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Case report: Enteral nutritional supplement as a likely cause of false-positive galactomannan testing ☆

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ABSTRACT

The detection of galactomannan (GM) in the serum of in immunocompromised patients is widely used for the early diagnosis of invasive aspergillosis. We report a case of a false-positive GM test presumably caused by the enteral nutritional supplement given to a non-neutropenic patient with intestinal graft-versus-host disease after a hematopoietic stem cell transplant. Clinicians should be alert to the possibility of false-positive GM results in patients on nutritional supplements.

1. Introduction

Galactomannan (GM) is a major constituent of *Aspergillus* cell walls that is released during growth of hyphae [1]. The detection of GM in the serum of immunocompromised patients is widely used for the early diagnosis of invasive aspergillosis (IA), and in the practice of pre-emptive anti-fungal therapy in febrile neutropenia [2].

We report a case of a false-positive serum GM result caused by the oral supplemental nutrition given to the patient. To the best of our knowledge, this is the first case of non-soy based enteral nutritional supplement implicated.

2. Case

The patient is a 29-year old Chinese woman with acute lymphocytic leukemia who had undergone a matched unrelated donor hematopoietic stem cell transplant. Neutrophil and platelet engraftment occurred on the 21st and 25th days post-transplant respectively. She was subsequently diagnosed with graft-versus-host-disease of the upper intestinal tract and was put on budesonide, tacrolimus and mycophenolate. She was found have CMV pp65 antigenemia on the 30th post-transplant day and was started on foscarnet.

On the 51st post-transplant day, a computerized tomography (CT) thoracic scan was ordered as part of an evaluation for suspected cervical lymphadenopathy. The scan demonstrated a few tiny pulmonary nodules which had not been seen on a CT thoracic scan 5 days prior.

The patient had no respiratory symptoms or signs. Her white blood cell count was 5320 mm^{-3} with 81.4% neutrophils hemoglobin 8.0 g/dl and platelet count 21,000 mm^{-3}. Chest X-ray (CXR) was normal.

The GM antigen index, assayed on the day after the CT thoracic scan was negative at 0.19 (positive cut-off 0.5). Nevertheless, in view of the CT findings, caspofungin was started.

On the 10th day of caspofungin, a check GM index became positive at 2.11 and remained positive at 0.69 4 days later. A repeated CT thoracic scan did not demonstrate any pulmonary nodules.

A thorough history and physical examination did not reveal symptoms or signs to suggest extra-pulmonary aspergillosis. In particular, she had no symptoms to suggest sinusitis, and a full neurological exam was normal. Patient's medications included budesonide, mycophenolate, trimethoprim/sulfamethoxazole, lamivudine, and ciprofloxacin. She had not been on other antibiotics during this time.

The patient was being managed as an inpatient and adhered to a strict hospital diet. She had been put on a supplemental nutritional product, Nepro® (Abbott Nutrition) 4 days prior to the positive GM result due to poor oral intake.

Because of concerns that the result was a false-positive, a sample from an unopened can of Nephro® was sent for GM testing

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and was positive at a high of 5.80 (see below for further details of testing).

Her supplemental feeds were stopped and within 10 days, the GM antigen index of patient's serum fell to below 0.20; the cut-off for a positive result being 0.50. Caspofungin was discontinued soon after – a total course of 19 days.

The nodules on the initial CT thoracic scan were present before the introduction of the Nepro®. Subsequent CXR and CT scans did not demonstrate the said lesions nor was there any re-appearance. Radiological artefacts could not be ruled out. We do not believe that the supplement could be the cause of the transient pulmonary nodules.

Follow-up of the patient up to 1 year later did not reveal any indication of Aspergillus infection.

3. Test procedure

Our laboratory uses a commercial GM test which employs a sandwich enzyme-linked immunosorbent assay (EIA) (Platelia™ *Aspergillus* Ag; Bio-Rad). The assay uses the rat monoclonal antibody EBA-2, which is directed against *Aspergillus* GM. The monoclonal antibody is used to sensitize the wells of the microplate, bind the antigen as the detector antibody in the conjugate reagent (peroxidase-linked monoclonal antibody).

Briefly, 300 μL of test serum was mixed with 100 μL of 4% EDTA treatment solution and boiled for 3 min, to dissociate immune complexes and to precipitate any serum proteins that might interfere with the test. After centrifuging at 10,000*g* for 10 min, 50 μL of the supernatant was added to 50 μL of the reaction mixture containing conjugated anti-GM EB-A2 antibody, and the mixture was incubated in microtiter plates precoated with the same antibody for 90 min at 37 °C. The plates were washed before adding 200 μL of a substrate-chromogen solution containing tetramethylbenzidine. After incubation for another 30 min in the dark at room temperature, 100 μL of 1.5 N sulfuric acid was added to stop the reaction. The plates were read at a wavelength of 450 nm using a reference filter of 630 nm. The index for each sample was calculated by dividing its optical density (OD) by the cutoff value (mean OD) of the threshold control. Indices of >0.5 were considered positive per the cutoff values for serum samples noted in manufacturer's package insert.

