Non-Sporulating Chrysosporium: An Opportunistic Fungal Infection in a Neutropenic Patient

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Summary
We report a case of an invasive infection with non-sporulating Chrysosporium species in a patient who was treated with chemotherapy for relapsed acute lymphoblastic leukemia. This patient presented with a persistent lobar pneumonia, skin lesions, and possible involvement of the central nervous system. The patient responded to treatment with amphotericin B and oral itraconazole.

Key Words: Neutropenic, Non-sporulating Chrysosporium, Blood culture

Introduction
Systemic fungal infections are a major cause of morbidity and mortality in neutropenic patients. Approximately 25% of patients with leukemia have evidence of fungal infection at autopsy. The incidence of fungal infection is increasing in immunosuppressed patients especially in the hemato-oncology patients. This is probably due to several factors including prolonged period of neutropenia, the use of broad spectrum antibacterial therapy, the increasing use of central venous access devices, and disruption of the normal mucosal barrier in patients post-chemotherapy. Common fungi isolated from this group of patients are Aspergillus, Candida, Penicillium marneffei and Mucor. Increasingly, infections with fungus that were not previously recognised as pathogenic are being described. We present a case of systemic infection of a rare fungal organism, non-sporulating Chrysosporium species in a neutropenic patient following an induction chemotherapy for relapsed acute lymphoblastic leukemia. We were unable to determine the exact genus of Chrysosporium species. To our knowledge, with the clinical findings and the significant blood cultures, this is the first reported case in Malaysia.

Case Report
A 50 year old female with a prior history of acute lymphoblastic leukemia in remission was admitted to the hospital for lobar pneumonia. She was on oral maintenance chemotherapy consisting of daily mercaptopurine and weekly methotrexate started about a year ago. The induction chemotherapy used to treat the acute leukemia 2 years previously was of the BFM...
protocol which consisted of daunorubicin, prednisolone, vincristine and L-asparaginase. She developed Citrobacter sepsis as well as Escherichia coli bacteriuria post-chemotherapy. Other complication from the induction chemotherapy was drug induced diabetes mellitus which recovered after chemotherapy was completed.

The patient presented with one week duration of productive cough. On physical examination at admission, she was febrile with a temperature of 38°C, and crepitations were audible over her right lung. A chest X-ray revealed a lobar opacity in the right lower lung field. The initial laboratory findings revealed Hb of 5.4g/dl, WCC of 1.4 x 10^9/L (neutrophils 66% with no blast seen) and platelet of 105,000. The blood sugar level was elevated (19.0mmol/L) and insulin therapy was commenced. Her oral maintenance chemotherapy was stopped. Intravenous antibacterial therapy consisting of ampicillin/sulbactam 1.2g three times a day and azithromycin 500mg daily were commenced. The antibiotic was later changed to cefepime at a dosage of 2g twice daily. There was no growth from 2 different sets of blood cultures. The fever settled after 3 days of antibiotics and there was symptomatic improvement. Treatment with cefepime was continued for a total of 10 days.

As her blood count did not improve, a bone marrow examination was performed which showed a relapse of acute lymphoblastic leukemia (blast count of 78%). Induction chemotherapy comprising of idarubicin and cytosine arabinoside was started. She remained quite well and afebrile despite becoming neutropenic on the 8th day following chemotherapy (+8). Subcutaneous granulocyte colony stimulating factor (G-CSF) was started.

On the 10th day post-induction chemotherapy (+10), she became febrile and complained of feeling of increasingly unwell with difficulty in breathing. On examination, there was hepatomegaly, bilateral axillary skin lesions and left nasal ulceration. These were not previously present on admission. A repeated blood (Bactec 9240, Becton Dickinson) and sputum cultures were taken on the same day. Repeat chest X-ray showed persistent right lower lung opacity. A bronchoscopy was performed the next day. The skin lesions were biopsied and broad spectrum antibiotic therapy, including imipenem, vancomycin and amphotericin B (1 mg/kg/day) were instituted. A CT scan of thorax and abdomen showed collapse consolidation in the apical segment of the right lower lung. CT scan of the brain and paranasal sinuses showed bilateral maxillary sinusitis and an irregular hypodense area extending from the vertex down to the temporal region which was reported as possibility due to a fungal abscess or a resolving subdural hematoma (Fig. 1). The patient did not exhibit any neurological signs.

After 48 hours, a mold was isolated from the blood cultures drawn on day +10. The isolate was subcultured onto SDA and incubated at 37°C. On day 4, a fluocose, white colony with a lobulated
centre appeared. The reverse side of the colony was also whitish in color. Microscopic examination of the colony on day 7 using tease mount technique with a drop of lactophenol cotton blue, thin segmented and irregularly branched hyphae were seen. There was no production of conidia. A subculture from SDA was made onto blood agar and incubated at 37°C for evidence of sporulation. A slide culture was also made using Potato Dextrose agar and incubated at 37°C, the slide culture was examined periodically at low power (10x) objective until day 10. The coverslip was then removed and stained with lactophenol cotton blue (Fig. 2). The fine structure of this fungus consisted of segmented hyphae with no conidia formation. The original SDA after 2 weeks of incubation at 37°C and the subculture on blood agar plate failed to show evidence of sporulation after prolonged incubation. The fungus isolated from blood was therefore identified as non-sporulating Chrysosporium based on the whitish texture of the colony, branching hyphae and absent of conidia production.

