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Comparative analysis of galactomannan lateral flow assay, galactomannan enzyme immunoassay and BAL culture for diagnosis of COVID-19-associated pulmonary aspergillosis

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Abstract

Background: Galactomannan Enzyme Immunoassay (GM-EIA) is proved to be a cornerstone in the diagnosis of COVID-19-associated pulmonary aspergillosis (CAPA), its use is limited in middle and low-income countries, where the application of simple and rapid test, including Galactomannan Lateral Flow Assay (GM-LFA), is highly appreciated. Despite such merits, limited studies directly compared GM-LFA with GM-EIA. Herein we compared the diagnostic features of GM-LFA, GM-EIA and bronchoalveolar lavage (BAL) culture for CAPA diagnosis in Iran, a developing country.

Materials/Methods: Diagnostic performances of GM-LFA and GM-EIA in BAL (GM indexes ≥1) and serum (GM indexes >0.5), i.e. sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and areas under the curve (AUC), were evaluated using BAL ($n = 105$) and serum ($n = 101$) samples from mechanically ventilated COVID-19 patients in intensive care units. Patients were classified based on the presence of host factors, radiological findings and mycological evidences according to 2020 ECMM/ISHAM consensus criteria for CAPA diagnosis.

Results: The *Aspergillus* GM-LFA for serum and BAL samples showed a sensitivity of 56.3% and 60.6%, specificity of 94.2% and 88.9%, PPV of 81.8% and 71.4%, NPV of 82.3% and 83.1%, when compared with BAL culture, respectively. GM-EIA showed sensitivities of 46.9% and 54.5%, specificities of 100% and 91.7%, PPVs of 100% and 75%, NPVs of 80.2% and 81.5% for serum and BAL samples, respectively.

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Conclusion: Our study found GM-LFA as a reliable simple and rapid diagnostic tool, which could circumvent the shortcomings of culture and GM-EIA and be pivotal in timely initiation of antifungal treatment.

KEYWORDS

COVID-19-associated pulmonary aspergillosis, culture, galactomannan enzyme immunoassay, galactomannan lateral flow assay

1 | INTRODUCTION

In summary, invasive pulmonary aspergillosis (IPA) is a life-threatening infection in patients with impaired immune systems and associated with mortality rate of 30%–60%.[1–4] Over the recent years, IPA also continued to emerge in the intensive care unit (ICU) population,[5] where IPA prevalence rates of 16%–23% have been reported among patients with severe influenza.[6] Although IPA reports among patients with severe COVID-19 were noted as a sporadic incidence early on during the pandemic, the recent multicentre studies suggest a notable median prevalence COVID-19-associated pulmonary aspergillosis (CAPA) (10% and 15%) with marked differences from centre to centre and mortality rates reaching 50%.[7–9]

In the absence of highly specific radiological findings, classification of CAPA in patients with COVID-19-associated acute respiratory failure relies mostly on mycological criteria. Conventional methods such as culture and microscopy are limited by less than perfect sensitivity and long turn-around time. To achieve an early diagnosis, which is essential for initiating early treatment and to reducing mortality, other non-culture-based techniques, particularly detection of galactomannan (GM), have become a cornerstone in diagnosing CAPA.[10–12] While galactomannan is usually detected with an enzyme immunoassay (EIA), the EIA is not broadly available in low and middle-income countries (LMICs), and turnaround time may be a limitation.[13] The *Aspergillus* specific galactomannan lateral flow assay (GM-LFA) is a simple and rapid test that may overcome some of those limitations, as it only requires rudimentary laboratory facilities and is featured by rapid turn-around time. While the LFA has recently been shown to reliably diagnose IPA from bronchoalveolar lavage (BAL) in various patient cohorts, including ICU patients,[14] only few studies to date have evaluated the LFA for diagnosis of CAPA.[15]

In this single-centre study, we evaluated performances of galactomannan (GM) EIA and GM-LFA in serum and BAL samples, as well as BAL culture for the diagnosis of CAPA.

