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Diagnostic Values and Limitations of (1,3)-β-D-Glucans and Galactomannan Assays for Invasive Fungal Infection in Patients Admitted to Pediatric Intensive Care Unit

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Abstract The relationship among (1,3)-β-D-glucans (BG), galactomannan (GM), and the risk of developing invasive fungal infections (IFI) has been observed in adult ICU and in children with hematological malignancies. Only scant data evaluated the value of BG/GM assays for diagnosis of IFI in patients with nonhematological diseases in pediatric intensive care unit (PICU). In this study, we assessed the diagnostic value of these markers for IFI in PICU. The records of 230 patients were retrospectively evaluated. Out of 117 patients (7 proven, 23 probable, and 87 cases without evidence of IFI) performed GM and BG assays. The results showed many factors were associated with false-positive test results. Patients who aged over 3 years had higher levels of GM and BG than younger infants. The levels of BG were higher in subjects with dairy, human blood products, antibiotics, and corticosteroids therapy than in cases without these treatments. Unlike BG assay, GM assay was less susceptible to above-mentioned factors expect blood products. The levels of BG and GM in IFI cases were dramatically higher than in controls. The diagnostic performance of these assays showed that GM assay had better results when compared with BG assay. On the whole, negative predictive value in both GM and BG assays was dramatically higher than other diagnostic parameters. In conclusion, BG assay was highly susceptible to many factors, and GM assay could be useful for diagnosis of IFI for its high sensitivity, but the over benefit of this assay limited in its inadequate specificity. The comparative advantage of BG and BG assays lied in excluding IFI in non-hematological PICU patients.

Keywords Fungal infection · Galactomannan · Glucan · Pediatrics

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Introduction

Invasive fungal infections (IFI) are an increasing problem in children and are associated with high attributable morbidity and mortality rates, as well as early and late onset complications [1]. The high mortality rate is partly due to difficulties and delays in the diagnosis based on clinical, radiological, and mycological methods [2]. Early mycological detection of fungal species is the cornerstone for a prompt diagnosis, best treatment strategy, and improved prognosis of patients with IFI [3]. As a consequence of the difficulties with diagnosis, significant effort has been developed including the detection of antigenic

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markers, such as galactomannan (GM) and β-1,3-D-glucan (BG) [1]. The revised definitions of invasive fungal disease and specifically invasive aspergillosis incorporated GM and BG positive results as mycological criteria supportive of a probable diagnosis [4].

The revised definitions mainly apply to hematological patients, particularly in those with hematological malignancies or after HSCT [5, 6]. Recently, some reports have suggested that the GM assay had low diagnostic value for IFI in non-hematological adult patients [2]. However, in the adults critical care setting, BG assay based on a blood sample drawn at the sepsis onset may guide the decision to start antifungal therapy early in patients at risk of candida infection [7, 8]. The results of several studies have shown that the level of GM in serum, especially in bronchoalveolar lavage, enhances the identification of Aspergillus species as a cause of pulmonary disease in ICU patients [9, 10]. Previous studies have emphasized the increased risk of IFI in children with congenital immunodeficiency or acquired immunodeficiency after immunosuppressive therapy, virus infection, antibiotic therapy, or prolonged use of steroids [11, 12]. However, there are few data from GM and BG assays of non-hematological and critically ill pediatric patients. Only one prospective study on neonatal fungal infection in the neonatal intensive care unit showed that *C. parapsilosis* (61.9 %) was the most frequent isolated species and BG assay as an adjunct diagnostic test in the diagnosis of IFI [13]. Despite the fact that GM and BG indexes have been important markers for diagnosis of IFI, false-positive and false-negative results have been reported, which are major drawback of these markers.

The purpose of this study was to assess the factors associated with false positive of BG and GM assays in non-hematological pediatric patients admitted in PICU. We also analyzed the utility of the BG and GM assays for the diagnosis of IFI.

