fluid for *Aspergillus* species or GM assay of serum or BAL fluid). The criteria of host factors included those used in criteria for EORTC/MSG[4] and AIDS.[5,6] Among patients with proven or probable IPA, those with constantly negative GM results from serum and positive *Aspergillus* spp. culture from sputum or BAL were classified into the GM-negative IPA group. We defined GM-negative IPA as cases in which GM results were persistently negative in one or more GM tests performed. We randomly selected GM-positive and culture-negative IPA cases for comparison at a 1:2 (GM-negative/positive) ratio.

GM antigen levels in serum were measured as described previously (Platelia *Aspergillus* EIA; BioRad Redmond, WA).[8,9] Serum and BAL fluid samples were considered positive if the GM index was $\geq$0.5 GM antigen level. Neutropenia was defined as $<0.5 \times 10^9$ neutrophils/L for >10 days temporally related to the onset of IPA. Appropriate antifungal therapy was defined as use of one of following drugs: voriconazole, liposomal amphotericin B, amphotericin B deoxycholate, caspofungin, micafungin, or posaconazole.

CT evaluation

Chest CT scans with or without contrast enhancement (usually high resolution CT) were performed when patients at high risk for invasive aspergillosis had symptoms (persistent fever despite the use of broad-spectrum antibiotics, cough, dyspnea, hemoptysis, or pleuritic chest

pain), positive galactomannan assay, or positive sputum/BAL culture for *Aspergillus*. CT evaluation was performed by two experienced thoracic radiologists in a retrospective manner. Where there was disagreement, the final decision was made by consensus. We used a glossary of CT imaging definitions to categorize pulmonary lesions. We categorized the main CT findings into angioinvasive form and airway-invasive form. Angioinvasive form was defined if at least two of the following features were found in the predominant CT image: a halo sign, an infarct-shaped consolidation, or an internal low-attenuation, cavity, or air-crescent sign.[3] Airway-invasive form was defined if at least two of the following features were found: small airway lesions, a peribronchial consolidation, or a bronchiectasis.[3] It was classified as a combined form when CT findings met the criteria for both types and it was designated undetermined when the findings did not fulfill any of the above criteria. To simplify the patterns, we then reassigned these forms to two types, angioinvasive (including the combined form) and non-angioinvasive (including the airway invasive and undetermined forms).

Statistical analysis

Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate, and continuous variables were compared using the Mann—Whitney U-test, as appropriate. All tests of significance were two-tailed, and a P value < 0.05 was considered to indicate statistical significance. Risk factors for 30-day mortality and GM-negative IPA were analyzed by performing a backward stepwise logistic regression analysis. Calculations were performed with SPSS for Windows, version 21K (SPSS, Chicago, IL).

Results

Patient characteristics and radiologic findings of GM-negative IPA

A total of 260 patients who met the criteria of proven or probable IPA were identified during the study period. Of these, one patient was excluded because GM results were not available. Of the remaining 259 patients, 158 (61%) were classified as GM-positive, culture-negative IPA; 67 (26%) were GM-positive, culture-positive IPA; and 34 (13%) were GM-negative, culture-positive IPA. These latter 34 patients were compared with 68 randomly selected patients from the 158 with GM-positive, culture-negative IPA. Of the 34 patients with GM-negative IPA, 29 received GM assay more than twice (interval was more than 4—7 days). These 29 (85%) patients revealed constantly negative GM results. The remaining five (15%) patients including two patients with proven IPA received GM assay once either at the day of CT scan or after CT scan, and revealed negative GM results.

Patients with diabetes mellitus, chronic kidney disease, and steroid use were more common, whereas those with hematological malignancy, neutropenia, or who had received cytotoxic chemotherapy were less common, in the GM-negative IPA group than in the GM-positive IPA group (Table 1). Interestingly, prior receipt of mold-active antifungal drugs before GM assay, which was correlated with neutropenia, was less common in GM-negative IPA than in GM-positive IPA (27% vs. 79%; $P < 0.001$). The median number of days from diagnosis to appropriate antifungal therapy was higher in GM-negative IPA than in GM-positive IPA (4 days vs. −1 days; $P < 0.001$).

