



# Impact of posaconazole prophylaxis and antifungal treatment on BAL GM performance in hematology malignancy patients with febrile neutropenia: a real life experience

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## Abstract

**Background** Diagnostic accuracy of galactomannan measurements is highly variable depending on the study population, diagnostic procedures, and treatment procedures. We aimed to evaluate the effect of posaconazole prophylaxis and empiric antifungal treatment upon diagnostic accuracy of GM measurements in bronchoalveolar lavage (BAL), bronchial lavage (BL), and serum in hematological malignancy population.

**Methods** Patients hospitalized in a single tertiary care center with hematologic malignancies undergoing fiberoptic bronchoscopy (FOB) with a preliminary diagnosis of IPA were retrospectively included.

**Results** In all the study population ( $n = 327$ ), AUC for BAL, BL, and serum GM were as follows: 0.731 [0.666–0.790], 0.869 [0.816–0.912], and 0.610 [0.540–0.676] with BL samples having the best diagnostic value. GM measurements in patients under posaconazole prophylaxis ( $n = 114$ ) showed similar diagnostic performance. While specificity was similar between patients with and without posaconazole prophylaxis, sensitivity of GM measurements was lower in patients with prophylaxis. Analyses with patient classified according to antifungal treatment at the time of FOB procedure ( $n = 166$ ) showed a decreased diagnostic accuracy in serum GM and BAL GM measurements related with the duration of treatment. However, BAL, BL, and serum GM measurements presented similar sensitivity and specificity in higher cut-off values in longer durations of antifungal treatment.

**Conclusion** Our study shows that posaconazole prophylaxis and active short-term (3 days) antifungal treatment do not significantly affect overall diagnostic performance of GM measurements in bronchoalveolar lavage and bronchial lavage samples. However, using different cut-off values for patients receiving active treatment might be suggested to increase sensitivity.

**Keywords** Galactomannan diagnostic performance · Bronchoalveolar lavage · Antifungal therapy

## Introduction

Diagnosis and treatment of invasive pulmonary aspergillosis (IPA) are ongoing clinical challenges, especially in immunocompromised patients [1]. Primary antifungal prophylaxis is effective in reducing incidence of IPA and results in improved clinical outcomes in high-risk hematology patients

[2, 3] and therefore it is recommended in clinical guidelines [4–6]. However, controversy over early empirical treatment and diagnostic-driven treatment strategies is ongoing [7–9]. A recent randomized trial concluded that preemptive diagnostic-driven approach is safe and diagnostic-driven treatment strategy results in less antifungal therapy without increased mortality [10]. Effective therapy with diagnostic-driven treatment strategies relies upon the diagnostic value of early indicators of fungal infection such as galactomannan and aspergillus polymerase chain reaction (PCR) tests.

Clinical utility of galactomannan antigen (GM) measurement in serum and bronchoalveolar lavage/bronchial lavage is acknowledged. However, reported diagnostic accuracy of galactomannan measurements is highly variable depending

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**Article's main point** Posaconazole prophylaxis and active short-term (3 days) antifungal treatment do not significantly affect diagnostic accuracy of GM measurements in BAL and BL samples. However, choosing new cut-off values might be needed.

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on the study population, diagnostic procedures, and treatment procedures [1].

We aimed to evaluate the effect of posaconazole prophylaxis and empiric antifungal treatment upon diagnostic accuracy of GM measurements in bronchoalveolar lavage, bronchial lavage, and serum in hematological malignancy patient population.

## Method

### Patient population

Patients aged > 18 years old hospitalized in a single tertiary care center with hematologic malignancies undergoing bronchoscopy with a preliminary diagnosis of invasive fungal disease (IFD) between January 2009 and December 2019 were reviewed and included in this retrospective study. Demographic and clinical characteristics were obtained from the hospital database.

Patients were classified on the basis of EORTC/MSGERC 2020 criteria. According to definitions, there are three levels of probability of IFD diagnosis as “proven,” “probable,” and “possible.” EORTC/MSGERC 2020 criteria consists of three domains: host factors, clinical features, and mycological evidence. Mycological criteria in EORTC/MSGERC 2020 includes direct microscopy of fungal culture, aspergillus PCR, and positive optic indexes of galactomannan antigen. Single serum or plasma GM  $\geq 1.0$  or cerebrospinal fluid (CSF) GM  $\geq 1.0$  or bronchoalveolar lavage (BAL) fluid GM  $\geq 1.0$  or single serum or plasma GM  $\geq 0.7$  and BAL fluid GM  $\geq 0.8$  are considered as positive mycological criteria [11].

### Definitions

Neutropenia and fever were defined according to American Society of Clinical Oncology and Infectious Diseases Society of America Clinical Practice Guideline [12].

Septic shock and multiple organ dysfunction syndrome (MODS) were defined according to Sepsis-1 criteria. Sepsis is characterized as systemic inflammatory response to infection. Septic shock is defined as persistent hypotension related with sepsis and hypo-perfusion related in organ dysfunction is present. MODS is defined as otherwise unexplained organ dysfunction in critically ill patients [13].

