Galactomannan antigen detection using bronchial wash and bronchoalveolar lavage in patients with hematologic malignancies

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Abstract

Background: The diagnosis of invasive pulmonary aspergillosis is challenging. It is unclear whether galactomannan (GM) results from bronchial wash (BW) and bronchoalveolar lavage (BAL) samples differ in a clinically meaningful way.

Results: Ninety-six paired (BAL and BW) samples from 85 patients were included. The average age was 53 years, 61% of the patients were male, and 74.1% had an underlying diagnosis of AML/MDS (ALL 7.1%, other hematologic malignancy 18.8%). 57 (67.1%) patients were neutropenic, and 56 (65.9%) patients were receiving mold-active drugs at least 48 h prior to bronchoscopy. The overall agreement between GM detection from BW and BAL was 63.5% (K = 0.152; 95% CI 0.008–0.311) and 73% (K = 0.149; 95% CI 0.048–0.348) at cut off ≥0.5 and ≥1.0, respectively. Among 43 positive samples, using a GM cut-off of 0.5, 39 (90.5%) were positive in BW samples whereas 12 (29.3%) were positive in BAL samples. The median level of GM in BW (0.28) samples was significantly higher than in BAL (0.20) samples among 53 samples with negative results (P = 0.001). There was no statistically significant difference in the median GM values between the BW and BAL samples with positive results (P = 0.08). There was no significant difference in GM detection between samples with positive and negative results with regard to antifungal, beta lactam antibacterial treatment or neutropenia (60.5 vs 56.6%; 53.9 vs 46%; 65.1 vs 54.7%, respectively).

Conclusion: This retrospective study examining two collection techniques suggests that BW may have higher diagnostic yield compared to bronchoalveolar lavage for GM detection.

Keywords: Galactomannan antigen, Bronchial wash, Bronchoalveolar lavage, Invasive pulmonary aspergillosis

Findings

The mortality rate of invasive pulmonary aspergillosis (IPA) remains high in part due to barriers to early diagnosis [1–3]. A definite diagnosis of IPA requires histological evidence of invasive disease or positive cultures from sterile sites [4]. However, respiratory cultures have poor sensitivity and tissue biopsy is not always feasible [5, 6]. Therefore, current diagnostic approaches include non-culture/histopathologic modalities including combination of chest CT imaging and non-invasive, non-culture based tools such as galactomannan (GM) antigen detection in bronchoalveolar lavage (BAL) and bronchial wash (BW) [7–11]. BW samples are defined as the first obtained aliquot of BAL fluid after instillation of small amount of saline into a major airway; BAL samples are collected from the distal bronchioles and alveoli following instillation of a large volume of saline often greater than 140 ml in several aliquots [12]. It is unclear whether GM results from BW and BAL samples differ in a clinically meaningful way. It is hypothesized that bronchial airways may contain more aspergillus hyphae with more subsequent GM release than in alveoli. Alternatively, GM in BAL fluid may be diluted by lavage to an undetectable level by ELISA [13, 14].

In this study, we aimed to compare the performance characteristics of GM antigen tests obtained from BW and BAL samples. This retrospective study included all adult patients with hematologic malignancies at the
University of Maryland Greenebaum Cancer Center who underwent bronchoscopy for evaluation of new pulmonary infiltrates while receiving broad spectrum antibiotics or progression of existing lung infiltrates from October 2010 to September 2013. Only patients who had concurrent paired BAL and BW samples separately tested for GM antigen during the same procedure were included. The Platelia Aspergillus enzyme immunoassay (Bio-Rad Laboratories, Hercules, CA, USA) was used to measure GM antigen levels, using different cutoffs for positivity (≥0.5 and ≥1). A patient who underwent repeated sampling in separate bronchoscopic procedures was included in the analysis if bronchoscopy had been performed on the basis of a new pulmonary infiltrate at least 2 months after the first procedure. Plasmalyte solution was not used to perform BAL. Kappa statistics were used to assess the agreement between the GM results obtained from BW and BAL samples. The Wilcoxon test and McNemar’s test were used, as appropriate. All data analyses were performed using SAS V.8.0. 96 paired samples (BAL and BW) were analyzed from 85 patients (AML/MDS 74.12 %, ALL 7.06 % and the others 18.82 %) comprising 52 males and 33 females with an average age of 53 years (range 20–81). 57 (67.06 %) patients were critically reviewed the manuscript. All authors read and approved the final manuscript.

Abbreviations
GM: galactomannan antigen; BW: bronchial washing; BAL: bronchoalveolar lavage; OD: optical density.

Authors’ contributions
MT and PR designed the study. MT, EW and PR collected the data; MT analyzed and interpreted the data. MT and PR wrote the manuscript. BG and MK critically reviewed the manuscript. All authors read and approved the final manuscript.

