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Diagnostic yield of sequential bronchoalveolar lavage fluid galactomannan assay in patients with negative serum galactomannan results suspected with invasive pulmonary aspergillosis

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Revised Abstract

Background: There are limited data in real clinical practice on the diagnostic value of bronchoalveolar lavage (BAL) fluid galactomannan (GM) assay in patients with suspected invasive pulmonary aspergillosis (IPA) who had negative serum GM results.
Method: This study was performed at a tertiary-care hospital, Seoul, South Korea between May 2008 and April 2019. All patients with suspected IPA whose serum GM assays revealed negative results and sequentially underwent BAL were enrolled in this study. Patients were classified as proven, probable, possible or not IPA by the revised 2019 EORTC/MSG definition.
Results: A total of 341 patients with suspected IPA including 4 proven IPA, 38 probable IPA, 107 possible IPA, and 192 not IPA were enrolled. Of these 341 patients, 107 (31%) with possible IPA were excluded from the final analysis. Of 42 patients with proven and probable IPA who had initial negative serum GM results, 24 (57%) revealed positive BAL GM results (n = 24) or BAL fungal culture (n = 8). Among the remaining 18 (43%), 2 (5%) were diagnosed as proven IPA by the histopathologic exam from transbronchial lung biopsy, 6 (14%) as probable IPA by subsequent sputum fungal culture, and 12 (29%) as probable IPA by repeated serum GM assay after BAL. Of 192 patients with not IPA, 14 (7%) revealed positive BAL GM results (n = 14) or BAL fungal culture (n = 8).
Conclusion: Sequential BAL in patients with suspected IPA who had initial negative serum GM results provided additional diagnostic yield in about half of patients.

Introduction

Invasive pulmonary aspergillosis (IPA) is a common and fatal opportunistic infection in patients with prolonged neutropenia, hematologic malignancy and hematologic stem cell transplantation, caused by the filamentous fungi of the genus *Aspergillus* [1]. Invasive aspergillosis is characterized by progression of the infection across the tissue, but lung biopsy to prove histopathological confirm for the diagnosis of proven IPA is often too great burden for the immunocompromised patients. Since the sensitivity of mycologic culture is as low as 20% [2], other mycologic evidence such as serum galactomannan has been widely used for the diagnosis of IPA. .
However, the sensitivity of serum GM assay is still suboptimal, which is 65% at a cut-off value 1.0 [3]. The previous study reported that the sensitivity of bronchoalveolar lavage (BAL) fluid GM assay was 91.3% at a cutoff value 1.0 [4]. Therefore, BAL has been considered as sequential diagnostic procedure in patients with negative serum GM but suspicious of IPA because of the relative invasiveness of the procedure. However, there are limited data in real clinical practice on the diagnostic value of subsequent BAL GM assay in patients with suspected IPA who had negative serum GM results. We thus investigated the diagnostic performance of BAL GM assay in patients with negative serum galactomannan assay who were suspected with IPA.

Methods

Study population and patient selection

A retrospective study was conducted for patients who were admitted to hematology unit and underwent subsequent BAL for suspected IPA at a 2700 bed tertiary-care teaching hospital, Seoul, South Korea. All patients with suspected IPA were reviewed through electronic medical records and patients who revealed positive serum GM before BAL were excluded. In case of patients who underwent BAL more than once, only the GM assay from first BAL was analyzed. This study was approved by the institutional review board in our hospital.

Definitions

Patients were classified as proven, probable, possible and not IPA by the revised 2019 EORTC/MSG definition [5]. Proven IPA was defined as histopathological evidence of tissue invasion of hyphae with morphologically consistent with *Aspergillus*. Probable IPA was defined as the presence of host factors with clinical features such as dense, well-circumscribed lesions with or without a halo sign, air crescent sign, cavity and wedge-shaped and segmental or lobar consolidation in CT, and mycological evidence of fungal infection by culture or galactomannan assay.
Neutropenia was defined as recent history of neutropenia (<500 neutrophils/mm3 for >10 days) related to present infection. Steroid use was defined as ≥0.3 mg/kg of methylprednisolone for ≥3 weeks in the past 60 days. Immunosuppressant use was defined as treatment with T-cell or B-cell immunosuppressants during the past 90 days.

