Assessment of Serum (1→3)-β-D-Glucan as a Measure of Disease Burden in a
Murine Model of Invasive Pulmonary Aspergillosis

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ABSTRACT

Serum (1→3)-β-D-glucan concentrations were serially measured in the presence and absence of antifungal therapy in a murine model of invasive pulmonary aspergillosis. Serum (1→3)-β-D-glucan was detected early during the course of infection, and reductions in this biomarker were associated with improved survival in animals treated with antifungal agents.
Early diagnosis and initiation of antifungal therapy have been demonstrated to improve outcomes in invasive aspergillosis (2, 3). Serial screening of serum for galactomannan and computed tomography have been shown to result in the earlier initiation of antifungal therapy in patients with invasive aspergillosis while avoiding unnecessary empiric antifungal use (5). However, the sensitivity of galactomannan is reduced in patients receiving antifungal agents (6). An assay for serum (1→3)-β-D-glucan is available and has been shown to be a useful diagnostic marker for invasive fungal infections (7, 8). However, there are few data with this assay in the diagnosis of invasive aspergillosis and its performance in the setting of drug therapy.

The objectives of our study were to measure the serum concentrations of (1→3)-β-D-glucan over time following pulmonary inoculation in an animal model of invasive pulmonary aspergillosis, and to examine the utility of this assay as a biomarker of disease burden in the presence of antifungal therapy. To achieve these objectives outbred ICR mice (Harlan), weighing between 18 - 22 grams, were immunosuppressed by cortisone acetate and cyclophosphamide and inoculated via an inhalation chamber on day 0 with either Aspergillus fumigatus clinical isolate AF 293 or CEA10 as previously described (9). To assess changes in serum (1→3)-β-D-glucan concentrations over time, mice were randomly chosen on days 1, 3, 5, and 7 post-inoculation, euthanized, and blood collected by cardiac puncture. The effects of antifungal therapy on serum (1→3)-β-D-glucan concentrations were measured in mice randomly assigned to one of four treatment regimens beginning one day after inoculation: 1) control, 2) amphotericin B deoxycholate 3 mg/kg IP QD, 3) posaconazole 40 mg/kg PO QD, or 4) liposomal amphotericin B 10 mg/kg IV QD. In the survival arm, therapy was continued until day 7 post-inoculation.
and animals were monitored off therapy until day 12. For fungal burden, therapy was continued until day 4. Mice were euthanized on day 5 and blood and lungs collected.

Serum (1→3)-β-D-glucan concentrations were measured using a commercially available kit (Fungitell, Associates of Cape Cod). Five microliters of each sample was transferred in duplicate to a 96 well cell culture tray and processed according to the manufacturer’s instructions (4). The mean rate of change in O.D. at 405 nm for each sample was read over 40 minutes with a microplate spectrophotometer (Synergy HT, Biotek Instruments, Inc., Winooski, VT) and unknowns interpolated from a standard curve. To measure pulmonary fungal burden lungs were homogenized in sterile saline and DNA was extracted using a commercially available kit (QIAamp DNA Mini Kit, Qiagen, Valencia, CA). Fungal DNA was measured by a real-time PCR assay using a probe and primers specific for the *A. fumigatus* *FKS* gene (GeneBank Accession No. U79728) and reported as conidial equivalents per gram of lung tissue (10). Each study was conducted in duplicate on separate occasions to ensure reproducibility. Survival was plotted by Kaplan-Meier analysis, and differences in percent survival were analyzed by the chi-square test. Differences in serum (1→3)-β-D-glucan and conidial equivalents were assessed for significance using the Kruskal-Wallis test with Dunn’s test for multiple comparisons. The Spearman rank correlation test was used for correlations between serum (1→3)-β-D-glucan and conidial equivalents.

