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DIAGNOSTIC PERFORMANCE OF PCR AND GALACTOMANNAN ANTIGEN TESTING IN RESPIRATORY SPECIMENS FOR DIAGNOSIS OF ASPERGILLOSIS

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Abstract

Introduction: Aspergillosis in immunocompromised individuals is a serious and potentially life-threatening infection. Early diagnosis of aspergillosis is a great diagnostic challenge. Therefore, there is a need for more sensitive methods for early detection of aspergillosis.

Aim: The aim of the study was to compare the diagnostic value of PCR and galactomannan antigen tests performed on respiratory specimens for the diagnosis of aspergillosis.

Material and methods: Respiratory specimens from 125 patients divided into 4 groups, according to EORTC/MSG criteria, were analysed with PCR and galactomannan antigen tests, at the Institute of Microbiology and Parasitology during a period of two years.

Results: PCR performed in respiratory specimens yielded the following sensitivity and specificity percentages in all four groups: 67.86 % and 85.71 %, 72.73 % and 75 %, 40.91 % and 50 %, 50 % and 50 % in all groups, respectively. Sensitivity and specificity of galactomannan antigen assay in respiratory specimens were: 75 % and 57.14 %, 86.36 % and 62.5 %, 81.82 % and 0 %, 80 % and 70.83 % in all 4 groups, respectively.

Conclusion: The findings of our study support the use of galactomannan antigen testing in respiratory specimens for the diagnosis of aspergillosis, noting its potential superiority to PCR in specific cohorts. Although PCR provides optimal sensitivity for early detection and treatment initiation, the greatest diagnostic accuracy is achieved by combining both methods. Therefore, a multimodal diagnostic strategy, integrating galactomannan and PCR testing, is recommended to enhance early detection and guide management, particularly in high-risk patients.

Keywords: *Aspergillus*, aspergillosis, PCR, galactomannan, respiratory specimen

Introduction

Aspergillosis, an opportunistic fungal infection due to *Aspergillus* species, presents a wide clinical spectrum. This ranges from allergic manifestations, including allergic bronchopulmonary aspergillosis, to progressive chronic diseases such as chronic pulmonary aspergillosis. The most acute and life-threatening form is invasive aspergillosis, which carries the highest mortality[1]. Historically associated with severely immunocompromised patients, such as neutropenic patients, patients with stem cell and solid organ transplantation, invasive aspergillosis is now also recognized in non-neutropenic, immunocompetent critically ill patients treated in intensive care units (ICU) for a prolonged time, without traditional risk

factors[2]. Predisposing conditions in this group include COPD, broad-spectrum antibiotic or corticosteroid use, diabetes, renal or liver failure, and malignancy[3].

Diagnostic challenges persist in the laboratory diagnosis of invasive aspergillosis. Conventional mycological techniques, which are time-consuming and insensitive (positive in <30 % of cases), remain the standard in many mycology laboratories. The diagnostic delays caused by these methods contribute directly to increased mortality[4].

The potential of PCR for detecting *Aspergillus* DNA has been recognized for years, yet it was not part of the EORTC/MSG consensus definitions until recently[5,6]. A shift has now occurred, driven by data demonstrating its utility for etiological diagnosis across patient groups. Consequently, PCR has been incorporated into the revised EORTC/MSG criteria for invasive disease and is also recommended by ESCMID/ERS guidelines for non-invasive forms like chronic pulmonary aspergillosis and ABPA[7]. As a result, PCR-based methods, long used in clinical practice, are now formally endorsed for diagnosing or ruling out suspected invasive fungal infections[8]. Their implementation is essential for achieving the accurate and reliable mycological diagnosis required to manage these serious infections effectively.

The shortcomings of conventional diagnostics have also accelerated the development of non-culture-based assays, including serological tests. These tests often target biomarkers released by fungi during growth. A prominent example is galactomannan (GM), an *Aspergillus*-specific polysaccharide antigen shed into bodily fluids early in the infectious process, which is detectable with ELISA[9]. The diagnostic utility of galactomannan bronchoalveolar lavage (BAL) fluid is well-documented, as a sensitive test for diagnosing IPA, both in hematology and nonhematology patients, including solid-organ transplant recipients and critically ill patients. A 2019 meta-analysis in lung transplant recipients reported a sensitivity of 71% and specificity of 85% for serum GM[10]. The BAL GM test demonstrated higher but more variable performance (sensitivity: 60-100%; specificity: 40-95%). Data from the same study showed BAL GM had a sensitivity of 80% and a specificity range of 38.5-81.8%[10].