Regarding radiologic findings, the presence of infarct-shaped consolidation (3% vs. 21%; P = 0.02), a halo sign (29% vs. 52%; P = 0.04), an air bronchogram (27% vs. 52%; P = 0.02), and/or pleural effusion (32% vs. 54%; P = 0.04) were less common in patients with GM-negative IPA than in those with GM-positive IPA (Table 2). Angioinvasive type was also less frequent in patients with GM-negative IPA than in those with GM-positive IPA (12% vs. 35%; P = 0.01), and the undetermined form and the presence of cluster of centrilobular nodule were more common in patients with GM-negative IPA than in those with GM-positive IPA (68% vs. 47%; P = 0.049, 50% vs. 28%; P = 0.047, respectively). The CT findings in non-neutropenic patients with IPA shared similar characteristics with those with GM-negative IPA. The CT findings revealed that mass-like consolidation (33% vs. 54%; P = 0.03), halo sign (28% vs. 56%; P = 0.005), and air bronchogram (26% vs. 56%; P = 0.002) were less common in non-neutropenic patients with IPA than in neurotropenic patients with IPA, while cavitary lung lesion (42% vs. 20%; P = 0.02) and centrilobular nodules (49% vs. 25%; P = 0.02) showed an opposite trend (Supplemental Table 1).

Risk factors for GM-negative IPA

We analyzed the risk factors for GM-negative IPA in the 102 patients with IPA (Table 3). Multivariate analyses using variables with P values < 0.1 in univariate analyses indicated that neutropenia (adjusted odd ratio [aOR], 0.10 [95% CI, 0.04 to 0.31]; $P < 0.001$) and prior receipt of mold-active antifungal drugs before GM assay (aOR 0.12 [95% CI, 0.04 to 0.37]; $P < 0.001$) were inversely associated with GM-negative IPA. As shown in Supplemental Figures 1 and 2, scatterplots showing the line of best fit between absolute neutrophil count and GM titer at diagnosis day (Supplemental Figure 1) and between days from diagnosis to receipt of mold-active agents and GM titer at diagnosis day had negative slopes (Supplemental Figure 2). We further analyzed the association between prior receipt of mold-active antifungal drugs and GM-negative IPA stratified by the presence of neutropenia (Supplemental Table 2). In patients without neutropenia, GM-negative IPA was more common in patients who had not received such prior agents than those who had (19% vs. 81%, $P < 0.001$). Conversely, in patients with neutropenia, GM-negative IPA tended to be more common in patients who had received them than in those who had not (57% vs. 43%, $P = 0.21$).

Outcomes of GM-negative IPA

The 30-day and 90-day mortality were similar between the two groups (24% versus 28%; $P = 0.81$, and 41% versus 54%; $P = 0.29$, respectively) (Table 1). We analyzed the risk factors for 30-day mortality in 102 patients with IPA (Table 4). Multivariate analyses indicated that chronic

Table 1 Baseline clinical and mycological characteristics, treatments, and outcomes of 34 patients with serum GM-negative IPA and 68 patients with serum GM-positive invasive pulmonary aspergillosis.