### Procedures

Patients presenting with ongoing fever and diagnostic or therapeutic challenges were evaluated by a multidisciplinary committee consisting of pulmonologists, infectious diseases specialists, microbiologists, and hematologists, thus tested

or treated accordingly. Empiric or diagnostic-driven antifungal treatment was started according to the patient’s clinical features and the attending physician’s decision. In case of antifungal treatment before FOB, patients were grouped according to duration of treatment as  $\geq 3$  days and  $\geq 6$  days.

Patients diagnosed with acute myeloid leukemia (AML) had received posaconazole prophylaxis for high-risk patients as recommended by Infectious Diseases Society of America (IDSA) [14]. Between 2009 and 2015, posaconazole prophylaxis was made by oral suspension [15]; beginning from 2015, the posaconazole prophylaxis has been made by oral tablet form. Dosage for posaconazole prophylaxis was 200 mg three times daily for oral suspension form and 300 mg daily after an initial 300 mg 2 times/day for tablet form. Serum galactomannan measurements were monitored bi-weekly [16, 17].

Patients with persistent fever for over 72 h under appropriate antibiotic treatment were evaluated with chest computed tomography (CT). In case of clinical and radiological findings consistent with IPA, fiber optic bronchoscopy (FOB) was scheduled for appropriate patients. Patients with severe hypoxemia or hemodynamic instability and platelet count  $< 20,000$  were not eligible for FOB procedure. FOB procedure was performed by an experienced chest disease specialist team. Bronchoalveolar lavage and bronchial lavage samples were taken from the effected lung lobe/segments according to thorax CT images and approximately 75–100 cc sterile isotonic saline solutions were used. Obtained bronchoalveolar lavage and bronchial lavage samples were submitted to bacterial, fungal, and mycobacterial cultures and tested for galactomannan levels. Tissue biopsies via FOB were obtained only if a visible tracheobronchial mucosal lesion were present such as mucosal necrosis, mucosal plaques, or uneven mucosa.

The galactomannan-ELISA was performed according to the manufacturer’s instructions (Platelia® Aspergillus; Bio-Rad, Marnes-la-Coquette, France). According to manufacturer manual (number 62794) positive GM result for bronchoalveolar lavage, bronchial lavage, and serum specimens, an OD index of  $\geq 0.5$  was considered positive.

### Statistical analyses

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 22 and MedCalc. Means and standard deviations were reported for normally distributed continuous data, and medians and 25–75 percentiles for non-normally distributed continuous data. The difference between two groups’ means and medians in independent samples was analyzed with Student’s *t*-test and Mann–Whitney *U* test, respectively. Categorical variables were compared by chi-square test. Correlation between numerical variables was assessed by Spearman analysis. Diagnostic value of GM

measurements to differentiate no IPA and possible IPA patients from probable IPA and proven IPA was calculated. Receiver operating characteristic (ROC) curve analysis was used to calculate the area under the curve (AUC) and diagnostic accuracy. Diagnostic accuracy of different cut-off values for different sites was compared by MedCalc analyze system. In order to avoid incorporation bias, we performed repeated analysis with patients stratified by criteria EORTC/MSGERC 2020 criteria excluded of GM as reference standard. Values of  $p < 0.05$  were considered statistically significant.

The institutional ethical committee approved the study protocol (2020–16/19).

## Results

Study population consisted of 327 patients with a mean age of  $46.8 \pm 15.0$  years old. Patients were classified according to 2020 EORTC/MSGERC definitions, as result 22 (6.7%) patients were in no IPA, 155 (47.4%) patients were in possible IPA, 141 (43.1%) patients in probable IPA, and 9 (2.8%) patients were in proven IPA group. Fiberoptic bronchoscopy (FOB) was performed under antifungal treatment in 166 (50.8%) patients and median duration of antifungal treatment was 1 [0–6] days. Within 93 positive fungal cultures, 97.8% had *Aspergillus fumigatus*. Characteristics of the study population are presented in Table 1.

### Galactomannan measurement results in study population

BAL GM, BL GM, and serum GM levels were evaluated by ROC curve analysis in order to determine the diagnostic value for differentiating proven and probable IPA patients against possible and non-IPA patients in the study population (Table 2). Calculated area under curve for BAL, BL, and serum GM were as follows: 0.731 [0.666–0.790], 0.869 [0.816–0.912], and 0.610 [0.540–0.676]. Comparison between ROC curves revealed a significant difference between prognostic values of galactomannan measurements from different sites, indicating a better diagnostic performance with bronchial lavage samples (Fig. 1). In addition, false negative results of galactomannan measurements, meaning negative serum and BAL or BL galactomannan (GM) results with fungal culture growth, were observed in 20 (6.1%) patients.

Although serum GM measurement at one time has the worst diagnostic value compared to BAL and BL GM measurements, repeated (bi-weekly) measurements of serum GM showed significant changes ( $p < 0.001$ ) in patients with proven and probable IPA compared to possible and no IPA patients (Fig. 2).

