

COMPARISON OF DIAGNOSTIC VALUES OF SERUM PCR AND SERUM GALACTOMANAN ANTIGEN ASSAY FOR DIAGNOSIS OF ASPERGILLOSIS

Mirchevska G.

¹Institute of Microbiology and Parasitology, Faculty of Medicine Skopje, Ss Cyril and Methodius University Skopje, Republic of North Macedonia

Abstract

Introduction: Aspergillosis is a major threat for immunocompromised hosts, but also for immunocompetent patients. Early diagnosis is still a diagnostic challenge, and there is a need for rapid and sensitive diagnostic methods.

Aim: The aim of the study was to compare the diagnostic value of serum PCR assay with serum galactomannan antigen test, for diagnosis of both invasive and non-invasive aspergillosis.

Material and methods: Sera from 125 patients divided into 4 groups (group I-immune deficiency, group II-prolonged stay in ICU, group III-chronic aspergillosis, group IV-cystic fibrosis), classified according to clinical diagnosis and EORTC/MSG criteria, were analysed at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, with molecular (PCR) and serological methods (galactomannan), during a period of two years (2014-2016).

Results: PCR in serum demonstrated the following sensitivity and specificity: 53.57% and 100% in group I, 36.36% and 100% in group II, 9.09% and 100% in group III, respectively. Serum PCR was not performed in the group with cystic fibrosis. The sensitivity and specificity of galactomannan in serum were: 64.29% and 57.14%, 40.91% and 62.5%, 40.91% and 62.5%, in groups I, II and III, respectively.

Conclusion: Our results indicate that both PCR and galactomannan in serum could be useful adjunct tests for diagnosis of both invasive and non-invasive aspergillosis, so an early antifungal treatment is initiated in order to achieve more favorable clinical outcome.

Key words: *Aspergillus; aspergillosis serum galactomannan; serum PCR.*

Introduction

Invasive fungal infections (IFIs) are severe systemic infections with high mortality rates, particularly in immunocompromised patients. Invasive pulmonary aspergillosis (IPA) is the most severe form of aspergillosis with the highest mortality rate. These infections are very common in patients with hematologic malignancies, chemotherapy-induced neutropenia, haematopoietic stem cell transplantation (HSCT), and solid organ transplantation (1,2). Many studies recently have demonstrated that IPA is more frequently registered in non-neutropenic, immunocompetent patients without classic risk factors, who have been treated in intensive care unit (ICU) (3). Most of these patients have chronic obstructive pulmonary disease (COPD), have received broad-spectrum antibiotic treatment or systemic corticosteroids, have impaired mucociliary clearance, diabetes, chronic renal disease, liver failure, but IPA can also be found in patients without underlying diseases (4). IPA is more frequently registered in critically ill patients treated in intensive care units (3). Chronic aspergillosis is locally invasive form of aspergillosis and is seen mainly in patients with mild immunodeficiency or with a chronic lung disease. Aspergilloma and allergic bronchopulmonary aspergillosis (ABPA) are noninvasive forms of aspergillosis (4).

Diagnosis of IPA is still a laboratory challenge, since clinical symptoms and signs are non-specific. The gold standard method for diagnosis of IPA is detection of fungi by histopathological examination of lung tissue. Standard mycological methods with culture on fungal media are time-consuming and insensitive. Diagnosis is also difficult as most of the diagnostic tools either lack specificity or sensitivity in the early phase of the infection. Over the recent years, novel molecular and serological methods have been developed to improve diagnosis of IPA in patients at high risk. Rapid, noninvasive, culture-independent diagnostic methods have contributed towards faster and better detection of invasive IFI and are essential for timely antifungal treatment. Among these methods, serological diagnostic tests focus on detecting biomarkers such as galactomannan (GM) from *Aspergillus*, in serum or BAL, and detection of DNA from the fungus in primarily sterile specimens. These diagnostic approaches have gained importance in the mycology laboratory (5).

The aim of the study was to compare the diagnostic value of serum PCR assay with serum galactomanan antigen test, for diagnosis of both invasive and non-invasive aspergillosis.

Material and methods

Study design

The study was performed at the Laboratory for diagnosis of fungal infections at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, Macedonia, during a 2-year period, as part of an ongoing PhD study during the period 2014-2016.

