ANSWERING REVIEWERS

October 9, 2019

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 80090-Edited.doc).

Title: Cytomegalovirus ileo-pancolitis presenting as toxic megacolon in an immunocompetent patient: Case report

Author: Joon Hyun Cho, Joon Hyuk Choi

Name of Journal: *World Journal of Clinical Cases*

ESPS Manuscript NO: 80090

Thank you very much for your kind comments.
We tried to revise the manuscript as much as possible according to the suggestions made by the reviewers, and enclosed revision detail and revised manuscript.
We hope all these revisions will be satisfactory.

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Answers to Reviewer No. 03276926
(highlighted by yellow and green color in the updated version of the manuscript)
This paper reports an interesting clinical presentation of toxic megacolon probably related to an ileo-colitis consecutive to a cytomegalovirus (CMV) infection in an apparently immunocompetent patient. Although the presentation of the case is well documented with a good iconography and a solid discussion, some points merit to be improved substantially.

(1) First, the authors give the feeling that CMV infection is only severe in immunocompromised patients. Even if the severity of the diseases is more pronounced in this category, CMV infection is not always an asymptomatic or mild disease in immunocompetent adults (lines 2 and 3 of Introduction and also discussion), as illustrated by a lot of literature. Even if we limit the discussion to the intestinal tract, different studies, in addition to those cited by the authors, report severe CMV colitis in non-immunosuppressed patients. (You DM, Johnson MD. Cytomegalovirus infection and the gastrointestinal tract. Curr Gastroenterol Rep 2012 ; 14 : 334-42; Hasegawa T, Aomatsu K, Nakamura M, Aomatsu N, Aomatsu K. Cytomegalovirus colitis followed by ischemic colitis in a nonimmunocompromised adult: a case report. World J Gastroenterol 2015 ; 21 : 3750-4.; Bernard S, Germi R, Lupo J, et al. Symptomatic cytomegalovirus gastrointestinal infection with positive quantitative real-time PCR findings in apparently immunocompetent patients: a case series. Clin Microbiol Infect 2015 ; 21 : 1121. e1-7).

Answer) Thank you for your advice. As your comments, CMV infection is not always an asymptomatic or mild disease in immunocompetent adults. Therefore, we described the sentence "Although gastrointestinal involvement is uncommon in immunocompetent patients, CMV colitis is being

increasingly recognized in apparently immunocompetent patients with immunomodulating factors, such as …" in the line 2 – 7 of page 9 in the "Discussion". In addition, we added three references that you suggested about severe CMV colitis in non-immunosuppressed patients in the sentence.

Another important point is the relationship between ulcerative colitis (UC) and CMV infection of the gut; patients with UC cannot be considered as immunodeficient, even if immunological disorders are probably linked to this disease (for a review, see for example this paper from our team: Pillet S, Pozzetto B, Jarlot C, Paul S, Roblin X. Management of cytomegalovirus infection in inflammatory bowel diseases. Dig Liver Dis. 2012;44:541-8).

Answer) Thank you for your thoughtful advice. We read your great paper well, and rewrote the confusing sentence in the line 2 – 3 of page 10 in the "Discussion".

In addition, no exploration of immunity was performed in this 70 year-old patient. CMV is a major agent of immunosenescence (as illustrated in this recent paper: 'From immunosenescence to immune modulation': a re-appraisal of the role of cytomegalovirus as major regulator of human immune function. Moss P. Med Microbiol Immunol. 2019;208:271-280). So the absence of clear immunodeficiency in this patient is not so surprising. It may reflect a consequence of aging. These different points merit to be discussed.

Answer) Our patient had no history of immunosuppressive disease or therapy, and despite of the extensive laboratory and radiologic work-up, he was confirmed to be no associated diseases. Therefore, we considered the patient to be immunocompetent. As your comment and the content of the recent paper references that you suggested, the absence of clear immunodeficiency in this patient is not so surprising, however, this case report illustrates that CMV-induced colitis should be considered as a possible differential diagnosis in an elderly immunocompetent patient with intractable symptoms of enterocolitis or megacolon of unknown cause. Although the reference you provided is not in accord with the contents of our manuscript exactly, the contents about immunosenescence and an age-related weakening of the immune system were described in the line 19 – 26 of the page 9 of "Discussion". We hope your generous understanding.

