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Paradoxical Increase in Circulating *Aspergillus* Antigen during Treatment with Caspofungin in a Patient with Pulmonary Aspergillosis

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A paradoxical increase in circulating *Aspergillus* antigen was observed during treatment with caspofungin in a patient with proven invasive aspergillosis. With the exception of treatment with the echinocandin, no other factors were found that might explain this clinical observation, which was supported by experiments done in vitro.

Case report. A 29-year-old man in remission of myelodysplastic syndrome was referred to our hospital to receive a T cell–depleted hematopoietic stem cell transplant (HSCT) from an unrelated donor. The patient became neutropenic after administration of cyclophosphamide, antithymocyte globulin, and total body irradiation; developed fever 12 days before transplantation; and was treated with meropenem. Blood cultures yielded *Staphylococcus epidermidis*, and teicoplanin was added to the treatment regimen. Plasma samples were tested twice weekly for circulating galactomannan (Platelia *Aspergillus* EIA; BioRad). Although the EIA also detects molecules other than galactomannan that bear the reactive epitope released by *Aspergillus* species, the term “galactomannan” is used in this report. An increase in the galactomannan index from 0.6 to 2.6 was noted 1 day before transplantation. High-resolution CT (HRCT) also revealed diffuse infiltrates in both lungs, with a halo sign in the upper left lobe. There were no pulmonary symptoms, although there was dull percussion of the left upper lung. These findings met the definition of probable invasive

aspergillosis, for which our protocol prescribed voriconazole. However, because the patient had not tolerated treatment with this azole for possible aspergillosis during an earlier admission to another hospital, intravenous treatment with caspofungin (70 mg on the first day, followed by 50 mg once daily) was started the day of the HSCT. Cyclosporin A treatment was also started to prevent graft-versus-host-disease, according the standard protocol. After 3 and 6 days of caspofungin treatment, the galactomannan index increased to 17.4 and 32.6, respectively; after the sixth day, the index decreased (figure 1). During this period, there was no evidence of clinical deterioration, blood cultures yielded only *S. epidermidis*, and follow-up HRCT performed 1 week after the start of caspofungin treatment showed slight progression of the infiltrate in the left upper lobe. After 2 weeks of caspofungin therapy, kidney and liver function remained normal, but a second HRCT revealed further progression of the lesion in the left lung, despite a galactomannan index of 4.6. The patient developed respiratory insufficiency and multiorgan failure on day 24 of caspofungin treatment, coinciding with granulocyte recovery, and was transferred to the intensive care unit. Antifungal treatment was then changed to liposomal amphotericin B (200 mg iv once daily), but the patient's condition deteriorated. He died 6 days later, 30 days after the allogeneic HSCT. The galactomannan index at death was 5.7. Evaluation of tissue of the upper lobe of the left lung obtained at autopsy revealed invasion by septate hyphae, and cultures yielded *Aspergillus fumigatus*. The minimal effective concentration (MEC) of caspofungin was 0.125 mg/L, and the MIC of voriconazole was 0.5 mg/L. The MEC was determined as described previously [1]. The MIC was determined using the microdilution technique according to the CLSI (formerly known as the NCCLS) M38-A guidelines [2].

The ability of caspofungin and voriconazole to enhance the release of galactomannan by the *A. fumigatus* strain isolated from tissue was investigated using an in vitro model in which the release of galactomannan was correlated with fungal biomass. Six growth experiments, each using a single concentration of caspofungin (MEC, 0.125 mg/L; final concentrations, 0.625 mg/L [5 × MEC], 1.25 mg/L [10 × MEC], and 2.5 mg/L [20 × MEC]) and voriconazole (MIC, 0.50 mg/L; final concentrations, 2.5 mg/L [5 × MIC], 5 mg/L [10 × MIC], and 10 mg/L [20 × MIC]), were conducted. The growth experiments included 6 incubation times (0, 16, 24, 40, 48, and 72 h). A single growth experiment was conducted without the addition of an antifungal agent and served as a control. Each culture that represented a time point consisted of 200 mL of liquid medium

