The learning curve, interobserver, and intraobserver agreement of endoscopic confocal laser endomicroscopy in the assessment of mucosal barrier defects

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Background and Aims: Confocal laser endomicroscopy can dynamically assess intestinal mucosal barrier defects and increased intestinal permeability (IP). These are functional features that do not have corresponding appearance on histopathology. As such, previous pathology training may not be beneficial in learning these dynamic features. This study aims to evaluate the diagnostic accuracy, learning curve, inter- and intraobserver agreement for identifying features of increased IP in experienced and inexperienced analysts and pathologists.

Methods: A total of 180 endoscopic confocal laser endomicroscopy (Pentax EC-3870FK; Pentax, Tokyo, Japan) images of the terminal ileum, subdivided into 6 sets of 30 were evaluated by 6 experienced analysts, 13 inexperienced analysts, and 2 pathologists, after a 30-minute teaching session. Cell-junction enhancement, fluorescein leak, and cell dropout were used to represent increased IP and were either present or absent in each image. For each image, the diagnostic accuracy, confidence, and quality were assessed.

Results: Diagnostic accuracy was significantly higher for experienced analysts compared with inexperienced analysts from the first set (96.7% vs 83.1%, $P < .001$) to the third set (95% vs 89.7, $P = .127$). No differences in accuracy were noted between inexperienced analysts and pathologists. Confidence (odds ratio, 8.71; 95% confidence interval, 5.58-13.57) and good image quality (odds ratio, 1.58; 95% confidence interval, 1.22-2.03) were associated with improved interpretation. Interobserver agreement κ values were high and improved with experience (experienced analysts, 0.83; inexperienced analysts, 0.73; and pathologists, 0.62). Intraobserver agreement was >0.86 for experienced observers.

Conclusion: Features representative of increased IP can be rapidly learned with high inter- and intraobserver agreement. Confidence and image quality were significant predictors of accurate interpretation. Previous pathology training did not have an effect on learning. (Gastrointest Endosc 2016;83:785-91.)

In the normal bowel, mucosal barrier integrity is maintained by a monolayer of epithelial cells held together by tight junctions.[1,2] Under homeostatic conditions, epithelial cells are shed from the tips of the villi in a controlled process, involving reorganization of the tight junctions to maintain integrity of the epithelial barrier.[3] However,

Abbreviations: CDO, cell dropout; CI, confidence interval; CJE, cell-junction enhancement; CLE, confocal laser endomicroscopy; CLS, confocal leak score; eCLE, endoscopic confocal laser endomicroscopy; FL, fluorescein leak; IBD, inflammatory bowel disease; IP, intestinal permeability; OR, odds ratio.

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when pathological cell shedding increases and exceeds the rate of repair, large gaps lead to loss of epithelial integrity and increased intestinal permeability (IP). The resultant exposure of luminal antigens to host immune cells leads to immune activation and inflammatory cell recruitment, which further drive cell shedding.[4] Previous studies have suggested that impaired epithelial barrier function is present in both inflammatory bowel disease (IBD) and irritable bowel syndrome, with possible pathoetiological implications of disease.[2,5-7] Indirect assays for measuring IP have quantified urinary excretion of chromium ethylenediamine tetraacetic acid or saccharide probes such as sucrose, lactulose, and mannitol after ingestion.[8] These methods have illustrated that both Crohn's disease and ulcerative colitis have higher IP.[9] Although simple and noninvasive, these tests have the limitations of low sensitivity and specificity, without the capacity to discriminate between inflamed and noninflamed tissues. Furthermore, inaccuracies could occur due to confounding factors of differential rates of bacterial degradation, intestinal transit time, and renal excretion.[10]

Endoscope-based confocal laser endomicroscopy (eCLE) integrates a confocal laser microscope within the tip of a standard flexible endoscope, allowing simultaneous macroscopic and microscopic assessment of GI mucosa. It uses a blue laser emitter to scan focal planes of target mucosa at 1000-fold magnification at varying depths of up to 250 μm to allow real-time in vivo histology assessments.[11,12] When this is used to obtain virtual biopsy specimens, CLE provides images equivalent to histological assessment in Barrett's esophagus, colorectal cancer, and celiac disease.[13-15] Learning characteristics and observer reliability of CLE in assessing varying diseases have been previously reported.[20-24] Although the learning characteristics of static images are established, no previous study has investigated the learning curve and inter- and intraobserver reliability for assessing eCLE features of a dynamic process such as increased IP. Also, the extent to which an analyst's experience and confidence may influence diagnostic accuracy and reliability remains unclear. Pathologists who are trained in the recognition of static histopathology may not have any advantage in the learning of the dynamic features of increased IP. Dynamic features representative of increased IP include cell-junction enhancement (CJE), fluorescein leak (FL) and cell dropout (CDO), but they cannot be visualized on conventional histopathology.[16]

The primary aim of this study was to evaluate the diagnostic accuracy, learning curve, inter- and intraobserver agreement for correctly identifying eCLE images of increased IP between analysts with and without previous training and pathologists. The secondary aim was to examine the impact of analysts' experience, image quality, and analysts' confidence as predictors of diagnostic accuracy.

METHODS

Data collection

Previously, 101 patients (80 patients with IBD, 21 control patients) prospectively recruited from the IBD Service of Bankstown-Lidcombe Hospital, Sydney, Australia, underwent eCLE (Pentax EC-3870FK; Pentax, Tokyo, Japan) with 5 mL of intravenous 10% fluorescein sodium given in increments. Diagnosis of IBD was made according to clinical, radiological, and histological findings. Patients were invited to participate in the study if they were older than 18 years of age, able to provide informed consent, and required a colonoscopy for clinical indications. Predetermined exclusion criteria were pregnancy, kidney disease, or known allergies to fluorescein. All eCLE images were taken from macroscopically normal tissue from the terminal ileum confirmed with paired histology yielding a total of 45,882 deidentified images. These were stored in a digital database at Bankstown-Lidcombe Hospital, of which 180 images (57 control, 123 increased IP) were selected by the reference eCLE analyst (R.W.L.) for this study. All of the 57 control images were derived from the 21 control patients, with the 123 increased IP features derived from 36 of the 80 patients with IBD, who represented those with the most significant barrier dysfunction. No more than 5 images were selected from an individual patient. Each image was assessed for the presence or absence of features of increased IP, as well as image quality by the same reference eCLE analyst who did not participate in the subsequent evaluation. A "good quality" image had at least one-third of usable material without blurred movement artifacts. The study was approved by Human Research Ethics Committee of Sydney South Western Area Health Service (reference number 14/327).

Reference standard

The 3 eCLE features of increased IP described previously are CJE, a buildup of fluorescein between 2 epithelial cells representing impaired tight-junction proteins before breakage of the final basal tight junction releasing the fluorescein into the lumen; FL, a fluorescein plume entering the lumen from between 2 enterocytes representing loss of apposition between 2 adjacent cells; and CDO, shedding of an apoptotic enterocyte into the luminal space (Fig. 1).[17] All features were initially derived through bench-top intravital 2-photon confocal laser microscopy of explant murine small intestines bathed in luminal fluorescein. Mice were given intravenous Hoechst 33258 to stain epithelial cellular nuclei as well as either intraperitoneal phosphate buffer solution as controls or 5 μg of tumor necrosis factor-α to induce intestinal mucosal cell shedding. Under confocal laser microscopy imaging (Carl Zeiss 7MP; Carl Zeiss, Oberkochen, Germany and Chameleon Vision II Ti:Sa laser; Coherent Scientific, Hilton, South Australia,