Group of patients and mycological analyses

Sera from 125 patients divided into 4 groups (group I-immune deficiency, group II-prolonged stay in ICU, group III-chronic aspergillosis, group IV-cystic fibrosis), classified according to clinical diagnosis and EORTC/MSG criteria, were analysed at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, with molecular and serological methods, during a period of two years (2014-2016). Samples were frozen and stored at -70°C for retrospective testing. These groups included patients with primary immune deficiency, critically ill patients treated in ICUs, patients with chronic aspergillosis and cystic fibrosis patients. IPA was defined according to the revised definitions by the EORTC/MSG (European organization for research and treatment of cancer/mycoses study group) consensus group (6).

Molecular detection of *Aspergillus* DNA

Extraction of DNA from serum. A total of 3-5 ml of peripheral blood was mixed with 5 volumes of buffer for lysis of eritrocytes (0,155 M NH_4Cl , 0,01 M NH_4HCO_3 , 0,1 mM EDTA (pH 7,4)), and this mixture was incubated 10 minutes at 4°C . After lysis of eritrocytes, the specimen was centrifuged at $300\times g$ for 10 minutes. The supernatant was discarded, and leucocytes were washed once $1\times$ PBS solution (1,4 M NaCl, 50 mM KCl, 90 mM $\text{Na}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 20 mM KH_2PO_4 (pH 7,4)) and centrifuged again. The leucocyte pellet was resuspended in $300 \mu\text{l}$ of $1\times$ PBS solution and the mixture was incubated with 100-125 U lyticase (lyticase-50.000 U; Sigma) for 30 minutes at 37°C . The residual material of human and fungal

cells was treated with 500-1.000 µg proteinase K (Boehringer) and 0.5% SDS (Natrium dodecyl sulphate) (Sigma) at 55°C during 1 hour. The residual cell material was then lysed while incubated with additional 100 µl 2×*Aspergillus* buffer for extraction (400 mM Tris-Cl, 1 M NaCl, 20 mM EDTA, 2% Natrium dodecyl sulphate) during 30 minutes at 65°C. Purification of fungal and human DNA was performed with phenol-chlorophorm extraction. The precipitation of DNA was performed with addition of 0.7 volume of isopropanol, further washed with 70% ethanol, and afterwards dried on air. The concentration of DNA was analyzed with spectrophotometer at 260 and 280 nm. The DNA extracts were freezed at -20°C until the PCR procedure (7).

Controls for extraction. Negative controls were purified water without DNA. Positive controls were included for every extraction and verification, with inoculation of saline solution with approximately 150 CFU of *A. fumigatus* conidial suspensions, in a volume of 500 µl. To determine the total number of injected CFU, 100 µl of the suspension containing around 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 reaction was performed in a volume of 25 µl mixture containing approximately 50-150 nanograms of total DNA. This PCR mixture contained around 0.5 U Taq DNA polymeraze, 6.25 nmol DNTP, 10 pmol primers (for the first PCR step – first set of primers: AFU 7S-AFU 7AS; for the second PCR step – another set of primes AFU 5S-AFU 5AS), which derived from sequences of *A. fumigatus* 18S rRNA gene). PCR was performed in an automated termocycler. The PCR products were separated with 2.5% agarose gel electrophoresis, dyed with ethidium bromid, and visualized with UV light. Control 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 (7).

Detection of galactomannan

A commercially available sandwich ELISA tect for detection of GM antigen was performed according to instructions of the manufacturer (8). Each sample was tested in duplicate, and the mean value was determined. The optical density of each well was read at 450 nm. The microtiter

plates were read within 30 minutes after addition of a stop solution. The optical density was determined spectrophotometrically with a microplate 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 for serum samples.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows. The results of our study are presented as numbers and percentages. The sensitivity, specificity, positive predictive value and negative predictive value were evaluated. 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

Sera from 125 patients were divided in 4 groups (patients with primary immune deficiencies, critically ill patients treated in intensive care units (ICUs), patients with chronic aspergillosis and cystic fibrosis (CF)) according to clinical diagnosis and EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study group) criteria (Fig. 1).