2 | MATERIAL AND METHODS

2.1 | Design of the study and samples collection

This single-centre prospective study was conducted at the Mazandaran University of Medical Sciences in Sari, Iran. During 1st May and 30th September 2020, among 302 intensive care units admitted COVID-19 PCR-confirmed patients, 105 patients who required mechanical ventilation for ≥4 days were included in the study. A total of 105 BAL and 101 sera samples were collected 3–4 days after mechanical ventilation from all included patients.

This study was approved by the ethics committee of the Mazandaran University of Medical Sciences (Code: IR.MAZUMS.REC.1399.233).

2.2 | BAL culture and mould identification

Bronchoalveolar lavage samples (10–15 ml) were centrifuged for 10 min at 800 × *g*. The supernatant was stored at −80°C for GM detection. The sediment was inoculated onto Sabouraud-Chloramphenicol dextrose Agar (SC) (QUELAB) plates and incubated for 5–7 days at 27°C. Mould colonies were sub-cultured onto SC and identified at species level by sequencing beta-tubulin and ITS loci as described previously.[16] The partial DNA sequence data from both genes were subjected to BLAST query of three online databases, including Centraalbureau voor Schimmelcultures (CBS-KNAW) Fungal Biodiversity Center, Utrecht, the Netherlands (http://www.cbs.knaw.nl), the National Center for Biotechnology Information, Bethesda, MD (http://www.ncbi.nlm.nih.gov) and *Fusarium* ID (http://isolate.fusariumdb.org/blast.php).

2.3 | Galactomannan assay

For evaluation of the GM in serum and BAL of all included patients, two commercially available techniques were used, including IMMY Sona *Aspergillus* GM lateral flow assay (GM-LFA, IMMY) and an enzyme immunoassay (EIA) technique by Dynamiker *Aspergillus* GM Assay DNK-1402-1 (GM-EIA, Dynamiker, Biotechnology).

Galactomannan lateral flow assay testing was performed on 300 μl each of BAL and serum samples following manufacturer's instructions. The LFA was performed by a single operator, using a GM index (GMI) >0.5 and ≥1.0 in serum and BAL as a threshold for positivity, respectively. To remove subjectivity, confirm validity and provide a GM index, the Sona LFA cube reader (IMMY Diagnostics) was used when reading each LFA.

For the GM-EIA, samples were tested in duplicate and the mean value used for interpretation, following the manufacturer's instructions.

Aspergillus PCR was not performed on the BAL samples as one of the mycological evidences.

2.4 | COVID-19-associated pulmonary aspergillosis (CAPA) definition

In this current study, we used the 2020 ECMM/ISHAM consensus criteria[17] for definition of COVID-19-associated pulmonary aspergillosis (CAPA). Patients were categorised as probable or possible or no CAPA.

Patients were classified as probable CAPA based on the presence of host factors (requiring ICU admission for respiratory distress with a positive SARS-CoV-2 PCR temporally related to ICU admission), radiological factors (pulmonary infiltrate, preferably documented by chest CT or cavitating infiltrate (not attributed to another cause)), clinical worsening and mycological criteria. The mycological criteria were defined as the presence of at least one of the following: serum GM index >0.5 or BAL GM index ≥1.0 (using GM-EIA or GM-LFA) or positive respiratory specimen culture for *Aspergillus*. If respiratory culture grows *Aspergillus* spp or other moulds, patients fulfilling above criteria will be classified as CAPA or COVID-19-associated pulmonary mould infections (CAPMI), respectively.

