Claudin-2 simplifies histological assessment of activity/remission of ulcerative colitis in real-life daily practice

Gabrio Bassotti[a], Rachele Del Sordo[b], Francesco Lanzarotto[c], Sara Mino[d], Chiara Ricci[c] and Vincenzo Villanacci[d]

Objectives Mucosal healing, and in particular histological mucosal healing, represents the new frontier as a treatment goal for inflammatory bowel diseases. However, the assessment of mucosal healing is presently somewhat limited by the numerous pathological scores available, and the lack of a global consensus on how to best assess it. For this reason, the availability of a simple and rapid test to evaluate the inflammatory state of the mucosa after treatment would be useful, especially for the daily routine.

Methods To exploit the above purpose, we evaluated the possible usefulness of antibodies against claudin-2, a protein of intestinal epithelium tight junctions, as a possible test to assess the presence of activity in ulcerative colitis following treatment. Biopsies from 28 patients with distal localization of the disease and clinical and endoscopic remission were tested for claudin-2 reactivity.

Results Claudin-2 reactivity was always negative in noninvolved segments and displayed a variable staining intensity in concordance with the histological activity. There was a highly significant ($P < 0.0001$) correlation between histological score and claudin-2 expression in the colonic segments involved (descending, sigmoid colon, and rectum).

Conclusions Our results suggest that the use of claudin-2 in the routine daily practice could simplify and corroborate the results of current histological evaluations, especially in clinical practice and posttreatment follow-up. Eur J Gastroenterol Hepatol 37: 409–413

Introduction

At present, one of the main therapeutic goals to reach in the treatment of ulcerative colitis is represented by the so-called mucosal healing [1]. This is a term initially used to evaluate endoscopic appearance after treatment [2], but more recently it was implemented to assess the actual reaching of histological remission [3,4]. Although, at least for research trials, there is some agreement attempt [5,6], the definition of histological mucosal healing in ulcerative colitis patients is still under debate [7]. The matter is further complicated and limited by the fact that the literature concerning this topic features varying definitions and multiple histologic indices, at present more than 30 for ulcerative colitis [4,8].

European Journal of Gastroenterology & Hepatology 2025, 37:409–413

Keywords: claudin-2, histology, mucosal healing, remission, ulcerative colitis

[a]Gastroenterology and Hepatology Section, [b]Anatomic Pathology and Histology Section, Department of Medicine and Surgery, University of Perugia, Perugia, [c]Gastroenterology Unit, Department of Clinical and Experimental Sciences, University of Brescia and [d]Department of Pathology, Institute of Pathology, ASST Spedali Civili and University of Brescia, Brescia, Italy

Correspondence to Gabrio Bassotti, MD, PhD, Clinica di Gastroenterologia ed Epatologia, Ospedale 'Santa Maria della Misericordia', Piazzale Menghini, 1, 06156 San Sisto, Perugia, Italy
Tel: +39 0755784434; e-mail: gabassot@gmail.com

Received 11 September 2024 **Accepted** 11 January 2025.

Thus, there is the obvious need of a scoring system that should be both simple and easy to use globally [9], especially in the daily clinical practice that represents the bulk of diagnostic approaches. This has also an important clinical meaning, since it has been shown that persistent histological activity in ulcerative colitis patients is associated with a greater likelihood of relapsing [10]. We have recently proposed a simplified histological score for inflammatory bowel diseases (IBDs) for an easy, fast assessment of the routine biopsies [11]. The performance of this score subsequently showed that it might simplify the routine pathologists' workload while yielding an accurate reporting of the pathological features [12].

However, even using a simplified score there is still a discrete amount of subjectivity and time needed in evaluating the pathological slides. For this reason, a reliable and simple marker of disease activity would be welcome to facilitate such assessment in the daily practice. Thus, to this purpose, we recently become interested in the assessment of claudin-2, a transmembrane protein of the intestinal tight junctions, involved in the regulation of the intestinal epithelial barrier [13]. Claudin-2 is not detected in the normal human colon, but is highly expressed in IBDs [14], with its expression positively correlated with disease activity [15].

