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CASE REPORT

Trichosporon beigelii pneumonitis following busulphan-induced leucopenia

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Trichosporon beigelii pneumonitis following busulphan-induced leucopenia. K. Yamauchi, T. Sato.
ABSTRACT: We present a 41 yr old male, who died of respiratory failure due to infiltration of *Trichosporon beigelii (cutaneum)* into the pulmonary interstitial spaces with systemic dissemination. Busulphan-induced leucopenia in the chronic phase of chronic myelogenous leukaemia had progressed before this infection developed. In leukaemic patients with profound leucopenia, interstitial pneumonitis due to *T. beigelii* should be considered as a possible cause of otherwise unexplained hypoxaemia.
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Trichosporon beigelii (cutaneum) is known to be the causative agent of a superficial infection of the hair shaft called white piedra and hypersensitivity pneumonitis [1]. *T. beigelii* has recently been recognized as causing a fatal infection in immunocompromised patients with acute leukaemia. We encountered a rare case of fatal respiratory failure due to *T. beigelii* interstitial pneumonitis. Busulphan-induced leucopenia in the chronic phase of chronic myelogenous leukaemia (CML) had progressed before this infection developed. The defective neutrophil function induced by CML itself is also discussed, for the possible role of the susceptibility to this agent.

Case report

In November 1985, a 41 yr old male was diagnosed as having chronic phase CML, on the basis of the following findings: a leucocyte count of $13.7\times10^9\cdot l^{-1}$, with 60% neutrophils ($8.2\times10^9\cdot l^{-1}$), 2% metamyelocytes, 5% myelocytes, 5% eosinophils, 6% basophils, 1% monocytes and 21% lymphocytes; hyperplastic bone marrow without maturation arrest of the myeloid series; and the presence of Philadelphia chromosome (Ph^1). Busulphan therapy was given as 2 mg daily and discontinued after 4 weeks' therapy when the leucocyte count was $10.2\times10^9\cdot l^{-1}$. Five months later, the leucocyte count increased to $18.4\times10^9\cdot l^{-1}$. For subsequent repeated leucocytoses, busulphan therapy was performed another 5 times, at intervals of 4–8 months, until June 1988, with a total dosage of 28 or 56 mg busulphan per course. The leucocytes were maintained at $8.1–18.4\times10^9\cdot l^{-1}$. In total, 280 mg of busulphan was administered over a period of 30 months. Progressive pancytopenia lasted during the following year without busulphan administration.

To confirm the presence of Ph^1 chromosome-positive clone at the deoxyribonucleic acid (DNA) level, Southern blot analysis, employing 3' and 5' breakpoint cluster region (bcr) probes, was performed by using DNA samples obtained from blood leucocytes and marrow cells. Rearranged restriction fragments of the bcr gene were not demonstrated with the conventional method. Scanty fragments were detected by amplifying the DNA samples obtained from marrow cells with polymerase chain reaction [2], but not from blood leucocyte samples. These results indicated that most of the blood leucocytes originated from Ph^1 chromosome-negative clone, which indicates an almost normal clone.

In October 1989, the leucocyte count was $0.9\times10^9\cdot l^{-1}$, with 36% neutrophils ($0.32\times10^9\cdot l^{-1}$), 1% eosinophils, 1% monocytes and 62% lymphocytes. Repeated bone marrow aspiration revealed hypoplasia without an increase in blasts, which suggested the acute transformation of CML.

From October 31, the patient's body temperature rose to 38.0–39.0°C without any local symptoms. Surveillance cultures were repeatedly performed. Chest roentgenograms showed interstitial infiltrate in the right lower lung field, despite normal breathing sounds. Systemic administration of trimethoprim/sulphamethoxazole with sulbactam/cefoperazone (SBT/CPZ), and inhalation of polymyxin B and amphotericin B, were used empirically as initial therapy. Eight days later, imipenem/cilastatin was substituted for SBT/CPZ because of a persistent fever. Despite these therapies, dyspnoea and an increased respiratory rate developed

without productive cough, in the middle of November, in addition to the fever. A chest roentgenogram, on November 15 (fig. 1), revealed a further spread of the interstitial infiltrate to the right lower field and left hilar and mediastinal adenopathy. Arterial blood gas analysis in room air revealed a pH of 7.51, a carbon dioxide tension (Pco_2) of 2.9 kPa and an oxygen tension (Po_2) of 6.8 kPa.

