Non-valvular Infective Endocarditis Caused by *Sarocladium kiliense* in an Immunocompromised Patient with Aplastic Anemia: A Case Report

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Abstract:
*Sarocladium kiliense* is ubiquitous in the human environment and is an emerging opportunistic pathogen, especially among immunocompromised hosts. A 77-year-old man diagnosed with aplastic anemia suffered from non-valvular endocarditis. After he passed away, fungal hyphae were observed in several lesions on a postmortem examination. Polymerase chain reaction (PCR) and a DNA sequence analysis revealed *S. kiliense* as the causative organism. This is the first case report of non-valvular fungal endocarditis caused by *S. kiliense* identified by PCR and a DNA sequence analysis in an immunocompromised patient. Although rare, invasive fungal infection caused by *S. kiliense* should be considered in immunocompromised hosts.

Key words: *Sarocladium kiliense*, infective endocarditis, non-valvular vegetation, aplastic anemia, immunocompromised host

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Introduction

Fungal endocarditis (FE) is rare, accounting for approximately 1%-6% of all cases of infective endocarditis (IE); however, it is a devastating disease with high mortality (1, 2). It occurs predominantly in patients who are immunocompromised; in intravenous drug abusers; in recipients of long-term antibiotic treatment, parenteral nutrition, or prosthetic heart valves; or in those who have received reconstructive cardiac surgery. *Candida* species are the most common cause of FE (53%-68% of cases), followed by *Aspergillus* (20%-25% of cases). Other less-common causes include *Coccidioides*, *Cryptococcus*, *Histoplasma*, and *Blastomyces* (3). However, modern medical techniques and new pathologies impacting the immune system may make humans more susceptible to infections caused by emerging agents, including *Sarocladium* species (4).

We herein report what is to our knowledge the first case of non-valvular FE caused by *Sarocladium kiliense*, previously known as *Acremonium kiliense* (5), identified by polymerase chain reaction (PCR) and a DNA sequence analysis after an autopsy of an immunocompromised patient with aplastic anemia (AA).

Case report

A 77-year-old man with hypertension presented to our hospital with pancytopenia. He had no history of heart disease. He was diagnosed with acquired idiopathic AA. Be-
cause he engaged in agricultural work, he chose outpatient treatment with cyclosporine (CsA) and eltrombopag (EPAG). Regular transfusions of red blood cells and platelets were also required. Two months later, he was hospitalized and received treatment with anti-thymocyte globulin (ATG). Computed tomography (CT) revealed no obvious focus of infection. Transthoracic echocardiography (TTE) revealed moderate aortic valve regurgitation, mild pulmonary hypertension, and left atrial enlargement. The patient was treated with ATG plus dose-escalation CsA and EPAG (intravenous infusion of ATG: 2.5 mg/kg/day from Day 1 to 5; oral CsA 6 mg/kg/day from Day 1; and oral EPAG, 75 mg/day from Day 1). Oral itraconazole (ITCZ; 100 mg/day) was given for fungal prophylaxis, and oral trimethoprim-sulfamethoxazole was given to prevent Pneumocystis jirovecii pneumonia. Treatment was effective, and the patient was discharged from the hospital on Day 36.

However, at 1.5 months post-ATG treatment, the patient complained of a fever lasting for a few days, fatigue, and anorexia. His temperature was 38.5 °C. A blood analysis revealed the following: hemoglobin (Hb) concentration, 70 g/L; white blood cell (WBC) count, 1.4×10^9/L (52.8% neutrophils, 19.4% lymphocytes, and 27.8% monocytes); platelet count, 14×10^9/L; creatinine, 89.21 μmol/L; and C-reactive protein (CRP), 143.5 mg/L. Serum 1,3-β-D glucan, Candida mannan antigen, Aspergillus galactomannan antigen, and Cryptococcus neoformans antigen levels were negative. T-SPOT® and Cytomegalovirus pp65 antigen (C7-HRP) tests were negative. Two sets of blood cultures were also negative.

CT revealed consolidation in the apex of the left lung (Fig. 1A), consolidation with a cavity in the right middle lung (Fig. 1B), multiple low-density splenic lesions (Fig. 1C), and right non-enhancing renal lesions (Fig. 1D). He strongly requested outpatient treatment because of agricultural work. Since he was considered to be immunosuppressed due to ATG plus CsA treatments, oral levofloxacin (250 mg/day) and amoxicillin/clavulanic acid (1,125 mg/day) were administered according to the recommendations for outpatient management of cases with a fever and neutropenia in adult patients treated for malignancy (6). ITCZ was replaced with voriconazole (VRCZ; 400 mg/day) in consideration of the possibility of invasive aspergillosis based on the CT findings of lung lesions, although the results of fungal serologic tests were negative. Fortunately, his temperature temporarily decreased after the treatments; however, a low-grade fever occasionally occurred for a week.