Mycological and other microbiologic evaluation

The Platelia *Aspergillus* EIA from Bio-Rad laboratories and Sanofi Diagnostics was used to detect the presence of GM from serum and BAL samples. The BAL GM result larger than 10 was calculated as 10, because the exact value over 10 could not be detected in this assay. The cut-off value of serum GM and BAL GM assay for the diagnosis of IPA was 1.0 and the combination of serum GM value more than 0.7 and BAL GM value more than 0.8, according to 2019 EORTC-MSG criteria [5].
Other infections were defined as significant pathogens identified only from BAL fluid other than nasopharyngeal swab/aspirate or sputum culture with following ones: *Pneumocystis jirovecii*, Cytomegalovirus (CMV), and respiratory virus. *P. jirovecii* pneumonia (PCP) was defined as positive immunohistochemistry stain or cycle threshold (Ct) value of quantitative PCR <31 [6] with clinical and radiological evidence of pneumonia. The diagnosis of probable CMV pneumonia was done through the detection of CMV in culture of BAL fluid or quantitation of CMV DNA in BAL fluid with clinical symptoms and/or signs of pneumonia [7]. Though a definite cut-off for CMV DNA load to differentiate pneumonia from pulmonary shedding did not exist, quantitative PCR titer of CMV DNA >3.9 from BAL fluid was used to represent pneumonia in our study [8].

Results

Baseline clinical characteristics

A total of 585 patients who were admitted to the hematology unit and underwent bronchoscopy for suspected IPA over the period between May 2008 and April 2019 were retrospectively reviewed. A total of 341 patients who had classical host factors for IPA and negative serum GM result before BAL were enrolled in this study. Of these 341 patients, 4 proven IPA, 38 probable IPA, and 192 not IPA were included for the final analysis and 107 (31%) with possible IPA were excluded. The baseline clinical characteristics are presented in Table 1.

Table 1. Clinical and hematologic characteristics of study population included in the final analysis

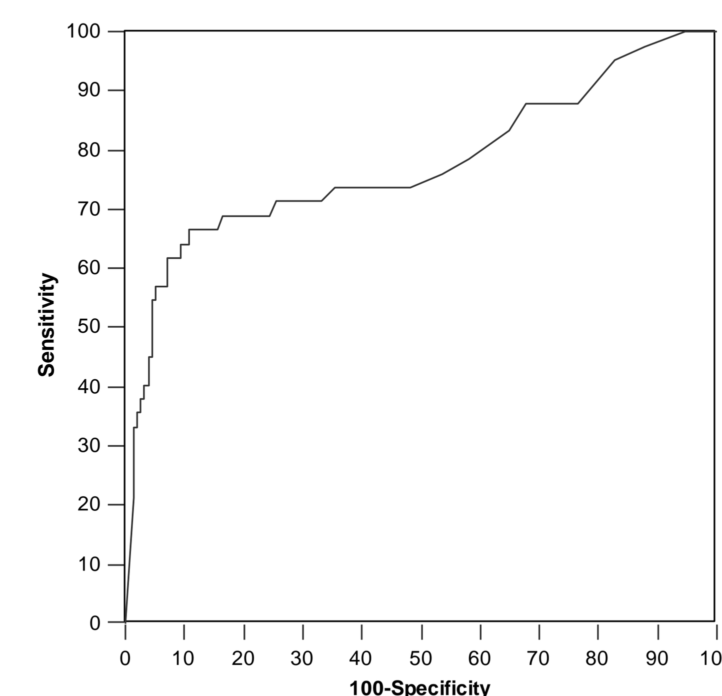
Characteristics	Total (n = 234)	Proven and Probable IPA (n = 42)	Not IPA (n = 192)	*p* value
Age, median years (IQR)	55 (45-64)	61 (52-66)	54 (44-63)	0.10
Female gender	88 (38)	21 (50)	67 (35)	0.07
Neutropenia (ANC <500/m^3)	65 (28)	15 (36)	50 (26)	0.21
Steroid use	22 (9.4)	6 (14)	16 (8.3)	0.23
Median dose (mg) per kilogram (IQR)	0.70 (0.40-0.95)	1.50 (1.25-1.75)	0.70 (0.40-0.85)	0.94
Immunosuppressant use	53 (23)	8 (19)	45 (23)	0.54
GVHD	49/86 (57)	10/15 (67)	39/71 (55)	0.40
Type of GVHD				
Acute	26/86 (30)	4/15 (27)	22/71 (31)	1.0
Chronic	23/86 (27)	6/15 (40)	17/71 (24)	0.21
Underlying diseases				
Myelodysplastic syndrome	34 (15)	6 (14)	28 (15)	0.96
Acute myeloid leukemia	101 (43)	18 (43)	82 (43)	0.76
Acute lymphoblastic leukemia	33 (14)	4 (10)	29 (15)	0.35
Myeloproliferative neoplasm	9 (4)	1 (2)	8 (4)	1.0
Lymphoma	32 (14)	7 (17)	25 (13)	0.53
Multiple myeloma	11 (5)	2 (5)	9 (5)	1.0
Others	14 (6)	3 (7)	11 (6)	0.72
Allogenic HSCT	86 (37)	15 (36)	71 (37)	0.88
Type of HSCT				
Autologous	6/92 (7)	3/18 (17)	3/74 (4)	0.09
Allogenic, full-matched	45/92 (49)	4/18 (22)	41/74 (55)	0.01
Allogenic, half-matched	41/92 (45)	11/18 (61)	30/74 (41)	0.12
Mold-active antifungal agents before BAL	121 (52)	23 (55)	98 (51)	0.66
Median days from antifungal agents to BAL (IQR)	7 (2.5-18)	9 (4.0-16)	5.5 (2.0-19)	0.21
Amphotericin B	73 (31)	13 (31)	60 (31)	0.74
Caspofungin	11 (5)	2 (5)	9 (5)	1.0
Micafungin	3 (1)	0 (0)	3 (2)	1.0
Itraconazole	7 (3)	1 (2)	6 (3)	0.36
Voriconazole	18 (8)	4 (10)	14 (7)	0.54
Posaconazole	9 (4)	3 (7)	6 (3)	0.21
Other (opportunistic) infections identified by BAL				
Pneumocystis jirovecii	26/190 (14)	5/30 (17)	21/160 (13)	0.60
Cytomegalovirus	9/188 (5)	2/29 (7)	7/159 (4)	0.63
Respiratory virus	12/193 (6)	1/29 (0)	11/164 (7)	0.22
Adenovirus	2 (17)	0 (0)	2 (18)	
Metapneumovirus	1 (8.0)	0 (0)	1 (9)	
Parainfluenza virus	7 (58)	1 (100)	6 (55)	
Respiratory syncytial virus	2 (17)	0 (0)	2 (18)	
Rhinovirus	2 (17)	0 (0)	2 (18)	