**Table 1** Population characteristics ( $n = 327$ )

Age, years	46.8 ± 15.0
Male gender, $n$ (%)	231 (70.6)
Underlying hematological malignancy, $n$ (%)	
<i>AML</i> , $n$ (%)	162 (49.5)
<i>ALL</i> , $n$ (%)	67 (20.5)
<i>MM</i> , $n$ (%)	27 (8.3)
<i>NHL</i> , $n$ (%)	43 (13.1)
Non-remission status, $n$ (%)	265 (81.0)
Posaconazole prophylaxis, $n$ (%)	114 (34.9)
Comorbidities, $n$ (%)	
<i>DM</i> , $n$ (%)	40 (12.2)
<i>CAD</i> , $n$ (%)	17 (5.2)
<i>COPD</i> , $n$ (%)	7 (2.1)
Radiological involvement in HRCT	
<i>Bilateral involvement</i> , $n$ (%)	194 (59.3)
<i>Nodule</i> , $n$ (%)	177 (53.2)
<i>Halo sign</i> , $n$ (%)	92 (28.1)
<i>Air crescent</i> , $n$ (%)	7 (2.1)
<i>Consolidation</i> , $n$ (%)	205 (62.7)
<i>Cavity</i> , $n$ (%)	38 (11.6)
FOB changed antibiotherapy, $n$ (%)	117 (35.8)
FOB changed antifungal therapy, $n$ (%)	98 (30.0)
Clinical and radiological response to antifungal treatment, $n$ (%)	162 (49.5)
Progression, $n$ (%)	95 (29.1)
Pleural effusion, $n$ (%)	44 (13.6)
MODS, $n$ (%)	26 (8.0)
Septic shock, $n$ (%)	81 (24.8)
<i>NIVM</i> , $n$ (%)	24 (7.3)
<i>IMV</i> , $n$ (%)	105 (32.1)
In-hospital mortality, $n$ (%)	100 (30.6)
30-Day mortality, $n$ (%)	106 (32.4)
Length of stay, days	38 [29–56]
Serum GM, OI	0.41 ± 1.51
BAL GM, OI	0.33 [0.20–0.92]
BL GM, OI	0.71 [0.31–3.23]

Data were expressed as numbers (percentages), mean ± SD, or median [IQR]

*AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *NHL* non-Hodgkin lymphoma, *MM* multiple myeloma, *IPA* invasive pulmonary aspergillosis, *GM OI* galactomannan optic indexes, *HRCT* high-resolution computed tomography, *NIVM* non-invasive mechanical ventilation, *IMV* invasive mechanical ventilation, *BAL* bronchoalveolar lavage, *BL* bronchial lavage

### Galactomannan measurement results in patients receiving posaconazole prophylaxis

Within the study population, 114 (34.8%) patients received posaconazole prophylaxis in-line with current guidelines. Calculated area under curve for BAL, BL, and

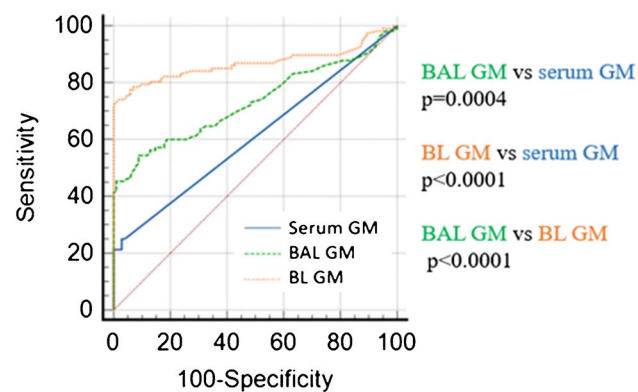
**Table 2** Diagnostic value of galactomannan measurements differentiating proven and probable IPA patients against possible and non-IPA patients in different populations

