Rasamsonia sp: An emerging infection amongst chronic granulomatous disease patients. A case of disseminated infection by a putatively novel Rasamsonia argillacea species complex involving the heart

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\textbf{ABSTRACT}

Chronic granulomatous disease (CGD) is a heterogeneous condition due to defects in NADPH oxidase characterized by granuloma formation and increased susceptibility to invasive infections. The use of broad-spectrum, mould-active antifungal prophylaxis has improved mortality. However rare resistant moulds have emerged as important pathogens. Diagnosis of these rare fungi requires molecular techniques, and treatment data are limited. Herein, we present a case of with disseminated Rasamsonia infection involving the heart.

1. Introduction

Chronic granulomatous disease (CGD) is a heterogeneous condition due to defects in NADPH oxidase characterized by granuloma formation and increased susceptibility to invasive infections. The defect in NADPH oxidase and the subsequent inability to generate microbicidal reactive oxidants predispose CGD patients to a wide array of infections. Most common pathogens which cause disease include, \textit{Staphylococcus aureus}, \textit{Serratia marcescens}, \textit{Burkholderia cepacia} complex, \textit{Nocardia} and \textit{Aspergillus} spp \cite{1}. Among fungi, filamentous species, especially \textit{Aspergillus} spp., have emerged as a major cause of mortality and morbidity amongst CGD patients. This has led to the routine use of anti-mould prophylaxis which has improved mortality \cite{2}. However rare moulds with reduced antifungal susceptibility profiles have emerged as important pathogens. Diagnosis of these rare fungi requires molecular techniques, and treatment data are limited. The use of DNA sequence analysis and the increasing number of fungal sequences available in databases have permitted improved identification. Herein, we present a case of with disseminated Rasamsonia infection involving the heart.

2. Case

A 35-year-old male with CGD and ulcerative colitis presented with persistent fevers, night sweats, weight loss, fatigue, and cough. His symptoms had been ongoing for a period of several months after being off prophylactic antimicrobials for several years (day 0). Initial imaging at that time had shown pulmonary nodules. Repeat imaging at time of presentation showed increase in the size of the pulmonary nodules, and new chest and abdominal lymphadenopathy, splenomegaly, as well as a lytic lesion in the left acetabulum (day +165). Attempts to establish a diagnosis by a sampling of the left acetabular lesion were unsuccessful. Pathology of the acetabular lesion revealed non-specific micro-abscesses and non-necrotizing granulomas. However, staining and cultures for bacteria, fungal, and mycobacteria were negative (day +179). He was resumed on trimethoprim-sulfamethoxazole, voriconazole prophylaxis, and interferon-gamma, which resulted in a resolution of his symptoms (day +284). Serial imaging over the next 1.5 years showed decreasing size of the lymphadenopathy and nodules. Two years after the resolution of symptoms, he was started on azathioprine due to worsening ulcerative colitis (day +797). However, he developed similar symptoms soon after (day +879). Computed tomography (CT) scan showed an increase in the size of pulmonary nodules and a new infiltrating right ventricular mass extending to the pericardial space that measured $4.4 \times 3.6$ cm (Fig. 1) (day + 902). He underwent a sternotomy and debridement of the cardiac mass and wedge resection of a lung nodule (day +919). Operative cultures of both the lung nodule and the cardiac mass grew mould (Figs. 2 and 3). Operative pathology revealed septate hyphae (Fig. 4).
Initially, his operative cultures were thought to be either *Penicillium* or *Paecilomyces* species based on the morphological examination. He was placed on intravenous (IV) liposomal amphotericin B (5 mg/kg/day), IV caspofungin (150 mg/day), and high dose oral posaconazole extended release tablets (500 mg/day for target levels of 3–5 μg/mL) with improvement in symptoms (day + 928).

The isolate was sent to the Fungus Testing Lab at the University of Texas Health at San Antonio, Texas for species identification and was accessioned as UTHSCSA DI19-1. Macroscopic and microscopic examination of the culture, showed phenotypic features salient to *Rasamsonia* species i.e. cream to tan colonies on potato flake agar (PFA, prepared inhouse) and sabouraud dextrose agar (SDA, Bio-Rad), growth at 37°C, roughened conidiophores and phialides, smooth, hyaline, cylindrical conidia in chains (Fig. 3). Isolate UTHSCSA DI19-1 was identified as a *Rasamsonia* species based on these characteristics [3]. The ITS region and partial beta tubulin gene (TUB) gene were chosen for sequencing to compare with sequences of the same loci in previous studies [3]. DNA extraction was done as previously described [4]. PCR and sequencing were carried out using primer pairs ITS1F and ITS4R for ITS and BT2a and BT2b for partial TUB [5,6]. The generated sequences, GenBank accessions MK630678 (ITS), MK636872 (TUB) were used to perform BLASTn searches in GenBank [7]. BLASTn search results were considered significant with an E-value of 0.0, at 97–100% identity and from 90% query coverage. Phylogenetic analyses were conducted independently with each locus and combined to assess the relationship of isolate UTHSCSA DI19-1 to species in the *R. argillacea* species complex.

