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Case Report

Granulocyte Colony Stimulating Factor-induced Exacerbation of Fungus-related Immune Restoration Inflammatory Syndrome: A Case of Chronic Disseminated Candidiasis Exacerbation

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Chronic disseminated candidiasis is a complication of the intensive therapies of hematological malignancies revealed during hematopoietic recovery, a context reminiscent of the immune restoration inflammatory syndrome in human immunodeficiency virus patients receiving antiretroviral therapy. We report a case of severe exacerbation of chronic disseminated candidiasis after pegylated granulocyte-colony stimulating factor administration. We emphasize the major inflammatory substrate of the disease and suggest that immune-modulating strategies such as hematopoietic growth factors, should be used cautiously in such patients.

KEYWORDS: chronic disseminated candidiasis, hematopoietic growth factors, hepatosplenic candidiasis, immune restoration inflammatory syndrome

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Introduction

Bodey et al first described hepatosplenic candidiasis, also referred as chronic disseminated candidiasis (CDC), in 1969.[1] It since has been recognized as a complication of the intensive therapies used in hematological malignancies, occurring in about 5% of acute myeloid leukemia (AML) cases.[2]

The pathophysiology of CDC is not yet fully understood. It is hypothesized that an initial colonization of

the gastrointestinal tract by *Candida* species, followed by blood translocation through the portal venous system, induces fungal hepatic and splenic dissemination. Large probabilistic antifungal therapy, justified by the aplasia-induced high dose chemotherapy, prevents the onset of a clinically apparent invasive fungal infection (IFI). CDC is then only revealed in the course of neutrophil recovery with fever, liver pain, cholestasis, disturbance of inflammatory parameters and disseminated hepatic and splenic abscess-like nodular lesions.[3] A major characteristic is the absence of evidence for an active IFI as judged by the absence of *Candida* sp. growth on liver biopsy specimens, blood or elsewhere. In contrast, non-specific inflammatory lesions, most usually a typical granulomatous reaction, are revealed.[4,5] Thus, CDC appears to share several features with IFI-associated immune reconstitution inflammatory syndrome (IRIS) also described in newly treated human immunodeficiency virus (HIV)-infected patients, or solid organ-transplanted patients.[4,6]

Until now, CDC was reported to require long-term systemic antifungal therapy. However, some cases of persisting, relapsing or even life-threatening manifestations, unresponsive to antifungals, have been reported, severely alleviating the prognosis.[2] In such patients, steroids may represent a useful adjuvant therapeutic procedure.[5]

We report a case of severe exacerbation of CDC following the use of pegylated granulocyte-colony stimulating factor (G-CSF), as confirmed by a significant worsening of hepatic lesions with extensive granulomas and necrosis. We discuss the potential promoting impact of hematopoietic growth factors in the development and severity of CDC and also illustrate the beneficial impact of corticosteroids (CST) in severe forms of CDC.

Case Report

In November 2006, a 75-year-old man was diagnosed with AML M0 with normal cytogenetics. Chronic lymphocytic leukemia Binet stage A had been diagnosed 10 years prior to AML, not necessitating any treatment. He received induction chemotherapy for AML with intravenous idarubicin (9 mg/m^2/day) for 4 days + cytosine arabinoside (200 mg/m^2/day) continuously for 7 days, supported with a single injection of pegylated G-CSF (pegfilgrastim, 6 mg on day 9). During the neutropenic period, and after first receiving conventional broad-spectrum antimicrobial therapy and empirical caspofungin for persistent fever, he was diagnosed with a possible pulmonary aspergillosis (Day 20 post-chemotherapy). He was then switched to voriconazole, 200 mg twice daily, with serum residual therapeutic levels > 1 ng/mL.