2.5 | Data analysis

Descriptive analysis of quantitative and qualitative variables was done using mean ± standard deviation and frequency respectively. The diagnostic performance of GM assay in BAL (GM indexes ≥1) and serum (GM indexes >0.5) was evaluated by calculating sensitivity, specificity, positive predictive value and negative predictive value and by receiver operating characteristics (ROC) curve analysis, with areas under the curve (AUCs) displayed with 95% confidence intervals and compared using the method by Hanley McNeil. Non-normally distributed data reported as medians and interquartile ranges (IQR) and changes of GM antigen level were examined using Wilcoxon Rank Test. We used Chi-square test for comparison of diagnostic value of GM-LFA and GM-EIA in BAL (GM indexes ≥1) and serum (GM indexes >0.5) samples. For correlation analyses between the LFA, EIA and culture, the Phi correlation coefficient (Phi) test as well as Spearman rho was used. The Phi test is one of a number of correlation statistics developed to measure the strength of association

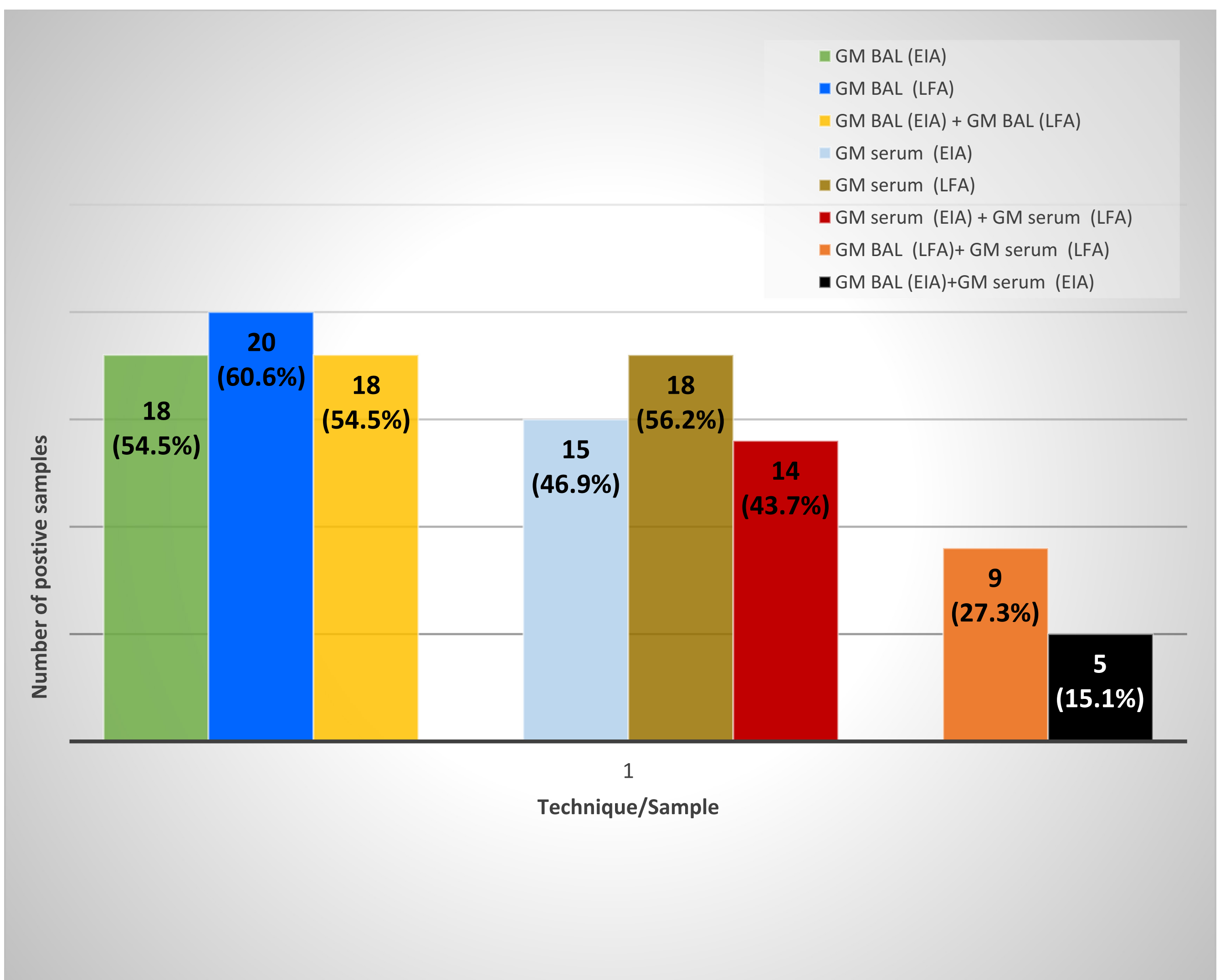


FIGURE 1 The frequency of positivity of galactomannan lateral flow assay (GM-LFA) and galactomannan enzyme immunoassay (GM-EIA) in bronchoalveolar lavage (BAL) (n = 33) and serum (n = 32) samples from COVID-19-associated pulmonary aspergillosis.

between two variables. The Phi is a nonparametric statistic used in cross-tabulated table data where both variables are dichotomous.

All statistical analysis was done by SPSS 25 (IBM) and Medcal20 at the 5% significance level.

3 | RESULT

