False-positive *Aspergillus* Antigenemia Due to Blood Product Conditioning Fluids

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The presence of *Aspergillus* antigens in blood transfusion components from different manufacturers was analyzed. Galacomannans were found in transfused patients, pooled platelet concentrates, fresh frozen plasma, and packed red cells collected using Fresenius Kabi bags. Galacomannans were also found in blood collection anticoagulant and platelet additive solution from this manufacturer.

Knowledge of serum galactomannan (GM) levels is useful in the early diagnosis of invasive pulmonary aspergillosis in at-risk patients [1]. False-positive and false-negative results for GM have been reported. A Cochrane systematic review revealed an overall sensitivity of 78% (61%–89%) and an overall specificity of 81% (72%–88%) using a GM optical density index (GM-ODI) of 0.5 as a cutoff. Higher cutoff values gave higher specificity at the cost of lower sensitivity [2].

Currently the primary recognized cause of false-positive GM results is nonintravenous administration of piperacillin-tazobactam and other betalactam antibiotics [3–7]. Other causes have been described [8–26].

We recently treated a patient with suspected pulmonary aspergillosis in whom evident clinical improvement was not consistent with rising serum GM levels. At that time the patient was receiving frequent platelet transfusions, which we suspected could be the source of false positivity.

Our hypothesis was that blood derivatives contained antigens that reacted in the Platelia *Aspergillus* assay and that their use might cause false-positive GM results in the recipients of such blood products. We aimed to assess the presence of GM in blood derivatives ready for transfusion and in fluids used in preparation of these derivatives. We also tested whether the amounts found could cause false-positive serum results in patients who had undergone transfusion.

Case Description

A 67-year-old woman with Henoch-Schönlein purpura and chronic renal disease under long-term treatment with corticosteroids and cyclophosphamide was referred from a different hospital to our intensive care unit with respiratory distress and neutropenia. Her chest radiograph showed diffuse bilateral infiltrates. Cytomegalovirus systemic infection was confirmed. The patient was treated with imipenem-cilastatin, clarithromycin, trimethoprim-sulfamethoxazole, ganciclovir, and supportive therapy. *Aspergillus* antigenemia determined on days 11, 19, 28, and 40 yielded GM-ODI readings of 1.67, 3.10, 0.91, and 0.14, respectively. Voriconazole therapy was given for 30 days, commencing from the first positive result. After 20 days of intensive care, the patient’s condition improved and she was transferred to a ward. During the first 11 days of admission, the patient received 10 units of pooled platelets and 4 bags of packed red blood cells (PRCs). On the following 12 days, 5 units of pooled platelets, 2 units of apheresis platelets, and 4 PRC were given. Tomography scans performed on admission and 1 month later did not reveal lung consolidations suggestive of aspergillosis. Testing of 1 of the pooled platelet concentrates that was administered revealed a GM-ODI of 6.18.

MATERIALS AND METHODS

Our institution is a 1570-bed public general university hospital. Blood is collected from healthy altruistic donors using multibag systems (CompoFlow and CompoFlow Select, Fresenius Kabi, Bad Homburg, Germany) and processed at the Madrid Central Blood Bank. The stock is shared with the Spanish Red Cross and other institutions’ blood banks as needed.

We evaluated the presence of reacting GM antigens in the following cases:
The supernatant of centrifuged blood products ready for administration: PRC, platelet concentrates, and fresh frozen plasma used in our hospital.

The fluids used for blood processing, including saline-adenine-glucose-mannitol (SAG-M), different citrated anticoagulant solutions (ACD-A, CPD, CPD-A), and platelet additive solution. The composition of these fluids is shown in Table 1.

Serum samples from 8 patients in whom Aspergillus infection was considered unlikely and who were scheduled to receive platelet therapy were tested before (up to 6 hours before) and after platelet transfusion (within 30 minutes of finishing the infusion). Two patients also received 2 bags of PRC immediately before the platelets. All patients were adults, with no cognitive impairment; none had fever or received betalactam antibiotics in the previous 7 days. Our hospital ethics committee approved the study protocol, and the patients gave their written informed consent.

**RESULTS**

**Blood Products**

We tested 66 blood products that were ready for transfusion, all of which were collected in bags from different manufacturers (Table 2). Pooled platelet concentrate and fresh frozen plasma collected in Fresenius Kabi bags had a high GM-ODI (median >5.0), while single-donor platelet concentrates had a GM-ODI of <0.5. The supernatant of PRC concentrates had variable GM levels.

**Conditioning Fluids**

Table 3 summarizes the different types of anticoagulant and preservation fluid evaluated, the manufacturers, and test results. The anticoagulant present in the blood processing bags manufactured by Fresenius Kabi and the platelet additive solution from the same company had a GM-ODI >3.0. Tested samples from other manufacturers had a GM-ODI <0.6. The anticoagulants used during the apheresis procedure were negative for GMs.
**Transfused Patients’ Sera**

Eleven pretransfusion and posttransfusion paired blood samples were obtained from 8 patients (Table 4). Median preinfusion GM-ODI was 0.20 (range, 0.10–0.89). Postinfusion levels were positive in all patients receiving pooled platelets (mean, 1.54; range, 0.96–2.99).

