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Hepatosplenic fungal infection during induction remission therapy for acute promyelocytic leukemia: a case report

Le infezioni epatospleniche fungine durante trattamento di induzione della remissione per leucemia acuta promielocitica: un caso clinico

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Summary

Hepatosplenic fungal infections (HSF) are the primary type of invasive fungal infections (IFI) identified among patients with acute leukemia. We describe a case of HSF acquired during remission induction for acute promyelocytic leukemia (APL) initially misdiagnosed as all-trans retinoic acid syndrome. Computed tomography (CT) identified a number of low-density lesions within the liver and spleen. Pathology following splenectomy was positive for fungal spores, indicating a deep-tissue fungal infection. The patient responded well to antifungal therapy and is currently in complete remission. HSF is a rare and life-threatening complication among APL patients undergoing chemotherapy. Rapid diagnosis and effective treatment are crucial to preventing the high mortality rate that accompanies HSF. Examination of tissue following surgical excision is currently an effective approach for the definitive diagnosis of IFI, and a high index of suspicion should be maintained in

Riassunto

Le infezioni epatospleniche fungine (ISF) rappresentano le infezioni fungine invasive (IFI) primarie riscontrate in pazienti affetti da leucemia acuta. Viene descritto un caso di ISF che si è presentato durante il trattamento di induzione della remissione per leucemia acuta promieolocitica (LAP) inizialmente mis-diagnosticata con la sindrome da acido trans-retinoico. La tomografia computerizzata (TC) evidenziava un numero di lesioni a bassa densità tra il fegato e la milza. L'istopatologia successiva alla splenectomia era positiva per la presenza di spore fungine, a dimostrazione di una infezione fungina a livello tissutale profondo. Il paziente rispondeva bene alla terapia antifungina e attualmente è in completa remissione. La ISF è una rara e pericolosa complicazione riscontrabile tra i pazienti affetti da LAP in trattamento chemioterapico. La diagnosi tempestiva ed un efficace trattamento sono requisiti essenziali per prevenire l'elevato tasso di mortalità che caratterizza le ISF. La valutazione del tes-

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suto ottenuto da asportazione chirurgica rappresenta attualmente l'approccio ottimale per poter effettuare diagnosi di IFI ed è consigliato mantenere un alto livello di attenzione nei pazienti neutropenici. Eur. J. Oncol., 17 (1), 49-54, 2012

Parole chiave: **leucemia acuta promielocitica; sindrome da acido trans-retinoico; infezioni fungine epatospleniche**

Introduction

A gradual, annual increase in the incidence of invasive fungal infections (IFI) following the onset of acute leukemia and subsequent chemotherapy has been observed. Hepatosplenic fungal infection (HSF) is the primary type of IFI, and difficulties still remain in the early diagnosis and treatment of these infections. This is especially true for HSF in patients with acute promyelocytic leukemia (APL), where the onset of fever secondary to all-trans retinoic acid (ATRA) syndrome must be differentiated from active infection. Failure to correctly diagnose HSF infection early may result in significant morbidity and mortality (1, 2). We report the successful management of one case of HSF in a patient with APL during remission induction with ATRA and consolidation treatment.

Case report

A 38-year-old male presented with a one-month history of fever, anorexia, chest pain, and postprandial abdominal discomfort; however, there was no documented weight loss. Upper endoscopy identified multiple gastric ulcers with minimal bleeding suggestive of *Helicobacter pylori*. Biopsies revealed no evidence of malignancy. The patient was treated with a two-week course of rabeprazole (10 mg), clarithromycin (500 mg), and metronidazole (40 mg) twice daily for 2-3 weeks. Unfortunately, pancytopenia occurred, and the patient was admitted for further evaluation. Complete blood count (CBC) showed a white blood cell (WBC) count of 0.74×10^9/L, hemoglobin (Hb) of 69 g/L, and platelet count of 16×10^9/L. Alkaline phosphatase and liver function tests were normal. Physical examination revealed pale mucous membranes and conjunctiva and bruising was observed over the right hip. On June 14th, bone marrow examination showed predominately promyelocytes (86%), which is consistent with APL. Assessment for the fusion gene transcript promyelocytic leukemia-retinoic acid receptor-alpha (PML-RAR-α) was positive. Blood coagulation tests, including platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen, were all normal. Chest computed tomography (CT) and abdominal ultrasonography revealed no abnormalities. Treatment with 20 mg of oral ATRA BID was initiated, and 200-400 mL of plasma and 10 units (U) of platelets per day were administered to alleviate the patient's dangerous bleeding symptoms which were related to APL.

