Statement on Best Practices in the Use of Pathology as a Diagnostic Tool for Celiac Disease

A Guide for Clinicians and Pathologists

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Abstract: Small intestinal biopsy interpretation has been the cornerstone for the diagnosis of celiac disease for over 50 years. Despite the existence of sensitive and specific serological tests, duodenal mucosal biopsies continue to be obtained in the vast majority of patients in whom a diagnosis of celiac disease is being considered. The accurate evaluation of these biopsies requires coordination and information sharing between the gastroenterologist, laboratory, and pathologist in order to optimize tissue sampling, preparation and interpretation. This document, a collaboration between the Rodger C. Haggitt Gastrointestinal Pathology Society and the North American Association for the Study of Celiac Disease, is intended to provide clinicians and pathologists with a summary of best practices in the use of endoscopy and biopsy for patients with suspected celiac disease. The authors present a comprehensive and critical appraisal of the literature with respect to the topics of endoscopic findings, best methods for the obtaining biopsies, completing the pathology form and pathologic assessment, including evaluating intraepithelial lymphocytes and villous architecture. A discussion of conditions with overlapping pathologic findings in duodenal mucosal biopsies is presented. In order to provide additional guidance for challenging situations, the authors include an appendix containing practical suggestions. This review may be utilized in interdisciplinary discussions to optimize care for patients with possible celiac disease.

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Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

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Key Words: celiac disease, tissue transglutaminase, intraepithelial lymphocytes, duodenal biopsy, drug injury

(*Am J Surg Pathol* 2018;42:e44–e58)

Celiac disease is an immune-mediated inflammatory disorder of the small intestine that develops when genetically susceptible individuals are exposed to dietary gluten. Histopathologic documentation of small intestinal injury is widely considered the gold standard method of establishing a diagnosis as virtually all correctly sampled patients with symptomatic celiac disease have diagnostic findings in mucosal biopsies. Characteristic histologic features include variable villous blunting, crypt hyperplasia, plasma cell-rich inflammation in the lamina propria, epithelial injury, and increased intraepithelial T lymphocytes; the latter are uniformly present whenever other histologic changes are identified. In fact, small intestinal biopsy interpretation has been utilized as the foundation for the diagnosis of celiac disease for over 50 years. While duodenal biopsy may be avoided in some children with symptomatic celiac disease, it is still considered the cornerstone of diagnosis. Optimizing histologic preparation is vital to providing an accurate diagnosis for affected patients, and, equally important, to excluding celiac disease when appropriate, as well as identifying other inflammatory conditions in patients with malabsorption symptoms.

The accurate evaluation of duodenal biopsy samples requires coordination between the endoscopist, endoscopy suite personnel, pathology laboratory, and surgical pathologist. The endoscopist is responsible for proper patient selection and for providing adequate samples; the pathologist is responsible for providing a clear, accurate interpretation of histologic findings and addressing relevant entities in the differential diagnosis. Treating clinicians should understand the scope and limitations of pathologic interpretation of these biopsies.

This document is the result of a collaboration between The Rodger C. Haggitt Gastrointestinal Pathology Society (GIPS) and The North American Association for the Study of Celiac Disease (NAASCD) and has been approved by both organizations. It is intended to present

best practices in common use in the biopsy evaluation of patients with suspected celiac disease, and to provide workable guidelines that can be utilized in daily practice. It is not a discussion of scientific methods used in the context of clinical trials or research endeavors.

METHODS FOR DEVELOPING THE RECOMMENDATIONS

The then Presidents of the Rodger C. Haggitt Gastrointestinal Pathology Society (M.E.R.) and the North American Association for the Study of Celiac Disease (J.A.M.) obtained approval from their respective executive boards to jointly enumerate best practices for the use of endoscopy and duodenal mucosal biopsy in the diagnosis of celiac disease. A team of pathologists and gastroenterologists with expertise in celiac disease was selected. Using contemporary search engines, these authors undertook a critical review of the literature addressing technical issues related to tissue acquisition at endoscopy, triage and histologic processing, and the diagnostic approach to duodenal biopsy interpretation. The authors then compiled the data to formulate best practice recommendations. In order to provide additional guidance for challenging situations as well as examples of phrasing of reports in common scenarios, the authors include Appendix 1, which summarizes practical suggestions for evaluating patients and samples when a diagnosis of celiac disease is suspected. These comments represent the collective opinions of the authors. They should not to be considered absolute rules for practice, but rather examples of how reporting may be approached.

This document was evaluated by the Executive Committees of the GIPS and NAASCD and was subsequently reviewed by an additional group of pathologists and gastroenterologists with expertise in the field (see the Acknowledgments section). After final approval by the GIPS and NAASCD Executive Committees, the recommendations were distributed to the society memberships via http://usgips.com; http://www.nasscd.org for a comment period. All comments received from the general membership, along with the authors' responses, were posted to the society websites, and appropriate modifications were incorporated into these recommendations.

