Serum galactomannan detection for early diagnosis of invasive pulmonary aspergillosis in children.

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Abstract

The aim of this study was to investigate the value of serum Galactomannan (GM) detection for the early diagnosis of Invasive Pulmonary Aspergillosis (IPA) in children as a reference for clinical diagnosis and treatment. A total of 80 high-risk children with IPA were selected (including three confirmed cases, 20 clinically diagnosed cases, 25 suspected cases, and 32 non-IPA patients) for the detection of serum GM concentration by enzyme-linked immunosorbent assay. Confirmed, clinically diagnosed, and suspected cases were set as the IPA group, and the Absorbance Index (AI) value for GM detection in the IPA group was 1.52 ± 0.74, while that in the non-IPA group was 0.57 ± 0.23; the difference was statistically significant (t=7.03, P<0.05). When the AI value for GM detection was ≥ 1, the diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were 81.25% (39/48), 93.75% (30/32), 95.12% (39/41), and 96.92% (30/39), respectively; when the AI value of GM detection was ≥ 0.5 and positive, the diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were 91.67% (44/48), 75.00% (24/32), 84.62% (44/52), and 85.71% (24/28), respectively. The positive cut-off value of the GM test had the strongest effect on detection accuracy. When the positive cut-off value was 1.0, the diagnostic sensitivity and specificity increased.

Introduction

Invasive Pulmonary Aspergillosis (IPA) is one of the most common causes of death due to invasive mycoses in the US and many other regions worldwide [1]. As the number of immunocompromised patients' increases, the incidence of fungal infections has also increased in recent years [1,2]. The number of patients in Intensive Care Units (ICUs) with respiratory tract samples positive for *Aspergillus* culture increases every year [3]; however, there is no non-invasive gold standard for the diagnosis of IPA for these patients. IPA is one of the most frequently underdiagnosed infections in critically ill patients [4] and remains an important cause of morbidity, mortality, and high hospital costs [4,5]. Including Bronchoalveolar Lavage Fluid (BALF) serum Galactomannan (GM) as an additional entry criterion for the AspICU clinical algorithm may increase the diagnostic sensitivity for IPA in ICU patients [6,7]. The role of GM in the serum or BALF for the diagnosis of IPA has been extensively evaluated in hematological patients; however, its performance in non-hematological patients is not well-established [7-10]. We suggest that including GM detection in the BALF into the diagnostic algorithm would increase the sensitivity of diagnosis. In this study, we prospectively evaluated the AspICU algorithm for critically ill patients with positive *Aspergillus* culture and tested patients with negative *Aspergillus* culture but a positive BALF antigen test. IPA has a high prevalence in the patients with cancer chemotherapy or organ transplantation [11,12], and its prevalence in pediatrics department has increased in recent years, which may be related to antibiotic abusage and low immunity in children, among other factors. Clinically, serum GM detection is typically applied for adult patients with IPA and has shown a high clinical value. However, its application for pediatric patients with IPA has been limited, and thus the associations between using GM to detect IPA between children and adults remain unclear. Based on the detection of serum GM in LPA high-risk children admitted to our hospital in recent years, we investigated the value of serum GM detection for the early diagnosis of IPA in children, thus providing a reference for clinical diagnosis and treatment.

Materials and Methods

Study subjects

A total of 80 pediatric patients with high-risk IPA admitted to our hospital from January 2010 to June 2015 were selected based on the following inclusion criteria: persistent fever (>38.0°C) and non-responsive to sufficient-quantity antibiotic treatment for 3 days or more. Diagnosis was performed based

on clinical symptoms and the diagnostic criteria of "treatment guidelines for children with invasive pulmonary fungal infections (2009)" issued by the respiration committee, Chinese Society of Pediatrics [4]. The study subjects included three confirmed cases, 20 clinically diagnosed cases, 25 suspected cases, and 32 non-IPA patients (37 boys and 43 girls, age ranging from 2 months and 18 days to 12 years, with an average age of 6.39 ± 1.47 years). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Children's Hospital of Zhengzhou. Written informed consent was obtained from all participants.

Diagnostic criteria [4]

1) Confirmed diagnosis: At least one item met the host factors and one met the microbiological diagnostic criteria for *Aspergillus*. 2) Clinically diagnosed: other invasive fungal infections except IPA. 3) Suspected: met at least one host factor and one of the microbiological diagnostic criteria of *Aspergillus*, or the possible infection site met one major or two minor clinical diagnostic criteria.

Methods

Serum samples were collected from the enrolled pediatric subjects with high-risk IPA before antifungal therapy; 5 ml of peripheral blood was extracted and then centrifuged at 1800 r/min for 10 min to separate the serum. The serum was then stored -80°C for the detection of serum GM by enzyme-linked immunosorbent assay (ELISA) using a detection kit from Bio-Rad (Platelia *Aspergillus* ELA kit, Hercules, CA, USA), and the operations were performed in accordance with the kit instructions. The testing instrument was an automatic ELISA detector (Bio-Rad). A total of 81 serum samples were obtained, and 11 patients were sampled twice at intervals of 1-2 weeks. The mean A value of the cut-off quality control serum was controlled within 0.3-0.8 A, that of the negative cut-off quality control serum was <0.4, and that of the positive cut-off quality control serum was >2. For determination of the test results, absorbance index (AI) ≥ 1 and ≥ 0.5 were determined as positive, and Al=A value of the tested serum/mean A value of the cut-off quality control serum.

