Confocal Laser Endomicroscopy Gives GIs a Powerful New Tool

Jeffrey Conklin, MD and Laith H. Jamil, MD

Confocal laser endomicroscopy (CLE) is a new tool for endoluminal imaging of gastrointestinal epithelia. The technology provides very high-resolution images of the mucosal layer by illuminating tissue with a low-power laser and detecting the reflected fluorescent light through a pinhole. CLE is being used to help detect Barrett esophagus, gastric cancer, colon polyps and many other conditions.

CLE can be performed with either a dedicated endoscope or a probe that passes through the accessory channel of a standard endoscope or colonoscope. Both devices offer very high magnifications, with resolutions in the range of 0.7 to 1.0 μm, and optical penetration of the epithelium to a depth of 250 μm, a level of resolution that rivals light microscopy. CLE also allows real-time, in vivo histopathological evaluation of suspected dysplastic and neoplastic epithelial lesions identified by other optically enhanced imaging modalities such as narrow-band imaging.

Initial studies using CLE identified epithelial abnormalities that reliably distinguished high-grade dysplasia (HGD) in Barrett esophagus and early esophageal adenocarcinoma from nondysplastic Barrett epithelium. These abnormalities included an irregular epithelial lining, dark areas indicating irregular fluorescein uptake, and irregular, dilated vessels. Using these criteria, probe-based CLE (pCLE) detected HGD and early adenocarcinoma with sensitivity of 80 percent and specificity of 94 percent.

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Figure 1: A is a histopathological image demonstrating Barrett esophagus with no dysplasia. The surface, columnar epithelial cells are aligned in a neat row with small, basally oriented nuclei. The black arrow points out a goblet cell—the rounded bluish cell—that is the diagnostic feature of intestinal metaplasia of Barrett esophagus. B is a confocal laser endomicroscopy image of the corresponding tissue in vivo. The whiter areas are the lamina propria where fluorescein is in the highest concentration. Orderly, columnar epithelial cells line the lamina propria as seen in the histopathological image. The white arrow indicates a goblet cell.
percent (Note 1). The positive predictive value was 44 percent and the negative predictive value was 99 percent.

In another study, CLE did not outperform the Seattle biopsy protocol – four quadrant biopsies every centimeter from the gastro-esophageal (GE) junction to the Z-line – as a screening tool for patients at high risk for HGD or early adenocarcinoma (Note 2). However, the use of CLE significantly decreased the number of biopsies needed to obtain the same screening result.

At Cedars-Sinai, we are using CLE to identify suspicious mucosal lesions for definitive therapy, as shown in the first case study below. In the second case study, CLE was used to guide surgical resection when endoscopy failed to identify a gross tumor or a sufficiently clear margin between normal and abnormal tissue.

Case study 1
A 76-year-old male presented with a 20-year history of intermittent dysphagia to solid foods that was not progressive. He never complained of heartburn, but for about a decade suffered from episodic regurgitation of gastric contents when lying down after large meals. In late 2008, an endoscopy revealed an irregular Z-line, and what was described as a small “pyramidal” lesion just below the GE junction. Biopsies of this lesion were interpreted as high-grade dysplasia arising in Barrett esophagus. After a work-up (including endoscopic ultrasound and PET/CT scan) was negative for invasive esophageal adenocarcinoma, he was referred to Cedars-Sinai for treatment.

Endoscopy demonstrated short salmon colored tongues of mucosa and a small area of “lumpy-bumpy” mucosal change just below the squamocolumnar junction. The patient was given intravenous fluorescein, and the irregular area and other parts of the Barrett mucosa were examined with the CLE probe. Intestinal metaplasia without dysplasia (Figure 1) was identified. High-grade dysplasia was present in the suspicious area and at another location where no mucosal irregularity had been identified with narrow-band imaging (Figure 2). These areas were injected with a mixture of epinephrine and saline, and removed piecemeal using a Duette device. Pathological examination of the tissue revealed “two foci of high-grade dysplasia, probably arising in a background of Barrett esophagus.”