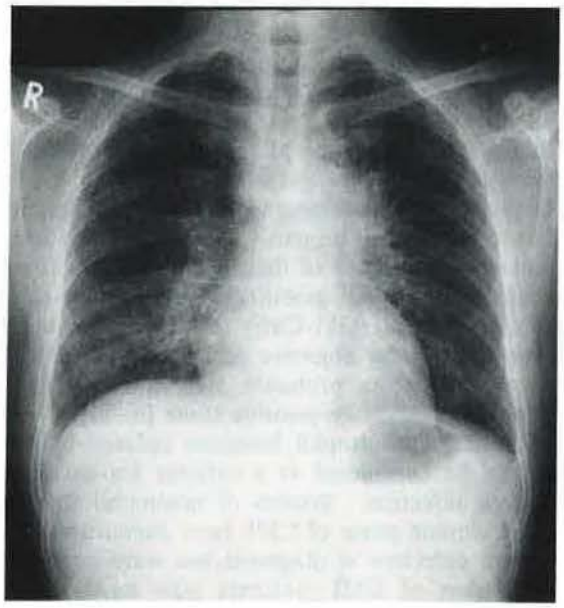


Fig. 1. – Posteroanterior view of chest roentgenogram showing interstitial infiltrate in right lower field and left hilar and mediastinal adenopathy.

Because of the persistent fever unresponsive to the antibiotics, a fungal infection was suspected. Intravenous miconazole was administered for 18 days, from the 13th of November until the patient's death, at a dose of 400 mg·day^{-1}. Simultaneously, amphotericin B syrup was administered at a dose of 1,200 mg·day^{-1} for the prevention of intestinal mycoses. Rifampicin and isoniazid were also administered orally for suspected tuberculosis. Despite the intensive supportive care, the patient's condition deteriorated and he died of respiratory failure on December 1, 1989. The final leucocyte count was $0.1\times10^{9}\cdot l^{-1}$, containing only lymphocytes.

The autopsy and microbiological findings were as follows:

1. The bone marrow was hypoplastic without any apparent increase in leukaemic blasts. There was also no evidence of infiltration of leukaemic blasts into the other organs.

2. The lungs were heavy and congested. Small nodules of <4 mm in diameter were widely disseminated, with fungal thromboemboli and haemorrhage in the lungs (fig. 2). Similar nodules were found in the liver, spleen, kidneys, bone marrow, myocardium, thyroid gland, testis and oesophagus. Remarkable thickness of the alveolar septa and disappearance of alveolar cavities were seen throughout the lungs. A host inflammatory response was strikingly absent from all organs.

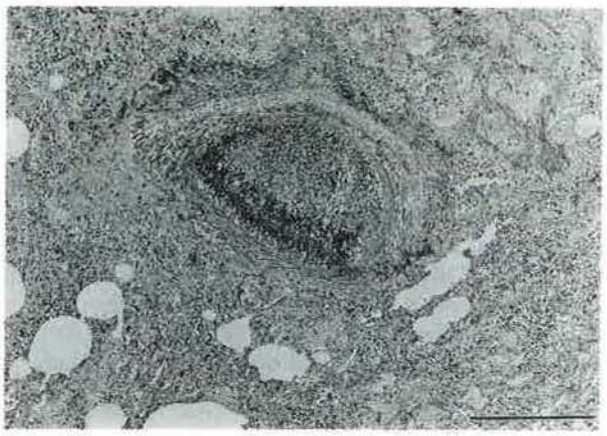
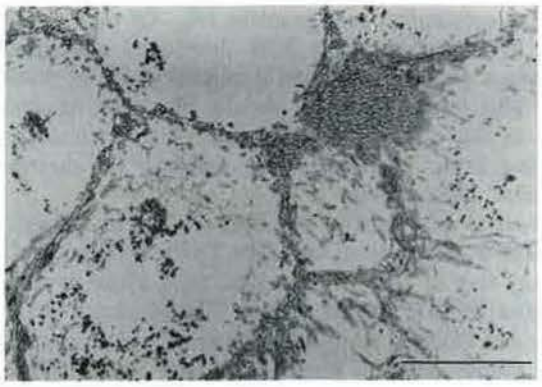


