False-positive *Aspergillus* real-time PCR assay due to a nutritional supplement in a bone marrow transplant recipient with GVH disease

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PCR screening for circulating DNA, especially when combined with antigen testing, has shown promise for the definitive diagnosis of invasive aspergillosis. False positives for *Aspergillus* real-time PCR assays have been described in several reports, but no sources of fungal DNA contamination could be clearly identified. We report a false-positive case for both galactomannan (GM) antigenemia and *Aspergillus* PCR due to nutritional supplement intake in a bone marrow transplant recipient with digestive graft-versus-host disease. Our case report also suggests that fungal DNA can pass into the serum from the intestinal tract in the same way as fungal GM. Clinicians should be aware of this possibility, so that the administration of costly, unnecessary antifungal treatments with potential adverse side-effects can be avoided.

**Keywords**  Aspergillosis, real-time PCR, false-positive

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**Introduction**

Invasive aspergillosis (IA) is a life-threatening infection; its incidence in immunocompromised patients continues to increase. While there has been a significant decrease in mortality in recent years in IA patients who have undergone hematopoietic cell transplantation, average death rates continue to exceed 50% [1]. Early diagnosis and treatment are essential for improvement in prognosis. Because of reports which indicate that galactomannan (GM) can be detected before the clinical signs of IA, serial testing for GM in serum has been proposed as a diagnostic screening tool [2,3]. However, false-positive GM results frequently occur, mainly due to the use of some antibiotics, some bacterial membrane-associated molecules, and the digestive absorption of GM [4,5]. PCR screening for circulating DNA, especially when combined with antigen testing, has shown potential in the definitive diagnosis of IA [6–8]. False-positive results from PCR assays have also been reported and clear identification of the sources of DNA detected in serum with these reactions has proven to be difficult [9,10]. We report here a case of false-positive results from both GM antigenemia and circulating *Aspergillus* DNA assays due to a nutritional supplement intake in a bone marrow transplant recipient with graft-versus-host disease (GVHD).

**Case report**

A 42-year-old man diagnosed with acute myeloid leukemia in January 2005, received an allogenic hematopoietic stem cell transplantation from an HLA-matched sibling donor in June 2006 (D0). At day 15 (D15), he presented a stage 3 (grade II) cutaneous acute GVHD which was treated by high-dose steroid therapy. He was discharged on D30 and was followed as an outpatient. He developed digestive chronic GVHD after D150 and was treated with a combination of corticosteroid and tacrolimus. At D167 he developed severe diarrhea and denutrition. Stool samples were negative for *Cryptosporidium* and other intestinal viruses and...
parasites. Corticotherapy was increased and after D178 he was placed on parenteral nutrition associated with an oral hypercaloric supplement, i.e., Scandishake Mix®, Nutricia (1 or 2 bags per day). At D190, the patient was admitted for pulmonary syndrome with productive cough and moderate dyspnea without fever. Serum had been tested for GM antigenemia as part of the twice-a-week systematic surveillance of all the hematology unit patients with host factors. The GM test was performed using the Platelia® Aspergillus sandwich enzyme-linked immunosorbent assay (Biorad, Marnes-la-Coquette, France), using a 0.5 index as the positive threshold. Real-time PCR was performed as previously described [11] with a 44-cycle threshold.

The first positive serum sample for GM and real-time PCR was observed at D190 (Fig. 1). The second serum sample, taken at D192, was also found positive by both GM and PCR. Disseminated aspergillosis with digestive origins was suspected, and the nutritional supplement was stopped that same day. We immediately started empirical antifungal treatment (voriconazole, 400 mg/day and caspofungin at 70 mg, then 50 mg/day). Thoracic computed tomography revealed unilateral lung consolidation with pleural effusion; Pseudomonas aeruginosa was isolated from sputum culture. Antibiotic therapy with antimicrobial coverage against P. aeruginosa was immediately associated with the antifungal therapy.

GM testing of serum sampled at D195 was negative, but PCR on the same serum was positive. Voriconazole was stopped due to neurological adverse events including hallucinations and visual disturbances, and was replaced by itraconazole (400 mg/day). Caspofungin treatment was also stopped. At D201 and D204, GM and PCR were negative and progressive clinical improvement was observed.

A Scandishake Mix® bag from the batch taken by the patient was sent to the mycological laboratory. The powder (85 g) was dissolved in 240 ml sterile distilled water and serially diluted. GM tests and real-time PCR were positive until dilution of 1/1000 (Table 1). Two other batches were also obtained from the manufacturer for laboratory testing and both were GM-positive until dilution 1/1000 and PCR-positive only at first dilution. Samples of 1 ml from each of the three batches of Scandishake Mix® solutions were cultured in three different media, i.e., thioglycollate broth, tryptone soya broth and Sabouraud medium. Cultures were incubated for 14 days at 37°C, 30°C and 25°C, respectively. They were checked every day for signs of microbial growth but they remained negative.