Measurements	Cut-off (GM-OI)	Sensitivity	Specificity	PPV	NPV	LR
Study population						
BAL	0.61	62.6 [54.5–70.4]	93.2 [88.5–96.4]	88.7 [81.7–93.2]	74.7 [70.5–78.4]	9.24 [5.2–16.1]
BL	0.85	79.3 [70.8–86.3]	93.6 [87.4–97.4]	92.9 [86.4–96.4]	81.3 [75.2–86.1]	12.5 [6.1–25.9]
Serum	0.59	27.3 [20.1–35.5]	98.1 [94.7–99.6]	92.7 [80.0–97.6]	61.0 [58.5–63.5]	14.6 [4.6–46.4]
Within patients receiving posaconazole prophylaxis						
BAL	0.61	56.7 [39.5–72.9]	94.9 [87.5–98.6]	84.0 [66.0–93.4]	82.4 [66.0–93.4]	11.2 [4.14–30.3]
BL	0.69	75.8 [56.5–89.7]	87.7 [75.2–95.4]	78.6 [62.8–88.9]	86.0 [76.2–92.2]	6.2 [2.8–13.4]
Serum	0.78	27.7 [14.2–45.2]	100 [95.3–100.0]	100.0	74.5 [70.5–78.2]	–
Within patients not receiving posaconazole prophylaxis						
BAL	0.80	60.1 [50.5–69.3]	97.9 [92.8–99.8]	97.1 [89.5–99.3]	68.1 [62.9–72.8]	29.4 [7.4–117.1]
BL	0.88	82.7 [73.2–90.0]	95.1 [86.5–99.0]	96.0 [88.8–98.6]	79.7 [71.2–86.2]	17.1 [5.6–51.8]
Serum	0.59	26.2 [18.0–35.8]	98.8 [93.6–100.0]	96.4 [78.9–99.5]	52.5 [49.6–55.4]	22.2 [3.0–160.6]
Within patients receiving antifungal treatment at the time of FOB procedure < 3 days						
BAL	0.54	59.0 [47.7–69.7]	91.6 [84.8–96.1]	84.5 [74.0–91.3]	74.4 [69.1–79.1]	7.0 [3.7–13.5]
BL	0.75	76.9 [64.8–86.5]	89.7 [79.9–95.8]	87.7 [77.8–93.6]	80.3 [72.1–86.5]	7.4 [3.6–15.2]
Serum	0.55	19.2 [11.2–29.7]	98.9 [94.4–100.0]	93.8 [66.9–99.1]	60.4 [57.7–63.0]	18.6 [11.2–29.7]
Within patients receiving antifungal treatment at the time of FOB procedure ≥ 3 days						
BAL	0.61	70.1 [57.7–80.7]	92.7 [83.9–97.6]	90.4 [79.9–95.7]	76.2 [68.8–82.3]	9.6 [4.1–22.8]
BL	0.94	84.3 [71.4–93.0]	100.0 [91.8–100.0]	100.0	84.3 [74.0–91.0]	–
Serum	0.55	37.7 [25.6–51.0]	95.3 [86.9–99.0]	88.5 [70.8–96.0]	61.6 [56.7–66.3]	8.0 [2.5–25.4]
Within patients receiving antifungal treatment at the time of FOB procedure ≥ 6 days						
BAL	0.77	70.4 [54.8–83.2]	97.5 [87.1–99.9]	96.9 [81.6–99.5]	75.5 [66.0–83.0]	28.8 [4.13–202.1]
BL	0.94	87.8 [71.8–96.6]	100.0 [85.8–100.0]	100.0	85.7 [70.5–93.8]	–
Serum	0.78	30.7 [17.0–47.6]	100.0 [90.5–100.0]	100.0	57.8 [52.6–62.8]	–

Data were expressed as % and 95% CI

GM-OI galactomannan optical index, PPV positive predictive value, NPV negative predictive value, LR likelihood ratio, BAL bronchoalveolar lavage, BL bronchial lavage

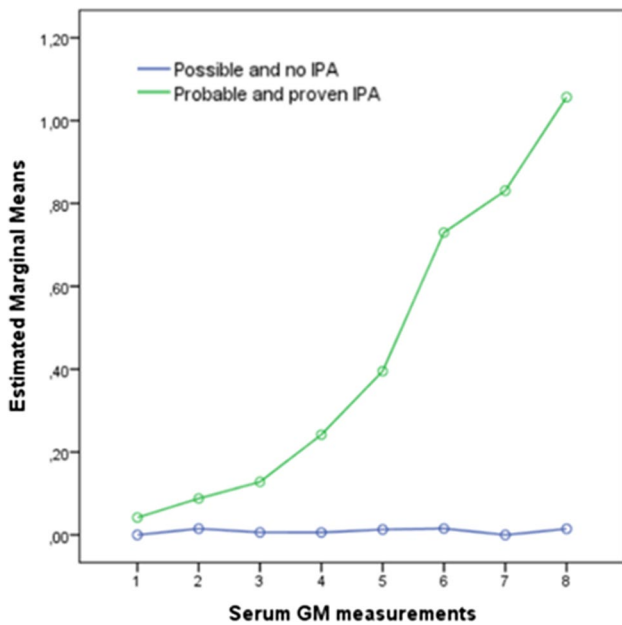
serum GM were as follows: 0.756 [0.649–0.865], 0.858 [0.754–0.961], and 0.643 [0.524–0.761]. While specificity is similar between patients with and without posaconazole



**Fig. 1** ROC analysis performed for bronchoalveolar lavage, bronchial lavage, and serum GM measurements. Abbreviations: GM galactomannan, BAL bronchoalveolar lavage, BL bronchial lavage

prophylaxis, sensitivity of GM measurements is lower in patients with prophylaxis (Table 2). Comparison of ROC curve analysis between patients grouped according to prophylaxis status showed no statistically significant difference in terms of the diagnostic performance of GM measurements (Fig. 3). However, as presented in Fig. 4, repeated measurements of serum GM levels in patients receiving posaconazole prophylaxis do not tend to show a linear progression and do not reach the high values compared to patients not receiving posaconazole prophylaxis. Fungus growth in culture was present in 8 (7.0%) patients and false negativity of GM measurement was observed in 4 (4.9%) patients.

When patients were classified without GM data, analyses showed no statistically significant difference in terms of the diagnostic performance of GM measurements between patients grouped according to prophylaxis status (supplement figure).