The aim of the study was to compare the diagnostic value of PCR and galactomannan antigen tests performed in respiratory specimens, for etiological diagnosis of aspergillosis.

Material and methods

Study design, specimens and methods

A prospective study was performed at the Mycology laboratory at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, Republic of Macedonia, in the period of 2 years (2014-2016), as part of a PhD study. Respiratory specimens (sputum, tracheal aspirate, BAL) from 125 patients divided into four groups, according to clinical diagnosis and risk factors for the development of invasive fungal infection, were analyzed at the Mycology laboratory. Invasive aspergillosis was defined according to the revised EORTC/MSG consensus definitions[5]. Specimens from the respiratory tract were homogenized and divided into two parts. One part of the specimen (1-5 ml) was centrifuged, and the suspended pellet (500 μl) was used for conventional method, and the second part was frozen at -80°C and used for molecular analysis and retrospective galactomannan testing.

Molecular detection of *Aspergillus* DNA

Extraction of DNA from respiratory specimens

Respiratory specimens were treated with N-acetylcysteine-Na hydroxide, and centrifuged at 13,000 rpm. The leukocyte pellet was resuspended in 300 μl of 1×phosphate buffered saline solution and the mixture was incubated with 100-125 U lyticase, during 30 minutes at 37°C for fungal cells degradation. The residual material was further treated with

500-1000 μg of proteinase K and 0.5 % SDS (Natrium dodecyl sulphate) at 55°C for 1 hour. The residual cell material was then lysed by incubation with an additional 100 μl 2×*Aspergillus* buffer for extraction for 30 minutes at 65°C. Purification of DNA was performed with conventional phenol-chloroform extraction. The precipitation of DNA was performed with 0.7 volume of isopropanol, to obtain a pellet, which was further washed with 70 % ethanol and dried on air. Analysis of the DNA concentration was performed with spectrophotometer at wavelength 260 and 280 nm. The DNA extracts were frozen at -20°C until PCR analysis[11].

Controls for extraction. Negative controls consisted of tubes with purified water without DNA, for evaluation of contamination during extraction. Positive controls were included for every extraction and verification of efficacy, and consisted of 500 μl of saline solution inoculated with approximately 150 CFU of *A. fumigatus conidial suspensions*. To determine the total number of injected CFU, 100 μl of the suspension containing approximately 30 CFU, was inoculated on the surface of the Sabouraud dextrose agar, which was incubated for 72 hours at 30°C.

PCR for *Aspergillus*. PCR was performed in 25 μl mixture containing 50-150 nanograms of DNA like a template. This PCR mixture contained approximately 0.5 U Taq DNA polymerase, 6.25 nmol DNTP, 10 pmol primers (for the first PCR step - first pair of primers: AFU 7S-AFU 7AS; for the second PCR step - second pair of primers AFU 5S-AFU 5AS), which were derived from sequences of the *A. fumigatus* 18S rRNA gene (Table 1).

Table 1. PCR primers for *Aspergillus*

Primers	Sequences (5'→ 3')	Amplicons
AFU 5S	AGG GCC AGC GAG TAC ATC ACC TTG	1436-1459
AFU 5AS	GG G (AG)GT CGT TGC CAA C(CT)C (CT)CC TGA	1648-1771
AFU 7S	CGG CCC TTA AAT AGC CCG	1296-1313
AFU 7AS	GA CCG GGT TTG ACC AAC TTT	1681-1700

The PCR products were separated with 2.5 % agarose gel electrophoresis dyed with ethidium bromide, and visualized with UV light. Control specimens included all components of the reaction mixture, except genomic DNA. As positive and negative controls for PCR, DNA of a human cell line T47D and diluted solution of *A. fumigatus* were used as templates[12].

