Beta-d-Glucan Detection as a Diagnostic Test for Invasive Aspergillosis in Immunocompromised Critically Ill Patients with Symptoms of Respiratory Infection: an Autopsy-Based Study

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Beta-(1,3)-β-D-glucan (BG) detection is an emerging tool to diagnose invasive fungal infections (IFIs). Invasive aspergillosis (IA) is the second most common IFI in immunocompromised intensive care unit (ICU) patients. We retrospectively analyzed the serum BG concentration (Fungitell; Associates of Cape Cod) in immunocompromised ICU patients with proven IA and in immunocompromised ICU patients in whom autopsy failed to show IFI. The study was performed in a 17-bed medical ICU in a 1,900-bed referral hospital. Patients at risk for IA were eligible for inclusion when at least two additional clinical signs were present. Patients with other IFIs were excluded. Fourteen patients with IA and 33 patients who had no IFI were eligible for inclusion. Serum BG levels were significantly higher in patients with IA than patients without an IFI (P < 0.01). Using a cutoff of 140 pg/ml, the sensitivity and specificity were 85.7 and 69.7%, respectively; the positive and negative predictive values were 54.5 and 92.0%, respectively. The positive and negative likelihood ratios were 2.83 and 0.21, respectively. Although serum BG concentrations were higher in immunocompromised ICU patients with IA than in patients with the same risk factors who did not have IFI on autopsy, the moderate performance characteristics of this test limit its use as a diagnostic test for IA in this population.

The incidence of invasive fungal infections (IFIs) has markedly increased in recent decades. Despite development of new antifungal drugs, the mortality rate from IFIs remains high, particularly in intensive care unit (ICU) patients. Invasive aspergillosis (IA) is the second most common IFI in immunocompromised ICU patients. The prognosis of IA is poor; mortality rates up to 100% have been reported in mechanically ventilated chronic obstructive pulmonary disease patients with IA (2), and in nonneutropenic critically ill patients with IA, rates up to 89% were published (5). A retrospective study in our medical ICU showed an observed mortality rate of 80% in patients suffering from IA, compared to a predicted mortality rate of 48% (14).

An important determinant of the prognosis is the time lag between the onset of infection and the administration of antifungal drugs (3, 25). This phase can be long due to diagnostic difficulties. Classical methods such as culture and biopsy are invasive and time-consuming tools (9). Detection of fungal cell wall components is a rapid and attractive tool to diagnose IFIs. Beta-(1,3)-β-D-glucan (BG) is one of those components and is present in a wide variety of pathogenic fungi such as Candida spp., Fusarium spp., Aspergillus spp., and Pneumocystis spp. The cell wall of Zygomycetes does not contain BG; Cryptococcus spp. have a capsule that captures BG before it is released into the bloodstream (12). BG is also present in the fungal wall of Penicillium spp. and Paecilomyces spp., which can cause contamination of the sample.

Aspergillus spp. have galactomannan (GM) as another important cell wall component. GM is released into the bloodstream during intravascular hyphal growth. However, neutrophils seem to capture GM, resulting in a low serum concentration (4). Consequently, the sensitivity of GM detection in serum is significantly lower in nonneutropenic patients than in neutropenic patients. Compared to serum GM detection, detection of GM in bronchoalveolar lavage (BAL) fluid has an increased sensitivity in critically ill (13) and hematology (11) patients. However, this test is invasive, requiring bronchoscopy, which is not always feasible in critically ill patients. In this study, we focus on the role of BG detection in serum as a diagnostic tool for IA. In order to do so, we compared the serum BG level in patients with IA to the levels in patients without an IFI. We also investigated the role of combined BG detection and GM detection in BAL fluid.

MATERIALS AND METHODS

Study population. A previous study compared the diagnostic value of detection of GM in BAL fluid with the detection of GM in serum samples in immunocompromised ICU patients at risk for IA. From July 2005 to December 2006, immunocompromised ICU patients with two additional clinical signs of fungal infection, such as fever, pulmonary infiltrate, or clinical respiratory symptoms, were included in this study. The study was performed in a 17-bed closed medical ICU. Host factors used as inclusion criteria in the original study were the following: (i) a hematologic malignancy, unless the patient had already been treated with antifungals for a presumed or proven IA; (ii) cancer and receipt of