TTE was performed for the further examination of bilateral leg edema. Unexpectedly, a mobile vegetation (20×27 mm) was found attached to the basal interventricular septum, close to the left ventricular (LV) outflow tract (Fig. 2). No mitral valve regurgitation was observed. He was diagnosed with possible IE according to the modified Duke criteria (7) and re-admitted to the hospital.

His temperature was 36.2 °C. A physical examination was unremarkable, except for the bilateral leg edema. Blood tests
revealed the following: Hb concentration, 81 g/L; WBC count, 6.6×10^9/L (59.2% neutrophils, 24.0% lymphocytes, 16.4% monocytes, 0.2% eosinophils, and 0.2% basophils); platelet count, 13×10^9/L; creatinine level, 160.13 μmol/L; CRP level, 191.6 mg/L; and brain natriuretic peptide level, 162.0 ng/L. No antinuclear antibodies were detected. After considering the perioperative risk, medical management with antibiotics was chosen.

No common pathogen of IE was isolated from two sets of blood cultures at the time of admission. Empirical antibiotic treatment with a combination of intravenous ampicillin/sulbactam and vancomycin was initiated due to suspicion of bacterial endocarditis. VRCZ was discontinued considering the low probability of fungal infection due to the negative results of fungal serologic tests. His temperature increased to 39 °C. Another six sets of blood cultures taken on Days 2, 4, and 8 post-admission remained negative. Unfortunately, multiple organ failure progressed rapidly, and the patient passed away on Day 8 post-admission (Fig. 3).

An autopsy was performed to identify the infecting organism and the direct cause of death. A vegetation (47×35 mm) was identified, attached mainly to the LV wall (Fig. 4A). Grocott’s methenamine silver staining revealed branched fungal hyphae (Fig. 4B and C). They were also observed in the right pulmonary lesion. Gram and Ziehl-Neelsen staining revealed no bacteria. These findings provided clues that led to a diagnosis of FE. A vegetation (15×7 mm) was also identified in the tricuspid valve. Subacute myocardial infarction due to coronary embolization caused by vegetations was noted. In addition, multiple organ infarctions following dissemination of the vegetations were observed in the ectopic thyroid, right lung, liver, spleen, and right kidney.

Consequently, a QIAamp DNA Mini Kit (Qiagen) was used to extract DNA from two sections of formalin-fixed, paraffin-embedded tissue obtained from the vegetations. DNA was also prepared from another two sections using a newly purchased QIAamp DNA FFPE Tissue Kit (Qiagen). PCR assays targeting the 28S ribosomal RNA D1-D2 and ITS1-5.8S-ITS2 regions were performed to identify the fungal species (8, 9). Sequencing the PCR products using the Basic Local Alignment Search Tool (BLAST) showed that the DNA sequences were 100% and 99.6% homologous with S. kiliense-type strains (accession nos. NG_057887 [28S ribosomal RNA D1-D2] and NR_130684 [ITS1-5.8S-ITS2 region], respectively). The 546 and 554 base pair amplicons were submitted to GenBank (accession nos. LC580004 and LC580005, respectively). The fungus in the vegetation was identified as S. kiliense. Scanning electron microscopy

![Figure 2. A transthoracic echocardiogram taken using a four-chamber view shows a vegetation (arrow) attached to the basal interventricular septum. LA: left atrium, LV: left ventricle, MV: mitral valve, RA: right atrium, RV: right ventricle](image)

![Figure 3. Clinical course after the first visit to our hospital. ABPC/SBT: ampicillin/sulbactam, AMPC/CVA: amoxicillin/clavulanic acid, ATG: anti-thymocyte globulin, CRP: C-reactive protein, CsA: cyclosporine, EPAG: eltrombopag, ITCZ: itraconazole, LVFX: levofloxacin, VCM: vancomycin, VRCZ: voriconazole, WBC: white blood cell](image)
Figure 4. Findings of a Sarocladium kiliense vegetation at autopsy. A A vegetation attached to the left ventricular wall (arrow). B, C Grocott’s methenamine silver stain of the vegetation. D–F Scanning electron microscopy photographs of the vegetation.