Characteristic	GM-negative, culture-positive IPA (n = 34)	GM-positive, culture-negative IPA (n = 68)	*P* value
Age, years, median (IQR)	53 (47–63)	53 (43–62)	0.45
Male gender	25 (74)	48 (71)	0.82
Underlying disease			
Hematologic malignancy	9 (27)	36 (53)	0.01
Solid organ transplant	11 (32)	10 (15)	0.07
Hematopoietic stem cell transplant	8 (24)	20 (29)	0.64
Diabetes mellitus	15 (44)	8 (12)	0.001
Chronic kidney disease	9 (27)	6 (9)	0.03
AIDS	2 (6)	0 (0)	0.11
Underlying condition			
Steroid use[a]	27 (79)	26 (38)	<0.001
Immunosuppressant use[b]	16 (47)	30 (44)	0.84
Neutropenia (ANC < 500/m^3)	7 (21)	52 (77)	<0.001
Cytotoxic chemotherapy within 1 month	9 (27)	46 (68)	<0.001
Median time from transplantation, days, median (IQR)[c]	177 (59–314)	117 (32–379)	0.70
Prior receipt of mold-active antifungal drugs before GM assay	9 (27)	54 (79)	<0.001
Itraconazole	1 (3)	17 (25)	0.006
Polyene	5 (15)	26 (38)	0.02
Micafungin	0 (0)	1 (1)	>0.99
Caspofungin	0 (0)	3 (4)	0.55
Voriconazole	3 (9)	6 (9)	>0.99
Posaconazole	0 (0)	1 (1)	>0.99
Diagnostic category of IPA			
Proven	3 (9)[e]	1 (1)[f]	0.07
Probable	31 (91)[g]	67 (98)[h]	
Positive BAL GM assay	7/13 (54)	3/10 (30)	0.25
Antifungal therapy at diagnosis day			
Polyene	19/30 (63)	34 (50)	0.27
Voriconazole	8/30 (27)	25 (37)	0.36
Itraconazole	1/30 (3)	3 (4)	0.8
Caspofungin	0 (0)	4 (4)	0.58
None	4 (12)	0 (0)	0.004
Median days from diagnostic day to appropriate antifungal therapy, median (IQR)[d]	4 (0–9)	−1 (−9∼0)	<0.001
Delayed therapy[i]	21 (62)	10 (15)	<0.001
Outcome			
30-day mortality	8 (24)	19 (28)	0.81
90-day mortality	14 (41)	37 (54)	0.29

[a] Use of corticosteroids at a mean minimum dose of 0.3 mg/kg/day prednisolone equivalent for >3 weeks.
[b] Treatment with other recognized T cell immunosuppressants such as cyclosporine, TNF-a blockers, or specific monoclonal antibodies (such as alemtuzumab) during the past 90 days.
[c] Analysis limited to transplant recipients.
[d] Use of voriconazole, liposomal amphotericin B, amphotericin B, echinocandin, or posaconazole.
[e] The 3 patients with GM-negative IPA had positive *Aspergillus fumigatus* cultures from sterile pulmonary tissue.
[f] One patient with GM-positive IPA exhibited a histomorphology compatible with aspergillosis and had positive results with the anti-*Aspergillus* antibody but negative results with the anti-*Rhizopus arrhizus* antibody.
[g] The 31 patients with GM-negative IPA had positive *Aspergillus* spp. cultures (9 reported as *A. fumigatus*, 1 reported as *A. niger*, the other reported as *Aspergillus* spp.) from sputum, and one patient also had a positive BAL culture (*Aspergillus* spp.).
[h] No one had positive sputum or BAL cultures.
[i] Delayed therapy was defined as appropriate therapy given at least 3 days after diagnosis.
Data are presented as No. (%) unless indicated otherwise. GM, galactomannan; IPA, invasive pulmonary aspergillosis; IQR, interquartile range; ANC, absolute neutrophil count; BAL, bronchoalveolar lavage.

Table 2 Comparison of computed tomography findings between 34 patients with serum GM-negative IPA and 68 patients with serum GM-positive IPA.