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chemotherapy within the last 3 months before admission; (iii) receipt of a solid organ transplant; (iv) steroid use consisting of at least 4 mg methylprednisolone (or equivalent) a day for at least 7 days in the 3 weeks before admission or during the course of the ICU stay for at least 5 days or a cumulative dose of at least 250 mg of methylprednisolone (or equivalent) in the 3 months before enrollment; (v) receipt of any other immunosuppressive treatment (tacrolimus, cyclosporine, methotrexate, cyclophosphamide, sirolimus); (vi) child class C cirrhosis; and (vii) HIV infection. The patients had to have at least two of the three following clinical features: (i) fever refractory to at least 3 days of appropriate antibiotics or fever relapse after a period of defervescence of at least 48 h while still receiving antibiotics; (ii) clinical signs and/or symptoms suggestive of invasive mycosis, such as pleuritic chest pain, physical finding of pleural rub, or one of the symptoms of lower respiratory tract infection (new sputum secretions, dyspnea, or hemoptysis); and (iii) development of new pulmonary infiltrates on chest X-ray (13). On the basis of the study protocol, bronchoscopy with BAL was done immediately after inclusion, and further analysis to detect GM in the BAL fluid was performed. The 110 included patients were categorized as having possible, probable, or proven IA following the criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG) (1). Of the patients included in the original study, we selected the patients with proven IA according to revised EORTC/MSG criteria (6) and the patients in whom autopsy failed to show an IFI. Patients with other IFIs, such as invasive zygomycosis, Pneumocystis jirovecii pneumonia, and invasive candidiasis, were excluded. Patients could be included if a serum sample was available on the day of, the day after, or the day before the GM BAL test was performed. BG detection was retrospectively done on the stored serum sample taken on the day of the first GM BAL or, if that was not available, the stored serum sample taken on the day after the first GM BAL. If this sample was not available, the serum sample taken on the day before the first GM BAL was analyzed. At the moment of sampling, none of the patients was receiving anti-fungal therapy other than fluconazole. One serum sample per patient was analyzed. The serum samples were stored at −20°C (data on file at Associates of Cape Cod [East Falmouth, MA]) show that BG is stable at −20°C. BAL fluid GM analyses were previously performed with the Platelia enzyme immunoassay (EIA; Platelia Aspergillus; Bio-Rad, Berkeley, CA). The study was approved by the Institutional Review Board of University Hospitals Leuven, and informed consents were obtained for all patients. We collected additional information according to the protocol provided by the manufacturer using an Ascent Multiskan Glucan assay. The BG concentration was determined with the Fungitell test (Associates of Cape Cod, East Falmouth, MA). All samples were tested according to the protocol provided by the manufacturer using an Ascent Multiskan instrument (Thermo Fisher Scientific, Waltham, MA). The tests were performed in duplicate in the same run following the instructions. Interference was avoided by the use of glucan-free materials. The mean of the two results for the same sample was calculated. The analysis was repeated when the coefficient of variation was greater than 100% with a mean BG concentration of 211.16 pg/ml. The coefficient of variation was more than 20% for BG concentrations below 200 pg/ml; for BG concentrations above 200 pg/ml, the analysis was repeated when the coefficient of variation was more than 100%. The analysis was repeated in one patient because the coefficient of variation was greater than 100% with a mean BG concentration of 378.20 pg/ml.

**Statistical analysis.** Data are presented as medians and interquartile ranges (IQRs) for variables that are nonnormally distributed. \( P \) values were calculated with the Mann-Whitney U test. Subanalyses were done in hematology and nonhematology patients. Hematology patients were defined as patients with a hematological malignancy, severe myelodysplastic syndrome, or stem cell transplantation. We investigated the impact of *Candida* colonization, bacteremia, and antibiotic treatment with amoxicillin and/or piperacillin-tazobactam on the serum BG concentration in the patients without IFI.

**RESULTS.** Of the 110 patients included in the original study, 47 were eligible for inclusion. Fourteen patients with IA and 33 patients with no IFI were included. Three patients with *Pneumocystis jirovecii* pneumonia, two patients with invasive candidiasis, and two patients with invasive zygomycosis were excluded. The overall patient characteristics are summarized in Table 1, and the test properties for the different cutoff levels are shown in Table 2.