ENDOSCOPIC FINDINGS IN CELIAC DISEASE

Endoscopic features of celiac disease described in the duodenum include paucity or loss of mucosal folds, effacement of folds with inflation, presence of a mosaic pattern, scalloping, nodularity, and increased visibility of the vascularity (Fig. 1). Some workers have suggested that in normal-appearing duodenal mucosa there is no need to take biopsies, as they do not reveal villous atrophy. However, this concept has been disproven by numerous studies.[1–16]

In one large, prospective, multicenter study of pediatric and adult patients with positive serology, the sensitivity, specificity, positive predictive value, and negative predictive value of endoscopic findings were good in adults (100%, 84.6%, 94.2%, and 100%, respectively), but much less reliable in children (86.8%, 9.1%, 82.1%, and 12.5%).[17] The authors concluded that endoscopic markers have low reliability for celiac disease, and their diagnostic value in selecting patients for biopsy is unacceptable, especially in populations with low disease prevalence.

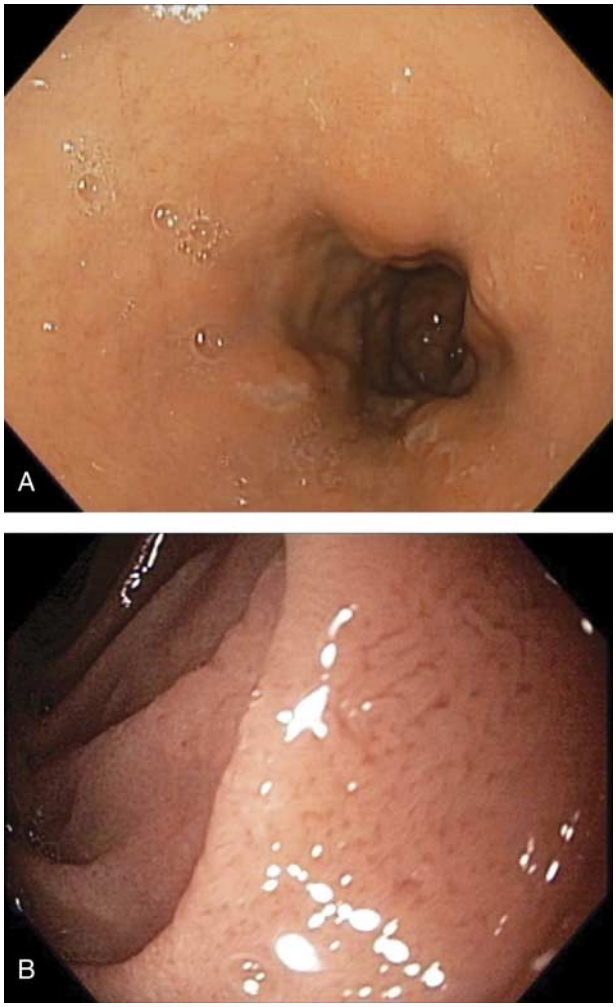

FIGURE 1. Positive endoscopic findings in celiac disease. A, An image from the second duodenum illustrating a scalloped appearance with effacement of folds in a 55-year-old woman at the initial diagnosis of celiac disease. B, Effacement of folds with a mosaic pattern and prominent vessels in the duodenum of a patient with celiac disease.

A retrospective study in the pediatric age group further confirmed the poor reliability of endoscopic markers.[18] The investigation addressed the general issue of concordance between endoscopic and histologic findings in 1000 pediatric esophagogastroduodenoscopies, concluding that for all diagnoses eventually established by histology, if biopsy specimens had only been obtained when the endoscopist identified abnormal mucosa, 48.5% of the pathologic findings would have been missed. In patients with histologic findings indicative of celiac disease, 43% had normal-appearing mucosa.

The use of narrow-band imaging or other forms of endoscopic light adjustment may enhance sensitivity.[19] Additional techniques to enhance the sensitivity of endoscopic

markers, such as immersion technique or zoom, may increase their diagnostic value, however, other than the immersion technique, such approaches have not been widely studied, and are not currently available in most medical centers.[7,11,13,14,20,21]

Summary and Recommendations

- The endoscopic findings of reduction or loss of duodenal folds, mosaic pattern, and scalloped folds are associated with villous blunting; however, as villous blunting is not specific to celiac disease, biopsies are recommended even when suggestive endoscopic findings are seen.
- The diagnostic sensitivity of endoscopy is low, ranging between 50% and 76% (average ~60%). It is lower in pediatric patients than in adults and varies according to pretest selection criteria.
- The specificity of endoscopic findings is higher, ranging between 80% and 100% (average ~93%).
- The finding of visually normal duodenal mucosa does not preclude a diagnosis of celiac disease, especially as some patients with celiac disease have normal villous morphology. Therefore, it is recommended that duodenal biopsies be taken during esophagogastroduodenoscopy whenever celiac disease is considered, regardless of endoscopic appearance.

