UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

Debrecen, 01 October 2023.

Lian-Sheng MA, MD, Prof.
Founder and Chief Executive Officer
World Journal of Gastroenterology
Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160,
Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: editorialoffice@wjgnet.com

Andrzej S Tarnawski, DSc, MD, PhD, Prof.
Editor-in-Chief
World Journal of Gastroenterology
Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160,
Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: editorialoffice@wjgnet.com

Gastroenterology Research Department,
University of California Irvine and the
Veterans Administration Long Beach
Healthcare System, Long Beach, CA 90822,
United States
E-mail:atarnawski@yahoo.com

Dear Professor Lian-Sheng MA, MD,
Dear Professor Andrzej S Tarnawski DSc, MD, PhD,
Dear Editorial Board,
Dear Editorial Office,

Please find attached the revised version of our manuscript entitled:

Invited Manuscript ID: 86628

Original Title: Fifty shades of Crohn's disease – when the location starts to sing via novel serological marker, anti-Chitinase 3-like 1 autoantibodies

Proposed new title based on the comments of the reviewers: A location-based prediction model for Crohn's disease regarding a novel serological marker, anti-chitinase 3-like 1 autoantibodies

Authors: Nora Sipeki, Patricia Kovats, Claudia Deutschmann, Peter Schierack, Dirk Roggenbuck, Maria Papp

Once again thank you for inviting us (Reviewer ID: 04213404) to contribute to the *World Journal of Gastroenterology* with the above paper and offering to waive the publication fee in case of final acceptance.

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

We are grateful to the reviewers for their in depth and important comments as well as for the critiques that help in improving the manuscript. We revised the manuscript according to the concerns raised.

Answers to reviewers' comments:

Reviewer 1 (ID: 02447059)

The authors aimed to investigate anti-CHl3L1 in IBD and its frequency and relation to clinical parameters, severity, and relapse of disease. They recruited 257 Crohn's disease (CD), 180 ulcerative colitis (UC) patients, and 86 healthy controls. They detected anti-CHl3L1 by in-house ELISA. They found that sIgA subtype aCHI3L1 positivity was higher in both CD and UC patients than in HCONT and the presence of both IgA and sIgA aCHI3L1 antibodies was associated with colonic involvement in patients with CD. They concluded that CHI3L1 is a novel neutrophil autoantigenic target in IBD. The consideration of antibody classes along with location-based prediction may transform the future of serology in IBD. They also discussed its potential as a therapeutic target. The study is very interesting and very important in the field, well-designed and well-written. But few concerns were raised during revision.

Scientific Quality: Grade A (Excellent)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)
Novelty of This Manuscript: Grade B (Good)
Creativity or Innovation of This Manuscript: Grade B (Good)
Scientific Significance of the Conclusion in This Manuscript: Grade A (Excellent)

Comment 1: *The title feels more as philosophical.*

We agree with *Reviewer 1* that the first impression of the title is more philosophical (we intended to trigger the readers' curiosity with the original title); therefore, we propose a more realistic and simpler data-based title: **A location-based prediction model for Crohn's disease regarding a novel serological marker, anti-chitinase 3-like 1 autoantibodies**.

Comment 2: *Figure 2 footnote is very confusing. Please present in a simpler way.*

Figure 1 summarises the results of the Kaplan-Meier survival analysis for the probability of internal penetrating/stricturing (IP/S) complication development in CD patients with non-stricturing and non-penetrating (NSNP) disease at diagnosis. Figure 2 summarises the results in the same manner for a subgroup of patients with colonic involvement at diagnosis. Information on the Montreal classification shown in Figure 1 and Figure 2's footnotes was omitted to present the data in a simpler manner. Descriptions of complications and Montreal equivalents are common clinical knowledge; therefore, *Reviewer 1*'s comment on the redundancy of the information is appropriate.

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

Footnotes of Fig.1. and Fig. 2. were rewritten according to the following:

"Fig.1. Kaplan-Meier survival analysis for the probability of internal penetrating/stricturing (IP/S) complication development in CD patients with non-stricturing and non-penetrating (NSNP) disease at diagnosis"

"Fig.2. Kaplan-Meier survival analysis for the probability of internal penetrating/stricturing (IP/S) complication development in CD patients with non-stricturing and non-penetrating (NSNP) disease and colonic involvement at diagnosis"

Comment 3: *association between the antibody status and clinical or endoscopic disease activity and duration need to be presented even as supplementary.*

The manuscript has been updated with a detailed description of the evaluation of aCHI3L1 status and levels with clinical and endoscopy activity indices, along with data regarding disease duration at sample procurement in CD patients in the RESULTS section - "Correlation of aCHI3L1 antibodies formation and overall disease duration in Crohn's disease: stability of aCHI3L1 antibodies in Crohn's disease" subsection. Information on these data is provided in Supplementary Tables 1 and 2.

