Cytomegalovirus and Clostridium Difficile co-infection in severe ulcero-hemorrhagic colitis during induction chemotherapy for acute lymphoblastic leukemia

Here we describe the first case of a biopsy-proven *Cytomegalovirus* ulcero-hemorrhagic colitis, associated with *Clostridium Difficile* co-infection, occurring during standard induction chemotherapy for common B cell acute lymphoblastic leukemia. We discuss the case and focalize clinical management and diagnostic issues arising from it.

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A previously healthy 39-year-old Caucasian male was admitted to the hospital because of the diagnosis of common B-cell acute lymphoblastic leukemia (ALL), associated with t(1;19) chromosomal translocation. He was commenced on induction chemotherapy with daunorubicin, vincristine and prednisone, according to the LAL-2000 protocol for ALL proposed by Italian multicentre group GIMEMA. On day 14 after the beginning of chemotherapy, owing to the onset of febrile neutropenia, intravenous antibiotic therapy with ceftazidime and vancomycin was initiated, obtaining a rapid clinical improvement. On day 26, the patient developed abdominal pain and several episodes of diarrhea, with bright red blood mixed to semiformed stool, followed by profuse rectorrhagia. The patient was afebrile, neither nausea nor vomiting were referred, and no signs of peritoneal irritation were found. Stool samples were collected for microbiological analyses. Urgent laboratory investigations were as follows: hemoglobin level lowering from 9.5 g/dL to 7.8 g/dL in 24-hours; white blood cell count of 3.7×10^9/L, with a differential leukocyte count showing severe lymphocytopenia; platelet count of 133×10^9/l, with normal coagulation clotting times and unremarkable blood chemistry profile. Packed erythrocytes were transfused; then an urgent colonsigmoidoscopy permitted to identify three large linear ulcerations, the widest approximatively measuring 4 ×1 cm, localized at the transition from the discending colon to the sigmoid colon, in the transverse colon, and in the coecum, respectively. The lesions were covered by fibrinous-necrotic exudates and fibrin clots, but no pseudomembranes were found (Figure 1A).

Microscopic examination of multiple endoscopic biopsies performed at the borders of the lesions revealed an inflammatory granulation tissue, containing distinct giant cells with prominent intranuclear inclusion bodies. Immunohistochemical staining with anti-Cytomegalovirus (CMV) monoclonal antibody (clone CCH2; Dako) resulted focally positive (Figure 1B). PCR amplification of DNA extracted from formalin-fixed, paraffin-embebbed bioptic specimens was positive for CMV DNA. All other tissutal searches for herpesviruses, mycotic forms, acid-fast bacilli and for common-B blast cells infiltration yielded negative results. On day 27, both CMV-pp65 antigenemia (2 positive cells/slide) and qualitative PCR amplification for CMV DNA on whole blood resulted positive. At the same time, microbiological analyses of stool samples showed negative results for common intestinal pathogenic bacteria, virus, ova and parasites, except for detection of Clostridium Difficile (CD) A and B toxins by enzyme-linked immunoassay (EIA). The patient was promptly started on a three-week course of antiviral therapy with Foscarnet (180 mg/kg/die), preferred to ganciclovir because of the reported minor hematotoxicity; moreover, intravenous vancomycin and ceftazidime were withdrawn, and oral metronidazole (1g/die) was started. The patient returned to his normal bowel habits in ten days, with stool samples negative for CD toxins and with persistently negative CMV-pp65 antigenemia. The patient completed the standard course of induction chemotherapy without other relevant complications, obtaining hematological and molecular complete remission of leukemia. After discharge, control colonoscopy revealed healing ulcerations and immunohistochemical assay for CMV antigen tested invariably negative in all biopsy specimens.

On day 70 from the beginning of induction chemotherapy, the patient developed fever and interstitial pneumonia with highly positive bronchoalveolar lavage fluid for CMV-pp65 antigenemia, for which he was successfully treated with ganciclovir (5 mg/kg twice a day, for three weeks). At that time, he was not receiving chemotherapy and his blood cell count was normal, except for mild lymphocytopenia. Then he underwent consolidation chemotherapy and autologous bone marrow transplantation, without further complications, and with a persistently negative CMV antigenemia, during the whole post-transplant period. The patient is still in hematologic and molecular remission, 20 months after the diagnosis.

Discussion

CMV colitis is a well recognized complication in acquired immunodeficiency syndrome (AIDS) as well as in solid organ and bone marrow transplant (BMT) settings. In other subsets of immunocompromised (IC) patients, CMV colitis is a rare but equally serious disease. To the best of our knowledge, only few cases have described the occurrence of CMV colitis during anti-neoplastic treatment for solid tumors and hematologic malignancies.[1-5] In all of these cases, the diagnosis of CMV disease was obtained by an endoscopic biopsy of colonic lesions, allowing tissutal identification of the virus. In one of these cases, the diagnosis was also supported by the positivization of high-level CMV antigenemia and, after treatment of CMV colitis, the patient also developed CMV-negative CD-associated pseudomembranous colitis.[4] Here we report the first case of biopsy-proven CMV colitis occurring in the course of standard induction chemotherapy for B-cell ALL. Our diagnosis, according to the definition of CMV gastrointestinal disease,[6] was based on the identification of clinical and endoscopic features associated with bioptical demonstration of CMV infection by immunohistochemical analysis, also supported by the positivization of CMV antigenemia. Nevertheless, a simultaneous CD infection was disclosed by the isolation of both fecal CD toxins with highly specific enzyme immunoassay (EIA) test. It is known that a problematic issue in the diagnostic process of CMV end-organ disease arises when other pathogens are identified together with CMV infection.[7] Concerning the gastrointestinal disease, there are no clear indications for specific and reliable evaluation of CMV and CD co-infection. Indeed, in the case reported here, while CMV seems to be strongly involved in the genesis of the colitis, leaving no doubt about the occurence of an inflammatory disease of the colonic mucosa driven by the virus, it appears much more difficult to assess the pathogenic contribution and clinical relevance of CD infection. In favour of a causative role of CD is the evidence that CD is a common cause of nosocomial diarrhea in IC patients. Consistent with this, in a large retrospective study, 7% of hematologic patients treated with myelosuppressive chemotherapy showed clinical symptoms with EIA positivity for CD-toxins.[8] Moreover, our patient presented major risk

