Case report

Late bioprosthetic valve endocarditis caused by *Phialemonium aff. curvatum* and *Streptococcus sanguis*: a case report

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Dual fungal and *Streptococcus sanguis* endocarditis is reported in a 63-year-old woman 7 months after placement of a porcine aortic valve prosthesis. Both microorganisms were isolated by blood cultures, and the patient succumbed after a full course of antibacterial chemotherapy without having received antifungal chemotherapy. The best possible designation of the fungus was *Phialemonium aff. curvatum* W. Gams & W. B. Cooke, as represented by CBS 331.93. At autopsy hyphae were revealed in the porcine valve tissue by conventional staining. A hyperimmune rabbit antiserum raised towards strain CBS 331.93 and extensively absorbed with heterologous fungal antigens reacted strongly with hyphae in the valve tissue by indirect immunofluorescence technique. We consider it most likely that the *Phialemonium* infection evolved insidiously from the time of open heart surgery and led to a haematogenous streptococcal infection of a more fulminant course.

**Keywords** immunohistochemistry, *Phialemonium aff. curvatum*, Prosthetic valve endocarditis, *Streptococcus sanguis*, taxonomy.

Introduction

The risk of endocarditis after placement of a cardiac valve prosthesis has been estimated at 1.4–3.0% [1]. Gram-positive bacterial infections are most common [1] but a diverse group of fungi also plays a role [2]. Fungal endocarditis should be suspected in patients with a cardiac valve prosthesis when a fungus is isolated repeatedly by blood culture. New imaging techniques, including transesophageal echocardiography may be helpful in confirming the diagnosis of infectious endocarditis. In rare cases of dual bacterial and fungal infection, however, presence of a mycosis may remain obscure until after the death of the patient. We present a case of endocarditis caused by *Phialemonium aff. curvatum* and *Streptococcus sanguis* involving a porcine aortic valve prosthesis with emphasis on mycological and immunohistochemical aspects.

Case report

The patient was a 63-year-old woman with arterial hypertension for 14 years and a systolic murmur for 6 years. A partial colectomy had been performed 9 months previously for an adenocarcinoma. The patient was admitted for a syncope, and Doppler echocardiography showed a valvular aortic stenosis. Invasive study confirmed a calcified valvular aortic stenosis with no regurgitation, the peak-to-peak gradient was 80 mm Hg and the left ventricular ejection fraction 0.70. The coronary arteries were patent without any significant stenosis.

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An operation was performed in extracorporal circulation with local and systemic hypothermia. The aortic valve was excised, an annuloplastic operation was performed with autologous pericardium, and a porcine prosthetic valve was inserted (MITROFLOW A23, Symbion Medical of Canada). Only autologous blood cells were transfused. After the operation the patient was normothermic and stable without inotropic support. Except for a solitary embolus of an arcadian artery of the right retina the postoperative period was uneventful. Echocardiography showed the prosthesis to be competent with an antegrade flow of 2 m s\(^{-1}\), that is within the expected range. At follow-ups 2 and 4 months after the operation the patient was well, but after 7 months she was admitted to a local hospital for dyspnoea, sweating and tiredness. On admission she was anaemic, in moderate distress, and the temperature was 39.0 °C. An acute anterior myocardial infarction was diagnosed by electrocardiography and an elevated level of serum creatine phosphokinase B (max. 46 U l\(^{-1}\), normal < 6 U l\(^{-1}\)) was determined.

Two sets of blood cultures (Colorbact, Statens Seruminstitut, Denmark) grew *Streptococcus sanguis* that was sensitive to penicillin and gentamicin. Pyrexia subsided 3 days after commencement of intravenous therapy with penicillin and gentamicin. No permanent intravenous line was placed. Transesophageal echocardiography revealed a paravalvular cavity (1.5 x 2.5 cm) in the thickened and oedematous aortic root close to the left atrium. The prosthetic valve was competent, and the left ventricular function was normal. There were no definite vegetations on the valvular leaflets.