Statistical analysis

SPSSl7.0 (SPSS, Inc., Chicago, IL, USA) was used for data analysis; the measurement data were expressed as the mean $\pm$ standard deviation and evaluated using the t-test. P<0.05 was considered significant.

Results

Comparison of serum GM between group IPA and group non-IPA

The confirmed, clinically diagnosed, and suspected cases were set as the IPA group; the AI value of GM detection in the IPA group was 1.52 ± 0.74 and that in the non-IPA group was 0.57 ± 0.23. The difference was significant (t=7.03, P<0.05).

AI value of GM detection ≥ 1 and positive (diagnostic sensitivity and specificity of GM in IPA pediatric patients)

The confirmed, clinically diagnosed, and suspected cases were considered positive, while non-IPA was considered negative. GM detection revealed 39 positive cases and 9 negative cases in the IPA group and 2 positive cases and 30 negative cases in the non-IPA group. The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were 81.25% (39/48), 93.75% (30/32), 95.12% (39/41), and 96.92% (30/39), respectively (Table 1).

When the AI value of GM detection was ≥ 0.5 and positive, GM detection revealed 44 positive cases and 4 negative cases in the IPA group and 8 positive cases and 24 negative cases in the non-IPA group. The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were 91.67% (44/48), 75.00% (24/32), 84.62% (44/52), and 85.71% (24/28), respectively (Table 2).

Table 1. *Diagnostic sensitivity and specificity of GM in detecting IPA children when AI ≥ 1 (n).*

Results of GM detection	Group IPA	Group non-IPA	Sum
Positive	39	2	41
Negative	9	30	39
Sum	48	32	80

Table 2. *Diagnostic sensitivity and specificity of GM in detecting IPA children when AI ≥ 1 (n).*

Results of GM detection	Group IPA	Group non-IPA	Sum
Positive	44	8	52
Negative	4	24	28
Sum	48	32	80

Discussion

Aspergillus causes fungal infections, typically hospital-acquired opportunistic infections, but its clinical infection rate is lower than that of *Candida albicans*; however, based on clinical reports, the rate of IPA infection has increased in recent years, and the *Aspergillus* infection-caused mortality rate has also increased and is the leading cause of fungal infections [11-14]. Lung tissues show the highest sensitivity to *Aspergillus* infection and high post-infection mortality, ranking the first among various infection sites. *Aspergillus* infection in children mainly occurs in new-borns, premature children, and those with immune deficiency and critically ill conditions; because of its high mortality, *Aspergillus* infection should be given attention by medical workers [14,15].

Aster *Aspergillus* infects lung tissues by releasing toxic products and antioxidants, which can interfere with and reduce the body's immune responses, protecting *Aspergillus* from being damaged by free radicals; *Aspergillus* can also interfere with the immunity of T cells by releasing interleukin (IL)-10, IL-4, or other cytokines, infection, and disease progression [16,17]. Currently, the diagnosis of IPA is mainly based on clinical symptoms, X-ray, or pathogen detection, among which opening the chest for lung tissue biopsy or thoracoscopic lung tissue pathological inspection is considered the gold standard diagnostic criteria; however, this test causes trauma to patients, and thus this method cannot be conducted clinically at the large-scale [18,19]. Non-culture methods for the diagnosis of IPA mainly use sputum, blood, or BALF, which show high diagnostic specificity and sensitivity [20-24]. Previous studies compared the diagnostic values of BALF with the serum GM test for diagnosis of IPA [25-29], and the results showed that when 1.0 was used as the positive cut-off value, the detection specificities and positive predictive values of BALF and serum GM were both 100.00%, but the detection sensitivity and negative predictive value of serum GM were less than those of BALF. When 0.5 was used as the positive cut-off value, the sensitivities of serum and BALF were both significantly increased, but the specificities were decreased. Because it is difficult to obtain phlegm specimens and BALF from children that may cause trauma and bucking easily in them, the serum GM detection method is used in more frequently in children to detect *Aspergillus* infection. Zhe analysed 20 reports from among 2658 studies and found that the early diagnostic value of serum GM for IPA was higher when 0.7 was used as the cut-off value [30,31]. This study investigated the diagnostic value of GM in children with early IPA. The results showed that the AI value in the IPA group (including confirmed, clinical diagnosed, and suspected cases) was 1.52 ± 0.74, which was significantly higher than that in the non-IPA group (0.57 ± 0.23), indicating the feasibility of diagnosing IPA by detecting serum GM. When the positive cut-off value of GM was ≥ 1, the diagnostic sensitivity, specificity, positive predictive value, and negative predictive value toward children with IPA were 81.25% (39/48), 93.75% (30/32), 95.12% (39/41), and 96.92% (30/39), respectively. When the cut-off value was set as ≥ 0.5, the diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were 91.67% (44/48), 75.00% (24/32), 84.62% (44/52), and 85.71% (24/28), respectively. Diagnostic sensitivity was lower when the positive cut-off was ≥ 1, but the diagnostic specificity, positive predictive value, and negative predictive value were higher, indicating that a positive cut-off ≥ 1 has higher diagnostic value, consistent with the results of previous studies. Although the sample size was limited, our results provide useful information regarding the early diagnosis of IPA in children.

In summary, detecting serum GM has diagnostic value for the early detection of IPA in children, and its positive cut-off value affects detection accuracy. When the cut-off value is set to 1.0, diagnostic sensitivity and specificity are increased.

Conflicts of Interest

All of the authors declare that they have no conflicts of interest regarding this paper.

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