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Utility of serum galactomannan antigen testing combined with chest computed tomography for early diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies with febrile neutropenia after antifungal drug treatment

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Abstract

Objective: To investigate the value of serum galactomannan antigen (GM) testing combined with chest computed tomography (CT) as a noninvasive method for early diagnosis of invasive pulmonary aspergillosis (IPA) in patients with hematological malignancies with febrile neutropenia after antifungal drug treatment.

Methods: We retrospectively analyzed the data of 376 patients with febrile neutropenia from January 2015 to August 2017. All patients were given broad-spectrum antibiotics and divided into the control group (effective antibiotic treatment, no antifungal drugs given) and the observational group (ineffective antibiotic treatment, antifungal drugs given). The serum GM testing, chest CT, and microbiological examination findings were compared between the two groups.

Results: The false-positive rates of GM testing for IPA in the control and observational groups were 4.04% and 8.65%, respectively, and the false-negative rates in the two groups were 1.10%

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and 9.62%, respectively. Sixty-five patients in the observational group and 11 in the control group had typical features of CT imaging.
Conclusion: Clinical weekly screening of serum GM and chest CT may be an effective combined approach to the early diagnosis of IPA in patients with febrile neutropenia, even if they have undergone antifungal treatment.

Keywords
Galactomannan antigen testing, invasive pulmonary aspergillosis, hematological malignancies, febrile neutropenia, antifungal treatment, computed tomography

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Introduction

Patients with hematological malignancies undergoing cytotoxic chemotherapy or hematopoietic stem cell transplantation commonly develop neutropenia, which makes these patients vulnerable to fungal infections. Fungal infections can occur in many locations. The incidence of invasive pulmonary aspergillosis (IPA) has rapidly increased in recent years. IPA is a life-threatening fungal infection that requires prompt diagnosis and treatment because it is associated with significantly high morbidity and mortality rates.[1] The traditional approach to diagnosing IPA includes a combination of clinical signs, radiographic evidence, and histopathological examination of specimens obtained via invasive procedures. However, patients with febrile neutropenia often have impaired hemostasis and thrombocytopenia, making diagnostic lung biopsy difficult to perform. Galactomannan antigen (GM) testing offers a potential noninvasive diagnostic method. However, some antifungals or antibiotics may influence the GM testing sensitivity, and false-positive or false-negative results are sometimes obtained. This retrospective study was performed to evaluate the utility of serum GM testing combined with computed tomography (CT) for the monitoring of IPA in patients who have hematological malignancies with febrile neutropenia after antifungal treatments.

Methods

Patients and study design

We retrospectively analyzed the role of serum GM testing combined with CT scans for identifying IPA in patients who had hematological malignancies with febrile neutropenia from January 2015 to August 2017. The inclusion criteria were the presence of hematological malignant disease, neutropenia (<500 neutrophils/mm^3), and fever (axillary temperature of $>38^{\circ}$C for ≥ 1 hour) and application of broad-spectrum antibiotics such as carbapenem or piperacillin/tazobactam for the febrile neutropenia. The exclusion criteria were an age of <12 years, no use of broad-spectrum antibiotics for febrile neutropenia, and anticipation that the patient would be unavailable to contact during the 30-day follow-up period. All patients with febrile neutropenia were given broad-spectrum antibiotics. According to their response to the antibiotic treatment, the patients were divided into two groups.

In the control group, the broad-spectrum antibiotics were effective and the patients were given no antifungal drugs. In the observational group, the broad-spectrum antibiotics were ineffective or the body temperature increased again during antibiotic treatment, and the patients were given antifungal drugs such as voriconazole, posaconazole, itraconazole, caspofungin, or amphotericin-B. The study was approved by the ethics board of the medical faculty in the First Affiliated Hospital of Xi'an Jiaotong University. All patients provided written informed consent.

GM testing

Serum GM was measured using the Platelia Aspergillus EIA (Bio-Rad Laboratories, Marnes-la-Coquette, France) according to the manufacturer's instructions. All serum samples were taken from patients in their neutropenic period, and the first sample was collected at the first occurrence of a fever of $>38^{\circ}C$ for ≥ 1 hour. The serum GM level was then measured once weekly until neutrophil recovery. Serum samples were considered positive if the GM index was equivalent to a 0.5 GM antigen level.

CT evaluation

Chest CT scans with or without contrast enhancement (usually high-resolution CT) were performed at three time points: after a fever of $>38^{\circ}C$ had persisted for ≥ 1 hour in patients with neutropenia, after the body temperature had returned to normal, and at the time of neutrophil recovery. CT scans were performed using a 64-multislice scanner (Philips, Best, the Netherlands). The main CT findings were divided into angio-invasive and airway-invasive. Angio-invasive findings were determined to be present if at least two of the following features were seen: a halo sign, an infarct-shaped consolidation, or an internal low-attenuation, cavity, or air-crescent sign. Airway-invasive findings were determined to be present if at least two of the following features were seen: small airway lesions, peri-bronchial consolidation, or bronchiectasis. Dense nodules were considered a typical sign of *Aspergillus*. CT evaluation was performed by two experienced thoracic radiologists in a retrospective manner. Where disagreement occurred, the final decision was made by consensus.