**Fig. 2** Repeated serum galactomannan measurements in different patients’ populations. Abbreviation: IPA invasive pulmonary aspergillosis

**Galactomannan measurement results in patients receiving antifungal treatment at the time of FOB procedure**

Fiberoptic bronchoscopy (FOB) was performed under antifungal treatment in 166 (50.8%) patients and median duration of antifungal treatment was 1 [0–6] days. Patients with  $\geq 3$  days and  $\geq 6$  days of antifungal treatment presented 41.0% and 26.0% of the study population.

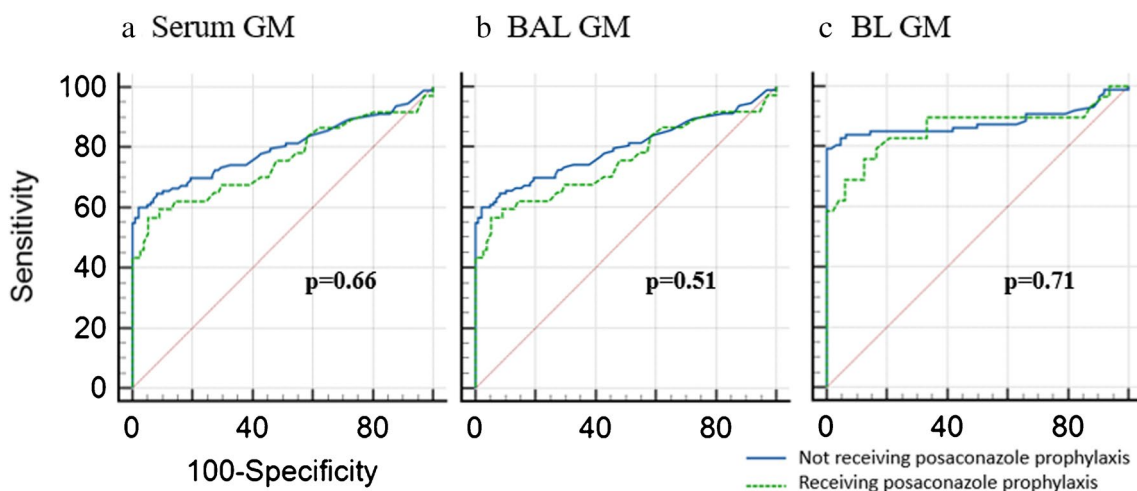
Comparison of ROC curve analysis between patient groups classified according to duration of antifungal treatment at the time of FOB procedure showed a decreased diagnostic accuracy in serum GM and BAL GM measurements (Fig. 5). It is observed that BAL, BL, and serum GM measurements present similar sensitivity and specificity in higher cut-off values in longer durations of antifungal treatment (Table 2).

When patients were classified without GM data, analyses showed statistically significant difference in terms of the diagnostic performance of GM measurements in all samples between patients grouped according to active anti-mold treatment for  $\geq 3$  days (supplement figure).

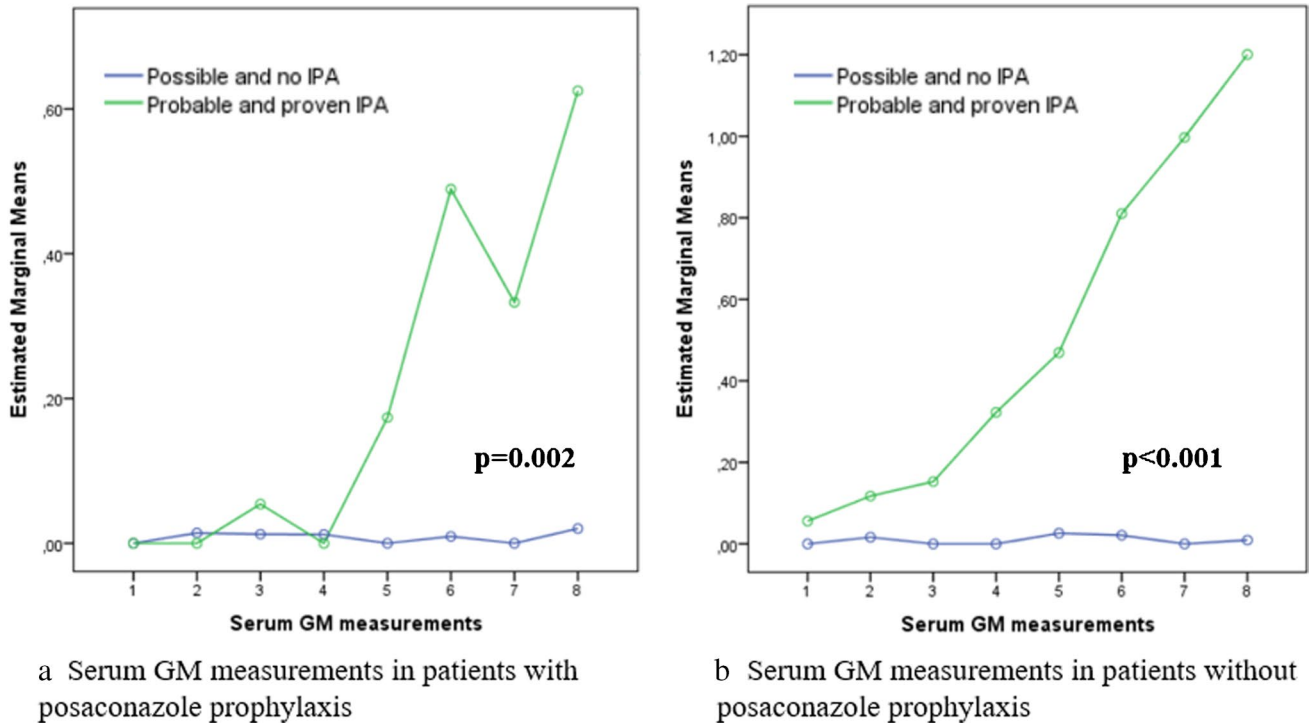
**Correlations with and within GM measurements in different patients’ groups**

