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To:
Jing Yu,
Science Editor, Editorial Office
Baishideng Publishing Group Inc.
World Journal of Gastroenterology

RE: 17907 revised

Erlangen, 29.06.2015

Dear Editor Dr. Jing Yu,

thank you for the response to our manuscript " Exacerbation of ulcerative colitis after anti-IL-6R salvage therapy" (Manuscript NO.: 17907) and the opportunity to resubmit a revised version for publication in "*World Journal of Gastroenterology*".

We thank the reviewer and the editor for the comments which have been taken into account for the revision of the manuscript. We have thoroughly considered and addressed all points raised by the reviewer and the editor. We have changed the manuscript format to "case report". We have moreover included more and detailed clinical information to our case report.

We hope you share our contentment and find the manuscript suitable for publication in its current form. For a detailed address of the reviewers' and editors' comments you can find our attached point-by-point reply.

We hope that this first reported case of a severe ulcerative colitis patient treated with a neutralizing anti-IL-6R antibody will meet the requirements for publication in "*World Journal of Gasroenterology*". We are looking forward to hearing from you.

Sincerely yours,

Raja Atreya
for the authors

Point-to-point reply to the editor

Authors are required to make these statements in the manuscript's title page (please see sample wording in attachment).
Besides, please provide these files, which are necessary for final acceptance, each in a separate PDF file, signed by the Correspondence author or a copy of Institution approval document(s)/letter(s) or waiver of confirmation. For sample wording and detailed information, please see the Revision policy in the attachment or Instruction to authors on our website. Thank you!
As requested by the editor, the required statements have been made in the manuscript's title page. Ethics approval and informed consent of the patient for analysis of the mucosal biopsies have been enclosed in the submission process of the revised manuscript. The anti-IL-6R antibody (tocilizumab) was used as compassionate use treatment in a patient with treatment refractory ulcerative colitis.

Core tip: Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.
To address this point a short summary has been included in the manuscript (all changes in the revised manuscript are underlined).

Audio Core Tip
In order to attract readers to read your full-text article, we request that the author make an audio file describing your final core tip, it is necessary for final acceptance. Please refer to Instruction to authors on our website or attached Format for detailed information.
We recorded an audio file of the core tip according to the specification given in the instruction to authors.

Comments
Please provide the "Highlighted contents" here, which is a necessary content.
The "Highlighted contents" have been inserted by the author according to the given writing requirements for comments.

Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please revise throughout. For those references that have not been indexed by PubMed, a printed copy of the first page of the full reference should be submitted.
We have added PubMed citation numbers and DOI citation to the reference list.

Point-to-point reply to the reviewer

Thank you for give me an opportunity to review this interesting paper. Authors presented well this manuscript, with correct English.
Some considerations have to be made:
In my opinion the manuscript should be changed for "case-report" with the following format: Abstract, Introduction, case-report and discussion.
The authors sincerely thank the reviewer for finding our paper interesting and classifying it as a well presented manuscript. According to the suggestion of the reviewer we have changed the manuscript to a "case report" and structured it according to the existing format.

Introduction: It's better to describe the clinical case with details after ("The patient received biweekly (…)"should be after)
We thank the reviewer for this comment and agree to describe the clinical case after the introduction. We therefore erased the sentence "The patients received biweekly…" in the introduction. This sentence was already included on page 5 in the case report section.

Material and methods:
First sentence: should be rewritten. The definite diagnosis of ulcerative colitis it's made by histopathological criteria.
We thank the reviewer for his thoughtful comment and agree that histopathology enables definite diagnosis of ulcerative colitis. We have changed the sentence accordingly.

It´s missing important information of the patient clinical history:

- Aminosalicylates: oral and enema? Doses?
The patient was initially treated with combined therapy of oral (3g) and local (2g) aminosalicylates. We agree that this important information was missing and have added it to the manuscript in the beginning of the case report section.

- How many cycles of corticosteroids made then patient before she developed a steroid dependent disease?
As the patient was treated outside of our institution by different physicians when she developed steroid dependent disease, we were unable to exactly find out how many cycles of corticosteroids were given to her before this diagnosis was made. We could only find out that she definitely received at least three treatment courses. As we do not have sufficient information regarding the amount of corticoid treatment courses before the development of steroid dependent disease, we could not include more detailed information in the manuscript.

- Steroid dependent disease? Value of prednisolone? 10 mg?
The patient had steroid dependent disease when she first visited our institution, with a requirement for steroid therapy $\geqslant$10 mg/day. This additional information has been inserted in the case report section of the manuscript on page 4.