### Conclusions

To our knowledge only two studies have compared GM antigen detection yield in BAL and BW with conflicting results [15, 16]. Similar to the results observed by Seyfarth et al., our study demonstrated that BW and BAL results are not comparable in terms of detection of GM [15]. The poor concordance between the results of GM detection from the two sample methods in our prospective single center study suggests that the addition of BW to BAL GM detection enhances positive results or it might be a better single diagnostic sample for GM antigen detection. However, we were unable to establish test specificity/sensitivity due to lack of a gold standard, insensitivity of aspergillus species culture and lack of concurrent serum GM assay (proven and probable cases). Further studies are needed to confirm the complementary role of the BW and to evaluate sensitivity, specificity of GM detection in BW and BAL in IPA diagnosis.

### Availability of supporting data

The data supporting the results of this study are included within this article.

### Table 1 Frequency of positive galactomannan antigen in BAL or BW samples at different cut-off galactomannan OD indexes

| Sample, n = 96 | BAL+, total | BW+, total | BAL+ or BW+ |
|---------------|------------|-----------|-------------|
| GM index ≥ 0.5 | 12 (12.5 %) | 39 (40.62 %) | 43 (44.79 %) |
| GM index ≥ 1.0 | 10 (10.42 %) | 26 (27.08 %) | 31 (32.29 %) |

OD optical density, BAL: bronchoalveolar lavage, BW: bronchial washing, GM: galactomannan
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Competing interests
The authors declare that they have no competing interests.

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References
1. Nivoix Y, Velten M, Letscher-Bru V, Moghaddam A, Natarajan-Amé S, Fohrer C, et al. Factors associated with overall and attributable mortality in invasive aspergillosis. Clin Infect Dis. 2008;47:1176–84.
2. Gallien S, Fournier S, Porcher R, Bottero J, Ribaud P, Sulahian A, et al. Therapeutic outcome and prognostic factors of invasive aspergillosis in an infectious disease department: a review of 34 cases. Infection. 2008;36:533–8.
3. Denning DW. Therapeutic outcome in invasive aspergillosis. Clin Infect Dis. 1996;23:608–15.
4. De Pauw B, Walsh TJ, Donnelly JP, 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(2):1813–21.
5. Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. Lancet Infect Dis. 2005;5:609–22.
6. Reichenberger F, Habicht JM, Gratwohl A, Tamm M. Diagnosis and treatment of invasive pulmonary aspergillosis in neutropenic patients. Eur Respir J. 2002;19:743–55.
7. Musher B, Fredricks D, Leisenring W, Balajee SA, Smith C, Marr KA. Aspergillus galactomannan enzyme immunoassay and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. J Clin Microbiol. 2004;42:5517–22.
8. Kono Y, Tsushima K, Yamaguchi K, Kurita K, Soeda S, Fujiwara A, et al. The utility of galactomannan antigen in the bronchial washing and serum for diagnosing pulmonary aspergillosis. Respir Med. 2013;107:1094–100.
9. Guo YL, Chen YQ, Wang K, Qin SM, Wu C, Kong JL. Accuracy of BAL galactomannan in diagnosing invasive aspergillosis: a bivariate metaanalysis and systematic review. Chest. 2010;138:817–24.
10. 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;61:492–8.
11. Norkin M, Wingard JR. Diagnostic strategies for invasive fungal infections in patients with hematologic malignancies and hematopoietic stem cell transplant recipients. J Natl Compr Canc Netw. 2013;11:941–9.
12. Baselski VS, Wunderink RG. Bronchoscopic diagnosis of pneumonia. Clin Microbiol Rev. 1994;7:533–58.
13. Klont RR, Menrinn-Kersten MA, Verweij PE. Utility of Aspergillus antigen detection in specimens other than serum specimens. Clin Infect Dis. 2004;39:1467–74.
14. Kauffman HF, Beaumont F, Meurs H, van der Heide S, de Vries K. Comparison of antibody measurements against Aspergillus fumigatus by means of double-diffusion and enzyme-linked immunosorbent assay (ELISA). J Allergy Clin Immunol. 1983;72:255–61.
15. Seyfarth HJ, Nenoff P, Winkler J, Krahl R, Haustein UF, Schauer J. Aspergillus detection in bronchoscopically acquired material. Significance and interpretation. Mycoses. 2001;44:356–60.
16. Racil Z, Kocmanova I, Toskova M, Buresova L, Weinbergerova B, Lengerova M, et al. Galactomannan detection in bronchoalveolar lavage fluid for the diagnosis of invasive aspergillosis in patients with hematological diseases—the role of factors affecting assay performance. Int J Infect Dis. 2011;15:e874–81.