The maximum likelihood method was used based on the Tamura 3-parameter evolutionary model with a Gamma distribution (TN92+G) for ITS and the Kimura 2-parameter with Gamma distribution for TUB [8,9]. The evolutionary models were determined by the Finding Model program as implemented in Molecular Evolutionary Genetics Analysis ver. 7 software (MEGA 7) [10]. BLASTn search results for ITS and TUB showed UTHSCSA DI19-1 closest to species in the *Rasamsonia argillacea* species complex. The maximum likelihood method was used based on the Tamura 3-parameter evolutionary model with a Gamma distribution (TN92+G) for ITS and the Kimura 2-parameter with Gamma distribution for TUB [8,9]. The evolutionary models were determined by the Finding Model program as implemented in Molecular Evolutionary Genetics Analysis ver. 7 software (MEGA 7) [10]. BLASTn search results for ITS and TUB showed UTHSCSA DI19-1 closest to species in the *Rasamsonia argillacea* species complex. The ITS BLAST search showed 98% identity with *R. argillacea* CBS 101.69T (NR_103623), 98% with *R. eburnea* CBS 100538T (NR_119934), 99% with *R. piperina* CBS 408.73T (NR_120176) and 99% with *R. aegroticola* IHEM 22641T (NR_111447) and in the TUB BLASTn search, 98% identity with *R. argillacea* CBS 101.69T (JF417456), 98% with *R. eburnea* CBS 100538T (JF417462), 97% with *R. piperina* CBS 408.73T (JX273000) and 97% with *R. aegroticola* DTO 137C3 (JX273007). Independent phylogenetic analysis of the ITS and TUB showed the isolate placed within the *R. argillacea* complex but not grouping with any of the 4 species clades in the complex (data not shown). The maximum likelihood tree of combined ITS and TUB also showed that our isolate is phylogenetically distant from the other species in the complex (Fig. 5) and could represent a new species (day 948). Additional genes would need to be included in the phylogenetic analysis and other phenotypic features need to be

![Fig. 1. CT chest with contrast demonstrating lung nodule.](image1)

![Fig. 2. Macroscopic morphology of *Rasamsonia* sp. UTHSCSA DI19-1 on Sabouraud dextrose agar.](image2)

![Fig. 3. Microscopic morphology of *Rasamsonia* sp. UTHSCSA DI19-1 showing asymmetric biverticillate penicillus and cylindrical to ovoid shaped conidia on SDA.](image3)

![Fig. 4. Grocott methenamine staining of cardiac mass pathology showing septate hyphae fungi.](image4)
investigated to confirm that UTHSCSA DI19-1 is a novel species within the *R. argillacea* species complex. Minimum inhibitory concentrations (MICs) against amphotericin B, posaconazole, and voriconazole were measured by broth microdilution according to the methods in the CLSI M38-A2 standard, and were 1.0, 1.0, and > 16.0 μg/mL, respectively. The minimum effective concentration (MEC) of caspofungin was determined to be 0.03 μg/mL.

Over the following four to eight weeks, the patient had radiographic resolution of his cardiac mass and pulmonary nodules. After six months of combination therapy with IV liposomal amphotericin B, IV caspofungin, and oral posaconazole, he was transitioned to lifelong monotherapy with high dose oral posaconazole (day +1150) and is currently doing well (day +1468).

### 3. Discussion

Among fungi, filamentous species, especially *Aspergillus* spp., have emerged as a major cause of mortality and morbidity amongst patients with CGD. This has led to the routine use of mould-active azole prophylaxis, which has improved mortality, though breakthrough infections with atypical moulds have been known to occur [2,11].

*Rasamsonia argillacea* is a thermotolerant fungus, previously classified as *Geosmithia argillacea* [12]. Routine identification using phenotypic methods can be challenging because of its similarity to *Aspergillus* spp., have emerged as a major cause of mortality and morbidity amongst patients with CGD. This has led to the routine use of mould-active azole prophylaxis, which has improved mortality, though breakthrough infections with atypical moulds have been known to occur [2,11].

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the patient's treatment course.

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