As neutrophil recovery occurred (December 16, 2006 on Day 24 post-chemotherapy), the patient was again febrile. Complete remission was documented on bone marrow examination. Computed tomography scanning showed regression of the suspected aspergillosis-related pulmonary nodules but the presence of multiple abscess-like liver and spleen lesions consistent with CDC (Figure 1A). At that time, screening for infectious agents, including *Mycobacteria*, was unsuccessful. Tests for *Candida* sp. carriage (urine, stools, blood, and mouth) as well as anti-Candida antibodies and serum mannan antigenemia detection were negative. Histological analysis of a liver lesion biopsy specimen revealed a nonspecific inflammatory infiltrate and fibrosis of portal spaces with no infectious agent (PAS or periodic acid Schiff was used to detect carbohydrate containing structures, including some fungi, *Actinomyces* sp. and macrophage inclusions of Wipple disease; the Grocott stain was used to detect fungal elements, and the Ziehl-Nielsen stain was used to detect bacteria, notably *Mycobacteria*) as well as bacterial and fungal cultures. There was no underlying metabolic or viral hepatic disease. Voriconazole alone was maintained, since the fever decreased spontaneously and the patient could go on chemotherapy.

In February 2007, after receiving another pegylated G-CSF injection (pegfilgrastim, 6 mg) following his first consolidation chemotherapy (Idarubicin 9 mg/m^2/day on day 1 + cytosine arabinoside 100 mg/m^2 subcutaneously twice per day for 5 days), he was hospitalized with severe asthenia, high fever (40 °C) and shivers despite a normal neutrophil count (6.6×10^9/L) and empirical oral antimicrobial therapy of more than 48 hours. No specific infection (e.g. *Mycobacteria* and *Bartonella henselae*) was documented but abdominal ultrasound and computed tomography scan revealed a significant worsening of hepatosplenic lesions (Figure 1B). Histological findings were significantly modified, consisting of typical granulomatous lesions surrounding a necrotic center and a peripheral inflammatory reaction associated with the same pre-existing fibrotic

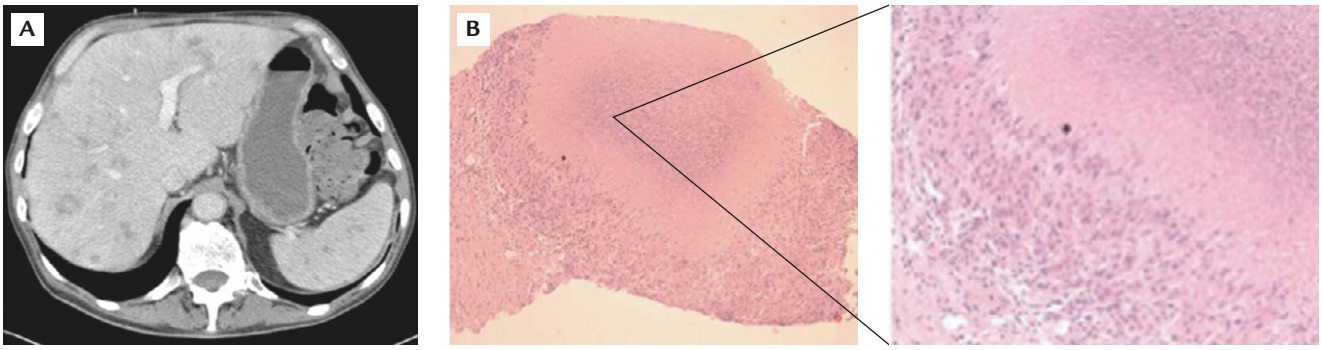

Figure 1. (A) Computed tomography scan showing exacerbation of hepatosplenic lesions. (B) Liver biobsy specimen recealing a typical epithelioid reaction surrounding a necrotic center.

lesions. Once again, there was no detectable infectious agent on the liver biopsy specimen after specific staining, fungal culture and immunohistochemical analysis for *Candida* (streptavidine peroxydase, Biogenesis, Argene). A molecular analysis towards bacteriological agents (16S rDNA PCR) was also negative. At that time, while maintaining the same antifungal therapy, we decided upon an adjuvant CST therapy due to the severity of the clinical manifestations. The patient initially received 0.5 mg/kg/day with a rapid tapering over 3 weeks. However, after an initial improvement, he relapsed while the CST was being decreased; CST had to be increased again to 1 mg/kg/day and maintained for 1 month. After this, the CST was slowly decreased according to clinical symptoms (fever, asthenia, abdominal pains) and serum levels of liver enzymes (alkaline phosphatase and γGT, which were 4 and 8 times the normal values, respectively, at CST initiation; Figure 2). It is worth noting here that the patient undertook his chemotherapy program concomitantly with approximately monthly consolidation courses (total of 6 identical courses), but G-CSF was not re-administered due to the complications associated with this patient.

