World Journal of Gastroenterology Manuscript NO: 46380 - Manuscript revision

Dear Editors,

In the following, we provide point-to-point answers to the reviewers‘ comments. The changes made in the manuscript can be viewed using the Track Changes function of Microsoft word.

Number ID 02566971:

Thank you very much for your valuable comments.

1. **Reviewer’s comment:** ***“The primary study end point was clinical response to tacrolimus salvage therapy, indicated by the possibility to successfully discharge the patient from the hospital.” Secondary endpoints were clinical response under tacrolimus therapy, colectomy rate, time to colectomy, and the occurrence of side effects." Not only the primary and secondary endpoints were overlapping, but the primary end-point is not optimal.***

   Answer:

   We changed the Methods section of the abstract and the main text accordingly, so that endpoints do not overlap any longer. Clinical response including the possibility of discharge from the hospital is the primary endpoint and is not listed as a secondary endpoint any longer. Also, we included a critical comment in the Discussion section of the manuscript concerning the quality of our endpoint:

   “Due to the retrospective character of the study, the term of “clinical response” was not clearly defined by quantitative parameters or cut-off values and depended much on the assessment by the treating physicians. This is why we chose to also incorporate the possibility to discharge the patient from the hospital into the definition of “clinical response”, as this is a relatively “hard” clinical endpoint in the “real world”.”

2. **Reviewer's comment:** ***"Is this their standard protocol to use FK as the first-line rescue medication for steroid-resistant acute severe UC? Looks like there was no pilot study. What's the rationale? Does CsA have worse side-effects?"***

Answer: Yes, indeed, it has been our standard procedure for about the last two decades to use tacrolimus as the first-line rescue medication for steroid-resistant acute severe ulcerative colitis. The following sentence has now been incorporated in the Methods section in the paragraph "Treatment algorithm" to make this clear for the reader:

"Tacrolimus has been used as the standard first-line rescue medication of the department in patients with steroid-refractory acute severe ulcerative colitis over the last two decades."

In the Discussion section, it is elaborated more thoroughly why tacrolimus was preferred over ciclosporin and infliximab at out department in the clinical setting of steroid-resistant acute severe ulcerative colitis. The explaining paragraph is displayed in the following:

"The question may be raised why we used tacrolimus as our standard medical salvage therapy in steroid-refractory acute severe ulcerative colitis. At our department, tacrolimus is preferred over ciclosporin in patients who underwent liver transplantation. This choice is made for the following reasons: liver-transplant patients treated with tacrolimus were less likely to experience acute rejection than those receiving ciclosporin[19]; mortality and graft loss at one year were significantly reduced in tacrolimus-treated liver-transplant recipients[20]; and finally, conversion from ciclosporin to tacrolimus has been shown to improve the cardiovascular risk profile in patients after liver transplantation[21]. Owing to our experience with tacrolimus, which is based on the relatively large number of liver-transplant patients followed up at our department, the administration of tacrolimus to patients with steroid-refractory acute severe ulcerative colitis has become our standard approach

over the last one to two decades. The most important reason for the preference of tacrolimus over anti-TNFa was its shorter elimination half-time. Thus, ciclosporin and infliximab were used much less frequently than tacrolimus in steroid-refractory acute severe ulcerative colitis."

A pilot study was indeed not performed. This is why we suggest in our conclusion that a prospective study comparing tacrolimus and ciclosporin in steroid-resistant acute severe ulcerative colitis would be valuable:

"For future research projects, a direct prospective comparison of ciclosporin and tacrolimus – as has already been performed in transplantation medicine – would also be interesting in the setting of steroid-refractory acute severe ulcerative colitis."

3. **Reviewer's comment:** ***"Among the 22 patients, 3 were on Infliximab, vedolizumab, and thiopurine, respectively upon admission. Should these 3 patients be excluded? I am wondering if this will affect the overall results."***

Answer:

That is a reasonable doubt, thank you for this important comment.

The patient who had received anti-TNF (infliximab) had already undergone the full induction scheme when he was admitted to the hospital with a flare. The time span between his third infliximab infusion and the admission to the hospital was 23 days. The patient who was on azathioprine at admission had been on azathioprine for 32 months prior to admission. Looking again into the data of the third patient, who was indicated to be on vedolizumab at admission, we found that we had made a mistake and had to revise the data, as this patient started vedolizumab in the ward after start of tacrolimus therapy and was by mistake also indicated to have had the therapy at admission. In the revised version of the manuscript, we deleted that line from **Table 1**. For the other two patients, the information on the duration of their therapies was added in detail in **Table 1**. Also, we incorporated a comment on your question in the Discussion section of the revised manuscript:

"Two patients were already on thiopurine or anti-TNFa (infliximab) therapy, respectively, when they were admitted to the hospital. As they had been on their therapies for 9 weeks (infliximab) and 32 months (azathioprine) when they were admitted to the hospital with acute severe ulcerative colitis, we do not think that their prior therapies interfered with our results of response to tacrolimus therapy."