Fig. 2. – Fungal thromboembolus due to *T. beigelii* in the lung. Large thromboembolus attached to an arterial wall contains numerous organisms that are invading the vessel wall in the absence of inflammatory reaction. (Haematoxylin and eosin stain. Bar scale=400 μm).

a



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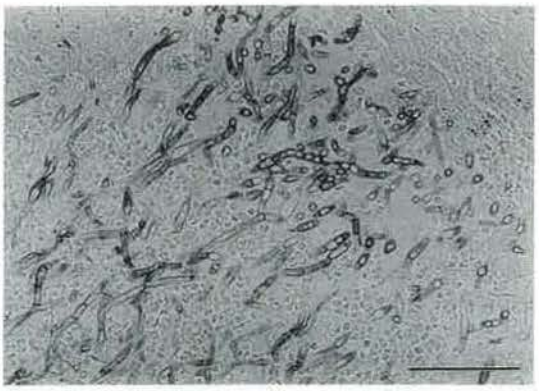

Fig. 3. – a) Invasion of alveolar septa in the lung by numerous hyphae of *T. beigelii* (Grocott's methenamine silver stain. Bar scale=200 μm). b) Branching, septate hyphae and variation in the width of hyphal elements consistent with *T. beigelii* in the lung. (Grocott's methenamine silver stain. Bar scale=40 μm).

3. The fungal elements exhibited branching, septate hyphae, marked variation in the width of hyphal elements, and arthrospores consistent with *T. beigelii* (fig. 3 a and b). Isolates from microbiological cultures of blood on November 6, and from nodules of the lungs

and spleen at autopsy, assimilated all the sugars included in the yeast identification system of API 20C Auxanogram (API System S.A., France) [3]. These isolates were identified as *T. beigelii* by the above characteristics.

4. Atypical mycobacteria appeared as polypoid lesions in the left upper trunk of the bronchus and disseminated in the bilateral pulmonary hilar and bronchopulmonary lymph nodes, but they were not seen in any section of the lungs. These organisms were positive in both Ziehl-Neelsen stain and immunostain to BCG.

Alveolar-capillary block, due to invasion of *T. beigelii* into the pulmonary interstitial space, was determined to be the direct cause of death.

Discussion

The fungus Trichosporon was found to be a colonizer of the respiratory, gastrointestinal or urinary tract in 3.7% of a series of patients who were undergoing either bone marrow transplantation or intensive chemotherapy for haematological malignancies [4]. Of those patients with Trichosporon, only 23% developed disseminated infections [4], and they had a mortality rate of 60–78% [5]. This fungal infection should, therefore, be considered in the differential diagnosis of infectious agents causing fever even relatively early in the course of profound neutropenia.

Absence of positive surveillance cultures does not exclude a diagnosis of invasive Trichosporon infection. In only five of nine patients who were found at autopsy to have lung infiltrates due to Trichosporon, was the organism isolated from the sputum [6]. Transbronchial lung biopsy or open lung biopsy may reveal fungi for the remaining patients of negative sputum culture. Thus, the diagnosis of this infection depends on positive cultures or histological evidence.

It is sometimes difficult to distinguish this fungus from *Candida* spp. in tissue sections. The immunoperoxidase method, using monoclonal antibodies specific to *T. beigelii* in the tissue section, was reported to be useful for the accurate diagnosis of trichosporonosis, as compared with polyclonal antibodies to the fungus, that often have cross-reactivity with other fungi [7]. However, the determination of the serum antibodies specific to *T. beigelii* is not useful for the diagnosis, because the production falls markedly in the immuno-compromised patients with profound leucopenia. When disseminated *T. beigelii* infection is suspected, determination of antigens or metabolites of the organism in the blood, as evaluated in disseminated candidiasis, may be a useful diagnostic procedure.

