

Original Article

Yield and Role of Surveillance Serum Galactomannan Assay for Early Detection of Invasive Pulmonary Aspergillosis in Adult Hematologic Malignancies and Hematopoietic Stem Cell Transplantation Patients

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Abstract:

Background: Invasive pulmonary aspergillosis (IPA) is a common life-threatening complication of patients with hematologic malignancy and hematopoietic stem cell transplant (HSCT) with prolonged neutropenia. Serum galactomannan (SGM) is currently used for early detection of IPA; however, the sensitivity and the specificity of SGM vary among laboratories. Therefore, we conducted this study to determine the yield of SGM assay in early detection of IPA among adult patients with hematologic malignancies and HSCT. **Method:** The retrospective study included 137 consecutive adult patients with hematologic malignancy and HSCT with IPA. All patients had the results of twice weekly SGM test and chest computed tomography scan performed on days 5-7 of persistent neutropenic fever and at IPA diagnosis. The performance of SGM was analyzed using the receiver operating characteristic (ROC) curve. **Result:** The 137 patients with 178 IPA episodes included 90, 25, 9 and 13 patients with acute leukemia, lymphoma, other hematologic malignancies, and HSCT, respectively. Median patients' age was 48 years (17-91 years). Median neutropenia duration before IPA diagnosis was 13 days (0-742 days). The sensitivity and the specificity of SGM at a cutoff level of 0.5 ng/mL were 31% and 100%, respectively. Based on ROC curve analysis, we found that a cut-off SGM level at 0.31 ng/mL had sensitivity and specificity of 50% and 49%, respectively. **Conclusion:** The yield of SGM surveillance at a cutoff level of 0.5 ng/mL for early detection of IPA among Thai patients with hematologic malignancy and HSCT was relatively low. A new cut-off SGM level of 0.31 ng/mL may facilitate the early diagnosis of IPA.

Keywords : ● Invasive pulmonary aspergillosis ● Serum galactomannan ● Chest computed tomography
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นิพนธ์ต้นฉบับ

ประสิทธิภาพและบทบาทของการตรวจ Serum Galactomannan ในการวินิจฉัยโรคเชื้อราในปอดชนิด Invasive Aspergillosis ในระยะเริ่มต้นในผู้ป่วยที่เป็นมะเร็งโรคเลือดและผู้ป่วยที่ได้รับการรักษาด้วยการปลูกถ่ายเซลล์ต้นกำเนิดเม็ดเลือด

