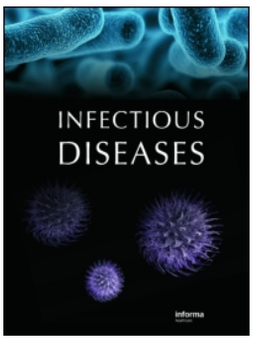

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Scandinavian Journal of Infectious Diseases

ISSN: 0036-5548 (Print) 1651-1980 (Online) Journal homepage: http://www.tandfonline.com/loi/infd19

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To cite this article: Nam Su Ku, Sang Hoon Han, Jun Yong Choi, Sun Bean Kim, Hye-Won Kim, Su Jin Jeong, Chang Oh Kim, Young Goo Song & June Myung Kim (2012) Diagnostic value of the serum galactomannan assay for invasive aspergillosis: It is less useful in non-haematological patients, Scandinavian Journal of Infectious Diseases, 44:8, 600-604, DOI: 10.3109/00365548.2012.657672

To link to this article: https://doi.org/10.3109/00365548.2012.657672

Published online: 04 Mar 2012.

Scandinavian Journal of Infectious Diseases, 2012; 44: 600–604

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ORIGINAL ARTICLE

Diagnostic value of the serum galactomannan assay for invasive aspergillosis: It is less useful in non-haematological patients

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Abstract

Background: The serum galactomannan assay (GMA) has been widely used for the diagnosis of invasive aspergillosis (IA). GMA is mainly used in patients with haematological malignancies or in those who have undergone haematopoietic stem cell transplantation (HSCT). However, there are few data from non-haematological patients. We evaluated whether GMA is useful for the diagnosis of IA in non-haematological patients. *Methods:* Patients who were subjected to serum GMA testing from January 2007 to December 2009 were evaluated retrospectively. Patients with haematological diseases or who underwent HSCT were excluded from our analysis. According to the criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group revised in 2008, the patients were categorized as proven, probable, possible, or non-IA. Proven and probable cases were defined as IA in this study. *Results:* Out of 778 patients, 13 (1.6%) had proven ($n = 9$) or probable ($n = 4$) IA. The sensitivity of the GMA was 23.1% (95% confidence interval (CI) 6.1–54.0%) and the specificity was 76.1% (95% CI 72.9–79.0%). The positive predictive value was 1.6% (95% CI 0.4–5.0%) and the negative predictive value was 98.3% (95% CI 96.8–99.1%). The likelihood ratios of a positive and negative test were 0.96 (95% CI 0.35–2.62) and 1.01 (95% CI 0.75–1.36), respectively. *Conclusions:* In this study, the sensitivity of the GMA for the diagnosis of IA was very low in non-haematological patients. Although the GMA test is considered useful for the diagnosis of IA in haematological patients, it had low diagnostic value for IA in non-haematological patients.

Keywords: *Galactomannan assay, invasive aspergillosis, non-haematological patients*

Introduction

Invasive aspergillosis (IA) is an important cause of morbidity and mortality in patients with haematological malignancies and profound immunosuppression [1,2]. Thus, early diagnosis and prompt antifungal therapy are very important for decreasing the high mortality rate of IA.

The serum galactomannan assay (GMA), which uses enzyme-linked immunosorbent assay (ELISA) technology, helps in the early diagnosis of IA [3,4]. However, studies evaluating the diagnostic value of serum GMA for the diagnosis of IA have largely been conducted in haematological patients, particularly in those with haematological malignancies or after haematopoietic stem cell transplantation (HSCT) [4–7].

Recently, some reports have suggested that non-haematological patients, such as solid organ recipients, patients receiving chemotherapy, those with chronic obstructive pulmonary disease, and those taking corticosteroids, are also at high risk for IA [8–10]. However, to our knowledge, there are few data from GMA testing of non-haematological patients. Therefore, we evaluated whether the GMA was useful for the diagnosis of IA in non-haematological patients.