The aim of the present study was to evaluate claudin-2 expression in ulcerative colitis patients in clinical and endoscopic remission, to assess the concordance with histological activity and to determine whether it

could represent a simple marker to detect histological remission/activity in the daily routine.

Patients and methods

Biopsy samples were obtained from 28 ulcerative colitis patients [18 men, 10 women, median age 51 years (95% confidence interval (CI): 44–55)], with left-side involvement (E2 of the Montreal classification) [16] and in clinical and endoscopic remission after treatment. Five of these patients participated in another study on claudin-2 in IBDs [17]. All patients were in clinical remission (partial Mayo score <3 with no individual subscore >1) and endoscopic remission [assessed by a Mayo Endoscopic Score (MES) of 1 or less].

Biopsies were obtained according to European Crohn's and Colitis Organization guidelines [18], with at least two specimens collected from each colonic segment (cecum, ascending, transverse, descending, sigmoid, rectum). The samples were correctly oriented on acetate cellulose filters, fixed in formalin, and embedded in paraffin. Sections stained with hematoxylin and eosin were firstly evaluated.

Histological activity was defined as: quiescent, when the biopsies of the different segments involved by the diseases showed signs of chronic disease (crypt distortion, basal plasmacytosis) without presence of neutrophils (score 0); mild (neutrophils present in the lamina propria and in the crypt epithelium, cryptitis, score 1); moderate (neutrophils present in crypt lumina, crypt abscesses, score 2); severe (presence of erosions/ulcerations, score 3).

Claudin immunohistochemistry (Claudin-2 antibody, Cell Signaling Technology, Danvers, Massachussets, USA, clone E1H90, dilution 1 : 100) was performed on 4 µm sections. The primary antibody was detected by means of a biotin-free polymeric-horseradish peroxidase-linker antibody conjugate system (Bond Polymer Refine Detection, Leica Biosystems, Newcastle upon Tyne, UK) with heat-induced epitope retrieval, using the Bond III automated immunostainer (Leica Biosystems, Melbourne, Australia).

We considered claudin-2 expressed when we observed staining with variable intensity along the cytoplasmatic cell membranes of glandular and of surface epithelium. The stain intensity was semi-quantified as follows [17]: 0, absence of immunostaining in glandular epithelium; 1, immunostaining of <10% epithelial cells in the glands; 2, immunostaining of at least 50% epithelial cells in the glands; 3, immunostaining of more than 90% epithelial cells in the glands (Fig. 1b, d and f; Fig. 2b and d).

Using these criteria we have previously reported that the sensitivity of claudin-2 to detect active disease was 100% and the sensitivity was 91%, with a positive predictive value of 98% and a negative predictive value of 100%. The inter-rater agreement ranged from good to very good for all colonic segments [17].

Statistical analysis

Since the data were not normally distributed, nonparametric tests were used for statistical analysis. In the descending, the sigmoid colon, and the rectum, the correlation between histological score and claudin-2 expression, between MES and histological score, and between MES and claudin-2 expression was assessed by means of the Kendall's rank correlation coefficient [19]. Values of $P < 0.05$ were chosen for rejection of the null hypothesis.

Ethical considerations

The study protocol was approved by the Ethical Committee of Spedali Civili di Brescia (no. 5923, v.3 of 24/05/2023) and carried out according to the principles of Declaration of Helsinki (1964, updated in 2013).

Results

All patients had left-side (E2) disease. Median MES was 1 (95% CI: 0–1). Expression of claudin-2 was detected on the lateral cell membranes and tight junctions in the surface epithelium, with no expression in the nuclei of epithelial and inflammatory cells. Claudin-2 was not expressed in noninvolved colonic segments, and showed variable staining intensity only in those areas with active disease (Figs. 1 and 2). The correlation between histological score and claudin-2 expression resulted highly significant for the descending (tau = 0.894, $P < 0.0001$), the sigmoid colon (tau = 1.0, $P < 0.0001$), and the rectum (tau = 0.94, $P < 0.0001$). There was also a significant correlation between the MES and histological score in the sigmoid colon and the rectum (for both segments, tau = 0.48, $P = 0.0004$) but not in the descending colon (tau = 0.16, $P = 0.25$). Concerning the correlation between the MES and claudin-2 expression, this was again significant in the sigmoid colon (tau = 0.40, $P = 0.0004$) and the rectum (tau = 0.52, $P = 0.0001$), but not in the descending colon (tau = 0.203, $P = 0.14$).

