ORIGINAL ARTICLE

Comparison of Bronchoalveolar Lavage Fluid Galactomannan and Aspergillus PCR for Diagnosis Invasive Pulmonary Aspergillosis in Neutropenic Patients

Itthiphat Arunsurat, MD[1], Apichart So-Ngern, MD[2], Paramet Bumroongsawat, MD[1], Wipa Reechaipichitkul, MD[1], Atibordee Meesing, MD[3], Pailin Ratanawatkul, MD[1], Worawat Chumpangern, MD[1]

[1] Subdivision of Pulmonary Medicine and Pulmonary Critical Care, Division of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[2] Subdivision of Sleep Medicine, Division of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[3] Subdivision of Infectious diseases and Tropical medicine, Division of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Background: Aspergillus polymerase chain reaction (PCR) represents a novel method for diagnosing invasive pulmonary aspergillosis (IPA). However, studies of comparison of bronchoalveolar fluid (BALF) galactomannan (GM) and Aspergillus PCR remain limited.

Objective: To compare the BALF GM and Aspergillus PCR for diagnosis of IPA.

Materials and Methods: A cross-sectional study was conducted at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, between January 1, 2019, and December 31, 2022. The study enrolled neutropenic patients aged ≥18 years with radiological findings suggestive of IPA, serum galactomannan <0.5, and who underwent bronchoscopy with BAL.

Results: Forty-five subjects were recruited. 23 (51.1%) were male. The mean (SD) age was 52.6 (17.8) years. Common underlying diseases included lymphoma (37.8%), leukemia (33.3%), and systemic lupus erythematosus (20.0%). The median (IQR) absolute neutrophil count was 192 (32 to 132) cell/mm^3. BALF GM ≥1.0 occurred in 19 subjects (42.0%), while BALF Aspergillus PCR occurred in 37 subjects (82.2%). The diagnostic sensitivity of BALF Aspergillus PCR was significantly higher than that of BALF GM ($p<0.001$), and the highest diagnostic sensitivity was achieved with the combined use of BALF Aspergillus PCR and BALF GM, the sensitivity was 93.3%.

Conclusion: Aspergillus PCR enhances the diagnostic yield of IPA. BALF GM and Aspergillus PCR should be utilized for diagnosing IPA in neutropenic patients with suspected radiological findings.

Keywords: Bronchoalveolar fluid; Galactomannan, Aspergillus PCR

Received 28 February 2024 | Revised 1 July 2024 | Accepted 8 July 2024

J Med Assoc Thai 2024;107(Suppl.1):S95-9

Website: http://www.jmatonline.com

Invasive pulmonary aspergillosis (IPA) commonly occurs in individuals experiencing prolonged neutropenia or those receiving corticosteroids and immunosuppressive agents, leading to a severe fungal infection with a high mortality rate[1-3]. The diagnostic process is challenging and intricate, often relying on chest imaging that demonstrates single or multiple nodules, which may or may not be surrounded by ground-glass opacity, a characteristic known as a halo sign. A definite diagnosis is required[4-6]. The European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC consensus in 2008 classified the diagnosis of IPA into proven IPA, probable IPA, and possible IPA based on evidence of *Aspergillus* spp. infection[1]. Proven IPA requires histological, cytopathological, or direct microscopic examination presenting with fungal hyphae suggestive of Aspergillus, accompanied by evidence of associated tissue damage and/or culture-growing *Aspergillus* spp. Probable IPA mandates all three criteria: 1) a host susceptible to IPA (e.g. neutropenia, prolonged corticosteroid use, receipt of an allogeneic stem cell transplant, treatment with

Correspondence to:

Chumpangern W.

Subdivision of Pulmonary Medicine and Pulmonary Critical Care, Division of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.