The majority of neutropenic patients with disseminated *T. beigelii* infection, including our patient, die despite the use of antimycotic agents [5]. Most Trichosporon strains were demonstrated in many *in vitro* studies to be susceptible to amphotericin B, miconazole and ketoconazole but frequently resistant to flucytosine. However, most of the reported patients have shown progression of trichosporonosis whilst receiving these agents. In the presence of antifungal drugs, the fungi seem to be inactive but may be able to survive if the organisms are not phagocytosed by macrophages or neutrophils. When progressive profound neutropenia (0.1×10^9 cells$\cdot l^{-1}$), as in this case, is expected with the primary disease and/or the administered cytotoxic drugs, prophylactic antifungal treatment should be considered in order to prevent mycosis. Because the most likely portals of entry for Trichosporon are the alimentary tract and lungs, oral administration and inhalation of an antifungal drug, as with the use of amphotericin B in this patient, are recommended. When febrile development is resistant to broad-spectrum antibiotics, empirical systemic administration of antifungal drugs should be performed, even without the evidence of profound fungal infection [8].

The resolution of infection in leukaemic patients with neutropenia is related primarily to bone marrow recovery following remission of their leukaemia [5]. Therefore, administration of granulocyte/macrophage-colony stimulating factor (GM-CSF) or G-CSF should be considered, with the objective of increasing the leucocyte count, despite probable stimulation of these cytokines to residual Ph-positive clone [9, 10].

The defective neutrophil functions induced by CML itself may be considered as a cofactor for developing *T. beigelii* infection. Studies of neutrophil functions from the chronic phase of CML have demonstrated that they were defective at diagnosis but were normalized in a number of CML patients who had achieved haematological remission after chemotherapy with busulphan or hydroxyurea [11, 12]. On the other hand, molecular analysis of this patient demonstrated a remarkable decrease of leukaemic clone bearing a hybrid bcr/abl DNA in bone marrow. This result indicates that most of the circulating leucocytes originate from normal clone. Although the neutrophil function was not measured in this patient, the possibility of the defective function as a cofactor for developing *T. beigelii* infection was not positively supported.

A minority of patients with an adequate number of neutrophils develop pneumonia without evidence of dissemination. Severely neutropenic patients are more likely to have disseminated infection. Our patient had a localized pulmonary infection in the early stage and, thereafter, may have disseminated systemically *via* fungal emboli as the leucopenia progressed. Pulmonary infiltration due to Trichosporon occurred in 13 of the previously reported 38 patients with trichosporonosis [13]. Three of 11 patients with postmortem evidence of pulmonary trichosporonosis had negative chest radiographs [13]. The other eight patients had X-ray findings ranging from bronchopneumonia to a patchy nodular pattern or lobar consolidation [13]. Recently, two cases with a cavitation pattern were reported [6, 14]. Thus, a majority of patients with pulmonary trichosporonosis showed pneumonic shadow in chest roentgenogram.

Our case and two cases reported in the literature [15] showed interstitial markings on chest roentgenograms, hypoxaemia, progressive dyspnoea and a dry cough.

These findings indicate interstitial pneumonitis rather than pneumonia. Differential diagnosis from pneumonitis induced by *Pneumocystis carinii* or cytomegalovirus will also be important for deciding the appropriate therapy.

Busulphan administration itself may be considered as another cause of interstitial pneumonitis induced in this patient. Chest roentgenograms of busulphan lung show diffuse intra-alveolar or interstitial patterns or a combination of both [16]. However, microscopic examination of lung tissue in this patient did not demonstrate large, bizarre and granular pneumocytes with hyperchromatic nuclei that were damaged by the antimitotic effects of busulphan [17]. Pulmonary toxicity has not been reported at a total dose of busulphan <500 mg and has occurred whilst the busulphan was being administered [17]. In our patient, clinical pulmonary disorder developed 11 months after stopping this drug, and the total dose of busulphan administered was only 280 mg. For these reasons, the possibility that the interstitial pneumonitis in this patient was induced by busulphan is very small.