factors for CD colitis, such as prolonged treatment with third generation cephalosporine, long-term hospital admission and recent receipt of antineoplastic chemotherapy.[8,9] Against a major role of CD is the lacking of endoscopic evidence of typical psudomembranous colitis, which is commonly associated with the severe clinical presentation of CD infection with frank hematochezia.[10] Moreover, other case series reported CD-toxins EIA positivity in 57% of patients with asymptomatic CD infection,[11] suggesting that, in the presence of colitis with tissutal CMV isolation, CD-toxins EIA positivity could be a non-diagnostic finding. On the basis of these considerations, CD colitis was categorized as a *possible disease* in our patient, and we decided to start combined antiviral and antimicrobial therapy.

Abdominal infectious diseases are common in patients with acute leukemia receiving standard chemotherapy, and bacterial and fungal enterocolitis are much more frequent than viral colitis.[8,12] However, a proven diagnosis of viral colitis is difficult to obtain. Relevant to this, CMV gastrointestinal disease can be only defined by the histologic demonstration of CMV on biopsy materials obtained by endoscopy.[6] The importance of searching for the viral involvment in hematological patients with severe colitis, even in the presence of other pathogens, has been underlined by the case, described by Kottaridis *et al.,*[13] of an autologous BMT recipient who developed life-threatening diarrhea associated with fecal CD toxins and bowel pseudomembranes but unresponsive to initial oral metronidazole. Because of the worsening of the clinical conditions of the patient, a second colonoscopy with rectal biopsy was performed, allowing the definitive diagnosis of CMV colitis, for which the patient was successfully treated with antivirals. Similarly, in our patient, urgent endoscopic biopsy evidenced CMV colitis that, otherwise, could have been diagnosed with delay, as a consequence of the misleading detection of CD toxins.

While allogeneic bone marrow recipients and even autologous recipients of selected CD34 positive cells should be treated with anti-virals at any level of antigenemia,[14] information about the clinical relevance of low-level antigenemia in patients receiving standard chemotherapy is scarce. The detection of 2 positive cells/slide in our case of biopsy proven CMV colitis suggests that low-level antigenemia should not be overlooked but considered as a possible indicator of CMV disease in patients with severe hemorrhagic diarrhea during standard chemotherapy for hematologic malignancies. Therefore, we think that even a low-level positivization of CMV antigenemia could be decisive to perform urgent endoscopy with biopsy and, whenever invasive diagnostic tools are not promptly feasible, this could become a hint for evaluation of empirical antiviral treatment in patients in whom no other pathogens have been identified or previous specific antimicrobial therapy has failed. Of interest, Mori *et al.*[15] reported that all the examined 19 allogeneic hematopoieitic stem cell transplant patients with the CMV gastrointestinal disease developed positive CMV antigenemia tests during their clinical course, with the values remaining at a low-level in 9 patients (47%). However, only 4 of these 19 patients (21%) developed a positive CMV antigenemia test before developing the CMV gastrointestinal diseases, suggesting that CMV antigenemia testing has limited value in prediction or early diagnosis of the CMV gastrointestinal disease after BMT. Real-time PCR could have a more diagnostic significance in this setting, being this test positive in 50% of the patients, before the developing of CMV gastrointestinal disease.[15] In conclusion, our case shows that not only BMT recipients or AIDS patients, but also acute leukemic patients can develop severe CMV colitis while receiving the first standard induction chemotherapy. A direct implication of our observation is that IC patients affected with severe hemorrhagic diarrhea should be considered for endoscopic bowel examination and tissutal search for CMV, even if fecal CD toxins are positive, in order to obtain a reliable diagnosis and start proper treatments as soon as possible. The successful clinical outcome in our patient suggests that, despite the occurence of a severe CMV ulcero-hemorrhagic colitis followed by a CMV pneumonia during and immediately after the induction chemotherapy course, autologous BM/PBSC transplant procedures can be safely performed in leukemic patients.

Figure 1. A. Endoscopic presentation of the severe colitis. Transverse colon: large ulceration with raised edges and irregular borders, indicated by arrows. **B.** Immunostaining of colonic biopsy. Cluster of giant cells with large intranuclear CMV inclusions (original magnification, x40; counterstaining with Giemsa). Inset: typical *owl eyes* aspect of a CMV infected cell (original magnification, x100; counterstaining with Giemsa).

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