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LETTER TO THE EDITOR

Febrile pancytopenia as uncommon presentation of disseminated toxoplasmosis after BMT

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Toxoplasmosis disease is a life threatening complication after hematopoietic SCT (HSCT). Indeed, in a recent prospective EBMT survey of *Toxoplasma gondii*-seropositive recipients, its incidence was estimated at 6%. The incidence of toxoplasmosis infection, defined as detection of *T. gondii* DNA by PCR, was evaluated at 16%.[1] Unfortunately in a previous retrospective study half of the toxoplasmosis disease cases reported after HSCT were diagnosed post-mortem.[2] We present here evidence for a misleading case of disseminated toxoplasmosis.

A 34-year-old Moroccan woman with very severe aplastic anemia unsuccessfully treated with immunosuppressive therapy underwent sibling allogeneic BMT. The conditioning regimen was fludarabine 30 mg/m^2 i.v. for 4 days, CY 30 mg/kg i.v. for 4 days and anti-thymocyte globulin 5 mg/kg for 4 days. She received CYA as GVHD prophylaxis. The patient was seropositive for *T.gondii* while the donor was seronegative. She experienced very slow and incomplete neutrophil and platelet engraftment impeding use of cotrimoxazole for toxoplasmosis prophylaxis, and requiring repeated treatment with PEG G-CSF. A semi-monthly follow-up of toxoplasmosis reactivation by PCR analysis (LightCycler Roche Diagnostics, Basel, Switzerland) in peripheral blood was performed and remained negative. The method used was a TaqMan-based real-time PCR assay that amplifies a 529-bp (AF487550) unit repetitive DNA sequence in the *T. gondii* genome. In our hands, this technique can detect as few as three copies/mL.

Post-transplant course was complicated by CMV reactivation (positive PCR at 4.2 log/mL N < 2.7) on day +20 treated with valganciclovir and tonsillar EBV-associated lymphoproliferative disease on day +30 successfully treated with four courses of rituximab. The recipient did not present any GVHD. On day +100, she was hospitalized for fever, asthenia and a 1-week history of back pain with dermatomal distribution at T12/L1 level. Clinical examination did not show any sign of neurologic deficit, meningeal irritation, skin rash, or organomegaly. Ocular fundi were normal. Laboratory data included elevated C-reactive protein at 130 mg/L, acute renal insufficiency with creatinin at 110 μmol/L (N < 70) and high-lactate dehydrogenase at 892 U/L (N < 350 μmol/L). She was slightly pancytopenic (WBC 3.2×10^9/L, ANC 3×10^9/L, platelet 109×10^9/L, Hb level 120 g/L). Blood cultures remained negative for fungi and bacteria as well as blood *T. gondii* PCR. EBV DNA was detectable in the plasma at 2.78 log/mL (N < 2.7). Abdominal ultrasonography was normal and there was no infiltrate on chest X-ray. Spinal cord magnetic resonance imaging was normal. A lumbar puncture yielded cerebrospinal fluid (CSF) with normal protein concentration (0.40 g/L), and pleocytosis (mononuclear cells at 5×10^6/L including 98% activated lymphocytes), *T. gondii* PCR was negative and positive EBV PCR at 3.88 log/mL (*N* < 2.4) suggested a viral reactivation.

In spite of an empiric therapy with piperacillin-tazobactam, vancomycin and EBV treatment with rituximab, the patient's condition deteriorated rapidly with increased fever, headache, lethargy and diffuse myalgia. We observed significant rise of C-reactive protein up to 270 mg/L, lactate dehydrogenase to 4435 UI/L, aldolase to 16.4 U/L (N < 7.6 U/L) and severe deterioration of renal function (creatinin 258 μmol/L). Worsening pancytopenia (WBC 0.3×10^9/L, platelet 9×10^9/L and Hb 73 g/L) led us to perform a BM examination. BM smears were hypocellular with nearly complete absence of the granulocytic lineage and an increased proportion of eosinophils. The same day, that is 8 days after the first lumbar puncture, a new CSF analysis was performed and *T.gondii* PCR was positive at 3 copies/mL, as well as a strongly positive at plasma control 12000 copies/mL. This prompted us to re-examine BM smears where we founded 5–10 isolated tachyzoïtes per smear (5–6 μm structure with incurved shape and excentered nucleus) without any cysts. Importantly, some tachyzoïtes were observed inside macrophages (Figure 1). Overall, these findings allow us to classify this episode as definite disseminated toxoplasmosis according to the EBMT-IDWP definitions.[3] Head magnetic resonance imaging scan identified the same day bilateral focal lesions predominantly in the deep left sylvian region, compatible with toxoplasmosis diagnosis (Figure 2a). Considering the absence of GVHD and severe infectious complications, immunosuppressive therapy was definitively stopped. Therapy consisting of pyrimethamine and clindamycin was introduced and allowed a fast remission of the main symptoms. *T.gondii* DNA decreased exponentially, but remained detectable in serum up to 3-weeks later. Blood cell counts rapidly improved. Cerebral magnetic resonance imaging controls have shown fast regression of cerebral lesions until complete disappearance (Figure 2b and c). Two years after HSCT, the patient is alive and remains in good condition without having experienced any *T. gondii* reactivation.

Toxoplasmosis is a common infection after HSCT in European countries, yet it is rarely considered in the differential diagnosis of pancytopenia in this context.