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บทคัดย่อ

ความเป็นมา โรคเชื้อราในปอดชนิด *invasive aspergillosis* เป็นภาวะแทรกซ้อนที่ทำให้เสียชีวิตได้ในผู้ป่วยมะเร็งเม็ดเลือดที่มีเม็ดเลือดขาวต่ำ ปัจจุบันมีการใช้ระดับกาแลคโตแมนแนนในเลือดเพื่อช่วยวินิจฉัยโรคเชื้อราในปอดชนิดนี้ในระยะเริ่มต้น โดยมีความไวและความจำเพาะของการตรวจแตกต่างกันในแต่ละห้องปฏิบัติการ จึงได้ทำการศึกษาผู้ป่วยผู้ใหญ่จำนวน 137 คน ที่เป็นมะเร็งเม็ดเลือดและผู้ป่วยที่ได้รับการปลูกถ่ายเซลล์ต้นกำเนิดเม็ดเลือดที่ได้รับการวินิจฉัยเป็นเชื้อราในปอดชนิดนี้ **วัตถุประสงค์** เพื่อศึกษาประสิทธิภาพของการตรวจกาแลคโตแมนแนนในเลือดในการวินิจฉัยโรคเชื้อราในปอดชนิดนี้ในระยะเริ่มต้น **วิธีการ** มีผู้ป่วยที่เป็นมะเร็งเม็ดเลือดที่ได้รับการวินิจฉัยเป็นเชื้อราในปอด *invasive aspergillosis* จำนวน 137 คน ในช่วงเวลาตั้งแต่เดือนมิถุนายน พ.ศ. 2553 ถึง เดือนมิถุนายน พ.ศ. 2558 ผู้ป่วยทั้งหมดมีผลตรวจเลือดกาแลคโตแมนแนนและมีผลตรวจเอกซเรย์คอมพิวเตอร์ทรวงอกในวันที่ 5-7 ของการมีไข้และมีเม็ดเลือดขาวต่ำอย่างต่อเนื่อง และในวันที่วินิจฉัยเชื้อราในปอด *invasive aspergillosis* **ผลการศึกษา** ผู้ป่วย 137 คน แบ่งเป็นมะเร็งเม็ดเลือดขาว 90 คน มะเร็งต่อมน้ำเหลือง 25 คน มะเร็งเม็ดเลือดชนิดอื่น 9 คนและผู้ป่วยที่ทำการปลูกถ่ายเซลล์ต้นกำเนิดเม็ดเลือด 13 คน อายุเฉลี่ย 48 ปี (17-91 ปี) ระยะเวลาเฉลี่ยของภาวะเม็ดเลือดขาวต่ำก่อนวินิจฉัยเชื้อราที่ปอด จำนวน 13 วัน (0-742 วัน) พบว่าระดับกาแลคโตแมนแนนในเลือด 0.5 ng/mL มีความไวและความจำเพาะของการตรวจกาแลคโตแมนแนนในเลือดร้อยละ 31 และ ร้อยละ 100 แต่ถ้าใช้ค่า 0.31 ng/mL มีความไวและความจำเพาะของการตรวจกาแลคโตแมนแนนในเลือดร้อยละ 50 และ 49 **สรุป** ผลของการตรวจกาแลคโตแมนแนนในเลือด เพื่อใช้ในการวินิจฉัยโรคเชื้อราในปอด *invasive aspergillosis* ในระยะเริ่มต้นในผู้ป่วยไทยที่เป็นมะเร็งเม็ดเลือดและผู้ป่วยที่ได้รับการปลูกถ่ายเซลล์ต้นกำเนิดเม็ดเลือดพบว่ามีความไวอยู่ในระดับต่ำมาก ดังนั้นค่าคัดกรองของผลตรวจกาแลคโตแมนแนนในเลือดที่สร้างใหม่อาจช่วยในการวินิจฉัยเชื้อราในปอด *invasive aspergillosis* ระยะเริ่มต้นได้มากขึ้น

คำสำคัญ : ● *Invasive pulmonary aspergillosis* ● *Serum galactomannan* ● *Chest computed tomography*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2560;27:261-8.

Introduction

Invasive pulmonary aspergillosis (IPA) is a common complication among patients with hematologic malignancy who develop prolonged neutropenia after chemotherapy or hematopoietic stem cell transplantation (HSCT).¹⁻³ Although the definitive diagnosis for proven IPA requires histopathological documentation of infection or a positive result of lung tissue culture,⁴⁻⁶ fiberoptic bronchoscopy with tissue biopsy or transthoracic needle biopsy is an invasive intervention that is not applicable for the patients who have severe thrombocytopenia. Over the last decade, the galactomannan (GM) assay has become a useful noninvasive test to detect invasive aspergillosis (IA) infection. The GM is the composition of *Aspergillus* cell wall, and its presence in serum is strongly suggestive of IA. Serum GM (SGM) with a cutoff level of 0.5 ng/mL has been approved by the US Food and Drug Administration to diagnose IPA. However, the sensitivity and the specificity of SGM with a cutoff level of 0.5 ng/mL varies in the range of 60-84% and 21-38%, respectively.⁷⁻⁹ The broncho-alveolar lavage (BAL) GM is another test that facilitates early recognition of IPA with a sensitivity from 60-100% at a cutoff value of 1.0 ng/mL.¹⁰⁻¹³ Data is lacking on SGM assay for early detection of IPA among Thai patients with hematologic malignancy and HSCT. We therefore conducted this study to determine the yield of SGM assay in early detection of IPA among adult patients with hematologic malignancies and HSCT.

