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

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 vincristine and prednisone, according to the LAL-2000 protocol for ALL proposed by Italian multicentre group GIMEMA, was started. On day 15, the patient developed abdominal pain and several episodes of diarrhea, with bright red blood mixed to semiformed stool, followed by profuse rectorrhagia. The 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 3.7×10⁹/L, with a differential leukocyte count showing severe lymphocytopenia; platelet count of 138×10⁹/L, with normal coagulation clotting times and unremarkable blood chemistry profile. Packed erythrocytes were transfused; then an urgent colonoscopy was performed. A biopsy of the colon was taken and confirmed the diagnosis of CMV colitis.

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 inclusions 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-embbeded biopsic 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 and CMV-pp65 antigenemia (2 positive cells/slide) and qualitative PCR amplification for CMV DNA on whole blood resulted positive. At that time, 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 3.7×10⁹/L, with a differential leukocyte count showing severe lymphocytopenia; platelet count of 138×10⁹/L, with normal coagulation clotting times and unremarkable blood chemistry profile. Packed erythrocytes were transfused; then an urgent colonoscopy was performed. A biopsy of the colon was taken and confirmed the diagnosis of CMV colitis.

One 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. 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 CMV antigenemia. 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 CMV antigenemia. 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 CMV antigenemia. 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 CMV antigenemia. 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 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. 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 occurrence 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. 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. Against a major role of CD is the lacking of endoscopic evidence of typical pseudomembranous colitis, which is commonly associated with the severe clinical presentation of CD infection with frank hematochezia. Moreover, other case series reported CD-toxins EIA positivity in 57% of patients with asymptomatic CD infection, suggesting that, in the presence of colitis with tissueal 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. 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. The importance of searching for the viral involvement in hematological patients with severe colitis, even in the presence of other pathogens, has been underlined by the case, described by Kottaridis et al., 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, 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. reported that all the examined 19 allogeneic hematopoietic 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 disease, 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. In conclusion, our case shows that not only

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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