Detection of galactomannan

A commercially available sandwich ELISA test for detection of GM antigen of *Aspergillus* species was performed according to manufacturer's instructions (Platelia *Aspergillus* protocol, Bio-Rad Laboratories, France)[13]. Respiratory specimens were tested in duplicate, and the mean value was determined. Three hundred microliters of each respiratory specimen and control were pipetted into individual 1.5 ml polypropylene tubes; 100 μL of sample treatment solution was added to each tube, and the tubes were vortexed. The tubes were heated for 3 minutes at 100^0C.

After centrifugation at 10.000 x g for 10 minutes, the supernatant was used for detection of galactomannan. Fifty microliters of the treated supernatant were added to each well. The plate was incubated in a dry microtiter plate incubator for 90±5 minutes at 37°C, and then washed 5 times. Two hundred microliters of the chromogenic solution were added to each well. The plate was incubated in dark at room temperature, for 30 minutes. One

hundred microliters of the stop solution were added to each well in the same order as the chromogenic solution. The optical density of each well was read at 450 nm. The microtiter plates were read within 30 minutes. The optical density was determined spectrophotometrically with a reader. The results were interpreted based on the index calculated from the measured OD at a wavelength of 450 nm. Although the threshold for a positive GM test result is still controversial, we used an optical density index cut-off of 0.5. An optical density index (ODI) cut-off value of 0.5 was used, based on the manufacturer's recommendation and its common use in bronchoalveolar lavage studies for optimizing sensitivity in diagnosing invasive pulmonary aspergillosis.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 17.0) for Windows. The results of our study are presented in numbers and percentages. Differences in distribution of proven, probable and possible fungal infections with *Aspergillus* were compared by Pearson Chi square test. P value less than 0.05 was considered statistically significant.

Results

Respiratory tract specimens from 125 patients were analyzed in four groups according to clinical diagnosis and EORTC/MSG criteria (Figure 1). The sensitivity and specificity of PCR performed on respiratory specimens across the four patient groups were as follows: 67.86% and 85.71% in group I (primary immune deficiency), 72.73% and 75% in group II (critically ill patients), 40.91% and 50% in group III (chronic aspergillosis), and 50 % and 50 % in group IV (cystic fibrosis). Corresponding sensitivity and specificity values for the galactomannan antigen assay in respiratory specimens were: 75% and 57.14% in group I, 86.36 % and 62.5% in group II, 81.82% and 0% in group III, and 80% and 70.83% in group IV. The complete lack of specificity (0%) for galactomannan in group III resulted from all eight patients classified as having "possible" aspergillosis testing positive by this assay, yielding false-positive results when compared with the definitive diagnostic standard.

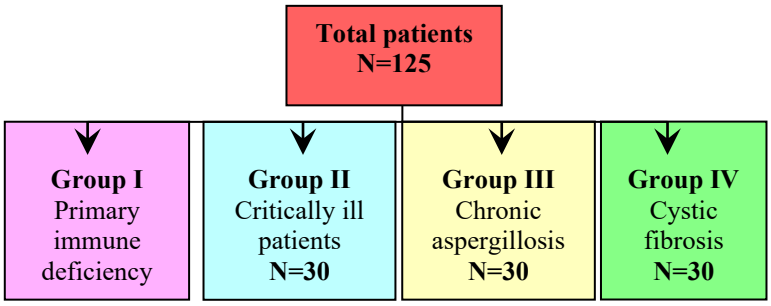

Fig. 1. Classification of patient groups according to clinical diagnosis and EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study group) criteria

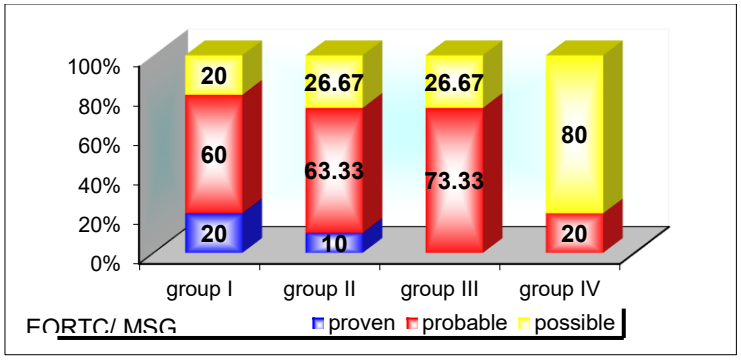

Fig. 2. Distribution of fungal infections according to EORTC/MSG criteria in all groups

Figure 2 illustrates the distribution of the patients according to diagnosis following the EORTC/MSG criteria. Only a small percentage of patients had proven infection according to these criteria. Twenty percent of these patients had some form of primary deficiency, and 10 % were hospitalized in the intensive care unit for a prolonged period.

