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Much ado about very little (lamina propria)?

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Mucosal biopsies are biopsies of the mucosa. Practicing pathologists and gastroenterologists are painfully aware of this, but not infrequently, our colleagues in internal medicine inquire as to whether there are, for example, diagnostic features of scleroderma on a given patient's mucosal biopsy samples. We patiently explain that the classic histologic features of this condition are found in the muscularis propria and that we can search only for secondary lesions on mucosal samples. “Why wasn't my GI stromal tumor/mesenteric fibromatosis/gastric glomus tumor diagnosed when I had endoscopy?” a patient might ask. It is all about what an endoscopic/colonoscopic mucosal biopsy can sample. Can it sample enough? That is the key issue raised by Gupta et al¹ in this issue of *Gastrointestinal Endoscopy*.

Throughout the GI tract, the mucosa consists of epithelium, lamina propria, and muscularis mucosae. Beneath the muscularis mucosae is the submucosa. Ideal mucosal samples obtained by endoscopic/colonoscopic biopsies contain mucosa and a bit of superficial submucosa. Sometimes they contain muscularis propria, which, in our hospitals, prompts a telephone call to ensure that the gastroenterologist knows that a perforation may have complicated the procedure. In most areas with columnar mucosa, the endoscopist can easily obtain samples that contain the full thickness of the mucosa and a small amount of superficial submucosa.

The situation in the esophagus is different. In the absence of columnar metaplasia, the squamous epithelium is tough, elastic, and slippery. It is more difficult to grasp with the biopsy forceps than columnar epithelium. When pathologists review slides of biopsy specimens of squamous esophagus that are from relatively healthy tissue, they often note that such biopsy samples are typically smaller than other mucosal biopsy samples—it is difficult to obtain a good “bite” of healthy, normal, or near-normal squamous esophagus. Consequently, esophageal squamous mucosal biopsies from normal or near-normal

squamous mucosa often lack associated lamina propria. On the other hand, endoscopists can easily visualize columnar epithelium and obtain a generous sample.

None of this would matter much had the concern not arisen about neoplastic columnar mucosa “buried” beneath apparently healthy squamous mucosa.² This finding has been the subject of several studies and reviews and has, to date, predominantly been observed in patients who have had photodynamic therapy, such that their buried neoplasias were detected beneath neosquamous epithelium. Buried Barrett's-type mucosa also can be found *de novo* in the absence of prior ablation treatment (this presumably results from cycles of damage and repair from the patient's reflux disease). This possibility is obviously concerning, except that, in the largest available photodynamic therapy trial, this finding was not an issue, in that all patients with buried dysplasia after treatment also had non-buried dysplasia.³ To date, there are limited data regarding this possibility in patients who have received radiofrequency ablation, but “buried Barrett's” may be a lesser concern.⁴

It is often not clear in various reported studies addressing the issue of buried Barrett's whether the samples of neosquamous esophagus actually contain sufficient tissue to determine whether any buried glands are present, because such glands are found in the lamina propria, and, thus, lamina propria must be sampled in the biopsy in order to detect them. As such, Gupta et al¹ arranged for pathology colleagues to blindly review squamous samples obtained from a cohort of patients who had been treated with various ablation techniques and compare them with squamous biopsy samples from untreated patients. In both groups, expert pathologists estimated the amount of lamina propria in these squamous samples. The authors learned that in over 60% of samples, regardless of whether or not the patients had undergone ablation therapy, there was no lamina propria to study. Based on these data, the authors raised the concern that sampling in patients who have had ablation therapy is simply not adequate to detect buried Barrett's mucosa and buried neoplasia.

Does this mean that in a few years, a large number of the patients who did so well after a year in a well-controlled 2009 radiofrequency ablation trial⁵ will return with buried cancers? Short-term follow-up of these patients to 3 years suggests not. Two of 106 patients treated with radiofrequency ablation progressed within 3 years to esophageal adenocarcinoma, but both carcinomas were visible and treatable by “rescue” EMR.⁶ Or is this concern akin to the fear that carcinoid tumors (well-differentiated neuroendocrine tumors) would begin cropping up when large numbers of people began taking proton pump inhibitors and inducing hypergastrinemia (that did not happen⁷; it was only a theoretical concern)? At first blush, the notion that most squamous samples contain essentially no lamina propria is quite concerning, but digging a little deeper, there may be less cause for concern than the data suggest.