Material and Methods

This retrospective study was approved by the Local Ethics Committee on Human Rights related to research involving human subjects at our institution with a waiver of patient consent. Between June 2010 and June 2015, all adult patients (> 15 years old) with hematologic malignancies and HSCT with or without neutropenia (ANC < 1,000/mL), diagnosed with IPA at Ramathibodi Hospital and had both serial twice weekly SGM data and chest computed tomography (CT) scan performed at the time of the diagnosis of IPA, were included. The

patients who had no results of twice weekly SGM or chest CT scan performed at the time of IPA diagnosis were excluded from the analysis. IPA episode was defined as the whole period of IPA diagnosis to disease completely resolved after treatment. The IPA was diagnosed with European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) criteria^{5,6} as described below. First, proven IPA comprised histopathological or cytopathological results of lung tissue demonstrated hyphae with evidence of associated tissue damage or positive culture for *Aspergillus* from pulmonary tissue sample obtained by sterile technique and exhibited a clinically and radiologically abnormal site consistent with infection. Second, probable IPA comprised a positive antigen assay either SGM ≥ 0.5 ng/mL or BAL GM ≥ 1.0 ng/mL with a clinically and radiologically abnormal site consistent with infection. Finally, possible IPA comprised clinically and radiologically abnormal site consistent with infection.

Microbiological and radiological methods

A serial twice weekly SGM antigen test using Platelia *Aspergillus* EIA kit, Bio-Rad Company was performed among all patients with hematologic malignancy and transplant during the period of febrile neutropenia. This assay was also performed among non-neutropenic transplant patients who developed persistent fever ≥ 5 -10 days after antibiotics therapy. Blood samples were collected twice weekly until 1-2 weeks after antifungal therapy. BAL GM testing was performed among all patients who underwent bronchoscopy. Chest CT scans were performed in all patients with neutropenia/non-neutropenia who had persistent fever after 5-7 days of antibiotics therapy and at the time of IPA diagnosis. Repeat chest CT scan was performed on days 12-14 in cases of persistent neutropenic fever when a patient had a normal CT finding on days 5-7. All patients diagnosed with IPA (proven, probable and possible) also had a repeat chest CT scan obtained 3-4 weeks after antifungal therapy for IPA. All chest CT scans were performed on a multi-detector CT scanner and were

reconstructed with a 2- or 1-mm slice thickness. Excluding three recurrent episodes of proven IPA, all chest CT findings in proven IPA episodes were retrospectively reviewed by an experienced chest radiologist. Chest CT findings were classified as a pulmonary nodule(s) with or without the halo sign, pulmonary consolidation, cavity and the air-crescent sign.

Statistical analysis

The sensitivity, specificity, positive and negative predictive values of the SGM assay were determined using PASW statistics, version 18. The receiver operating characteristic (ROC) curve was analyzed using PASW statistics, software, version 18.

Results

Between June 2010 and June 2015, 124 patients with hematologic malignancies and 13 patients with HSCT met our inclusion criteria totaling 73 (53%) women and 64 (47%) men. The median age of the 137 patients was 48 years (range, 17-91 years). Of 124 patients with hematologic malignancies, 90 (66%), 25 (18%) and 9 (7%) patients presented acute leukemia, lymphoma and other hematologic malignancies, respectively. The baseline characteristics of all patients are shown in Table 1. Patients exhibiting negative SGM by repeat testing and having no evidence of pulmonary abnormality by chest CT scan were not considered having an IPA

Table 1 Characteristics of hematologic malignancy and HSCT patients with IPA

Characteristic	Number (%)
Age (years), median: 48 (17-91 years)	N = 137
15-40	46 (34)
41-65	71 (52)
> 65	20 (15)
Sex	N = 137
Female	73 (53)
Male	64 (47)
Disease	N = 137
Acute Leukemia	90 (66)
- Acute myeloid leukemia (AML)	72 (53)
- Acute lymphoblastic leukemia (ALL)	18 (13)
Chronic myeloid leukemia, blastic phase (CML-BP)	4 (3)
Lymphoma	25 (18)
- Diffuse large B cell lymphoma (DLBCL)	13 (9)
- Primary CNS lymphoma (PCNSL)	3 (2)
- T-cell lymphoma**	4 (3)
- Hodgkin lymphoma (HL)	1 (1)
- Others	4 (3)
Multiple myeloma (MM)	2 (1)
Myelodysplastic syndrome (MDS)	3 (2)
Hematopoietic stem cell transplantation	13 (9)
Type of chemotherapy in AML/ALL	N = 99
- Induction/ Salvage regimen	58 (58.6) / 13 (12)
- Consolidation	14 (14)
- Palliative / supportive therapy	14 (14)
Duration of neutropenia before diagnosis (days), median: 13 (0-742 d)	N = 178
- 0-15	102 (57)
- 16-30	48 (27)
- 31-60	14 (8)
- > 60	14 (8)