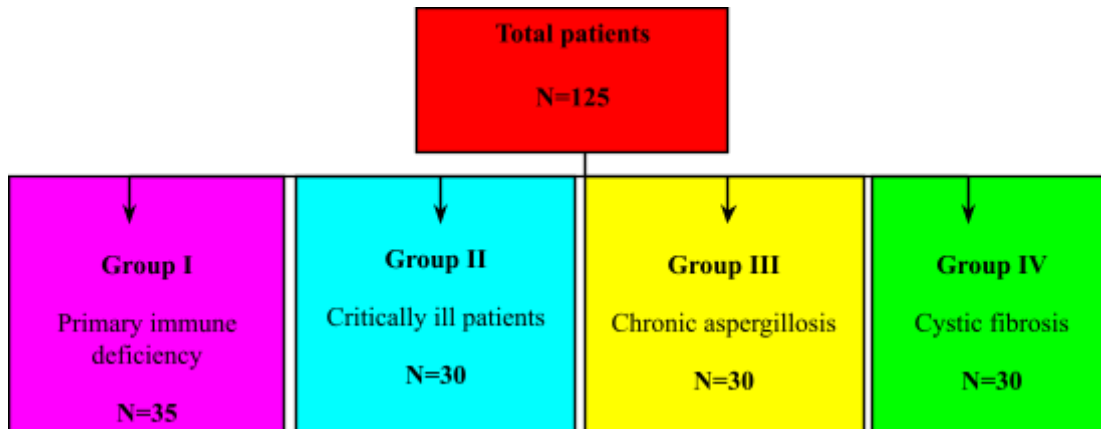


Fig.1: Classification of patient groups according to EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study group) criteria

According to gender analysis of participants, men were more frequently distributed in I, III and IV group (60%, 60%, 53.33% respectively), whereas in the II group, both genders were equally

distributed. Average age of all patients were: 40.8±17.7, 59.7±13.3, 64.7±6.3, and 28.9±8.5 years in all four groups, respectively (table 1).

Table 1. Characteristics of patients according to gender and age

<i>Aspergillus</i>				
	Group I N=35	Group II N=30	Group III N=30	Group IV N=30
Gender	n (%)	n (%)	n (%)	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

^ap(Chi-square test)

Distribution of the participants, according to diagnosis for proven, probable and possible fungal infection, with EORTC/MSG criteria (European Organization for Research and Treatment of Cancer/Mycoses Study group), are presented in figure 2. According to EORTC/MSG criteria, only a small percentage of patients had proven infection with *Aspergillus*. Of these, 20% (7/35) patients had some type of primary deficiency, and 10% (3/30) patients had a prolonged stay in ICU.

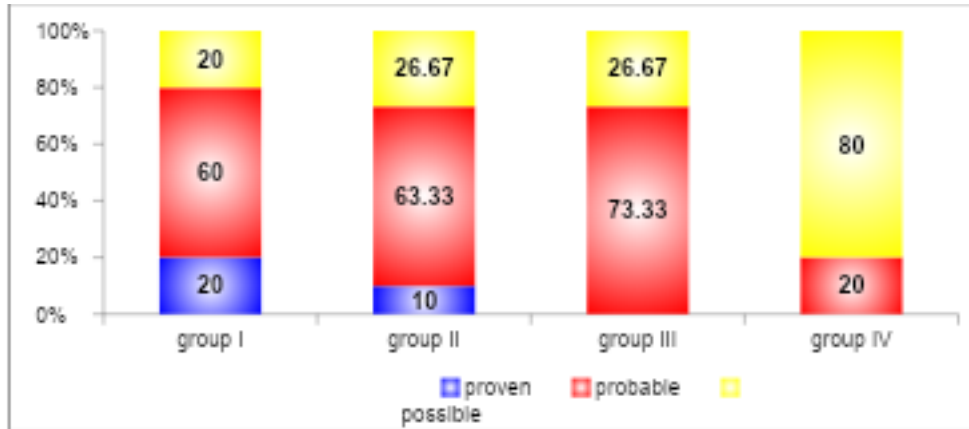


Fig.2. Distribution of fungal infections according to EORTC/MSG criteria in all groups

Differences in distribution of proven, probable and possible infections with *Aspergillus* were statistically significant between group I versus groups III and IV, and between group II versus groups III and IV (Table 2).