After the initial defervescence, the patient had a relapse of fever and was intermittently febrile during the ensuing approximately 3 weeks. The patient remained on intravenous penicillin and gentamicin, and blood cultures at 7 days after admission were sterile. A fungus, however, was isolated from four sets of blood cultures obtained after day 15. The patient was transferred, and penicillin monotherapy was continued as transthoracic and transesophageal echocardiography showed no signs of progression. On day 25 the patient suddenly developed left-sided paralysis, and a CT scan of the cerebrum demonstrated a massive haemorrhage. The patient died on day 26 from cerebral herniation without antifungal therapy being instituted.

**Mycology**

**Primary isolation**

The fungal isolate (CBS 331.93) was obtained by blind subculture from six of eight aerobic culture bottles with a nominal content of 10 ml of blood each. Bacteriological media, including 5\% horse blood agar were incubated at 35 °C along with Sabouraud dextrose agar (Statens Seruminstitut, Copenhagen) at 30 °C. After overnight incubation of the plates, growth was recognized, and by direct microscopy elongated ellipsoid fungal cells were seen that were interpreted and initially reported as 'yeast cells'. The blood culture bottles revealed a flocculent growth at the bottom after extended incubation at room temperature.

**Description of CBS strain 331.93 (Fig. 1)**

Colonies reaching 9 mm diameter in 8 days on 2\% malt extract agar (MEA) at 21 °C, 17–19 mm at 24–27 °C, and 26–28 mm at 30–33 °C; temperature minimum for growth around 18 °C, maximum slightly above 36 °C. Colonies were whitish, moist and smooth, with the colony centre becoming pale sulphur yellow. On oatmeal agar (OA) after 10 days becoming ochraceous-grey (hazel) due to pigmented submerged vegetative hyphae. Vegetative hyphae were 0.5–2.5 μm wide. The odour was pronounced musty.

Chlamydospores were absent. Sporulation was observed on 2\% MEA, OA and, particularly, potato–carrot agar after 7–10 days; short phialidic necks were seen arising from superficial, almost submerged, or simple aerial hyphae, sometimes also with an integrated phialide terminating a hypha, more rarely discrete phialides arising in lateral position. Conidia were hyaline, smooth-walled, elongate ellipsoid 3–5 x 1–1.8 μm, length/width 3.0–3.5, or allantoid, 4–7 x 1–1.8 μm; the former predominating at temperatures up to 27 °C, the latter at higher temperatures.

The fungus is a typical member of genus *Phialemonium* because of its pattern of sporulation and very narrow.
vegetative hyphae. It is closest to *Phialemonium curvatum* by commonly producing allantoid conidia, but deviates by a more frequent occurrence of straight, ellipsoid conidia, and a higher temperature optimum (24-30 °C in *Phialemonium curvatum*). In *Phialemonium dimorphosporum* W. Gams & W. B. Cooke, straight ellipsoid conidia differ rather sharply from the allantoid ones, they are shorter, 2.5-3.5 × 1-1.5 μm, and the colonies tend to develop a vinaceous-buff reverse; the temperature optimum is equally lower.

**Histopathology**

At autopsy bilateral myocardiac hypertrophy was conspicuous. Neither myocardiac infarction nor fibrosis was apparent, and the coronary arteries were patent. The endocardium and native valves were normal. The aortic valve prosthesis was competent with small vegetations along the free valvular edge. No abscesses were seen in the annular region or the aortic root. Other significant findings included a pyramidal splenic infarction, a solitary tumour of the liver, and extensive haemorrhage of the right cerebral hemisphere.

Sections of the prosthetic valve revealed fibrin and inflammatory cells at the surface and infiltration by hyphal elements (Fig. 2). In sections of the aortic root formation a pseudoaneurysm was seen with ulceration of the endothelium, destruction of the media and micro-abscesses of both media and adventitia. No bacterial or fungal elements were seen. The liver tumour was diagnosed as a metastasis probably from the resected colonic adenocarcinoma. Cerebral vessels showed marked hypertensive alterations with perivascular evasion of blood, cerebral infarction with vacuolization and a macrophage reaction with no acute inflammatory response and no bacterial or fungal elements. Bacterial and mycological cultures obtained at autopsy were without significant growth.