T cell lymphoma: **angio-immunoblastic T cell lymphoma, lymphoblastic T cell lymphoma, peripheral T cell lymphoma

episode. Therefore, a total of 178 IPA episodes were identified among the 137 patients studied, including 30 proven, 85 probable and 63 possible, respectively (Table 2). IPA occurred commonly among the patients with acute leukemia (99 episodes), especially among patients receiving induction chemotherapy (58 episodes). Median duration of neutropenia before the diagnosis of IPA was 13 days (range, 0-72 days), and 52% of 178 IPA episodes developed early within the first two weeks of neutropenia. Few MDS patients with IPA had prolonged neutropenia longer than one year. Proven IPA patients, exhibited co-infection organisms, including mucormycosis or candida pneumonia (n = 5), bacterial pneumonia (n = 5), multiple organisms (n = 3) and both viral and pulmonary tuberculosis (n = 1) (Table 2). Because of three recurrent proven IPA episodes, only chest CT scan findings in 27 proven IPA episodes were reviewed. The main CT findings consisted of pulmonary nodules (100%). An associated halo sign was found among 25 patients with pulmonary nodules (92%) and pulmonary consolidation was found in 8 patients (30%).

Results of GM detection in serum and new cut-off SGM level

A total of 754 samples were tested for SGM (mean 7 samples/patient), 126 samples (16.7%) were found to be positive for SGM, and the median SGM index was 0.14 ng/mL (range, 0.01-29.0 ng/mL). With the cut-off level of 0.5 ng/mL at time of IPA diagnosis (178 IPA episodes), the sensitivity of the SGM assay was 31.5% while its specificity was 100%, and the PPV and NPV were 100% and 48%, respectively. The area under ROC curve of a cut-off SGM level at 0.5 was 0.758 [95%CI: 0.688 to 0.829] (Figure 1). The median neutropenia duration before documented IPA were 11, 10 and 14 days for proven, probable and possible IPA, respectively. In the proven and probable IPA groups, only 30% (9/30) and 40% (34/85) had SGM level $\geq$ 0.5 ng/mL. Four (13.3%) proven and 24 (28.2%) probable IPA episodes had positive SGM results with the median follow up of 5 days (range, 3-20 days) and 7 days (range, 2-30 days) after diagnosis of IPA, respectively (Table 3). In the possible IPA group, 5 (7.9%) patients developed positive

Table 2 Types of IPA and investigative procedures in 178 episodes of patients with IPA

Variable	Number of event (%)
IPA episodes	N = 178
- Proven	30 (17)
- Probable	85 (48)
- Possible	63 (35)
Co-infection with other organisms in proven IPA	15 (8)
- Fungal pneumonia (mucormycosis/ candida)	4 (2) / 1 (1)
- Viral pneumonia	1 (1)
- Bacterial pneumonia	5 (3)
- Pulmonary tuberculosis	1 (1)
- Multiple organism pneumonia	3 (2)
Invasive investigations	N = 117
- Bronchoscopy	44 (25)
- Bronchoscopy with transbronchial biopsy	44 (25)
- Transthoracic needle biopsy	20 (11)
- Wedge resection or lobectomy	4 (2)
- Paranasal sinus biopsy	8 (4)
- Splenectomy	2 (2)