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

<i>Aspergillus</i>	group I N=35	group II N=30	group III N=30	group IV N=30
n (%)	n (%)	n (%)	n (%)	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			

^ap(Chi-square test) ^b(Fisher exact test) *p<0.05 **p<0.01

Regarding the application of PCR in serum, presence of *Aspergillus* DNA was confirmed in 42.86% patients with primary deficiency, 26.67% patients with prolonged ICU stay, and in 6.67% patients with chronic aspergillosis. Statistically significant differences were confirmed

between group I versus group III ($p=0.0014$) and group IV ($p<0.0001$), and between group II versus group IV ($p=0.0046$) (Table 3).

Table 3. PCR in serum

Ggroup <i>Aspergillus</i>				
	Group I N=35	Group II N=30	Group III N=30	Group IV N=30
Serum PCR	n (%)	n (%)	n (%)	n (%)
No 100 (80%)	20 (57.14%)	22 (73.33%)	28 (93.33%)	30 (100%)
Yes 25 (20%)	15 (42.86%)	8 (26.67%)	2 (6.67%)	0
	Chi-square: 23.09 ^a $p < 0.000039$ I vs II ^a $p=0.17$ II vs III ^b $p= 0.08$ III vs IV ^b $p= 0.5$ I vs III ^b $p= 0.0014^{**}$ II vs IV ^b $p= 0.0046^{**}$ I vs IV ^b $p<0.001$			

^a p (Chi-square test) ^b(Fisher exact test)

The results from comparative diagnostic performance of serum PCR test and serum galactomannan antigen test in the group of patients with immunodeficiency are shown in Table 4. Sensitivity, specificity, positive and negative predictive values of serum PCR test were: 53.57% / 100% / 100% / 35%. Sensitivity, specificity, positive and negative predictive values of serum galactomannan antigen test were: 64.29 % / 57.14% / 85.71% / 28.57%, in group I, respectively.

Table 4. Diagnostic performances of serum PCR test and serum galactomannan antigen test in the group of patients with primary immunodeficiency

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Serum PCR	53.57	100	100	35
Galactomannan in serum	64.29	57.14	85.71	28.57

The results from comparative diagnostic performance of serum PCR test and serum galactomannan antigen test in the group of critically ill patients and prolonged hospital stay in ICU are presented in Table 5. Sensitivity, specificity, positive and negative predictive values of serum PCR test were: 36.36% / 100% / 100% / 36.36%. Sensitivity, specificity, positive and

negative predictive values of serum galactomannan antigen test were: 40.91% / 62.5% / 75% / 27.78%, in group II, respectively.

Table 5. Diagnostic performances of serum PCR test and serum galactomannan antigen test in the group of patients with prolonged hospital stay in the ICU

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Serum PCR	36.36	100	100	36.36
Galactomannan in serum	40.91	62.5	75	27.78

The results from comparative diagnostic performance of serum PCR test and serum galactomannan antigen test in the group of patients with chronic aspergillosis are presented in Table 6. Sensitivity, specificity, positive and negative predictive values of serum PCR test were: 9.09% / 100% / 100% / 28.57%. Sensitivity, specificity, positive and negative predictive values of serum galactomannan antigen test were: 40.91% / 62.5% / 75% / 27.78%, in group III, respectively.

Table 6. Diagnostic performances of serum PCR test and serum galactomannan antigen test in the group of patients with chronic aspergillosis

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Serum PCR	9.09	100	100	28.57
Galactomannan in serum	40.91	62.5	75	27.78

In the group with cystic fibrosis, only galactomannan in serum was performed, so comparison between both methods was not analyzed.

Discussion

Aspergillus is considered as one of the most common causes of death in immunocompromised patients, with mortality rates up to 40% to 50% in patients with acute leukemia and recipients of hematopoietic stem cells transplantation. From recently, immunocompetent patients without any

risk factors for invasive fungal infections, suffer from IPA as well. Early diagnosis of IPA can significantly improve patient prognosis and increase the chances for survival of the patient, if treatment is started earlier. This still presents a big challenge for both clinicians and laboratory workers (1).

Non-invasive and nonculture-based methods diagnostic strategies could present a great benefit for earlier diagnosis of aspergillosis. Screening for circulating *Aspergillus* DNA for the diagnosis of aspergillosis has been the subject of many studies for many years (8), and has shown potential in the definitive diagnosis of aspergillosis, especially in combination with other biomarkers. Detection of galactomannan (GM) can also contribute to earlier mycological diagnosis (10).

