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

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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

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...
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 7.79×10⁶ 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 cmH₂O, 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 of the blood, sputum, and CSF.

All CSF cultures after the initiation of FLCZ 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 nonspecific 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 Pneumocystis jirovecii, 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 monother-

| Table. Laboratory Data on Initial Admission. |
|---------------------------------------------|
| LDH 168 IU/L | WBC 8300/μL |
| BUN 26 mg/dL | CD40 26/μL |
| CRE 0.68 mg/dL | CD80 103/μL |
| Gla 112 mg/dL | Hgb 19.1 g/dL |
| Na 136 mEq/dL | MCV 87.3 fl |
| K 3.4 mEq/dL | Plt 15.8 × 10⁹/μL |
| Cl 99 mEq/dL | Ferritin 1671 ng/mL |
| Ca 9 mEq/dL | CRP 2.05 mg/dL |

Abbreviations: BUN, blood urea nitrogen; Ca, calcium; CD4+, cluster of differentiation 4 T-cell count; CD8+, cluster of differentiation 8 T-cell count; Cl, chloride; CRL, 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.
therapy 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-

apy and before Day 14 were positive for *C. neoformans*, suggesting that FLCZ was ineffective as acute-phase chemotherapy 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

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 H2O (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-fluorocytosine (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.
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 re-initiation 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 adenaliitis, 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 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).

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Authorship statement
All authors meet the ICMJE authorship criteria.

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