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Original article

COMPARISON OF SERUM GALACTOMANNAN TEST WITH TRADITIONAL CULTURE FOR DIAGNOSIS OF ASPERGILLOSIS

Mirchevska Gordana[1], Kaftandzieva Ana[1], Mojsova Mijovska Maja[2], Trajkovska Vanja[3], Andonovska Biljana[3], Antova Emilija[4], Babinkostova Zoja[5], Cvejoska Cholakovska Valentina[6]

[1]Institute of Microbiology and Parasitology, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
[2]Faculty of Medical Sciences, Goce Delcev University, Stip, Republic of North Macedonia
[3]University Clinic for Traumatology, Orthopedic Disease, Anesthesiology, Reanimation and Intensive Care Medicine and Emergency Department, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
[4]University Clinic for Cardiology, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
[5]University Clinic for Psychiatry, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
[6]University Children's Hospital, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
e-mail: gordana.mirchevska@medf.ukim.edu.mk, gordmir@gmail.com

Abstract

Introduction: Invasive aspergillosis is a severe and often fatal fungal infection caused by *Aspergillus* species, and its early diagnosis remains a significant clinical challenge.

This study aimed to evaluate and compare the sensitivity and specificity of the serum galactomannan antigen test with conventional culture techniques for the early identification of *Aspergillus* infections.

Material and methods: Over a two-year period, respiratory samples and sera were collected from 125 patients at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, North Macedonia. Patients were stratified into four clinical categories according to EORTC/MSG criteria. All samples were analyzed using both traditional fungal culture and a serological ELISA test for galactomannan detection.

Results: Traditional fungal culture identified 71 *Aspergillus* isolates, with the highest positivity rate observed in patients with chronic aspergillosis (63.33%), followed by those with cystic fibrosis (56.67%), primary immunodeficiency (51.43%), and ICU patients (43.33%). Sensitivity and specificity for fungal culture across the groups ranged from 54.55% to 100% and 12.5% to 100%, respectively. For the galactomannan assay, sensitivity and specificity ranged from 0% to 64.29% and 57.14% to 87.5%, respectively. The galactomannan test demonstrated moderate accuracy and lower sensitivity than culture in critically ill patients, though performance was comparable in immunocompromised individuals.

Conclusion: Although the serum galactomannan test shows variable performance, it may serve as a valuable complementary tool to traditional culture methods, potentially enabling earlier initiation of antifungal treatment and contributing to improved patient outcomes.

Keywords: aspergillosis, galactomannan, diagnosis, serum, culture

Introduction

The incidence of invasive fungal infections has risen considerably in recent decades. Invasive aspergillosis is a severe deep-seated infection associated with high mortality, particularly among immunocompromised individuals such as those with hematological malignancies, transplant recipients, or patients on prolonged immunosuppressive therapy[1,2].

Recently, non-neutropenic populations, such as patients with COPD, bronchiectasis, or critically ill patients treated in intensive care units, have also been recognized as susceptible groups for development of pulmonary aspergillosis[3]. Despite advances in antifungal therapies, delayed diagnosis contributes to poor outcomes, highlighting the need for accurate and timely diagnostic tools[4]. Histopathologic demonstration of microorganisms in tissue samples or fungal growth on fungal media is the "gold standard". Conventional mycological methods are limited by low sensitivity and slow turnaround times. Consequently, non-culture-based assays such as antigen and molecular detection methods have gained importance[5]. Among these, the galactomannan antigen test, targeting a polysaccharide component of the Aspergillus cell wall, has emerged as a valuable serological tool[6]. Released into circulation during early infection, galactomannan can be detected via ELISA, offering a potential advantage for early diagnosis[7]. Although included in the EORTC/MSG diagnostic criteria for neutropenic patients, its utility in non-neutropenic and critically ill populations requires further validation[8].

This study aimed to evaluate and compare the sensitivity and specificity of the serum galactomannan antigen test with traditional culture methods for the early identification of infections with *Aspergillus* species.