Despite CST and chemotherapy, the patient did not suffer any other serious infectious complications. In October 2007, CST and voriconazole were withdrawn, based on the normalization of inflammatory (C-reactive protein and fibrinogen) and liver parameters, (except γGT, which was 1.5 times higher than the normal value, possibly owing to voriconazole-related toxicity; Figure 2). The hepatic and splenic lesions had been replaced by non-evolving sequelae. After 18 months, the patient showed no signs of relapse of either hematological or inflammatory disease.

Discussion

CDC is a well-recognized entity corresponding to an inflammatory complication of systemic infections by *Candida* sp., mostly of the liver, spleen and, more rarely, the lung and the kidneys. It can arise during the course of hematopoietic recovery after intensive chemotherapy.[2,3] However, the pathophysiology of CDC is unclear and the management of patients with CDC in current clinical practice is still challenging. The original nature of the case presented here led to a reliance on the identification of a potentially very strong immune risk factor predisposing to severe forms of CDC, i.e. the use of hematopoietic growth factors.

Evolving *Candida* sp.-related invasive infections are preferentially associated with an unbalanced Th2-type immune response.[7,8] This is deleterious, since an effective response against *Candida* sp. infections requires a Th1-type immune response, with a major role for TNF-α (tumor necrosis factor-alpha) and IFN-γ (interferon-gamma) production.[9,10] In contrast, CDC is thought to result from an excessive Th1 response,[5] as testified by the presence of necrotizing granulomas and T cell-type lymphoid infiltrates, and the absence of fungal documentation, apart from potential debris from dead fungi. This last implies that the pre-existing *Candida* sp.-related IFI is perfectly well-controlled at the time of CDC diagnosis. Finally, the onset of the disease during hematopoietic restoration following intensive chemotherapy is also a major criterion in favor of an excessive immune reaction and justifies the inclusion of CDC within the spectrum of IFI-related immune restoration inflammatory syndrome, a disease typically mediated through an uncontrolled Th1-type immune

Years	2006		2007							Normal range
Day/month	16/11	19/12	21/01	04/03	20/03	04/04	20/05	29/06	16/08	
PMN	0.6	7.7	7.2	24.0	10.6	6.6	11.4	7.5	3.9	2–7.5 × 10^9/L
CRP	12	137		240	146	86	61	12	24	< 10 mg/L
ALP	88	166	561	516	622	376	421	200	210	< 135 IU/L
GGT	14	130	133	405	413	231	139	151	147	< 50 IU/L
Fibrinogen		6.8	7		7			3.4		3–4 g/L

