ANSWERING REVIEWERS

August 06, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format

Title: Growth and bone health in paediatric patients with Crohn ́s disease receiving subcutaneous tumor necrosis factor antibody

Author: Judith Pichler, Wolf Dietrich Huber, Prof. Christoph Aufricht, Bettina Bidmon-Fliegenschnee

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 12369

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

(1) First Reviewer:

Pichler et al. describes the effect of Adalimumab on the growth and bone health in 18 pediatric Inflammatory Bowel Disease. Most of the children in the trial loss Infliximab (murine monoclonal antibody linked to the constant domains of human kappa and immunoglobulin) efficacy and once switched to Adalimumab (human recombinant IgG1 monoclonal antibody) showed improvement in Pediatric Crohn's Disease Activity Index with some children showing catch-up growth.

The children also underwent bone densitometry measurements—it is these measurements that appear to be unique to this study. There are at least 13 published papers (2008-2014) on Crohn's disease and the use of Adalimumab and its effect on children's growth. The authors do attempt to compare their results to some of these previously published results with all eighteen pediatric patients receiving Adalimumab between 2007 and 2011. Thus, the timeline of treatment in this study correlates with the other publications. In all the study complements the other studies and supports the role for inflammation in Crohn's disease.

Dear Reviewer, thank you so much for your kind comments. We definitely agree that the timeline of treatment correlates with the other studies. We have already planned to perform a follow up study to investigate the long- term effect of adalimumab on growth and bone.

(2) Second Reviewer:

This retrospective cohort study, is well planned and well executed. The most major problem is the statistical analysis method because of the smaller sample size although the author has pointed it out.

Dear Reviewer, thank you so much for this helpful comment. We completely agree with you that statistical analyses are difficult to interpret in such a small cohort of patients. We have therefor added this comment in the limitation part to highlight these concerns.
We have therefor changed the manuscript as follows:

The sample size of the study was too small to draw a final conclusion on bone mineralisation and ADA. In addition, with such a limited number of patients statistical analyses are difficult to interpret.

1. The sample size is just 18 that is too small to use the mean±SE, especially in Table 3 and Table 4, let alone these analysis methods(?2- test, paired t-test, t-test and ANOVA). Generally, more than 30 samples are treated as a large sample in statistical field.
   Thank you so much for this comment. We have now changed the tables and used median and range instead of mean values.

2. Median and quartile should be used for statistical description.
Non-parametric test should be used for hypothesis testing. Multivariate analyses includes too few variables, which makes the results unreliable. My suggestion is that qualitative analysis should be applied in this paper, and quantitative analysis has many limitations.

2. P7. What is 400IE cholecalciferol (vitamin D3)? You mean 400 IU?
   Thank you so much, this is a typo and we have now changed it to 400IU.
3. P13, the author seemed to take more in explaining the reason affecting the results of the study. This may be put in the limitation part.
   Thank you for this helpful advice. We have now moved this part into the limitation section.

4. In 'Conclusion' section, the author stated that "A better nutritional status is positive predictor for improved growth and bone mineralisation". How does this point come from the present study?

   Thank you for this comment. We agree that this sentence may be misleading. We could demonstrate that inflammation has an impact on growth since children in remission showed significantly better weight and height SDS compared to those with mild or moderate to severe disease activity. Disease related factors influencing growth were weight and BMI SDS at start. The improvement of nutritional status, hence improved BMI and weight, could be partly explained by the positive effect of ADA on the intestinal mucosa similar to IFX with reduction of excessive loss of nutrients from the inflamed gut.

   To make it cleared we have now changed the abstract as follows:

   A better nutritional status with improvement in BMI and weight is positive predictor for improved growth and bone mineralisation.

(3) Overall a very nice presentation of the data. I agree with the authors that the small sample size is likely the main limiting factor. The authors nicely point out that there is an overall dearth of evidence for growth on adalimumab. Thus even small studies are valuable.

A few suggestions:

1. The section on "Predictors for improvement in growth and bone mineralisation" is confusing. I would recommend re-writing this section, with more information, to be more readable.

Thank you, we fully agree that this section is difficult to read, we have now amened the text as follows:

*Linear regression analysis was performed to assess simultaneously the effect of several potential predictors of growth and bone health after treatment with ADA (**Table 5**). There was a strong association between ΔHeight SDS post ADA and 25-OHD levels. Higher 25-OHD levels were positive predictors for better growth with the use of ADA. When bone health was analysed using ΔBMD SDS, PCDAI at start was the only significant predictor for bone mineralisation. With a lower disease activity it is more likely to have a better bone mineralisation.*

2. In the conclusion in both the abstract and the paper, the authors conclude that adalumumab induced and maintained remission in children with CD. However the design of the trial was a retrospective, observational study without a control group. The study was not designed nor powered to assess adalumumab for the induction and maintenance of remission. I would recommend removing this from the conclusions and focusing only on the growth catch up which the authors primarily evaluated.

Thank you so much for this comment, we completly agree with you. Therefore we have deleted this from the abstract and manuscript.

3. It is odd steroid cessation was not associated with an improvement in anthropometry and bone health. This may be due to small sample size. Given the pathophysiology of steroids and bone disease, the authors should comment on this in the discussion briefly.

Thank you for highlighting this important point. We have now included this in the discussion as follows:

In order to attenuate inflammation, glucocorticoids are used in a majority of patients with moderate to severe disease-activity. Glucocorticoids may impair growth through a variety of mechanism including a reduction of growth hormone secretion and its action [4,5] and are known to have effects on calcium and bone metabolism [43]. Although we found a trend in reduction, however not significantly, of daily and cumulative steroid dose, we could not found any association with improved growth or bone health in our limited number of patients. In line with this, also other clinical studies of the role of steroids in IBD and growth retardation have reported conflicting data [5].

Overall nicely done. This study is consistent with prior published literature and adds to the growing body of literature on anti-TNF use in children.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Judith Pichler
Department of Paediatric and Adolescent Medicine
Medical University Vienna
Währinger Gürtel 18-20
1090 Vienna
Austria
judith.pichler@meduniwien.ac.at