Two days after ATRA treatment, the patient's temperature increased to 40°C and was accompanied by a sore throat, shortness of breath, and chest tightness. CBC revealed a WBC of 1.26 x 10^9/L, Hb of 67 g/L, and a platelet count of 15 x 10^9/L. A C-reactive protein and endotoxin level were both normal. Additionally, blood and throat cultures were negative. Chest CT identified small, bilateral pleural effusions, and ATRA syndrome was diagnosed based upon the CT findings and the patient's symptoms. Treatment involved a combination of dexamethasone (5 mg QD) and imipenem (1 g TID). The dexamethasone was continued for 43 days. After 8 days, the WBC count dramatically increased to 26.3×10^9/L, and the platelet count was 28×10^9/L.

The ATRA dose was tapered to 30 mg. Furthermore, low-dose HA (homoharringtonine [HHT]) at 0.25-0.5 mg/m^2 and cytarabine (Ara-C) at 25-50 mg/m^2 were initiated and daily adjusted based on the WBC count. When the WBC count dropped to 2.1 × 10^9/L, the dose of ATRA was increased to 20 mg BID for 20 days. However, the patient again developed a high fever (42°C), and 10 mg of dexamethasone was administered for 14 days. On July 20th (46 days from the time of APL diagnosis), blood tests returned to the normal values, and bone marrow examination showed complete remission (CR).

Subsequently, arsenious acid was administered as a consolidation treatment; however, the patient's temperature remained elevated despite the cessation of ATRA treatment. Repeat blood cultures, an endotoxin test, and a (1-3)-β-D-glucan test were all normal. An assay for anti-tuberculosis antibodies was negative. On August 10th, a chest CT showed an increase in the size of the pleural effusions compared with previous scans, and multiple low-density lesions of varying size were identified in the spleen (Figure 1A). Chemotherapy with a combination of daunorubicin (40 mg/m^2) and Ara-C (100 mg/m^2) was started. Oral itraconazole (5 mg/kg/day) was added to the regimen for fungal prophylaxis and continued for the duration of chemotherapy.

In September, repeat CT scans continued to show multiple low-density lesions in the spleen, and an ultrasound-guided splenic biopsy was performed. Pathologic examination identified necrotic tissue at the edge of the inflammatory fibrous tissue, but no evidence of fungal infection was found. After two courses of chemotherapy, CT scan showed an increase in the number of lesions within the liver and spleen.

Three months after CR, the patient still had an intermittent, low-grade fever. Blood cultures performed at this time were negative. Repeat blood tests showed an endotoxin level at 233.7 pg/mL and (1-3)-β-D-glucan level at 254.65 pg/mL (normal range 2-20 pg/mL). CT continued to show an increased number of hepatic and splenic lesions (Figure 1B). Caspofungin (50 mg/day) and ciprofloxacin (200 mg/day) were initiated at this point. After 2 weeks on this treatment regimen, his positive response (*ie*, afebrile) suggested the presence of a deep fungal infection involving the liver