(2) Second, the authors stress the presence of fever at admission. For how long time? CMV infection is frequently associated with long-lasting fever.

Answer) As described in the manuscript, intravenous ganciclovir was started on day 11, and subsequently, the fever gradually reduced and subsided.

Before the hemorrhagic episode, an infection was discussed since antibiotics were prescribed and stool cultures were performed. This part of the observation is not enough detailed. I suppose that blood cultures were also performed. A high leukocyte count is mentioned. What was the formula of white cells in blood? In addition to CRP, were other inflammatory markers studied? The "infectiology" part of the observation must be more described in more details.

Answer) As your comment, the "infectiology" part of the observation was more refined and described in details in "*Laboratory examinations* "of the "Case Presentation".

(3) Third, the virological investigations are poorly described (sorry I am a virologist!). Immunochemical studies are convincing but, nowadays, the diagnosis of CMV colitis relies on the intestinal viral load. It seems that the viral load of 3360 DNA copies/ml corresponds to circulating blood (serum? whole blood? by using which technique?). It would be interesting to analyze the kinetics of the CMV intestinal viral load from the different biopsies taken during the follow-up of the patient (if samples have been stored frozen, it can be done retrospectively). The presence of CMV DNA within the mucosa

and its decrease at the convalescence phase would permit to prove definitively that CMV was the cause of the colitis.

Answer) As your comments, currently, the diagnosis of CMV colitis relies on the intestinal viral load. Especially to the patients with ulcerative colitis, quantitative real-time tissue (inflamed mucosa) PCR assay is useful for early detection of cytomegalovirus infection. In our case, unfortunately, we did not use quantitative tissue PCR method because we did not strongly suspect CMV enterocolitis in our patients at first. Furthermore we don't have any frozen biopsy sample now. Therefore, we cannot retrospectively analyze the kinetics of the CMV intestinal viral load during the follow-up. We used quantitation of CMV DNA in whole-blood samples (The tests that use whole blood have higher sensitivities than serum), and described it in the line page 28 of "*Colonoscopic and further diagnostic work-up on clinical time course*" of the "Case Presentation". Please understand us generously.

(4) Fourth, the discussion about the need for treating or not by ganciclovir the patient because he was immunocompetent is very surprising. As documented in previous paper, the delay for treating this infection in immunocompetent patients may explain the severity of the evolution. In the present case, given the severity of the clinical picture and the absence of alternative diagnosis, it seems evident for me that the correct attitude was to treat by ganciclovir (in the absence of contra-indication) without delay, what has been done successfully.

Answer) We agree with your precious advice and added your valuable comments in the end of "Discussion"

If the authors are able to amend correctly these 4 important points, this observation merits to be reported.

Answer) We hope all these revisions would be satisfactory to you.

Answers to Reviewer No. 00038617
(highlighted by sky blue color in the updated version of the manuscript)
In this paper, the authors report the case of a 70-year-old male with a non-immunocompromised state that presented with toxic megacolon and subsequently developed massive hemorrhage as a complication of CMV ileo-pancolitis. CMV enterocolitis presenting as toxic megacolon in an immunocompetent patient is rarely encountered. This manuscript was written well overall, and this case certainly provides a new perspective to the gastroenterologist.

Minor comments)

(1) For the description of blood tests, it is better to describe only abnormal values.

Answer) We deleted normal values and furtherly refined the laboratory findings

(2) Where is D-2 in Figure 2? It should not be the terminal ileum. Please describe the location.

Answer) Thanks for your delicate suggestion. We corrected the "Figure legend" and the associated sentence.

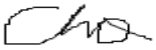
(3) In the blood test of this case, an increase in leukocytes is observed from the beginning. Is it explained only by virus infection? I am concerned that the CMV enterocolitis in this case may be secondary to acute bacterial enterocolitis.

Answer) We are very sympathetic to your intellectual point. We also thought about the possibility of

the CMV enterocolitis secondary to acute bacterial enterocolitis. However, stool cultures for Clostridium difficile and enteric pathogens and blood cultures were all negative. We found that the increase in leukocytes, CRP and ESR were not related to bacterial infection but to toxic megacolon.

Thank you again for publishing our manuscript in the *World Journal of Clinical Cases*

Sincerely yours,



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