Novelties on non-invasive biomarkers for the assessment of intestinal permeability and gut barrier integrity in patients with inflammatory bowel diseases

Novelties on non-invasive biomarkers for the assessment of intestinal permeability and gut barrier integrity in patients with inflammatory bowel diseases

Gian P. CAVIGLIA [1] *, Davide G. RIBALDONE [1, 2], Sharmila FAGOONEE [3]

[1]Department of Medical Sciences, University of Turin, Turin, Italy; [2]Unit of Gastroenterology, Molinette Hospital, Città della Salute e della Scienza, Turin, Italy; [3]Institute of Biostructure and Bioimaging, National Research Council, Molecular Biotechnology Center (CNR), Turin, Italy

*Corresponding author: Gian P. Caviglia, Department of Medical Sciences, University of Turin, 10100 Turin, Italy. E-mail: gianpaolo.caviglia@unito.it

Inflammatory bowel diseases (IBD) are chronic gastrointestinal disorders consisting in two main phenotypes, ulcerative colitis (UC) and Crohn's disease (CD), both characterized by an immune-mediated pathogenesis and a clinical relapsing and remitting course.[1] The exact etiology of IBD is unknown, but it is supposed that impaired intestinal permeability, together with genetic, microbial, immunological, and environmental factors contribute to their onset.[2, 3] Consistently, it has been reported that an increase in small intestinal permeability preceded colitis development in the interleukin-10 gene-deficient (IL-$10^{-/-}$) mouse.[4] Furthermore, in the clinical setting, altered gut barrier integrity was already recognizable in individuals who later developed CD,[5] and still detectable in ongoing symptomatic IBD patients who achieved mucosal healing,[6] or in those with moderate to severe disease activity that achieved clinical remission following biologic therapy.[6, 7] Taken together, these observations are consistent with the hypothesis that increased intestinal permeability may be a necessary but not sufficient condition for the development of IBD and that the restoration of gut barrier function may be a late event in the improvement of the disease. In this scenario, a crucial aspect is represented by the methods available for the assessment of intestinal barrier integrity and for the measurement of intestinal permeability. Functional tests, such as ^{51}Cr-Ethylenediaminetetraacetic acid (EDTA) absorption test, Ussing chambers, and confocal laser endomicroscopy, have demonstrated good accuracy, but their invasiveness and some safety issues limit their use in humans.[8] Sugar absorption test (*i.e.*, lactulose/mannitol test) has been proposed as non-radioactive alternative to ^{51}Cr-EDTA test; however, both lactulose and mannitol can be affected by gastrointestinal dilution, motility, bacterial degradation, and renal function, thus rising some concerns on the specificity of the test.[9] To date, the identification of novel non-invasive and reliable tests for the assessment of intestinal permeability is an unmet need. Promising results have been reported for the measurement of tight junction structural proteins and modulators. Above all, zonulin, the human analogue of the zonulae occludens toxin by *Vibrio colerae*, has been widely studied in the last 2 decades in different clinical settings as non-invasive biomarker of intestinal permeability.[10] Zonulin acts as local modulator of intestinal tight junctions, regulating antigens passage through the paracellular pathway of

intestinal epithelial.[10] We previously observed that patients with IBD had higher serum zonulin concentration in comparison to healthy subjects, showing an area under the curve of 0.98 for IBD detection; interestingly, no correlation was observed between serum and fecal zonulin.[11] Recently, Szymanska *et al.* observed that fecal zonulin values were higher in pediatric IBD patients compared to control group (CG), reporting a significant correlation between fecal zonulin and fecal calprotectin, particularly in CD patients (r=0.73); however, no significant difference was observed in serum zonulin concentration between children with IBD and CG.[12] Finally, another study that investigated zonulin in IBD patients and its relation to the disease localization, behaviour, and smoking status, reported that both fecal and serum zonulin levels were elevated in patients with active CD but not in those with UC.[13] Taken together, these results may raise the question whether zonulin should be measured in serum or feces. Possibly, the answer could be partially retrieved by the study of Wegh *et al.* investigating which biological sample among serum and feces was most relevant to assess intestinal permeability in UC patients in clinical remission compared to 5-sugars absorption test. In agreement with our findings, the authors observed no correlation between serum and fecal zonulin; more interestingly, the urinary sucrose excretion was significantly correlated with serum zonulin (r=0.62) and fecal calprotectin (r=0.55), but not with zonulin measured in feces.[14] Nevertheless, caution should be used in interpreting these results since sugar absorption test is reliable for the assessment of small intestinal permeability rather than whole intestine. Apart from zonulin, promising results have been reported for a broad spectrum of biomarkers reflecting intestinal epithelial integrity and bacterial translocation.[15] The former includes claudins and occludins that constitute the extracellular structural proteins of tight junctions, plasma citrulline, an amino acid produced by small intestine enterocytes which has been proposed as a marker of functional enterocyte mass, and intestinal fatty acid binding protein (I-FABP), a protein involved in the transport of fatty acids from the apical membrane of the enterocyte to the endoplasmic reticulum, that has been reported to reflect the turnover rate of enterocytes. The latter includes indirect markers of impaired intestinal barrier function, such as the measurement of circulating bacterial endotoxins (lipopolysaccharides, LPS; limulus amebocyte lysate, LAL assay) and related antibodies (anti-LPS; endogenous endotoxin-core antibody, endoCAb), and fecal butyrate concentration, a short chain fatty acid able to decrease bacterial translocation and modify tight junction proteins expression in favor of a barrier preservation. Overall, currently available results are preliminary and need to be further investigated. Each of the above-mentioned biomarkers owns specific limitations; possibly, the combination of several biomarkers reflecting different structural and functional aspects of intestinal barrier integrity could be useful to define a novel non-invasive tool able to reliably detect and measure intestinal permeability not only in patients with IBD but also in different clinical conditions where impaired intestinal permeability represents a valuable new target for disease prevention and therapy.

