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# Seroprevalence of galactomannan antigen in Erzurum and comparison of two different test kits for galactomannan detection

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**Abstract:** Early diagnosis of aspergillosis is important to initiate antifungal therapy and improve the prognosis of the disease. One of the commonly used tests for early diagnosis is the galactomannan antigen test. In this study, we aimed to determine the prevalence of galactomannan antigen in various risk groups; We aimed to compare the results of two different test kits used in diagnosis and to determine false positive rates. Bio-Rad Platelia Aspergillus Ag kit was used to detect Aspergillus galactomannan antigen in serum samples of patients who were hospitalized in various clinics or admitted to hospital for serious diseases. In order to detect false positives, some of the samples found positive in this test were studied with Bio Bio-Rad" kits as well as "Dynamiker Biotechnology (Tianjin) DNK-SM-1402-1" test kits for the second time. The same procedure was repeated for the third and fourth times. Galactomannan antigen was searched in 735 different cases. In 306 (41.6) cases were obtained in at least one positive result study. Galactomannan antigen was the most common in septicaemia (75.0%); the lowest rate was found in patients with pre-diagnosis of neoplasm (21.8%). Galactomannan antigen positivity was highest in patients over the age of 65. Galactomannan antigen positivity was found to be very similar in the second and third studies of positive samples. In the fourth repetition, both firms gave 100% similar results. From the first to the last, GM positivity rates gradually decreased and GM positivity of 98 (32.0%) out of 306 positive cases in the first study has continued. It was determined that the kits belonging to two companies can be used with the same reliability and the positive rates of both tests gradually decreased. 

**Keywords:** Aspergillosis; Anemia; Galactomannan Antigen; Lymphoma; Leukemia; Septicemia.

## 1. Introduction

Aspergillus, which is mostly formed by Aspergillus species like Aspergillus fumigatus and Aspergillus niger, causes morbidity and mortality especially in immunosuppressed patients, and those who undergo solid and liquid organ transplantation and in hospitalized patients treated for serious diseases. It has been reported recently that the increase in fungal infections has reached alarming rates (1). Invasive

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Pulmonary Aspergillus (IPA), which is characterized by aspergillus hypha that invades lung tissue, is one of the most important fungal infections that can be mortal. The change of IPA incidence according to populations who are at risk and the diagnostic criteria make it difficult to show the incidence of this disease with numbers. In a meta-analysis, it has been shown that 29 studies covering the 2000-2018 period had an average of 16.3% (2.5%-57.1%) invasive Aspergillus prevalence based on PCR blood test results (2). It was reported that the incidence of IPA is 10%; in patients with Acute Myeloblastic Leukemia, between 3-15% in patients who undergo solid organ transplantation; and the mortality associated with IPA is approximately 45% (3). Actually, it was reported that despite the important advances in treatment and prevention, the incidence of IPC continues to increase and the mortality rate exceeds 50%.

The timely diagnosis of opportunistic fungal diseases, which often accompany serious diseases, is important for starting antifungal treatment. However, the diagnosis of fungal infections is difficult because of the symptoms, which are not partially featured. Although traditional diagnostic methods like histopathological examination and culture, which are still considered as a gold standard, maintain their importance in diagnosis, new serological and molecular techniques were also developed because the traditional ones have low sensitivity in detecting fungal pathogens (4). One of the oldest serological tests is the Aspergillus Platelia Aspergillus Enzyme-Linked GM Immunoassay (Bio-Rad, Hercules, CA) Antigen Test (5). GM is an exo-antigen that is released from cell walls during in vivo and in vitro reproductions of aspergillus species (6). GM, which is a soluble polysaccharide, is a biological marker that can be shown in samples like urine, cerebrospinal fluid (BOS), Broncho Alveolar Lavage (BAL) apart from GM serum samples (7). This test has the capability of detecting approximately 1 ng/mL antigen in serum. The sensitivity of this test, which does not necessitate an invasive procedure, varies between 50 and 92.6%, and its specificity varies between 94 and 99.6% in patients with hematological malignancies (7). Among the factors that affect the performance of this test include the use of antibiotics like piperacillin/tazobactam, especially the cross-reactions with other microorganisms and natural or parenteral foods (8-10). It was reported that GM test could give 38% false positivity among non-neutropenic patients when BAL samples were used (11).

