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MESSAGE

Barrier healing represents a novel therapeutic target in ulcerative colitis (UC), although its assessment remains challenging and lacks standardisation. This exploratory study evaluates the ability of ultra-high magnification endocytoscopy to guide tissue sampling and drive automated quantification of tight junction (TJ) proteins to assess intestinal barrier integrity and predict major adverse outcomes (MAOs). 34 UC patients in clinical remission prospectively underwent assessment with endocytoscopy and machine learning-enabled intestinal barrier protein evaluation. The combination of endocytoscopy with Claudin-2 expression showed promise in accurately predicting MAOs over 12 months. This integrative approach holds promise in identifying deep healing and enhancing treat-to-target strategy in UC.

DETAIL

Barrier healing is attracting fresh attention as a therapeutic target in UC.[1 2] However, its evaluation is subjective and not standardised. It has generally depended on probe permeability with considerable variability, thus highlighting an unmet need for novel tools to accurately and objectively assess deep healing and predict clinical outcomes, including endocytoscopy, histology and intestinal barrier proteins. Endocytoscope (Olympus, Japan) is a commercially available endoscope capable of achieving up to 520-fold magnification, enabling real-time, in vivo assessment of intestinal cellular components and accurately guiding tissue sampling.[3] Furthermore, automated spatial multispectral imaging pathology is promising for precisely and objectively quantifying intestinal barrier proteins.[4]

This exploratory study aims to combine endocytoscope with intestinal barrier proteins assessment through machine learning-enabled multispectral spatial imaging (MSI) (figure 1) to assess the ability of this integrative approach to define deep healing and predict MAOs over a 12-month follow-up.

Patients with an established diagnosis of UC in clinical remission, defined as a partial Mayo score ≤3 without any subscore ≥1 and undergoing surveillance colonoscopy at two tertiary referral centres were prospectively enrolled (online supplemental table 1). In all patients, ascending and descending colon were assessed using high-definition white light endoscopy followed by ultra-high magnification endocytoscope. Our previously developed endocytoscopy score assessed mucosal healing by considering crypt architecture, cell infiltration between crypts, distance between crypts and visibility of superficial microvessels (table 1; online supplemental 2).[3] Endocytoscope was used to guide tissue sampling in both ascending and descending colon. Immunohistochemistry (IHC) followed by multiplex immunofluorescence aided by MSI (Akoya Biosciences, USA) was performed. Three TJ proteins, including Claudin-2, Occludin and the Junctional Adhesion Molecule A (JAM-A), studied as gut permeability regulators,[5] were assessed. A machine learning software (inForm digital platform, V.3.0) was used for objective protein quantification in epithelium and lamina propria (online supplemental 3). Patients were followed up for 12 months after index colonoscopy, and MAOs, including flare-up, hospitalisation, need for colectomy and change of treatment, were recorded. Details of statistical analysis are provided in online supplemental 4.

34 UC patients were included in this exploratory study, of whom 67% (23/34) had concomitant primary sclerosing cholangitis (PSC) (online supplemental table 1). Seven out of 34 patients (20%) had a MAO during the follow-up. An endocytoscopy score >5 significantly correlated with MAOs, showing a Kendall's tau coefficient of 0.35 (p=0.042) (table 2). The Kaplan-Meier curve confirmed an endocytoscopy score >5 to predict a lower MAO-free survival (p<0.001) (figure 2A). Regarding TJ proteins, Claudin-2 and Occludin showed significant epithelial localisation (online supplemental 3), with only Claudin-2 significantly correlating with MAOs. Specifically, we found