We hope we are right for the sake of our patients, but we suspect that this particular concern is overstated. Presumably, neosquamous mucosa that is not on top of Barrett's mucosa is just as tough and healthy as native squamous mucosa and, thus, is difficult to biopsy in a fashion that yields abundant lamina propria for study. On the other hand, we encounter buried Barrett's mucosa daily on mucosal biopsy samples from our own busy endoscopy clinic. The authors do not actually tell us whether they found buried Barrett's esophagus-type glands in

their samples. It appears that they counted those fragments as Barrett's mucosa rather than squamous fragments and chose "squamous-only" fragments for analysis, which would be expected to have negligible lamina propria. From the images in the current study, the illustrated squamous fragments had reflux changes but not particularly striking ones.¹ When Barrett's mucosa is buried beneath squamous mucosa, the lamina propria in that region (presumably a function of stromal-epithelial interactions⁸) probably becomes more cohesive and resembles the lamina propria of the intestines, with a full complement of inflammatory cells and stroma (Fig. 1A-D). This may allow a better biopsy "bite" to be obtained, in contrast to squamous-only mucosa, from which the lamina propria readily tears away, as seen in the authors' illustrations.¹ As a result, one may pose the question: Is the absence of lamina propria indicative of no buried Barrett's? The real issue is more one of simply being able to screen the entire neosquamous esophagus well enough. This is not a new issue; it always has been *the* issue for long-segment Barrett's esophagus. Unfortunately, this issue is currently addressed (albeit imperfectly) in clinical practice only by aggressive random biopsy protocols. Additionally, the lesions that arise on follow-up may derive from stem cells anyway,⁹ and we may not detect the key cells with current techniques, regardless of how we sample.

The authors were asked by their reviewers to conclude that cutting-edge imaging, specifically optical coherence tomography (OCT), might solve this problem that may or may not actually exist. However, such techniques would not solve the problem in their current state. For example, confocal laser endomicroscopy allows an "optical biopsy" to a depth of about 250 μm and can be very helpful in better targeting areas of columnar mucosa to sample or to remove by endoscopic resection.¹⁰ But, as noted in Figure 2A and B, this imaging depth may not be deep enough to solve the problem (that may or may not exist) of detecting buried Barrett's mucosa in the absence of obvious surface Barrett's mucosa. Endoscopic OCT can image the esophageal wall sufficiently deeply, but the image quality has been poor, and clinically available OCT probes are too insensitive for detecting esophageal intestinal metaplasia. It would seem unlikely for probe-based OCT or other very high-resolution imaging techniques to be appropriately sensitive to detect a gland or two of buried Barrett's epithelium over a large area of esophagus. However, large foci of buried Barrett's glands also are likely to have an associated surface allocation of persistent or recurrent Barrett's epithelium.

This is an exciting time to be caring for patients with Barrett's esophagus-associated dysplasia. In the past, we had to offer them esophagectomy because we had nothing better. Now we have something better, but we have yet to learn how durable our endoscopic treatments really are. Imaging will become more sensitive, and we will develop better protocols for sampling. We will learn just how often dysplasia rears its head again after ablation, and (we hope) we will learn that buried Barrett's mucosa is infrequent but still readily sampled on post-ablation biopsies because it adheres to the overlying squamous epithelium. Ultimately, what really matters is how important buried Barrett's esophagus is, in the context of the individual patient and his/her risk for cancer development. Careful examination with current high-definition, white-light imaging also can lead to visualization of clinically relevant subsquamous nodules with neoplasia. Finally, the number of buried

Barrett's cancers is still impressively low, despite thousands of patients treated with endoscopic ablation. All the hype about lamina propria (or lack thereof) in post-ablation esophageal specimens may be much ado about very little.

Abbreviation

OCT optical coherence tomography

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Figure 1.

A, In this EMR section, there is squamous mucosa at the right and an area of Barrett's mucosa at the top of the sample. At the left, there is a zone of buried Barrett's mucosa; both Barrett's foci demonstrate a duplicated muscularis mucosae. Note that the lamina propria (the supporting tissue around the glands) in the area of the Barrett's mucosa is cellular, whereas the lamina propria beneath the pure squamous epithelium has little cellularity. When such a focus is biopsied, negligible lamina propria tissue is grasped, and little lamina propria tissue appears on small biopsy samples (H&E, orig. mag. $\times 20$). **B**, Note the higher magnification of the zone at the left part of Figure 1A at the interface between the buried Barrett's mucosa and squamous mucosa. Some of the buried glands are intimately associated with the squamous epithelium and embedded in cellular stroma that would be biopsied as a unit (H&E, orig. mag. $\times 10$). **C**, This is a mucosal biopsy sample showing buried nondysplastic Barrett's mucosa. Again, note the abundant lamina propria (H&E, orig. mag. $\times 20$). **D**, Another mucosal biopsy detecting buried high-grade dysplasia. There is abundant

lamina propria, and the glands are closely apposed to the squamous mucosa and thus were readily sampled (H&E, orig. mag. $\times 20$).

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Figure 2.

A, Confocal laser endomicroscopy would detect the buried dysplastic glands, because they are about 250 μm below the surface (H&E, orig. mag. $\times 20$). **B**, Only the squamous component would be apparent, but the reactive squamous changes might prompt a biopsy. Note that the buried lamina propria neoplasia has a dense supporting lamina propria that would be readily grasped by biopsy forceps in addition to the overlying squamous epithelium (H&E, orig. mag. $\times 20$).