Overall, the time needed to evaluate claudin-2 expression on histological slides was about 30 s.

Discussion

The need of having available a simple test for the daily routine evaluation of mucosal healing in ulcerative colitis patients following treatment prompted us to investigate whether such test might be somewhat represented by the use of claudin-2 staining in biopsy samples of these patients, to assess its effectiveness and confirm the remission of colonic inflammation. We selected to test claudin-2 since there was previous evidence of its high expression in active IBD patients [14], expression positively correlated with the inflammation degree [15]. Moreover, in a previous experience we have demonstrated that claudin-2 expression is highly specific (does not feature in other inflammatory colitides), sensitive, and displays almost no interobserver variability in the evaluation of IBD patients [17]. These features would thus represent an ideal marker to test the residual activity/mucosal healing in these patents.

In the present study, we wanted to investigate the possible usefulness of adding claudin-2 immunohistochemistry to the routine evaluation of biopsies of distal ulcerative colitis patients in clinical/endoscopic remission. The decision to evaluate distal ulcerative colitis patients was due to the fact that this group represented a quite homogeneous one from a clinical/endoscopic point of view, and that usually ulcerative colitis pathological findings are

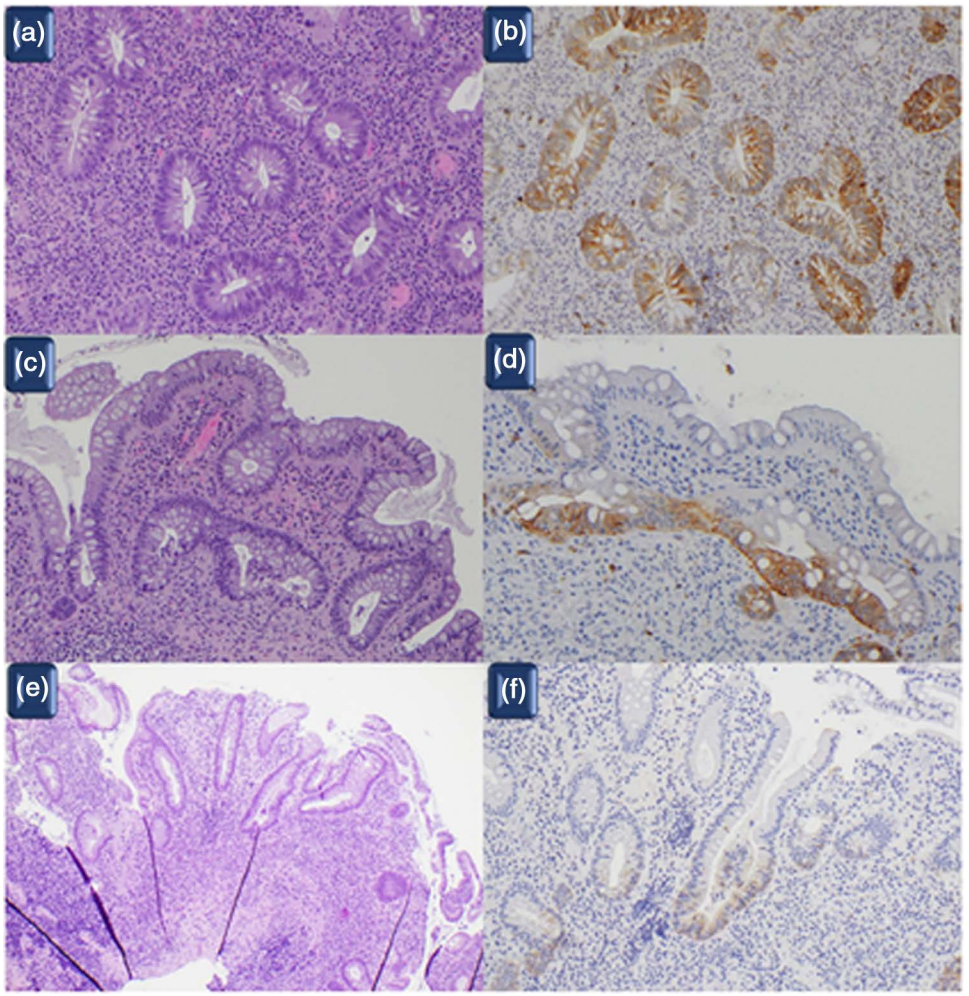

Fig. 1. Representative images of UC patients with different histological severity and claudin-2 expression. (a) Severe activity, H&E ×20. (b) The same area with a diffuse positivity (score 3) for claudin-2, ×20. (c) Moderate activity, H&E ×10. (d) The same area with a moderate positivity (score 2) for claudin-2, ×20. (e) Low activity, H&E ×4. (f) The same area with focal positivity (score 1) for claudin-2, ×20. Note: (a) and (b) are shown only for comparison, and do not belong to patients investigated in this study. H&E, hematoxylin and eosin; UC, ulcerative colitis.

less variable than those found in Crohn's disease [20]. Moreover, patients in clinical/endoscopic remission are those more likely to have minimal histological activity that might be missed by inexperienced pathologists.