Microbiological examination

Before use of broad-spectrum antibiotics, every patient underwent a microbiological examination of their sputum and blood for the presence of fungal microorganisms. Plates were incubated for fungal growth at $30^{\circ}C$ for 14 days and inspected regularly.

Definitions of IPA

Patients were diagnosed with IPA according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) 2008 criteria.[2] Patients were classified as having proven, probable, possible, or no IPA.

Statistical analysis

Continuous variables were compared using a t test, and categorical variables were compared using the chi-squared test. SPSS for Windows, Version 13.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and a P value of <0.05 was considered statistically significant.

Results

Patient characteristics

In total, 376 patients were included in the study. The demographic and clinical characteristics of all enrolled patients are

presented in Table 1. In total, 814 blood samples were collected from patients with febrile neutropenia. The median number of tests per patient was two. There were no statistically significant differences in the demographic data between the two groups. All patients in the observational group were diagnosed with IPA; 5 cases were proven, 86 were probable, and 13 were possible. Twenty patients in the control group were diagnosed with IPA (Table 2). Among the patients with IPA, 103 had leukemia, 10 had non-Hodgkin's lymphoma, and 11 had plasma cell disease.

GM testing

Serum GM testing was positive in 91 patients in the observational group and 20 patients in the control group. The median GM titer was significantly higher in the observational group than in the control group ($P < 0.05$). An interactive scatter diagram displaying the distribution of GM values in the observational group with a cut-off point of 0.5 ng/mL is presented in Figure 1. The false-positive rates of GM testing for IPA in the control and observational groups were 4.04% and 8.65%, respectively. The false-negative rates of GM testing for IPA in the control and observational groups were 1.10% and 9.62%, respectively.

Chest CT scans

Among all patients with IPA, 65 patients in the observational group (6 patients with

Table 1. Demographic and clinical characteristics of enrolled patients.

Characteristic	Observational group (n = 104)	Control group (n = 272)
Sex		
Male	56 (53.8)	131 (48.2)
Female	48 (46.2)	141 (51.8)
Age, years	49 (16–65)	45 (15–71)
Diagnosis		
AML	31 29.8)	104 (38.2)
ALL	30 (28.8)	93 (34.1)
CLL	6 (5.8)	2 (0. 7)
NHL	20 (19.2)	65 (23.8)
MM	15 (14.4)	7 (2.5)
Waldenstrom macroglobulinemia	1 (1.0)	0 (0.0)
Plasmacytoma	1 (1.0)	1 (3.7)
Duration of neutropenia, days		
Mean	14.5	14.1
Median	15	13
Range	7–29	5–34
Patients given antifungal drugs		
Fluconazole	34 (32.8)	0 (0.0)
Voriconazole	56 (53.8)	0 (0.0)
Itraconazole	6 (5.8)	0 (0.0)
Caspofungin	4 (3.8)	0 (0.0)
Amphotericin-B	4 (3.8)	0 (0.0)

Data are presented as n (%) or median (range) unless otherwise indicated

AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; MM, malignant melanoma

3 typical features of CT imaging, 27 patients with 2 typical features of CT imaging, and 32 patients with 1 typical feature of CT imaging) and 11 patients in the control group (9 patients with 2 typical features of CT imaging and 2 patients with 1 typical feature of CT imaging) had typical features of CT imaging. There was a significant difference in the frequency of typical *Aspergillus* signs between the observational and control groups ($P < 0.05$) (Table 3).

Table 2. Invasive pulmonary aspergillosis in the two groups.

Invasive pulmonary aspergillosis	Observational group (n = 104)	Control group (n = 272)
Proven	5 (4.8)	0 (0.0)
Probable	86 (82.7)	20 (7.4)
Possible	13 (12.5)	0 (0.0)

Data are presented as n (%)

Microbiological examination

Aspergillus species were detected in cultures of sputum or blood samples (observational group) in four patients (1.06% of all study patients). The isolated species were *A. fumigatus* (two patients), *A. flavus* (one patient) and *A. niger* (one patient). Histological

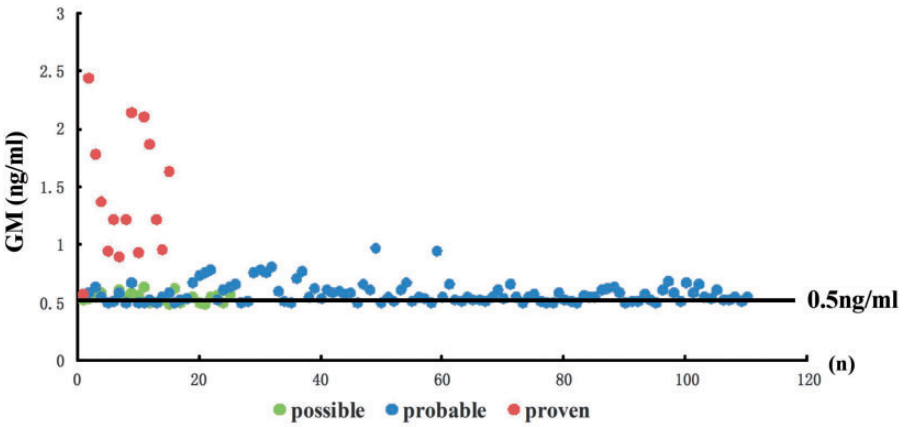

Figure 1. Scatter diagram displaying the GM distribution among the observational group with a cut-off point of 0.5 ng/mL. GM, galactomannan antigen

Table 3. Results of computed tomography scans in the two groups.