Data are given as number (percentage) of patients unless otherwise indicated.
Abbreviation: IQR, interquartile range; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation

Table 2. Diagnostic performances of various tests

Proven and Probable IPA vs Not IPA	Sensitivity % (n/N[a], 95% CI)	Specificity % (n/N[b],95% CI)	PPV % (95% CI)	NPV % (95% CI)	Positive Likelihood (95% CI)	Negative Likelihood (95% CI)
BAL GM or fungal culture	57 (24/42, 41–72)	93 (178/192, 89-96)	63 (49–75)	91 (88–94)	8.4 (4.8–15)	0.46 (0.32–0.65)
BAL GM	57 (24/42, 41–72)	93 (178/192, 89-96)	63 (49–75)	91 (88–94)	8.4 (4.8–15)	0.46 (0.32–0.65)
Subsequent repeated serum GM	29 (12/42, 16–45)	93 (178/192, 88-96)	46 (30-63)	86 (83–88)	3.9 (2.0–7.9)	0.77 (0.63–0.94)
Sputum fungal culture	21 (9/42, 10-37)	97 (187/192, 94-99)	64 (39–84)	86 (83-87)	8.2 (2.9-23)	0.81 (0.69-0.95)
Tissue biopsy	40 (4/10, 9.6–70)	100 (11/11, 100-100)	100 (100-100)	65 (42–87)	Not Applicable	0.6 (0.36–1.0)

[a]Number of patients with a positive test result/number of patients tested among diagnosed as proven and probable IPA.
[b]Number of patients with a negative test result/number of patients tested among diagnosed as not IPA.



BAL GM	
Area under the ROC curve	0.78 (95% CI, 0.72-0.83)
Cut-off value by Youden Index	0.72
Sensitivity	67% (95% CI, 55-84%)
Specificity	89% (95% CI, 64-78%)

Figure 1. ROC curve for BAL GM assay in patients suspected with IPA who had negative serum GM results.

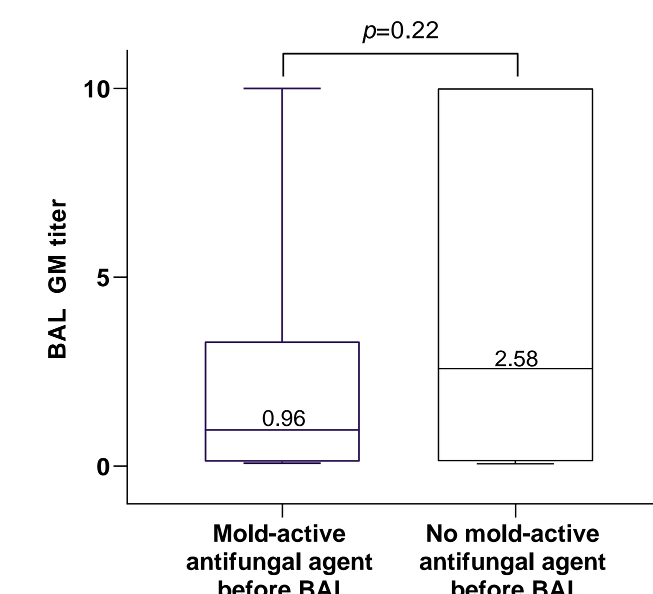


Figure 2. Box-and-whisker plot showing BAL GM titer by prior receipt of mold-active antifungal agent.