	GM-negative, culture-positive IPA (n = 34)	GM-positive, culture-negative IPA (n = 68)	*P* value
Macronodule (≥1 cm in diameter)	18 (53)	33 (49)	0.83
Single	6 (18)	10 (15)	0.78
Multiple	12 (35)	23 (34)	>0.99
Consolidation, mass shaped	15 (44)	31 (46)	>0.99
Single	11 (32)	24 (35)	0.83
Multiple	4 (12)	7 (10)	0.82
Consolidation, infarct shaped	1 (3)	14 (21)	0.02
Single	1 (3)	8 (12)	0.14
Multiple	0 (0)	6 (9)	0.07
Halo sign	10 (29)	35 (52)	0.04
Clusters of centrilobular nodules (<1 cm)	17 (50)	19 (28)	0.047
Consolidation, peribronchial	11 (32)	30 (44)	0.29
Ground glass opacity	17 (50)	38 (56)	0.67
Small airway lesion	2 (6)	9 (13)	0.26
Smooth bronchial wall thickening	11 (32)	22 (32)	>0.99
Air-crescent sign	3 (9)	4 (6)	0.58
Cavitary lesion	13 (38)	17 (25)	0.18
Internal low attenuation	6 (18)	18 (27)	0.46
Reverse halo sign	4 (12)	8 (12)	>0.09
Air bronchogram	9 (27)	35 (52)	0.02
Atelectasis	0 (0)	14 (21)	0.004
Hilar/mediastinal lesion	2 (6)	8 (12)	0.35
Pleural effusion	11 (32)	37 (54)	0.04
Pericardial effusion	2 (6)	9 (13)	0.26
Angioinvasive type	4 (12)	24 (35)	0.01
Angioinvasive form[a]	4 (12)	19 (28)	0.07
Combined form[b]	0 (0)	5 (7)	0.17
Non-angioinvasive type	30 (88)	54 (79)	0.27
Airway-invasive form[c]	7 (21)	22 (32)	0.25
Undetermined form[d]	23 (68)	32 (47)	0.049

[a] Designated when the predominant CT image had at least two of the following findings: a halo sign, an infarct-shaped consolidation, an internal low attenuation, cavity, or air-crescent sign.
[b] Designated when CT findings included both angioinvasive and airway-invasive features.
[c] Designated when the predominant CT image had at least two of the following findings: small airway lesions, a peribronchial consolidation, a bronchiectasis.
[d] Designated when the pattern did not conform to the above criteria.
Data are presented as No. (%) unless indicated otherwise.

Table 3 Univariate and multivariate analyses of GM-negative IPA in 102 patients with IPA.

Variable	Univariate analyses	Multivariate analyses	
	OR (95% CI)	aOR (95% CI)	*P* value
Neutropenia	0.08 (0.03–0.22)	0.10 (0.04–0.31)	<0.001
Solid organ transplantation	2.77 (1.04–7.41)		
Prior receipt of mold-active antifungal drugs before GM assay	0.09 (0.04–0.24)	0.12 (0.04–0.37)	<0.001
Computed tomography findings			
Halo sign	0.39 (0.16–0.95)		
Air bronchogram	0.34 (0.14–0.83)		
Centrilobular nodules	2.58 (1.10–6.07)		
Pleural effusion	0.40 (0.17–0.95)		

kidney disease (HR, 7.16 [95% CI, 1.30 to 39.44]; $P = 0.02$), neutropenia (HR, 9.05 [95% CI, 1.88 to 43.70]; $P = 0.006$), intensive care unit care (HR, 2.75 [95% CI, 0.87 to 8.74]; $P = 0.09$), presence of pleural effusion (HR, 3.48 [95% CI, 1.01 to 11.99]; $P = 0.048$), and presence of a reverse halo sign (HR, 10.12 [95% CI, 1.73 to 59.35]; $P = 0.01$) were

Table 4 Hazard ratios for 30-day mortality in 102 patients with IPA.