4. **Reviewer's comment:** ***"Clinical response was defined as a significant decrease of stool frequency, rectal bleeding, and plasma CRP concentration....."***. ***This is too arbitary. Are there any quantitative parameters to define "significant decrease"?***

Answer: As this is a retrospective study, clear cut-off values for the definition of the term "clinical response" were unfortunately not defined. In our opinion, it would be inappropriate to retrospectively introduce them. This study is a real-world observation, reflecting everyday decisions. In the revised version of the manuscript, we changed the paragraph "Definitions" as indicated below:

"*Clinical response was defined as a significant decrease of stool frequency, rectal bleeding, and plasma CRP concentration, as well as an amelioration of general well-being as documented in the patient curve,* ***which resulted in the possibility to discharge the patient from the hospital to continue the therapy on an outpatient basis."***

We also added a sentence considering this limitation of the study in the Discussion section:

"Due to the retrospective character of the study, the term of "clinical response" was not clearly defined by quantitative parameters or cut-off values and depended much on the assessment by the treating physicians. This is why we chose to also incorporate the possibility to discharge the patient from the hospital into the definition of "clinical response", as this is a relatively "hard" clinical endpoint in the "real world"."

To make it clearer for the reader, it has been outlined in the paragraph "Short-term efficacy of tacrolimus" of the Results section to what extent single parameters as CRP values or stool frequency changed during the hospital stay.

"Directly prior to the first administration of tacrolimus, the mean plasma CRP concentration was 87.5 ± 12.2 mg/L, and it decreased to 24.3 ± 10.5 mg/L at discharge from the hospital (n = 20; the two patients who were transferred to the surgery department were excluded). It was 51.5 ± 11.4 mg/L at day 5 of tacrolimus therapy and 42.9 ± 11.8 mg/L at day 7 of tacrolimus therapy. The occurrence of blood in stool was documented in 100% of the patients at admission to the hospital, while blood in stool was documented in 11/20 (55%) patients at discharge from the hospital. The mean stool frequency was 13.5 ± 1.4 at admission (n = 22) and decreased to 5.4 ± 0.6 at discharge (n = 20). The mean body temperature at admission was 38.0 ± 0.2°C at admission, decreasing to 36.9 ± 0.1°C at discharge from the hospital."

5. **Reviewer's comment:** ***"The decisions to start salvage therapy with tacrolimus, and whether oral versus intravenous treatment would be used, were made on a per-case basis by the treating physicians' team." This is also confusing. Is the desicion to start, switch or stop FK treatment simply based upon clinical experience? Is there any objective criterion?"***

Answer: Changes were made accordingly in the Methods section:

"In patients with steroid-refractory disease, the treating physicians' team (always including a senior consultant in gastroenterology with experience in IBD therapy) decided on the basis of disease severity, comorbidities, patient age, prior medications, and patients' wishes which rescue therapy was most appropriate. Usually, a visceral surgeon was involved in the decision-making process. Tacrolimus has been used as the standard first-line rescue medication of the department in patients with steroid-refractory acute severe ulcerative colitis over the last two decades."

There was no single objective criterion which was in all cases used for making treatment decision.

6. **Reviewer's comment:** "*The whole treatment process and follow-up of individual patient is very complex and I do suggest the authors set up a new table and provide with detailed information.*"

   Answers: We understand your comment very well. As due to the retrospective character of the study, the follow-up was not well structured as in a prospective study, and as the follow-up was indeed very complex with medications being started and stopped, we decided to focus on colectomy rates, time to colectomy, and reasons for tacrolimus therapy discontinuation in order to depict the middle-to long-term outcome. We tried different ways to summarize and simplify the data but do not think we would add more value to the manuscript by describing all therapies that were administered after hospital discharge in more detail. We feel that add more information here would be confusing and distract from the main aspects of the study.

The reviewers with the ID numbers 00505564 and 00503322 did not request any changes to be made.

We hope we could provide helpful and sufficient answers to the comments and are looking forward to your reply.

Yours sincerely,

Annika Gauss