We compared the diagnostic value of serum PCR assay with serum galactomannan antigen test, for diagnosis of both invasive and non-invasive aspergillosis. We have demonstrated the highest sensitivity of *Aspergillus* PCR in serum in the first group, with selected high-risk patients with immunodeficiencies. Sensitivity of galactomannan antigen test in our study was 64.29%, and comparing it with the molecular method, serum PCR demonstrated slightly lower sensitivity (53.57%). Sensitivity of PCR ranging from 72 to 88% and specificity from 75 to 98.7% were demonstrated by other studies. Sensitivity of as low as 26% has been demonstrated by some studies (11). A meta-analysis of 16 studies with 1618 patients, demonstrated an overall sensitivity of 88% and a specificity of 75%. If two consecutive tests are used to define positivity, the sensitivity and specificity would be 75% and 87%, respectively. The results of this study concluded that two positive tests are necessary to confirm the diagnosis, while one negative PCR result is sufficient to exclude the diagnosis (12). As a screening tool, a negative PCR result can help to rule out invasive aspergillosis. Most previous studies have focused on sensitivity of serum PCR for high-risk patients, like hematological malignancies or hematopoietic stem cell transplants (13). Raad have demonstrated a sensitivity of 100% for proven IPA infections, but only 57% for probable or possible invasive aspergillosis, in a study analyzing solid cancers (14). Studies which included patients with solid organ transplants or cancers have demonstrated lower sensitivity of serum PCR. In a meta-analysis of diagnostic performance of *Aspergillus* PCR, the presence of at least two positive whole-blood PCR specimens in a high-risk patient should be considered very indicative, if not confirmatory, of IA (15). Similar sensitivity of 58% of serum GM test was demonstrated in the study of Pfeiffer (16). Leeflang registered sensitivity of 78% (17) and Arvanitis demonstrated sensitivity of 92% with the serum GM test (11). It has been

demonstrated that detection of galactomannan levels in serum is inferior compared to BAL GM detection, which is considered to be a better way for distinguishing IPA from other infectious lung diseases.

Lower sensitivity of *Aspergillus* PCR in serum (36.36%) was demonstrated in the group with critically ill patients with prolonged stay in ICU. Bocci and coworkers also demonstrate that sensitivity is lower in serum samples than in respiratory specimens (1). According to the 2019 meta-analysis published by Cruciani, pooled sensitivity and specificity of *Aspergillus* PCR from blood is reported to be between 79% and 80% for a single positive result and 60% and 94% for two consecutive positive test results in immunocompromised people (18). But, the performance of PCR in blood decreases during systemic mould-active prophylaxis or treatment, and in non-neutropenic patients (13). According to Boch and coworkers, serum PCR has a sensitivity as low as 11% in ICU patients, although the sensitivity improved to 56% in BAL specimens (19). In a retrospective single-center analysis for comparison of galactomannan, PCR and mycologic analysis of pulmonary samples in both neutropenic and nonneutropenic patients, PCR sensitivity tended to be better in neutropenic patients (82.1%) than in nonneutropenic patients (62.5%). Sensitivity of serum GM test in our group of critically ill patients with prolonged stay in ICU was 40.91%. According to Eigl, galactomannan is often not present in the serum of non-neutropenic patients, in which airway-invasive growth is more typical (20). Thus, GM testing from BAL is preferred in this setting. Some studies have published results on the combined diagnostic capabilities of the GM and PCR assays in non-hematological patients, which is significantly improved compared to using only one of these methods solely. In the study by Lahmer and co-workers, serum GM levels have been elevated above the cutoff of >0.5 , in 3 patients only (10%) (21). The sensitivity of the serum GM test was lower than in our study (30%). One of the problems with serum GM determination is occurrence of false positive findings, which lowers its specificity, because critically ill patients are sometimes treated for bacteraemia, that could be an additional reason for false positive results.

Very low sensitivity of *Aspergillus* PCR in serum (9.09%) was demonstrated in the group with chronic aspergillosis. In the study by Imbert and coworkers, among 16 patients with noninvasive forms of aspergillosis, 10 were immunocompetent, 3 had metastatic malignancy, 2 had solid organ transplants and 1 had alcoholic liver cirrhosis. Clinical forms were simple aspergilloma ($n = 7$), colonization ($n = 4$), chronic cavitory aspergillosis ($n = 4$) and chronic bronchitis ($n = 1$).

