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...
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, 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 $<2 \times 10^6$ 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 agranulocytosis associated with conscience disorders, pleuropneumonia and ascites). Voriconazole levels (residual level at 0.33 mg l$^{-1}$) 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 excavation 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
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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date of collection/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>
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<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>

*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.*

![Aspergillus serological kinetics in transplant patient](http://jmmcr.sgmjournals.org/3/)

**Fig. 1.** Serum kinetics of galactomannan ELISA assay (Ag), anti-*Aspergillus* antibodies ELISA assay (Ab) and WB. Ag and Ab ELISA were expressed as a ratio to their respective cut-off values, whereas the WB results are expressed in arbitrary units related to the intensity of reactive bands. MAS, macrophage activation syndrome.
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**