**BG levels in ICU patients with IA.** In our study population and with the cutoff proposed by the manufacturer (80 pg/ml), the sensitivity and specificity to detect IA were 85.7% (95% confidence interval [CI], 60.1 to 96.0%) and 36.4% (95% CI, 22.2 to 53.4%), respectively. Increasing the cutoff value to 140 pg/ml did not affect sensitivity but improved specificity to 69.7% (95% CI, 52.7 to 82.6%). The area under the receiver operating curve was 0.76 (Fig. 1). GM detection in BAL fluid had a 92.9% sensitivity and an 81.8% specificity to detect IA in the studied group.

All patients with proven IA and a positive BG serum sample had a positive BAL fluid GM test. When positivity was defined as a combination of a positive serum BG test and a positive BAL fluid GM test, the sensitivity was 85.7% (95% CI, 60.1 to 96.0%), with specificity rising to 87.9% (95% CI, 72.7 to 95.2%).

Subanalyses were performed in hematology (n = 20) and nonhematology (n = 27) patients. With a cutoff set at 140 pg/ml, the sensitivity was 72.7% (95% CI, 43.4 to 90.3%) and the specificity was 77.8% (95% CI, 45.3 to 93.7%) in the hematologic patients. In the nonhematologic patients, the cutoff value of 140 pg/ml showed a sensitivity of 100.0% (95% CI, 100.0 to 100.0%) and a specificity of 69.6% (95% CI, 49.1 to 84.8%).

**BG levels in patients without IFIs.** There were four patients with bacteremia (two patients with *Staphylococcus epidermidis*, one patient with *Enterococcus faecalis*, and one with *Streptococcus pneumoniae* bacteremia). The BG level was, although not statistically significant, higher in patients with bacteremia (median, 191.8 pg/ml; IQR, 69.5 to 303.9 pg/ml) than in those who did not have bacteremia (n = 29; median, 89.8 pg/ml; IQR, 50.59 to 144.2 pg/ml) (P = 0.62). In two patients with bacteremia (1 patient with *S. epidermidis* and 1 patient with *E. faecalis* bacteremia), the BG concentration was higher than 140 pg/ml, resulting in a false-positive test when a cutoff of 140 pg/ml was used.

There was no difference in the BG level in serum samples of patients colonized with *Candida* yeasts (n = 14; median, 89.5 pg/ml; IQR, 55.3 to 153.9 pg/ml) than those not colonized (n = 19; median, 99.7 pg/ml; IQR, 49.65 to 255.7 pg/ml) (P = 1.00).

There were no patients without an IFI who received amoxicillin. In the six patients who received piperacillin-tazobactam, the BG level (median, 72.7 pg/ml; IQR, 49.3 to 1,704.9 pg/ml) did not differ from the BG level in patients who did not receive this antibiotic (median, 99.7 pg/ml; IQR, 51.8 to 150.7 pg/ml) (P = 0.96).

**DISCUSSION.** In this retrospective study, we examined whether determination of the serum BG level is a useful diagnostic test to detect IA. The serum BG level was higher in patients with IA than in patients without IFI. Our data are comparable to the data of Presterl et al., who investigated the BG concentration in long-term-intensive-care patients (23), and can explain the association between elevated serum BG levels and death in ICU patients with suspected ventilator-associated pneumonia (8).
At a 140-pg/ml cutoff, the sensitivities were 85.7%, 100.0%, and 72.7% in the overall groups of ICU patients, the nonhematologic ICU patients, and the hematologic ICU patients, respectively. The specificities in those groups were 69.6%, 69.6%, and 77.8%, respectively.

As far as we know, this is the first autopsy-based study evaluating serum BG in critically ill patients at risk for IA. This is important because biopsy and/or autopsy the “gold standard” used to diagnose IA. A previous autopsy-based hospital-wide study in a cancer center was done by Obayashi et al., who examined 54 autopsy-proven IFI cases and 402 cases without evidence for IFI on postmortem examination (18). They found a sensitivity of 95.1% with a specificity of 85.7% using the Fungitec GTest MK.

In our population, we used a cutoff of 140 pg/ml, which is considerably higher than the 80 pg/ml that is suggested by the manufacturer. Odabasi et al. used 60 pg/ml as a cutoff and found a sensitivity and specificity of 100 and 90%, respectively, to detect IFI in patients with acute myeloid leukemia and myelodysplastic syndrome. Twenty patients had proven or probable IFI, and only four of them had evidence of IA (19). Our data contrast highly with the results of Ostrosky-Zeichner et al., who investigated the serum BG concentration in a hospital-wide multicenter evaluation. They found a sensitivity of 70% and a specificity of 87% to detect IFI at a cutoff of 60 pg/ml (20). If this cutoff is used in our study population, the sensitivity was higher (85.7%) and the specificity was markedly lower (36.4%).