**Immunohistopathology**

Immunohistopathology was applied to ascertain the pathogenic role of strain CBS 331.93 isolated from blood.

**Antigen**

Strain CBS 331.93 was grown in agitated Czapek-Dox broth at 25 °C for 14 days. The mycelial mat was disintegrated with an X-press (Biox AB, Sweden) and the somatic antigen was processed as previously described [3]. The protein content of the antigen preparation was 5.6 mg ml⁻¹. Four strains from the culture collection of CBS were processed in the same way (Table 1).

**Immunization schedule**

Two New Zealand rabbits were immunized for 4 months with subcutaneous doses of somatic antigen every third week. Freund's complete adjuvant was used initially and was substituted by Freund's incomplete adjuvant for
Table 1 Reactivity of rabbit antiserum raised against *Phialemonium* aff. *curvatum* CBS 331.93 as assessed by crossed immunoelectrophoresis (XIE) and indirect immunofluorescence (IIF) technique with homologous and heterologous fungal antigens

<table>
<thead>
<tr>
<th>Antigens*</th>
<th>XIE (No. of precipitin lines)</th>
<th>IIF† (Reactivity of staining)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unabsorbed antiserum</td>
<td>Unabsorbed antiserum</td>
</tr>
<tr>
<td><em>Phialemonium</em> aff. <em>curvatum</em> CBS 331.93</td>
<td>&gt; 25</td>
<td>+ + +</td>
</tr>
<tr>
<td><em>Phialemonium</em> curvatum CBS 490.82</td>
<td>8-9</td>
<td>nd</td>
</tr>
<tr>
<td><em>Fusarium</em> solani CBS 166.87</td>
<td>7-8</td>
<td>nd</td>
</tr>
<tr>
<td><em>Phialemonium</em> obovatum CBS 902.85</td>
<td>1</td>
<td>nd</td>
</tr>
<tr>
<td><em>Acremonium</em> kiliense CBS 535.86</td>
<td>1</td>
<td>nd</td>
</tr>
<tr>
<td><em>Aspergillus</em> fumigatus</td>
<td>3</td>
<td>+ + +</td>
</tr>
<tr>
<td><em>Aspergillus</em> flavus</td>
<td>3</td>
<td>+ + +</td>
</tr>
<tr>
<td><em>Aspergillus</em> niger</td>
<td>0</td>
<td>+ +</td>
</tr>
<tr>
<td><em>Absidia</em> corymbifera</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><em>Rhizopus</em> oryzae</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida</em> albicans</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida</em> tropicalis</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><em>Geotrichum</em> capitatum</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><em>Geotrichum</em> candidum</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

nd: not done.

*For sources of *Aspergillus* spp., Zygomycetes and yeast isolates see [7].
†IIF was carried out with experimentally infected murine tissues. Intensity of staining was graded on a scale from 0 to + + + [7].

Experimental infection of mice

Conidia were harvested from plates of Sabouraud dextrose agar pH 7.0 (Difco, USA) after 21 days of incubation at 25 °C. Infections were established in 6-week-old BABL/c ABom mice treated subcutaneously with prednisolone 5 mg per animal at days −3, −2, −1 and 0. An intravenous challenge of conidia (0.5 × 10⁸ colony forming units per mouse) in 0.5 ml of sterile 0.9% NaCl was given. The mice were sacrificed after 7 days and histopathological lesions were found in the cerebrum, lungs and myocardium that grew the *Phialemonium* strain in pure culture. Tissues were processed as previously described [4].

Immunofluorescence staining

The procedure used for indirect immunofluorescence (IIF) staining of formalin-fixed tissues was as previously described [3,4]. FITC-conjugated swine anti-rabbit IgG antiserum (F205, DAKO, Denmark) was used at a dilution of 1:20. For the most reactive of the two anti-*Phialemonium* aff. *curvatum* antisera, the plateau endpoint was 1:64 and the staining endpoint 1:256. Heterologous absorption was carried out at a dilution equal to the plateau endpoint with aliquots of somatic antigen of *Absidia* corymbifera, *Candida* albicans and *Geotrichum* candidum as well as a cell wall fraction of *Aspergillus* fumigatus, respectively. A second and third absorption was also carried out (1:60) with the *A. fumigatus* somatic antigen (Table 1).