(SEM) photographs revealed irregularly shaped hyphae showing plump, shriveled, or constricted patterns with different widths. They also identified Y-shaped, T-shaped, or highly branched hyphae (Fig. 4D-F). The direct cause of death was noted as vegetations and septic embolism caused by S. kiliense.

Discussion

We reported the clinical course of FE caused by S. kiliense in an immunocompromised patient with AA. The patient suffered from life-threatening IE despite receiving empirical antibiotic treatment. In this case, IE was unlikely to be a differential diagnosis of his persistent fever because there were no obvious physical signs. Ultimately, he died, and PCR and a DNA sequence analysis of a vegetation following an autopsy revealed S. kiliense as the causative pathogen. The present case was therefore ultimately diagnosed as one of FE caused by S. kiliense.

Summerbell et al. reviewed the taxonomy of Acremonium and reported that in 2011, some species, including S. kiliense, were transferred to the genus Sarocladium (5). Sarocladium species are widespread in the environment, where they exist as saprobes in soil; they can be opportunistic pathogens in immunocompromised patients, although the incidence is extremely low (10). S. kiliense is expected to become a new emerging nosocomial fungal pathogen (11). In the present case, agricultural work might have been an unexpected source of infection.

Recently, both localized and systemic infections have been recognized. For example, keratitis, endophthalmitis, mycetoma, onychomycosis, and cutaneous infections caused by localized infection by Sarocladium species have been reported in immunocompetent patients. In contrast, Sarocladium species may cause disseminated infections, such as catheter-related bloodstream infections in immunocompromised patients; such infections may involve multiple organs in severe cases (12, 13).

Regarding S. kiliense infection in hematological diseases, Junior et al. reported invasive fungal infections in patients with hematological malignancies. They identified a rare mold infection, and fungemia caused by S. kiliense was confirmed in 1 of 19 patients (14). Furthermore, FE caused by S. kiliense is rare (a single case of endocarditis in a prosthetic heart valve was reported in Portuguese in 1981 (15)). Therefore, little is known about FE caused by S. kiliense. In the present patient, we confirmed fungal hyphae in Grocott’s methenamine silver staining and identified S. kiliense by PCR and a DNA sequence analysis. It was also found in another organ. Unfortunately, PCR with a blood sample could not be performed because no antemortem blood specimens were available. In addition, two kinds of DNA extraction kits (one of them newly purchased) were used. Therefore, we consider that the detection of S. kiliense was not the result of contamination during sample preparation. We also present SEM images of S. kiliense endocarditis for the first time.

The sensitivity of blood cultures for detection of Candida species is 50%-75%. In contrast, only 4% of Aspergillus species can be detected in blood cultures in cases of Aspergillus endocarditis (AE) (3). The percentage of positive blood cultures is not very high. Therefore, as in the present case, blood cultures may not be able to contribute to the detection of the causative organism of FE.

Anatomically, the mitral or aortic valve is the most common site of FE (16). Non-valvular vegetations have been shown to be significantly associated with AE (17). In our case, TTE revealed a vegetation attached to the basal inter-
ventricular septum, which presented as a non-valvular vegetation. The autopsy revealed that it was attached mainly to the LV wall, although the mechanism underlying the non-valvular vegetation development remains unclear. Non-valvular vegetation may also be a clinical characteristic of FE caused by \textit{S. kiliense}.

According to therapeutic data based on a few case reports, \textit{S. kiliense} is not very sensitive to amphotericin B. As a result, VRCZ or PSCZ may be better therapeutic alternatives (12-14). In general, mortality rates for FE are incredibly high despite aggressive treatment with a combination of medical and surgical interventions (18). In our case, antifungal treatment with VRCZ should have been continued, considering the possibility of FE at the time when the patient was admitted; however, even if he had continued to receive antifungal therapy, a poor clinical course would have been likely without surgical intervention.

In conclusion, although early recognition of FE is difficult, it should be considered as a differential diagnosis when non-valvular vegetation is suspected. In addition, \textit{S. kiliense} should be considered as a causative pathogen of emerging infections, particularly in immunocompromised hosts who present with clinical signs and symptoms suggestive of fungal infection.

The authors state that they have no Conflict of Interest (COI).

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Informed consent

Informed consent was obtained from the patient’s next of kin for publication of this case report.

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