None of these patients had positive serum GM or PCR (22). Low sensitivity for detecting *Aspergillus* DNA in chronic aspergillosis patients has been previously demonstrated in many studies. This has been explained by the hypothesis that DNA of *Aspergillus* could be more easily demonstrated in respiratory specimens from the site of infection than in blood or serum. A possible explanation for the lower sensitivity or a negative PCR in serum is that the specimen is not taken from the site of infection and the transient DNA-emia.

The utility of the serum GM antigen detection test for the diagnosis of pulmonary aspergillosis other than IPA is controversial. In our group of patients with chronic aspergillosis, GM antigen test in serum demonstrated sensitivity of 40.91%, which was similar to the findings in the study by Park and collaborators who demonstrated sensitivity of 38%. Hemoptysis was suggested to be a possible reason for the high rate of serum GM positivity (9), probably due to bleeding from bronchial artery, allowing GM to reach the circulation. Patients with slowly progressive pulmonary aspergillosis are likely to demonstrate serum GM positivity because of the release of fungal antigens into the bloodstream due to angioinvasive growth of *Aspergillus* (23). Kono and co-workers demonstrated sensitivity of serum GM levels as low as 14.3% for the diagnosis of chronic aspergillosis and ABPA (24). Park and co-workers found that the sensitivity was only 38% for serum GM (25). Kitasato and co-workers reported a sensitivity of serum GM of 21.4% at a cut-off ≥ 1.5 and 50% at a cut-off ≥ 0.5 for chronic aspergillosis (26). Fujiuchi et al. reported that at a cut-off level of ≥ 0.5 , the sensitivity for CNPA were 63.4% (27). In contrast to these results, Sehgal and coworkers demonstrated poor sensitivity of serum GM for diagnosing chronic aspergillosis. According to Sehgal, the poor performance of serum GM test is likely due to the less invasive nature of chronic aspergillosis (28). In a recent study, the sensitivity of serum GM was only 23%. Therefore, serum GM antigen test could not be used for diagnosis of CPA (29).

Our study demonstrate that PCR is useful both in nonneutropenic patients and nonhaematologic populations. As previously reported, our result indicates that performing both GM antigen test and PCR on the same serum, the sensitivity of the diagnostic approach increases. Our study has few limitations, and the main one is that it is a single-center study. Additionally, we used only a single serum sample for investigation, which didn't allow assessment of reproducibility of the methods. Financial constraints prevented these analyzes to be performed twice.

Conclusion

Results of this study indicate that a single method, either molecular or serological, could not provide definite diagnosis of invasive or non-invasive aspergillosis. As these methods detect different biomarkers of the disease, combining them is likely to prove more useful.

Implementation of different microbiological methods, as well as appropriate interpretation of results, in collaboration with clinicians, is the most important aspect towards accurate and precise etiological diagnosis of aspergillosis and earlier start of antifungal treatment in order to achieve favorable clinical outcome.

Ethics approval protocol number and date of approval: This study was approved by the Institutional Review Board of the Faculty of Medicine, University "Ss Cyril and Methodius", Skopje, Republic of North Macedonia (at the 10th regular session of the Institutional Review Board) (Decision No. dated 27 October 2011).

Author contributions statement: Gordana Mirchevska is the sole author of this work and was responsible for all aspects of the study, including conception, design, data acquisition, analysis, interpretation, manuscript drafting, and critical revision. The author approved the final version and takes full responsibility for the integrity and accuracy of this work.

Conflicts of interest declaration: None declared

Funding information: This research received no external funding.

References:

1. Bocci MG, Cascarano L, Capecchi G, Lesci A, Sabatini V, Rubino D, Stazi GV, Garotto G, Carrara S, Vulcano A, Gori C, Del Nonno F, Colombo D, Falasca L, Caraffa E, Cicalini S, Fontana C. Pulmonary Aspergillosis in Immunocompromised Critically Ill Patients: Prevalence, Risk Factors, Clinical Features and Diagnosis-A Narrative Review. *J Fungi (Basel)*. 2025;11(9):617. doi: 10.3390/jof11090617.