**DISCUSSION**

We demonstrated that 2 products used in processing donated blood, namely, CPD (the anticoagulant of Compo Flow and Compo Select) and Composol platelet additive solution (Fresenius Kabi, Bad Homburg, Germany), contained

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**Table 2. Galactomannan Optical Density Index Readings of Blood Products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Blood Collection Bag Manufacturer</th>
<th>Number of Samples Tested</th>
<th>GM-ODI Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed red cells</td>
<td>MacoPharmaa</td>
<td>4</td>
<td>0.18 (0.11–0.28)</td>
</tr>
<tr>
<td></td>
<td>Fenwalb</td>
<td>2</td>
<td>0.22–0.30</td>
</tr>
<tr>
<td></td>
<td>Fresenius Kabic</td>
<td>13</td>
<td>0.95 (0.31–4.43)</td>
</tr>
<tr>
<td>Frozen fresh plasma</td>
<td>Fresenius Kabic</td>
<td>18</td>
<td>&gt;5.00 (&gt;5.00)</td>
</tr>
<tr>
<td>Pooled plateletsd</td>
<td>Fresenius Kabic</td>
<td>19</td>
<td>&gt;5.00 (2.90–&gt;5.00)</td>
</tr>
<tr>
<td>Apheresis plateletsg</td>
<td>Haemoneticsg</td>
<td>10</td>
<td>0.25 (0.09–0.47)</td>
</tr>
</tbody>
</table>

Abbreviation: GM-ODI, galactomannan optical density index.

* MacoPharma, Mouvaux, France.
* Fenwal Inc., Lake Zurich, IL.
* Fresenius Kabi AG, Bad Homburg, Germany.
* Blood was collected in Fresenius Kabi bags and platelets were pooled in empty bags manufactured by Caridian BCT (Lakewood, CO).
* The Haemonetics extended storage platelet apheresis set contained no fluids (Haemonetics Corporation, Braintree, MA). The donors’ blood was anticoagulated with ACD-A manufactured by Grifols (Grifols, Las Torres de Cotillas, Murcia, Spain).

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**Table 3. Galactomannan Readings of Fluids Used in Blood and Platelet Donation**

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Product</th>
<th>Manufacturer</th>
<th>Number of Lots</th>
<th>GM-ODI, Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrated anticoagulant</td>
<td>ACD-A</td>
<td>Fresenius Kabi</td>
<td>2</td>
<td>0.16–0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baxter</td>
<td>2</td>
<td>0.13–0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grifols</td>
<td>2</td>
<td>0.23 (0.12–0.33)</td>
</tr>
<tr>
<td></td>
<td>CPD-A</td>
<td>MacoPharma</td>
<td>1</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Terumo Penpol</td>
<td>1</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>CPD</td>
<td>Fenwal</td>
<td>3</td>
<td>0.14 (0.12–0.20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fresenius Kabi</td>
<td>5</td>
<td>&gt;5.00 (3.07–&gt;5.00)</td>
</tr>
<tr>
<td>Platelet additive solution</td>
<td>Composol PS</td>
<td>Fresenius Kabi</td>
<td>2</td>
<td>3.52–&gt;5.00</td>
</tr>
<tr>
<td></td>
<td>Intersol PS</td>
<td>Fenwal</td>
<td>2</td>
<td>0.16–0.21</td>
</tr>
<tr>
<td></td>
<td>SSP</td>
<td>MacoPharma</td>
<td>1</td>
<td>0.35</td>
</tr>
<tr>
<td>Erythrocyte stabilizing solution</td>
<td>SAG-M</td>
<td>Fresenius Kabi</td>
<td>6</td>
<td>0.19 (0.10–0.38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fenwal</td>
<td>3</td>
<td>0.17 (0.013–0.19)</td>
</tr>
</tbody>
</table>


* Fresenius Kabi Norge AS, Halden, Norway.
* Baxter Healthcare Corporation, Deerfield, IL.
* Laboratorios Grifols, Las Torres de Cotillas, Murcia, Spain.
* MacoPharma, Mouvaux, France.
* Terumo Penpol Limited, Trivandrum, India.
* Fenwal Inc., Lake Zurich, IL.
* Fresenius Kabi AG, Bad Homburg, Germany.
high amounts of antigens that reacted in the Platelia-Aspergillus assay. Donated platelets and plasma prepared using these products had a high GM-ODI. Serum samples from patients receiving these products became false positive after infusion. Because a previous platelet infusion had been given a few days earlier, the antigen cleared in less than 7 days in most of these patients. Two patients had a positive baseline GM-ODI, probably reflecting a carryover effect from a previous transfusion. Both were negative in follow-up serum samples, were not treated for aspergillosis, and survived more than 2 months. The supernatant of PRC, which is expected to contain primarily SAG-M, may contain a small but variable proportion of plasma and citrated anticoagulant, thus, potentially explaining the variable antigen levels found.