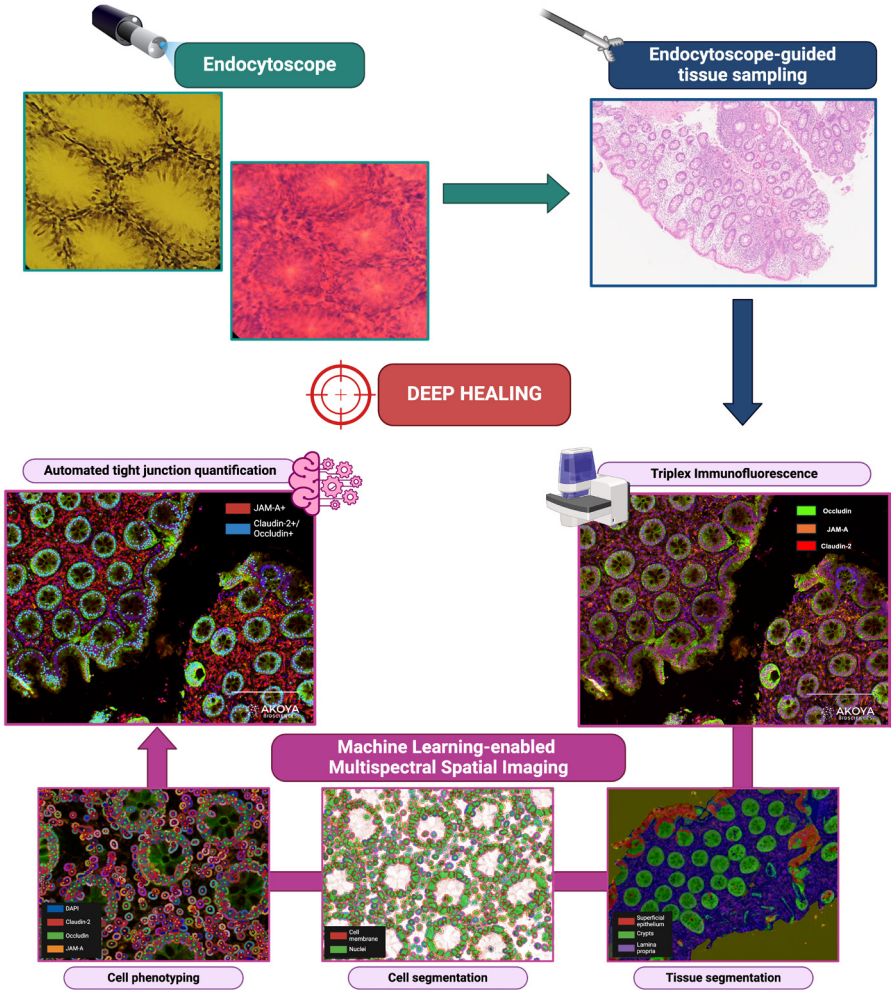

Figure 1 Ultra-high magnification endocytoscopy driving machine learning-enabled multispectral spatial intestinal barrier protein imaging. Created with BioRender.com.

that patients with higher cell density and mean expression of Claudin-2 showed a higher cumulative probability of MAOs (p=0.045 and p=0.041, respectively) (figure 2B,C). Notably, the integrated assessment of endocytoscopy score with Claudin-2 mean expression and cell density showed higher correlations with MAOs compared with endocytoscopy score alone: 0.43 (p=0.014) and 0.51 (p=0.003), respectively (table 2; figure 2D,E).

Table 1 Endocytoscopy scoring system

Endocytoscopy scoring system[3]	
Crypts architecture	
Normal or elongated	1
Irregular	2
Necrosis	3
Infiltration of the cell between the crypts	
≤50%	1
>50%	2
Distance between the crypts	
Normal (≥3 crypts in a VF)	1
Elongated (<2 crypts in a VF)	1
Intermediate (2–3 crypts in a VF with infiltrating cells in LP)	2
Drop-out/necrosis	3
Visibility of superficial microvessels	
Not visible	0
Visible	1
Total score	3–9

LP, lamina propria; VF, visual field.

COMMENTS

We demonstrated an innovative strategy integrating endocytoscopy with a machine learning-enabled automated quantification of intestinal barrier proteins to assess deep healing and accurately predict MAOs in UC patients.

While the current therapeutic targets in UC are endoscopic and histologic remission,[6] advanced endoscopic tools can help

Table 2 Correlations of endocytoscopy score, Claudin-2 cell density and Claudin-2 mean expression with major adverse outcomes

	Kendall's tau coefficient	P value
Endocytoscopy score>5	0.35	0.042
Claudin-2 CD ≥4170.87	0.25	0.153
Claudin-2 ME ≥5.79	0.27	0.121
Endocytoscopy+Claudin-2 CD	0.43	0.014
Endocytoscopy+Claudin-2 ME	0.51	0.003

CD, cell density; ME, mean expression.

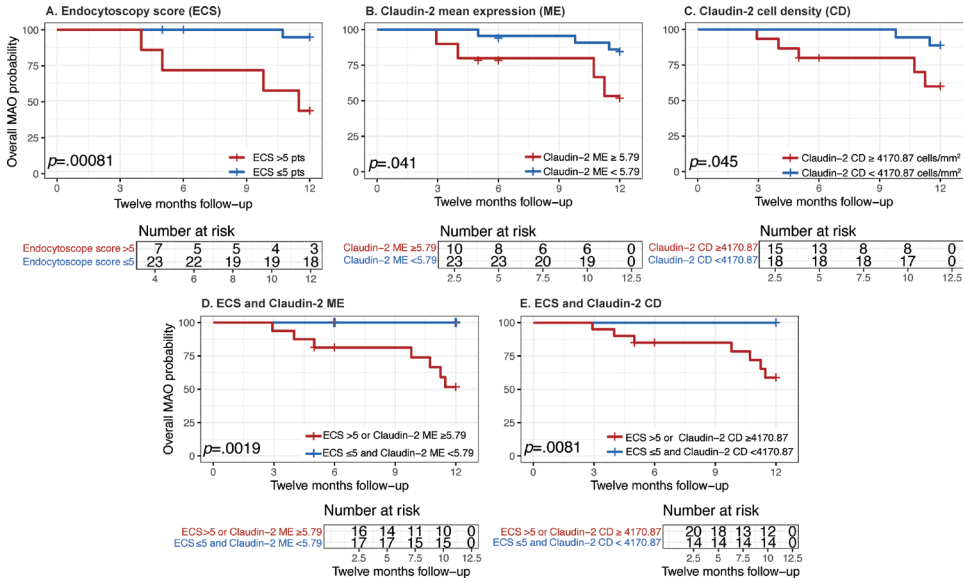

Figure 2 Kaplan-Meier for the cumulative probabilities of being relapse-free according to endocytoscopy score (A), mean expression of Claudin-2 (B), Cell density of Claudin-2 (C) and combination of endocytoscope with mean expression and cell density of Claudin-2 (D) Created with BioRender.com. CD, cell-density; ECS, endocytoscopy score; MAO, major adverse outcome; ME, mean expression.

