

## **Answering reviewer 1**

Thank you very much for reviewing our manuscript and giving us very valuable comments and suggestions. We have revised the manuscript according to your comments and suggestions as follows. We would appreciate it very much if you would review the revised manuscript.

**Q1:** Legend of figures – it is not necessary to include an explanation of findings as demonstrated in the figures, as they are included in the text.

**A:** We have deleted the explanations of findings in the Figures.

**Q2:** If possible include the results of neutrophils and monocytes, comparing before and after treatment to assess the effect of the proposed treatment. And also fecal calprotectin, PCR?

**A:** We have shown the data of neutrophil count, monocyte count, CRP level, and serum albumin concentration before and after CAP in a Table. Thus, we have added a new Table as Table 2 and some sentences in “Materials and Methods” and “Results” sections as follows. As a result, Table 2 in the previous version has changed to Table 3 in the revised version. We did not examine fecal calprotectin in this study.

“Laboratory data (C-reactive protein [CRP] level, serum albumin concentration, neutrophil count, and monocyte count) before and after CAP were also examined in 28 patients treated between April 2008 and December 2019.” (**“Materials and Methods” section**)

“Laboratory data before and after CAP are shown in Table 2. As shown in Table 2, the inflammatory parameter (CRP) and the nutritional parameter (serum albumin

concentration) significantly improved after CAP. Neutrophil count significantly decreased after CAP therapy. Monocyte count tended to decrease after CAP, but no significant difference was observed.” (**Results section**)

**Q3:** Review the format of references

**A:** We have reviewed and revised the format of references.

**Q4:** Discuss the impact of this treatment in the clinical practice, comparing to the others drugs treatment, including costs and quality of life.

**A:** We have added the following sentences in the section of “Discussion”. As a result, we have added 3 references (reference No 62, 63, 64).

“There have been several studies comparing the impact of CAP in the clinical practice with the conventional pharmacotherapy for UC <sup>[53, 62-64]</sup>. A meta-analysis showed that GMA is effective for inducing clinical remission in patients with UC compared with CS (OR, 2.23; 95% CI: 1.38-3.60) and that the rate of adverse events by apheresis was significantly lower than that by CS (OR, 0.24; 95% CI: 0.15-0.37) <sup>[62]</sup>. Another meta-analysis showed that comparing with conventional pharmacotherapy including CS, leukocytapheresis supplementation presented a significant benefit in promoting a response rate (OR, 2.88, 95% CI: 1.60-5.18) and remission rate (OR, 2.04, 95% CI: 1.36-3.07) together with significant higher steroid-sparing effects (OR, 10.49, 95% CI: 3.44-31.93) in patients with active moderate-to-severe UC <sup>[63]</sup>. In this regard, Domènech et al showed that the addition of 7 weekly sessions of GMA to a conventional course of oral prednisolone did not increase the proportion of steroid-free remissions in patients with active steroid-dependent UC <sup>[53]</sup>. On the other hand, Tominaga et al <sup>[64]</sup> showed that

GMA produced efficacy equivalent to prednisolone and was without safety concern. Although they also showed that the average medical cost was 12739.4€/patient in the GMA group and 8751.3€ in the prednisolone group ( $P < 0.05$ ), they concluded that the higher cost of GMA vs prednisolone should be compromised by good safety profile of GMA.”

**Q5:** Also, I have some questions: Is UC patients with proctitis included?

**A:** As we show in Table 1, patients with proctitis were not included in the study.

**Q6:** Do you have in the medical records data about surgical rate in the short- and long-term?

**A:** We have shown the surgical operation rate of our patients within 6 months, 3 years, and throughout the observation period after the first course of CAP in the sections of “Materials and Methods” and “Results” as follows.

