PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease in comparison with non-celiac, non-IBD subjects (HERMES): A case-control study protocol
AUTHORS	Hegyi, Péter; Szakács, Zsolt; Csiszár, Beáta; Kenyeres, Péter; Sarlós, Patrícia; Erőss, Bálint; Hussain, Alizadeh; Nagy, Ágnes; Kőszegi, Balázs; Veczák, Ibolya; Farkas, Nelli; Bódis, Emőke; Márta, Katalin; Szentesi, Andrea; Tőkés-Füzesi, Margit; Berki, Tímea; Vincze, Áron; Toth, Kalman; Bajor, Judit

VERSION 1 - REVIEW

REVIEWER	Tamara Milovanovic School of Medicine, University of Belgrade Clinical Center of Serbia, Clinic for Gastroenterology and Hepatology, Belgrade Serbia
REVIEW RETURNED	27-Sep-2018

GENERAL COMMENTS	With great pleausure I read this study proposal and I am looking forward to read the results in the future. The topic is very interesting and has imprortant clinical consequences.

REVIEWER	Prajwal Gyawali The University of Newcastle
REVIEW RETURNED	09-Oct-2018

GENERAL COMMENTS	1) Introduction Can be shortened focusing on complex interrelationship between CeD/IBD, hemorheology and coagulopathy and vascular risks. 2) Please mention what indices of hemorheological parameters will be used for data analysis and also please mention at what shear rate is WBV will be measured 3) Broad aims needs to be presented in primary and secondary objective section rather than telling what assessment you do. Like associating/correlating the rheological profiles with vascular risk factors or coagulopathy or immunological markers of the diseases etc. Other than comparing markers between case and control, Are you also trying to see the link between diseases/diseases specific parameters or cardiovascular complications with rheological and coagulopathy markers. If yes, this needs to be stated as objectives (primary or secondary). If yes, this needs to be also clarified in statistical analysis section.

	4) BIOBANK: Is it a single group biobank or multiple group can approach for the analysis of the sample. Nature of the biobank along with ethical aspects needs to be clarified. 5) 1 or 2 paragraphs of discussion will enhance the quality of the protocol paper (focusing on big picture of the trial, how the data helps to advance treatment or change clinical guidelines etc)

REVIEWER	Igor Dumic Icahn School of Medicine at Mount Sinai, New York City, NY, USA
REVIEW RETURNED	12-Nov-2018

GENERAL COMMENTS	I have read with interest this study protocol. The study will attempt to address important questions and could provide valuable information in order to better understand the risks associated with hypercoagulability in patients with IBD and celiac disease. It is well written. I do have several concerns listed below. Those would need to be addressed before it is considered for publication. Introduction: Please be more specific in the definition of celiac disease. It should be mentioned that celiac disease have gastrointestinal and extra intestinal manifestation and more should be elaborate about it's silent and atypical presentation. The prevalence- it is higher in Scandinavian country and authors might consider describing the range of prevalence rather than just average worldwide. Lines 117-119 - needs updated reference, there is a more recent review of literature on thrombosis associated with celiac disease and theories of pathogenesis. Please review the following article Igor Dumic, Scott Martin, Nadim Salfiti, Robert Watson, and Tamara Alempijevic, “Deep Venous Thrombosis and Bilateral Pulmonary Embolism Revealing Silent Celiac Disease: Case Report and Review of the Literature,” Case Reports in Gastrointestinal Medicine, vol. 2017, Article ID 5236918, 8 pages, 2017. https://doi.org/10.1155/2017/5236918. Line 135- a word is incomplete, please fix that Line 213- I would suggest excluding patient who are on anticoagulation for any reason, patient on anti-thrombotic therapy (aspirin, clopidogrel etc) and finally those who are on oral contraceptive pills and steroids. All of these are known to be associated with altered coagulation profile. People with recent (within one year VTE) and lupus patients should be excluded as well. Line 266-267- GOLD standard for diagnosis of CD is small intestinal biopsy, and I believe that biopsy should be mandatory in all patients with suspected CD in order to confirm diagnosis before enrollment in the study Line 268-271. I would refer authors to the following article : Hindryckx P, Levesque BG, Holvoet T, et al. Disease activity indices in coeliac disease: systematic review and recommendations for clinical trials. Gut 2018;67:61-69. I am not confident that serum antibodies are reliable test to monitor disease activity and certainly they are nod FDA approved. Line 282- I would also add folic acid levels, please see the following article: S. Saibeni, A. Lecchi, G. Meucci et al., “Prevalence of hyperhomocysteinemia in adult gluten-sensitive enteropathy at diagnosis: Role of B12, folate, and genetics,”