Material and methods

Study design and population

A two-year diagnostic evaluation was conducted at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, North Macedonia, as part of a doctoral research project. The study involved 125 patients at risk for invasive aspergillosis, divided into four clinical cohorts: primary immunodeficiency, critically ill patients admitted to intensive care units (ICU), chronic aspergillosis, and cystic fibrosis.

Classification followed the revised EORTC/MSG consensus definitions for invasive fungal diseases[8].

Specimen collection and processing

Respiratory tract specimens, including sputum, tracheal aspirates, and bronchoalveolar lavage (BAL) were collected alongside serum samples. All specimens were processed in the laboratory for diagnosis of fungal infections. Following initial culture, remaining specimens were stored at -70°C for subsequent galactomannan antigen testing.

Traditional mycological culture

Respiratory specimens were inoculated onto Sabouraud dextrose agar and chromogenic medium (Oxoid). Cultures were incubated under appropriate conditions, and *Aspergillus* isolates were identified based on macroscopic colony morphology and microscopic examination of conidial structures using lactophenol cotton blue staining.

Serum galactomannan detection

Galactomannan antigen was measured in serum using a commercially available sandwich ELISA kit (Platelia™ Aspergillus Ag, Bio-Rad, France), performed according to the manufacturer's instructions (9). Briefly, 300 µL of each serum sample and control was treated with 100 µL of pretreatment solution, heated, and centrifuged. The supernatant was assayed in duplicate wells for detection of the GM antigen. Following incubation with conjugate and

chromogenic substrate, optical density (OD) was measured at 450 nm (reference 620/630 nm). Results were expressed as an optical density index (ODI), with a cut-off of ≥ 0.5 considered positive.

Statistical analysis

Data were analyzed using SPSS for Windows (version 20.0). Descriptive statistics were reported as frequencies, percentages, and means ± standard deviation. Group comparisons for categorical variables were performed using Pearson's chi-square or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

Results

This study evaluated 125 patients stratified into four clinical categories: primary immunodeficiency, ICU-treated critical illness, chronic aspergillosis, and cystic fibrosis. Demographic analysis indicated a predominance of male participants in three of the four groups, with mean ages varying according to underlying condition (Table 1).

Table 1. Characteristics of patients according to gender and age

Aspergillus **Gender**	**Group I N=35 n (%)**	**Group II N=30 n (%)**	**Group III N=30 n (%)**	**Group IV N=30 n (%)**
Men 70(56%)	21(60%)	15(50%)	18(60%)	16(53.33%)
Women 55(44%)	14(40%)	15(50%)	12(40%)	14(46.67%)
	[a]p = 0.81			
Age (years) mean±SD, min-max				
	40.8±17.7 5-69	59.7±13.3 4-78	64.7±6.3 52-76	28.9±8.5 18-52

[a]p(Chi-square test)

According to EORTC/MSG classifications, proven aspergillosis was infrequent, observed mainly in immunodeficiency and ICU groups, whereas probable and possible infections were more common across cohorts (Table 2).

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

Aspergillus **n (%)**	**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

Culture-based detection of *Aspergillus* was most frequent in patients with chronic aspergillosis (63.33%), followed by those with cystic fibrosis (56.67%), primary immunodeficiency (51.43%), and ICU-treated patients (43.33%). However, the differences in

positive respiratory cultures among the four groups were insufficient for statistical significance (p=0.46). Serum galactomannan positivity was highest in the immunodeficiency group (60%) and lowest in cystic fibrosis (10%). Statistical comparisons revealed significant differences in galactomannan detection rates between immunodeficiency and cystic fibrosis groups (p=0.000003), as well as between ICU/chronic aspergillosis and cystic fibrosis groups (p=0.007) (Table 3).