ICU patients seem to have more false-positive results at lower cutoffs than patients who are not critically ill. A recent study showed a 25% rate of false positivity during the first 3 days of ICU admission (17). The origin of the false-positive results is not completely known. We investigated whether colonizion with Candida spp. and bacterial bloodstream infec-
tions as well as the administration of beta-lactam antibiotics are associated with positive BG levels. In patients colonized with Candida spp., the BG levels were not higher than those in patients who were not colonized, confirming previous reports (10, 19, 21).

Some reports suggest that bacterial bloodstream infections can cause elevated serum BG levels (22, 23). Digby et al. found no difference in the serum glucan level of critically ill patients with IFI and patients with bacterial infections (7). In our study, there were four patients without IFI whose blood cultures were positive, and all of the cultures showed Gram-positive bacteria. Due to the small sample size, we cannot draw any conclusion concerning this topic.

There are some reports describing higher fungal antigen levels in serum samples after administration of beta-lactam antibiotics. The administration of beta-lactam antibiotics such as amoxicillin-clavulanic acid and piperacillin-tazobactam is a known cause of falsely elevated serum and BAL fluid GM levels (13, 24). Mennink-Kersten et al. found higher serum BG levels in patients treated with amoxicillin-clavulanic acid and a high concentration of BG in the antibiotic batches themselves. They found no glucan reactivity in tested batches of piperacillin-tazobactam (15). We found no association between the serum BG level and piperacillin-tazobactam administration in the six patients who were treated with piperacillin-tazobactam. However, the number of patients is not sufficient to be able to draw any conclusion. There was no association between the serum BG level and the administration of piperacillin-tazobactam in a previous study in patients without risk factors for IFI (16).

The sensitivity to detect IA was 85.7% in the overall ICU population. Recently, Meersseman et al. showed that detection of GM in BAL fluid has a higher sensitivity than detection of GM in serum. They found a sensitivity of 88% in a medical
In our study population, a subgroup of the study by Meersseman et al., the sensitivity to detect IA with the GM BAL fluid test was 92.9%. Compared to detection of GM in BAL fluid, the combination of a positive serum BG result and detection of GM in BAL fluid does improve the specificity from 81.8 to 89.5%, but the sensitivity decreases from 92.9 to 85.7%. As all patients with IA and a positive BG test result were positive for detection of GM in BAL fluid, the combined use of positive serum BG and BAL fluid GM results did not improve the sensitivity compared to that of a positive BAL fluid GM result alone. A positive BG test cannot be an indicator to start preemptive therapy, especially since the treatment strategies for other IFIs that cause elevated BG levels are different. Another reason not to start antifungal therapy on the basis of BG concentrations is the low positive predictive value of the test.

Although the BG serum level is higher in patients suffering from IA than in patients with no IFI, the properties of detection of the BG serum level are insufficient to use the test as a diagnostic tool for IA. In the predefined population, the prevalence of IFI is 28.6%. With this prevalence, a positive BG serum test increases the probability to 46.6%; a negative test decreases the probability to 8.8%. These results indicate the constraints of a positive BG test and that further investigation of this test is necessary. The detection of the BG serum level itself is expensive and requires additional investigations to differentiate the type of IFI, further increasing cost.

We recognize that our study has limitations. First of all, the study was performed in a medical ICU. Consequently, the results cannot be applied to surgical ICU patients. Second, we analyzed only one serum sample from each patient. Previous reports suggest that the specificity increases when two or three sequential positive serum samples were required for a true-positive test result (19, 21). Further investigations are necessary to define the role of serial sampling in critically ill patients. Finally, since the study is autopsy based, we cannot exclude a bias due to inclusion of patients with infections more severe than those in the patients who survived and thus were not included.

In conclusion, the serum BG level is significantly higher in critically ill patients suffering from IA than in patients with the same risk factors but with no arguments for IFI on autopsy. At the cutoff of 140 pg/ml, the sensitivity to detect IA was 85.7%; the negative predictive value was 69.7%; the positive predictive value of the test.

REFERENCES