Demographic characteristics and underlying diseases of the study population and culture results and identified fungal species were presented in our *previous report*.[17] In summary, of 105 patients, 58 (55.2%) were male. The patients' ages ranged from 25 to 95 years with a mean of 65.2 years. Of 105 patients, underlying conditions were well-known in 91 cases, out of which hypertension (48/105; 45.7%) was *frequent*. *Aspergillus* (22/29, 75.9%), *Fusarium* (6/29, 20.6%) and *Diaporthe foeniculina* (1/29, 3.4%) were isolated from COVID-19 patients.

Out of 40 cases with probable COVID-associated mould infection, 33/40 (82.5%) were diagnosed as CAPA, of which 22 patients had positive BAL culture for *Aspergillus* species. Using the GM-EIA, 18/33

TABLE 1 Distribution of galactomannan antigen level in patients with and without COVID-19-associated pulmonary aspergillosis.

		Wilcoxon's rank test		
		Mean rank ODI		
	Median ODI (IQR)	Positive rank	Negative rank	*p*-value
CAPA patients (*n*/*N* = 33/33)				
Serum GM-EIA	0.5 (0.35–0.75)	15.48	10.83	<.001
Serum GM-LFA	0.6 (0.5–0.9)			
BAL GM-EIA	1.05 (0.45–1.75)	15.93	3	<.001
BAL GM-LFA	1.45 (0.6–2.85)			
Culture positive CAPA patients (*n*/*N* = 22/33)				
Serum GM-EIA	0.4 (0.3–0.6)	11.74	4	<.001
Serum GM-LFA	0.5 (0.5–0.9)			
BAL GM-EIA	1.4 (0.65–1.95)	9.5	0	<.001
BAL GM-LFA	2.5 (0.85–3.8)			
Culture negative CAPA patients (*n*/*N* = 11/33)				
Serum GM-EIA	0.6 (0.6–0.8)	4.07	7.5	.13
Serum GM-LFA	0.8 (0.5–0.9)			
BAL GM-EIA	0.6 (0.4–0.9)	6.4	2.0	.005
BAL GM-LFA	0.8 (0.6–1.1)			
No CAPA patients (*n*/*N* = 72/105)				
Serum GM-EIA	0.3 (0.2–0.3)	44.2	67.02	<.001
Serum GM-LFA	0.4 (0.3–0.5)			
BAL GM-EIA	0.3 (0.2–0.4)	40.6	82.5	<.001
BAL GM-LFA	0.4 (0.2–0.4)			

TABLE 2 The sensitivity, specificity and positive and negative predictive values for galactomannan lateral flow assay and galactomannan enzyme immunoassay in bronchoalveolar lavage (GM indexes ≥1) and serum (GM indexes >0.5) samples.

