Pulmonary Protothecosis in a Pediatric Liver Transplant Patient

Ronald M. R. Tan, Marion M. Aw, Seng Hock Quak, and Si Min Chan
Department of Paediatrics, Khoo Teck Puat - National University Children’s Medical Institute, National University Hospital, Singapore

Corresponding Author: Si Min Chan, MBBS, FRCPCH, PGDip PID, Department of Paediatrics, Khoo Teck Puat - National University Children’s Medical Institute, National University Hospital, National University Health System, 1E Kent Ridge Rd, NUHS Tower Block Level 12, Singapore 119228. E-mail: si_min_chan@nuhs.edu.sg.

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Immunocompromised patients are susceptible to infections from common and unusual microorganisms. Protothecosis is seldom suspected on clinical grounds, yet it is readily diagnosed once detected in the laboratory. We report the first pediatric liver transplant recipient with pulmonary protothecosis, detected during an episode of Pneumocystis jirovecii pneumonia, and we conducted a review of the available literature.

Key words. algae; amphotericin B; liver transplant; opportunistic infection; protothecosis.

Protothecosis is an unusual infection in humans and is caused by achlorophyllous algae of the genus Prototheca. Most are isolated cutaneous infections with low-grade inflammation and an indolent course. However, immunocompromised patients may develop systemic infections with florid symptoms.

CASE REPORT
A 4-year-old Chinese girl presented with 2 weeks of productive cough, 1 week of intermittent low-grade fever, and 3 days of breathlessness, lethargy, and poor oral intake. Intermittent coughing episodes had been noted in the previous 4 months.

She had received a living-related liver transplant 8 months previously for congenital biliary atresia, after a Kasai procedure at 50 days of life. Her immunosuppressants comprised 1 mg of tacrolimus 2 times a day and 1 mg of prednisolone once a day. Tacrolimus level at presentation was 5.7 micrograms (mcg)/L (range, 4–20 mcg/L). One week earlier, the dose had been reduced from 2 mg every morning and 1 mg every night, due to a high serum tacrolimus level of 22.9 mcg/L. This reaction may have been due to inconsistent compliance with medication. Four months earlier the tacrolimus level was 12 mcg/L; there had been no dose changes for the last 5 months. Co-trimoxazole prophylaxis had been discontinued 3 months after transplant, per local protocol. The patient did not have any post-transplant rejection.

The patient’s father and elder sister recently recovered from a cough. There was no recent travel or known tuberculosis contact. The patient denied exposure to animals, farms, rural areas, or natural water bodies. The patient’s vaccinations were up to date, including varicella-zoster and pneumococcal conjugate vaccines.

Upon admission, the patient’s temperature was 37.5°C, her heart rate was 115/min, her respiratory rate was 65/min, and her SpO2 was 88% on room air. Physical examination revealed retractions, bilateral crepitations, and a generalized erythematous papular-pustular rash over her face, body, and upper limbs.

Arterial blood gas showed the following: pH 7.39, pCO2 28 mmHg, pO2 67 mmHg, HCO3 17 mmol/L, base excess –8. Chest x-ray (CXR) revealed bilateral patchy infiltrates. Full blood count and renal and liver function tests were unremarkable, except for albumin (28 g/L) and lactate dehydrogenase (1045 U/L). Erythrocyte sedimentation rate was 37 mm/h and C-reactive protein was 6 mg/L. Plasma Epstein-Barr virus (EBV) polymerase chain reaction (PCR) was positive (660 copies viral DNA/mL); a repeat level 1 week later was negative. Blood cultures, blood cytomegalovirus (CMV) PCR, serum galactomannan index, nasal swab for influenza A/B PCR, and urine streptococcus pneumoniae antigen were negative.
Bronchoalveolar lavage (BAL) showed normal airway appearance. Cytology revealed *P. jirovecii* cysts on Gomori methamine staining (GMS). No organisms were seen on Gram stain; bacterial culture had mixed growth with methicillin-sensitive *Staphylococcus aureus* ($1 \times 10^4$). Cytomegalovirus, EBV, and rhinovirus PCR were positive. Bronchoalveolar lavage studies for other viruses, *Mycoplasma*, and mycobacteria were negative.