Differences in the distribution of proven, probable and possible fungal infection were statistically significant between the primary immunodeficiency group and the chronic aspergillosis and cystic fibrosis group, as well as between the critically ill patient group and the chronic aspergillosis and cystic fibrosis group (Table 2).

Table 2. Distribution of proven, probable and possible fungal infections according to EORTC/MSG criteria

Aspergillus	**group I N=35 n (%)**	**group II N=30 n (%)**	**group III N=30 n (%)**	**group IV N=30 n (%)**
Proven 10 (8%)	7(20%)	3(10%)	0	0
Probable 68 (54.4%)	21(60%)	19(63.33%)	22(73.33%)	6(20%)
Possible 47 (37.6%)	7(20%)	8(26.67%)	8(26.67%)	24(80%)

[b]p<0.001, I *vs*. II p=0.3, II *vs.* III p=0.345, III *vs*. IV p<0.001, I *vs.* III p=0.03*, II *vs*. IV p<0.001, I *vs.* IV p< 0.001, [a]p(Chi-square test), [b](Fisher exact test), *p<0.05, **p<0.01

PCR in respiratory specimens confirmed 20 (57.14 %) positive specimens with *Aspergillus* in patients with primary immune deficiency, 18 (60 %) in patients with prolonged stay in ICU, 13 (43.33 %) positive specimens in patients with chronic aspergillosis and 15 (50 %) in patients with cystic fibrosis. *A. fumigatus* was confirmed as etiological agent in all positive specimens (Table 3).

Table 3. PCR in respiratory specimens and *Aspergillus* DNA recovery

group *Aspergillus*	**group I N=35**	**group II N=30**	**group III N=30**	**group IV N=30**
PCR in RT	**n (%)**	**n (%)**	**n (%)**	**n (%)**
no 59 (47.2%)	15(42.86%)	12(40%)	17(56.67%)	15(50%)
yes 66 (52.8%)	20(57.14%)	18(60%)	13(43.33%)	15(50%)
	Chi-square: 2.06 p = 0,6			
PCR in RT				
A. fumigatus n=66	20	18	13	15
p(Chi-square test)				

The results of the diagnostic performance of PCR and galactomannan in respiratory specimens in the group of patients with immunodeficiency are shown in Table 4. Sensitivity, specificity, positive and negative predictive values of PCR in respiratory specimens were: 67.86% / 85.71% / 95% / 40%. Sensitivity, specificity, positive and negative predictive values of galactomannan antigen test in respiratory specimens were: 75% / 57.14% / 87.5% / 36.36%.

Table 4. Diagnostic performances of PCR and galactomannan in respiratory specimens in the group with immune deficiency

Test	**Se (%)**	**Sp (%)**	**PPV (%)**	**NPV (%)**
PCR in RT	67.86	85.71	95	40
GM in RT	75	57.14	87.5	36.36

The results of the diagnostic performance of PCR and galactomannan in respiratory specimens in the group of critically ill patients with prolonged ICU stay are shown in Table 5. Sensitivity, specificity, positive and negative predictive values of PCR in respiratory

specimens were: 72.73% / 75% / 88.89% / 50%. Sensitivity, specificity, positive and negative predictive values of galactomannan antigen test in respiratory specimens were: 86.36% / 62.5% / 86.36% / 62.5%.

Table 5. Diagnostic performances of PCR and galactomannan in respiratory specimens in the group with prolonged ICU stay

Test	Se (%)	Sp (%)	PPV (%)	NPV (%)
PCR in RT	72.73	75	88.89	50
GM in RT	86.36	62.5	86.36	62.5

The results of the diagnostic performance of PCR and galactomannan in respiratory specimens in the group with chronic aspergillosis are shown in Table 6. Sensitivity, specificity, positive and negative predictive values of PCR in respiratory specimens were: 40.91% / 50% / 69.23% / 23.53%. Sensitivity, specificity, positive and negative predictive values of galactomannan antigen test in respiratory specimens were: 81.82% / 0% / 69.23% / 0%.