Materials and Methods

Patients and Clinical Data Collection

All pediatric patients who underwent BG and GM assays from April 2013 to April 2014 were evaluated. Patients were enrolled if they were aged under 18 year and required a stay in the PICU of 3 or more days, and had not been diagnosed with hematological disease, including malignancies, or those who have not undergone HSCT. The patients were identified from a computerized database compiled by the diagnostic laboratory at Union Hospital, Tongji Medical college, Huazhong University of Science and Technology. Informed consents were provided by their parents. These patients were classified as proven or probable cases of IFI according to the criteria of the EORTC/MSG revised in 2008 [4]. The patients with IFI had compatible symptomatic and radiological features.

Various clinical data were collected through retrospective review of the electronic medical records, and these data included age, sex, APACHE and SOFA scores, underlying diseases, the length of PICU, various treatment regimens, site of infection, EORTC classification, pathology specimen, culture specimen, and mortality.

Galactomannan Detection in Serum

Aspergillus galactomannan antigen was detected by 1-stage immunoenzymatic sandwich microplate assay (Platelia Aspergillus; Bio-Rad, Marnes-la-Coquette, France). Samples were processed according to the manufacturer's instructions. Briefly, each test serum (300 µl) was mixed with 100 µl of treatment solution and placed in a boiling water bath for 3 min. After centrifugation, the supernatant was used for further testing. 50 µl supernatant and 50 µl horseradish peroxidase-labeled monoclonal antibody (clone EBA-2) were incubated in EBA-2-coated microplates for 90 min at 37 °C. After 5 washing steps, 200 µl of the substrate buffer was added to each well, and the plates were incubated for 30 min at room temperature. The enzymatic reaction was terminated by stopping solution. Optical density (OD) was read at 450 nm with Microplate Spectrophotometer. Positive and negative controls were included in each assay. The result was considered positive for an index value $\geq$ 0.5 on duplicate tests.

(1,3)-β-D-Glucans Assay in Serum

BG antigen was detected with the Fungitell® test kit according to the manufacturer's protocols. Briefly, serum samples were treated for 10 min at 37 °C with a solution containing 0.6 M KCl and 0.125 M KOH. The absorbance was then read at 405 nm. The

concentration of BG in each sample was automatically calculated using a calibration curve with standard solutions ranging from 6.23 to 100 pg/ml. The BG level $\geq$100 pg/ml was considered positive. Serum assays were performed in duplicate. All positive samples were retested and considered positive only if the repeat test was also positive.

Data Analysis

Data were analyzed using GraphPad Prism 5.0 software (USA). GM and BG indexes were reported as medians with ranges. The following diagnostic performance of GM and BG indexes was calculated: their 95 % confidence intervals (CIs), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). Student's t-tests were used to compare continuous variables, and Chi-square or Fisher's exact tests were used to compare categorical variables. All P values were two-tailed, and a P value of less than 0.05 was considered statistically significant.

Results

Patients' Clinical Characters

During the study period, out of 312 admitted pediatric patients to our PICU, 230 patients fulfilled the inclusion criteria above specified and 117 patients who performed GM and BG assays were enrolled as participants. Characteristics of the 117 subjects (30 patients in the IFI group and 87 patients in the group without evidence of IFI) were collected. The overall prevalence of IFI was 25.6 % (30/117). In repeat testing cases, only the first results were used. The clinical characteristics of patients are shown in Table 1. The median age was 3 months (range 2–9 months), and 55.5 % of children were female. The median PICU length of stay was 14 days (range 3–30 days). The median APACHE II score and SOFA score were 16 (range 5–38) and 5 (range 0–9), separately. The most common underlying disease was pneumonia (86.3 %), followed by congenital heart disease (67.5 %). All patients suffered from congenital heart disease were complicated with pneumonia.