The patient was treated with face mask oxygen, 3 weeks of co-trimoxazole, 1 week of hydrocortisone, and 2 weeks of oral prednisolone. Tacrolimus was discontinued. She received piperacillin-tazobactam for 1 week. Oseltamivir was stopped after 1 day. After 14 days of ganciclovir, she developed febrile neutropenia, which improved after stopping ganciclovir and administering granulocyte-colony stimulating factor for 5 days and cefepime for 1 week.

Two weeks later, creamy yeast-like colonies were cultured on Sabouraud agar from the BAL specimen. Wet mount preparation showed round-oval *Prototheca wickerhamii* organisms with a characteristic symmetrical endosporulating appearance (Figure 1). The species was identified using the Vitek yeast identification database (bioMerieux). Minimum inhibitory concentration values on susceptibility testing were as follows: $>256$ mg/L fluconazole, 1.5 mg/L voriconazole, and 0.19 mg/L amphotericin B.

By then, the patient had clinically improved except for persistent cough and worsened forehead and nose rash. Further investigations were done to determine the extent of protothecal infection. Blood fungal cultures for *Prototheca* were negative. Repeat CXR showed improvement. Computed tomography (CT) scan of the thorax showed diffuse ground-glass opacification, centrilobular nodules, and prominent mediastinal lymphadenopathy.

Figure 1. *Prototheca wickerhamii* isolated from our patient. A, Creamy white yeast-like colonies of *P. wickerhamii* on Sabouraud agar. B, Wet mount preparation (unstained) showing *P. wickerhamii* with symmetrical endosporulating appearance. Note that the organisms are of variable size.

DISCUSSION

*Prototheca* organisms are ubiquitous in nature. There are 5 species of *Prototheca*, but only *P. wickerhamii* and *Prototheca zopfii* are known to be pathogenic in humans [1].

Over 100 cases of human protothecosis have been described since 1964, mainly from Japan, the United States, and Europe [1, 2]. Two to 5 new cases are reported yearly, suggesting a constant incidence [2]. Most patients were over 30 years old or elderly [1]. Only 8 pediatric cases have been reported [3, 4].

The pathogenesis of protothecosis is not well understood. *Prototheca* can colonize the fingernails, skin, and respiratory and gastrointestinal tracts of humans [5]. Infection may occur after contact with potential sources such as water, soil, animals, or food; or traumatic inoculation with or without existing breach of skin integrity [1]. Human to human transmission is not known [1]. Defects
in cellular immunity may predispose to protothecosis, although the specific circumstances under which *Prototheca* infection develops are not always predictable [1]. Recognized risk factors for pathogenic protothecosis include diabetes mellitus, peritoneal dialysis, local or systemic steroid use, acquired immune deficiency syndrome (AIDS), renal transplantation, and lymphocyte or neutrophil defects (although no specific immunodeficiency has been identified). Our patient had poor cell-mediated immunity, reflected by low adenosine triphosphate levels (24 ng/mL) on Cylex ImmuKnow assay.

*Prototheca* are considered rare opportunistic pathogens of moderate pathogenicity and virulence [1]. In a review of 108 cases of protothecosis, overall attributable mortality rate was reported as 2.2%, much lower than that for candidemia [2]. However, individual outcome may vary depending on the clinical context. In a case series for protothecosis complicating cancer, overall mortality was 54% and attributable mortality was 14% [6], but in adult transplant recipients this rate was 88% and 85%, respectively [5]. In disseminated protothecosis treated with amphotericin B, 2 of 3 patients with cancer died [6] and all 4 transplant recipients died [5].

*Prototheca* species are spherical unicellular organisms that reproduce by endospore formation [1]. They grow well at 25–37°C, appearing as soft wet yeast-like white-to-tan colonies on Sabouraud-dextrose agar [1]. On Gram stains, *Prototheca* may be confused with yeasts; however, *Prototheca* species stain well with fungal stains such as GMS, Grocott, and Periodic acid-Schiff [5]. Morphologically, *Prototheca* are similar to *Lacazia loboi*, *Coccidioides immitis*, *Histoplasma duboisi*, *Blastomyces dermatitidis*, and *P jirovecii* [1]. After a retrospective review of our BAL cytology specimen, we determined that the organisms seen were more consistent with *P jiroveci*—uniformly-sized, boat-shaped, associated with casts, and no endospores or complex internal structures—whereas *Prototheca* organisms are more variable in size and shape.