Phone: +66-43-348360 ext. 3746, 3664, **Mobile:** +66-88-5638864

Fax: +66-43-203767, +66-43-348399

Email: worawat@kku.ac.th

How to cite this article:

Arunsurat I, So-Ngern A, Bumroongsawat P, Reechaipichitkul W, Meesing A, Ratanawatkul P, Chumpangern W. Comparison of Bronchoalveolar Lavage Fluid Galactomannan and Aspergillus PCR for Diagnosis Invasive Pulmonary Aspergillosis in Neutropenic Patients. J Med Assoc Thai 2024;107(Suppl.1):S95-9.

DOI: 10.35755/jmedassocthai.2024.S01.S95-S99

© 2024 JOURNAL OF THE MEDICAL ASSOCIATION OF THAILAND

T cell immunosuppressants), 2) the presence of dense, well-circumscribed lesions with or without a halo sign, air-crescent sign, or cavity, 3) mycological evidence of *Aspergillus* spp. such as microscopical detection of a fungal element in respiratory specimens (sputum, bronchoalveolar lavage (BAL) or bronchial brush), culture growing *Aspergillus* spp. in a respiratory specimen, or positive serum or BAL galactomannan (GM). Possible IPA requires two criteria: 1) a host susceptible to IPA as described earlier and 2) the presence of dense, well-circumscribed lesions with or without a halo sign, air-crescent sign, or cavity[6]. Patients diagnosed with possible IPA necessitate thorough assessment due to the absence of mycological evidence of IPA. Consideration must be given to excluding other causes similar to possible IPA, such as pulmonary mucormycosis, candidiasis, herpes simplex virus, Kaposi sarcoma, and mycobacterial infections[7,8].

While pathological evidence of fungal invasion provides a definitive diagnosis, performing tissue biopsy poses challenges in several cases due to treatment-induced thrombocytopenia or underlying disease[4]. GM is the antigen derived from Aspergillus cell wall and serves as indirect evidence of *Aspergillus* spp. infection. However, false-positive results have been observed in patients receiving piperacillin-tazobactam[9,10] or amoxicillin-clavulanic acid[11]. Additionally, false-positive results may occur in patients infected with other fungi, including *Fusarium* spp.[12], *Penicillium* spp.[13], *Histoplasma capsulatum*[14]. The false-negative result could be found in patients who concurrently administered mold-active antifungal therapy[15-17]. In the clinical practice of our center, empiric antifungal therapy particularly amphotericin B was initiated in a neutropenic host who suspected IPA. Otherwise, these patients might receive piperacillin-tazobactam due to febrile neutropenia. In such circumstances, GM decreased the performance for diagnosis. The PCR for aspergillus was developed for helping IPA diagnosis. A meta-analysis revealed the sensitivity and specificity were 57% (95% CI of 75 to 91%) and 99% (95% CI of 65 to 84%) for IPA diagnosis[18]. The diagnostic performance is good. The EORTC/MSGERC revised the criteria for IPA diagnosis in 2020 and endorsed the PCR of aspergillus for mycological evidence of probable IPA[19]. This increases the diagnosis from possible IPA to probable IPA. In our center, diagnosis of IPA remains problematic.

In many cases suspected IPA received empiric antifungal therapy that causes a low level of GM either in serum or BAL. In our experience, the PCR aspergillus is useful in diagnosing IPA in such cases. To date, there is no study in Thailand comparison BALF GM and Aspergillus PCR to diagnosis IPA in high-risk patients. The objective of this study is to compare the BALF GM and Aspergillus PCR for diagnosis of IPA.

Materials and Methods

A cross-sectional study was conducted at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, a tertiary hospital in Northeast Thailand, from January 1, 2019, to December 31, 2022. The study received approval from the Human Research Ethics Committee, Khon Kaen University (approval number HE641228).