	GM-EIA		GM-LFA	
	Serum (*n*/*N* = 32/101)	BAL (*n*/*N* = 33/105)	Serum (*n*/*N* = 32/101)	BAL (*n*/*N* = 33/105)
GM index	>0.5	≥1	>0.5	≥1
Sensitivity	46.9	54.5	56.3	60.6
Specificity	100	91.7	94.2	88.9
PPV	100	75	81.8	71.4
NPV	80.2	81.5	82.3	83.1
Likelihood ratios	40.6	26.1	31.5	27.3
AUC	0.832	0.808	0.859	0.801

Abbreviations: AUC, area under the curve; GM, galactomannan; GM-EIA, galactomannan enzyme immunoassay; GM-LFA, galactomannan lateral flow assay; NPV, negative predictive value; PPV, positive predictive value.

(54.5%) patients with CAPA had a positive BAL GM result and 15/32 (46.9%, serum sample was not available for one patient) had a positive GM result in one or more serum samples (Figure 1). The GM-LFA resulted positive in 20/33 (60.6%) BAL samples, and results were mostly consistent with the GM-EIA except for two samples that were positive by LFA but negative by GM-EIA. Serum GM-LFA resulted positive in 18/32 (56.2%); four samples that resulted negative with the EIA had a positive result by LFA, while other results were consistent between the assays. Of 33 BAL samples, 18 (54.5%) showed positivity in both two techniques GM-EIA and GM-LFA while in serum samples ($n = 32$), 14 (43.7%) were positive in both techniques (Figure 1).

Of six patients with *Fusarium*-positive BAL samples, five had BAL GM index ≥1; however, none showed GM positivity in serum samples.

3.1 | Comparison of GM-EIA and GM-LFA for CAPA diagnosis

For the GM-EIA, the Median ODI (IQR) was 1.05 (0.45–1.75) for BAL and 0.5 (0.35–0.75) for serum, while for the GM-LFA Median ODI (IQR) was 1.45 (0.6–2.85) for BAL and 0.6 (0.5–0.9) for serum. Spearman rho showed 0.92 and 0.74 Correlation Coefficients in BAL and serum between two methods (p-value = <.001; Table 1).

According to comparison of GM BAL and GM serum obtained by GM-EIA and GM-LFA in CAPA patients who had a positive culture for *Aspergillus* species, Wilcoxon Rank Test showed the positive/negative mean rank as 9.5/0 (BAL-EIA/BAL-LFA) and 11.7/4 (serum-EIA/serum-LFA) for BAL and serum GM, respectively. There was a significant correlation in both methods (p-value = <.001). Whereas in the CAPA group with negative culture, the positive/negative mean ranks of GM BAL and GM serum as 6.4/2.0 and 4.07/7.5 were reported by two methods, respectively. In contrast to serum samples, the correlation in BAL samples was significant (p-value = <.001; Table 1).

Table 2 shows the sensitivity, specificity and positive and negative predictive values for galactomannan lateral flow assay and galactomannan enzyme immunoassay in BAL (GM indexes ≥1) and serum (GM indexes >0.5) samples. The sensitivity and specificity of GM serum EIA/LFA were reported as 46.9/100 and 56.3/94.2, respectively. The sensitivity and specificity of GM BAL EIA/LFA were also 54.5/91.7 and 60.6/88.9, respectively.

The sensitivity and specificity of GM serum EIA/LFA in CAPA patients with positive culture were 28.6/88.8 and 47.6/85.0 respectively. The sensitivity and specificity of GM BAL EIA/LFA were also observed as 72.7/90.4 and 77.2/86.7, respectively (Table 3).

3.2 | Correlation between galactomannan levels in serum and BAL samples (using GM-EIA and GM-LFA) with BAL culture

The serum/BAL GM-LFA were positive in 47.6% and 77.2% of CAPA cases with positive BAL culture and 72.7% and 27.2% of CAPA cases with negative BAL culture, respectively. The serum/BAL GM-EIA was also positive in 28.6% and 72.7% of cases with culture positive CAPA and in 81.8% and 18.2% of CAPA cases with negative culture results, respectively.

