Poznań, 31-10-2016

Dear Editor,

Thank you for giving us a chance to submit a revision of our paper entitled: "Trefoil factor-3 for mucosal healing monitoring in Crohn's disease treated with anti-TNF-α antibodies".

We have revised the manuscript thoroughly and followed each comment of the reviewers and editors.

The manuscript has been checked by a native-speaking person and some minor edition has been done. The data were also checked by a professional biostatistician and an appropriate certificate has been provided. "Comments" section has been added, according to the comments of the editor.

Please find below the comments of the reviewers and our reply to them. Each change in the manuscript was marked in red in the file containing revised version of our article.

We would also like to thank the reviewers and editors for the assessment and all comments. We did our best to response to each comment adequately.

Yours sincerely,

Piotr Eder, MD, PhD

Corresponding author

RESPONSE TO THE REVIEWERS' COMMENTS

REVIEWER # 1

Comment to authors: Dear Author, I read your manuscript. Overall it is a globally good designed study and well written manuscript, although it's small sample size. It is acceptable for publication

Reply: Thank you for this comment. We discussed the sample size as a possible limitation of our study in the Discussion section. However, according to our sample size calculations that took into account the number of Polish CD patients starting each year anti-TNF therapy and the percentage of them undergoing colonoscopy both before and after induction therapy, the study group of 30 patients was enough to draw meaningful conclusions (see also response to Reviewer 2).

REVIEWER # 2

Comment to the authors No. 1: Firstly I think the title of the study should be modified to clearly state the negative finding of the study (as it stands it appears at first glance to suggest the marker is useful).

Reply 1: The title of the manuscript has been changed, according to the comment of the reviewer #2.

Comment to the authors No. 2: The authors should also discuss whether a sample size calculation was performed in advance of the study and how the sample size was decided. There is always a concern with a negative study of the potential for type 2 error due to small sample size.

Reply 2: Thank you for this comment. The small sample size was listed as a potential limitation of a study in the Discussion section. The number of patients analyzed was affected mainly by a limited number of patients in whom repeated colonoscopic examination could be performed within a short period of time due to the invasiveness of endoscopy. Nevertheless, a sample size calculation was performed, which was based on the data from the National Health Fund and a Polish Crohn's disease registry. According to these data – each year about 300 Polish CD patients receive anti-TNF therapy. While colonoscopic examination before the initiation of treatment is obligatorily performed in about 2/3 of patients (with luminal CD), control colonoscopy after the induction period of anti-TNF therapy is not obligatory and clinical decisions are based on clinical indices and biochemical analysis. It has been estimated that approximately 15-20% (n=45-60) of Polish CD patients undergo full colonoscopic assessment both before and after induction anti-TNF therapy with endoscopic assessment of mucosal healing. For study population n=45, minimal sample size should be n=31 (absolute precision of 0,1 with CI 95%, α error = 0,05). For study population n=60, minimal sample size is n=37 (absolute precision of 0,1 with CI 95%, α error = 0,05) or n=25 (taking absolute precision of 0,15). That is why we concluded that a study group of 30 patients would be large enough to perform the study.

Comment to the authors No. 3: Also, the authors should discuss how they selected the time points for endoscopic evaluation for mucosal healing, and why the time points differed for adalimumab and infliximab. I would argue that at week 10, after just three doses of infliximab, one would hope to see endoscopic mucosal improvement but not expect to see optimal healing for 4-6 months.

Reply 3: Thank you for this comment. The most appropriate time to assess mucosal healing in Crohn's disease is still a matter of debate. According to current European guidelines (as well as our national guidelines), anti-TNF induction regimen is defined as 3

doses of IFX (0-2-6 week) or 12-week treatment with ADA. This is also in accordance with IFX and ADA prescribing information (product characteristics). Although there is no commonly accepted strict definition on when the assessment of mucosal healing should be performed, the ECCO guidelines suggest that primary response "may be determined within 12 weeks" (Dignass A, et al. J Crohns Colitis 2010). This was additionally confirmed by the "ECCO pathogenesis workshop on anti-TNF therapy failures in IBD" (Allez M, et al. J Crohns Colitis 2010). In the most important clinical trials concerning the efficacy and safety of anti-TNF antibodies in Crohn's disease, week 10 for IFX and 12-14 week for ADA are commonly used for the assessment of induction therapy.

The examples may include the endoscopic substudy of ACCENT-1 trial (for IFX), in which the authors assessed mucosal healing at week 10 and then decided, whether the patients can be considered as responders and non-responders (Rutgeerts P, et al. Gastrointest Endosc 2006). In the EXTEND study, which was designed to assess the efficacy of ADA for inducing and maintaining mucosal healing in Crohn's disease (Rutgeerts P, et al. Gastroenterology 2012), the primary end point was the percentage of patients with mucosal healing at week 12 (induction therapy with ADA).

The aforementioned observations are reflected in our national guidelines and the recommendations of a National Health Fund, which oblige us to assess the efficacy of induction anti-TNF therapy in Crohn's disease at week 10 and at week 12-14 in case of IFX and ADA, respectively.

Thus, although we fully agree that time-points for the assessment of mucosal healing after induction anti-TNF therapy are not strictly defined, we believe that they were chosen appropriately. We made an additional comment on that as a possible limitation of a study and some adequate references are added.