The inclusion criteria were as follows: 1) age ≥18 years, 2) absolute neutrophil count <500 cell/mm^3, 3) abnormalities observed in the chest computed tomography (CT) scan consistent with IPA, including: a) dense, well-circumscribed nodule with or without halo sign or reverse halo sign, b) air crescent sign, c) cavity, d) wedge-shape and segmental or lobar consolidation, 4) serum GM optical density (OD) index <0.5, 5) patient who underwent bronchoscopy with BAL. The exclusion criteria were: 1) suspicious of aspergilloma or chronic necrotizing pulmonary aspergillosis and 2) patients who underwent transbronchial biopsy or bronchial brushing.

Medical records were systematically collected, including demographic data, serum GM, complete blood count results, radiological findings from imaging studies, and bronchoalveolar lavage fluid (BALF). The BALF data encompassed microbiological profiles, GM level, Aspergillus PCR, and cytological findings.

The diagnostic sensitivity was determined by the number of patients with the BALF GM OD index ≥1.0 or positive BALF Aspergillus PCR, divided by the total number of patients diagnosed with IPA. Additionally, patients meeting the possible criteria were classified as having IPA if they responded positively to effective antifungal therapy. This approach assessed the diagnostic sensitivity of the selected criteria in cases where the IPA diagnosis was based on possible criteria. The primary objective of the present study is to compare the BALF GM and Aspergillus PCR for diagnosis of IPA.

Statistical analysis

The sample size for the study was calculated with 80% power, a type I error rate of 5%, a diagnostic sensitivity of BALF Aspergillus PCR based on meta-analysis at 57%[18], and an estimated diagnostic sensitivity in our center of 75%. The calculated total study population was 55. Categorical data were expressed as numbers and percentages. Normal distributed continuous data were presented as mean and standard deviation (SD), while non-normal distributed data were presented as the median and interquartile range (IQR). The comparison of diagnostic sensitivity was assessed using McNemar's Chi-square, and a p-value of less than 0.05 was considered statistically significant. To assess the

impact of empirical antifungal therapy, a subgroup analysis was conducted on patients who received effective empirical antifungal treatment prior to bronchoscopy. Statistical analysis was performed using Stata version 10.1 (StataCorp, Texas, USA).

Results

In the present study, 45 patients were recruited, with 51.1% (23/45) being male. The mean age was 52.6 years. Underlying diseases were 37.8% (17/45) lymphoma, 33.3% (15/45) leukemia, and 20% systemic lupus erythematosus (9/45). The median absolute neutrophil count (ANC) was 192/mm^3 (IQR 32 to 362). Radiographic findings revealed that 82.2% (37/45) had halo nodules, and 64.4% (29/45) had less than 3 nodules. Before bronchoscopy, 35.6% (16/45) of patients had received effective antifungal therapy. Additional baseline patient characteristics are detailed in Table 1.

The study observed a median BALF GM OD index was 0.73 (IQR 0.35 to 1.3). At a cutoff value of 0.5, BALF GM was positive in 62.2% (28/45) of cases; at 0.7, 53.3% (24/45) were positive, and at a cutoff value of 1.0, 42% (19/45) were positive. The median serum GM OD index was 0.29 (IQR 0.2 to 0.4). BALF Aspergillus PCR was positive in 82.2% (37/45) of cases. All patients met the criteria for IPA, either by possible or probable criteria, as outlined in Table 2.

Figure 1 illustrates the laboratory investigation results for the IPA diagnosis. The combination of BALF GM and BALF Aspergillus PCR demonstrated a higher diagnostic sensitivity than BALF Aspergillus PCR alone or BALF GM alone (93.3% vs. 82.2%, p=0.03), (93.3% vs. 42.2%, p<0.001), respectively. Moreover, BALF Aspergillus PCR exhibited a significantly increased diagnostic sensitivity for the diagnosis of IPA compared with BALF GM alone (82.2% vs. 42.2%, p<0.001).

In a subgroup analysis of patients who received effective empirical antifungal therapy before bronchoscopy, our findings revealed a significantly higher diagnostic sensitivity of BALF Aspergillus PCR compared to BALF GM at a cutoff value of 1.0 (75.0% vs. 12.5%, p=0.004).