Table 1 Demographics and clinical characteristics of studied groups

Characteristics	Patients (n = 117)
Age, median month {range}	3 (2–9)
Sex	
Male	52
Female	65
APACHE II, median (range)	16 (5–38)
SOFA, median (range)	5 (0–9)
PICU stay, median days (range)	14 (3–30)
Underlying diseases	
Congenital heart disease	79
Pneumonia	101
Severe sepsis	18
Surgery	23
Diarrhea	5
Other	2

Effect of Various Factors on the GM and BG Assays

We observed the GM and BG levels from 117 pediatric patients (Fig. 1). Patients who aged over 3 years had higher levels of GM and BG in serum than younger infants($P < 0.05$). The patients who treated with corticosteroids, blood products, and dairy products (infant formula) showed significantly higher BG level than those who were absent of treatments mentioned above($P < 0.05$). The level of GM in serum did not affected by treatments with corticosteroids and dairy products, but that was different in populations with treatment of blood products. Patients with treatment of blood products had higher level of GM than those without the use of blood product support($P < 0.05$).

Analysis of GM and BG in samples infection with bacterium showed that this factor had no significant effect on those two assays. No significant difference was found in BG levels between positive rates in Gram negative and positive groups ($P = 0.65$), nor in GM levels between these two groups ($P = 0.53$). The same results were obtained from antibiotics (meropenem and teicoplanin) samples. Those two factors (bacteremia and antibiotics) had no significant impact on the level of GM and BG.

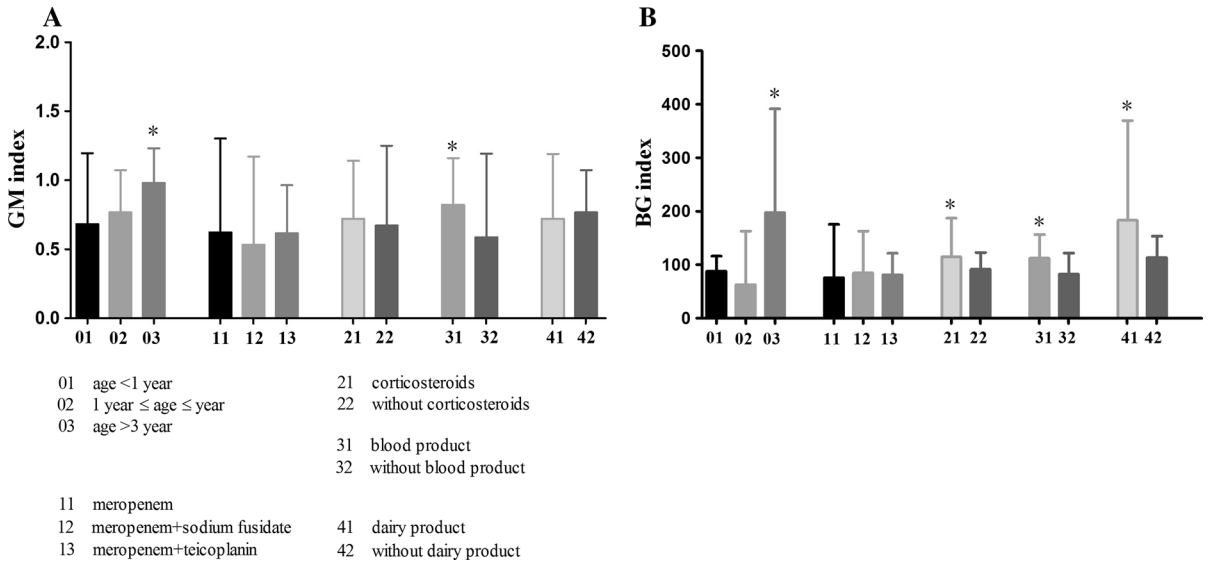

Fig. 1 Effect of various factors on the GM and BG assays. Data from all 117 patients were used to analyze GM (**a**) and BG (**b**) performance. Patients who aged over 3 years had higher levels of GM and BG in serum than younger infants (01, 02, 03), and BG levels were higher in subjects with antibiotics (11, 12, 13), corticosteroids (21), human blood products (31), and dairy therapy (41) than non-therapy with these treatment programs. Unlike BG assay, GM levels in serum seem to be less susceptible to above-mentioned factors expect intravenous therapy with blood production. Student's *t* tests were performed to determine statistical significance between samples ($*P < 0.05$)