Phi coefficients were used to evaluate the correlation between the techniques. This coefficient in relation to GM-EIA and GM-LFA in BAL sample was respectively $\Phi = 0.51$ (p-value < .001) and $\Phi = 0.52$ (p-value < .001),and in serum sample $\Phi = 0.57$ (p-value < .001) and $\Phi = 0.55$ (p-value < .001; Table 4). İn cases with culture positive CAPA, Phi coefficient in relation to GM-EIA and GM-LFA in BAL sample was respectively $\Phi = 0.61$ (p-value < .001) and $\Phi = 0.59$ (p-value < .001), and in serum sample, $\Phi = 0.20$ (p-value = .05) and $\Phi = 0.32$ (p-value < .001; Table 4).

3.3 | ROC curve analyses

In ROC curve analysis (Figure 2), the GM-LFA and the GM-EIA showed a similar diagnostic performance in serum (GM-LFA$_{AUC}$ =0.859,

TABLE 3 The sensitivity, specificity and positive and negative predictive values for galactomannan lateral flow assay and galactomannan enzyme immunoassay in bronchoalveolar lavage (GM indexes ≥1) and serum (GM indexes >0.5) samples for cases with culture positive CAPA.

	GM-EIA		GM-LFA	
	Serum (n/N = 21/101)	BAL (n/N = 22/105)	Serum (n/N = 21/101)	BAL (n/N = 22/105)
GM index	>0.5	≥1	>0.5	≥1
Sensitivity	28.6	72.7	47.6	77.2
Specificity	88.8	90.4	85.0	86.7
PPV	40.0	66.7	45.5	60.7
NPV	82.6	92.6	86.1	93.5
Likelihood ratios	3.5	34.5	9.2	33.3
AUC	0.721	0.823	0.767	0.808

Abbreviations: AUC, area under the curve; GM, galactomannan; GM-EIA, galactomannan enzyme immunoassay; GM-LFA, galactomannan lateral flow assay; NPV, negative predictive value; PPV, positive predictive value.

TABLE 4 The comparison of diagnostic value of galactomannan lateral flow assay and galactomannan enzyme immunoassay in bronchoalveolar lavage (GM indexes ≥1) and serum (GM indexes >0.5) samples.

	Serum GM-EIA				Serum GM-LFA				BAL GM-EIA				BAL GM-LFA			
	+	-	*p*-value	Correlations coefficient (Phi and Cramer's)	+	-	*p*-value	Correlations coefficient (Phi and Cramer's)	+	-	*p*-value	Correlations coefficient (Phi and Cramer's)	+	-	*p*-value	Correlations coefficient (Phi and Cramer's)
Patients with CAPA ($n = 33$)	15	17	<.001	0.57	18	14	<.001	0.55	18	15	<.001	0.51	20	13	<.001	0.52
Patients without CAPA ($n = 72$)	0	69			4	65			6	66			8	64		
Patients with culture positive CAPA	6	15	.05	0.20	10	11	<.001	0.32	16	6	<.001	0.61	17	5	<.001	0.59

Abbreviations: GM, galactomannan; GM-EIA, galactomannan enzyme immunoassay; GM-LFA, galactomannan lateral flow assay.

In general a total of 105 BAL and 101 sera samples were collected from 105 mechanically ventilated COVID-19 patients.