Variable	Death (n = 27)	Survival (n = 75)	Unadjusted		Adjusted	
			Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age > 65 years	7 (26)	11 (15)	2.04 (0.70–5.95)	0.19		
Chronic kidney disease	7 (26)	8 (11)	2.93 (0.95–9.08)	0.06	7.16 (1.30–39.44)	0.02
Proven IPA	0 (0)	4 (5)	–	0.57		
Serum GM-negative IPA	8 (30)	26 (35)	0.79 (0.31–2.06)	0.81		
Use of corticosteroid	10 (37)	43 (57)	0.44 (0.18–1.08)	0.08		
Neutropenia	20 (74)	39 (52)	2.64 (0.997–6.98)	0.07	9.05 (1.88–43.70)	0.006
Intensive care unit care	16 (59)	20 (27)	4.00 (1.59–10.06)	0.004	2.75 (0.87–8.74)	0.09
Macronodule	11 (41)	40 (53)	0.60 (0.25–1.47)	0.37		
Halo sign	16 (59)	29 (39)	2.31 (0.94–5.66)	0.07		
Multiple mass-shaped consolidation	6 (22)	5 (7)	4.00 (1.11–14.43)	0.03		
Infarct-shaped consolidation	5 (19)	10 (13)	1.48 (0.46–4.80)	0.51		
Cavitary lesion	5 (19)	25 (33)	0.46 (0.15–1.34)	0.22		
Pleural effusion	18	30 (40)	3.00 (1.19–7.56)	0.02	3.48 (1.01–11.99)	0.048
Internal low attenuation	5 (19)	19 (25)	0.67 (0.22–2.02)	0.60		
Reverse halo sign	8 (30)	4 (5)	7.47 (2.03–27.50)	0.001	10.12 (1.73–59.35)	0.01
Centrilobular nodules	8 (30)	28 (37)	0.71 (0.27–1.83)	0.64		
Small airway lesion	0 (0)	11 (15)	–	0.04		
Angio-invasive form	11 (41)	16 (21)	2.54 (0.99–6.53)	0.07		
Airway-invasive form	7 (26)	22 (29)	0.84 (0.31–2.28)	0.81		
Prior receipt of mold-active antifungal drugs before GM assay	19 (70)	44 (59)	1.67 (0.65–4.31)	0.36		
Delayed therapy[a]	4 (15)	27 (36)	0.31 (0.10–0.99)	0.048		
Initial amphotericin-B	13 (48)	32 (43)	1.25 (0.51–3.07)	0.65		
Initial voriconazole	9 (33)	24 (32)	1.06 (0.41–2.72)	>0.99		

[a] Delayed therapy was defined as appropriate therapy given at least 3 days after diagnosis.
CI, confidence interval; GM, galactomannan; IPA, invasive pulmonary aspergillosis.

independently associated with 30-day mortality. GM-negative IPA was not associated with 30-day mortality.

Discussion

Neutropenia and prior receipt of mold-active antifungal drugs before GM assay were independently associated with GM positivity among patients with proven/probable IPA. In addition, the 30-day and 90-day mortality were similar between GM-negative IPA and GM-positive IPA. To the best of our knowledge, this is the first study to have compared the characteristics, risk factors, and outcomes between GM-negative IPA and GM-positive IPA.

In line with previous reports,[1,10–12] we observed that neutropenia, hematologic malignancy, and receipt of cytotoxic chemotherapy were less common in GM-negative IPA. Previous meta-analyses that investigated the performance of the GM assay have shown that its sensitivity varies according to host factors. In one such analysis, among patients with hematological malignancies, the pooled sensitivity of the GM assay was 0.70 (95% CI, 0.62–0.77) in proven IPA, 0.82 (95% CI, 0.70–0.90) in those undergoing HCT, and 0.22 (95% CI 0.03–0.60) in SOT recipients.[1] Given that activation of neutrophils is the dominant host defense against invasion of conidia into the bloodstream, neutropenia may lead to angioinvasion and higher fungal burden,[10,13,14] and macrophage deficiency could limit GM clearance from the bloodstream.[15] On the other hand, inflammatory reactions in non-neutropenic patients may decrease the GM index. We reaffirmed that the presence or absence of neutropenia is an important factor determining the sensitivity of the GM assay.