Materials and methods

Study period and population

Non-haematological patients who underwent GMA testing from January 2007 to December 2009 were evaluated retrospectively. Non-haematological patients were defined as patients without haematological disease, including malignancies, or those who have not undergone HSCT. Patients who were aged 18 y or older were eligible for this study. The patients were identified from a computerized database compiled by

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(Received 23 November 2011; accepted 9 January 2012)

ISSN 0036-5548 print/ISSN 1651-1980 online
DOI: 10.3109/00365548.2012.657672

the diagnostic laboratory at Severance Hospital, a 2000-bed tertiary-care teaching hospital in Seoul, Korea. Each of these patients was classified according to the criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC) revised in 2008 [11]. Thus, the patients were categorized as proven, probable, possible, and non-IA. Proven and probable IA cases were defined as IA in this study. The definition of IA was not based on the GMA test [12].

Various clinical data were collected through retrospective review of the electronic medical records; these data included age, sex, underlying diseases, various predisposing factors, site of infection, EORTC classification, pathology specimen, culture specimen, and mortality.

Galactomannan detection

Aspergillus galactomannan antigen was detected by 1-stage immunoenzymatic sandwich microplate assay (Platelia Aspergillus EIA; Bio-Rad Laboratories, Marnes-la-Coquette, France). Samples were processed according to the manufacturer's instructions. Briefly, 300 µl test serum was added to 100 µl 4% ethylenediaminetetraacetic acid (EDTA) solution. After vigorous homogenization, the tubes were heated to 100°C in a water bath for 3 min, followed by centrifugation of the tube at $10{,}000 \times g$ for 10 min. Next, 50 µl supernatant and 50 µl horseradish peroxidase-labelled monoclonal antibody (clone EBA-2) were incubated in EBA-2-coated microplates for 90 min at 37°C. After 5 washes, the plates were incubated with 200 µl substrate chromogen reaction solution for 30 ± 5 min in the dark, with the temperature ranging from 18 to 25°C. The reaction was stopped with 1.5 N sulphuric acid solution. Optical density (OD) was read at 450 nm with a 620 nm reference filter. Positive, negative, and cut-off controls were included in each assay. An OD index of 0.5 was considered positive. All positive samples were retested and considered positive only if the repeat test was also positive.

Data analysis

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR−) were calculated in comparison with the reference diagnosis, which was based on cases of definite or probable aspergillosis. The 95% confidence intervals (CIs) were calculated using the Wilson score method. Student's *t*-tests were used to compare continuous variables, and Chi-square or Fisher's exact tests were used to compare categorical variables. All *p*-values were 2-tailed, and a *p*-value of less than 0.05 was considered statistically significant.

Results

Study population

During the study period, 778 patients were tested by serum GMA. In cases with multiple tests, only the first result was used. The clinical characteristics of the patients are shown in Table I. The median age was 61 y (range 18–97 y) and 59.1% of the patients were male. The most common underlying disease was solid organ malignancy (44.5%), followed by diabetes mellitus (19.2%) and solid organ transplantation (SOT; 11.3%). The most frequent route of infection was pulmonary infection (628 cases, 80.7%).

Table II shows the characteristics of 13 (1.6%) patients with proven ($n = 9$) or probable ($n = 4$) IA. Diabetes mellitus (DM) (8/13) was the most common underlying condition of patients with IA. Five patients were SOT recipients. Of these, 2 were liver transplant recipients and 3 were kidney transplant recipients. The most common site of infection was

Table I. Clinical characteristics of 778 patients who underwent a galactomannan assay for the diagnosis of invasive aspergillosis.

	IA ($n = 13$), *n* (%)	Study cases ($n = 778$), *n* (%)
Age, y, median (range)	65 (36–83)	61 (18–97)
Male	8 (61.5)	460 (59.1)
Underlying disease		
Solid organ transplantation	5 (38.5)	88 (11.3)
Solid organ malignancy	0	346 (44.5)
Rheumatologic disease	2 (15.4)	56 (7.2)
Diabetes mellitus	8 (61.5)	149 (19.2)
Old pulmonary tuberculosis	3 (23.1)	10 (1.3)
Liver disease	0	16 (2.1)
Cardiovascular disease	1 (7.7)	71 (9.1)
Pulmonary disease	1 (7.7)	65 (8.4)
HIV infection	0	13 (1.7)
Prolonged neutropenia (≥10 days)	0	7 (0.9)
Long-term steroid treatment (≥3 weeks)	2 (15.4)	20 (2.6)
Immunosuppressive therapy	5 (38.5)	88 (11.3)
Site of infection		
Pulmonary infection	6 (46.2)	628 (80.7)
Sinonasal infection	4 (31.0)	14 (1.8)
CNS	2 (15.4)	10 (1.3)
Disseminated infection	0	73 (9.4)
Others	1 (7.7)[a]	53 (6.8)

IA, invasive aspergillosis; HIV, human immunodeficiency virus; CNS, central nervous system.
[a]Chest wall abscess.