"In addition, no association was detected between the antibody status and clinical or endoscopic disease activity (actual HBI or SES-CD), or disease duration at the time of sample procurement (Supplementary Table 1). aCHI3L1 IgG levels were not analysed further because of the low prevalence of antibody positivity in the CD cohort. IgA and sIgA aCHI3L1 antibody levels did not differ according to clinical activity (p=0.385 and 0.6830, respectively). Nevertheless, the actual CDAI, HBI, and SES-CD indices were also not correlated with IgA and sIgA aCHI3L1 antibody levels, as determined by Spearman correlation analysis (Supplementary Table 2). The levels of aCHI3L1 antibodies were not associated with disease duration (Kruskal-Wallis test)."

The METHODS section was updated with the additional statistical methods used during the analysis: "The Spearman's nonparametric rank correlation test was used to determine correlations."

Comment 4: *Table 4, Why there is no testing correlation between anti-CHl3L1 and Anti-GP2 IgA and complicated disease behaviour?*

The aforementioned data from Table 4 were omitted because statistically significant differences were not obtained for a given parameter. At the request of Reviewer 1, Table 4 has been updated using these data.

Comment 5: *Table 5, though important, is difficult to follow.*

We agree with *Reviewer 1* that Table 5 is a very complex summary of the uni- and multivariate Cox regression analyses evaluating the association between clinical and serologic variables and the study endpoint events; however, we believe that all the information is necessary to

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

understand the main message of the article. Based on the robust data presented, it is difficult to simplify the table content without losing key information.

Comment 6: *Cox regression description need to be transferred from results section to methodology section.*

According to Reviewer 2's request, descriptions of the Cox regression analyses have been transferred from the Results section to the Methodology section.

Comment 7: *The relation to anti-CHl3L1 negativity to treatment type need to be addressed.*

Regarding autoantibodies against CHI3L1, we did not find any association between aCHI3L1 formation and response to therapy. This finding is concordant with previous observations in IBD, where, in contrast to other disorders, serum antimicrobial and autoantibody formation and status remain largely unaffected by the actual inflammatory burden and are not altered by anti-inflammatory therapy or surgical resection. Unlike other autoimmune diseases such as ANCA-associated vasculitides, classic and newly discovered autoantibodies (anti-neutrophil cytoplasmic antibodies [ANCA], anti-pancreatic autoantibodies [PAbs], antiphospholipid antibodies [APLAs], anti-chitinase 3-like 1 autoantibodies [aCHI3L1]), and antimicrobial antibodies (anti-Saccharomyces cerevisiae antibodies [ASCA], anti-OMP PlusTM IgA) have no proven value in monitoring the efficacy of IBD therapy. [1] The Discussion section has been updated with these relevant information.

Comment 8: *The change of anti-CHl3L1 to positivity or to negativity during follow up period need to be explained.*

There were no clinically significant differences in the evaluated study endpoints when considering stability data. Further studies with sequential sampling and serial measurements both before and after the study endpoint events are needed for a more detailed evaluation of aCHI3L1 antibody stability in CD, which is beyond the limits of the current work.

Comment 9: *Discussion is long and narrative. It needs to be more focused.*

The Discussion section have been shortened according to the Reviewer's suggestions.

Reviewer 2 (ID: 03700188)

The title reflects the main subject of the manuscript The abstract summarizes and reflect the work. The key words reflects well the focus of the manuscript The manuscript describes methods (e.g., experiments, data analysis, surveys, and clinical trials, etc.) in adequate detail. The research objectives were achieved and it brought some progress in this field. The figures, diagrams, and tables were sufficient, good quality and appropriately illustrative. The manuscript appropriately cited the latest, important and authoritative references in the Introduction and Discussion sections. The author self-cited once as first author and twice as second one. The manuscript is well, concisely, and coherently organized.