	Clinical Gastroenterology and Hepatology, vol. 3, no. 6, pp. 574–580, 2005. Line 286- could authors include TAFI measurements? I don't think it is essential but would add to the strength of the study. Please see : N. H. van Tilburg, F. R. Rosendaal, and R. M. Bertina, "Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis," Blood, vol. 95, no. 9, pp. 2855–2859, 2000. Line 311- I would add secondary outcomes also to be folic acid and homocysteine levels Lines 316-316. It would be worthwhile of considering to divide patients with IBD to the UC group and CD group. While both are associated with hypercoagulability those are distinct disorders and variations in hypercoagulability do exist. References 22-24 are little outdated, they can be kept in my opinion but new ones need to be added

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Tamara Milovanovic

Institution and Country: School of Medicine, University of Belgrade Clinical Center of Serbia, Clinic for Gastroenterology and Hepatology, Belgrade Serbia

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

With great pleausure I read this study proposal and I am looking forward to read the results in the future. The topic is very interesting and has improrant clinical consequences.

[Authors' comment] We appreciate your kind comment. Thank you.

Reviewer: 2

Reviewer Name: Prajwal Gyawali

Institution and Country: The University of Newcastle

Please state any competing interests or state 'None declared': None

Please leave your comments for the authors below

1) Introduction Can be shortened focusing on complex interrelationship between CeD/IBD, hemorheology and coagulopathy and vascular risks.

[Reply] We shortened and restructured the introduction to make it more consised and focused (Pgs 3-5, Lns 88-152).

2) Please mention what indices of hemorheological parameters will be used for data analysis and also please mention at what shear rate is WBV will be measured

[Reply] Please, find all the hemorheological parameters measured in Supplementary material with a brief summary of methodological details. We adhere to the manual of use of the tools when performing the measurements. Viscosity is measured at 90 s-1 shear rate.

3) Broad aims need to be presented in primary and secondary objective section rather than telling what assessment you do. Like associating/correlating the rheological profiles with vascular risk factors or coagulopathy or immunological markers of the diseases etc. Other than comparing markers between case and control, Are you also trying to see the link between diseases/diseases specific parameters or cardiovascular complications with rheological and coagulopathy markers. If yes, this needs to be stated as objectives (primary or secondary). If yes, this needs to be also clarified in statistical analysis section.

[Reply] We rephrased the objectives (Pg 5, Lns 137-152) and the statistical section accordingly (Pgs 12-13, Lns 338-353).

4) BIOBANK: Is it a single group biobank or multiple group can approach for the analysis of the sample. Nature of the biobank along with ethical aspects needs to be clarified.

[Reply] The Biobank receives samples from all the research projects run by the Centre for Translational Medicine (headquarter: University of Pécs). Frozen samples from each project (including ours) are deidentified, numbered, and kept in locked cabinets. Access to the cabinets containing biological material from HERMES is granted for the Principal Investigator of the study and those personnel involved in the maintenance of the facility, exclusively. Access to unauthorized personnel to the samples stored is strictly prohibited.

The operation of the Biobank has been approved by the National Public Health and Medical Officer Service, Hungary (Ref No 666-34/2009). The collection, storage, and analysis of biological samples (whole blood, plasma, and urine) from HERMES were approved by the Scientific and Research Ethics Committee of the Medical Research Council, Medical School, University of Pécs (Ref No 6917). The committee has approved the analysis pre-planned within the study protocol submitted for publication. Other non-genetic investigations require modification of the ethical approval by submitting a modified study protocol to the board. Genetic investigations of the stored samples can only be performed if patients consented subsequently; however, the recent study protocol does not include any genetic studies.

