Case Report

Galactomannan antigen and Aspergillus antibody responses in a transplant recipient with multiple invasive fungal infections

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Introduction: We report Aspergillus antigen and antibody responses in a case of multiple invasive fungal diseases.

Case presentation: The patient, a double-lung transplant recipient, had candidaemia and invasive pulmonary aspergillosis with cerebral involvement. The follow-up of the serum Aspergillus galactomannan antigen by ELISA showed a level increase that correlated with the patient's aggravation. It was retrospectively completed by kinetics analysis using different anti-Aspergillus antibody assays (ELISA and Western blotting).

Conclusion: A balance between the Aspergillus antigen and anti-Aspergillus antibodies was shown using different anti-Aspergillus antibody assays. The results of these two antibody techniques appeared rather congruent.

Keywords: antibody; Aspergillus; ELISA; galactomannans; Western blotting.

Introduction

Candidaemia and invasive aspergillosis are the two most common invasive fungal infections (IFIs) reported worldwide (Bitar et al., 2014; Neofytos et al., 2010). Their incidences have been increasing markedly since the beginning of the 21st century and their prognosis relies mainly on early and appropriate therapy. Diagnosis is usually based on host and clinical factors, as well as microbiological data collected from different types of sample (De Pauw et al., 2008). The diagnosis of IFIs relies mostly on standard microbiological cultures (in respiratory tract samples, for example) and antigen-based assays (e.g. galactomannan for invasive aspergillosis) performed in serum or body fluid samples, such as bronchoalveolar lavage (BAL) and, possibly, cerebrospinal fluid (CSF). We report here a case of multiple IFI, comprising candidaemia and invasive pulmonary aspergillosis (IPA) with cerebral involvement. In addition, we describe the full kinetics of the galactomannan antigen and of the anti-Aspergillus antibodies during the course of this patient’s disease. To the best of our knowledge, this case is the first to allow the study of the Aspergillus antigen and antibody kinetics, as well as a comparison of two different antibody detection techniques such as ELISA and a newly commercialized anti-Aspergillus Western blotting (WB) method.

Case report

A 34-year-old woman presented initially in April 2013 with a 4-month history of renal failure associated with convulsive seizures. She had been a double-lung transplant recipient for idiopathic pulmonary fibrosis for 5 months. On the basis of magnetic resonance imaging, a posterior reversible encephalopathy syndrome was diagnosed. Medication on that day was cyclosporin, prednisolone, ganciclovir, trimethoprim/sulfamethoxazole and levetiracetam. She was then transferred to the nephrology ward (day 0) where a macrophage activation syndrome was diagnosed based on bone-marrow aspiration. The patient shifted

Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; IFI, invasive fungal infection; IPA, invasive pulmonary aspergillosis.
towards multiple organ failure and was then transferred to the intensive care unit on day 23. On presentation, the patient had hypotension and a Glasgow coma scale score of 8. Generally, the patient had septic shock in a context of agranulocytosis associated with conscience disorders, pleuropneumonia and ascites. Voriconazole (Vfend, 200 mg twice a day) was added to the broad-spectrum antibiotic therapy, as IPA was suspected on the basis of host and clinical factors such as persistent antibiotic-refractory fever. CSF as well as blood cultures, BAL and an ascites puncture were taken on the same day (day 23) in the intensive care unit. CSF showed a glucose level of 3.65 mmol l⁻¹ and total protein of 0.37 g l⁻¹ with a nucleated cell count of <2 × 10⁶ l⁻¹. On day 26, the blood cultures, BAL and ascites puncture were positive for Candida albicans. Cultures of the BAL also showed a pulmonary aspergillosis with the isolation of numerous Aspergillus fumigatus colonies. Galactomannan assayed by ELISA (ELISA Platelia Aspergillus Ag; BioRad) was positive in CSF on day 26 (index 5.55) and in serum on day 30 (index >6 after control). No anti-Aspergillus antibody was detected by ELISA (ELISA Platelia Aspergillus IgG; BioRad) in this serum. In view of this multiple IFI (disseminated candidiasis and IPA associated with cerebral aspergillosis) and of the unusual localization, liposomal amphotericin B (Ambisome) was added to the voriconazole on day 30. The patient’s clinical condition was stable and her immunosuppressive therapy was adjusted (reintroduction of intravenous cyclosporin and prednisolone), as well as the dosing of voriconazole (100 mg twice a day on day 32 due to severe cholestasis and toxic voriconazole level at 5.59 mg l⁻¹). On day 39, after 5 days of non-invasive ventilation, the patient again required intubation after isolation of a multidrug-resistant Klebsiella pneumoniae strain in blood cultures. With regard to hepatotoxicity and subtherapeutic voriconazole levels (residual level at 0.33 mg l⁻¹), persistent A. fumigatus-positive mycological cultures of two other BAL samples and the results of the antifungal MICs (0.125 µg ml⁻¹ for voriconazole, 0.019 µg ml⁻¹ for itraconazole, 0.125 µg ml⁻¹ for posaconazole, 0.064 µg ml⁻¹ for caspofungin and 2 µg ml⁻¹ for amphotericin B), the antifungal therapy was switched to liposomal amphotericin B with caspofungin (Cancidas) on day 58. On day 64, a favourable outcome was not achieved, with multiple organ failures, physical exhaustion and severe denutrition. CT imaging showed a bilateral pleuropneumonia and excavated lesions of the superior pulmonary lobe, as well as suspected cerebral abscesses that were confirmed by magnetic resonance imaging. Lumbar puncture was advocated but could not be performed due to persistent low platelets. Intra-alveolar and digestive tract haemorrhages were also diagnosed on day 64. By day 70, facing ineffectiveness of antifungal treatments and persistent multiple organ failure, the patient’s state rapidly deteriorated to a state of septic shock, eventually leading to death.