Analysis of the results showed that claudin-2 expression displayed a highly significant correlation with the grade of histological activity, being on the other hand invariably negative in non-affected areas. We found that the use of claudin-2, requiring on average 30 s by the pathologist to assess its expression, did not delay the routine activity. Conversely, its use resulted in an easier evaluation of the areas involved by active inflammatory processes, without (or at least reducing) the need for more complex scores, therefore speeding the diagnostic process. In fact, as previously shown, experienced dedicated pathologists need on average 5.5 min to score IBD biopsies with the Geboes/Nancy systems (the more frequently adopted for clinical trials), whereas other scores such as the Robarts need at least 4 min, and the Extent Chronicity Activity Plus 7.5 min, and a simplified score needs 2 min of assessment [11]. Of course, these times are proportionately longer for moderately/scarcely experienced or general pathologists. Thus, the availability of a simple, reproducible, and easily interpretable test (hopefully coupled with a simplified scoring system) even in less experienced hands could represent a bonus for the daily routine assessment of ulcerative colitis activity following treatment in high-volume centers, and minimized the likelihood of missing small or focal areas of residual inflammatory activity. Of course, it remains to be established whether this approach could be applied for research trials.

In conclusion, we feel that the routine use of claudin-2 staining could represent a useful adjunct in the interpretation of the inflammatory state in patients with ulcerative colitis after treatment. Should these findings be replicated in more and larger studies, since the pathologists' work represents an essential element in the multidisciplinary approach to IBD [21], this test could contribute to a better approach to the goal of reaching a truly complete mucosal healing in such condition [22].

Acknowledgements

G.B. contributed in the conceptualization (equal), data curation (equal), formal analysis (equal), methodology (equal), writing – original draft (lead), and review and