Table 6. Diagnostic performances of PCR and galactomannan in respiratory specimens in the group with chronic aspergillosis

Test	Se (%)	Sp (%)	PPV (%)	NPV (%)
PCR in RT	40.91	50	69.23	23.53
GM in RT	81.82	0	69.23	0

In group III (chronic aspergillosis), the galactomannan assay showed high sensitivity (81.82 %) but a specificity of 0 %. This was due to all eight patients classified as having "possible" aspergillosis according to EORTC/MSG criteria testing positive for galactomannan, resulting in false-positive results when evaluated against the definitive diagnostic standard.

The results of the diagnostic performance of PCR and galactomannan in respiratory specimens in the group with cystic fibrosis are shown in Table 7. This method had the following diagnostic performances: sensitivity 50 %, specificity 50 %, positive predictive value 20 %, negative predictive value 80 %. Sensitivity, specificity, positive and negative predictive values of galactomannan antigen test in respiratory specimens were: 80 % / 70.83 % / 30 % / 85 %.

Table 7. Diagnostic performances of PCR and galactomannan in respiratory specimens in the group with cystic fibrosis

Test	Se (%)	Sp (%)	PPV (%)	NPV (%)
PCR in RT	50	50	20	80
GM in RT	80	70.83	30	85

Discussion

Invasive mold infections are systemic diseases caused by the dissemination of molds in deep-seated tissues. Among these, *Aspergillus* is a predominant opportunistic pathogen, associated with high mortality rates of 40-50 % in severely immunocompromised cohorts, such as patients with acute leukemia and hematopoietic stem cell transplant recipients[1]. More recently, it has been identified as the most common fungal infection in the critically ill population[2]. The morbidity and mortality of aspergillosis remain unacceptably high (50-70 %) despite available antifungals, underscoring that early diagnosis is imperative but remains a significant hurdle. The conventional diagnostic mainstay, culture of respiratory specimens, is limited by prolonged turnaround times and low sensitivity. This has led to the increased integration of molecular methodologies, particularly PCR, for detecting microbial DNA in

high-risk patients[5-8]. While PCR-based screening for *Aspergillus* DNA holds considerable potential for definitive diagnosis, its reported performance is inconsistent, with sensitivities ranging from 72-88 % (and as low as 26 % in some studies) and specificities from 75-98.7 %. These results are similar to previously published data reporting sensitivities ranging from 71 to 82 % and specificities from 92.8 to 98%[12].

In the primary immunodeficiency patient group, a PCR assay was assessed on respiratory specimens for the early detection of *Aspergillus* DNA in high-risk patients. The sensitivity and specificity of our PCR tests differed from some previously published reports. Specifically, the sensitivities for PCR of respiratory specimens across our 4 groups were 67.86%, 72.73%, 40.91%, and 50%, respectively, with corresponding specificities of 85.71%, 75%, 50%, and 50%. While some studies report BAL PCR sensitivities between 80% and 100 %, our results indicated a lower sensitivity, aligning more closely with other authors who also documented reduced performance[14]. In this group, the ELISA galactomannan (GM) test on respiratory specimens showed a higher sensitivity (75%), but lower specificity than PCR in the respiratory specimens (57.14%). This sensitivity is comparable to that reported by Lahmer *et al.,* (70%), although their specificity was higher (94%)[12]. Detection of GM in bronchoalveolar lavage (BAL) fluid is widely regarded a superior method for diagnosing invasive pulmonary aspergillosis (IPA). This is because BAL samples are collected directly from the site of infection, containing higher concentrations of galactomannan antigen, especially in non-neutropenic patients. In two separate studies, sensitivities of 58.3 % in neutropenic mice and 70% in immunosuppressed rats, with *Aspergillus* PCR, were reported[15,16]. However, the specificity in our first two groups is consistent with the high specificities (84-100%) found in the literature[17]. Commercial kits also show variability; one kit demonstrated 81% sensitivity and 100% specificity, while the MycoGENIE *A. fumigatus* kit showed 71% sensitivity and 100% specificity[18]. Overall, performance of both in-house and commercial PCR assays in respiratory specimens varies significantly, with sensitivities reported from 65.5% to 100% and specificities from 80% to 100%, influenced by factors such as PCR target, DNA extraction methods, and patient population[18,19]. Several factors may explain the lower sensitivity observed in our study. Unlike other studies that focused on specific high-pre-test-probability cohorts (patients with acute myeloid leukemia or those with radiographic pneumonia), our study population was more heterogeneous, and clinical information was often lacking. Furthermore, the potential impact of concurrent anti-mold treatment or prophylaxis in our patients, which could reduce the fungal load in BAL fluid and impair DNA detection, cannot be ruled out[20].