References

1. Bertani L, Caviglia GP, Antonioli L, Pellicano R, Fagoonee S, Astegiano M, *et al.* Serum Interleukin-6 and -8 as Predictors of Response to Vedolizumab in Inflammatory Bowel Diseases. J Clin Med 2020;9:1323. PubMed https://doi.org/10.3390/jcm9051323

2. Ribaldone DG, Pellicano R, Actis GC. The gut and the inflammatory bowel diseases inside-out: extra-intestinal manifestations. Minerva Gastroenterol Dietol 2019;65:309–18. PubMed https://doi.org/10.23736/S1121-421X.19.02577-7

3. Caviglia GP, Rosso C, Ribaldone DG, *et al.* Physiopathology of intestinal barrier and the role of zonulin. Minerva Biotecnol 2019;31:83–92. https://doi.org/10.23736/S1120-4826.19.02554-0

4. Arrieta MC, Madsen K, Doyle J, Meddings J. Reducing small intestinal permeability attenuates colitis in the IL10 gene-deficient mouse. Gut 2009;58:41–8. PubMed https://doi.org/10.1136/gut.2008.150888

5. Turpin W, Lee SH, Raygoza Garay JA, Madsen KL, Meddings JB, Bedrani L, *et al.*; Crohn's and Colitis Canada Genetic Environmental Microbial Project Research Consortium; CCC GEM Project recruitment site directors include Maria Abreu. Increased Intestinal Permeability Is Associated With Later Development of Crohn's Disease. Gastroenterology 2020;159:2092–2100.e5. PubMed https://doi.org/10.1053/j.gastro.2020.08.005

6. Chang J, Leong RW, Wasinger VC, Ip M, Yang M, Phan TG. Impaired intestinal permeability contributes to ongoing bowel symptoms in patients with inflammatory bowel disease and mucosal healing. Gastroenterology 2017;153:723–731.e1. PubMed https://doi.org/10.1053/j.gastro.2017.05.056

7. Caviglia GP, Rosso C, Stalla F, Rizzo M, Massano A, Abate ML, *et al.* On-Treatment Decrease of Serum Interleukin-6 as a Predictor of Clinical Response to Biologic Therapy in Patients with Inflammatory Bowel Diseases. J Clin Med 2020;9:800. PubMed https://doi.org/10.3390/jcm9030800

8. Marlicz W, Skonieczna-Żydecka K, Dabos KJ, Łoniewski I, Koulaouzidis A. Emerging concepts in non-invasive monitoring of Crohn's disease. Therap Adv Gastroenterol 2018;11:1756284818769076. PubMed https://doi.org/10.1177/175628481

9. Michielan A, D'Incà R. Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. Mediators Inflamm 2015;2015:628157. PubMed https://doi.org/10.1155/2015/628157

10. Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. Tissue Barriers 2016;4:e1251384. PubMed https://doi.org/10.1080/21688370.2016.1251384

11. Caviglia GP, Dughera F, Ribaldone DG, Rosso C, Abate ML, Pellicano R, *et al.* Serum zonulin in patients with inflammatory bowel disease: a pilot study. Minerva Med 2019;110:95–100. PubMed https://doi.org/10.23736/S0026-4806.18.05787-7

12. Szymanska E, Bierla J, Dadalski M, Wierzbicka A, Konopka E, Cukrowska B, *et al.* New non-invasive biomarkers of intestinal inflammation and increased intestinal permeability in pediatric inflammatory bowel diseases and their correlation with fecal calprotectin: a pilot study. Minerva Gastroenterol 2022. [Epub ahead of print]. PubMed https://doi.org/10.23736/S2724-5985.22.03156-4

13. Malíčková K, Francová I, Lukáš M, Kolář M, Králíková E, Bortlík M, *et al.* Fecal zonulin is elevated in Crohn's disease and in cigarette smokers. Pract Lab Med 2017;9:39–44. PubMed https://doi.org/10.1016/j.plabm.2017.09.001

14. Wegh CA, de Roos NM, Hovenier R, Meijerink J, Besseling-van der Vaart I, van Hemert S, *et al.* Intestinal Permeability Measured by Urinary Sucrose Excretion Correlates with Serum Zonulin and Faecal Calprotectin Concentrations in UC Patients in Remission. J Nutr Metab 2019;2019:2472754. PubMed https://doi.org/10.1155/2019/2472754

15. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, *et al.* Intestinal permeability—a new target for disease prevention and therapy. BMC Gastroenterol 2014;14:189. PubMed https://doi.org/10.1186/s12876-014-0189-7

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions.—Gian P. Caviglia, Davide G. Ribaldone, and Sharmila Fagoonee have given substantial contributions to the study conception, methodology and to data collection; Gian P. Caviglia contributed to the manuscript draft; Davide G. Ribaldone and Sharmila Fagoonee contributed to the manuscript revision and editing. All authors read and approved the final version of the manuscript.

History.—Manuscript accepted: May 16, 2022. - Manuscript received: May 11, 2022.

(*Cite this article as*: Caviglia GP, Ribaldone DG, Fagoonee S. Novelties on non-invasive biomarkers for the assessment of intestinal permeability and gut barrier integrity in patients with inflammatory bowel diseases. Minerva Gastroenterol 2023;69:1-3. **DOI: 10.23736/S2724-5985.22.03215-6**)