In study population, serum GM measurements at the day of FOB were moderately correlated with BAL GM ( $r=0.426, p<0.001$ ) and BL GM ( $r=0.364, p<0.001$ ) levels in addition to weakly correlation with duration of neutropenia before thorax CT imaging ( $r=0.148, p=0.01$ ) and duration of antifungal treatment before FOB ( $r=0.176, p=0.002$ ). BAL GM measurement was correlated with BL GM ( $r=0.607, p<0.001$ ), duration of neutropenia before thorax CT imaging ( $r=0.155, p=0.007$ ), and duration of antifungal treatment before FOB ( $r=0.099, p=0.073$ ).

In the patient population receiving posaconazole prophylaxis, serum GM was similarly correlated with BAL GM ( $r=0.391, p<0.001$ ) and BL GM ( $r=0.295, p=0.02$ ) levels but not with the duration of neutropenia before thorax CT imaging ( $r=0.169, p=0.08$ ) and duration of antifungal treatment before FOB ( $r=0.161, p=0.09$ ). In addition,

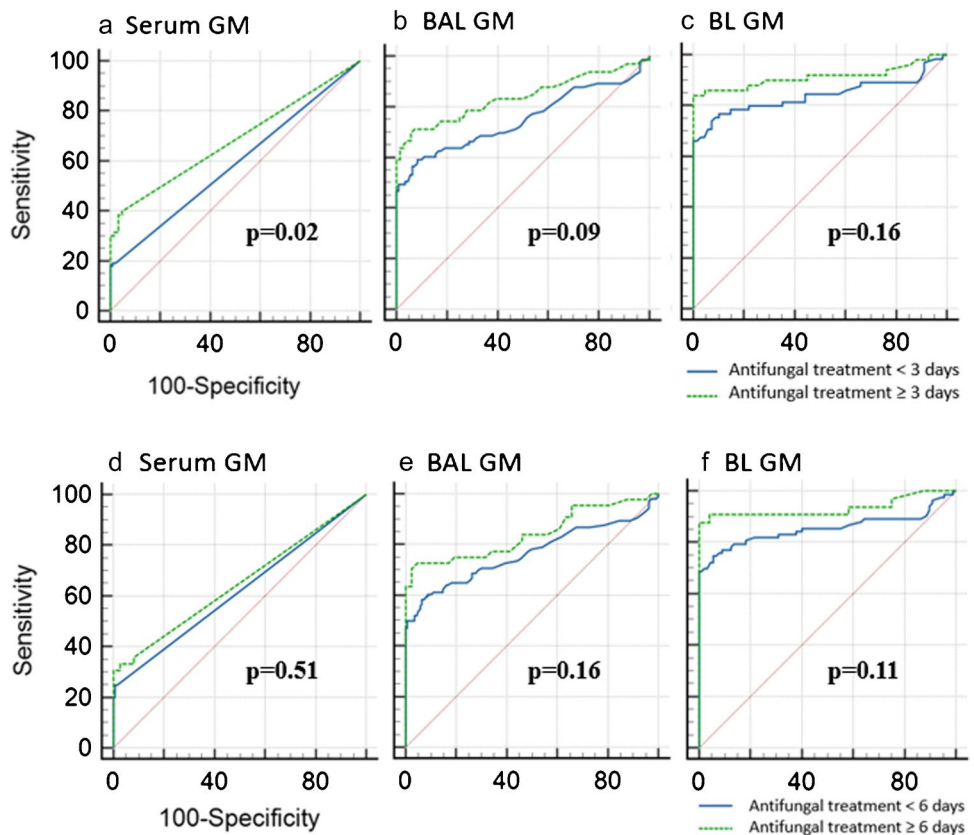


**Fig. 3** Comparison of ROC curve analysis between patients classified according to posaconazole prophylaxis status. Abbreviations: GM galactomannan, BAL bronchoalveolar lavage, BL bronchial lavage



**Fig. 4** Comparison of repeated serum GM measurements between patients classified according to posaconazole prophylaxis status. Abbreviation: IPA invasive pulmonary aspergillosis

**Fig. 5** Comparison of ROC curve analysis between patients classified according to antifungal treatment status at the time of FOB procedure. Abbreviations: GM galactomannan, BAL bronchoalveolar lavage, BL bronchial lavage



BAL GM measurement was only correlated with BL GM ( $r=0.443$ ,  $p<0.001$ ).

