

## NOTES

### Quantification of 1,3- $\beta$ -D-Glucan Levels in Children: Preliminary Data for Diagnostic Use of the $\beta$ -Glucan Assay in a Pediatric Setting<sup>∇</sup>

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**1,3- $\beta$ -D-Glucan serum levels have demonstrated good diagnostic sensitivity and specificity for the diagnosis of candidiasis in adult patients, but normal levels for children have not been established. We found higher 1,3- $\beta$ -D-glucan levels in children than those previously reported in adults.**

Accurate and rapid diagnosis of invasive candidiasis is critical because *Candida* species are the fourth-most-commonly isolated organisms in bloodstream infections in hospitalized patients and are associated with substantial morbidity and mortality across a wide range of patient populations (1, 4, 6). The current gold standard for diagnosis of invasive *Candida* infections is isolation of the organism in culture from normally sterile body fluids. However, the sensitivity of blood cultures for diagnosing invasive candidiasis has been shown to be <30% in some clinical situations in adult patients (2). The poor sensitivity of blood culture may be exacerbated in children, from whom much less blood can be collected for culture.

1,3- $\beta$ -D-Glucan ( $\beta$ -glucan) is a cell wall component found in several fungal pathogens, including *Candida* and *Aspergillus*, and can be detected through its ability to activate factor G in the coagulation cascade of the horseshoe crab (9). Two commercial kits are available for the detection of  $\beta$ -glucan, Fungitec G-test (Seikagaku Corporation, Tokyo, Japan) and Fungitell (Associates of Cape Cod, Inc., Falmouth, MA) (8).  $\beta$ -Glucan is present in small amounts in the serum of healthy adults, and knowledge of this level in uninfected patients is needed before testing this new assay in the setting of potential invasive fungal infection (8).

The quantification of  $\beta$ -glucan levels in uninfected and infected adult patients has been performed (10). Baseline  $\beta$ -glucan levels in uninfected pediatric patients are unknown, and therefore, this novel diagnostic test is unusable for children until these critical data are determined. In this study, we evaluated  $\beta$ -glucan levels in children specifically not at risk for invasive fungal infection, using the Fungitell assay, in order to

establish the necessary foundation for future randomized clinical trials of the assay with at-risk and infected children.

We collected serum samples from children who underwent venipuncture at Duke University Medical Center for routine clinical care. Subjects were excluded if they were immunosuppressed in any fashion, including patients with chronic renal failure or diabetes or patients receiving systemic immunosuppressive medications (steroids, chemotherapy, or immunosuppressants). In addition, patients were excluded if they were intubated, had central venous catheters in place, or had undergone recent surgery. We purposely used a broad definition of “immunosuppressed” to best guarantee that only immunocompetent and uninfected children were tested. The samples were obtained with the approval of the Duke University Medical Center Institutional Review Board.

The Fungitell assay was performed according to the manufacturer’s instructions. Previously determined reference values for the assay in adult patients are as follows: negative, <60 pg/ml; indeterminate, 60 to 79 pg/ml; and positive,  $\geq$ 80 pg/ml. The data were analyzed with STATA 8.2 (College Station, TX). Median values and interquartile ranges were calculated for this cohort. We used nonparametric testing with either Kruskal-Wallis or Wilcoxon signed-rank sum in order to calculate two-tailed *P* values.

We determined the  $\beta$ -glucan levels from 120 pediatric patients (Table 1). The median age was 9.2 years (range, 7 months to 8 years). The median  $\beta$ -glucan level was 32 pg/ml, and the mean value was 68 ( $\pm$ 128) pg/ml. The mean values did not vary significantly by age stratum or gender. The five highest observations were 348 pg/ml (12-year-old female), 374 pg/ml (2-year-old female), 491 pg/ml (14-year-old male), 754 pg/ml (13-year-old female), and 947 pg/ml (14-year-old male). Ninety-four (78%) of the patients had  $\beta$ -glucan levels of <60 pg/ml, 8 (7%) had levels of 60 to 79 pg/ml, and 18 (15%) had levels of  $\geq$ 80 pg/ml.

The observed mean  $\beta$ -glucan level in our cohort was higher than the levels previously reported in healthy adult patients (68

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TABLE 1.  $\beta$ -Glucan levels in nonimmunocompromised children

Patient characteristic	No. of patients	Mean $\beta$ -glucan level (pg/ml)	P value
Age (yr)			0.51
0.5–3	25	53	
4–9	33	47	
10–13	21	85	
14–18	21	85	
Gender			0.71
Male	60	64	
Female	60	72	
Total	120	68	

pg/ml versus 48 pg/ml) (10) but lower than the levels of all 15 adult candidemic patients in a previous study (11). The mean  $\beta$ -glucan level measured in two previous reports of 137 candidemic adult patients with the Fungitell assay was 1,246 pg/ml (8, 10). The specificities of the assay in this pediatric population were 79% and 85% using 60 pg/ml and 80 pg/ml as cutoff values. In adult patients, the Fungitell assay demonstrated 87% and 92% specificities using 60 pg/ml and 80 pg/ml as cutoff values (10).

Our elevated mean value may have resulted from several false-positive results. False-positive results have been observed for bacteremic patients (3, 11), patients receiving hemodialysis with membranes containing cellulose (5), patients receiving coagulation factors and albumin (12), hemolyzed serum specimens (5), and specimens contaminated from gauze (7). Neonates were purposely excluded from this analysis because of concern for contamination during heel-stick sampling, a common methodology for obtaining venous blood in that specific age population.

As the number of immunocompromised pediatric patients at risk for candidiasis grows, there is an increasing need for improved methods for detection. Previous work has shown  $\beta$ -glucan to be of potential benefit in immunocompromised adult patients, but there has been no testing reported for children.  $\beta$ -Glucan levels should be evaluated prospectively in a randomized clinical trial with pediatric patients at high risk for invasive candidiasis in order to determine the value of the assay as a diagnostic test for this patient population. However, our data suggest that the upper limit of normal for serum  $\beta$ -glucan levels may in fact be higher in pediatric patients than

in adults. This is critical information to assess before embarking on a clinical trial with infected children, because the cutoff value for children must be determined before widespread clinical use of this novel diagnostic surrogate in pediatric patients.

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