Protothecosis has 3 clinical forms: cutaneous lesions, olecranon bursitis, and disseminated or systemic infection [1, 3].

Cutaneous protothecosis usually manifests as a vesiculobullous ulcerative lesion with purulent discharge. Many different lesions have been reported including erythematous plaques, pustules, nodules, verrucous lesions, pyodermic and herpetiform lesions, ulcers, and hypopigmented lesions.

Olecranon bursitis is usually preceded by injury to the elbow and causes induration, tenderness, erythema, and production of serosanguinous fluid.

Systemic and disseminated infections mainly affect immunocompromised hosts with solid organ or hematopoietic stem cell transplants, cancer chemotherapy, and AIDS [1, 5, 6]. They involve the skin and subcutaneous tissue, gut, peritoneum, spleen, blood, meninges, and central venous catheters [5, 6]. To date, there are 3 other reported cases of protothecosis with respiratory tract involvement [1, 6–8]. A 39-year-old man with metastatic colon cancer had CT thorax findings of peribronchial nodules with mediastinal adenopathy and pneumonitis. *Prototheca* species were isolated from BAL. He responded clinically to 28 days of fluconazole [6]. A 58-year-old man with relapsed leukemia post-stem cell transplant developed fatal protothecosis with *P zopfii* despite long-term voriconazole for pulmonary aspergillosis. He was treated unsuccessfully with caspofungin and 5 days of Ambisome. An autopsy revealed disseminated protothecosis in the lung, kidney, heart, and liver [7]. A 24-year-old woman with diabetes mellitus developed ulcerative *P wickerhamii* nasopharyngeal soft-tissue masses after prolonged intubation. She was treated successfully with surgical excision and amphotericin B [8].

Protothecal infection often occurs with copathogens such as CMV, herpes simplex virus, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Cryptococcus spp*, *Candida glabrata*, and *Aspergillus spp* [1]. Our patient’s illness was consistent with *P jirovecii* pneumonia (PJP), treatment of which resulted in clinical and radiological improvement but not resolution. Her CT findings may not have been solely due to PJP. The main CT features in PJP are extensive ground-glass opacities; nodules and lymphadenopathy being less frequent [9]. The positive BAL result for CMV was probably not clinically relevant because the blood CMV PCR was negative. Both the BAL and blood were positive for EBV, but because the blood EBV PCR cleared rapidly within 1 week, we believed that this was unlikely to be a true pathogen causing the CT findings of nodules and lymphadenopathy. There was clinical uncertainty in differentiating between true infection, colonization, and contamination; however, in an immunocompromised child without full clinical recovery, considering the potential for severe morbidity and mortality due to this organism, we believed that isolation of *P wickerhamii* merited treatment. Even so, the direct clinical benefit to our patient remains unclear. We considered the possibility of BAL sample contamination, but we believed that this result was unlikely because there were no other laboratory-reported cases and no previous contamination concerns. Environmental cultures were not done. A skin biopsy of the patient’s rash may have provided evidence for disseminated protothecosis and aided therapeutic decisions.

Treatment of protothecosis is controversial, with no consistent clinical response [1]. Localized skin lesions are
usually surgically excised, and systemic treatment was added for deeper lesions. Systemic infections are treated with antifungal agents; usually azoles and amphotericin B. Most treatment failures are associated with azoles [1]. Amphotericin B has the best in vitro activity and appears to be the most effective modality for systemic protothecosis [1]. The optimal dose and duration are unknown. All 7 cancer patients who received amphotericin B for protothecosis for a median of 24 days (range, 6–60 days) responded to treatment [6]. One reported case of refractory intestinal protothecosis responded to interferon gamma and itraconazole after 6 months of treatment [10].

To our knowledge, this is the first reported case of protothecosis in a pediatric liver transplant recipient. Given the increasing use of immunosuppressants, clinicians should be aware of these rare yet potentially significant algae.

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