In the group with critically ill, non-neutropenic patients, the sensitivity and specificity of the PCR test was lower than the galactomannan test. High sensitivity of PCR in BAL (90%) was also confirmed in the study by Zhang *et al.*, who demonstrated that BAL provided high sensitivity for detection of DNA in non-neutropenic patients[21]. These data again confirm the hypothesis that DNA of *Aspergillus* can be more easily detected in respiratory specimens compared to other specimens. When comparing PCR results between neutropenic and non-neutropenic patients, similar data were obtained, in contrast to another study that reported higher PCR sensitivity in neutropenic patients (82.1%) than in nonneutropenic patients (62.5%)[22]. In the group with critically ill, non-neutropenic patients, the respiratory GM ELISA demonstrated high sensitivity (86.36%) and a specificity of 62.5%. These figures align closely with the study by Nuh *et al*., who reported a sensitivity of 86% and a specificity of 63 % for sputum galactomannan[23]. Another study by Sarae *et al.* found a comparable sensitivity of 83.3% with a slightly higher specificity of 74.2%[24]. Comparing the respiratory GM ELISA with PCR, respiratory GM ELISA test showed a higher sensitivity (86.36 %) compared to PCR in the respiratory specimens (72.73%), but lower specificity (62.5%) compared to PCR specificity (75%). In another study, when PCR and respiratory GM ELISA

were directly compared using BAL, PCR demonstrated higher sensitivity while maintaining comparable specificity. The superiority of PCR reached statistical significance at an ODI cut-off of 1.0, though not at a cut-off of 0.5[25]. The reported performance of BAL GM across the literature varies, with studies in solid organ transplant recipients, hematological patients, and non-immunosuppressed patients demonstrating sensitivities of 73%-100% [26-28]. This range is influenced by the use of different optical density index cut-off values (from 0.5 to 1.0). For instance, Maertens *et al.,* demonstrated high sensitivity for BAL GM in both neutropenic and non-neutropenic patients with proven IPA[29]. However, direct comparison between studies is challenging due to inconsistencies in the timing of specimen collection and the undefined impact of concurrent antifungal therapy in many study populations[26]. In 10 other studies reporting on both PCR and GM, the diagnostic performance of PCR in BAL fluid was non-significantly better than that of GM in BAL fluid using an ODI cut-off of 0.5. When PCR was performed in addition to GM in BAL fluid, the sensitivity of PCR significantly improved from 89% with GM alone to 97% with GM and PCR, with no loss of specificity[25].

Regarding chronic pulmonary aspergillosis, the recent joint guidelines from the European Society of Clinical Microbiology and Infectious Diseases and the European Respiratory Society (ESCMID/ERS) now recommend the use of *Aspergillus* PCR on bronchoalveolar lavage (BAL) fluid for diagnosing non-invasive forms of the disease[32]. In our study, the sensitivity and specificity of PCR on respiratory specimens in the CPA group were 40.91 % and 50 %, respectively. This is lower than the average sensitivity and specificity of approximately 80 % reported in various other studies[22,30,31].