- Azathioprine: doses? Clinical response? And endoscopy?
The azathioprine dose was 100 mg per day with a patient weight of 51 kg. This treatment was initiated outside our clinic and the clinical response was reported by the patient, who felt better for approximately 6 months. No endoscopic examinations were performed during that time. The mentioned information has been added to the manuscript.

- Why methotrexate in ulcerative colitis?
This therapy was initiated outside our clinic due to refractory disease course of the patient to conventional therapy. We indicated this in the manuscript. Although some reports indicate that methotrexate might have a therapeutic effect in some ulcerative colitis patients (Khan et al., 2013), a Cochrane review clearly showed no benefit for methotrexate over placebo to induce remission in these patients (Chande et al., 2014). Nevertheless two large ongoing placebo-controlled trials (METEOR and MERIT-UC) are currently further assessing the efficacy and safety of methotrexate treatment in patients with active ulcerative colitis.

- Why you continued azathioprine after 5 years of therapy and without a good response?
Azathioprine therapy was continued in the patient during our therapy with infliximab and adalimumab, as the patient reported that she felt better overall since initiation of azathioprine therapy in 2005. She said that her disease was more aggravated prior to the initiation of azathioprine therapy. She nevertheless still had signs of active clinical and endoscopic disease during the treatment period, but wished to continue that treatment. Azathioprine therapy was stopped in 2008 when methotrexate treatment was initiated. The patient reported that she had more severe clinical features of disease (stool frequency, rectal bleeding) during that time. Azathioprine therapy was therefore re-initiated outside our clinic when methotrexate therapy was stopped due to severe skin lesions. We have included this information in the manuscript. When anti-TNF therapy with infliximab was initiated, ongoing azathioprine therapy was continued. We hoped that combined azathioprine and anti-TNF therapy might have an enhanced therapeutic effect in the aggravated disease course in comparison to anti-TNF monotherapy. Our plan was to discontinue azathioprine treatment 6 months after response to anti-TNF treatment.

- How was the disease before starting anti-TNF therapy? Initial response? Adalimumab doses? When it was stopped?
The patient had a chronic active disease course when therapy with the anti-TNF antibody infliximab was initiated in our clinic in 2010. The patient initially responded to treatment with only slightly elevated stool frequency and absent rectal bleeding. This has been added to the text. After over one year of treatment there was secondary loss of response, which could not be sufficiently treated by intensified infliximab application. Infliximab was first given at 5 mg/kg every four weeks and then at 10 mg/kg every four weeks as stated in the manuscript. Infliximab treatment was stopped in 2012.
Adalimumab therapy was initiated in 2012 with an initial dose of 160 mg and then 80 mg after two weeks, followed by 40 mg every fortnight. There was no response to therapy. Adalimumab therapy was stopped after 3 months of therapy. This has been added to the manuscript.

- What was the Truelove and Witts score? Did the patient have tachycardia (N90 bpm) or temperature N 37.8 °C ?

We thank the reviewer for this important clinical question. The Truelove and Witts severity index indicated moderate disease. The patients had no tachycardia or pyrexia. This has been added to the text.

- Mayo score – 10- moderate disease? - 5 or more stools more than normal – 3 + Obvious blood with stool most of the time – 2 + Severe disease (spontaneous bleeding, ulceration) – 3? + Moderate disease – 2 ? = 10

The reviewer makes a very good and convincing point that a total Mayo score of 10 does not reflect moderate disease. We have changed the assessment in regard to the total Mayo score to severe disease in the text. We thank the reviewer for this important advice.

- Why Riley and not Geboes score? Score of 15 – what does it mean?

The Riley and Geboes score are currently regarded as the two main scoring methods for the histological assessment of UC. The Riley score mainly looks at density and distribution of neutrophils and at mucosal defects. The Geboes score is a grading system that furthermore also evaluates density of mononuclear cells and density of eosinophils. The Geboes score includes a more elaborate grading of crypt lesions and surface epithelial damage. Both scores have now been validated to have good intraobserver reproducibility and good interobserver agreement (Bressenot et al., 2014).

We decided to use the Riley score in our study as it has already been used in different clinical UC trials to evaluate the therapeutic efficacy of various therapeutic substances (Gibson et al., 2006; Feagan et al., 2005; Kruis et al., 2003). We therefore thought that it might be suitable to assess histological response to our initiated anti-IL-6R antibody treatment.

The histological criteria of the Riley score of the mucosal biopsies that we took before and after tocilizumab therapy were:

Riley Criteria	Before treatment	After treatment
Acute inflammation	3	3
Crypt abscesses	0	3
Mucin depletion	3	2
Surface integrity	3	3
Chronic inflammation	3	3
Crypt architecture	3	2
TOTAL SCORE	**15**	**16**

The Riley scores of 15 before and 16 after tocilizumab treatment reflect severe UC on a histological level.