Interestingly, prior anti-mold antibiotic use before GM assay was inversely associated with GM-negative IPA. In a previous study, using a cutoff value of 1.0, the sensitivity of the GM assay was lower in HCT recipients receiving antifungal compounds (20% for proven IPA) than in those not receiving such compounds (87.5% for proven IPA).[2] However, in our study, GM-negative IPA was more common in patients who had not received prior anti-mold antibiotics, especially in non-neutropenic patients, than in those who had (Supplemental Table 2). Furthermore, a longer period of use of prior antifungals was associated with high GM levels (Supplemental Figure 2). A possible explanation for this counterintuitive finding is that prior antifungal drug use in neutropenic patients may not affect GM-positive or GM-negative IPA (Supplemental Table 2), but prior antifungal drug use in non-neutropenic patients may delay the diagnosis and result in relatively high fungal burden or angioinvasive aspergillosis such as GM-positive IPA at the time of diagnosis (Supplemental Table 2). Another explanation could be that the suboptimal "mold-active antifungal drugs" such as itraconazole and polyene in neutropenic patients might result in GM-positive IPA. In contrast, non-neutropenic patients with infrequent antifungal therapy might result in lesser degree of angio-invasiveness, which could explain the GM-negative IPA. This indirectly indicates that early suspicion for IPA in non-neutropenic patients even in

negative GM results is important. In this context, our findings on the clinical characteristics and CT findings of patients with GM-negative IPA would provide useful clues in this difficult clinical situation. In addition, CT findings in non-neutropenic patients with IPA may give important clues for early diagnosis of IPA, especially in non-neutropenic patients in whom serum GM has limitation. That is, CT findings in non-neutropenic patients with suspected IPA should be interpreted with caution because so-called "well-circumscribed lesion" in EORTC criteria could be absent in non-neutropenic patients with IPA.

A recent study demonstrated that GM was released from *in vitro Aspergillus* culture during the exposure to echinocandin, and the blood GM level paradoxically increased in a patient with IPA during caspofungin therapy.[16] Therefore, the exposure to echinocandin before the diagnosis of IPA could paradoxically increase blood GM level in patients with suspected IPA. However, there were only 4 patients who received echinocandin before they were diagnosed as GM-positive IPA. Most of the patients received polyene (31/108, 29%) or itraconazole (18/102, 18%), which have suboptimal anti-*Aspergillus* activity. In this regard, our findings that prior antifungal use was independently associated with GM-positive IPA should be validated in other cohorts, and the biologic plausibility for these interesting findings should be further investigated.

The outcome of GM-negative IPA was not better than GM-positive IPA despite the fact that patients with GM-negative IPA were associated with non-neutropenia, which is a good prognostic factor for IPA.[17,18] A possible reason for this is the delayed diagnosis and treatment of IPA in patients with GM-negative IPA, which was supported by our findings in that the median number of days from diagnosis to appropriate antifungal therapy was higher in GM-negative than in GM-positive IPA (4 days vs. −1 days; $P < 0.001$). If repeated serum assays for GM are negative, it is necessary to undergo BAL assays for GM for early diagnosis.[12] In this study, only about 40% of patients with GM-negative IPA underwent BAL assays, of whom about half were positive for GM. Given that BAL assays for GM are more sensitive than BAL or sputum fungal cultures,[19] many patients with GM-negative IPA (in serum assays) might miss a chance at early diagnosis without invasive procedures such as BAL and be later recognized as GM-positive IPA (in serum assays).