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References

1. Yoshida K, Ando M, Sakata T, Araki S. – Environmental mycological studies on the causative agent of summer-type hypersensitivity pneumonitis. *J Allergy Clin Immunol*, 1988; 81: 475–483.
2. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. – Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 1988; 239: 487–491.
3. Dermoumi H. – Differentiation of yeast-like fungi isolated from clinical specimens with the API 20C auranogramm. *Arztl Lab*, 1979; 25: 289–291.
4. Haupt HM, Merz WG, Beschorner WE, Vaughan WP, Saral R. – Colonization and infection with Trichosporon species in the immunosuppressed host. *J Infect Dis*, 1983; 147: 199–203.
5. Hoy J, Hsu KC, Rolston K, Hopfer RL, Luna M, Bodey GP. – *Trichosporon beigelii* infection: a review. *Rev Infect Dis*, 1986; 8: 959–967.
6. Santhosh-Kumar CR, Al-Hedaithy SSA, El-Saghir NB, Ajarim DSS. – Cavitating pneumonia due to *Trichosporon beigelii* in a patient with acute myeloid leukaemia. *J Infect Dis*, 1989; 19: 65–68.
7. Takeuchi T, Kobayashi M, Moriki T, Miyoshi I. – Application of a monoclonal antibody for the detection of *Trichosporon beigelii* in paraffin-embedded tissue sections. *J Pathol*, 1988; 156: 23–27.
8. Hughes WT, Armstrong D, Bodey GP, Feld R, Mandell GL, Meyers JD, Pizzo PA, Schimpff SC, Shenep JL, Wade JC, Young LS, Yow MD. – Guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. *J Infect Dis*, 1990; 161: 381–396.
9. Vadhan-Raj S, Velasquez WS, Butler JJ, LeMaistre A, Lepe-Zuniga J, Keating MJ, Koller CA, Gutterman JU. – Stimulation of myelopoiesis in chronic lymphocytic leukemia and in other lymphoproliferative disorders by recombinant human granulocyte-macrophage colony-stimulating factor. *Am J Hematol*, 1990; 33: 189–197.
10. Teshima H, Ishikawa J, Kitayama H, Yamagami T, Hiraoka A, Nakamura H, Shibata H, Masaoka T, Takaku F. – Clinical effects of recombinant human granulocyte colony-stimulating factor in leukemia patients. A phase I/II study. *Exp Hematol*, 1989; 17: 853–858.
11. Whittaker JA, Khurshid M, Hughes HR. – Neutrophil function in chronic granulocytic leukaemia before and after busulfan treatment. *Br J Haematol*, 1974; 28: 541–549.
12. Carulli G, Azzara A, Papineschi F, Rizzuti A, Grassi B, Ambrogi F. – Neutrophil functions in chronic myeloid leukemia. *Leukemia Res*, 1990; 4: 823–824.
13. Walsh TJ, Newman KR, Moody M, Wharton RC, Wade JC. – Trichosporonosis in patients with neoplastic disease. *Medicine*, 1986; 65: 268–279.
14. Marin J, Chiner E, Franco J, Borras R. – *Trichosporon beigelii* pneumonia in a neutropenic patient. *Eur J Clin Microbiol Infect Dis*, 1989; 8: 631–633.
15. Saul SH, Khachatoorian T, Poorsattar A, Myerowitz RL, Geyer SJ, Pasculle AW, Ho M. – Opportunistic trichosporon pneumonia. Association with invasive aspergillosis. *Arch Pathol Lab Med*, 1981; 105: 456–459.
16. Ginsberg SJ, Comis RL. – The pulmonary toxicity of antineoplastic agents. *Semin Oncol*, 1982; 9: 34–51.
17. Taetle R, Dickman PS, Feldman PS. – Pulmonary histopathologic changes associated with melphalan therapy. *Cancer*, 1978; 42: 1239–1245.