Molecular diagnosis of *Aspergillus* endocarditis after cardiac surgery

Parisa Badiee, Abdolvahab Alborzi, Ealaheh Shakiba, Mazyar Ziyaeyan and Bahman Pourabbas

The prevalence of *Aspergillus* endocarditis (AE) is increasing in the hospital population. *Aspergillus* species contribute to approximately 25% of all cases of fungal endocarditis. This study is a descriptive report of the use of nested PCR to detect DNA specific for *Aspergillus* species in serum for the diagnosis of cardiac infections. Open heart surgery was performed on patients and collected samples were examined microscopically and cultured. Ten sera in total from five patients were extracted for *Aspergillus* DNA and nested PCR with *Aspergillus* species primers was carried out. The lowest limit of detection for the PCR assay was 1 c.f.u. (ml serum)^-1. The PCR was positive in three patients. Culture of valvular tissue confirmed the growth of *Aspergillus fumigatus* in one patient and *Aspergillus niger* in two patients. In this study we have demonstrated the presence of invasive aspergillosis in patients who had undergone open heart surgery and the usefulness of a molecular assay for the diagnosis of AE.

**METHODS**

Five cases of infected endocarditis have been recently diagnosed (within the 12 months preceding June 2008) at the Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, and were carefully investigated. A diagnosis of endocarditis required fulfillment of the Duke criteria and the presence of a vegetation (Durack et al., 1994). All the patients had undergone aortic valve replacement.

To determine the detection limit of the assay for fungal pathogens in the blood, suspensions of serum with *Aspergillus* (*Aspergillus flavus, Aspergillus niger* and *Aspergillus fumigatus*) conidia (1 to 10^5 conidia ml^-1) were diluted, and each solution was cultured for determining the colony count, and for DNA extraction and PCR.

Two blood specimens from each patient where there was a suspicion of fungal infection after heart surgery were examined for *Aspergillus*...
DNA. Aspergillus DNA was extracted from the sera of patients using the QIAamp DNA minikit (Qiagen) in accordance with the manufacturer’s recommendations. PCR was performed as Yamakami et al. (1996) suggested, which is a nested PCR with two sets of primers. It is worth mentioning that this PCR is able to identify all Aspergillus species. After examining the DNA bands of ethidium bromide-stained gels after electrophoresis, one patient received voriconazole immediately, and all patients underwent open heart surgery after 2 days. Infected valve specimens were cultured on Sabouraud dextrose agar (Merck) and other microbiological media, and examined microscopically in 10% potassium hydroxide solution. Definitive identification was made in all cases by microscopic examination in the specific culture medium with lactophenol cotton blue.

RESULTS AND DISCUSSION

The lower limit of detection of this PCR assay was 1 c.f.u. (ml serum)⁻¹. Nested PCR with Aspergillus species primers produced bands on ethidium bromide-stained gels in three patients (Fig. 1). Valvular vegetations were detected by echocardiography and fungal infections were documented in three patients by surgical exploration. Direct microscopic examination of the three samples was positive for fungal growth and on potassium hydroxide smear showed the characteristic pattern of Aspergillus (hyphae of 5–10 μm in width, which were branched with numerous septa distributed at regular intervals, consistent with typical Aspergillus species morphology). Culture of valvular tissue on Sabouraud dextrose agar confirmed the growth of A. fumigatus in one patient and A. niger in two patients. Two of the patients with AE that received late antifungal therapy (post-surgery) died and one patient lived. Staphylococcus aureus was isolated from the blood and valve replacement from two patients with negative PCR. The demographic and clinical features of the patients are listed in Table 1.

AE, most commonly found in open heart surgery cases, is associated with a high mortality rate in adults and children. Post-mortem studies indicate that AE is more invasive than Candida endocarditis (Feigin et al., 2004). The most common clinical features are fever, major peripheral emboli and a changing heart murmur (Rubinstein & Lang, 1995), as seen in all the presented cases. Non-specific laboratory findings, such as anemia, leukocytosis, elevated erythrocyte sedimentation rate and elevated C-reactive protein, are often present in patients with suspected PVE. The culture and histopathological samplings from embolic material for the diagnosis of AE are invasive. Because specimens must be obtained surgically, and since pathogenic fungi may require culturing times of 2–3 weeks or longer, definitive diagnosis may be delayed, thus impacting patient care. Aspergillus may be isolated from the infected valves in post-mortem or biopsy specimens. A positive blood culture is essential to establish an accurate microbiologic diagnosis and it is the major criterion in the Duke criteria. However, in patients with Aspergillus infection, isolation of the organism from blood cultures is exceedingly rare. Gumbo et al. (2000) reported endocarditis on a native or prosthetic valve or in a mural location characterized by peripheral embolization, and almost always there was a negative result from blood culture. However, Pemán et al. (2007) reported a case of fungal endocarditis in a native mitral valve with the isolation of A. fumigatus both in valve vegetation and in blood culture bottles. The patient underwent valve replacement and antifungal treatment with voriconazole and caspofungin, but he died on post-operative day 45 with disseminated aspergillosis confirmed by necropsy. Paradoxically, galactomannan antigen detection in the serum was negative. El-Hamamsey et al. (2004) reported three cases of Aspergillus aortic valve endocarditis caused by A. niger, A. fumigatus and Aspergillus spp. Despite attempts at combined medical and surgical therapy, mortality was 100% in their report. Sherman-Weber et al. (2004) reported the major pathogens that caused infective endocarditis in cardiac transplantsations were S. aureus (four cases) and A. fumigatus (three cases). Endocarditis-related mortality was 80%. All patients with AE died. Kotanidou et al. (2004) and McCracken et al. (2003) reported that for their patients with AE all serological tests for Aspergillus were negative, and did not help the diagnosis and follow-up.