Serum (1→3)-β-D-glucan was detected early during the course of infection, with significant increases in concentrations by day 3 post-inoculation (218 pg/mL, range 49.2 – 609 pg/mL) compared to the thresholds of 60 pg/mL and 80 pg/mL set by the manufacturer as the cutoffs for negative and positive results in humans, respectively, (4)
and negative controls (37.6 pg/mL, 0 – 68.3 pg/mL, p < 0.01) (Figure 1). Increases in this biomarker occurred 2 days prior to the first observed death in infected mice. (1→3)-β-D-glucan concentrations remained elevated on days 5 (635 pg/mL, 142 – 1375 pg/mL) and 7 (442 pg/mL, 225 – 510 pg/mL) post-inoculation.

Decreases in serum (1→3)-β-D-glucan were associated with improved survival in animals treated with antifungal agents. Posaconazole resulted in 100% survival and significant reductions in serum β-1,3-glucan concentrations in mice infected with AF 293 (28.5 pg/mL) or CEA 10 (0 pg/mL) compared to infected controls (758 pg/mL and 707 pg/mL, respectively; p < 0.01) (Figure 2). Although both amphotericin B formulations resulted in improved survival against both isolates (Figure 2 A and C), only liposomal amphotericin B significant reduced serum (1→3)-β-D-glucan concentrations (Figure 2 B and D), and neither formulation resulted in median concentrations below 127 pg/mL. Posaconazole also therapy resulted in significant reductions in conidial equivalents compared to controls against both isolates (Table 1). However, only liposomal amphotericin B treatment led to a reduction in pulmonary fungal burden and only against AF 293. Although good correlation was found between (1→3)-β-D-glucan and conidial equivalents for both isolates (AF 293 Spearman rho 0.81, p < 0.0001; CEA10 Spearman rho 0.48, p = 0.0019), no treatment regimen completely sterilized lungs when measured by conidial equivalents. This discrepancy between (1→3)-β-D-glucan serum concentrations and residual lung tissue fungal burden may possibly reflect colonization within the upper airways following pulmonary inoculation as previously described (1).

While the results of this study are promising, there are limitations that must be considered. Although clinical studies have shown this to be a useful diagnostic marker
for invasive fungal infections with a high negative predictive value (7, 8), it is not specific for *Aspergillus* species as (1→3)-β-D-glucan is a component of the cell wall of a number of pathogenic fungi. In addition, one study reported false positive reactions in patients with Gram-positive or Gram-negative bacteremia (8). Several substances can also result in false positives, including serum exposure to gauze, as well as cellulose filters used in patients undergoing hemodialysis (4). In addition, we did not evaluate the effects of echinocandins on serum (1→3)-β-D-glucan concentrations.

These data suggest the potential use of the serum (1→3)-β-D-glucan assay for screening and early diagnosis of invasive aspergillosis. Furthermore, the results from our model also suggest that this assay may be useful for monitoring treatment efficacy. Further clinical studies are warranted to confirm our results.

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TRANSPARENCY DECLARATIONS

RB, DM, MO, ACV, WRK – None to declare

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REFERENCES


FIGURE AND TABLE LEGEND

**Figure 1.** Change in serum (1→3)-β-D-glucan concentrations over time in mice following pulmonary inoculation with *A. fumigatus* clinical isolate AF 293. ‡ p < 0.01 vs. negative controls and infected mice 1 hour after inoculation (1 hr SAC) and on day 1 post-inoculation. N ≥ 5 per time point.

**Figure 2.** Survival (A and C) and serum (1→3)-β-D-glucan concentrations (B and C) in mice treated with antifungal agents following pulmonary inoculation with *A. fumigatus* clinical isolate AF 293 (A and B) and CEA 10 (C and D). Treatment groups consisted of infected controls (■), amphotericin B deoxycholate (AMB, ▼) 3 mg/kg IP QD, posaconazole (POS, ●) 40 mg/kg PO QD, liposomal amphotericin B (LAMB, ◆) 10 mg/kg IV QD, and uninfected controls (Neg. control). ‡ p < 0.01 vs. infected controls, AMB, and LAMB. * p ≤ 0.01 vs. infected controls. N = 10 per treatment group and infected controls.

**Table 1.** Pulmonary fungal burden as measured by quantitative real-time PCR and reported as conidial equivalents (CE).
Table 1.

<table>
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<th>Treatment Group</th>
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