	Observational group (n = 104)	Control group (n = 272)
Nodular infiltrate	22 (21.2)*	2 (0.7)
Halo sign	14 (13.5)*	0 (0.0)
Wedge-shaped pleura-associated consolidation	26 (25.0)*	6 (2.2)
Focal ground-glass opacities	25 (24.0)*	9 (3.3)
Caverns/air-crescent sign	8 (7.7)*	2 (0.7)
Tree-in-bud pattern/centrilobular nodules	2 (1.9)*	0 (0.0)
Peribronchial consolidations	2 (1.9)*	0 (0.0)
Diffuse ground-glass opacities	5 (4.8)*	1 (0.4)

Data are presented as n (%)
*$P < 0.05$ compared with control group

examination findings of specimens obtained from lung biopsy were available in four patients, including one patient with a negative sputum and blood culture and three with a positive sputum or blood culture.

Antimycotic treatment and outcome

Overall, 124 patients received antimycotic treatment for IPA. Voriconazole was the most commonly used antimycotic agent. Sixteen patients died of IPA, giving a mortality rate of 13.4% in the observational group and 0.7% in the control group.

Discussion

The EORTC/MSG revised criteria for defining IPA require a microbiological and or histopathological diagnosis to prove the presence of an infection. A lung tissue biopsy can be obtained by needle aspiration or thoracoscopic biopsy. Bronchial lavage specimens are commonly used in the clinical setting.[2,3] However, many patients who have hematological malignancies with thrombocytopenia or impaired coagulation cannot tolerate an invasive operation. Fungal culture is time-consuming; additionally, the fungal culture-positive rate is lower than the bacterial culture-positive rate, and if no fungal species is cultured, the diagnosis of IPA still cannot be excluded.[4] Therefore, a noninvasive diagnostic method for IPA is needed. Many reports have described assessment of the value of *Aspergillus* polymerase chain reaction (PCR) in the clinical setting. The sensitivity and specificity of *Aspergillus* PCR for diagnosing IPA are 84% and 76%, respectively.[5] The sensitivity of samples from bronchoalveolar lavage (BAL) fluid is higher than that from blood samples, but the specificity is lower.[6–11] In patients who have hematological malignancies with neutropenia and fever, obtaining a BAL fluid sample is difficult due to myelosuppression and thrombocytopenia. *Aspergillus* PCR is not recommended for routine use in the clinical setting because few assays have been standardized.

GM testing is a noninvasive diagnostic assay for detection of *Aspergillus*, and its sensitivity varies in different conditions according to host factors. In patients undergoing allogeneic hematopoietic stem cell transplantation, the sensitivity of GM testing was approximately 70% in serum and approximately 82% in BAL fluid.[12] In the present study, the sensitivity of serum GM testing in patients with neutropenia was much higher than the reported sensitivity in patients without neutropenia. This difference may be due to the fact that patients with neutropenia are vulnerable to fungal infection, and the fungal burden is high in these patients; additionally, macrophage deficiency limits the GM clearance from the blood.[12–15] False-positive results have been reported in patients given certain antibiotics such as piperacillin-tazobactam and amoxicillin clavulanate, and false-negative results have been reported in patients who undergo antifungal prophylaxis with agents such as echinomycin.[16–18] In the present study, the false-positive rates of GM testing for IPA in the control and observational groups were 4.04% and 8.65%, respectively. The false-negative rates of GM testing for IPA in the control and observational groups were 1.10% and 9.62%, respectively, which is in accordance with the literature. These findings show that antibiotic and antifungal treatment in patients with neutropenia does not significantly affect the sensitivity of GM testing.

Chest CT performed at fever onset may help to identify the cause of fever and provide clinical information before GM testing is positive.[19–21] In the present study, 65 patients in the observation group and 11 patients in the control group had typical features of CT imaging. There was a

significant difference in the frequency of typical *Aspergillus* signs between the two groups.

Our study has two main limitations. The number of patients with histologically proven IPA was small, and the different sensitivities of GM testing and CT scans among different antifungal treatments was not compared.

In conclusion, clinicians should be aware of the potential antifungal treatment effects on GM testing and with the appropriate GM cut-off optical density index values as well as the proper interpretation and identification of possible false-positive or false-negative results. The GM assay has the potential to become an effective tool for diagnosing IPA. Once-weekly screening of GM in serum and chest CT scans may be an effective combined approach to the early diagnosis of IPA in patients who have hematological malignancies with febrile neutropenia, even if they have accepted antifungal treatment.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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