detect deeper levels of healing and predict potential flare-ups. For instance, probe confocal laser endomicroscopy (pCLE) has demonstrated promise in real-time barrier healing assessment and MAOs prediction, likely superior to endoscopic and histological remission in UC patients.[1] Similarly, we have recently shown that endocytoscope is an innovative tool for mucosal healing assessment and strongly correlates with histological remission.[3] Unlike pCLE, the endocytoscope does not require the intravenous administration of fluorescein dye or expensive laser probes. Also, the endocytoscope includes a 2.8 mm working channel, allowing targeted biopsies at selected sites. We hypothesised that the endocytoscope offers a significant advantage in accurately assessing mucosal healing and patchy inflammation compared with standard endoscopic techniques, thereby enabling the identification of optimal sites for targeted biopsy sampling to assess barrier healing.

Starting from these premises, we first assessed the ability of endocytoscopy to predict MAOs over a 12-month follow-up in a cohort of UC patients in clinical remission. We found that an endocytoscopy score >5 has promise in accurately predict MAOs, consistently with previous studies.[7 8] However, to achieve a deeper and more accurate healing assessment capable of precisely forecasting outcomes, we developed a novel integrative model combining the endocytoscope-guided mucosal assessment and tissue sampling with the quantitative automated imaging pathology evaluation of intestinal TJ proteins.

The intestinal barrier assessment mainly relies on evaluating its molecular components using biopsy specimens.[2 9] Specifically, TJs are dynamic multiprotein complexes that interconnect epithelial cells and constitute the primary component of the epithelial intestinal barrier, preventing the translocation of luminal antigens. For this reason, we selected three representative TJs, namely Claudin-2, Occludin and JAM-A, previously associated with barrier impairment in inflammatory bowel disease (IBD).[10–12] This exploratory study did not assess other TJs, including the novel MARVELD3, since we focused on proteins directly involved in barrier function but not adherens proteins. We have previously comprehensively shown that TJs can predict outcomes in UC[13] despite the evaluation being performed with IHC, which is limited by observer subjectivity. Hence, for the first time, we used endocytoscope to guide biopsy site selection, with samples subsequently evaluated through a sophisticated machine learning-enabled automated analysis (inForm) for an objective quantification of TJ. Claudin-2, previously identified as an intestinal inflammatory marker,[10 14] emerged as our study's most specific marker of intestinal barrier integrity, exhibiting a strong correlation with MAOs. Notably, our analysis revealed specific thresholds for cell density (<4170.87 cells/mm^2) and for mean expression (<5.79 normalised count, total weighting) of Claudin-2 associated with a more favourable prognosis. Hence, combining endocytoscopy assessment with Claudin-2 automated quantification offered a standardised and comprehensive deep healing assessment in UC. This approach achieved a significantly strong correlation with MAOs, stronger than endocytoscope alone. Our novel approach holds promise for research and future clinical practice, wherein initial endocytoscope assessment can guide subsequent Claudin-2 quantification for risk stratification and optimal patient management. This approach holds particular relevance for UC patients complicated by PSC, representing the majority of our study population (67%), who face heightened risks of subtle inflammation and barrier damage, with consequential long-term outcomes, including colorectal cancer.[15] In these patients, achieving deeper healing may be paramount, and the combined use of endocytoscope and automated protein assessment offers a novel and promising avenue for tailored therapeutic management.

This exploratory study has some limitations, including the sample size and the absence of healthy controls. Also, we considered only three proteins for epithelial barrier assessment while other molecules could also be relevant. Nonetheless, these selected proteins have demonstrated a strong association with UC in prior research.[5]

To confirm our results, ongoing multicentre prospective studies with larger sample sizes and exploring a wider spectrum of intestinal TJ proteins to enhance deep healing assessment are underway. Nonetheless, our findings pave the way for a novel comprehensive assessment of healing in UC patients, using advanced endoscopy to assess in real-time cellular mucosal

details and guide tissue sampling combined with automated barrier protein evaluation through machine learning models. This integration represents an initial stride towards molecular personalised medicine in IBD.

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Contributors Conceptualisation: MI, SG; data collection, MI, SM, GS, YM, IZ, MP-T, ID, BH, RC, EF, UNS, ZA, RH, TLP, OMN; writing—original draft preparation SM, GS, YM, IZ, MP-T, ID; writing—review and editing MI, SG, SM, GS and YM; supervision: MI, SG, PM and LB. All authors have read and agreed to the published version of the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

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