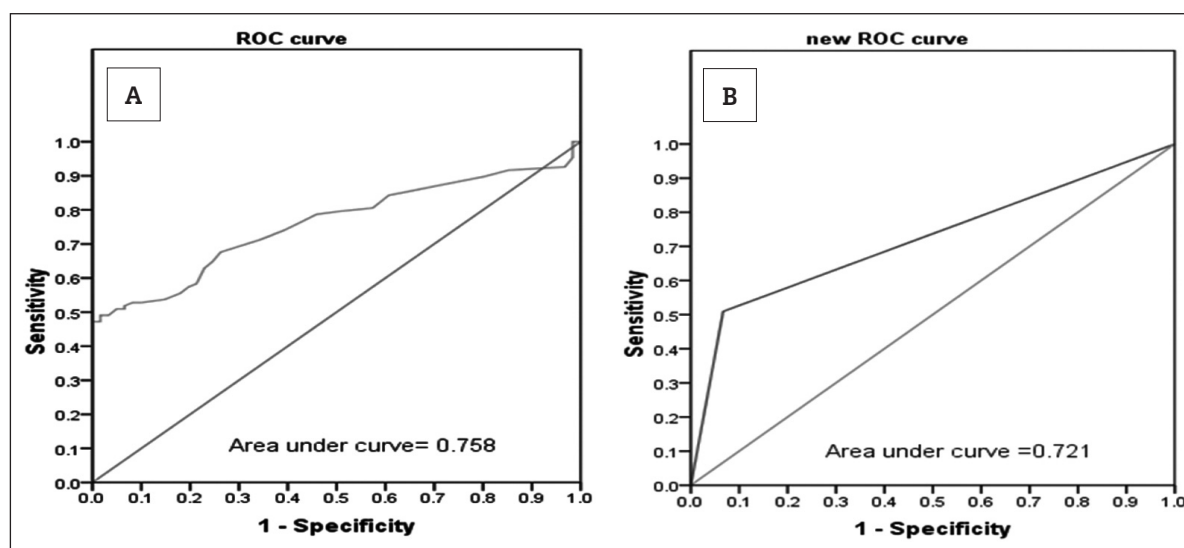


Figure 1 ROC of serum galactomannan assay at cut-off level of 0.5 ng/mL (A) and 0.31 ng/mL (B)

Table 3 Galactomannan assay and treatment for 178 IPA episodes

Variable	Proven IPA	Probable IPA	Possible IPA
	(N/%)	(N/%)	(N/%)
Number of events with SGM $\geq$ 0.5 ng/mL at the time of diagnosis	9 (30)	34 (40)	0
Median duration of neutropenia at the time of diagnosis (days)	11	10	14
Number of events with BAL GM $\geq$ 1.0 ng/mL	9 (69)	27 (69)	3 (97)
Median duration of neutropenia at the time of bronchoscopy (days)	6	11	15
Antifungal therapy	30	85	63
- Voriconazole	27 (90)	67 (78.8)	30 (47.6)
- Ambisome	-	5 (5.9)	1 (1.6)
- Amphotericin B	2 (6.7)	10 (11.8)	16 (25.4)
- Posaconazole	-	1 (1.2)	2 (3.2)
- Itraconazole	1(3.3)	2 (2.3)	14 (22.2)

GM in serum later with the median time after IPA diagnosis of 19 days (range, 4-29 days). Nine proven IPA patients died from IPA. The positive SGM was detected in 67% of deaths (6/9 episodes) and in 14.3% of surviving patients (3/21 episodes). The median SGM level among dead patients was 1.85 ng/mL (range, 0.88-9.15 ng/mL). Based on a ROC curve analysis, a cut-off SGM level at 0.31 ng/mL had sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of 50, 49, 93 and 51.4%, respectively. The area under ROC curve of the cut-off SGM level of 0.31 ng/mL was 0.721 [95%CI: 0.645 to 0.798] (Figure 1).

GM detection in broncho-alveolar fluid specimen

BAL fluids were tested for GM in 88 IPA episodes. The median duration of neutropenia before BAL GM

test were 6, 11 and 15 days for proven, probable and possible IPA episodes, respectively. Positive BAL GM results (index $\geq$ 1.0) were found among 40 IPA episodes (45.5%), including 69% (9/13), 69% (27/39) and 9.7% (3/31) in proven, probable and possible episodes, respectively (Table 3). The sensitivity and specificity of BAL GM test results were 73% and 86%, respectively. Double positive BAL GM and SGM results were found in 8 (61.5%) proven and 10 (28.6%) probable IPA episodes.