**“2.5.5. The surgical operation rate:** The surgical operation rates of the patients within 6 months, 3 years, and throughout the observation period after the first course of CAP were examined.” (**“Materials and Methods” section**)

“The surgical operation rate of the patients within 6 months after the first course of CAP was 9.1 % (5/55). The surgical operation rate within 6 months after the CAP was significantly lower in patients who achieved steroid-free remission after the first course of CAP (0%) compared with that in patients who had poor effectiveness in the first course of CAP (29.4%) ( $P = 0.0039$ ). The surgical operation rate within 3 years after the first course of CAP was 12.7 % (7/55). The surgical operation rate within 3 years after the CAP was significantly lower in patients who achieved steroid-free remission after the first course

of CAP (4%) compared with that in patients who had poor effectiveness (29.4%) (P=0.0209). The surgical operation rate throughout the observation period (18-193 months (81.5 [mean]  $\pm$ 47.3 [SD])) after the first course of CAP was 20 % (11/55). The surgical operation rate throughout the observation period after the CAP was significantly lower in patients who achieved steroid-free remission after the first course of CAP (12%) compared with that in patients who had poor effectiveness in the first course of CAP (41.2%) ((P=0.0293).” (**“Results” section**)

**Q7:** Do you think that UC patients with disease activity not responding to steroids can represent a refractory disease or a more time is necessary to conclude this?

**A:** As we described in the “Methods” section, we have defined steroid-refractory UC as active UC characterized by the failure to respond to appropriate dose (0.75–1.5 mg/kg/day) of prednisolone administered over at least 1 week.

## **Answering reviewer 2**

Thank you very much for reviewing our manuscript and giving us very valuable comments. We have revised the manuscript according to your comments as follows. We would appreciate it very much if you would review the revised manuscript.

**Q1.** Two different treatments, GMA and LCAP, are included in the study. Is it possible to compare the effects of these two methods in refractory ulcerative colitis?

**A:** We have added the following data concerning the efficacy of GMA and LCAP in the “Results” section.

“The rates of clinical remission, steroid-free remission, and poor effectiveness after GMA were 69.2%, 43.6%, and 30.8%, respectively. The rates of clinical remission, steroid-free remission, and poor effectiveness after LCAP were 68.8%, 50.0%, and 31.2%, respectively. There were no significant differences in the rates of both clinical remission and steroid-free remission after CAP between patients who received GMA therapy and patients who received LCAP.”

**Q2:** Does your patient’s medical records include CRP, albumin, neutrophils and monocytes? If so, they should be compared before and after treatment to assess the effect of Cytapheresis.

**A:** We have shown the data of CRP level, serum albumin concentration, neutrophil count, and monocyte count before and after CAP in a Table. Thus, we have added a new Table as Table 2 and some sentences in “Materials and Methods” and “Results” sections as follows. As a result, Table 2 in the previous version has changed to Table 3 in the revised version.

“Laboratory data (C-reactive protein [CRP] level, serum albumin concentration, neutrophil count, and monocyte count) before and after CAP were also examined in 28 patients treated between April 2008 and December 2019.” (**“Materials and Methods” section**)

“Laboratory data before and after CAP are shown in Table 2. As shown in Table 2, the inflammatory parameter (CRP) and the nutritional parameter (serum albumin concentration) significantly improved after CAP. Neutrophil count significantly decreased after CAP therapy. Monocyte count tended to decrease after CAP, but no significant difference was observed.” (**“Results section**)

### **Answering reviewer 3**

Thank you very much for reviewing our manuscript and giving us very valuable comments. We have revised the manuscript according your comments as follows. We would appreciate it very much if you would review the revised manuscript.

**Q1:** In line 2-3 page 5, "The mean DAI and CAI scores before CAP decreased significantly after CAP ( $P < 0.0001$ )" is quite confusing. Should it revised to "The mean DAI and CAI scores were significantly decreased after CAP ( $P < 0.0001$ )"?

**A:** We have revised "The mean DAI and CAI scores before CAP decreased significantly after CAP ( $P < 0.0001$ )" to "The mean DAI and CAI scores were significantly decreased after CAP ( $P < 0.0001$ )."

**Q2:** As said in the part of "Introduction", CAP is usually performed using two methods, namely GMA and LCAP. Could the authors compare the differences between these two methods either in the part of "Results" or "Discussion"?

**A:** We have added the following data concerning the efficacy of GMA and LCAP in the "Results" section.

"The rates of clinical remission, steroid-free remission, and poor effectiveness after GMA were 69.2%, 43.6%, and 30.8%, respectively. The rates of clinical remission, steroid-free remission, and poor effectiveness after LCAP were 68.8%, 50.0%, and 31.2%, respectively. There were no significant differences in the rates of both clinical remission and steroid-free remission after CAP between patients who received GMA therapy and patients who received LCAP."