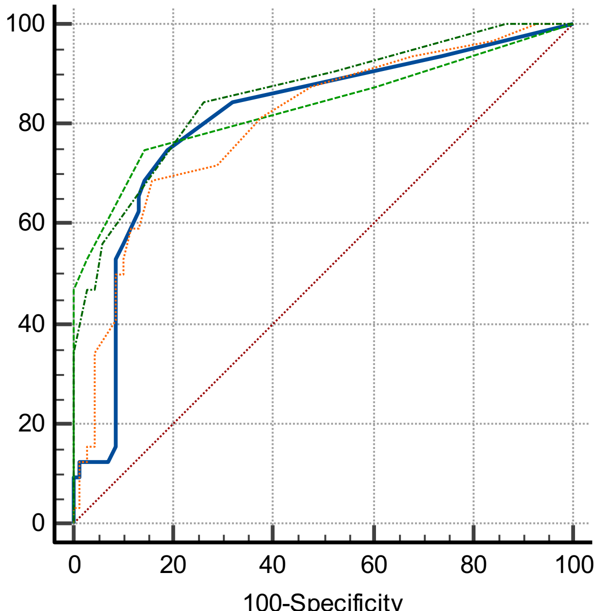
95% CI 0.776–0.920 vs GM-EIA$_{AUC}$ =0.832, 95% CI 0.745–0.899; *p*-value = .56) and BAL samples (GM-LFA$_{AUC}$ =0.801, 95% CI 0.710–0.874 vs GM-EIA$_{AUC}$ =0.808, 95% CI 0.717–0.879; *p*-value = .73). In CAPA group with positive culture, the GM- LFA and the GM-EIA showed a similar diagnostic performance in serum (GM-LFA$_{AUC}$ =0.767, 95% CI 0.651–0.883 vs GM-EIA$_{AUC}$ =0.721, 95% CI 0.592–0.850; *p*-value = .07) and BAL samples (GM-LFA$_{AUC}$ =0.808, 95% CI 0.693–0.924 vs GM-EIA$_{AUC}$ =0.823, 95% CI 0.715–0.930; *p*-value = .06; Figure 3).

4 | DISSCUSSION

COVID-19-associated pulmonary aspergillosis has emerged as a complication of COVID-19 associated with acute renal failure in the ICU. CAPA is mainly diagnosed in non-neutropenic patients and therefore presents with "atypical" clinical and radiological presentations due to primarily airway invasive growth of *Aspergillus* species.[18] As a result, diagnosis of IPA in non-neutropenic patients strongly relies on mycological findings. Conventional mycological diagnostics, however, may have insufficient sensitivities, as shown in a large autopsy study,[19,20] where only one-fourth of autopsies proven IPA were diagnosed in vivo by culture-based methods. Due to the imperfect sensitivity of conventional diagnostics,[19,20] serological and molecular methods have become a cornerstone in diagnosing IPA.[21] Particularly GM testing from BAL and serum is now widely used for diagnosis and treatment stratification in IPA.[22,23] Sensitivities and specificities of the GM-EIA found in our study were comparable with previous studies; however, turn-around time for the GM EIA may vary. In this present study, the *Aspergillus* LFA test has shown to be a reliable alternative with results that strongly correlate with GM-EIA testing, and similar sensitivity (60.6%) and specificity (88.9%) in BAL fluid and serum (sensitivity 56.3%) for diagnosing CAPA. These findings are mostly in line with those of a recent multicentre study evaluating the LFA in respiratory specimens for diagnosis of CAPA,[15] and IPA in non-neutropenic patients.[24] Jenks et al.[24] also concluded *Aspergillus* GM- LFA and *Aspergillus*-specific LFD test in BAL as two point-of-care assays in non-neutropenic patients.

In contrast, performance of the GM-LFA for diagnosing CAPA in serum was superior in this study vs prior studies,[15,25] despite similar strong correlations with serum GM-EIA indexes, potentially indicating more advanced *Aspergillus* disease at the time of diagnosis. Together with previous studies, our results indicate that the GM-LFA may serve as a valuable tool for informing early treatment decisions as well as preventing of overtreatment in settings that do not have access to fast GM-EIA results.