Bronchoalveolar lavage did not grow any organism. The skin biopsy showed local ulceration and inflammation and stained negative for any fungal elements or acid fast bacilli, unfortunately, the biopsy specimen was not submitted for fungal culture. The patient improved and became afebrile after 15 days of antibacterial and antifungal therapy. The antibacterial therapy was stopped but amphotericin B was continued. Her skin and nasal lesion resolved and she was discharged on day +51 with thrice weekly intravenous amphotericin B (1mg/kg/day) and oral itraconazole 400mg daily.

A repeat bone marrow examination 2 weeks after discharge showed that the patient did not achieve remission even though her blood counts had improved. Her diabetes is now controlled with oral hypoglycemic agents. The patient remained well and afebrile. After discussion with her family, a decision was made to withhold any further chemotherapy in view of possible persistent fungal infection. A repeat CT of brain and thorax 2 months after the initial CT scan showed the previous subdural collection has resolved and the consolidation of the right lung has decreased in size. She is still receiving the intravenous amphotericin B three times a week as an outpatient basis.

Discussion

Opportunistic fungal infections typically occur in immunocompromised patients. Disseminated candidiasis and aspergillosis are the most common fungal infections in neutropenic

![Fig. 2: Macroscopic and microscopic structure of non-sporulating Chrysosporium.](image)
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patients. All are considered ubiquitous in nature. These patients have extremely poor outcome and often the poor survival is attributed to a delay in diagnosis.

Members of the genus *Chrysosporium* that are human pathogens include *Chrysosporium parvum*, and *C. parvum var. cresens*. The fungi are common soil saprophytes. The colonies are predominantly white to tan, varied from waxy to powdery. The reverse is mostly colorless to tan. The conidiophores are not easily differentiated from irregularly branched hyphae. The members produced aleurioconidia terminally, at the end of short or long lateral 2 or 3 conidia. The fungus isolated from blood is filamentous, grew rapidly on SDA producing a white fluffy mycelium with a lobulated centre within 4 days. The colonial morphology and microscopic structure are very similar to species of members of genus *Chrysosporium*. However, as the fungus failed to sporulate after prolonged incubation of SDA or growing on Potato Dextrose Agar incubated at 37°C for 14 days, the isolate was called a non-sporulating *Chrysosporium*.

In human, there are only rare reports of deep infection caused by *Chrysosporium* species, and most of which are difficult to treat. The first confirmed case of human chrysosporial infection, or adiaspioromycosis was reported in 1964. Adiaspiromycosis is usually confined to the lung because inhaled conidia do not replicate or disseminate. Both solitary and diffuse forms of pulmonary disease have been described. However, in our patient, the adiaspiondia may have form endospores or changed to mycelia as her own defenses were destroyed, the mold could have spread hematogenously throughout her body. The source of her infection may have originated from the lung.

Her persistent fever was treated empirically with broad spectrum antibacterial therapy and amphotericin B. The skin lesions and the nasal lesion recovered with the treatment. The blood culture which grew the organism was unlikely to be due to contamination in view of her clinical findings and significant improvement of the symptoms after commencement of amphotericin B.

There are only a few reports on extrapulmonary disease in humans caused by *chrysosporium*. Stillwell et. al.³ found *chrysosporium parvum* as a cause of osteomyelitis in a 24 year old man. They treated the patient with amphotericin B with complete resolution of the disease. In immunocompromised patients, Warwick et. al.² described an invasive *chrysosporium* infection of the paranasal sinuses extending into the central nervous system in a young girl following bone marrow transplantation. She subsequently died from the infection. Roilides et. al.³ described a 15 year old boy with chronic granulomatous disease who had disseminated *chrysosporium* infections causing pneumonia and osteomyelitis of the tibia. The disease recurred when amphotericin B was stopped despite on oral maintenance of oral itraconazole. The disease finally resolved after a year of liposomal amphotericin B.

In summary, we reported a case of disseminated infection caused by non-sporulating *Chrysosporium* in a patient with acute lymphoblastic leukemia. The treatment of this disseminated infection is unknown, however, the combination of amphotericin B and oral itraconazole appeared to be effective in controlling the infection. Like *Chrysosporium* infection, this fungus may cause an aggressive and disseminated disease and may be fatal especially in an immunocompromised patient. The prolonged therapy with amphotericin B may be the standard of care. Although the Colony-stimulating factors, for examples, the granulocyte, granulocyte-macrophage colony stimulating factors may have a favourable effect on the incidence and outcome of this group of patients, further study is needed.
CASE REPORT

References