Table 3. Detection of GM antigen in serum with ELISA test

ELISA galactomannan	Group I N=35 n (%)	Ggroup II N=30 n (%)	Group III N=30 n (%)	Group IV N=30 n (%)
negative 77 (61.6%)	14(40%)	18(60%)	18(60%)	27(90%)
positive 48 (38.4%)	21(60%)	12(40%)	12(40%)	3(10%)
	I *vs.* II *vs.* III [a]p=0.11		II / III *vs.* IV [a]p=0.007**	
	I *vs.* IV [a]p=0.00003**			

[a]p(Chi-square test) [b]p (Fisher exact test) **p<0.01

Diagnostic performance analysis revealed that conventional culture exhibited variable sensitivity (ranging from 54.55% to 100%) and variable specificity (12.5% to 100%) across the different patient groups. In contrast, serum galactomannan demonstrated moderate to low sensitivity, particularly in ICU and chronic aspergillosis patients (40.91%), and no sensitivity in cystic fibrosis patients. The specificity of the galactomannan assay ranged from 57.14% to 87.5% (Table 4).

Table 4. Comparative diagnostic performance of culture *versus* serum galactomannan by patient group

Patient Group	Diagnostic test	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV, %)	Negative predictive value (NPV, %)
Primary immunodeficiency	Culture (Respiratory Sample)	64.29	100.00	100.00	41.18
	Serum Galactomannan (ELISA)	64.29	57.14	85.71	28.57
ICU-treated patients	Culture (Respiratory Sample)	59.09	100.00	100.00	41.18
	Serum Galactomannan (ELISA)	40.91	62.50	75.00	27.78
Chronic aspergillosis	Culture (Respiratory Sample)	54.55	12.50	100.00	41.18
	Serum Galactomannan (ELISA)	40.91	62.50	75.00	27.78
Cystic fibrosis	Culture (Respiratory Sample)	100.00	54.17	100.00	41.18
	Serum Galactomannan (ELISA)	0.00	87.50	0.00	77.78

Discussion

Findings of this study are consistent with previous reports indicating that serum galactomannan detection is more reliable in hematological and neutropenic patients compared

to non-neutropenic or anatomically predisposed groups, such as those with cystic fibrosis. The limited sensitivity observed in the latter populations may be attributed to localized pulmonary infection without systemic antigen release, rapid clearance of galactomannan by circulating neutrophils, or intermittent antigen shedding in chronic colonization states. In critically ill ICU patients, the reduced sensitivity of the assay could also be influenced by concurrent bacterial infections, prior or ongoing antifungal therapy or atypical fungal invasion patterns that limit antigen dissemination. Notably, in cystic fibrosis patients, despite frequent airway colonization by *Aspergillus*, systemic galactomannan was rarely detected, likely reflecting intact immune containment and a predominantly mucosal, non-angioinvasive disease phenotype.

Invasive aspergillosis remains a diagnostic challenge due to its nonspecific clinical presentation, particularly in non-neutropenic patients. Delayed diagnosis can lead to severe complications and increased mortality, underscoring the need for reliable diagnostic tools[10]. Although serum galactomannan detection is well-established in neutropenic populations, its utility in non-neutropenic and critically ill patients requires further evaluation[11].

The variable performance of conventional culture observed in this study (sensitivity 54.55-100%, and specificity 12.5-100%), aligns with prior reports. Tashiro *et al.* similarly noted that a significant proportion of respiratory samples with *Aspergillus* growth represented colonization rather than invasive disease[12]. This inconsistency highlights the need for complementary diagnostic approaches. Therefore, we evaluated the sensitivity and specificity of serum galactomannan with ELISA test.

Among immunodeficient patients in our cohort, serum galactomannan demonstrated a sensitivity of 64.29%. This exceeds the lower sensitivities (24.32-37.84%) reported by Zhou *et al*., at comparable cut-offs but aligns with ranges reported in other meta-analyses[8]. In ICU patients, sensitivity was 40.91%, consistent with Lahmer *et al*., findings, who reported sensitivities of 30-35%[13]. The occurrence of false-positive results in our study, particularly in patients with possible aspergillosis, led to reduced specificity (57.14-62.5%), a recognized limitation in critically ill patients who frequently have concurrent bacterial infections or other sources of antigen cross-reactivity.