Our findings showed an excellent correlation between the GM detection by two applied methods and culture in BAL samples with good to excellent discriminatory power (Φ = 0.51 (GM-EIA) and Φ = 0.52 (GM-LFA)) in differentiating of probable CAPA from patients with no CAPA, while performance in serum was less discriminatory (Φ = 0.57 (GM-EIA) and Φ = 0.55 (GM-LFA)) which was concordant with a study by Cai et al.,[26] who showed that the serum



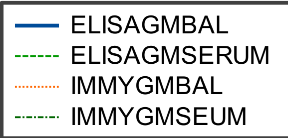


FIGURE 2 The receiver operating characteristic curves of serum and BAL galactomannan in diagnosing COVID-19-associated pulmonary aspergillosis.

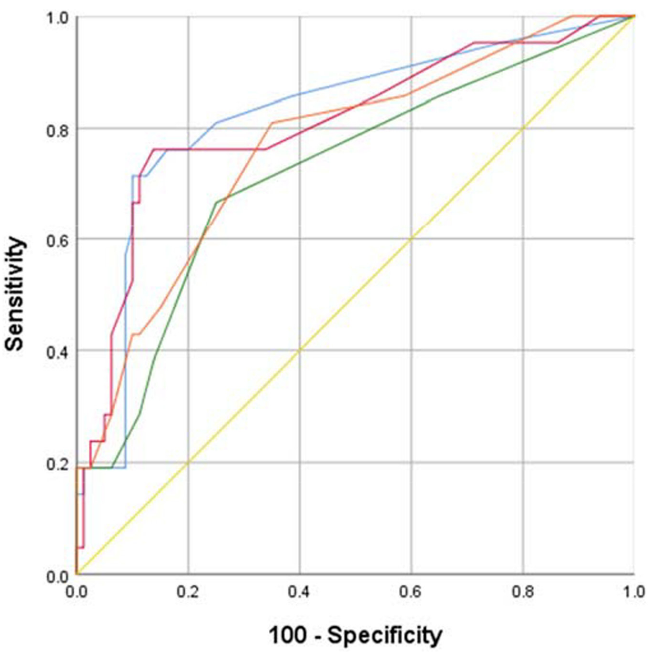


FIGURE 3 The receiver operating characteristic curves of serum and BAL galactomannan in diagnosing COVID-19-associated pulmonary aspergillosis (cases with culture positive CAPA compared with other cases).

GM assay was less useful for the diagnosis of IPA in non-neutropenic patients. Comparison of the likelihood ratios of the BALF GM and serum GM tests in this study indicated that the BALF GM test was more helpful for the diagnosis of probable CAPA than the serum GM test which is also in concordance with other previous reports.[27,28] This finding may also be explained by the observation that GM is cleared by neutrophils, resulting in lower sensitivity of GM when tested in serum from non-neutropenic patients when compared with GM tested from BAL samples which are usually taken at the primary location of infection.[27,29] Our finding showed a higher rate of serum GM positivity in comparison to some other previous studies, which may have to do with diagnosis of CAPA occurring at a later stage of disease in our population vs previously published populations, as outlined by the extremely high 90.9% mortality rate in our cohort of CAPA patients.

Limitations of our study include the single-centre, partly retrospective design and the fact that according to current consensus definitions BAL GM and serum GM results were utilised for CAPA

classification, definitely resulting in an overestimation of GM performance for diagnosing CAPA.

5 | CONCLUSION

According to our results, BAL GM detection using both EIA and LFA is a promising approach for early diagnosis of CAPA with the LFA method being an attractive option for settings that lack fast turnaround for GM-EIA testing.

AUTHOR CONTRIBUTIONS

M.T.H., L.D., W.P. and A.A. were involved in the concept and design of the study. M.G., J.Y.C., M.M., M.A., I.H., S.M. and M.H. were involved in the acquisition, analysis and/or interpretation of the data. All authors participated in drafting the manuscript and its critical revisions for important intellectual content. All authors approved the final submitted article.

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CONFLICT OF INTEREST

Martin Hoenigl received research funding from Astellas, Euroimmune, Pfizer, Gilead, Scynexis, MSD and NIH. All other authors declared no potential conflict of interest of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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