IIF staining of prosthetic valve tissue

The immunological specificity of the staining was confirmed with sections of tissue from mice experimentally infected with a range of fungal agents (Table 1). Heterologously absorbed anti-*Phialemonium* antiserum reacted strongly with the fungal elements in the prosthetic valve (Fig. 3). Preimmune sera from the two rabbits were unreactive.

Discussion

This case history is remarkable for the concurrent bacterial and fungal endocarditis associated with a porcine bioprosthetic valve and affecting the paravalvular area and the aortic root. Both agents were isolated by blood
culture, but fungal elements only were demonstrated by postmortem examination of the porcine valve. Cardiac and aortic lesions were probably devoid of streptococci because of the intensive and prolonged antibacterial therapy that had been associated with a clear-cut, but temporary clinical response.

The isolation of Phialemonium was unexpected and no antifungal chemotherapy was instituted as its significance became understood only after the postmortem examination. This taxon is rarely seen in clinical microbiological laboratories. The species so far described in Phialemonium are supported by considerable numbers of isolates which seem to fall into morphologically discrete clusters; but they are still rather difficult to distinguish, and several isolates of similar taxa have become available since the publication of the genus by Gams & McGinnis [5] which makes identification difficult. Therefore it does not seem warranted to describe additional taxa at this time based on single isolates without, at least, producing molecular evidence for their distinctness. Therefore the best possible designation of the present fungus is Phialemonium aff. curvatum W. Gams & W. B. Cooke, as represented by CBS 331.93. The identity of the fungus infecting the prosthetic valve and the blood isolate was substantiated by immunohistochemistry. Several taxa of Acremonium which are close to Phialemonium on morphological grounds have been associated with opportunistic infections, e.g. in relation to exit sites of intraperitoneal or intravascular catheters [6-9]. It must be emphasized that our patient did not have a permanent intravascular catheter at any time.

Species of the genus Phialemonium have been linked to human infection in two patients who were renal transplant recipients: a granulomatous foot infection in a 50-year-old woman and peritonitis in a 5-year-old girl [10]. McGinnis et al. [11] reported a cutaneous Phialemonium obovatum infection, which had probably become disseminated, in a child with extensive thermal burn injuries. Moreover, in the formal description of the genus Gams & McGinnis [5] mentioned a number of isolates of clinical origin mostly from skin, nail and cornea. The case reported here adds information on the clinical spectrum, and Phialemonium spp. should be added to the expanding list of fungal agents that may infect prosthetic heart valves [2,12,13].

Lecso-Bornet et al. [12] have described an insidious course of endocarditis of a porcine aortic valve prosthesis due to a ubiquitous thermophilic hyphomycete Thermo-mycetes lanuginosus. The fungus was an unexpected isolate at autopsy 7 months after insertion of a valve prosthesis. Endocarditis caused by both Listeria monocytogenes and Acremonium (Cephalosporium) has been reported in a patient with a calcified native aortic valve [14]. The occurrence of dual infections is compatible with one agent predisposing for another, but the order of events should be judged cautiously from the blood cultures, as the agents may differ widely in numbers of colony-forming units released, growth rate and readiness of detection of growth by the blood culture system. Thus, the sequence of events remains speculative, but we find it most likely that the Phialemonium infection was primary and by endothelial damage formed a nidus for a more rapidly evolving streptococcal infection. As suggested by Lecso-Bornet et al. [12] and Opal et al. [13], who described a case of Aspergillus clavatus aortic valve endocarditis after coronary by-pass surgery, Phialemonium may have been introduced during open heart surgery as a contaminant of the bioprosthesis. Alternatively, airborne conidia may have impacted in the operation field during valve placement [15]. A low growth rate close to the temperature maximum along with other unfavourable factors in the milieu of the body may have retarded the evolution of the infection.

References