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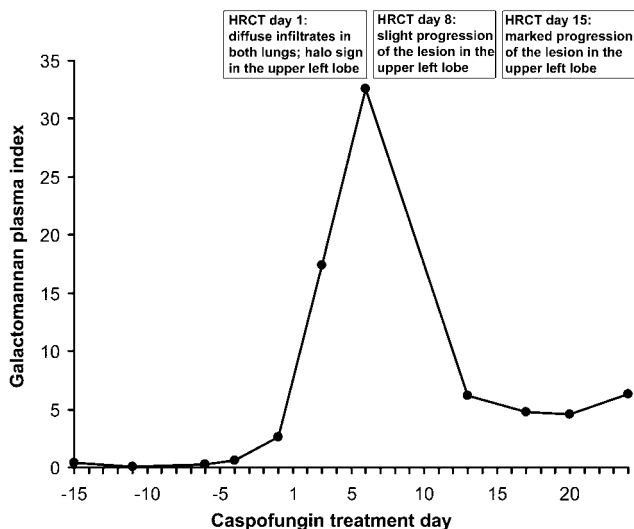


Figure 1. Galactomannan indices measured in the patient. Day 1 was the first day of caspofungin treatment and the day of transplantation. Treatment was changed to liposomal amphotericin B on day 24. HRCT, high-resolution CT.

(yeast nitrogen base, 100 mmol/L glucose, buffered with 3-[N-morpholino]propanesulfonic acid [MOPS]; pH, 7.4) that was inoculated with sufficient *A. fumigatus* conidia to yield a final concentration of 10^4 conidia/mL. The cultures were incubated at 37°C and shaken at 160 rpm. Each drug was added to the cultures after 16 h of incubation, resulting in the concentrations described above. At each time point, a culture was filtered (\emptyset pore 0.8 μ m), and the fungal biomass was determined by freeze-drying. Galactomannan in the culture filtrate was measured using the EIA (figure 2). A calibration curve was prepared 6 times with purified galactomannan (0–16 ng/mL; BioRad) to determine the antigen quantitatively. Reactivity was plotted against the fungal biomass for each concentration of antifungal agent. Subsequently, regression lines were calculated during the logarithmic growth phase, and the EIA reactivity was expressed as micrograms of galactomannan per milligram of dry weight.

When the *A. fumigatus* strain was exposed to caspofungin, the release of antigen in the culture supernatant increased from 8.4 (control value without caspofungin) to 19.2, 56.0, and 110.6 micrograms of galactomannan per milligram of dry weight at concentrations of 5 \times MEC, 10 \times MEC, and 20 \times MEC, respectively. There was no increase detected when the isolate was exposed to the various concentrations of voriconazole.

Discussion. The concentration of circulating galactomannan has been shown to correspond with clinical outcome in patients treated with amphotericin B or mold-active antifungal azoles, with decreasing levels in patients who respond to therapy and stable or increasing levels in patients for whom therapy failed [3]. This correlation is less clear for patients treated with

the echinocandin caspofungin. In the case presented, the level of circulating galactomannan did not correspond with a clinical response to caspofungin therapy. In fact, a paradoxical increase in circulating galactomannan was observed with a peak galactomannan index of 32.6. This is significantly higher than the indices observed among 22 patients with proven and probable invasive aspergillosis in our hospital who had been treated with drugs other than caspofungin for proven and probable invasive aspergillosis (mean \pm SD, 3.4 \pm 3.9; range 0.3–15.5; $P = .03$).

Our clinical observation was supported by in vitro experiments in which exposure of the patient's *A. fumigatus* strain to caspofungin, at concentrations comparable to those that can be achieved during therapy [4, 5], resulted in an increased release of galactomannan per milligram of biomass. This phenomenon was not observed when the strain was exposed to voriconazole. In experimental models of invasive aspergillosis using neutropenic rabbits, rats, and mice, persistently high levels of circulating galactomannan were measured during caspofungin therapy despite clinical improvement [6–8], although this was not observed among patients with proven invasive aspergillosis who received salvage therapy with the drug [9].