Our commercial GM assay has been approved for use by the United States Food and Drug Administration (FDA) for serum and bronchoalveolar lavage fluid only. For our analysis, we used 300 μL of undiluted nutritional supplement and the rest of the steps were in accordance with manufacturer's instructions for serum.

4. Discussion

Although the GM assay is popularly used to help clinicians make a diagnosis of IA, its sensitivity is variable. In one meta-analysis, the sensitivity of GM testing for IA varied from 30% to 100%, whereas specificity was $>75\%$ [3]. The wide range in sensitivity was attributable to design bias in most studies [4]. Test performance is generally accepted to be better for neutropenic patients who had received cytotoxic chemotherapy for hematological malignancies [3].

GM may be detected in serum before the presence of clinical signs or symptoms of IA. Serial GM testing may have a role in early detection and would allow pre-emptive treatment of IA in vulnerable patients [5]. Serial values may be used as a way of monitoring response to therapy, as radiologic findings may worsen with therapy, related to a return of leukocytes [6]. Hence, serial GM testing has been advocated in patients with documented IA as a prognostic marker and a measure of the efficacy of antifungal therapy [7].

One known drawback of GM testing is the potential for false-positive results. Cross-reactions with filamentous fungi like Fusarium species, Penicillium and Cladosporium are known [8]. Cross-reactivity with dimorphic fungi like Histoplasmosis has also been reported [9].

Notable false-positives have been reported following treatment with piperacillin–tazobactam [10] and amoxicillin–clavulanate, though more recent studies suggest that the modern preparations of piperacillin–tazobactam no longer cause this problem [11]. The false-positive results may persist for as long as 5 days after drug discontinuation. GM antigen has been detected in caspofungin preparations but was not shown to cause false positives in patients' sera [12].

Plasmalyte, a buffered intravenous solution containing sodium gluconate has also been implicated as a cause of false-positive GM results [13]. False-positive GM results have been shown in patients receiving Plasmalyte for intravenous hydration or if Plasmalyte is used for BAL collection. Cross-reacting antigens, introduced at the manufacturing stage of these commonly used healthcare formulations, has been postulated to be the cause.

Galactomannans form cell wall components of certain legumes and are used extensively in the food industry as thickening and stabilizing agents in various food products like as ice cream, cheese spreads, salad creams, processed meat products, and pie fillings [14]. Reports of food types being tested positive on diagnostic GM assays include canned vegetables, rice, pasta [15] and an infant formula with thickener [16].

Gastrointestinal translocation of fungal GM from food has also been thought to cause false-positive results [15,17]. This is especially pertinent in patients with impaired integrity of the intestine such as patients undergoing cytotoxic chemotherapy or patients with intestinal graft-versus-host disease, as in our case.

Despite the ubiquitous nature of GM products, there are only 2 case reports of false-positive GM from oral nutritional supplements causing confusion in clinical care. Both involved the administering of soy-based enteral nutrition supplements to patients with intestinal graft-versus-host disease [18,19]. The presumed product in our case is not soy-based and lists soy as an ingredient in less than 1% by amount.

Pertaining to our patient, several pointers led us to suspect that the GM result was a false-positive. Her pulmonary nodules were very small, and she had not been neutropenic in the days prior to their discovery. Furthermore, the clinical picture was not consistent with the elevated GM result.

Detection of high GM levels in the culprit product bolstered our case, albeit the assay was not validated for use on specimens other than serum and BAL. Discontinuing the supplemental feed allowed the GM levels to fall to normal levels within 10 days and gave us the confidence to discontinue an expensive anti-fungal agent.

We emphasize the need to review every single positive GM result, especially if the clinical picture is not consistent with the result (e.g. rising levels in a patient who is clinically responding to an intravenous anti-fungal medication).

Given the wide ranging drugs and foodstuff that have been implicated in GM false positives, clinicians should scrutinize patient's medications and diet actively. The relatively long half-life of circulating GM antigens in serum may mean one would need to review recently ingested or infused products up to at least 10 days or more.

Through this case report, we hope to inform clinicians of the false-positives of GM testing to avoid unnecessary administration of costly antifungal treatments and their attendant side-effects on our patients.

Conflict of interest

None.

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