Within patients receiving antifungal treatment for  $\geq 3$  days, serum GM was correlated with BAL GM ( $r=0.514$ ,  $p<0.001$ ) and BL GM ( $r=0.504$ ,  $p<0.001$ ) levels in addition to not statistically significant correlation with duration of neutropenia before thorax CT imaging ( $r=0.172$ ,  $p=0.06$ ) and time interval between thorax CT and FOB procedure ( $r=0.177$ ,  $p=0.0054$ ). BAL GM measurement was correlated with BL GM ( $r=0.720$ ,  $p<0.001$ ) and duration of neutropenia before thorax CT imaging ( $r=0.158$ ,  $p=0.07$ ).

### Sensitivity and specificity results with standardized cut-off values in different patient populations

For study population, galactomannan cut-off value of 0.5 in BAL, BL, and serum measurements has sensitivity of 65.3% [57.1–72.9], 84.3% [76.4–90.4], and 28.0% [20.7–36.3] in addition to specificity of 84.1% [77.9–89.2], 64.8% [55.2–73.6], and 96.8 [92.9–98.9], respectively. Changing the cut-off value to 1.0 resulted in decreased sensitivity to in all samples 51.3% [43.0–59.5], 73.0% [63.9–80.8], and 24.1% [13.8–27.7], respectively. However, cut-off value as 1.0 increased specificity in all samples to 100.0% [97.4–100.0] in BAL, 100.0% [96.7–100.0] in BL, and 100.0% [97.7–100.0] in serum measurement.

When using 0.5 as the cut-off value, posaconazole prophylaxis resulted in decrease of sensitivity and increase of specificity in BAL and BL GM measurements. However, in case of using 1.0 as the cut-off value, decrease of sensitivity was present in BAL, BL, and serum GM measurements while specificity did not change (presented in supplement table-S2).

In patients receiving antifungal treatment for  $\geq 3$  days, cut-off value of 0.5 had decreased sensitivity in BAL, BL, and serum GM measurements but similar specificity compared to patients with  $< 3$  days of antifungal treatment. However, cut-off value of 1.0 had better sensitivity in patients receiving antifungal treatment for  $\geq 3$  days (presented in supplement table-S2).

## Discussion

In our study, we showed that BAL and BL galactomannan measurements have better sensitivity than a single serum GM measurement at the same day in hematological malignancy patients. Posaconazole prophylaxis did not change the diagnostic value of GM measurement in BAL and BL however showed a decreased sensitivity in similar cut-off values. Repeated serum GM measurements did not show expected changes in patients receiving posaconazole

prophylaxis. In order to reach a similar sensitivity, the cut-off value for serum samples needed an increase. Performing FOB under antifungal treatment resulted in statistically nonsignificant increase in diagnostic value of BAL and BL GM measurements.

Heng et al. evaluated 783 high-risk invasive aspergillosis patients within adult hematology patients from 16 different studies in their meta-analysis. Using cut-off value as 0.5, they calculated higher sensitivity and specificity in BAL samples for differentiating proven and probable IPA patients from possible and no IPA patients compared to our study. Reported sensitivity was 82% and specificity was 92%. However, they noted a significant heterogeneity in their study population [18].

In our study population, GM measurements from BL samples had better diagnostic value compared to BAL samples. While Taremi et al. and Seyfarth et al. showed similar results [19, 20], Ağca et al. and Racil et al. showed opposite results concerning diagnostic accuracy between BAL and BL samples [21, 22]. Higher GM measurements in BL samples might be due to colonized *Aspergillus* species in patients' bronchial tree [21]. Given the larger area covered by BL, sampling penetration of the endothelial cell layer in a larger area might also result in higher GM measurements [23].

While many studies reported diagnostic value of GM measurement in BAL, BL, and serum samples, evaluating the effect of antifungal prophylaxis or treatment was not possible due to the low number of IPA patients within the study population [24–27]. In our study, using posaconazole prophylaxis resulted in nonsignificant decrease in sensitivity but increase in specificity compared to non-prophylaxis state, in all samples. For patients under posaconazole prophylaxis, using standardized cut-off value as ODI 1.0 results in moderate to large differences in sensitivity and no difference in sensitivity [28]. However, using a different cut-off value can overcome this issue. Active antifungal treatment for  $\geq 3$  days resulted in similar specificity but nonsignificant increased sensitivity in BAL and BL samples. Similar to patients with posaconazole prophylaxis, for patients under antifungal treatment, using standardized cut-off values of ODI 0.5 and 1.0 results in moderate and large differences in sensitivity and using a different cut-off value can also solve this problem [28].