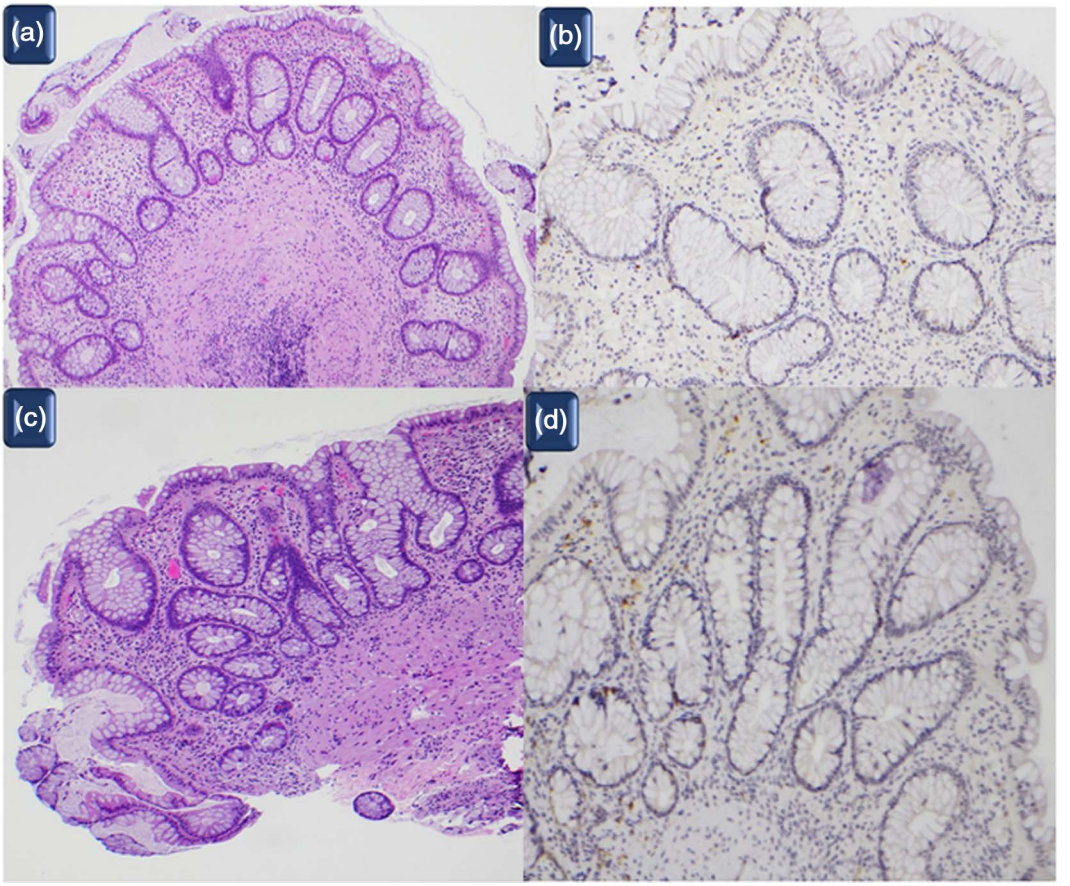

Fig. 2. (a and c) Representative images of UC patients with histological quiescence. H&E, ×10. (b and d) Negative expression (score 0) of claudin-2, ×20. H&E, hematoxylin and eosin; UC, ulcerative colitis.

editing. R.D.S., F.L., S.M., and C.R. contributed in the investigation (equal) and review and editing. V.V. contributed in the conceptualization (equal), investigation (equal), data curation (equal), formal analysis (equal), methodology (equal), and review and editing. All authors read and approved the final version of the manuscript.

The data underlying this article are available from the corresponding author on reasonable request.

Conflicts of interest

There are no conflicts of interest.