This study had several limitations of note. First, designating GM-positive and culture-negative IPA as a comparison group may not represent all patients with GM positive IPA. In fact, of 260 patients with IPA, 158 (67%) were GM-positive and culture-negative. We assume that GM-positive and culture-positive IPA might have characteristics between GM-negative, culture-positive IPA and GM-positive, culture-negative IPA. Therefore, we compared the clinical characteristics of GM-negative, culture-positive IPA with those of GM-positive, culture-negative IPA. Second, polymerase chain reaction (PCR) of *Aspergillus* was not performed. A recent study showed promising results for the use of *Aspergillus* PCR in the early diagnosis of IPA.[20,21] In addition, serum assays for GM and PCR are complementary for the diagnosis of IPA.[22,23] Therefore, the clinical characteristics of *Aspergillus* PCR-negative IPA may be interesting compared to those of GM-negative IPA (in serum assays). However, methodological issues regarding *Aspergillus* PCR have not been resolved, so further studies are needed in this area. Third, only 4 proven IPA cases were included in our study and no autopsies were performed on the patients who died. For this reason, some may argue that the patients with pulmonary mucormycosis (PM) could have been misclassified as those with probable IPA, given that some patients had reverse halo sign, a typical CT finding in patients with PM. However, our previous study clearly showed that reverse halo sign was more common in patients with PM (54%) than in those with IPA (6%; P value < 0.001).[24] In this context, the frequency of reverse halo sign (12%) in patients with IPA in this study is consistent with our previous study.[24] Furthermore, our previous study demonstrated that positive sputum culture result was highly correlated with positive immunohistochemistry result, which is regarded as the gold standard test in the absence of sterile fungal culture.[7] Therefore, it is unlikely that patients with PM revealed positive *Aspergillus* culture from their sputum while showing negative GM results. Fourth, we followed the clinical criteria of EORTC/MSG as "well-circumscribed lesions" such as macronodule or consolidation. In other words, if patients who met the host and microbiologic criteria also had macronodules with small airway lesions, they were classified as probable IPA. As for those who had small airway lesions without well-circumscribed lesions, they were not classified as probable IPA. Therefore, this study could underestimate the CT findings of patients with IPA who had only non-specific CT radiologic findings without any proven histologic and sterile culture evidence. Finally, one may argue that the GM level is dynamic, so it is possible that 5 (15%) of the 34 patients with GM-negative IPA who underwent GM assay only once might test positive on repeated tests. However, there is no consensus on the necessity of multiple GM tests. Indeed, 2008 EORTC/MSG criteria revised the mycologic criteria as one positive GM result,[4] while 2003 EORTC/MSG criteria used the mycologic criteria[25] as two consecutive positive GM results. Regarding such conflicting policies, further studies are needed to clearly define the positive and negative criteria for GM results.

In conclusion, clinicians should be aware of the possibility of GM-negative IPA especially in patients with the host factors of probable IPA but without neutropenia. For patients with the host factors of probable IPA but not on anti-mold prophylaxis (mostly due to non-neutropenia), negative GM could not exclude the possibility of IPA, even though chest CT scans showed images of lungs with abnormal infiltrates that were not specific to IPA features. Angioinvasive aspergillosis is less common in GM-negative IPA than in GM-positive IPA. Mortality was not significantly different between the groups.

Conflict of interest

There are no potential conflicts of interest for any authors.

Acknowledgments

Financial support: This study was supported by grants from the National Research Foundation of Korea (Grant NRF-2015R1D1A1A01059315) and the Asan Institute for Life Sciences (2015-0193).