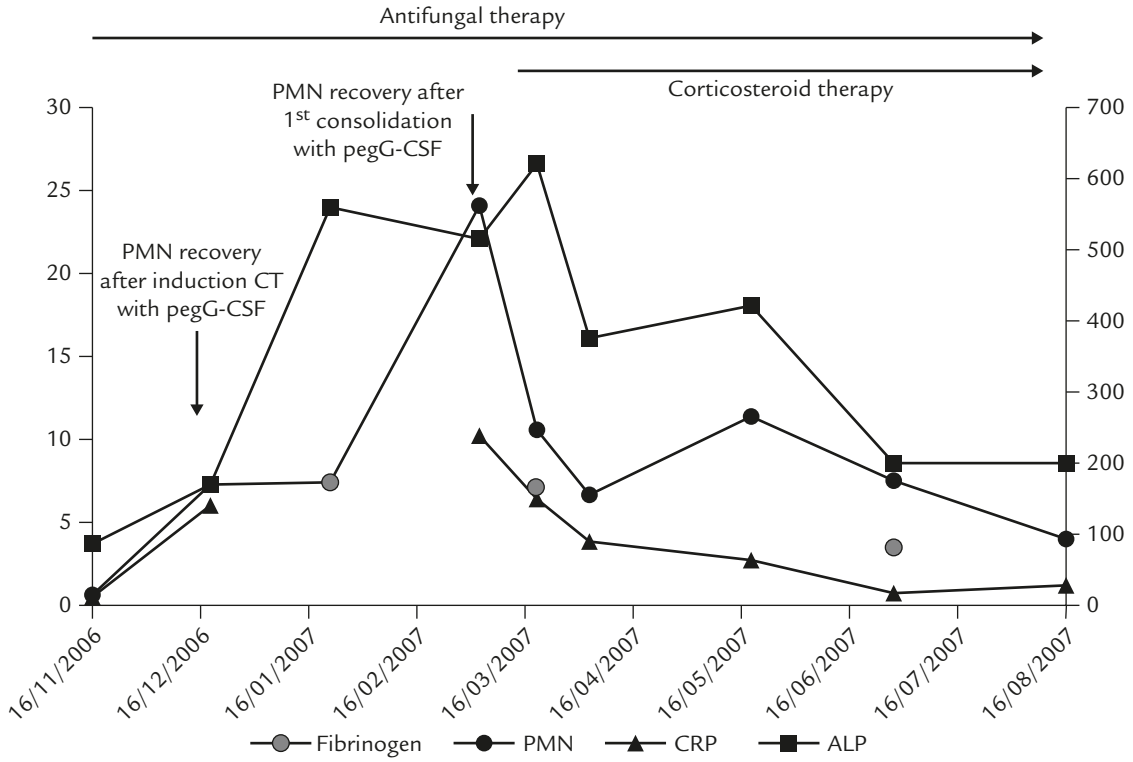

Figure 2. Biological parameters evolution according to pegylated G-CSF and corticosteroid therapy. PMN = Polymorphonuclears; ALP = alkaline phosphatases; CRP = C-reactive protien; GGT = gamma glutamyl transferase.

response and observed in various pathological conditions, including patients infected with HIV and/or *Mycobacterium tuberculosis*, or solid organ transplant recipients.[6,11] At present, however, specific studies are needed to precisely identify the immune substrate of CDC and to demonstrate the hypothesis of an excessive Th1 immune reaction described elsewhere for standard cases of IRIS.[4,11]

Filgrastim is a recombinant human G-CSF, which accelerates neutrophil recovery after hematotoxic chemotherapy and/or stimulates peripheral blood stem cell mobilization to prepare for autologous stem cell transplantation following high-dose chemotherapy. The pegylated form of filgrastim exhibits a major hematopoietic growth factor activity *in vivo* because of a prolonged half-life owing to a reduction in renal clearance. Pegfilgrastim is associated with earlier, and stronger, neutrophil recovery compared with filgrastim, as demonstrated by higher CD34 progenitor levels after hematotoxic chemotherapy, and its successful use for peripheral blood stem cell mobilization rescue in poor mobilizer patients with multiple myeloma.[12,13]

Filgrastim primarily affects neutrophil progenitors and its effects on other immune cells appear either limited or contradictory. However, G-CSF therapy probably has immune-modulating properties for monocytes/macrophages, i.e. Th1/2 lymphocyte-inducing dendritic cells,[14,15] and it apparently has the potential to induce disparate

immune responses depending on the underlying disease and/or the context.[16,17]

Little is known about the impact of G-CSF use on neutropenic patients treated for a *Candida* sp. invasive infection following hematotoxic chemotherapy. In a mouse model of disseminated candidiasis, Graybill et al[18] reported that mice treated with G-CSF and fluconazole showed increased survival and a reduced kidney tissue count of *Candida* sp. compared with mice receiving fluconazole or G-CSF alone. There are currently no human data available, either after recovery from neutropenia or in the context of CDC. Based on the pathophysiology of IFI-related IRIS in HIV-infected patients[4,6,11] and the consistency of a Th1-type immune response in CDC, it is tempting to postulate that CDC may be exacerbated through G-CSF therapy itself (i.e. increased immune cell recruitment at the abscesses sites) as well as through the promotion of an additive and potent Th1 immune response. Therefore, G-CSF, and particularly its long half-life pegylated form, should be used cautiously in recovering leukemic patients who have suffered, or are still suffering from an IFI, especially in a proven case of CDC.

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