The performance of PCR is highly variable. In one study, a PCR assay that could simultaneously detect triazole-resistance was evaluated. In this study a sensitivity of 71.4 % and specificity of 63.6 % were reported[32]. However, a lack of standardization has prevented consensus on its use. This variability is further illustrated by a study of 218 patients suspected of having CPA, where the sensitivity and specificity of both a commercial (AsperGenius) and an in-house PCR were approximately 50-53% and 30-31%, respectively[33]. The galactomannan ELISA test on respiratory specimens demonstrated high sensitivity (81.82%) but a specificity of 0%. This high sensitivity is consistent with literature; for instance, Park Seong *et al.* reported a BAL GM sensitivity of 92% [34], supporting its utility as an effective diagnostic tool. Other studies, such as those by Kono *et al.* and Nguyen *et al*., also reported high sensitivities (85.7% and 100 %, respectively) for BAL GM in diagnosing chronic forms of aspergillosis, reinforcing its diagnostic potential in this context[34,28]. Park *et al.* found a sensitivity of 92% for BAL GM, suggesting that the BAL GM is a more useful test for diagnosis of pulmonary aspergilloma[34]. The complete lack of specificity in our cohort occurred because all eight patients, classified as having a "possible" *Aspergillus* infection per EORTC/MSG criteria, tested positive for galactomannan. These were therefore considered false positives according to the definitive diagnostic standards. When comparing PCR with galactomannan test, PCR demonstrated lower sensitivity (40.91%) than galactomannan testing (81.82 %). Research suggests that the diagnostic value of BAL PCR for chronic pulmonary aspergillosis increases when it is used in combination with other tests. Algorithms where both a PCR and another assay yield positive results have been shown to provide higher specificity, positive predictive value (PPV), and more robust diagnostic confirmation. Despite its high sensitivity, the galactomannan assay has recognized limitations that can affect its specificity. False-positive results may occur in patients receiving certain β-lactam antibiotics (piperacillin-tazobactam) or in the context of infections with other fungi (*Penicillium, Histoplasma*) that exhibit cross-reactive antigens. These factors, combined with the high prevalence of colonization or non-invasive fungal airway involvement in some patient groups, may contribute to reduced specificity in certain clinical settings. In our study, the complete lack of specificity (0%) observed in the chronic aspergillosis group underscores that

galactomannan testing should not be used as a standalone diagnostic tool in this population, but rather as part of a multimodal diagnostic approach that includes clinical, radiological, and complementary mycological criteria.

In the cystic fibrosis group, the sensitivity and specificity of *Aspergillus* PCR were both 50%. In the literature, there is insufficient data regarding the efficacy of PCR testing in CF sputum samples. One study revealed considerable discrepancies between culture and PCR sensitivity (33% *vs*. 73%, respectively)[36]. In a study by Guegan *et al*., PCR offered much higher sensitivity than culture, with over 40 % of negative cultures yielding positive results with at least one PCR assay[37]. This study demonstrated that all PCR methods used proved to be substantially more sensitive than standard culture for identifying *Aspergillus* in sputum samples from CF patients. The respiratory GM test in our study showed higher sensitivity than PCR (80%) and a specificity of 70.83%. The thick pulmonary mucus traps *Aspergillus* conidia, allowing for significant fungal growth and the subsequent release of large amounts of GM antigen. As noted by Baxter *et al*., both PCR and GM tests demonstrate good reproducibility in CF sputum and can be positive even when cultures are negative, which makes them potentially useful for monitoring antifungal therapy response[36].

Many studies agree that PCR can provide excellent diagnostic accuracy, particularly when used in combination with GM. Studies have reported up to 100% sensitivity and 98% specificity when both tests are positive[38].

Our study has several limitations, the primary one being its single-center design. Furthermore, financial constraints permitted the analysis of only a single respiratory specimen per patient for diagnosing aspergillosis. This approach prevented an assessment of the methods' reproducibility, as the tests could not be performed in duplicate. Therefore, a combined diagnostic approach using both PCR and GM is recommended to optimize sensitivity and specificity across different patient populations.

Conclusion

The results of our study showed that the galactomannan antigen test in respiratory specimens is valuable for diagnosing aspergillosis and, in some patient groups, may be superior to PCR. The PCR test offers optimal sensitivity and could be a useful diagnostic tool for aspergillosis, suggesting earlier antifungal treatment for better clinical outcome. Combining molecular methods with galactomannan testing enhances the diagnostic performance of both tests and is more useful than any single test alone for diagnosing aspergillosis. Implementing tailored strategies is crucial for enhancing early detection of aspergillosis and improving clinical outcomes in high-risk populations.

Conflict of interest statement. None declared.

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