References

1 Akiyama S, Miyatani Y, Rubin DT. The evolving understanding of histology as an endpoint in ulcerative colitis. *Intest Res* 2024; 22:389–396.
2 Mazzuoli S, Guglielmi FW, Antonelli E, Salemme M, Bassotti G, Villanacci V. Definition and evaluation of mucosal healing in clinical practice. *Dig Liver Dis* 2013; 45:969–977.
3 Neurath MF, Vieth M. Different levels of healing in inflammatory bowel diseases: mucosal, histological, transmural, barrier and complete healing. *Gut* 2023; 72:2164–2183.
4 Pai RK, D'Haens G, Kobayashi T, Sands BE, Travis S, Jairath V, *et al*. Histologic assessments in ulcerative colitis: the evidence behind a new endpoint in clinical trials. *Expert Rev Gastroenterol Hepatol* 2024; 18:73–87.
5 Magro F, Doherty G, Peyrin-Biroulet L, Svrcek M, Borralho P, Walsh A, *et al*. ECCO Position Paper: harmonization of the approach to ulcerative colitis histopathology. *J Crohns Colitis* 2020; 14: 1503–1511.
6 Ma C, Sedano R, Almradi A, Vande Casteele N, Parker CE, Guizzetti L, *et al*. An international consensus to standardize integration of histopathology in ulcerative colitis clinical trials. *Gastroenterology* 2021; 160:2291–2302.
7 Villanacci V, Del Sordo R, Parigi TL, Leoncini G, Bassotti G. Inflammatory bowel diseases: does one histological score fit all? *Diagnostics (Basel)* 2023; 13:2112.
8 Mosli MH, Parker CE, Nelson SA, Baker KA, MacDonald JK, Zou GY, *et al*. Histologic scoring indices for evaluation of disease activity in ulcerative colitis. *Cochrane Database Syst Rev* 2017; 5:CD011256.
9 Lang-Schwarz C, Agaimy A, Atreya R, Becker C, Danese S, Fléjou JF, *et al*. Maximizing the diagnostic information from biopsies in chronic inflammatory bowel diseases: recommendations from the Erlangen International Consensus Conference on Inflammatory Bowel Diseases and presentation of the IBD-DCA score as a proposal for a new index for histologic activity assessment in ulcerative colitis and Crohn's disease. *Virchows Arch* 2021; 478:581–594.
10 Gupta A, Yu A, Peyrin-Biroulet L, Ananthakrishnan AN. Treat to target: the role of histologic healing in inflammatory bowel diseases: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2021; 19:1800–1813.e4.
11 Villanacci V, Antonelli E, Lanzarotto F, Bozzola A, Cadei M, Bassotti G. Usefulness of different pathological scores to assess healing of the mucosa in inflammatory bowel diseases: a real life study. *Sci Rep* 2017; 7:6839.
12 Caputo A, Parente P, Cadei M, Fassan M, Rispo A, Leoncini G, *et al*. Simplified Histologic Mucosal Healing Scheme (SHMHS) for inflammatory bowel disease: a nationwide multicenter study of performance and applicability. *Tech Coloproctol* 2022; 26:713–723.
13 Rosenthal R, Milatz S, Krug SM, Oelrich B, Schulzke JD, Amasheh S, *et al*. Claudin-2, a component of the tight junction, forms a paracellular water channel. *J Cell Sci* 2010; 123:1913–1921.
14 Prasad S, Mingrino R, Kaukinen K, Hayes KL, Powell RM, MacDonald TT, Collins JE. Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Lab Invest* 2005; 85:1139–1162.
15 Weber CR, Nalle SC, Tretiakova M, Rubin DT, Turner JR. Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and

may contribute to early neoplastic transformation. *Lab Invest* 2008; 88:1110–1120.
16 Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, *et al*. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; 19(Suppl A):5A–36A.
17 Villanacci V, Del Sordo R, Lanzarotto F, Ricci C, Sidoni A, Manenti S, *et al*. Claudin-2: a marker for a better evaluation of histological mucosal healing in inflammatory bowel diseases. *Dig Liver Dis* 2025. doi: 10.1016/j.dld.2024.08.001. Online ahead of print.
18 Adamina M, Feakins R, Iacucci M, Spinelli A, Cannatelli R, D'Hoore A, *et al*. ECCO topical review optimising reporting in surgery, endoscopy, and histopathology. *J Crohns Colitis* 2021; 15: 1089–1105.
19 Kendall MA. New measure of rank correlation. *Biometrika* 1938; 30:81–89.
20 Yantiss RK, Odze RD. Diagnostic difficulties in inflammatory bowel disease pathology. *Histopathology* 2006; 48:116–132.
21 Kim SH, Buhle A, Roberts A, Singh N, Mir A, Kesar V, *et al*. Multidisciplinary inflammatory bowel disease conference: the impact of the expert pathologist on patient care. *Inflamm Bowel Dis* 2024; 30:1482–1491.
22 Villanacci V, Del Sordo R, Mino S, Locci G, Bassotti G. Histological healing in IBD: ready for prime time? *Dig Liver Dis* 2025:S1590-8658(25)00040-4. doi: 10.1016/j.dld.2025.01.039. Online ahead of print.