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

Scientific Quality: Grade A (Excellent)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)
Novelty of This Manuscript: Grade B (Good)
Creativity or Innovation of This Manuscript: Grade A (Excellent)
Scientific Significance of the Conclusion in This Manuscript: Grade A (Excellent)

We are grateful for the Reviewer's positive feedback. To briefly reflect Reviewer 2's comment on self-citations, self-citations were needed to avoid self-plagiarism.

Reviewer 3 (ID: 02997260)

The manuscript presents the results of a robust and interesting study, it is written in good English, but needs some improvements.

Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision

Some technical inaccuracies:

Comment 1: *The STROBE Statement checklist is missing, the cover letter was uploaded instead.*

The cover letter was uploaded by mistake instead of the STROBE Statement checklist. This technical error will be corrected during the upload of the revised version of the manuscript.

Comment 2: *In the Certificate of Statistical Analysis the signature of Elek Dinya was just copied from another document and pasted in this certificate.*

The electronic signature on the Certificate of Statistical Analysis was replaced with a handwritten scanned back version. This updated document will be uploaded to the revised version of the manuscript.

Comments on the manuscript text:

Comment 3: *How to understand the results of the univariate analysis presented in Table 1: in the part 'Need for resective surgery' where the number of subjects was given as a fractional number in the column 'n of subjects' ?*

A total of In total, A total of Cross-sectional descriptive statistical data regarding the clinical characteristics of IBD patients at diagnosis and at the end of follow-up are presented in Table

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

1. This prospective follow-up study included 261 patients with CD and 183 with UC. 44.8% (n=117) of the CD patients underwent resective surgery and 12.6% (n=33) underwent multiple surgeries until the end of the follow-up period.

However, the Reviewer specifically asked about the table regarding univariate analysis, which has been described in Table 5: *"Univariate and multivariate Cox regression analyses evaluating the association between clinical and serologic variables and the study endpoint events (A: complicated disease course, B: need for resective surgery, C: reoperation after resection, and D: development of perianal complications) in CD patients. Subgroup analysis of B1 patients with colonic involvement is shown in the second half of the table."* The "n of subjects" in Table 5. Part A and B is 209, since those are the patients with follow-up and B1 Montreal classification at diagnosis – patients with a complicated disease course (B2 and B3 according to Montreal classification) at diagnosis were excluded from this evaluation (the disease has already been complicated at the beginning; therefore, evaluating this outcome in this subgroup of patients had no clinical value). This is the reason why "n of subjects" is less than the maximum number of CD patients with follow-up (n=261), since we evaluated these outcomes in a subgroup of CD patients with B1 at diagnosis. Similarly, in Table 5. Part C reoperation after resection ("Need for resective surgery in B1 patients with previous CD-related abdominal surgery") was only evaluated in patients with previous CD-related abdominal surgery (n=73). In Part D, the development of perianal penetrating complications (P1) was evaluated in patients with CD without perianal involvement (P0) at diagnosis (n=215).

Comment 4: *To better support the findings of the study, it would be useful to provide estimates of the sensitivity and specificity of the anti-CHI3L1 test in predicting the course of IBD.*

Association of serum antibodies and clinical phenotypes of Crohn's disease has been extensively assessed in cross-sectional single time point studies; however, prospective follow-up studies evaluating the prognostic potential of these antibodies are rare in the IBD literature. Cross-sectional single-time point studies have limited clinical value in predicting the disease course of IBD. [1] This is the reason why the authors chose a prospective study design and used uni- and multivariate time-dependent statistical analysis methods to evaluate the clinical utility of a newly discovered autoantibody (aCHI3L1) in the prediction of a complicated disease course in IBD. These statistical methods cannot allow us to estimate the sensitivity (SENS) and specificity (SPEC) of aCHI3L1 for these outcomes. Data regarding SENS and SPEC of aCHI3L1 antibodies as diagnostic tests were omitted from the manuscript. First, it was not the main focus of the manuscript. Second, similar data have already been published in the authors previous paper on aCHI3L1 autoantibodies in a paediatric IBD cohort. [2] Information on the

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

sensitivity and specificity of aCHI3L1 antibodies as diagnostic tests is available in Supplementary Table 1 (Diagnostic performance of antibodies against CHI3L1).