The Role of GM and BG Assays in Diagnosis of IFI

In our cohort, 7 had proven cases of IFI, 23 had probable cases of IFI, and 87 had controls from PICU wards. Proven cases included 3 mold infections (2 with *Aspergillus fumigatus* and 1 with *Fusarium solani*) and 4 candida infections (3 with *C. albicans* and 1 with *C. glabrata*). Table 2 shows the characteristics of 7 patients with proven IFI. The distribution of BG and GM index according to the type of IFI diagnosis is described in Fig. 2. The BG levels and GM index in cases of IFI were dramatically higher than in controls. The median of the BG levels for IFI patients was 479.8 and 60.7 pg/ml for controls. The median of the GM levels for IFI patients was 1.2 and 0.67 for controls.

The diagnostic performance of these two tests is presented in Table 3. GM assay had better results when compared with BG assay in all studied variables. In particular, the GM assay was found with high sensitivity [90.0 % (95 % CI 73.5–97.9 %)] and NPV [91.2 % (95 % CI 76.3–98.1 %)] than those of the BG

Table 2 Characteristics of 7 patients with proven IFI

No.	Age (month)/sex	Underlying disease	Fungal smear	Blood culture	GM	BG (pg/ml)
1	48/M	Severe sepsis	*Aspergillus fumigatus*	*Acinetobacter baumannii*	1.87	114.80
2	4/F	Severe pneumonia, CHD	Negative	Fusarium solani	1.49	282.00
3	5/M	Surgery; severe pneumonia	*C. albicans*	*Pseudomonas aeruginosa*	0.97	33.91
4	4.5/M	Severe pneumonia, CHD	*C. albicans*	Negative	0.95	284.30
5	6/F	Diarrhea	*C. albicans*	Negative	0.01	1310.00
6	3/M	Severe pneumonia, CHD	Negative	*Aspergillus fumigatus* *Staphylococcus aureus*	1.11	221.50
7	5/F	Severe pneumonia	Negative	*C. glabrata*	0.39	50.00

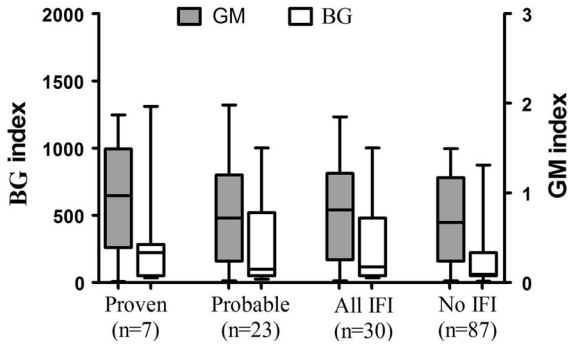

Fig. 2 EORTC/MSG criteria for IFI. Distribution of galactomannan index (GM, color bars) and (1 → 3) β-D-glucan levels (BG, *blank bars*) according to the type of IFI diagnosis. The *box* and *whiskers* plots display the median, 25th, and 75th percentile of the distribution (*box*), and whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the *box*. *Horizontal dashed lines* give the respective positivity thresholds

assay [53.3 % (95 % CI 34.4–71.6 %)] and [79.7 % (95 % CI 63.3–88.4 %)], respectively, for IFI diagnosis. Conversely, the specificity [35.6 % (95 % CI 25.7–46.6 %)] and PPV [32.5 % (95 % CI 22.7–43.7 %)] based on GM assay were lower than those on the BG assay (63.2 and 33.3 %, respectively). On the whole, NPV in both GM assay and BG assay was dramatically higher than other diagnostic test parameters, which indicated that the comparative advantage of BG assay and BG assay lied in excluding IFI.

The areas under the ROC curves (AUC) for GM and BG was 0.74 (95 % CI 0.65– 0.82) and 0.61 (95 % CI 0.52–0.70) (Fig. 3). The AUC of GM was higher than that of BG, but there was no statistical difference ($P = 0.08$).