There was no major complication during the bronchoscopy with bronchoalveolar lavage. The most frequently observed complication was hypoxemia. Additional complications are detailed in Table 3.

Table 1. Patient characteristics

Characteristics	n=45
Male (n, %)	23 (51.1)
Age (mean, SD)	52.6 (17.8)
BMI (mean ± SD)	21.98±4.1
Underlying disease (n, %)	
Lymphoma	17 (37.8)
Leukemia	15 (33.3)
Other haematologic disease	6 (13.3)
Systemic lupus erythematosus	9 (20.0)
ANC in cells/mm^3 (median, IQR)	192 (32 to 362)
Radiological findings (n, %)	
Nodules <3 nodules	29 (64.4)
Nodules ≥3 nodules	16 (35.6)
Halo nodule	37 (82.2)
Reverse halo nodule	4 (8.9)
Cavity	4 (8.9)
Alveolar infiltration	20 (44.4)
Received antifungal therapy before bronchoscopy (n, %)	16 (35.6)

SD=standard deviation; BMI=body mass index; ANC=Absolute neutrophil count; IQR=Interquartile range

Table 2. Laboratory investigation results for the IPA diagnosis

Laboratory parameters	n=45
Serum GM (median, IQR)	0.29 (0.2 to 0.4)
BALF GM OD index (median, IQR)	0.73 (0.35 to 1.3)
≥0.5 (n, %)	28 (62.2)
≥0.7 (n, %)	24 (53.3)
≥1.0 (n, %)	19 (42.0)
Positive BALF Aspergillus PCR (n, %)	37 (82.2)
BALF culture growing Aspergillus spp. (n, %)	22 (50.0)
BALF cytology revealed the presence of Aspergillus morphology (n, %)	7 (15.6)

GM=galactomannan; BALF=bronchoalveolar lavage fluid; OD=optical density; PCR=polymerase chain reaction

Discussion

IPA is frequently identified in immunocompromised individuals, particularly in neutropenic patients[4,5]. As per the revised 2020 criteria from the EORTC/MSGERC, the goal standard for diagnosis of IPA is mycological evidence of *Aspergillus* spp. from sterile material[19]. However, the practicality of obtaining tissue biopsies through transbronchial or open lung biopsy is often limited due to factors such as thrombocytopenia resulting from ongoing treatment or underlying diseases. Consequently, serum or BALF GM or Aspergillus PCR plays pivotal roles in aiding the diagnosis of this condition. Our study contributes additional evidence supporting the diagnostic yield of BALF Aspergillus PCR in neutropenic patients in Thailand.

Our study highlights a statistically significant increase in diagnostic sensitivity with the combined use of BALF Aspergillus PCR and BALF GM (at a cutoff value of 1.0), in comparison to using BALF Aspergillus PCR or BALF GM alone. This finding aligns with a prior study that reported a sensitivity of 62.5% and specificity of 94.6% when combining Aspergillus PCR and GM in BALF[20]. Similarly, another study demonstrated that BALF Aspergillus PCR alone provided a sensitivity of 88.6% and specificity of 95.5%[21]. Furthermore, BALF Aspergillus PCR contributed

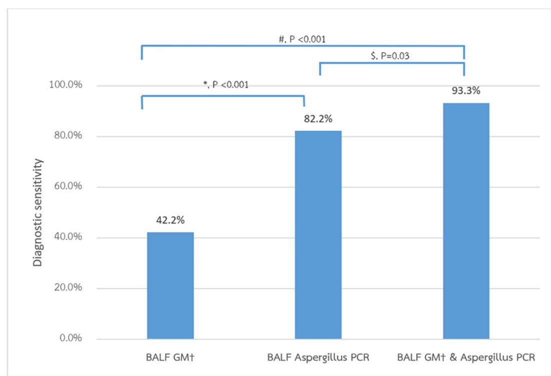

Figure 1. The diagnostic sensitivity of laboratory investigation results for the IPA diagnosis.