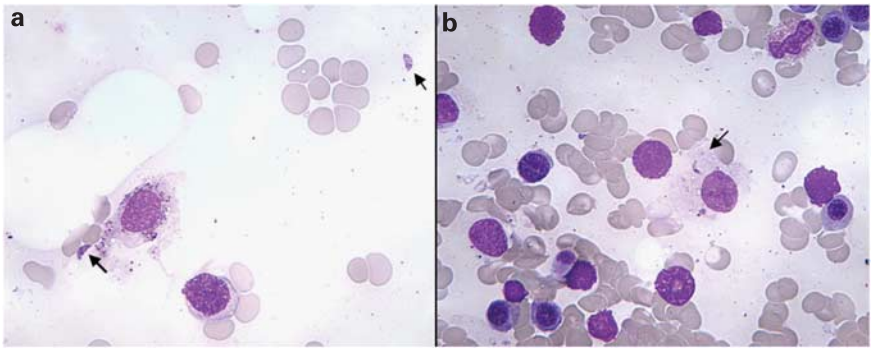

Figure 1 Representative images of BM smear showing *Toxoplasma* tachyzoite. (**a**) May Grunwald Giemsa (MGG) × 1000 showing 5–6 μm structure with incurved shape and excentred nucleus, (**b**) MGG, × 1000 representing tachyzoite inside macrophage.

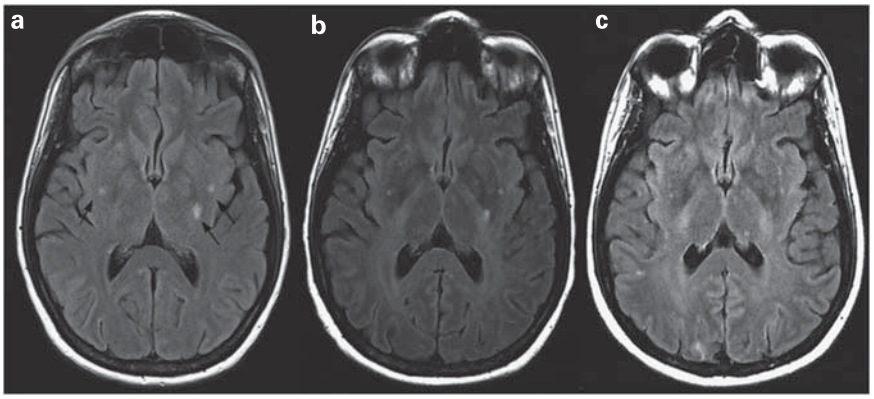

Figure 2 (**a**) HSCT + 108: transversal T_2*FLAIR*-weighted magnetic resonance imaging (MRI) demonstrates bilateral hyperintense signal consistent with a cerebral toxoplasmosis. (**b**) HSCT + 115 and (**c**) HSCT + 132: subsequent controls showing fast regression of the cerebral lesions.

Diagnosis in these patients is often difficult.[4] First clinical features of toxoplasmosis are not specific; fever remains, in most cases, the major sign and other symptoms depend on the dissemination of the infection. Moreover, the development of DNA-based diagnostic methods has greatly improved the sensitivity of the biological diagnosis, but reports of toxoplasmosis cases with negative *T.gondii* PCR in plasma are not rare.[5] In our case, first negative PCR results in serum and CSF were misleading. Diagnosis of definite disseminated toxoplasmosis was obtained secondarily thanks to the observation of tachyzoites by microscopic examination of Giemsa-stained smears of BM, associated with the late positivity of *T.gondii* PCR in CSF and plasma. Our finding supports performing *T.gondii* PCR with BM aspirate in such a situation. In addition, this case emphasizes the importance of reconsidering the possibility of toxoplasmosis disease in high-risk population patients with uncontrolled infection of unknown origin, even if first *T.gondii* PCR result is negative. Indeed, our patient presented four out of five risk factors of toxoplasmosis disease in the EBMT prospective survey of seropositive patients,[1] that is, (1) advanced disease status, (2) antithymocyte globulin in the conditioning regimen, (3) *T.gondii*-seronegative donor and (4) no appropriate prophylaxis with co-trimoxazole. The very slow hematopoietic recovery prevented us from prescribing recommended, but myelotoxic toxoplasma prophylaxis from day + 30, such as co-trimoxazole, combination of pyrimethamine-sulfadoxine or clindamycin-pyrimethamine.[6] In this situation, weekly *T.Gondii* PCR screening is required[7] and a prophylaxis with atovaquone could have been a good alternative, but its efficacy still has to be evaluated. To our knowledge, this is the second case reported of BM involvement by *T.gondii* after HSCT. The other case occurred in 1991 (ref. 8) and the diagnosis relied on tachyzoites and cyst examination in Giemsa-stained smears of BM aspirates confirmed by immunohistochemistry staining. Anti-toxoplasmosis treatment initiation allowed a very fast recovery. We decided to use the pyrimethamine-clindamycin combination, which is less myelotoxic than the standard treatment with pyrimethamine-sulphadiazine. It provided a fast clinical and biological improvement. In high-risk populations, a pre-emptive strategy has been advocated,[9] but initiation of anti-toxoplasmosis treatment before a diagnosis has been established in a patient at risk with a severe uncontrolled infection of unknown origin is a reasonable option.

Conflict of interest

The authors declare no conflict of interest.

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