2. Mirchevska G, Kotevska V, Kaftandzieva A, Kirovski I, Stojanoski Z, Trajkovska-Dokic EI. Evaluation of BAL PCR for diagnosis of aspergillosis. *Macedonian journal of anaesthesia*. 2022;6(2):10-21.
3. Zubovskaia A, Vazquez JA. Invasive Aspergillosis in the Intensive Care Unit. *J Fungi (Basel)*. 2025;11(1):70. doi: 10.3390/jof11010070.
4. Kousha M, Tadi R, Soubani AO. Pulmonary aspergillosis: a clinical review. *Eur Respir Rev* 2011;20(121):156-74. doi: 10.1183/09059180.00001011.
5. Barton RC. Laboratory diagnosis of invasive aspergillosis: from diagnosis to prediction of outcome. *Scientifica (Cairo)*. 2013;2013:459405. doi: 10.1155/2013/459405.
6. Bassetti M, Azoulay E, Kullberg BJ, Ruhnke M, Shoham S, Vazquez J, Giacobbe DR, Calandra T. EORTC/MSGERC Definitions of Invasive Fungal Diseases: Summary of Activities of the Intensive Care Unit Working Group. *Clin Infect Dis*. 2021;72(Suppl 2):121-127. doi: 10.1093/cid/ciaa1751.
7. Skladny H, Buchheidt D, Baust C, Krieg-Schneider F, Seifarth W, Leib-Mösch C, et al. Specific Detection of *Aspergillus* Species in Blood and Bronchoalveolar Lavage Samples of Immunocompromised Patients by Two-Step PCR. *J Clin Microbiol*. 1999;37(12):3865-3871. doi:10.1128/jcm.37.12.3865-3871.1999
8. Platelia™ *Aspergillus* Antigen from http://www.bio-rad.com/webroot/web/pdf/inserts/CDG/en/62784_881082_EN.pdf
9. Suarez F, Lortholary O, Buland S, Rubio MT, Ghez D, Mahé V, Quesne G, Poirée S, Buzyn A, Varet B, Berche P, Bougnoux ME. Detection of circulating *Aspergillus fumigatus* DNA by real-time PCR assay of large serum volumes improves early diagnosis of invasive aspergillosis in high-risk adult patients under hematologic surveillance. *J Clin Microbiol*. 2008;46(11):3772-7. doi: 10.1128/JCM.01086-08.
10. Hites M, Goicoechea Turcott EW, Taccone FS. The role of galactomannan testing to diagnose invasive pulmonary aspergillosis in critically ill patients. *Ann Transl Med*. 2016;4(18):353. doi: 10.21037/atm.2016.08.32.
11. Arvanitis M, Anagnostou T, Mylonakis E. Galactomannan and Polymerase Chain Reaction-Based Screening for Invasive Aspergillosis Among High-Risk Hematology Patients: A Diagnostic Meta-analysis. *Clin Infect Dis*. 2015;61(8):1263-72. doi: 10.1093/cid/civ555.

12. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. *Lancet Infect Dis*. 2009;9(2):89-96. doi: 10.1016/S1473-3099(09)70019-2
13. Egger M, Jenks JD, Hoenigl M, Prattes J. Blood Aspergillus PCR: The Good, the Bad, and the Ugly. *J. Fungi*. 2020;6(1):18. doi:10.3390/jof6010018.
14. Raad I, Hanna H, Sumoza D, Albitar M. Polymerase chain reaction on blood for the diagnosis of invasive pulmonary aspergillosis in cancer patients. *Cancer*. 2002;94(4):1032-1036. DOI 10.1002/cncr.10349.
15. Arvanitis M. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. *J Clin Microbiol*. 2014;52(10):3731-42. doi: 10.1128/JCM.01365-14.
16. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis*. 2006;42(10):1417-27. doi: 10.1086/503427.
17. Leeflang MM, Debets-Ossenkopp YJ, Wang J, Visser CE, Scholten RJ, Hooft L, Bijlmer HA, Reitsma JB, Zhang M, Bossuyt PM, Vandenbroucke-Grauls CM. Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev*. 2015;2015(12):CD007394. doi: 10.1002/14651858.CD007394.pub2.
18. Cruciani M., Mengoli C., Barnes R., Donnelly J.P., Loeffler J., Jones B.L., Klingspor L., Maertens J., Morton C.O., White L.P. Polymerase chain reaction blood tests for the diagnosis of invasive aspergillosis in immunocompromised people. *Cochrane Database Syst Rev*. 2019;9(9):CD009551. doi: 10.1002/14651858.CD009551.pub4.
19. Boch T, Reinwald M, Spiess B, et al. Detection of invasive pulmonary aspergillosis in critically ill patients by combined use of conventional culture, galactomannan, 1–3-beta-D-glucan and Aspergillus specific nested polymerase chain reaction in a prospective pilot study. *J Crit Care*. 2018;47:198–203.
20. Eigl S, Prattes J, Lackner M, et al. Multicenter evaluation of a lateral-flow device test for diagnosing invasive pulmonary aspergillosis in ICU patients. *Crit Care*. 2015;19(1):178.
21. Lahmer T, Rasch S, Schnappauf C, Beitz A, Schmid RM, Huber W. Comparison of Serum Galactomannan and 1,3-Beta-D-Glucan Determination for Early Detection of Invasive Pulmonary Aspergillosis in Critically Ill Patients with Hematological Malignancies and Septic Shock. *Mycopathologia*. 2016;181(7-8):505-11. doi: 10.1007/s11046-016-0011-x.