Although mold-active prophylaxis and antifungal treatment were various between and within study populations in the meta-analysis conducted by Heng et al., there was a statistically nonsignificant decrease in sensitivity of BAL GM measurements [18]. Lim et al. had 54.7% of the IPA population under mold-active treatment of a median 7 days and showed that BAL GM measurements had lower sensitivity compared to patients not receiving treatment, but the results were not statistically significant. BAL GM titer

and duration of treatment were not significantly correlated [29]. Some other studies also reported lower BAL GM levels, false negative patients, and a statistically not significant decrease sensitivity in patients receiving mold-active prophylaxis [30, 31]. While Heng et al. showed no difference in sensitivity of BAL GM after median 19 days mold-active antifungal agents before FOB [32], Racil et al. indicated that using antifungal treatment for 2 or more days results in decreased sensitivity for BAL, BL, and serum samples [22]. On the contrary, Musher et al. described a trend of better performance of BAL GM measurements in patients under antifungal therapy [33]. In light of recent literature and findings from study, we can argue that short-term antifungal treatment (< 3 days) does not significantly affect diagnostic accuracy of BAL and BL GM measurements. However, higher cut-off values might be needed to achieve similar sensitivity and specificity.

In hematology patients receiving effective anti-mold prophylaxis, serum galactomannan surveillance with repeated bi-weekly measurements is not recommended. However, serum GM measurement in clinically high-risk patients as a part of diagnostic-driven protocol is recommended [34, 35]. In our study, repetitive serum galactomannan measurements did not show a similar curve in patients receiving prophylaxis compared to patients not receiving prophylaxis.

In our study, patients under active anti-mold treatment for  $\geq 3$  days one serum GM measurement at the time of FOB procedure had better area under curve results compared to patients not receiving active anti-mold treatment. This result is more pronounced in patients' classification without the use of GM results. Similar to our results, Hoenigl et al. presented good sensitivity of serum galactomannan measurement in patients under antifungal prophylaxis and empirical therapy [36]. On the contrary to our results, Marr et al. showed that sensitivity of serum GM measurements is significantly lower in patients using mold-active prophylaxis or treatment at the day of testing. It is also shown that specificity did not change with antifungal therapy. All in all, in the study conducted by Marr et al., area under curve is not affected by treatment at the week prior to diagnosis with microbiological or histopathological tests. This indicating a short term of therapy with antifungals does not have a significant effect on diagnostic accuracy of serum GM [37]. With these data, we can conclude that while repetitive serum measurements lose validity for surveillance under anti-mold prophylaxis, serum GM measurements are still useful under active anti-mold treatment.

In our study, the duration of neutropenia is found to be weakly correlated with BAL and serum GM measurements while neutrophil count was not correlated with GM measurements. Heng et al. found no correlation between neutropenia and BAL GM measurement in their meta-analysis

[18]. Maertens et al. reported no difference in BAL GM levels between neutropenic and non-neutropenic cases within hematologic diseases [30]. Contrarily, Racil et al. reported better sensitivity in BAL, BL, and serum samples in neutropenic patients compared to non-neutropenic patients [22].

Limitations of this study reside in the retrospective nature of the study and the lack of available PCR tests for fungal pathogens. However, given the large number of patients undergoing FOB gives an important strength to our study. False positive results are defined as positive GM results without any radiological abnormalities and absence of clinical symptoms [38]. False positive results in GM, especially measured in serum, are present in enteral nutrition intake, solutions with sodium gluconate, and piperacillin/tazobactam treatment. Although there are studies confirming piperacillin/tazobactam relation with positive GM measurements, a more recent study showed that false positivity is related with manufacturer [39]. While serum GM false negativity is reported, BAL GM false negativity is not reported as frequently. Case reports describing false positive BAL GM results consists of enteral nutrition aspiration [40], nocardiosis [41], histoplasmosis [42], contamination of the container used for collection and transport of materials [43], and using Plasmalyte solution for BAL collection [44]. A recent study conducted by Farmakiotis et al. [45] included all GM measurements without clinical or radiological exclusion criteria and calculated 27% false positivity with BAL GM cut-off  $\geq 0.5$  and 14% false positivity with BAL GM cut-off  $\geq 1.0$ . In our study, we only included patients with fever and radiological involvement. Therefore, aimed to minimize false positivity.

## Conclusion

Bronchoscopic evaluation provides important diagnostic information in hematological malignancy patients with pulmonary infiltrates. Our study shows that posaconazole prophylaxis and active short-term (3 days) antifungal treatment do not significantly affect diagnostic accuracy of GM measurements in BAL and BL samples. Different cut-off values for patients receiving active treatment might be suggested to achieve similar sensitivity results.

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**Author contribution** HA takes responsibility as guarantor and is responsible for the content of the manuscript, data analysis and interpretation, study design, writing of the manuscript, and critical review. NAAÖ and HA had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. NAAÖ contributed to the study design, data collection, data analysis, interpretation of results, and the writing of the manuscript. BÖ, DÖT, and KVA are responsible for data collection



and data interpretation. ÖAG, AGD, ED, AU, İEP, EK, VÖ, FÖ, and RA contributed substantially to the study design, data analysis and interpretation, and the writing of the manuscript.

**Data availability** Data is available upon reasonable request.

## Declarations

**Ethics approval** Ethical committee approval was obtained prior to study from the institutional board (2020–16/19).

**Consent to participate** As a retrospective study, we did not apply for a consent to participate in the study from the patients.

**Consent for publication** All authors have read, approved, and have a consent for publication of this manuscript.

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