No studies were conducted before on galactomannan antigen seropositivity in risk groups in our region. Although it does not fall off the agenda with its false-positive results, knowing the performance of the GM antigen test, which is recommended to be used in the diagnosis of invasive Aspergillus, will be a guide in the test selection. For this purpose, it was planned to compare the results of the GM antigen kits from two different companies, and to determine the results of repeated tests in serum samples taken on different days from patients who had GM antigen positivity.

## 2. Material and Methods

*2.1.* Scope *of the Study, Cases and Clinical Samples*

The study was conducted between March 2016 and March 2018 at the Routine Microbiology of the Laboratory Atatürk University Research Hospital. The serum samples of 735 different cases whose GM antigen tests were requested, whose preliminary diagnosis was acute and chronic lymphoma, anemia, idiopathic thrombocytopenic purpura (ITP), pulmonary Aspergillus (PA), leukemia, multiple myeloma, neoplasms, and septicemia, who referred to our hospital for treatment. Galactomannan antigens in the serum samples were studied daily, and the samples that were not studied on the same day were stored in the refrigerator (at +4°C) to be studied in three days at the latest.. This study was conducted in accordance with the Declaration of Helsinki Principles. Ethics committee approval was obtained [20.06.2020-317]. Research and Publication Ethics have been complied.

*2.2.* Commercial *kits used to detect GM antigen in serum*

The "Bio-Rad Platelia Aspergillus Ag Kit" was used to detect Aspergillus GM antigen in the serum samples of all patients who were admitted to the hospital for the first time in two years. In this test, 239 patients who were positive and whose identities were obtained were studies for the second time with the "Dynamiter Biotechnology (Tianjin) DNK-SM-1402-1" test kit as well as the "Dynamiker Biotechnology (Tianjin) DNK-SM-1402-1" kit. The same procedure was applied for the third time to the patients who were positive and was repeated for the fourth time in patients who were positive after this application.

*2.3. Evaluation of* serum *GM antigen tests and of results*

The "Bio-Rad Platelia Aspergillus Ag Kit" and "Dynamiker Biotechnology (Tianjin) DNK-SM-1402-1" test kits that were used in the detection of GM antigen were used according to the recommendations of the manufacturers. Galactomannan levels were considered positive in patient samples when the optical sites of the samples were 0.90 or above, or 0.5 or above the optical density index. In this study, a total of 5640 clinical samples of 735 cases were examined in two years in terms of GM with Bio-Rad Company Kits, and the patient results were reported according to these data.

*2.4. Statistical Analysis*

Chi square test applied. $P<0.05$ was considered statistically significant.

## 3. Results

The GM antigen was examined 5640 times in 735 different cases whose ages ranged from 1 to 92, whose mean age was 54.5, whose 413 (56.2%) were male, and 322 (43.8) were female. Out of 179 (24.4) of the male cases, and 127 (17.3) of the female cases had GM antigen positivity in 306 (41.6) cases at least in one examination. The distribution of galactomannan positivity between the genders according to disease groups is given in Table 1. GM positivity was detected to be higher in men; however, this difference was not statistically significant compared to women (P=0.2872). The highest positivity was detected in septicemia patients at a rate of 75.0%; followed by leukemia, lymphoma, multiple myeloma, pulmonary Aspergillus, ITP, and anemia patients. The lowest positivity was detected in patients with neoplasms. GM antigen positivity was significantly higher in septicemia patients than the patients with neoplasms (c2=12.2354; SD=1; p=0.0005).

The distribution of Galactomannan positivity is given in Table 2 according to age groups. As it can be understood in the table, the highest positivity in total was detected in patients aged 66 and older, and the lowest positivity was detected in the young group aged 1-17 who represented young participants.

However, galactomannan positivity between the age groups did not show a statistically significant difference (c2=1.1268; SD=3; p=0.7706).

In the present study, 5640 clinical samples of 735 cases were examined in terms of GM with Bio-Rad Company Kits, and the patient results were reported according to these data. The 239 of the positive samples in this first study were re-examined for the second time, 153 for the third time, and 98 for the fourth time. The "Dynamiker Biotechnology (Tianjin) DNK-SM-1402-1" test kits were included in the repetitions. At the end of these re-studies, the changes detected in galactomannan positivity are given in Table 3. As you can see, positive results obtained from the tests belonging to the two companies decreased, provided that the results were close to each other parallel to the increase in the examinations. In the fourth examination, 98 (32.0%) of the 306 patients who were determined to be positive insisted on GM positivity in the 4th examination.