- 22.Imbert S, Gauthier L, Joly I, Brossas JY, Uzunov M, Touafek F, Brun S, Mazier D, Datry A, Gay F, Fekkar A. Aspergillus PCR in serum for the diagnosis, follow-up and prognosis of invasive aspergillosis in neutropenic and nonneutropenic patients. *Clin Microbiol Infect.* 2016;22(6):562.e1-8. doi: 10.1016/j.cmi.2016.01.027.
- 23.Sehgal IS, Dhooria S, Choudhary H, Aggarwal AN, Garg M, Chakrabarti A, Agarwal R. Utility of Serum and Bronchoalveolar Lavage Fluid Galactomannan in Diagnosis of Chronic Pulmonary Aspergillosis. *J Clin Microbiol.* 2019;57(3):01821-18. doi: 10.1128/JCM.01821-18.
- 24.Kono Y, Tsushima K, Yamaguchi K, Kurita N, Soeda S, Fujiwara A, Sugiyama S, Togashi Y, Kasagi S, To M, To Y, Setoguchi Y. The utility of galactomannan antigen in the bronchial washing and serum for diagnosing pulmonary aspergillosis. *Respir Med.* 2013;107(7):1094-100. doi: 10.1016/j.rmed.2013.04.007.
- 25.Park SY, Lee SO, Choi SH, Jeong JY, Sung H, Kim MN, Choi CM, Hong SB, Oh YM, Shim TS, Lim CM, Koh Y, Kim DS, Kim YS, Woo JH, Kim SH. Serum and bronchoalveolar lavage fluid galactomannan assays in patients with pulmonary aspergilloma. *Clin Infect Dis.* 2011;52(7):149-52. doi: 10.1093/cid/cir027.
- 26.Kitasato Y, Tao Y, Hoshino T, Tachibana K, Inoshima N, Yoshida M, Takata S, Okabayashi K, Kawasaki M, Iwanaga T, Aizawa H. Comparison of Aspergillus galactomannan antigen testing with a new cut-off index and Aspergillus precipitating antibody testing for the diagnosis of chronic pulmonary aspergillosis. *Respirology.*2009;14(5):701-8. doi: 10.1111/j.1440-1843.2009.01548.x.
- 27.Fujiuchi S, Yamamoto Y, Takeda A, Nishigaki Y, Fujit Y, Yamazaki Y, Fujikane T, Osanai S, Ohsaki Y. [Evaluation of galactomannan antigen and beta-D-glucan value for diagnosis of chronic necrotizing pulmonary aspergillosis]. *Nihon Kokyuki Gakkai Zasshi.* 2009;47(1):7-11.
- 28.Sehgal IS, Dhooria S, Choudhary H, Aggarwal AN, Garg M, Chakrabarti A, Agarwal R. Utility of Serum and Bronchoalveolar Lavage Fluid Galactomannan in Diagnosis of Chronic Pulmonary Aspergillosis. *J Clin Microbiol.* 2019;57(3):e01821-18. doi: 10.1128/JCM.01821-18.
- 29.Shin B, Koh WJ, Jeong BH, Yoo H, Park HY, Suh GY, Kwon OJ, Jeon K. Serum galactomannan antigen test for the diagnosis of chronic pulmonary aspergillosis. *J Infect.*2014;68(5):494-9. doi: 10.1016/j.jinf.2014.01.005.