Table II. Clinical characteristics of 13 patients with proven or probable invasive aspergillosis.

No.	Sex/age (y)	Underlying condition	Site of infection	EORTC/MSG for IA	Risk factor for IA	Specimen[a]	Antifungal prophylaxis	GMA
1	F/79	DM	Sinonasal	Proven	-	Surgical tissue	No	Positive
2	M/78	DM	Sinonasal	Proven	-	Surgical tissue	No	Negative
3	F/42	SLE/DM	Pulmonary	Probable	Steroid	BAL	No	Negative
4	M/65	Liver TPL/DM	Pulmonary	Probable	T-cell IST	Sputum	No	Negative
5	F/36	SLE/DM	Pulmonary	Probable	Steroid	BAL	No	Negative
6	M/65	Old pulmonary TB	Pulmonary	Proven	-	Surgical tissue	No	Positive
7	M/83	DM	Sinonasal	Proven	-	Surgical tissue	No	Positive
8	F/56	Old pulmonary TB	Pulmonary	Proven	-	Surgical tissue	No	Negative
9	M/76	Old pulmonary TB	CNS	Proven	-	Surgical tissue	No	Negative
10	M/67	Liver TPL	Sinonasal	Proven	T-cell IST	Surgical tissue	No	Negative
11	F/68	Kidney TPL	CNS, eye	Probable	T-cell IST	Eye aspirate	No	Negative
12	F/55	Liver TPL/DM	Chest wall	Proven	T-cell IST	Surgical tissue	No	Negative
13	M/61	Kidney TPL/DM	Pulmonary	Proven	T-cell IST	Surgical tissue	No	Negative

EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; IA, invasive aspergillosis, GMA, galactomannan assay; DM, diabetes mellitus; SLE, systemic lupus erythematosus; TB, tuberculosis; TPL, transplantation; CNS, central nervous system; IST, immunosuppressive therapy; BAL, bronchoalveolar lavage.
[a]The sample from where the Aspergillus species was isolated.

the lungs (6/13), followed by the sinonasal cavities (4/13). Only 1 of the 13 patients with IA died, and he had pulmonary aspergillosis. No patient received antifungal prophylaxis before diagnosis. In addition, no patients with IA had neutropenia at the time of IA diagnosis. Five patients had received T-cell immunosuppressive therapy and 2 patients had received steroid treatment prior to diagnosis.

Diagnostic value of the serum GMA

The results of the serum GMA are shown in Table III. The sensitivity of the test was 23.1% (95% CI 6.1–54.0%) and the specificity was 76.1% (95% CI 72.9–79.0%). The PPV was 1.6% (95% CI 0.4–5.0%) and the NPV was 98.3% (95% CI 96.8–99.1%). The LR + and the LR − were 0.96 (95% CI 0.35–2.62) and 1.01 (95% CI 0.75–1.36), respectively.

Ten of 13 patients with IA had false-negative results by GMA, including 5 patients with pulmonary aspergillosis, 2 patients with sinonasal aspergillosis, 2 patients with central nervous system aspergillosis, and 1 patient with chest wall aspergillosis.

Table III. Diagnostic value of serum galactomannan assay for invasive aspergillosis.

	Case of invasive aspergillosis ($n = 778$)
TP/(TP + FN)	3/(3 + 10)
TN/(TN + FP)	582/(582 + 183)
Sensitivity % (95% CI)	23.1 (6.1–54.0)
Specificity % (95% CI)	76.1 (72.9–79.0)
PPV % (95% CI)	1.6 (0.4–5.0)
NPV % (95% CI)	98.3 (96.8–99.1)
LR + (95% CI)	0.96 (0.35–2.62)
LR − (95% CI)	1.01 (0.75–1.36)

TP, true positive; FN, false negative, TN, true negative; FP, false positive; PPV, positive predictive value; NPV, negative predictive value; LR +, positive likelihood ratio; LR −, negative likelihood ratio; CI, confidence interval.