BALF=bronchoalveolar lavage fluid; GM=galactomannan; PCR=polymerase chain reaction

† BALF GM OD index cutoff value ≥1.0; * McNemar's Chi-Squared test between BALF GM (≥1.0) vs. BAL Aspergillus PCR, p-value <0.001; # McNemar's Chi-Squared test between BALF GM (≥1.0) vs. BALF GM (≥1.0) and BALF Aspergillus PCR, p-value <0.001; $ McNemar's Chi-Squared test between BALF Aspergillus PCR vs. BALF GM (≥1.0) and BALF Aspergillus PCR, p-value=0.03

Table 3. Complications of bronchoscopy with bronchoalveolar lavage

Complications	n=45 (%)
Hypoxemia (n, %)	12 (26.7)
Minor bleeding (n, %)	1 (2.2)
Significant bleeding (n, %)	0 (0.0)
Worsened respiration (n, %)	2 (4.4)
Needed invasive mechanical ventilation (n, %)	0 (0.0)
Pneumothorax (n, %)	0 (0.0)
Cardiac arrest (n, %)	0 (0.0)

to an increased diagnostic sensitivity for diagnosis compared to BALF GM alone, particularly in patients who had received effective antifungal therapy before bronchoscopy.

Aspergillus PCR is a novel and sensitive method for detecting nucleic acid of *Aspergillus* spp. This diagnostic tool can be applied to test either serum or BALF[18]. The positive results of Aspergillus PCR correlate significantly with the fungal load, demonstrating higher values in BALF from patients with aspergillosis compared to those with non-aspergillosis conditions[21]. These findings hold potential implications for enhancing the diagnosis of IPA.

The authors observed that BALF GM also exhibited good diagnostic performance for identifying IPA. Using a cutoff of ≥0.7, approximately half of cases were identified. However, increasing the cutoff to ≥1.0 improved specificity, albeit at the cost of reducing the number of identified cases. These findings align with results reported in another study[20-22].

Our study further indicates that complications arising from bronchoscopy with bronchoalveolar lavage predominantly manifest as hypoxemia, with fewer instances of respiratory compromise and an absence of serious complications such as pneumothorax or cardiac arrest. These findings align with those reported in previous study[23]. This suggests that the procedure may be considered safe for enhancing the diagnosis of IPA.

The present study has several limitations. Firstly, the study population was limited to neutropenic patients, while IPA can also occur in other contexts such as prolonged steroid use, solid organ or hematopoietic stem cell transplant, and immunosuppressive agent use. Secondly, the authors lacked cases of proven IPA based on the EORTC/MSGERC criteria for diagnosis. Thirdly, our study faced recruitment challenges and was underpowered due to the slow enrollment, partly attributed to the COVID-19 situation. Despite this, the primary endpoint reached statistical significance. Finally, the absence of non-IPA patients in this study precludes the determination of diagnostic yield.

Conclusion

Aspergillus PCR enhances the diagnostic yield of IPA. BALF GM and Aspergillus PCR should be utilized for diagnosing IPA in neutropenic patients with suspected radiological findings.

What is already known on this topic?

GM demonstrate a high diagnostic yield for IPA in neutropenic patients. However, GM testing is associated with both false positives and false negatives.

What this study adds?

BALF Aspergillus PCR proves to enhance the diagnostic sensitivity of IPA, especially in individuals who received effective antifungal therapy prior to bronchoscopy.

Acknowledgements

The authors are grateful to the Division of Medicine, Faculty of Medicine, Khon Kaen University for the publication support.

Conflicts of interest

The authors declare no conflict of interest.