Re-assessment of the slides according to the criteria of the Geboes Score was done in co-operation with a pathologist blinded to the clinical data of the patient. Please find the results of each score in the following table.

Geboes Criteria	Before treatment	After treatment
Structural	0.3	0.2
Chronic inflammatory infiltrate	1.3	1.3
Lamina propria Eosinophils	2A.3	2A.3
Lamina propria Neutrophils	2B.3	2B.3
Neutrophils in epithelium	3.2	3.2
Crypt destruction	4.3	4.3
Erosion or ulceration	5.4	5.4
TOTAL SCORE	**0.3/1.3/2A.3/2B.3/3.2/4.3/5.4**	**0.2/1.3/2A.3/2B.3/3.2/4.3/5.4**

The results of the Geboes score have been included in the manuscript.

- Did you check for clostridium difficile?
We excluded clostridium difficile infection in the patient by stool tests; this has now been specifically mentioned in the manuscript on page 5.

- Did you think in Leucocytapheresis therapy?
Our clinic did not have access to Leucocytapheresis therapy to ulcerative colitis patients at that time. The patient did not want to go to another clinic in Germany that offers this therapy. This has now been included in the manuscript.

- In all of this period the patient was with azathioprine and prednisolone 10mg/day?
There was no concomitant azathioprine therapy when the patient was treated with the anti-IL-6R antibody. Prednisolone was unchanged during the treatment period with 10 mg/day. This has been added to the text (page 5).

RESULTS:

Why do you think there was a normalization of the blood count and CRP level when the patient showed no clinical and endoscopic improvement, after the therapy of tocilizumab?
The reviewer makes a valid statement in pointing out that there was a normalization of the blood count and CRP-level in the course of tocilizumab treatment, while there was no clinical or endoscopic improvement of disease. Quantitative gene expression analysis showed that tocilizumab application was not able to suppress mucosal IL-6 levels. In accordance with the absent therapeutic response to tocilizumab treatment, there were instead even higher IL-6 levels after completion of therapy. We assumed that blockade of IL-6 signaling may induce compensatory IL-6 production on a mucosal level. The normalization of CRP levels may be explained by suppression of soluble IL-6 in the blood. It is known that CRP is predominately synthesized in hepatocytes as part of the acute phase response upon stimulation by IL-6 (Darlington *et al.,* 1986). Upregulation of IL-6 has been shown to result in higher CRP-concentrations. In our patient the normalization of CRP-levels could be explained by inhibition of systemic IL-6 levels in the blood. We did not measure IL-6 levels in the blood of

our patient to investigate if tocilizumab treatment led to a drop of IL-6 levels in the blood. There might therefore have been a discrepancy regarding enhanced mucosal IL-6 expression and suppressed IL-6 blood levels due to tocilizumab treatment in our patient. This possible explanation has now been included in the manuscript.
Our patient still had marked mucosal inflammation at the end of tocilizumab treatment although CRP-levels were normalized. This result is in line with studies that have demonstrated that there is no statistically significant correlation regarding the level of active mucosal inflammation and CRP levels (Henriksen et al., 2008). This reference has also been included in the revised manuscript (page 8).

The Riley histologic score rose? Did you mean rise?
We apologize for the mentioned orthographic mistake. We have changed it in the manuscript according to the reviewer's advice.

"(..) she remained on 10mg/day prednisolone therapy"- it should be explained earlier.
According to the reviewer's suggestion, we have earlier mentioned (page 4) that the patient had steroid dependent disease, with a requirement for steroid therapy ⩾10 mg/day.

4º paragraph: "Altogether (…)" again why there was a laboratory improvement?
We have changed the paragraph in the revised manuscript according to the possible explanation of laboratory improvement given in our reply to the first comment by the reviewer.

What happened with the patient? Agreed finally with the surgery?
We thank the reviewer for this question. After anti-IL-6R antibody treatment was stopped, the patient refused to undergo proctocolectomy and continued with steroid therapy. The patient was then put on therapy with the anti-adhesion molecule antibody vedolizumab in 2014. This therapy is continued till now and a partial response of the patient in clinical and endoscopic terms could be documented. The patient still refuses any surgical treatment options. We have included the recent development of the patient's therapies to the manuscript on page 5.

DISCUSSION:

The second paragraph should be the last one because it's a conclusion and because is the first case published in the English literature gives value to the manuscript (perhaps should be mentioned in the title?).
We thank the reviewer for this suggestion. We have moved the second paragraph to the end of the manuscript, summarizing the main content of the presented case. We have also taken the reviewers advice and emphasized that this is the first published case regarding anti-IL-6R antibody treatment in a severe ulcerative colitis patient in the title.