Case study 2
A 68-year-old male with vague upper GI complaints underwent an endo-gastro-duodenoscopy (EGD) with random biopsies, which showed evidence of adenocarcinoma. A PET CT was negative, so a repeat EGD was performed with the goal of pinpointing the location and the periphery of the tumor. No gross tumor was found, and multiple biopsies were performed. Pathological examination of these biopsies revealed evidence of HGD.
The patient was then referred for a third EGD to identify the margins of dysplasia for surgical resection. As before, the procedure found no gross malignant abnormality, but did reveal somewhat more prominent and erythematous folds 10 cm below the GE junction and distally (Figure 3). At this time, pCLE was employed to examine the area of the gastric body and the antrum very carefully. In the antrum, we noted multiple areas of complete disorganization and loss of structures in the tissue, suggestive of dysplasia (Figure 4). An area of normal pattern of gastric glands was noted approximately 10 cm below the GE junction and proximally (Figure 5). This normal-appearing area was injected in two locations with tattoo spot injection to serve as a clear margin to cut during surgery. Subsequently, the patient underwent distal gastrectomy at the margins of the tattoo, omentectomy and reconstruction by retrocolic Billroth II gastro-jejunostomy. Pathological examination noted minute focus of HGD involving the antrum, with the proximal margins negative for dysplasia or metaplasia.

**References**


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**Split-Dose of 6-MP/Azathioprine to Manage IBD Patients with Preferential 6-MMP Metabolism**

David Q. Shih, MD, PhD; Minh Nguyen, MD; Eric A. Vasiliasuskas, MD

Azathioprine (AZA) and 6-mercaptopurine (6-MP) have been shown to be effective in treating inflammatory bowel disease (IBD). These thiopurines are generally well-tolerated, continue to be widely utilized, and remain a cornerstone of IBD therapy despite the introduction of the newer class of biologic therapeutics.

The utility of 6-MP metabolite monitoring is increasingly recognized, as determination of levels can assist clinicians in optimizing clinical response to thiopurines and allows for the identification of individuals at increased risk for drug-induced toxicities. Prior studies suggest that 6-thioguanine (6-TGN) metabolite levels correlate with therapeutic efficacy in IBD patients, whereas abnormally high 6-methylmercaptopurine (6-MMP) levels are associated with dose-dependent toxicities (see references).

A significant portion of individuals (about 20 percent) with the “normal genotype” (TPMTH/TPMTH) exhibit preferential 6-MMP metabolism. When dosed in the traditional weight-based, once-a-day fashion, patients with this metabolic phenotype exhibit high 6-MMP levels, usually in the face of “subtherapeutic” 6-TGN levels. 6-MP/AZA dose escalation in this subset of patients – in an attempt to push the 6-TGN level into the “therapeutic range” – often results in dose-dependent leukopenia, transaminitis and/or flu-like symptoms (headaches, nausea, myalgias, fatigue, general malaise). Such patients are often deemed “partial responders,” in that the dose that leads to adequate control of IBD symptoms also causes the aforementioned toxicities. Overproduction of 6-MMP and side-effects resolve with dose reduction, but the lower dose often fails to adequately suppress disease activity, resulting in suboptimal, partial symptom control. The management of this subset of IBD patients thus remains a significant challenge to gastroenterologists.

Anecdotally, we observed that simply splitting the daily dose of thiopurine (e.g., 50 mg BID rather than 100 mg once daily) can reduce the 6-MMP metabolites while maintaining 6-TGN levels. This observation inspired a retrospective chart review (IRB #Pro00014002) of patients with baseline 6-MMP levels greater than 7,000 pmol/8×10⁸ RBC who underwent split dosing (n=16).

Dividing the daily thiopurine dose led to a significant reduction in 6-MMP levels (11,879 vs. 5,955 pmol/8×10⁸ RBC; p=0.0001) without adversely affecting clinical disease activity or 6-TGN levels (250 vs. 227 pmol/8×10⁸, p=NS) (Figure 1). Side effects associated with 6-MMP, such as increased liver function test (LFT), leukopenia and flu-like symptoms, improved in seven of eight patients. After mean follow-up of 42 months, seven of the patients remained on the split dose without adverse effect.

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16 patients were able to be maintained on a split dose of 6-MP with control of their IBD.

To our knowledge, this is the first study to demonstrate the effectiveness of dose splitting on preferential metabolism. This novel approach has several advantages over other commonly employed strategies. Dose splitting does not sacrifice potential efficacy associated with dose reduction, and may even allow for further upward titration of thiopurine to efficacy if needed. It avoids the introduction of possible additional medication side effects as can be seen with co-administration of allopurinol and the potential cost burden of designer biologic inventions. This maneuver is relatively simple for both patients and practitioners alike.

Split-dose administration of 6-MP/AZA rather than traditional single daily dosing represents a safe and effective treatment option for IBD patients with preferential 6-MMP metabolism who might otherwise not tolerate immunomodulator therapy and require ongoing steroid exposure and escalation of therapy to biologics, other immunosuppressives or surgery.

References: