Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.7282-21
Intern Med 61: 257-261, 2022
http://internmed.jp

【 CASE REPORT 】

Progressive Cytopenia Developing during Treatment of Cryptococcosis in a Patient with HIV Infection and Bone Marrow Cryptococcal Infection

Mai Katsura, Ayako Okuhama, Yoshiki Koizumi, Naokatsu Ando, Yasuaki Yanagawa, Daisuke Mizushima, Takahiro Aoki, Kunihisa Tsukada, Katsuji Teruya, Yoshimi Kikuchi, Shinichi Oka and Koji Watanabe

Abstract:

Cytopenia is a common complication in patients with human immunodeficiency virus (HIV) infection. Identifying the cause is demanding because of the wide range of possible diagnoses. We herein report an HIV-infected patient with disseminated cryptococcosis involving multiple organs including the blood, brain, lungs, and bone marrow, who developed progressive pancytopenia after initiation of anti-fungal treatment with liposomal amphotericin-B (L-AMB) and flucytosine (5FC). The pancytopenia persisted despite early 5 FC discontinuation. A bone marrow biopsy revealed cryptococcal infiltration and the blood examination findings recovered quickly after resuming L-AMB. Thus, this HIV-infected patient's pathological findings and clinical course suggested that the primary cause of the pancytopenia was bone marrow cryptococcosis.

Key words: *Cryptococcus*, HIV, cytopenia, adrenal insufficiency

(Intern Med 61: 257-261, 2022)
(DOI: 10.2169/internalmedicine.7282-21)

Introduction

Cryptococcus neoformans is an established cause of infection in human immunodeficiency virus (HIV)-infected patients with CD4+ T cell counts below 100/μL. These infections have a high mortality rate, accounting for 15% of acquired immunodeficiency syndrome-related deaths (1). The most susceptible organs are the central nervous system (CNS) and lungs. Cryptococcal infection can also disseminate systemically, infecting other sites, such as the skin, eyes, liver, bone marrow, and adrenal glands (2-7).

The most potent regimen for treating cryptococcal meningitis is a combination of liposomal amphotericin-B (L-AMB) and flucytosine (5FC); however, adverse events often necessitate modification of the initial regimen. In particular, 5FC reportedly induces pancytopenia in 27-50% of patients treated for cryptococcal meningitis. This high incidence may be an overestimation because of various confounding factors (8, 9). Cytopenia is generally attributable to one or more of the following: HIV infection itself, drug-induced myelosuppression, hematological malignancy, histoplasmosis, and mycobacteriosis. In addition, a few case reports have documented pancytopenia caused by cryptococcal infiltration of bone marrow in HIV-infected patients (3-6), the incidence being as yet unknown. The wide variety of possible diagnoses complicates determining the cause of cytopenia in HIV-infected patients with disseminated cryptococcosis.

We herein report a patient with disseminated cryptococcosis with bone marrow involvement who developed progressive pancytopenia after initiation of anti-fungal treatment and whose pancytopenia persisted despite discontinuation of the suspected drugs.

Case Report

An HIV-infected Japanese man in his 20s was referred to

AIDS Clinical Center of the National Center for Global Health and Medicine, Japan
Received: February 21, 2021; Accepted: June 20, 2021; Advance Publication by J-STAGE: July 30, 2021
Correspondence to Dr. Ayako Okuhama, ayokuhama@hosp.ncgm.go.jp

Table. **Laboratory Data on Initial Admission.**

LDH	168 IU/L	WBC	8,300 /μL
BUN	26 mg/dL	CD4#	26 /μL
CRE	0.68 mg/dL	CD8#	103 /μL
Glu	112 mg/dL	Hgb	10.1 g/dL
Na	136 mEq/dL	MCV	87.3 fL
K	3.4 mEq/dL	Plt	13.8×10^4 /μL
Cl	99 mEq/dL	Ferritin	1,671 ng/mL
Ca	9 mEq/dL		
CRP	2.05 mg/dL		

BUN: blood urea nitrogen, Ca: calcium, CD4#: cluster of differentiation 4-T-cell count, CD8#: cluster of differentiation 8-T-cell count, Cl: chloride, CRE: creatinine, CRP: C-reactive protein, Glu: glucose, Hgb: hemoglobin, K: potassium, LDH: lactate dehydrogenase, MCV: mean corpuscular volume, Na: sodium, Plt: platelet, WBC: white blood cell

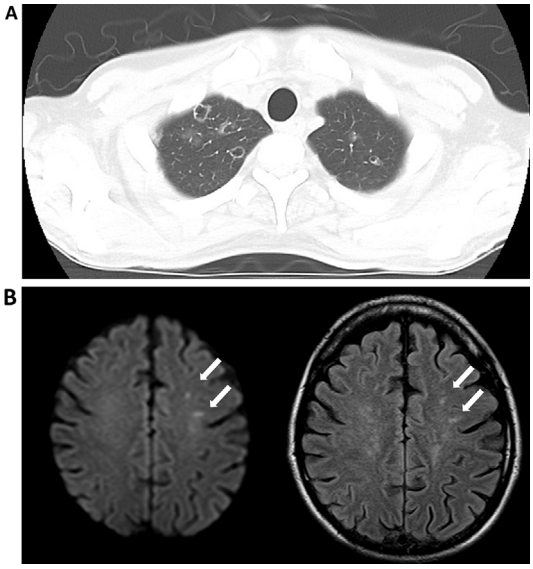

Figure 1. **Radiological findings on chest CT and brain MRI. (A) Chest CT showing multiple nodules with thin cavity wall formation. The lesions are suggestive of septic emboli. (B) Diffusion-weighted imaging (left) and fluid-attenuated inversion recovery (right) of brain MRI on admission. These findings show multiple hyperintensity lesions (arrows) in the left semi-oval center, which are interpreted as subacute infarction.**

our hospital after being diagnosed with cryptococcal meningitis and commencing anti-fungal treatment in another hospital. He was naïve to anti-retroviral therapy (ART) because he had not attended for follow-up after being diagnosed with HIV infection two years previously. He had not traveled overseas in the past few years. On admission to the previous hospital (Day 1), he had reported an intermittent fever and weight loss for the past two months, followed by persistent dry cough and headache for the past week. On admission to our hospital (Day 4), vital signs had been normal except for an axillary temperature of 38.4°C.

A physical examination had revealed nuchal rigidity and jolt accentuation. His CD4+ and CD8+ T lymphocyte counts were 26 (9.9%) and 103 (39.8%) cells/μL, respectively, and his HIV viral load was 7.79×10^5 copies/mL. A full blood count (FBC) had shown slightly increased leukocytes, mild anemia, and slightly decreased platelets (Table). A lumbar puncture had yielded clear cerebrospinal fluid (CSF), an opening pressure of 30 cmH_2O, a leucocyte count of 1/μL, protein of 4 mg/dL, glucose of 43 mg/dL, and numerous encapsulated yeasts on an India ink examination. Blood and CSF cultures taken on admission had yielded *C. neoformans var. grubii* on Day 4, identified by matrix-assisted laser desorption-ionization time of flight mass spectrometry. Chest computed tomography (CT) revealed multiple diffuse nodules in both lungs (Fig. 1A) and brain magnetic resonance imaging (MRI) showed multiple T2 high-intensity lesions in both frontal lobes (Fig. 1B). All of these lesions were interpreted as septic emboli. The patient was therefore diagnosed with disseminated cryptococcosis with involvement of the blood, CSF, cerebral parenchyma, and lungs.

Anti-fungal treatment had been started with 3.3 mg/kg L-AMB and 10 mg/kg 5FC daily on Day 1 at the previous hospital (Fig. 2), after which the patient rapidly developed progressive pancytopenia. To clarify whether or not he had drug-induced cytopenia, we switched his drug regimen to 3.3 mg/kg L-AMB monotherapy daily on Day 8 and then to 1,200 mg fluconazole (FLCZ) monotherapy daily on Day 13. Despite the discontinuation of these medications, his cytopenia continued to progress. To identify the cause for his progressive cytopenia, we obtained a bone marrow aspirate and performed a biopsy on Day 18. Giemsa-stained specimens showed non-specific hypocellularity with no evidence of hemophagocytic syndrome, hematological malignancy, or immune thrombocytopenia. In addition, no abnormalities were found on a flow cytometric analysis. Grocott staining revealed infiltration by budding yeasts (Fig. 3). The aspirate smear mostly showed the peripheral blood state, failing to capture the bone marrow tissue, and its culture was negative for *C. neoformans.*

We also noted that our patient had adrenal insufficiency (AI), with a serum adrenocorticotropic hormone (ACTH) concentration of 4,152 pg/L and cortisol 12.8 μg/dL. Although he seemed unwell on transfer to our hospital and was lethargic during treatment, he lacked other findings characteristic of AI, such as hyponatremia, hyperkalemia, hypoglycemia, and a low blood pressure. No masses in, or enlargement of, the adrenal glands were found on plain CT. However, AI was confirmed by a rapid ACTH stimulation test. Hydrocortisone supplementation (30 mg daily) was started on Day 16, after which the patient's lethargy and loss of appetite resolved rapidly. Besides *C. neoformans,* none of the documented causes of AI in HIV-infected patients including *Pneumocystic jirovicii*, cytomegalovirus, mycobacterium, and anti-adrenal antibodies were detected by an examination of the blood, sputum, and CSF.

All CSF cultures after the initiation of FLCZ monotherapy and before Day 14 were positive for *C. neoformans*, suggesting that FLCZ was ineffective as acute-phase chemo-

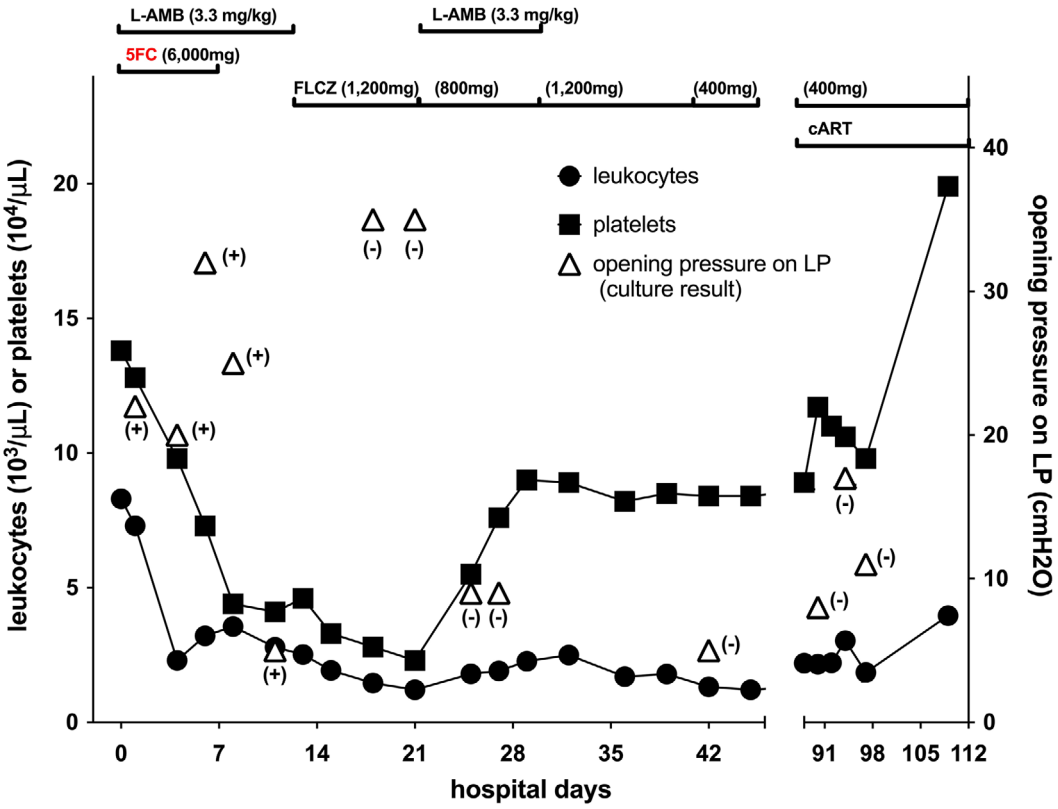

Figure 2. **Clinical course and laboratory findings in the present case. Leukocyte and platelet counts are presented per microliter (left Y axis). Opening pressure on lumbar puncture is presented as centimeters of H_2O (right Y axis). Results of culture of cerebrospinal fluid are presented adjacent to the opening pressures (+: positive, −: negative). Anti-fungal agents are presented at the top with their daily dose in parentheses. WBC: white blood cell count, L-AMB: liposomal amphotericin-B, 5FC: 5-flucytosine (daily dosage), FLCZ: fluconazole (daily dosage), cART: combination anti-retroviral therapy, LP: lumbar puncture**

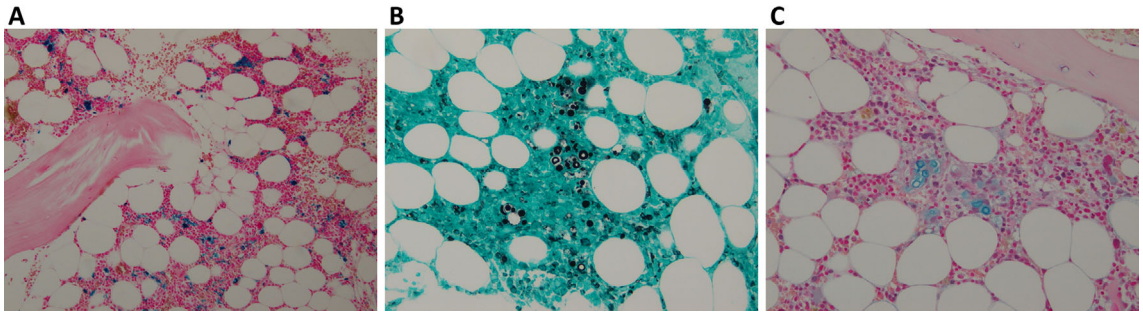

Figure 3. **Histopathological findings in bone marrow performed on Day 18. (A) Giemsa-stained section at 100× magnification showing non-specific hypocellularity. There was no evidence of immune thrombocytopenia, hemophagocytic syndrome, or hematological neoplasms. (B) The localized cluster of encapsulated yeasts is highlighted by Grocott methenamine silver staining (400× magnification). (C) Alcian blue stain at 400× magnification showing the mucopolysaccharide capsules of *C. neoformans*. The poor granuloma formation in this patient is typical in severely immunocompromised patients with cryptococcosis.**

therapy in this patient. We substituted a combination of 3.3 mg/kg L-AMB and 800 mg FLCZ daily for FLCZ monotherapy on Day 22, having concluded that our patient's myelosuppression was due to bone marrow cryptococcosis and not to L-AMB. As *C. neoformans* isolated from CSF culture was sensitive to L-AMB and FLCZ, peripheral blood cell counts started to recover soon after the reintroduction of L-AMB. By Day 29, the patient had developed acute L-AMB-related renal impairment, and 1,200 mg FLCZ monotherapy daily was substituted for L-AMB. After CSF cul-

tures collected on Days 17, 21, 22, and 25 all proved negative, we switched to consolidation treatment with 400 mg FLCZ daily on Day 35 before he was discharged on Day 46. It should be noted that his pancytopenia persisted at a moderate extent after L-AMB reintroduction during hospitalization. We subsequently initiated combination ART (cART) with co-formulated bictegravir, emtricitabine, and tenofovir alafenamide on Day 83. FBC on his latest follow-up visit showed significant recovery, with a leukocyte count of 3,960/L, hemoglobin level of 9.2 g/dL, and platelet count of 19.9×10^4/L.

Discussion

We herein report an instructive example of a patient with HIV infection and cryptococcosis who developed progressive pancytopenia after commencing anti-fungal treatment. Despite the fact that the pancytopenia developed after the initiation of that treatment, we concluded based on the clinical findings that the primary cause of the pancytopenia was bone marrow cryptococcosis.

It is worth noting that bone marrow cryptococcosis was only one of a number of possible primary causes of our patient's pancytopenia. Though our patient had a normal FBC on admission, all three blood cell components decreased rapidly soon after the introduction of L-AMB and 5FC (Fig. 1). Common causes of cytopenia in HIV-infected patients include HIV itself, lymphoproliferative disorders, and opportunistic infections, such as by mycobacteriosis and histoplasmosis. In addition, antibiotics-induced myelosuppression is another well-documented primary cause in patients undergoing treatment for cryptococcal meningitis. The overall incidence of leukocytopenia is reported by several studies to be 27-50% for 5FC, 15-20% for L-AMB, and < 1.5% for fluconazole, according to the prescribing information (10, 11). Considering this high occurrence of leukocytopenia caused by drugs, we discontinued 5FC, and then L-AMB. Nevertheless, the progressive pancytopenia persisted. Despite the fact that cryptococcus was identified in multiple organs, including the bone marrow, serological, microbiological, and histological tests yielded no evidence of other well-documented etiologies, suggesting that our patient's myelosuppression was attributable to bone marrow cryptococcosis. This diagnosis was consistent with his clinical course in that his FBC recovered quickly after reintroduction of L-AMB, the key drug for treating HIV-related cryptococcosis. Several *in vitro* studies have shown that cryptococcus infiltration can inhibit hematopoiesis. In one study, it was found that the polysaccharide capsule of *C. neoformans* inhibits hematopoiesis in patients with leukemia (12). Another reported that macrophages activated by this yeast suppress the development of erythroid progenitor cells in rats (13). In addition to cryptococcosis, HIV infection itself should also be considered as a cause of our patient's pancytopenia, as starting cART completely restored the FBC.

Of interest, our patient's pancytopenia progressed during the first two weeks, even after the initiation of antifungal therapy. This phenomenon may have represented a paradoxical upgrading reaction (PUR), an inflammatory rebound that is hypothetically attributable to the immunosuppressive effect of fungal capsular components released immediately after initiating antifungal treatment. The following facts strongly suggest that our antifungal therapy was effective in reducing the fungal load: blood cultures reverted to negative immediately after the initiation of antifungal therapy, CSF cultures reverted to negative from Day 18, and *C. neoformans* isolated from CSF culture was found to be sensitive to L-AMB and FLCZ. In another report of a patient with cryptococcal meningitis (14), antifungals were thought to have induced PUR, resulting in prolonged pancytopenia that paradoxically worsened with antifungal treatment. This may have occurred in our patient. However, his complicated clinical course prevented the clear identification of the impact of cryptococcosis and its treatment on hematopoiesis.

The cause of the patient's AI remained unknown. After most of the documented causes in HIV-infected patients, including cytomegalovirus and autoimmune adrenalitis, were excluded by laboratory findings, primary AI, HIV infection itself (15), FLCZ, and adrenal gland cryptococcosis remained as possible causes. High-dose FLCZ is reportedly associated with AI (16). Adrenal cryptococcosis can also cause AI, as reported in several cases, where an adrenal biopsy largely contributed to the diagnosis and treatment (17, 18). In our case, though, a biopsy was not indicated because of his low platelet count. Furthermore, we considered it unnecessary to make a definitive diagnosis for adrenal cryptococcosis because anti-cryptococcal treatment had been already initiated for the blood-culture-positive disseminated disease.

In summary, we encountered a patient with disseminated cryptococcosis whose pancytopenia persisted after discontinuation of the suspected causative drugs, ultimately being attributed to bone marrow cryptococcosis and HIV infection. Even in the presence of 5FC-related myelosuppression, bone marrow cryptococcosis is a possible concurrent cause of persistent cytopenia in patients with a massive fungal burden.

The authors state that they have no Conflict of Interest (COI).

Financial Support

This work was supported by Japan Agency for Medical Research and Development (AMED) [grant number 21fk0410038h 0001].

Acknowledgement

We thank all the staff of the National Center for Global Health and Medicine who were involved in this case, especially Dr. Maruyama, the endocrinologist who advised on the treatment of our patient's adrenal insufficiency; Dr. Togano, the hematologist who performed the bone marrow biopsies; and Drs. Misumi and Igari, the pathologists who performed the pathological examina-

tion of relevant specimens. We also thank Dr Trish Reynolds, MBBS, FRACP, for editing the English text of a draft of this manuscript.

References

1. World Health Organization. Rapid advice: diagnosis, prevention, and management of cryptococcal disease in HIV-infected adults, adolescents and children. 2011.
2. Maziarz EK, Perfect JR. Cryptococcosis. Infect Dis Clin North Am **30**: 179-206, 2016.
3. Vechi HT, Theodoro RC. Invasive fungal infection by *Cryptococcus neoformans* var. grubii with bone marrow and meningeal involvement in a HIV-infected patient: a case report. BMC Infect Dis **19**: 220, 2019.
4. Pantanowitz L, Omar T. Bone marrow cryptococcal infection in the acquired immunodeficiency syndrome. J Infect **41**: 92-94, 2000.
5. Ashiwini BR, Raghupathi A, Srinarthan A. Cryptococcosis of bone marrow: a case report with review of literature. J Clin Diagn Res **8**: 158-159, 2014.
6. Suner L, Mathis S. Disseminated cryptococcosis in bone marrow. Blood **123**: 3070, 2014.
7. Paolo WF Jr, Nosanchuk JD. Adrenal infections. Int J Infect Dis **10**: 343-353, 2006.
8. Chuck SL, Sande MA. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. N Engl J Med **321**: 794-799, 1989.
9. Stamm AM, Diasio RB. Toxicity of amphotericin B plus flucytosine in 194 patients with cryptococcal meningitis. Am J Med **83**: 236-242, 1987.
10. AmBisome (amphotericin B) liposome for injection. Astellas Pharma US, Inc. 2020 [Internet]. [cited 2021 Jun 4]. Available from: https://www.astellas.us/docs/ambisome.pdf.
11. DIFLUCAN. Roerig, Division of Pfizer Inc. 2011 [Internet]. [cited 2021 Jun 4]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/019949s051lbl.pdf.
12. Stypulkowski C, Jordan M, Dorezal M. Inhibition of proliferation of the bone marrow in cases of chronic lymphatic leukaemia with extracts of *Cryptococcus neoformans*. Folia Biol **15**: 65-68, 1967.
13. Roodman GD. Mechanisms of erythroid suppression in the anemia of chronic disease. Blood Cells **13**: 171-184, 1987.
14. Einsiedel L, Gordon DL, Dyer JR. Paradoxical inflammatory reaction during treatment of *Cryptococcus neoformans* var. gattii meningitis in an HIV-seronegative woman. Clin Infect Dis **39**: 78-82, 2004.
15. Chrousos GP, Zapanti ED. Hypothalamic-pituitary-adrenal axis in HIV infection and disease. Endocrinol Metab Clin North Am **43**: 791-806, 2014.
16. Riedl M, Maier C, Zettinig G, Nowotny P, Schima W, Luger A. Long term control of hypercortisolism with fluconazole: case report and *in vitro* studies. Eur J Endocrinol **154**: 519-524, 2006.
17. Ito M, Hinata T, Tamura K, et al. Disseminated cryptococcosis with adrenal insufficiency and meningitis in an immunocompetent individual. Intern Med **56**: 1259-1264, 2017.
18. Matsuda Y, Kawate H, Okishige Y, et al. Successful management of cryptococcosis of the bilateral adrenal glands and liver by unilateral adrenalectomy with antifungal agents: a case report. BMC Infect Dis **11**: 340, 2011.