The challenge we face today is to find a way to detect evidence of infection with non-invasive and high-accuracy innovative laboratory methods. Molecular identification of fungal infections has typically been presented in other research (Imai et al., 2000; Posteraro et al., 2000; Löffler et al., 1998; Einsele et al., 1997). A PCR assay for the detection of fungal nucleic acids may be the optimal diagnostic approach, because it offers the potential of being more sensitive than current culture-based methods and more applicable to a variety of specimen types (Van Burik et al., 1998; Badiee et al., 2007). A molecular approach to improve the microbiological diagnosis is reported in valvular heart disease cases by Breitkopf et al. (2005). Also, disseminated aspergillosis in one patient with
endocarditis diagnosed by a RFLP method was also reported (McCracken et al., 2003). In the present study all documented AE cases were positive with nested PCR. Because of the ubiquitous nature of the organism, establishing a definitive diagnosis of disease caused by Aspergillus is difficult. In order to maximize specificity, we require additionally the presence of valvular vegetation demonstrated by echocardiogram, the pathology of a surgical specimen, or documentation at autopsy. In our study, surgical specimens confirmed the definitive diagnosis by PCR test results.

One patient responded to treatment with an antifungal agent, but unfortunately in two patients, the diagnosis was too late and antifungal therapy was unsuccessful; the patients died. Two patients had negative PCR results and S. aureus was detected in both cultures from the blood and surgical samples. These patients were successfully treated and responded well to antibiotic therapy.

**Conclusion**

We demonstrated in the present study invasive aspergillosis in open heart surgery patients and the usefulness of a molecular assay for the diagnosis of AE. However, the power of such a study is quite limited because of the small number of cases studied.

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**REFERENCES**


**Table 1. Clinical features of patients with infected endocarditis after heart surgery**

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>Time of FIE after surgery (months)</th>
<th>No. of surgeries</th>
<th>Organism</th>
<th>Result of nested PCR</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>42/M</td>
<td>4</td>
<td>2</td>
<td><em>A. fumigatus</em></td>
<td>Positive</td>
<td>None</td>
<td>Dead</td>
</tr>
<tr>
<td>64/M</td>
<td>7</td>
<td>3</td>
<td><em>A. niger</em></td>
<td>Positive</td>
<td>Voriconazole + surgery</td>
<td>Alive</td>
</tr>
<tr>
<td>35/F</td>
<td>8</td>
<td>3</td>
<td><em>A. niger</em></td>
<td>Positive</td>
<td>Amphotericin + surgery</td>
<td>Dead</td>
</tr>
<tr>
<td>31/F</td>
<td>2.5</td>
<td>1</td>
<td><em>S. aureus</em></td>
<td>Negative</td>
<td>Antibacterial + surgery</td>
<td>Alive</td>
</tr>
<tr>
<td>25/M</td>
<td>3.5</td>
<td>1</td>
<td><em>S. aureus</em></td>
<td>Negative</td>
<td>Antibacterial + surgery</td>
<td>Alive</td>
</tr>
</tbody>
</table>

F, Female; M, male; FIE, fungal infected endocarditis.

**Table 1**. Clinical features of patients with infected endocarditis after heart surgery

- **Age/sex**: The age and sex of the patients
- **Time of FIE after surgery (months)**: The time interval between the heart surgery and the diagnosis of infected endocarditis
- **No. of surgeries**: The number of heart surgeries performed
- **Organism**: The type of organism causing the infected endocarditis
- **Result of nested PCR**: The result of the nested PCR test
- **Treatment**: The treatment administered for the condition
- **Outcome**: The outcome of the treatment (Alive or Dead)