Comment 5: *In the Discussion section, the authors named numerous inflammatory conditions when CHI3L1 participates without clear indication of its role in these conditions. Please indicate the differences, if any. In connection with this, why did the authors conclude that CHI3L1 is a marker of IBD progression, as it has been found to overexpress in many inflammatory conditions in addition to CD and UC? It is more likely to be a marker of systemic inflammation, is it not?*

We would like to thank the reviewer for this important reflection in the Discussion section. The authors evaluated the role of antibodies against CHI3L1 (aCHI3l) and not the glycoprotein itself (CHI3L1) in predicting the complicated disease course in IBD. Therefore, robust data regarding CHI3L1, the glycoprotein, in the discussion are not or only partially relevant to the topic, and recent reviews are available on the subjects as well. However, CHI3L1 itself plays numerous roles in other chronic inflammatory diseases, data on autoantibodies against it are scarce. aCHI3L1 positivity was observed only in rheumatoid arthritis (RA) and IBD patients. Detailed information on the similarities and differences between RA and IBD is available in the Discussion section. Unnecessary information on CHI3L1 has been omitted from the manuscript because it can cause confusion for the reader.

Reflecting on the other part of the comment, in our study no association was detected between the antibody status and clinical or endoscopic disease activity (actual HBI or SES-CD) at the time of sample procurement (Supplementary Table 1). Nevertheless, the actual CDAI, HBI, and SES-CD indices were not correlated with IgA and sIgA aCHI3L1 antibody levels, as determined by Spearman correlation analysis (Supplementary Table 2). These findings align with previous results and can be explained by disease activity-independent, persistent elevation of CHI3L1 glycoprotein, the autoantigenic target of these aCHI3L1 antibodies. However, from a general point of view, aCHI3L1 antibody positivity can be considered a phenomenon of chronic systemic inflammation, but it is not a clinically useful inflammatory biomarker, since it cannot reflect either the inflammatory activity of CD or the efficacy of anti-inflammatory therapy in CD patients. Regarding autoantibodies against CHI3L1, we did not find any association between aCHI3L1 formation and response to therapy, which is consistent with previous IBD studies. In contrast to other disorders, serum autoantibody and antimicrobial antibody formation and status remain largely unaffected by the actual inflammatory burden and are not altered by anti-inflammatory therapy or surgical resection in IBD. [1]

Considering that the CHI3L1 glycoprotein is a polyhistor in physiological and pathological conditions, instead of highlighting only its general role in inflammation, we would like to draw

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

attention to its role in tissue remodelling and fibrosis. [2-4] Fibrogenesis induced by chronic inflammatory processes in CD plays a pivotal role in the formation of strictures and fistulas (complicated B2 and/or B3 behaviour according to the Montreal classification). Previously, *Erzin et al.* showed that increased serum CHI3L1 was associated with intestinal stricture formation in patients with CD. [5] Based on our findings regarding the clinical utility of autoantibodies against CHI3L1 (aCHI3L1) in predicting the development of a complicated disease course, we would suggest that aCHI3L1 antibodies are biomarkers of fibrosis in CD settings.

Comment 6: *Since the authors found a higher concentration of anti-CHI3L1 in subjects having other antibodies, what about the possibility of overlapping? Had the authors checked the possibility of overlapping of different antibodies tests taking into account that YKL-CHI3L1 itself possesses domains for many mucosa (extracellular matrix) proteins?*

Presence of immunoglobulin (Ig) G, IgA and secretory (s) IgA (sub)type antibodies against CHI3L1 (aCHI3L1) in serum samples of IBD patients (n=257 in CD and n=180 in UC) and healthy controls (n=86) were determined by an “in house” enzyme-linked immunosorbent assay (ELISA) technique using recombinant human CHI3L1 as solid-phase antigen. A detailed description of the assay development is available in our previous publication. [2] Aspecific binding of other antibodies (e.g. anti-Gp2) to the assay and fals positive results were excluded during the development process of the assay.

Enhanced antibody formation in the serum is a well-known feature of inflammatory bowel disease (IBD) and is currently considered to be a reflection of the enhanced microbial challenge to the gut due to a disturbed gut innate immune system that triggers an exaggerated adaptive immune response. [1] A tendency for IgA-dominant antibody formation in IBD supports this hypothesis. The significant associations between the presence of certain IgA antimicrobial (ASCA, anti-OMP), antiphospholipid (anti-PS/PT), and autoantibody (anti-GP2) types and aCHI3L1 IgA and sIgA positivity found in our study would likely reflect an increased bacterial translocation and microbial overload of the mucosa, rather than fals positivity due to overlapping antibodies.

