A case of cutaneous penicilliosis in a child with acute myeloid leukaemia

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Introduction: We present a case of cutaneous penicilliosis in a paediatric patient with acute myeloid leukaemia (AML).

Case report: A 2-year-old boy with AML first developed probable pulmonary aspergillosis during induction chemotherapy in an overseas centre in May 2013, and was treated with AmBisome and voriconazole. When he was admitted to our centre with relapsed AML in October 2013, he was given a fifth course of chemotherapy, and treated with AmBisome for probable pulmonary aspergillosis in view of pulmonary nodular opacities on computed tomography. He thereafter developed an erythematous skin lesion with central eschar on his right hand and left calf. Serum and bronchoalveolar lavage galactomannan antigen (GM Ag) indices increased to a value of >10. AmBisome was changed to voriconazole, and caspofungin was added for 10 days. The left calf skin biopsy showed abundant fungal hyphae with septations. A skin culture grew Penicillium citrinum with MICs (μg ml⁻¹) of: caspofungin 0.016, itraconazole 0.5, amphotericin 1.5 and voriconazole >256. Caspofungin and itraconazole were commenced, and voriconazole was discontinued. The skin lesions and serial GM Ag indices improved. The patient later developed increasing GM Ag indices to a value of >10, which was attributed to Aspergillus flavus left pulmonary mycetoma, which was surgically resected. He eventually succumbed to relapsed AML after a bone-marrow transplant.

Conclusion: To the best of our knowledge, this is the first paediatric case of P. citrinum infection. Rising GM Ag indices were attributed to cross-reactivity of Penicillium spp. with GM Ag enzyme immunoassays.

Keywords: Penicillium citrinum; Penicilliosis; Galactomannan.

Introduction

With the increasing prevalence of primary and secondary immunodeficiency, e.g. AIDS, and the increasing use of...
immunosuppressive therapy, the incidence of opportunistic infections has been on the rise. Infections, particularly that of *Penicillium marneffei*, have been reported in multiple centres in South-east Asia. However, *Penicillium citrinum*, which is isolated mainly from the environment, has only rarely been reported to cause infection in humans. To the best of our knowledge, we report here what we believe is the first paediatric case of *P. citrinum* infection and we investigated the diagnosis and treatment of the infection in this patient.

**Case report**

A 2-year-old boy was diagnosed with acute myeloid leukaemia (AML; M5 subtype) at an overseas centre in May 2013. He received four chemotherapy cycles as per the Children Oncology Group Australia Acute Myeloid Leukaemia Protocol (COG AAML 531) consisting of cytarabine, daunorubicin, etoposide and mitoxantrone. During his induction chemotherapy, a computed tomography (CT) scan of his thorax showed a nodule containing an air-fluid level in the anterior right upper lobe and peripheral peribronchial changes, highly suggestive of a fungal infection. A bronchoalveolar lavage (BAL) sample was processed and was positive for galactomannan antigen (GM Ag) (the exact levels were not available to us), although the fungal cultures of the BAL fluid were negative. He was thus diagnosed with probable pulmonary aspergillosis and was initially treated with amphotericin B liposome (AmBisome) and voriconazole, followed by voriconazole monotherapy (uncertain dosage) for 3 months. At the end of the chemotherapy, he was in complete remission. A CT scan of his thorax also showed improvement. He was discharged on prophylactic oral co-trimoxazole.

He was subsequently readmitted to our centre in October 2013 for relapsed AML and was given a fifth chemotherapy course. A CT scan of his thorax revealed two pulmonary nodular opacities in the right middle and lower lobes. He was subsequently referred to another tertiary centre for bone-marrow transplant, which he underwent at the end of 2014. Unfortunately, his AML relapsed and he died in early 2015.

Subsequently, he developed two tender, erythematous skin nodules with central eschar on his left calf and right hand in November 2013, with no concomitant fever. Within 2 days, the nodules developed central necrosis, and his serum and BAL GM Ag level simultaneously increased to a value of > 10. AmBisome was then changed to voriconazole (18 mg kg\(^{-1}\) day\(^{-1}\)) and caspofungin (50 mg m\(^{-2}\) day\(^{-1}\)) was added for 10 days. An excisional biopsy of the skin lesion on the left calf was performed, and histology showed abundant fungal hyphae with septations, which were highlighted by periodic acid–Schiff and Gomori methenamine silver stain. There was only mild surrounding chronic inflammation (Fig. 1a). The tissue was cultured on Sabouraud dextrose agar (SDA) and in brain–heart infusion broth (BHIB) and incubated at 30 °C. Both the SDA and BHIB grew a mould identified as *Penicillium* spp. on microscopy. PCR and internal transcribed spacer sequencing were done to identify the species, with a result of *Penicillium citrinum* with a score of 99 % similarity using BLAST analysis. Antifungal susceptibility was done using an E-test on RPMI 1640 medium with MOPS solution and 2 % glucose (Sigma-Aldrich)]. The MICs (\(\mu\)g ml\(^{-1}\)) were 0.016 for caspofungin, 0.5 for itraconazole, 1.5 for amphotericin and > 256 for voriconazole. At this juncture, a CT of his thorax also showed a left upper lobe nodule with peripheral cavitation suggestive of mycetoma and right pleural thickening with a small pleural effusion. Voriconazole was promptly discontinued; caspofungin (50 mg m\(^{-2}\) day\(^{-1}\)) and itraconazole (5 mg kg\(^{-1}\) day\(^{-1}\)) were commenced. With the new antifungal regime, the skin lesion on the right hand improved. Serum GM Ag indices, which were regularly tested, also decreased to a value of 6.96.