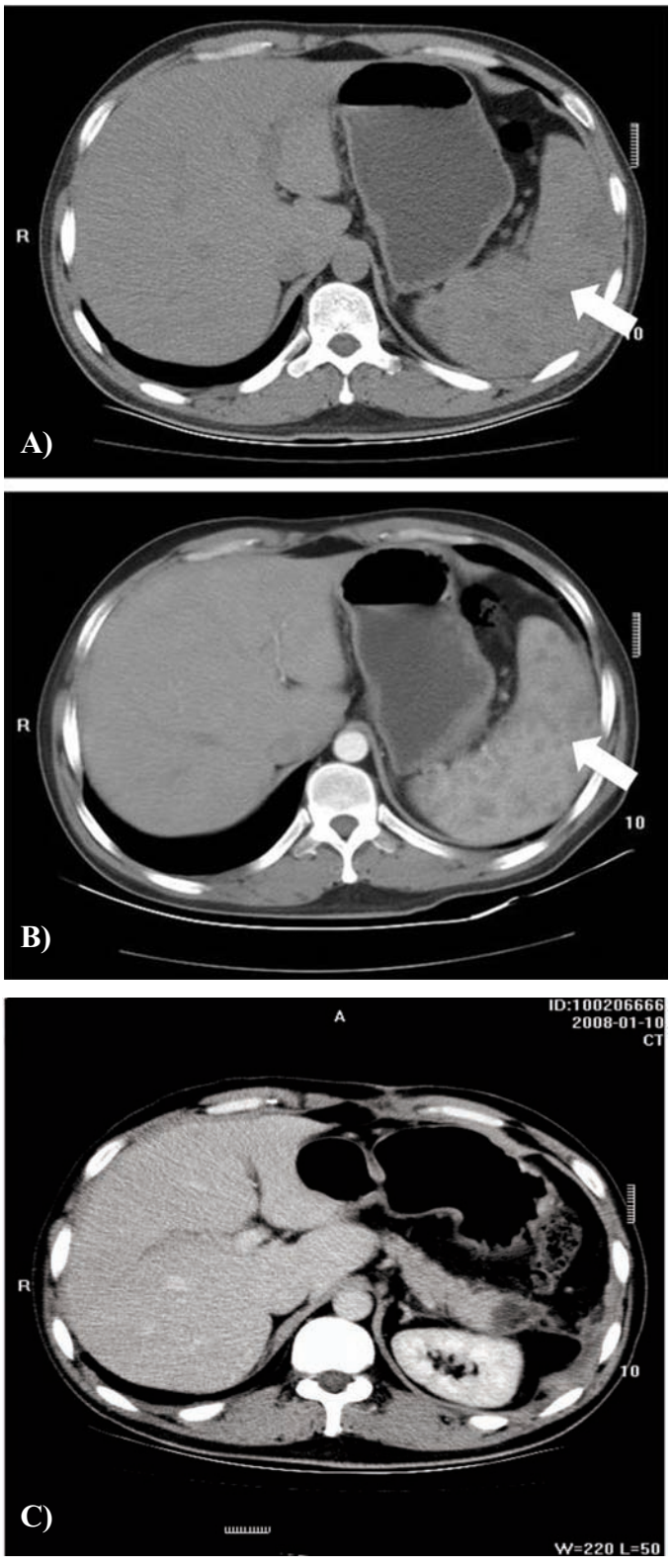

Fig. 1. The abdominal computed tomography (CT) images were taken 2 months after the treatment of APL (A); 5 months after the treatment of APL (B) and after splenectomy (C)

and spleen. Concerns about a worsening deep splenic infection and the risk of splenic rupture led to a splenectomy on December 14th (Figure 1C). An adhesion between the spleen and the stomach associated with some purulent discharge was observed. The spleen pathology report identified necrotic tissue, Langerhans cells, and positive PAS and silver staining for fungal spores (Figure 2). No hyphal forms were detected. Overall, the definitive diagnosis of ISI involving the spleen took 4 months (from the time of the abnormal CT to the final pathology report following splenectomy). Unfortunately, attempts to culture the fungus failed, presumably due to previous anti-fungal therapy.

Following the splenectomy, the patient was afebrile, and the (1-3)-β-D-glucan level decreased to within the normal range. Voriconazole 400 mg was orally administrated q12h and continued for >1 year. After 4 months on voriconazole, a CT scan indicated significant improvement in both the size and the number of hepatic lesions. Additional ATRA treatment did not cause any fever during continued chemotherapy. At present, the patient remains in CR and negative for the PML-RAR- gene fusion; he still receives regular chemotherapy. CT scans and (1-3)-β-D-glucan examinations are repeated once a month and continue to be normal. The alkaline phosphatase levels remained normal throughout the entire course of treatment.