Drugs that have been shown to cross react with the EIA (e.g., piperacillin-tazobactam and amoxicillin-clavulanate) were not administered to this patient [10]. In addition, the *S. epidermidis* isolated from blood cultures has not been shown to cross-react with the EIA test for galactomannan [11]. The initial increase in circulating galactomannan did not correspond with clinical deterioration, nor did it coincide with important changes in kidney or liver function, leaving treatment with caspofungin as the most likely explanation for the initial increase in circulating galactomannan. The concomitant initiation of cyclosporin A therapy could have had an effect on the release of

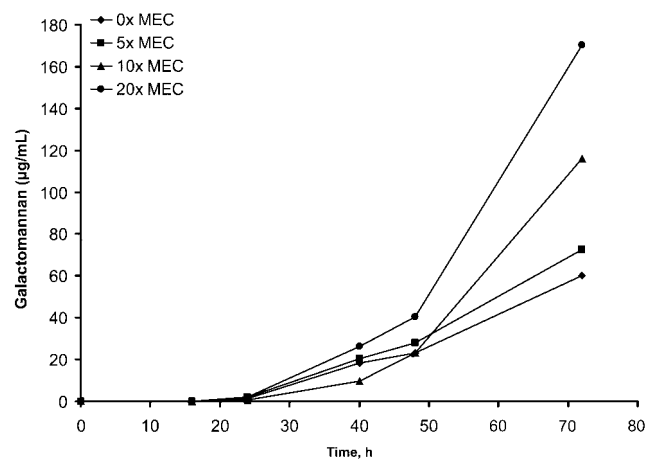


Figure 2. In vitro release of galactomannan by the causative *Aspergillus fumigatus* strain versus time, when incubated with 3 concentrations of caspofungin. Minimum effective concentration, 0.125 mg/L.

galactomannan, because the drug is known to increase exposure to caspofungin by up to 35% [12]. Furthermore, this drug exhibits antifungal activity against *A. fumigatus* and appears synergistic in vitro when combined with caspofungin [13, 14].

An initial increase in circulating galactomannan might be expected in patients treated with this class of antifungal agents as a result of the mechanism of action of the drug. In a rabbit model of invasive aspergillosis, exposure to caspofungin resulted in fragmentation of *Aspergillus* hyphae in the tissue and in an increase in the number of hyphal tips [6]. Some galactomannan is released at the hyphal tips, which may have resulted in the increased release of galactomannan in conjunction with degradation of the cell wall. Because some galactomannan is covalently bound to (1,3)- β -D-glucan in the fungal cell wall [15], exposure to caspofungin would result in a reduced amount of (1,3)- β -D-glucan molecules in the cell wall and, therefore, an increased release of galactomannan.

Amphotericin B and the azoles are cell membrane-active agents and have been shown to cause morphological alterations to the fungal cell wall [16, 17]. Persistently higher levels of circulating galactomannan were observed in mice with invasive aspergillosis that were given monotherapy with caspofungin and not in those treated with a combination of the echinocandin and amphotericin B [8]. Therefore, preexposure of the fungus to azoles or amphotericin B could have an impact on the release of galactomannan during subsequent caspofungin therapy. This might explain the lack of a paradoxical increase in galactomannan among patients given caspofungin for salvage therapy because they had already been exposed to other antifungal agents [9].

In conclusion, this is, to our knowledge, the first clinical report to describe a paradoxical increase in circulating galactomannan after caspofungin treatment for proven invasive aspergillosis. Further research is warranted to determine the frequency and significance of this clinical observation. In any event, this case shows that results of galactomannan monitoring among patients treated with caspofungin therapy should be interpreted with caution.

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