Discussion

IA is a leading cause of death among immunocompromised patients, especially those with haematological malignancy or those who have undergone HSCT or SOT [1,13,14]. Since the serum GMA is a non-invasive test and gives rapid results, it may aid in the early diagnosis of IA [3,4]. However, the serum GMA test is mainly used in haematological patients, particularly those with haematological malignancies or who have had an HSCT. According to a meta-analysis of GMA studies conducted from 1996 to 2005, 26 of the 29 studies were conducted in haematological patients [15]. In those 26 studies, the sensitivity of the GMA test ranged from 33% to 100%, and the specificity ranged from 60% to 100%. In patients without haematological malignancies or HSCT, GMA demonstrated a lower sensitivity [15–18]. In patients with liver transplantation, the sensitivity of GMA was 56%, and it was 25% in patients with lung transplantation.

To our knowledge, aside from 1 study examining the value of a single GMA test for the diagnosis of IA in non-haematological patients with clinical isolation of Aspergillus species [12], little is known regarding GMA conducted in non-haematological patients, regardless of their underlying conditions, such as neutropenia or isolation of Aspergillus species. Therefore, we designed our study to include all non-haematological patients, regardless of underlying conditions. Furthermore, our study included a large population of patients.

In this study, the sensitivity of serum GMA was 23.1% for the diagnosis of IA in non-haematological patients. False-negative results from GMA in patients with IA have yet to be explained; however, some studies have reported that GMA can produce negative results in patients without neutropenia, in those undergoing antifungal prophylaxis [19], and in those with localized IA, such as Aspergillus tracheobronchitis [17]. No patients with IA in our study received antifungal prophylaxis or had local IA, such as tracheobronchitis. In addition, we had no patients with IA who had neutropenia. Therefore, it is possible that the lack of neutropenia may have influenced our results, which indicated a low sensitivity of GMA. According to Walsh et al., the sensitivity of the test was 15.7% in non-neutropenic patients with chronic granulomatous disease, 16.7% in those with Job's syndrome, and 80% in other immunocompromised patients with neutropenia [20]. The low sensitivity of the test in non-neutropenic patients may be related to a lower fungal burden, as the ability to clear the fungal mannan protein from the bloodstream by macrophage mannosyl receptors remains unimpaired in patients without granulocytopenia [17,21].

The PPV of the serum GMA was very low in our study, likely due to the low prevalence of IA. The PPV of any test is known to decrease with decreasing prevalence of the outcome [15]. Therefore, we propose that GMA should be utilized only when there is a high prevalence of IA, such as in high-risk populations with neutropenia and malignancy or populations that have undergone transplantation.

False-positive GMA tests have been documented in 8–10% of patients with cancer and in HSCT recipients [6,19,22,23]. In our study, the false-positive rate was 23.5% (183/778). Among the patients with false-positive results, 71 received piperacillin–tazobactam ($n = 61$) or amoxicillin–clavulanate ($n = 10$) treatment, which are known to yield positive test results [24], and 3 had Penicillium or Fusarium infections, both of which may have cross-reactivity with Aspergillus galactomannan antigen [25]. Other causes of false-positive results are unclear, but transient antigenemia and induction of antigenemia by immunosuppressive therapy have been proposed to account for the false-positive results observed in such patients [23,26,27].

In this study, 8 of 13 patients with IA had DM, and all were taking medication for DM. Although uncontrolled DM, defined as a fasting blood glucose level ≥ 140 mg/dl or random blood glucose level ≥ 200 mg/dl [28,29], could be considered a risk factor for IA, this remains uncertain. According to Chakrabarti et al., however, uncontrolled diabetes is now recognized as one of the underlying diseases associated with IA [29]. In addition, 4 of 8 patients with DM in our study had uncontrolled diabetes. Therefore, further studies with a larger number of IA patients involving multiple centres will be necessary to more accurately ascertain the association of DM with IA.

Our study has several limitations. First, our patients were chosen from a single centre. Second, the prevalence of IA was low. Third, as in all retrospective studies, there is a potential for bias and inaccurate data collection. Further prospective studies with high-risk populations involving multiple centres are necessary to ascertain the diagnostic value of GMA for the accurate diagnosis of IA.

In conclusion, the sensitivity of GMA for the diagnosis of IA was very low in non-haematological patients. Although GMA tests are useful for the diagnosis of IA in haematological patients, serum GMA was less useful for the diagnosis of IA in non-haematological patients.

Acknowledgements

This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (No. A102065).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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