Discussion

HSF infection is a form of disseminated fungal infection with a predilection for the liver, spleen, and kidneys, affecting as many as 7% of acute leukemia patients (2). Early diagnosis remains a challenge because cytologic, histologic, and microbiologic findings are often negative despite the presence of active infection. The mortality rate has been reported as high as 40% in patients with IFI damaging multiple organs, 50% in patients with fungemia, and >90% in patients with invasive *Aspergillus* infection (3, 4). Furthermore, identification and treatment of HSF infections present therapeutic challenges that often require interruption of chemotherapy for the leukemia, leading to progression or relapse of the disease. The combination of ATRA and anthracycline-based chemotherapy is considered the standard induction treatment in APL (5).

In this case, APL was successfully treated with ATRA for remission induction and consolidation; however, recovery was complicated by HSF infection missed early in the course of treatment. The sudden onset of fever, accompanied by shortness of breath and chest tightness, shortly after initiating ATRA treatment, along with the finding of pleural effusion on CT, led to the erroneous diagnosis of ATRA syndrome. Also referred to as APL differen-

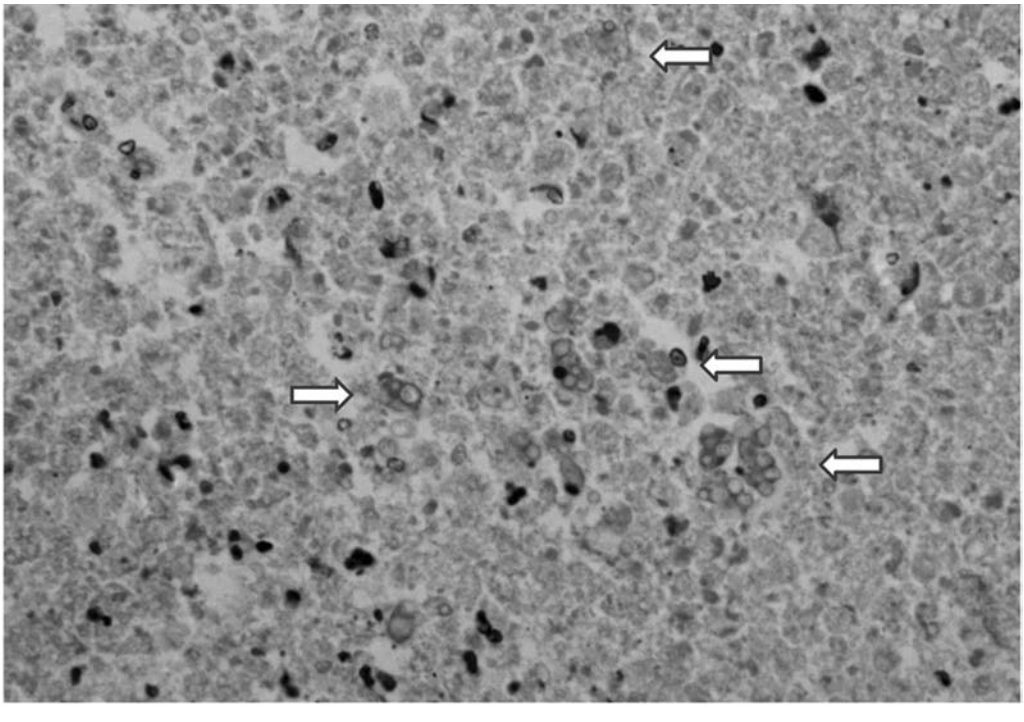

Fig. 2. Postoperative pathological examination (×400). The reported visible necrotic tissue, Langerhans cells, and PAS-positive fungal spores in the spleen, supporting the diagnosis of fungal infection

tiation syndrome and retinoic acid syndrome, ATRA syndrome is the major side effect of ATRA treatment and can include unexplained fever, weight gain, respiratory distress, interstitial pulmonary infiltrates, pleural and pericardial effusion, hypotension, and acute renal failure. The incidence of ATRA syndrome in APL patients has been reported between 10% and 16%, and the mortality rates vary between 2% and 10% (6,7). Carrillo-Esper et al. reported the incidence and mortality rate to be as high as 27% and 29%, respectively (8). Once suspected, treatment involves immediate cessation or reduction of the ATRA dose and initiation of corticosteroids in order to combat the systemic inflammatory response from the ATRA-induced effect on promyelocytic differentiation.