Confounder of the galactomannan index

The most common confounders for positive SGM were piperacillin and tazobactam therapy, administered to febrile neutropenic patients during the first week of empirical antibiotic treatment. In the group receiving piperacillin and tazobactam therapy (67 IPA

episodes), positive SGM was found among 13 of 33 probable IPA episodes, and in 5 patients diagnosed with proven IPA. On the other hand, the SGM was negative in all 29 possible episodes who received piperacillin and tazobactam therapy. Repeat SGM test and chest CT scan were performed in all cases producing positive SGM results. Amphotericin B was given before BAL for 7 proven and 15 probable IPA episodes. Median time for antifungal therapy in the proven and the probable IPA episodes were 5 days (2-8 days) and 4 days (2-18 days), respectively. Five (71.4%) proven and 13 (86.7%) probable IPA episodes were found to have GM positive results in BAL samples even after receiving prior antifungal therapy.

Antifungal therapy for IPA patients

The rationale for antifungal therapy of our national health policy during the study period recommended use of voriconazole only among proven and the probable IPA patients. Therefore, antifungal therapy in our practice relied on the clinical severity of febrile neutropenia, chest, paranasal sinus or abdominal CT scan findings, SGM, BAL GM, histopathological and cytopathological results. Chest CT scan and SGM were performed among all patients before starting antifungal therapy. In all, 90%, 79% and 48% of the 137 patients with proven, probable and possible IPA episodes received treatment with voriconazole, respectively (Table 3).

Discussion

This study demonstrated a relatively low sensitivity (31%) of twice weekly SGM assay in early detection of IPA among adult patients with hematologic malignancies and HSCT, and even the specificity for the SGM test was remarkably high (100%). Our results differed from other published data in western countries showing 60-80% of sensitivity and 21-38% of specificity.^{7-9,14-18} Moreover, the median duration of neutropenia at the time of diagnosis IPA in our proven and probable IPA were shorter than those documented in the previous reports; 11 days vs. 18-37 days and 10 days vs. 10-27

days, respectively.¹⁵⁻¹⁸ Although pulmonary nodules with the halo sign were found in 25 (92%) proven IPA episodes on chest CT scan, only 30% of these proven IPA episodes had positive SGM results. Nonetheless, in 72 proven and probable IPA episodes with negative SGM assay at IPA diagnosis, 28 (38.9%) developed positive SGM test results within the median follow up of 7 days after IPA diagnosis. We also found that the sensitivity of our BAL GM assay (73%) was twice as high as those in the serum. The results were similar to that reported in the study of Becker MJ, et al and Meersseman W, et al in which the SGM assay had two fold lower sensitivity than those for BAL GM; 44% vs 89% and 47% vs. 100%, respectively.^{14,15} Because the SGM and BAL GM assays in our laboratory were performed under a standard protocol (PlateliaAspergillus EIA kit, Bio-Rad no clear reason could explain the relatively low sensitivity of twice weekly SGM assays in this study. We speculated that the low Aspergillus fungal load in the serum of our IPA patients, for which almost all of our patients were investigated too early, either by chest computer scan and other invasive procedures, led to a low sensitivity of the SGM test. Although the rationale for antifungal therapy among our IPA patients drove the pre-emptive strategy, it did not cause the reduced GM sensitivity. Moreover, 71% of patients with proven and 87% of patients with probable IPA episodes were found to have positive BAL GM results even within 1-2 weeks of antifungal therapy. In addition, we were able to demonstrate that the cut-off SGM level at 0.31 provided a higher sensitivity of 50% and a specificity of 49% in early detection of IPA. A retrospective nature of the study and a small number of proven IPA cases may have affected the accuracy of the GM assay.

Conclusion

The yield of twice weekly SGM assays in early diagnosis of IPA among our patients with hematologic malignancies and HSCT was extremely low. The new cut-off SGM level of 0.31 ng/mL may facilitate early

diagnosis of IPA among these patients, especially those with abnormal chest CT findings.

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