Case Reports

Cross-reactivity in the Platelia™ Aspergillus enzyme immunoassay caused by blastomycosis

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It is well known that cross reactions with other fungal pathogens including Histoplasma capsulatum can occur with the use of the Platelia™ Aspergillus galactomannan assay. We report two patients with confirmed blastomycosis whose bronchoalveolar lavage (BAL) fluid tested positive for Aspergillus galactomannan despite no evidence of aspergillosis.

Keywords galactomannan, blastomycosis, cross-reaction

Introduction

The Platelia™ (Bio-Rad laboratories, Redmond, WA, USA) Aspergillus enzyme linked immunosassay (EIA) was designed to detect a galactomannan antigen produced by Aspergillus species but cross reactions have been reported with other fungal pathogens, including Histoplasma capsulatum [1]. In an in vitro analysis, culture supernatants of Blastomyces dermatitidis gave positive results in the Platelia™ Aspergillus EIA [2]. To the best of our knowledge, positive results have not been reported in patients with blastomycosis. Herein we report two patients with blastomycosis whose bronchoalveolar lavage (BAL) fluids tested positive in the Platelia™ Aspergillus EIA.

Case reports

Case study 1

A 68-year-old man with diabetes mellitus and rheumatoid arthritis, for which he was receiving infliximab, presented with a six-week history of worsening cough, dyspnea, and decreased exercise capacity. Chest radiograph and CT scan showed multiple bilateral pulmonary nodules and left hilar consolidation. Initial BAL specimen and lung biopsies were negative for pathogens and malignancy but the lung tissue showed organizing pneumonia without granulomas. The BAL fluid was positive at 0.52 index units in the Platelia™ Aspergillus EIA (cut-off is 0.5 index units) and 0.6 ng/ml in the MVista Histoplasma antigen EIA (Table 1). Antibodies to Histoplasma, Blastomyces and Coccidioides measured by immunodiffusion were negative.

After transfer to a tertiary care hospital, a second bronchoscopy was performed and showed yeast cells consistent with B. dermatitidis. A urine specimen was positive at 1.79 EIA units in the MVista second generation Blastomyces antigen EIA (Table 1). Liposomal amphotericin B was started for presumed blastomycosis. B. dermatitidis, confirmed by PCR, was eventually isolated from both BAL fluids, but no Aspergillus species or any other mold was recovered.

Case study 2

A 67-year-old man with history of myasthenia gravis treated with prednisone and azathioprine was admitted to the hospital for recurrent pneumonia. He had received two courses of levofloxacin during the six weeks prior to the current admission. Chest radiograph revealed left lower lobe consolidation. Bronchoscopy and BAL were performed and revealed broad budding yeast, 6–10 micrometers in diameter, compatible with B. dermatitidis (Fig. 1). Blastomyces antigen was detected in the BAL fluid (above limit...
Aspergillus galactomannan blastomycosis

Table 1  Aspergillus, Blastomyces and Histoplasma antigen results.

<table>
<thead>
<tr>
<th>Result</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plateia™ Aspergillus EIA</td>
<td>0.52 index units</td>
<td>0.88 index units</td>
</tr>
<tr>
<td>BAL</td>
<td>Not done</td>
<td>0.07 index units</td>
</tr>
<tr>
<td>Serum</td>
<td>Not done</td>
<td>0.07 index units</td>
</tr>
<tr>
<td>MVista® Blastomyces EIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL</td>
<td>Not done</td>
<td>Above limit</td>
</tr>
<tr>
<td>Urine</td>
<td>1.79 units&lt;sup&gt;3&lt;/sup&gt;</td>
<td>14.33 ng/ml</td>
</tr>
<tr>
<td>Serum</td>
<td>0.28 units</td>
<td>Above limit</td>
</tr>
<tr>
<td>MVista® Histoplasma EIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL</td>
<td>&lt; 0.6 ng/ml&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&gt; 39 ng/ml&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine</td>
<td>&lt; 0.6 ng/ml</td>
<td>19.07 ng/ml</td>
</tr>
<tr>
<td>Serum</td>
<td>&lt; 0.6 ng/ml</td>
<td>29.43 ng/ml</td>
</tr>
</tbody>
</table>

1 The cut-off for positive for the Plateia™ Aspergillus EIA is 0.5 index units. 2 MiraVista Diagnostics, Indianapolis, IN. 3 Second Blastomyces antigen generation assay, results of 1.0 unit or higher are positive. 4 Third-generation Blastomyces antigen assay, upper limit of quantification is 14.7 ng/ml. 5 Third-generation Histoplasma antigen assay, results more than three times the negative control are positive, and those between the cut-off and the 0.6 ng/ml standard are reported as positive, less than 0.6 ng/ml and those above the upper limit of quantification are reported as above 39 ng/ml.

Discussion

These cases illustrate, although not surprisingly, that the antigen detected in patients with blastomycosis may cause positive results in the Platelia™ Aspergillus EIA. In both patients the Aspergillus GM was barely above 0.5 index units, the FDA-cleared cut-off for positivity for serum and BAL. The physicians caring for these patients included aspergillosis or an endemic mycosis in the differential diagnosis, based upon underlying immunosuppression and consistent radiographic abnormalities.

Given the cross-reactivity of the antigen detected in blastomycosis and histoplasmosis [3] and demonstration that Blastomyces culture supernatant caused positive results in the Platelia™ Aspergillus EIA [2], cases of blastomycosis also could be expected to cause cross reaction in this assay. The mechanism for this cross-reactivity has not been fully studied. A GM is present in the urine, blood and of quantification (< 14.7 ng/ml), urine (14.33 ng/ml) and serum (above limit of quantification) in the third generation MVista Blastomyces antigen EIA, Table 1. BAL fluid also was positive for Aspergillus antigen (0.88 index units) but the serum was negative. Cultures of the BAL fluid eventually yielded B. dermatitidis, the identification of which was confirmed by thermal transformation from mold form when grown on Sabouraud and Brain-Heart Infusion agars at 25°C to the characteristic yeast on Cotton Seed Agar at 37°C. No Aspergillus species or any other mold was isolated from the BAL fluid.

BAL of patients with blastomycosis that is immunologically indistinguishable from that in histoplasmosis [3–6]. H. capsulatum, B. dermatitidis and Paracoccidioides brasiliensis contain immunologically and chemically similar GMs in their cell walls, in which galactofuranose is present at the non-reducing terminals [7]. Galactofuranose is the epitope detected by the monoclonal antibodies [8] used in the Platelia Aspergillus EIA. The GM found in A. fumigatus contains (1®5)-β galactofuranosyl side chains while Histoplasma GM has (1®6)-α-D-galactofuranosyl side chains. Chemical differences in the galactofuranosyl side chains probably account for the weak cross reactivity seen with Aspergillus spp. and H. capsulatum [1] and B. dermatitidis and the strong cross reactivity between H. capsulatum, B. dermatitidis and P. brasiliensis [4]. Our preliminary findings need to be confirmed in larger studies.

Misdiagnosis of aspergillosis in a patient with blastomycosis may cause errors in the selection of antifungal therapy. For example, the echinocandins are not active or recommended for the treatment of blastomycosis and the role of voriconazole is unclear [9]. Care must be taken to exclude blastomycosis in patients with a positive
Aspergillus antigen result in the appropriate epidemiological and clinical settings.

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References