**Table 1:** Galactomannan positivity according to disease groups.

| **Disease** | | **GM (+)** | | **GM (-)** | | **P value** |
|---|---|---|---|---|---|---|
| | | **Female** | **Male** | **Female** | **Male** | |
| | **n** | **n (%)** | **n (%)** | **n (%)** | **n (%)** | |
| Septicaemia | 8 | 4 (50.0) | 2 (25.0) | 1 (12.5) | 1 (12.5) | |
| Leukemia | 187 | 41 (21.9) | 68 (36.4) | 40 (21.4) | 38 (20.3) | |
| Lymphoma | 134 | 28 (20.9) | 48 (35.8) | 22 (16.4) | 36 (26.9) | |
| Multipl myelom | 44 | 12 (27.3) | 11 (25.0) | 11 (25.0) | 10 (22.7) | |
| Pulmoner aspergilloz | 8 | 1 (12.5) | 2 (25.0) | 2 (25.0) | 3 (37.5) | |
| ITP | 32 | 7 (21.9) | 5 (15.6) | 18 (56.3) | 2 (6.3) | |
| Anemia | 74 | 10 (13.5) | 13 (17.6) | 27 (36.5) | 24 (32.4) | |
| Neoplasm | 248 | 24 (9.7) | 30 (12.1) | 74 (29.8) | 120 (48.4) | |
| Total | 735 | 127 (17.3) | 179 (24.4) | 195 (26.5) | 234 (31.8) | 0.2872 |

GM: Galactomannan, ITP: Idiopathic thrombocytopenic Purpura

**Table 2:** Galactomannan positivity according to age groups.

| **Age Group** | | **GM (+)** | **GM (-)** | **P value** |
|---|---|---|---|---|
| | **n** | **n (%)** | **n (%)** | |
| 1-17 | 10 | 3 (30.0) | 7 (70.0) | |
| 18-45 | 204 | 81 (39.7) | 123 (60.3) | |
| 46-65 | 287 | 121 (42.2) | 166 (57.8) | |
| 66 and older | 234 | 101 (43.2) | 133 (56.8) | |
| Total | 735 | 306 (41.6) | 429 (58.4) | 0.7706 |

**Table 3:** Results obtained with the kits of two companies used to search for GM antigen.

| Period of study | n | BIORAD | | DNK-SM-1402-1 | |
|---|---|---|---|---|---|
| | | GM (+) n (%) | GM (-) n (%) | GM (+) n (%) (%) | GM (-) n (%) |
| Firs study | 735 | 306 (41.6) | 429 (58.4) | - | - |
| Second time study | 239 | 230 (96.2) | 9 (3.8) | 229 (95.8) | 10 (4.2) |
| Third time study | 153 | 151 (98.7) | 2 (1.3) | 152 (99.3) | 1 (0.7) |
| Four time study | 98 | 98 (100.0) | 0 (0.0) | 98 (100.0) | 0 (0.0) |

## 4. Discussion

In the present study, GM antigen seroprevalence was detected in several disease groups in line with the preliminary diagnosis of clinics that requested GM antigen tests. The patients consisted of those who were at risk for leukemia, lymphoma, multiple myeloma, ITP, anemia, neoplasms, septicemia, invasive fungal infections that were pre-diagnosed with PA.

Interestingly, in the present study, the highest positivity rate was detected in septicemia patients; and positivity was detected below the overall average in PA cases who were expected to be represented at a higher rate than other diseases. We believe that the low number of cases in these two disease groups might have played a role in this result. Apart from these two groups, the highest positivity rates were detected in leukemia, followed by lymphoma, multiple myeloma, ITP and anemia, and the lowest positivity was detected in patients with neoplasms. It is possible to speculate that these results are similar to the general literature data.