Comment 7: *Did controls with positive anti-CHI3L1 develop UC or Cd during follow-up?*

Two control subjects were positive for IgG, IgA, and sIgA aCHI3L1, and two more controls were positive for sIgA only. None of them developed any form of IBD (CD, UC, or IBD unclassified) during the follow-up period.

Comment 8: *What was the consequence of negative subjects at baseline becoming positive during follow-up?*

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

There were no clinically significant differences in the evaluated study endpoints when considering stability data. Further studies with sequential sampling and serial measurements both before and after the study endpoint events are needed for a more detailed evaluation of aCHI3L1 antibody stability in CD, which is beyond the limits of the current work.

We hope that data from this study can provide easily applicable information from basic science to the clinicians in the everyday practice decision making when treating a patient with IBD.

All authors have fulfilled the criteria of authorship and seen and approved the final version of the manuscript and they have authorized the first author to grant on behalf of all authors to transfer exclusive copyright to *World Journal of Gastroenterology* in case of acceptance.

We also state that the manuscript, including related data, figures and tables has not been previously published and that the manuscript is not under consideration elsewhere.

We hope that the article could provide useful new information to the readers of *World Journal of Gastroenterology*.

Sincerely yours,

Nora Sipeki, MD, PhD
Peer Reviewer
Reviewer ID: 04213404
World Journal of Gastroenterology

Assistant Lecturer
Division of Gastroenterology
Faculty of Medicine
University of Debrecen
98, Nagyerdei blv., Debrecen, H-4032
Hungary
Tel: +36-52-258-598
e-mail: sipeki.nora@med.unideb.hu; norasipeki@gmail.com

Maria Papp MD, PhD, DSc, FEBG
Professor of Medicine
Vice President for Medicine of the Clinical Center, University of Debrecen
Head of Division of Gastroenterology, Faculty of Medicine, University of Debrecen

University of Debrecen, Clinical Center, Institute of Gastroenterology, director
98, Nagyerdei blv., Debrecen, H-4032, Hungary
Phone: (+36) 52-258-598
Mobil: (+36) 30-405-4993
E-mail: papp.maria@med.unideb.hu

UNIVERSITY of DEBRECEN

DIVISION OF GASTROENTEROLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF MEDICINE
98, Nagyerdei blv.
Debrecen, H-4032, Hungary
Phone: +36-52-258-598

1 Papp M, Lakatos PL. Serological studies in inflammatory bowel disease: how important are they? *Current opinion in gastroenterology* 2014; **30**(4): 359-364 [PMID: 24811052 DOI: 10.1097/mog.0000000000000076]

2 Deutschmann C, Sowa M, Murugaiyan J, Roesler U, Röber N, Conrad K, Laass MW, Bogdanos D, Sipeki N, Papp M, Rödiger S, Roggenbuck D, Schierack P. Identification of Chitinase-3-Like Protein 1 as a Novel Neutrophil Antigenic Target in Crohn's Disease. *Journal of Crohn's & colitis* 2019; **13**(7): 894-904 [PMID: 30753386 PMCID: PMC6657965 DOI: 10.1093/ecco-jcc/jjz012]

3 Zhao T, Su Z, Li Y, Zhang X, You Q. Chitinase-3 like-protein-1 function and its role in diseases. *Signal Transduct Target Ther* 2020; **5**(1): 201 [PMID: 32929074 PMCID: PMC7490424 DOI: 10.1038/s41392-020-00303-7]

4 Pieczarkowski S, Kowalska-Deptuch K, Kwinta P, Wędrychowicz A, Tomasik P, Stochel-Gaudyn A, Fyderek K. Serum concentrations of fibrosis markers in children with inflammatory bowel disease. *Folia Med Cracov* 2020; **60**(1): 61-74 [PMID: 32658213 DOI: 10.24425/fmc.2020.133487]

5 Erzin Y, Uzun H, Karatas A, Celik AF. Serum YKL-40 as a marker of disease activity and stricture formation in patients with Crohn's disease. *Journal of gastroenterology and hepatology* 2008; **23**(8 Pt 2): e357-362 [PMID: 17725598 DOI: 10.1111/j.1440-1746.2007.05121.x]