References

1. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006;**42**(10):1417—27.
2. Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis* 2004;**190**(3):641—9.
3. Park SY, Lim C, Lee SO, Choi SH, Kim YS, Woo JH, et al. Computed tomography findings in invasive pulmonary aspergillosis in non-neutropenic transplant recipients and neutropenic patients, and their prognostic value. *J Infect* 2011;**63**(6):447—56.
4. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from 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) consensus group. *Clin Infect Dis* 2008;**46**(12):1813—21.
5. Segal BH, Walsh TJ. Current approaches to diagnosis and treatment of invasive aspergillosis. *Am J Respir Crit Care Med* 2006;**173**(7):707—17.
6. Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselaers N, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med* 2012;**186**(1):56—64.
7. Jung J, Park YS, Sung H, Song JS, Lee SO, Choi SH, et al. Using immunohistochemistry to assess the accuracy of histomorphologic diagnosis of aspergillosis and mucormycosis. *Clin Infect Dis* 2015;**61**(11):1664—70.
8. Park SY, Kim SH, Choi SH, Sung H, Kim MN, Woo JH, et al. Clinical and radiological features of invasive pulmonary aspergillosis in transplant recipients and neutropenic patients. *Transpl Infect Dis* 2010;**12**(4):309—15.
9. Park SY, Lee SO, Choi SH, Sung H, Kim MN, Choi CM, et al. Aspergillus galactomannan antigen assay in bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. *J Infect* 2010;**61**(6):492—8.
10. Cordonnier C, Botterel F, Ben Amor R, Pautas C, Maury S, Kuentz M, et al. Correlation between galactomannan antigen levels in serum and neutrophil counts in haematological patients with invasive aspergillosis. *Clin Microbiol Infect* 2009;**15**(1):81—6.
11. Maertens JA, Klont R, Masson C, Theunissen K, Meersseman W, Lagrou K, et al. Optimization of the cutoff value for the Aspergillus double-sandwich enzyme immunoassay. *Clin Infect Dis* 2007;**44**(10):1329—36.
12. Meersseman W, Lagrou K, Maertens J, Wilmer A, Hermans G, Vanderschueren S, et al. Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. *Am J Respir Crit Care Med* 2008;**177**(1):27—34.
13. Chamilos G, Luna M, Lewis RE, Bodey GP, Chemaly R, Tarrand JJ, et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003). *Haematologica* 2006;**91**(7):986—9.
14. Nucci M, Nouer SA, Cappone D, Anaissie E. Early diagnosis of invasive pulmonary aspergillosis in hematologic patients: an opportunity to improve the outcome. *Haematologica* 2013;**98**(11):1657—60.
15. Bennett JE, Friedman MM, Dupont B. Receptor-mediated clearance of Aspergillus galactomannan. *J Infect Dis* 1987;**155**(5):1005—10.
16. Klont RR, Mennink-Kersten MA, Ruegebrink D, Rijs AJ, Blijlevens NM, Donnelly JP, et al. Paradoxical increase in circulating Aspergillus antigen during treatment with caspofungin in a patient with pulmonary aspergillosis. *Clin Infect Dis* 2006;**43**(3):e23—25.
17. Fisher CE, Stevens AM, Leisenring W, Pergam SA, Boeckh M, Hohl TM. The serum galactomannan index predicts mortality in hematopoietic stem cell transplant recipients with invasive aspergillosis. *Clin Infect Dis* 2013;**57**(7):1001—4.
18. Miceli MH, Grazziutti ML, Woods G, Zhao W, Kocoglu MH, Barlogie B, et al. Strong correlation between serum aspergillus galactomannan index and outcome of aspergillosis in patients with hematological cancer: clinical and research implications. *Clin Infect Dis* 2008;**46**(9):1412—22.
19. Hoenigl M, Prattes J, Spiess B, Wagner J, Prueller F, Raggam RB, et al. Performance of galactomannan, beta-d-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. *J Clin Microbiol* 2014;**52**(6):2039—45.
20. Arvanitis M, Ziakas PD, Zacharioudakis IM, Zervou FN, Caliendo AM, Mylonakis E. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. *J Clin Microbiol* 2014;**52**(10):3731—42.
21. Avni T, Levy I, Sprecher H, Yahav D, Leibovici L, Paul M. Diagnostic accuracy of PCR alone compared to galactomannan in bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis: a systematic review. *J Clin Microbiol* 2012;**50**(11):3652—8.
22. Aguado JM, Vazquez L, Fernandez-Ruiz M, Villaescusa T, Ruiz-Camps I, Barba P, et al. Serum galactomannan versus a combination of galactomannan and polymerase chain reaction-based Aspergillus DNA detection for early therapy of invasive aspergillosis in high-risk hematological patients: a randomized controlled trial. *Clin Infect Dis* 2015;**60**(3):405—14.
23. Imbert S, Gauthier L, Joly I, Brossas JY, Uzunov M, Touafek F, et al. Aspergillus PCR in serum for the diagnosis, follow-up and prognosis of invasive aspergillosis in neutropenic and non-neutropenic patients. *Clin Microbiol Infect* 2016;**22**(6).562.e561—568.
24. Jung J, Kim MY, Lee HJ, Park YS, Lee SO, Choi SH, et al. Comparison of computed tomographic findings in pulmonary mucormycosis and invasive pulmonary aspergillosis. *Clin Microbiol Infect* 2015;**21**(7). 684.e611—688.
25. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002;**34**(1):7—14.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jmii.2017.05.007.