When all the cases were considered, the prevalence of invasive Aspergillus in our region was 41.6% according to the results of the first GM antigen test. The datum that IPA prevalence varies according to the disease risk groups and the testing methods used in the diagnosis and their sensitivity and specificity has become classical knowledge. Linke et al. reported that the epidemiology and treatment practices of invasive fungal diseases following allogeneic hematopoietic stem cell transplantation are in constant change (12). Melancon et al. argued that the sensitivity of the galactomannan test was 44.8%, and its specificity was 100% in the diagnosis of acute invasive fungal sinusitis and reported that there were no significant associations between galactomannan condition and mortality in this patient population (13). In a study conducted in our country, the sensitivity of the GM antigen test was found to be 68%; specificity was 77% according to 0.5 ng/ml cut-off value in neutropenic pediatric patients (14). Chan et al. reported that the galactomannan antigen seropositivity rates increased from PA (24.1%) to chronic PA (35.7%) and IPA (54.9%) (15). Cai et al. reported that the most common underlying disease of IPA patients was Chronic Obstructive Pulmonary Disease, and the sensitivity of the GM test was 40.7% and its specificity was 61.1% (16). As in these studies, in many other studies, the sensitivity of the GM test was found to be lower. These results mean that the GM antigen test detects those with real diseases at a very low rate. Its specificity was found to be high in some studies; however, it was found to have low rates in some studies. According to Cai et al., who reported the specificity of this test as 61.1%, patients were not correctly identified by nearly 40% of those who were detected as negative. Although it has an important place in early diagnosis of IPA, it is difficult to argue that GM antigen tests can detect a completely safe prevalence rate. However, the results that will be obtained from the test will be guiding together with another laboratory, radiological and clinical findings.

Many studies were conducted on the relation between GM antigen positivity and age groups and gender. In one of these, Kaur et al. identified PA prevalence to be at the highest level in 21-40 age group (13.3%) in HIV-positive patients who were admitted with lower respiratory infection in India; and reported the prevalence as 18.7% in women, and 7.7% in men (17). Sun et al. argued the average IPA incidence in Taiwan as 1.51 per million people on an annual scale and noted that this rate increased at the end of one year and observed male dominance (M/F: 1.85/1.15) in the IPA incidence (18). In a study conducted in the Netherlands, Chai et al. detected that GM positivity was 64.8% in men, and 35.2% in women when they considered the galactomannan index of 0.5%; however, they also reported that this high prevalence was not statistically significant in men compared to women (19). Parallel to these results, GM positivity was higher in men than in women; however, there was no statistically significant difference between the genders ($p = 0.2872$). In the present study, the rate of positivity increased gradually as the ages of the patients increased. In this context, the highest GM positivity was detected in patients who were over the age of 65. However, this difference was found to be not statistically significant between age groups ($p = 0.7706$).

In the present study, GM antigen presence was mainly examined with the kits of Bio-Rad Company, and the results obtained with these kits were reported to the relevant clinics. The second, third and fourth repetitions for the GM antigen search also included the "Dynamiker Biotechnology (Tianjin) DNK-SM-1402-1" test kits; and the results of these tests were compared. In the second repetition, the same results were obtained from the tests of the two companies.

Although these results showed that GM positivity could last up to two years in risk groups, maybe even longer, and the two companies had similar performance.

As a result, GM antigen prevalence was as high as 41.6% in patients with the risk of invasive fungal infections like leukemia, lymphoma, multiple myeloma, ITP, anemia, neoplasms, septicemia and PA in Erzurum region. Proportionally, IPA risk was higher in men compared to women, and higher in the elderly compared to the young population, and GM positivity is long-term. It was determined that the GM antigen kits of both "Bio-Rad" and "Dynamiker Biotechnology" could be used with the same safety level. Invasive Aspergillus causes hospitalization durations to be extended, and risky patients have to undergo expensive antifungal treatment processes, especially those with immunodeficiency, which also causes a financial burden on the patient and the economy of the country. For this reason, it is necessary that the diagnosis of invasive Aspergillus is made without delay.

## 5. Conclusions

Invasive Aspergillus causes hospitalization durations to be extended, and risky patients have to undergo expensive antifungal treatment processes, especially those with immunodeficiency, which also causes a financial burden on the patient and the economy of the country. For this reason, it is necessary that the diagnosis of invasive Aspergillus is made without delay.

**Limitations of the Study**

Two kits have been compared within the possibilities.

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None

**Conflict of Interests**

The authors declare no conflict of interest.

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**Author Contributions**

Writing and analyzing D.Ç, Statistics analyzing and interpretation Ö.Ç.

**Ethical Approval**

Ethics committee approval was received for this study from the ethics committee of Ataturk University.

**Data sharing statement**

None

**Consent to participate**

None

**Informed Statement**

None

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