According to the literature, patients receiving GM-containing antibiotics clear the GM antigen in 6 to 120 hours [3, 27–29]. Single-dose administration of Plasma-Lyte in healthy volunteers leads to a positive GM that persists for 10 to 24 hours. Experimental animal models have shown that after Plasma-Lyte infusion, GM-ODI becomes positive with a peak at 1 hour and then becomes negative after 8 hours. Repeated dosing resulted in accumulation, and serum levels remained positive for more than 36 hours [30]. Similar results were found using piperacillin-tazobactam [31]. In the reported case, GM-ODI readings 6 days after the last pooled platelet transfusion were 0.91.

Invasive aspergillosis has a high mortality rate. Early initiation of antifungal therapy is critical for survival, and, in most cases, in the absence of histopathological confirmation, antifungal therapy is initiated when possible aspergillosis criteria are fulfilled. Despite its limitations, the GM assay has been widely used in recent years as a surrogate marker in the definition of probable aspergillosis and is considered a useful adjunct for establishing an early diagnosis [1].

Currently recognized causes of false-positive GM can be categorized into four groups: (1) cross-reaction from an existing non-Aspergillus fungal infection (Histoplasma [8–10], Penicillium marneffei [11], Trichosporon [12], or Cryptococcus [11–13]); (2) intravenous administration of fungal-derived products as betalactam antibiotics [3–7] or gluconate-containing PlasmaLyte solutions [16, 17]; (3) poor postextraction management of samples in the laboratory [25, 26]; and (4) enteric absorption of ingested GM present in thickening gums or bacterial antigens (this has been proposed but not proven). In these cases, the relationship with mucositis is controversial [18–24]. To our knowledge, blood derivatives have never before been related to false-positive results in GM tests.

Gluconate-containing intravenous fluids are a known cause of false-positive Aspergillus antigenemia. Gluconate and citric acid are prepared industrially by Aspergillus niger carbohydrate fermentation [32, 33]. We suspect that the source of the reacting antigen that was detected may be remnant galactomannans present in citric acid and/or sodium citrate and/or gluconate as a result of the industrial purification procedure that was used.

To our knowledge, citric acid and citrate have not been associated with the presence of Aspergillus antigens, although citric acid is a commonly used preservative in canned vegetables, which have been found to contain GM antigens [34].

Patients at risk of aspergillosis, such as bone marrow recipients, patients with hematologic or rheumatologic disorders, and solid organ recipient patients, frequently need blood

### Table 4. Pre- and Posttransfusion Galactomannan Levels in Noninfected Patients

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>7</td>
<td>0.18</td>
<td>No</td>
<td>&gt;5.00</td>
<td>0.96</td>
</tr>
<tr>
<td>2</td>
<td>&gt;30</td>
<td>7</td>
<td>0.10</td>
<td>No</td>
<td>2.90</td>
<td>1.46</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5</td>
<td>0.18</td>
<td>No</td>
<td>&gt;5.00</td>
<td>1.62</td>
</tr>
<tr>
<td>4a</td>
<td>4</td>
<td>3</td>
<td>0.27</td>
<td>0.30</td>
<td>&gt;5.00</td>
<td>1.53</td>
</tr>
<tr>
<td>4b</td>
<td>4</td>
<td>4</td>
<td>0.27</td>
<td>No</td>
<td>&gt;5.00</td>
<td>1.28</td>
</tr>
<tr>
<td>4c</td>
<td>4</td>
<td>4</td>
<td>0.40</td>
<td>No</td>
<td>&gt;5.00</td>
<td>1.41</td>
</tr>
<tr>
<td>5a</td>
<td>&gt;30</td>
<td>5</td>
<td>0.17</td>
<td>No</td>
<td>2.90</td>
<td>1.15</td>
</tr>
<tr>
<td>5b</td>
<td>1</td>
<td>6</td>
<td>0.89</td>
<td>No</td>
<td>&gt;5.00</td>
<td>2.99</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>4</td>
<td>0.54</td>
<td>No</td>
<td>&gt;5.00</td>
<td>1.38</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>4</td>
<td>0.13</td>
<td>No</td>
<td>&gt;5.00</td>
<td>1.64</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>7</td>
<td>0.21</td>
<td>4.31</td>
<td>&gt;5.00</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Patients 4 and 5 volunteered on 3 and 2 different occasions, respectively.

Abbreviations: GM-ODI, galactomannan optical density index; PRC, packed red cells.
derivatives. The use of blood derivatives containing galactomannan antigens may explain a substantial number of previously unexplained false-positive *Aspergillus* antigen results. If this problem proves to be widespread, interpretation of positive results may need to be reevaluated whenever such platelet or plasma treatments are administered.

Caution must be exercised with all parenteral products using chemicals obtained from fungi. Efforts should be made to commercialize galactomannan-free certified parenteral drugs and blood products for use in patients at risk of invasive aspergillosis.

**Notes**

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**References**