**Outcome and follow-up**

In February 2014, the patient’s serum GM Ag index was noted to again rise to a value of > 10. Subsequent CT of the thorax showed a left upper lobe nodule with internal air-fluid level and a stable right-sided effusion. The patient underwent resection of the left lung mycetoma, which grew *Aspergillus flavus*, with MICs (\(\mu\)g ml\(^{-1}\)) of 0.006 for caspofungin, 1 for itraconazole, 2 for voriconazole and 4 for amphotericin. After surgical resection of the mycetoma, the serum GM Ag index decreased to a value of 0.91.

The patient was subsequently referred to another tertiary centre for bone-marrow transplant, which he underwent at the end of 2014. Unfortunately, his AML relapsed and he died in early 2015.

**Discussion**

*Penicillium* spp. are endemic in the environment, particularly in the South-east Asian region. Of these species,
**P. marneffei** has been the most commonly reported pathogen, especially among immunocompromised individuals. In contrast, **P. citrinum** has only been reported in a handful of cases, causing urinary tract infection (Guze & Haley, 1958), mycotic keratitis (Gugnani et al., 1978), bronchopulmonary infection (Mori et al., 1987), pneumonia with pericarditis in a patient with acute leukaemia (Mok et al., 1997) and chronic sinusitis (Twarzuk et al., 2014). It is noteworthy that all the studies so far have been reported only on adults and the cases of **P. citrinum** causing bronchopulmonary infection, pneumonia with pericarditis and urinary tract infection were identified mainly on autopsy. To the best of our knowledge, there have been no previous reports of **P. citrinum** infections in children, and we believe this to be the first reported paediatric case of **P. citrinum** infection.

In this study, we found that the temporal trend of GM Ag index levels could be used to monitor the disease activity of both *Penicillium* and *Aspergillus* infections. Studies have shown that the mAb present in the commercial sandwich ELISA cross-reacts with the heteropolysaccharide galactomannan complex antigen, which is present in the cell walls of *Aspergillus*, *Penicillium* and *Cryptococcus* spp. (Huang et al., 2007). One study reported the GM Ag indices (absorbance values) to be significantly higher in penicilliosis (with or without fungaemia) than in cryptococcosis (Huang et al., 2007). However, there are no studies that have compared GM Ag indices of *Penicillium* infections and *Aspergillus* infections. In our case study, the rising GM Ag level indicated increasing fungal activity and the patient probably had both invasive *Penicillium* and *Aspergillus* infections. The GM Ag index rose when he developed the cutaneous *Penicillium* infection and decreased appropriately after excision of the lesion and after starting appropriate antifungal drugs. The *Penicillium* sp. was obtained from an excisional biopsy, and the corresponding histological findings make it less likely to be a contaminant. Subsequently, the GM Ag index level rose again and correlated with radiological development of a left lung mycetoma due to *A. flavus*. It was therefore difficult to attribute the rising GM Ag levels preferentially to either the *Penicillium* activity or the concomitant *Aspergillus* activity.

Our study was a case report of paediatric penicilliosis. More studies with a substantial sample size are needed to evaluate further the role of the GM Ag assay in monitoring the disease activity of penicilliosis. Patients suffering from penicilliosis often present with non-specific symptoms, and definitive diagnosis is often by identification or isolation of *Penicillium* sp. in tissue biopsies. However, the invasive nature and cultural methods of these investigations often delay diagnosis, which may partly explain why *P. citrinum* was detected only on autopsy in the cases of urinary tract infection (Guze & Haley, 1958) and bronchopulmonary penicilliosis (Mori et al., 1987). Early serodiagnosis of penicilliosis would guide rapid treatment and reduce the mortality associated with disseminated penicilliosis. There have so far been no specific serodiagnostic tests available for *P. citrinum*, but several tests have been investigated thoroughly for *P. marneffei*. For instance, specific mAb-based ELISA tests have been developed with *P. marneffei* cell-wall antigens in serum and urine (Cao et al., 1999; Panichakul et al., 2002) to aid the serodiagnosis of penicilliosis. However, these tests are not available commercially. Clinical trials should be conducted to study further the applicability of these investigations.

So far, there have been no reports on effective antifungals against *P. citrinum*, possibly because the microbe is often identified only on post-mortem. There have, however, been studies demonstrating that other *Penicillium* sp., especially *P. marneffei*, can be treated with parenteral amphotericin B and oral itraconazole (Sirisantana et al., 1998). In addition, voriconazole, which is an extended-spectrum triazole, has been reported to be an effective therapeutic option for systemic *P. marneffei* infections in AIDS patients (Supparatpinyo & Schlamm, 2007). In our case, the empirical use of Ambisome and voriconazole was ineffective against *P. citrinum*. Instead, the use of caspofungin and itraconazole guided by the MIC results were effective in treating the infection. Therefore, early serodiagnosis, pathological diagnosis and appropriate antifungal susceptibility testing are essential for appropriate treatment in order to improve clinical outcomes.

In summary, to the best of our knowledge, we report the first paediatric case of *P. citrinum* infection in a child with AML. A high GM Ag index, although highly suggestive of *Aspergillus* infection, can also be caused by *Penicillium* infection, which could be a concomitant pathogen, especially in immunocompromised individuals. More studies are needed to elucidate the role of GM Ag in penicilliosis. Specific tests for serodiagnosis of *Penicillium* sp. infections may help early diagnosis, and thereby improve clinical outcomes in the future.

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This was a retrospective review of a case of penicilliosis and no experimental procedure was performed on the patient involved. None of the authors have any conflict of interest to report.

**References**