Two serum samples were analysed during this patient’s follow-up: the first, from day 25, was positive for galactomannan but negative for anti-Aspergillus antibodies; the second, from day 36, gave a lower index for the galactomannan antigen but was positive for antibodies by ELISA and WB. As a balance between antigen and antibody levels seemed to be observed between these two sera 11 days after the diagnosis, this event had to be further explored.

Additional BAL and sera were retrospectively recovered from different laboratory departments of the Hospices Civils de Lyon University Hospitals. In order to implement kinetics analysis, sera were screened in the same ELISA runs for galactomannans and for anti-Aspergillus antibodies. For antibody presence, WB (Aspergillus Western Blot IgG; LDBio Diagnostics, Lyon) was also performed. The BALs were only tested for galactomannan levels. The results are summarized in Table 1. A local semi-quantitative analysis of the WB was performed based on the number and intensity of the specific bands described by the manufacturer. The kinetics of the different serological parameters are summarized in Fig. 1. A marked increase in galactomannan levels followed sequentially by an increase in anti-Aspergillus antibodies was observed. The balance between Aspergillus antigen and anti-Aspergillus antibodies was then confirmed, as positive serology was concomitant with the decrease in Aspergillus antigen levels. Upon pulmonary lesion excava-
tion and cerebral abscess formation, the antibody levels eventually decreased in both the ELISA and WB, while a new antigen level increase occurred.

**Discussion**

We report here the case of a multiple IFI comprising candidaemia and IPA with cerebral involvement in a double-lung transplant recipient. Mixed fungal infections have a low incidence globally (<5%) and are due mainly to Aspergillus and Candida associations (Kume et al., 2011). Conversely, a higher incidence rate of multiple IFIs (approx. 17%) was described in solid-organ transplant recipients by Neofytos et al. (2010). The two sera sent for routine Aspergillus serodiagnosis indicated simultaneous and opposite-level variations, suggesting a balance between Aspergillus antigen and anti-Aspergillus antibodies that was confirmed later by the kinetics analysis. Although the antigen-to-antibody balance is well known for Candida antigen and anti-Candida antibodies (Sendid et al., 2002), it is rarely mentioned in aspergillosis (Verweij, 2009) and never, to the best of our knowledge, outside haematological patients. Moreover, the kinetics that we observed seemed congruent with the patient’s clinical condition.