Although ATRA syndrome should be considered in the differential diagnosis as a cause of fever in the early stage of remission induction treatment for APL, infection (specifically IFI), should top the list. HSF infection may occur in febrile neutropenic patients and should be considered in the presence of fever unresponsive to antibiotics and/or nausea/vomiting unresponsive to conventional antiemetics associated with generalized or right upper quadrant abdominal pain (1, 2, 9). According to a report by Masood, elevated serum alkaline phosphatase levels, up to 10-fold of the normal value, have also been observed in 60-91% of HSF infections (1).

The diagnosis of HSF also depends upon blood culture results, imaging studies, and tissue pathology from biopsy, when feasible. Unfortunately, blood cultures are positive in <50% of patients, and liver and/or spleen biopsies are often technically too difficult to perform and are not always successful in detecting fungal infection (10, 11). Several reports have compared the efficacy of ultrasonography, CT, and magnetic resonance imaging (MRI) in detecting hepatosplenic lesions (2, 12). Although MRI demonstrated the highest diagnostic accuracy, cost and availability limit its use in many areas (1). CT scan was found to be far superior to ultrasonography in the detection of hepatosplenic lesions (97% *vs* 65%, respectively) (9). In our case, abdominal ultrasound was used instead of CT and may have missed existing hepatosplenic lesions. However, imaging modalities might fail to identify fungal lesions when patients are neutropenic due to the inability to mount the inflammatory response required to generate radiologically visible findings (*eg*, infiltrate). In the case of a negative imaging study in a neutropenic patient with a high index of suspicion for HSF infection, repeated imaging is required, especially after hematologic recovery.

In our case, the initial imaging study (abdominal ultrasound) failed to identify any liver or spleen abnormalities, and subsequent CT studies several months later finally suggested the presence of hepatic and splenic lesions. Blood cultures and splenic biopsy were negative, and splenectomy was performed. Subsequent pathologic examination confirmed the diagnosis of HSF. Retrospectively, several factors predisposed our patient to HSF infection: 1) APL caused agranulocytosis and immune system dysfunction; 2) the gastrointestinal ulcers treated with a proton pump inhibitor may have caused a relative achlorydia, thereby promoting fungal colonization; 3) further immune system comprise occurred as a result of the leukemia treatment; 4) the administration of high-dose, broad-spectrum antibiotics may have altered the patient's normal microflora; and, 5) dexamethasone used to alleviate the symptoms associated with presumed ATRA syndrome further suppressed the immune system and promoted fungal growth.

Because the diagnosis of HSF infection is so challenging, a high degree of clinical suspicion must be maintained for all leukemia patients. New guidelines recommend a serum galactomannan test and (1,3)-β-D-glucan (GM/G) test for early diagnosis and preemptive treatment of IFI. This approach may help to identify IFI earlier and may also be used to dynamically follow the changes in the course of the infection (13, 14). Another emerging diagnostic strategy includes testing for serum D-arabinitrol, which has been identified in 40% of tissue-proven IFI patients with negative blood cultures (1). The use of polymerase chain reaction (PCR) to detect fungal DNA in the blood of neutropenic patients has also been reported (15). Ongoing research suggests a role for certain cytokines and immune system modulators, such as interleukin-10, tissue necrosis factor-alpha, and lymphotoxin-alpha, in the development of IFI, which could lead to the development of additional diagnostic tests (1).

As previously mentioned, routine microbiological examinations were consistently negative in this case. Although PCR testing was not performed in this case, hepatosplenic candidiasis is generally considered the most frequent IFI in this setting. Other etiologies, including cryptococcosis and histoplasmosis, were also considered. The pathology report after splenectomy confirmed the presence of fungal infection. Anti-fungal treatment for IFI must be targeted to the specific pathogen, when possible, and continued at a sufficient dose for an extended period of time (usually months) to achieve a long-term cure. Our patient continued treatment with oral voriconazole (400 mg) for more than 1 year, and the results of 1-3-β-D-glucan assays and abdominal CT scans following splenectomy remained normal.

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