In chronic aspergillosis patients, serum galactomannan was detected in 40% of cases (12/30), a rate consistent with previously reported positivity rates of 50% (5/12) by Kitasato *et al*.,[14] and 37.84% by Zhou *et al.,*[9]. Kitasato *et al*., reported a sensitivity of 21.4% at a cut-off ODI≥1.5, which increased to 50% when the threshold was lowered to ≥0.5; however, specificity data were not provided in their analysis[14]. Clinical factors such as hemoptysis may influence antigen detection, as demonstrated by Park *et al*., who observed significantly higher GM positivity in patients with hemoptysis compared to those without (52% *vs*. 9%, p=0.02)[15]. This suggests that vascular disruption may facilitate antigen entry into circulation, a mechanism supported by a documented case in which *A. fumigatus* was cultured from blood following bronchial artery embolization in a patient with pulmonary aspergilloma[16]. In chronic, slowly progressive aspergillosis, angioinvasive growth may also contribute to intermittent antigenemia[17]. Nevertheless, the diagnostic performance of serum GM in chronic aspergillosis remains modest overall, with sensitivities as low as 14.3% reported in some studies, including those assessing both chronic aspergillosis and allergic bronchopulmonary aspergillosis[18]. To date, few studies have systematically evaluated the utility of serum GM in this specific patient population.

Among cystic fibrosis patients, serum galactomannan sensitivity was 0%, despite frequent airway colonization. This aligns with Warren *et al.* and likely reflects the mucosal, non-angioinvasive nature of *Aspergillus* in CF, where systemic antigen dissemination is rare despite local antigen production[19]. The consistently low serum GM levels in CF likely reflect distinct pathophysiological mechanisms compared to invasive aspergillosis in immunocompromised hosts. In CF, thickened airway mucus promotes Aspergillus conidial

trapping and germination, leading to local antigen production that may contribute to conditions such as allergic bronchopulmonary aspergillosis[20]. However, systemic invasion is rare, possibly due to preserved immune cellular function despite impaired mucosal clearance. Consequently, GM release into circulation appears minimal and intermittent.

Supporting this, Baxter *et al*. found that while sputum GM was frequently positive in CF patients and demonstrated good reproducibility over time, serum GM was seldom elevated[21]. In their longitudinal assessment, 47% of sputum samples were GM-positive, suggesting that antigen detection in respiratory specimens may be more relevant for monitoring colonization or treatment response in CF than serum-based testing.

Our results support existing evidence that serum galactomannan is inferior to bronchoalveolar lavage detection, particularly in non-neutropenic patients. This may be due to localized infection without systemic antigen release, rapid clearance of antigen by neutrophils, or lower levels of angioinvasion. Bronchoalveolar lavage sampling offers direct access to the infection site, making it a more sensitive medium for galactomannan detection.

A limitation of this study is the lack of detailed clinical data, such as antifungal exposure or concurrent infections, which may influence galactomannan levels. Nevertheless, our findings reinforce that serum galactomannan has limited sensitivity in non-neutropenic and anatomically predisposed patients. It may serve best as an adjunct to culture, particularly when BAL is unavailable or culture results are pending.

Conclusion

This study indicates that serum galactomannan detection possesses moderate diagnostic accuracy for invasive aspergillosis but generally exhibits lower sensitivity compared to respiratory culture, especially in critically ill and cystic fibrosis patients. Its performance appears more favorable in immunocompromised individuals, such as those with hematological malignancies, where it may provide adjunctive value when culture results are negative. Future studies should incorporate clinical parameters and assess the impact of antifungal therapy on test performance.

While no single method suffices for definitive diagnosis, serum galactomannan can serve as a useful complementary tool, particularly when conventional mycological results are pending or unavailable. Integrating biomarker testing with clinical assessment remains essential for timely diagnosis, appropriate antifungal initiation, and improved patient outcomes.

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Conflict of interest statement. None declared.

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