DNA extracted from serum at D190 and D192, and from the three batches of Scandishake Mix bag, was amplified using primer AfumiR1/F1 as described by Haugland et al. [12]. PCR products were subsequently sequenced on an ABI 3130 DNA analyzer (Applied Biosystems, Courtaboeuf, France), with the same primers that were used in the PCR step. Sequences were compared to sequences available in the GenBank database using Blast software (http://www.
negative. DNA in the sample. Samples with crossing point above 44 cycles were baseline signal. Thus, the higher the Cp was, the lower the amount of fluorescence of the sample became significantly different from the threshold, or crossing point (Cp), which marked the cycle at which fluorescence of the sample became significantly different from the baseline signal. Thus, the higher the Cp was, the lower the amount of DNA in the sample. Samples with crossing point above 44 cycles were negative.

Table 1 GM and PCR testing of the nutritional supplement Scandishake Mix® (Nutricia)

<table>
<thead>
<tr>
<th>Batch 5353</th>
<th>Batch 5389</th>
<th>Batch 5345</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM*</td>
<td>PCR†</td>
<td>GM</td>
</tr>
<tr>
<td>1/10</td>
<td>6.11</td>
<td>34</td>
</tr>
<tr>
<td>1/100</td>
<td>6.37</td>
<td>36</td>
</tr>
<tr>
<td>1/1000</td>
<td>5.88</td>
<td>41</td>
</tr>
</tbody>
</table>

*Results are expressed in indexes for GM.
†Real-time PCR results were expressed by determination of the threshold of detection, or crossing point (Cp), which marked the cycle at which fluorescence of the sample became significantly different from the baseline signal. Thus, the higher the Cp was, the lower the amount of DNA in the sample. Samples with crossing point above 44 cycles were negative.

ncbi.nlm.nih.gov/blast). The same 136 bp sequence, showing 100% identity with part of A. fumigatus ribosomal DNA, was observed in the patient serum samples and the Scandishake Mix® batch given to the patient.

Discussion

Numerous causes of false positives of Platelia Aspergillus GM have been previously described. Among them, gastrointestinal translocation of fungal GM from contaminated food was described in patients with chemotherapy-induced mucosal lesions and in children with impaired integrity of the intestine [4]. Nutrients containing soybean protein have already been reported as a cause of false-positive GM in a patient with GVHD [13]. False-positive Aspergillus real-time PCRs have also been described in several reports. Fungal DNA contamination originating from intravenous antibiotics, and transient circulating DNA in patients with sub-clinical infections have been suspected as possible sources [7, 9,10,14]. However, this is the first report, to our knowledge, that fungal DNA passed into the serum in the same way as fungal GM, i.e., from the intestinal tract.

We assessed other explanations for our false-positive PCR results. Contamination from the environment or PCR reagents is unlikely because we used real-time PCR technology and commercial DNA extraction kits, and all our negative controls were negative. At the time of the false-positive result, the patient was receiving several intravenous drugs (antibiotics, corticoids, antivirals) which could have interfered with the GM and PCR assays. However, he received these therapies before this event, and previous weekly-tested samples were all negative. He received oral penicillin therapy during exactly the same period of nutritional support. However, oral penicillins have never been described as a cause of false-positive GM, and in vitro GM testing using Platelia Aspergillus assays has shown that oral penicillins exhibit no reactivity (index < 0.5) [5,15]. Therefore, the most probable hypothesis is that the Aspergillus DNA present in the nutritional supplement passed through the mucosa, in an amount large enough to be detected in serum. This could be related to the severe intestinal mucosal injury in this patient.

The possibility of contamination of the supplement by conidia, which can germinate and lead to DNA- and GM-emia, cannot be completely excluded. However, the Scandishake Mix was investigated for the presence of A. fumigatus by culturing in three different media. All culture media were negative. We therefore concluded that only non-viable particles of the fungus were present in the nutritional supplement.

The product contains glucose, saccharose, maltodextrin, partially hydrogenated vegetable oils (soybean and palm), medium chain triglycerides, and skimmed milk powder (cow). Real-time PCR investigation for Aspergillus DNA of the osidic components, vegetable oils, and milk powder could provide pertinent information for identifying the origin of Aspergillus DNA contamination. Aspergillus DNA-free ingredients could therefore be selected for the production of nutritional supplements that are given to immunocompromised patients.

In conclusion, consumption of nutritional supplements is safe for immunocompromised patients and is an essential element in controlling severe denutrition, which is frequent in such patients. When in vivo GM and PCR results are positive, early infection should be the first diagnosis to spring to mind, and clinicians should begin administration of specific antifungal therapy. However, if the patient is receiving nutritional supplements, they should also consider the possibility of false-positive results, and ask the mycological lab to test for PCR and GM reactivity to the nutritional supplement. If lab results support the false-positive hypothesis and the clinical status of the patient is satisfactory, administration of these costly treatments with potential adverse side-effects can be discontinued.

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References


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