Discussion

Since the EORTC/MSG definitions for all IFI and specifically invasion aspergillosis (IA) incorporated GM and BG positive results as mycological criteria supportive of probable diagnosis [4], both GM and BG assays have received the most attention in the medical literature. However, those two markers are mainly applied to hematological patients, neutropenia, or neutropenia with fever, particularly in those with hematological malignancies or after HSCT [5, 6]. In non-hematological patients, the diagnostic value of GM for IFI is controversial. Some reports suggested that GM assay had low diagnostic value for IFI in non-hematological adult patients [2]. However, some research showed the level of GM in serum and especially in bronchoalveolar lavage supported and improved the identification of Aspergillus species as a cause of pulmonary disease in ICU patients [9, 10]. In non-hematological and critically ill pediatric patients, the related research is scare. Only one prospective study showed that neonates with proven IFI were positive for BG assay, which indicated that BG assay could be regarded as an adjunct diagnostic test in the diagnosis of IFI [13]. Therefore, we designed this study to include all non-hematological and immunocompromised patients with non-neutropenia in PICU.

As we know, early diagnosis of IFI is challenging. Interest is increasing rapidly in use of surrogate markers, such as BG, GM, candida species-specific DNA, colonization index, and candida score as primary parameter in predicting the onset of IFI [7]. In an appropriate laboratory logistics, the results of BG and GM assays can be obtained more easily and rapidly than those of PCR techniques, which was the reason we focused on these two assays in study design. We noticed that the increasing evidence showed many factors could be related with false-positive results, including administration of human blood products (albumins and immunoglobulins) [5, 14], thrombocyte infusion, treatment with such antibiotics as piperacillin–tazobactam or amoxicillin–clavulanate [15, 16], presence of serious bacterial infections, use of surgical gauzes containing severe mucositis or glucan [17, 18].

Table 3 Performances of BG and GM in 117 patients with high risk of IFI

	SN (%) (95 % CI)	SP (%) (95 % CI)	PPV (%) (95 % CI)	NPV (%) (95 % CI)	PLR (%) (95 % CI)	NLR (%) (95 % CI)
BG	53.3 (34.4–71.6)	63.2 (52.2–73.3)	33.3 (20.4–48.4)	79.7 (68.3–88.4)	1.5 (0.9–2.2)	0.8 (0.5–1.2)
GM	90.0 (73.5–97.9)	35.6 (25.7–46.6)	32.5 (22.7–43.7)	91.2 (76.3–98.1)	1.4 (1.2–1.5)	0.3 (0.1–0.9)
BG/GM	46.7 (28.3–65.7)	52.1 (37.2–66.7)	37.8 (22.5–55.2)	61.0 (44.5–75.8)	0.9 (0.6–1.6)	1.1 (0.6–1.6)

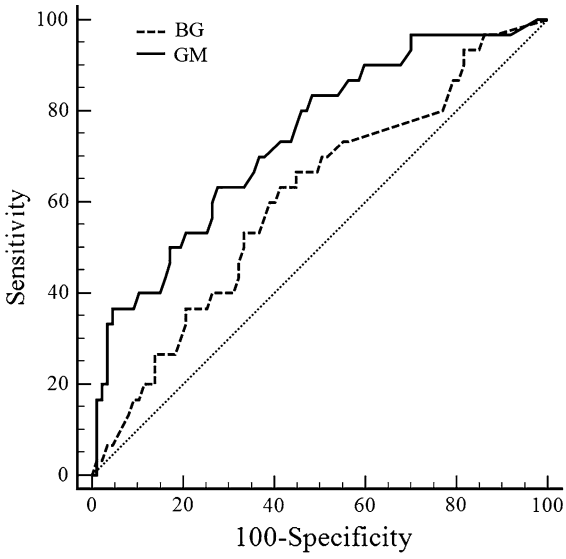

Fig. 3 ROC AUC curves of BG and GM assay for diagnosis of IFI. The AUC of GM was higher than that of BG, but there was no statistical difference ($P = 0.08$)