5) 1 or 2 paragraphs of discussion will enhance the quality of the protocol paper (focusing on big picture of the trial, how the data helps to advance treatment or change clinical guidelines etc)

[Reply] We agree. In line with this, we created a short discussion in the study protocol (Pgs 13-14, Lns 373-389).

[Authors' comment] We would like to thank Reviewer: 2 for the excellent comments, which have significantly improved the quality of our manuscript.

Reviewer: 3

Reviewer Name: Igor Dumic

Institution and Country: Icahn School of Medicine at Mount Sinai, New York City, NY, USA

Please state any competing interests or state 'None declared': None

Please leave your comments for the authors below

I have read with interest this study protocol. The study will attempt to address important questions and could provide valuable information in order to better understand the risks associated with hypercoagulability in patients with IBD and celiac disease. It is well written. I do have several concerns listed below. Those would need to be addressed before it is considered for publication.

Introduction: Please be more specific in the definition of celiac disease. It should be mentioned that celiac disease have gastrointestinal and extra intestinal manifestation and more should be elaborate about it's silent and atypical presentation.

[Reply] We added the information required to the 'Introduction' section (Pg 3, Lns 94-99).

The prevalence- it is higher in Scandinavian country and authors might consider describing the range of prevalence rather than just average worldwide.

[Reply] Indeed. Please, find the amended data based on the recent WGO guideline (Pg 3, Lns 95-96).

Lines 117-119 - needs updated reference, there is a more recent review of literature on thrombosis associated with celiac disease and theories of pathogenesis. Please review the following article Igor Dumic, Scott Martin, Nadim Salfiti, Robert Watson, and Tamara Alempijevic, "Deep Venous Thrombosis and Bilateral Pulmonary Embolism Revealing Silent Celiac Disease: Case Report and Review of the Literature," Case Reports in Gastrointestinal Medicine, vol. 2017, Article ID 5236918, 8 pages, 2017. https://doi.org/10.1155/2017/5236918.

[Reply] Thank you for the recommendation of this comprehensive summary article, which highlights the importance of our question of interest. We updated the reference list by adding the article mentioned (Ref No 25).

Line 135- a word is incomplete, please fix that

[Reply] We completely rearranged the section mentioned.

Line 213- I would suggest excluding patient who are on anticoagulation for any reason, patient on anti-thrombotic therapy (aspirin, clopidogrel etc) and finally those who are on oral contraceptive pills and steroids. All of these are known to be associated with altered coagulation profile. People with recent (within one year VTE) and lupus patients should be excluded as well.

[Reply] Thank you for the thought-provoking comment. We rephrased the exclusion criteria and exclude those patients having a thrombotic event within one year of recruitment, taking vitamin K antagonists or antiplatelet drugs, and those with verified lupus (Pg 7, Lns 172-202). Since there is a female predominance among celiac patients, and a significant fraction of the population is in childbearing age, the exclusion of patients using oral contraceptives may distort the generalizability of the population included. Exclusion of IBD cases receiving glucocorticoids may cause a distortion as well because we would lose a significant fraction of the active IBD cases, which will not allow us to perform the pre-planned subgroup analysis by disease activity. Based on this arguments, we do not think that the exclusion of these cases would be beneficial, but we will include these factors, as explanatory variables in the multivariate analysis (Pgs 12-13, Lns 344-346).

Line 266-267- GOLD standard for diagnosis of CD is small intestinal biopsy, and I believe that biopsy should be mandatory in all patients with suspected CD in order to confirm diagnosis before enrollment in the study

[Reply] Indeed. We rephrased the inclusion criteria of celiac disease accordingly (Pg 7, Ln 179)

Line 268-271. I would refer authors to the following article : Hindryckx P, Levesque BG, Holvoet T, et al. Disease activity indices in coeliac disease: systematic review and recommendations for clinical trials. Gut 2018;67:61-69.