Interestingly, ELISA antigen detection was first found to be positive in the CSF and then in sera and BAL. However, antigen levels in the serum collected 3 days before the lumbar puncture showed a value close to the cut-off level (0.46) retrospectively. If this serum had been tested on time with antigen assays performed every day, it would have contributed to an earlier diagnosis of aspergillosis. For the CSF sample, the antigen was found at a very high
Aspergillus serological kinetics in transplant patient

Table 1. Galactomannan ELISA and anti-Aspergillus antibody levels (ELISA and WB): antigen ELISA results are expressed in index (sample absorbance/cut-off value absorbance), antibody ELISA results are expressed in arbitrary units ml$^{-1}$, whereas WB results are expressed as positive (+) or negative (−). For WB, four specific bands were monitored and their intensity is scaled from 0 to 4, with two positive bands determining a positive result. BAL, Italic font indicates samples tested after patient’s death. NA, Not applicable.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date of collection/nephrology ward entry</th>
<th>Galactomannan ELISA antigen levels (index)</th>
<th>ELISA antibody titre (AU ml$^{-1}$)</th>
<th>Antibody WB (total intensity/number of specific bands)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>Day 23</td>
<td>5.55</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>BAL</td>
<td>Day 23</td>
<td>6.70</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>Day 47</td>
<td>6.91</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>Day 57</td>
<td>5.52</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Serum</td>
<td>Day 2</td>
<td>0.09</td>
<td>0</td>
<td>− (0.5/1)</td>
</tr>
<tr>
<td></td>
<td>Day 20</td>
<td>0.46</td>
<td>0</td>
<td>− (0/0)</td>
</tr>
<tr>
<td></td>
<td>Day 25</td>
<td>6.61</td>
<td>0</td>
<td>− (0/0)</td>
</tr>
<tr>
<td></td>
<td>Day 36</td>
<td>4.74</td>
<td>76</td>
<td>+ (10/4)</td>
</tr>
<tr>
<td></td>
<td>Day 44</td>
<td>4.24</td>
<td>47</td>
<td>+ (8.5/4)</td>
</tr>
<tr>
<td></td>
<td>Day 54</td>
<td>4.33</td>
<td>26</td>
<td>+ (6.5/4)</td>
</tr>
<tr>
<td></td>
<td>Day 61</td>
<td>6.57</td>
<td>16</td>
<td>+ (5/3)</td>
</tr>
</tbody>
</table>

level, but the culture remained negative, as often reported, with only 31 % of positive Aspergillus cultures in CSF (Antinori et al., 2013). Direct examination of the BAL samples quickly yielded ‘hyphae’, indicating a fungal infection, but several incubation days were needed before species identification based on macroscopic and microscopic...
characteristics was achieved. This case report highlights the need to test antigens in samples other than sera or BAL, such as CSF (Antinori et al., 2013; Piens et al., 2004), in patients at risk who are displaying clinical pulmonary and cerebral symptoms, as indicated by the European Organization for Research and Treatment of Cancer (De Pauw et al., 2008). However, BAL and sera are presently the only clinical samples validated by the manufacturer’s recommendations. Along with standard mycological procedures, regular monitoring of galactomannan twice weekly at least in the serum is paramount to an early diagnosis (Mennink-Kersten et al., 2004), but testing CSF and BAL as well as frequent laboratory antigen processing is also important. According to our retrospective serum kinetics analysis, the increase in *Aspergillus* antigen levels was concomitant with clinical deterioration, whereas the decrease was correlated to the response to therapy, as described in previous studies (Boutboul et al., 2002; Maertens et al., 2001; Miceli et al., 2008; Park et al., 2011; Penack et al., 2008; Sheppard et al., 2006; Woods et al., 2007).

This case report also analysed different anti-*Aspergillus* antibody assays that are rarely performed for this type of patient, thus allowing us to correlate the results from ELISA and WB, a newly commercialized technique. We observed a marked parallel increase as well as a synchronous decrease in both the antibody markers. An increase in antibody titres was observed after the patient came out of aplasia on day 30, and on day 61, a significant increase in *Aspergillus* antigen was observed. This might be related either to a higher release of galactomannans correlated to clinical deterioration and uncontrolled invasive aspergillosis or to a decrease in antibody titres resulting in a lower binding capacity of galactomannans (Herbrecht et al., 2002; Mennink-Kersten et al., 2004).

The present case report also highlights the interest in serological diagnosis (galactomannan and possibly anti-*Aspergillus* antibodies) in solid-organ transplant recipients, as described previously for haematological malignancies (Verweij, 2009; Persat, 2012). Better management of at-risk patients by simultaneous serum antigen and antibody follow-up would need larger studies. Furthermore, the results of the two antibody techniques (ELISA and WB) appear rather congruent, but larger studies are also needed to evaluate more precisely the kinetics of WB versus ELISA.


** References**