Recent data have suggested that false-positive results secondary to piperacillin–tazobactam are not specific to the antibiotic but rather the manufacturer of the antibiotic [19, 20] and other beta lactam antibiotics such as ampicillin–sulbactam as a cause of false-positive GM or BG assay [21]. Among these factors, we focused on age, diet, human blood products, antibiotics, and corticosteroids in our evaluation. Regarding the interacting effect of age, our results showed patients who aged over 3 years had higher levels of GM and BG in serum than younger infants, which disagreed with previous report that the mean BG values did not vary significantly by age stratum [22]. One possible reason for this inconsistency is that inclusion criteria are non-uniform in different cohort. Smith et al. [22] collected serum samples from immunocompetent and uninfected children who underwent venipuncture, while samples were collected from children who had suffered from infection disease in our cohort. Further analysis showed that the BG levels were higher in subjects with dairy, human blood products, antibiotics, and corticosteroids therapy than non-therapy with these treatment programs, which agreed with previous reports. Unlike BG assay, GM levels in serum seem to be less susceptible to above-mentioned factors expect intravenous therapy with blood production.

In our evaluation, patients included 30 subjects with IFI and 87 with no IFI. The BG level >100 pg/ml had sensitivity and specificity of 63.3 and 63.2 % for confirmed IFI, respectively. With respect to prior studies [7], the overall BG assay sensitivity and specificity were lower in our cohort. A major problem in performing a BG assay in children was lack of baseline levels for healthy children. Some study suggested that normal mean BG values in children were higher than those in adults tested (68 vs. 48 pg/ml for children and adults, respectively) [22], which raised questions about the cutoff values of BG in children. The low sensitivity and specificity in our cohort may be related with the high cutoff value of BG assay. In addition to the low frequency of cases of proven IFI, giving an evaluation of the prevalence of IFI in the target population allowed an assessment o f PPV and NPV by use of BG assay in our study. With a prevalence of fungal infection of 16.8 %, the PPV for the BG assay was 33.3 %, in spite of a very high NPV (approximately 80 %). Therefore, the comparative advantage of BG assay lies in excluding IFI according to earlier reported [23], while a challenge question about the BG assay remains variable factors that could enhance BG levels for some reasons other than IFI.

The other noninvasive diagnostic method we used in our study was the GM assay. We demonstrated that the sensitivity of serum GM was higher than that of BG assay. GM assay sensitivity for IFI has varied markedly among studies, from 30 to 100 % [19, 24, 25]. This variability in the assay has been attributed to several factors, including the site of infection, fungal localization, or angioinvasiveness [25]. The high sensitivity of the GM assay in our study may be related with some pediatric patients infected with *fumigatus*, which is similar to the results reported by Hachem et al. [15], while the overall sensitivity of GM assay was higher in patients with *A. fumigatus* than with non-*fumigatus Aspergillus* species groups. Furthermore, the sensitivity of detection for the antigen is notably higher for populations with disseminated aspergillosis than for those with pulmonary aspergillosis [26]. In our cohort, a considerable part of populations were suffered with disseminated and pulmonary fungal infection, which may be another possibility that contributed to the high sensitivity.

In addition, we found that a combination of BG and GM measurement in this setting failed to improve the sensitivity for the diagnostic performance, but it was

helpful for excluding IFI. Our study had several limitations. Firstly, we did not perform the GM or BG assay on samples of bronchoalveolar lavage fluid from the patients with invasive pulmonary aspergillosis. It is possible that these specimen assays may enhance the sensitivities of the diagnostic assays. Secondly, we did not evaluate values for the GM or BG assay among patients who responded to antifungal therapy than those among patients who failed antifungal therapy. Thirdly, samples were collected less frequently, which may have resulted in the lower detection level of the antigen.

To conclude, BG assay was more susceptible to many factors which could cause false-positive results than GM assay. These factors included age, diet, human blood products, antibiotics, and corticosteroids. Moreover, we considered the sensitivity of BG assay for the diagnosis of IFI was lower than that of GM assay. On the whole, the comparative advantage of BG assay and BG assay lied in excluding IFI in non-hematological pediatric patients.

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Compliance with Ethical Standards

Conflict of interest No conflict of interest exits in the submission of this manuscript.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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