[Reply] We added this comprehensive summary article to the reference list (Ref No 55).

I am not confident that serum antibodies are reliable test to monitor disease activity and certainly they are nod FDA approved.

[Reply] Agreed.Technically, we are lacking valid objective tools for measuring dietary adherence and disease activity of celiac patients, which is a common issue in celiac studies. Since celiac-specific antibodies are directly involved in the pathogenesis of the disease and tend to normalize on a long-term gluten-free diet, tTG seemed the most suitable for monitoring the activity of the immune response. Quoting Ludvigsson et al.: '…well validated IgA anti-TG2 and anti-DGP tests will be important assets to clinical studies by helping to monitor CD activity…'

Gut. 2018 Aug;67(8):1410-1424. doi: 10.1136/gutjnl-2017-314853. Epub 2018 Feb 13. Outcome measures in coeliac disease trials: the Tampere recommendations. Ludvigsson JF1,2, Ciacci C3, Green PH4, Kaukinen K5,6, Korponay-Szabo IR7,8, Kurppa K5,9, Murray JA10, Lundin KEA11,12, Maki MJ13,14, Popp A15,16, Reilly NR17,18, Rodriguez-Herrera A19, Sanders DS20, Schuppan D21,22, Sleet S23, Taavela J16, Voorhees K24, Walker MM25, Leffler DA26

Line 282- I would also add folic acid levels, please see the following article: S. Saibeni, A. Lecchi, G. Meucci et al., "Prevalence of hyperhomocysteinemia in adult gluten-sensitive enteropathy at diagnosis: Role of B12, folate, and genetics," Clinical Gastroenterology and Hepatology, vol. 3, no. 6, pp. 574–580, 2005.

[Reply] We strongly agree with the reviewers' point that the measurement of folic acid would be desirable in the study. Therefore, we added folic acid to the laboratory panel (Pg 10, Ln 263). However, it is important to highlight that serum folic acid is not a sensitive indicator of the long-term folic acid status. The most reliable way of the assessment would be the measurement of intracellular folic acid concentrations from the red blood cells. Unfortunately, this measurement is not available in our clinical laboratory.

Line 286- could authors include TAFI measurements? I don't think it is essential but would add to the strength of the study. Please see : N. H. van Tilburg, F. R. Rosendaal, and R. M. Bertina, "Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis," Blood, vol. 95, no. 9, pp. 2855–2859, 2000.

[Reply] Unfortunately, TAFI measurements are not available in our center; therefore, we would not prefer to measure. If you insist on measuring TAFI, we attempt to establish a collaboration with another laboratory.

Line 311- I would add secondary outcomes also to be folic acid and homocysteine levels

[Reply] We added homocysteine and folic acid to the secondary outcomes (Pg 11, Ln 296).

Lines 316-316. It would be worthwhile of considering to divide patients with IBD to the UC group and CD group. While both are associated with hypercoagulability those are distinct disorders and variations in hypercoagulability do exist.

[Reply] Agree. We divided IBD patients into UC and CD groups (Pg 13, Ln 351)

References 22-24 are little outdated, they can be kept in my opinion but new ones need to be added

[Reply] We added more recent references describing prothrombotic factors in celiac disease. (e.g., Ref No 15, 16, 25, 30)

[Authors' comment] We would like to thank Reviewer: 3 for the excellent comments, which have significantly improved the quality of our manuscript.

VERSION 2 – REVIEW

REVIEWER	Dr Prajwal Gyawali The University of Newcastle
REVIEW RETURNED	25-Jan-2019

GENERAL COMMENTS	The authors have addressed all the previous concern that I had with this protocol paper. This version is better and good for publication.

REVIEWER	Igor Dumic Mount Sinai School of Medicine, New York City, NY, USA
REVIEW RETURNED	21-Dec-2018

GENERAL COMMENTS	The authors have successfully answered all questions. I recommend to publish unaltered.