References

1. De Pauw B, Walsh TJ, Donnelly JP, 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;46:1813-21.
2. Franquet T, Müller NL, Giménez A, et al. Spectrum

of pulmonary aspergillosis: histologic, clinical, and radiologic findings. Radiographics. 2001;21:825-37.
3. Kim SH, Kim MY, Hong SI, et al. Invasive Pulmonary Aspergillosis-mimicking Tuberculosis. Clin Infect Dis. 2015;61:9-17.
4. Letourneau AR, Issa NC, Baden LR. Pneumonia in the immunocompromised host. Curr Opin Pulm Med. 2014;20:272-9.
5. Fukuda T, Boeckh M, Carter RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood. 2003;102:827-33.
6. Mikulska M, Furfaro E, Del Bono V, et al. Piperacillin/tazobactam (Tazocin™) seems to be no longer responsible for false-positive results of the galactomannan assay. J Antimicrob Chemother. 2012;67:1746-8.
7. Vergidis P, Razonable RR, Wheat LJ, et al. Reduction in false-positive Aspergillus serum galactomannan enzyme immunoassay results associated with use of piperacillin-tazobactam in the United States. J Clin Microbiol. 2014;52:2199-201.
8. Mattei D, Rapezzi D, Mordini N, et al. False-positive Aspergillus galactomannan enzyme-linked immunosorbent assay results in vivo during amoxicillin-clavulanic acid treatment. J Clin Microbiol. 2004;42:5362-3.
9. Tortorano AM, Esposto MC, Prigitano A, et al. Cross-reactivity of Fusarium spp. in the Aspergillus Galactomannan enzyme-linked immunosorbent assay.J Clin Microbiol. 2012;50:1051-3.
10. Huang YT, Hung CC, Hsueh PR. Aspergillus galactomannan antigenemia in penicilliosis marneffei. Aids. 2007;21:1990-1.
11. Wheat LJ, Hackett E, Durkin M, et al. Histoplasmosis-associated cross-reactivity in the BioRad Platelia Aspergillus enzyme immunoassay. Clin Vaccine Immunol. 2007;14:638-40.
12. Marr KA, Laverdiere M, Gugel A, et al. Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. Clin Infect Dis. 2005;40:1762-9.
13. Duarte RF, Sánchez-Ortega I, Cuesta I, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. Clin Infect Dis. 2014;59:1696-702.
14. Cornely OA. Galactomannan testing during mold-active prophylaxis. Clin Infect Dis. 2014;59:1703-4.
15. Arvanitis M, Ziakas PD, Zacharioudakis IM, et al. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. J Clin Microbiol. 2014;52:3731-42.
16. 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):641-649.
17. Verweij PE, Mennink-Kersten MASH. Issues with galactomannan testing. Med Mycol. 2006;44(Supplement_1):S179-S183.
18. Heng SC, Morrissey O, Chen SC, et al. Utility of bronchoalveolar lavage fluid galactomannan alone or in combination with PCR for the diagnosis of invasive aspergillosis in adult hematology patients: a systematic review and meta-analysis. Crit Rev Microbiol. 2015;41(1):124-134.
19. J Peter Donnelly, Sharon C Chen, Carol A Kauffman, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium, Clinical Infectious Diseases, Volume 71, Issue 6, 15 September 2020, Pages 1367–1376.
20. Eigl S, Hoenigl M, Spiess B, et al. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. Med Mycol. 2017 Jul 1;55(5):528-534.
21. Imbert S, Meyer I, Palous M, et al. Aspergillus PCR in Bronchoalveolar Lavage Fluid for the Diagnosis and Prognosis of Aspergillosis in Patients With Hematological and Non-hematological Conditions. Front Microbiol. 2018 Aug 14;9:1877.
22. Luong ML, Clancy CJ, Vadnerkar A, et al. Comparison of an Aspergillus real-time polymerase chain reaction assay with galactomannan testing of bronchoalvelolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. Clin Infect Dis. 2011 May;52(10):1218-26.
23. Stahl DL, Richard KM, Papadimos TJ. Complications of bronchoscopy: A concise synopsis. Int J Crit Illn Inj Sci. 2015;5:189-95.