Table 3. Diagnostic performance of BAL GM assay by cut-off values in patients with suspected IPA who had negative serum GM results

Proven and Probable IPA vs Not IPA	Sensitivity % (n/N[a], 95% CI)	Specificity % (n/N[b], 95% CI)	PPV % (95% CI)	NPV % (95% CI)	Positive Likelihood (95% CI)	Negative Likelihood (95% CI)
cut-off value						
≥ 0.5	67 (28/42, 50-80)	88 (168/192, 82-92)	54 (43-64)	92 (89-95)	5.3 (3.5-8.2)	0.38 (0.25-0.59)
≥ 0.69[c]	67 (28/42, 50-80)	89 (171/192, 84-93)	57 (46-68)	92 (89-95)	6.1 (3.9-9.6)	0.4 (0.24-0.58)
≥ 1.0	57 (24/42, 41–72)	93 (178/192, 89-96)	63 (49–75)	91 (88–94)	8.4 (4.8–15)	0.46 (0.32–0.65)
≥ 1.5	48 (20/42, 32-64)	95 (183/192, 91-98)	69 (52-82)	89 (86-92)	10 (5.0-21)	0.55 (0.41-0.73)

[a]Number of patients with a positive test result/number of patients tested among diagnosed as proven and probable IPA.
[b]Number of patients with a negative test result/number of patients tested among diagnosed as not IPA.
cThe optimal cut-off value of BAL GM assay from the ROC curve according to Youden index.

Discussion

In this hematology unit-based retrospective study comprising 234 patients suspected with IPA, our results showed that sequential BAL in patients with negative serum GM results provided additional diagnostic yield in more than half of patients. Our findings can provide important information for decision making of clinicians on the rationale of doing BAL in patients with negative serum GM assay.
For the demand of prompt and sensitive diagnostic tool, serum GM has been widely used as complementary to sputum fungal culture. But, the sensitivity of serum GM assay is still suboptimal, which is approximately 65% [3], for detecting IPA. As a result, the possibility of IPA in patients who had negative serum GM assay should be considered in the setting of classical host factors and BAL GM is recommended for further invasive test [9]. But there are limited data about the additional benefit of BAL GM assay in patients with suspected IPA who had negative serum GM results. Our study focused on this clinical question and identified that sequential BAL GM assay in patients with negative serum GM result had additional diagnostic yield in 57%.
It is worth noting that the sensitivity of BAL GM assay in our study was 57% and is lower than that of previous studies. In the study of Maertens et al, the sensitivity of BAL GM assay in patients with hematologic disease was 91% at a cutoff value 1.0 [4] and in another prospective study from patients in ICU, the sensitivity of BAL GM was 88% at a cut off value 0.5 [10]. The possible explanation for this finding is that prior receipt of mold-active antifungal agent before BAL might have influenced on BAL GM titer. Mold-active antifungal agent is a well-known factor affecting both serum GM assay [11, 12, 13] and BAL GM assay [14]. In a study with patients with hematological diseases, 71% of patients with proven and probable IPA received mold-active antifungal agent before BAL, and antifungal therapy for ≥2 days significantly decreased the sensitivity of BAL GM from 79% to 51% at a cut-off value 0.5 [14]. Likewise, about half of patients with proven and probable IPA received mold-active antifungal agent before BAL in our study, and the sensitivity of BAL GM assay decreased from 68% to 48% by use of mold-active antifungal agent before BAL.
Furthermore, we found that about one quarter of patients with suspected IPA had other (opportunistic) infections including P. jirovesi, CMV or respiratory viral infections (Table 1). Since IPA, P. jiroveci pneumonia (PCP), and CMV pneumonia share similar risk factors such as hematologic malignancy, hematopoietic stem cell transplantation, high dose steroid and immunosuppressant use [15, 16, 17], differential diagnosis for these infections is critical to start the most appropriate drug. As a result, considering this additional benefit of detecting other opportunistic infections, the recommendation for BAL in patients with suspected IPA who had negative GM results is warranted in terms of detecting other opportunistic infection or co-infection.
This study had several limitations. First, only 4 proven IPA were included in our study and no autopsies were performed. So, some misclassification bias could be possible. In addition, the definition of probable IPA included positive BAL GM results, so some verification bias might occur. Second, routine check-up of polymerase chain reaction of *Aspergillus* spp. was not possible in out hospital during the study period, so *Aspergillus* PCR was not considered in the diagnosis of IPA in our study. So, some IPA cases were missed or misclassified.

Conclusion

In conclusion, our results suggest that BAL GM assay can be considered as further diagnostic tool for IPA in patients with negative serum GM result. The prior use of mold-active antifungal agent before BAL decreased the sensitivity of BAL GM assay and the BAL GM titer but statistically insignificant degree.

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