LOCATION AND NUMBER OF BIOPSIES TO BE TAKEN

As an introductory comment, endoscopists should note that biopsy samples from the small intestine are exquisitely fragile and are easily disrupted during handling, potentially compromising subsequent histologic analysis. Special care is required when transferring specimens from the biopsy forceps to the specimen container.

The histologic abnormalities in the duodenum in celiac disease patients can be patchy in distribution, especially among children. A study of 110 symptomatic pediatric patients with supportive serologic studies and samples from at least 4 duodenal sites found that 93% of patients had mucosal abnormalities in at least 1 sample, but only 50% had such findings in all samples.[22] The same investigators later demonstrated that nearly half of pediatric patients with celiac disease displayed variable villous abnormalities in samples from different sites in the duodenum.[23] Another study evaluated duodenal biopsy samples from 67 children with suspected celiac disease and found that 64% of patients had patchy disease, and 12% of patients had variably severe changes in biopsy samples from the same location.[24] Similar findings have been described in adults. In a study of duodenal biopsy samples from 53 adults with celiac disease (all of whom had 1 sample from the duodenal bulb, 4 from the proximal duodenum, and 4 from the distal duodenum), 10 (19%) patients had patchy disease. However, obtaining samples from all 3 sites established a diagnosis in all affected patients.[25]

Duodenal Bulb Biopsies

Previous celiac disease guidelines mandated avoiding sampling the duodenal bulb because common morphologic changes found in that location, such as mild villous blunting and inflammation, often collectively referred to as "peptic" injury, may simulate celiac disease. In addition, it was believed that bulb samples rarely enhance diagnostic yield compared with postbulbar samples.[26] For example, a study of samples from the duodenal bulb and distal duodenal mucosae from 25 adults with serologic evidence of celiac disease found that specimens from the bulb did not improve detection of celiac disease.[27] However, multiple studies have shown that villous blunting and intraepithelial lymphocytosis can be restricted to the duodenal bulb in 2.5% to 13% of patients with celiac disease, most often in the pediatric population.[28–30] In an evaluation of 102 pediatric patients with celiac disease, all of whom had 5 duodenal mucosal biopsies, including one from the duodenal bulb, involvement of the duodenal bulb was present in all patients, and it was the only site of injury in 25% of patients.[31] Data from an additional study suggest that the severity of villous abnormalities varies by biopsy location, even within the duodenal bulb, with more pronounced villous blunting found in the 9 o'clock or 12 o'clock positions.[32] In contrast, results from a larger study (n = 268) demonstrated that a single specimen from any site within the duodenal bulb was sufficient to maximize sensitivity for the identification of villous shortening.[33] Overall, available data indicate that failure to sample the duodenal bulb may result in missed diagnoses. However, given the aforementioned common finding of mild blunting, sometimes associated with prominent Brunner glands and inflammatory changes, findings limited to the bulb should be correlated with serological and other evidence of celiac disease in order to avoid over diagnosis.[34,35] Current guidelines suggest that 2 biopsies from the duodenal bulb should be obtained when celiac disease is suspected.[36]

Number of Biopsy Samples

The number of specimens submitted overall also correlates with likelihood of detecting histologic evidence of celiac disease. In a series of 102 patients with celiac disease who had 4 specimens submitted, the diagnostic yield was 90% with 2 specimens, 95% with 3 specimens, while the remaining 5% required all 4 biopsies to achieve diagnosis.[37] The American College of Gastroenterology and the American Gastroenterological Association recommend obtaining 2 tissue samples from the duodenal bulb and at least 4 from the distal duodenum for evaluation of celiac disease.[36,38] We concur with these recommendations. At present, adherence to these guidelines in the United States appears to be low, and in one study the most common number of specimens submitted to a national pathology laboratory was 2.[39]

In addition to the need for biopsies from several sites, there is some evidence to suggest that obtaining a single biopsy per pass of the forceps improves the quality of the subsequent histologic specimen. One study evaluated specimen orientation in 86 patients who underwent

4 biopsies of the duodenum for evaluation of celiac disease. Two of the samples were obtained using a single-biopsy technique with 1 bite per pass and 2 were obtained with a double-biopsy technique (2 bites per pass of the forceps). They found that the double-biopsy technique was associated with fewer well-oriented specimens (42% vs. 66% of samples obtained with single-biopsy technique, $P < 0.01$).[40]

Completing the Pathology Form

Once duodenal biopsy samples have been obtained, detailed completion of the pathology requisition form by gastroenterologists is a crucial step in insuring accurate and complete diagnosis. All clinical information pertinent to the diagnosis, including the reason for endoscopy, endoscopic findings, medications (especially olmesartan and other angiotensin II receptor blockers, non–steroidal anti-inflammatory drugs [NSAIDs], and antineoplastic agents), supportive historical and laboratory data, current adherence to a gluten-free diet, and specific questions for the pathologist should be included on pathology requisition forms in order for pathologists to achieve complete and accurate reports that correctly direct patient care. If tTG testing has been carried out the titer should be specifically included, which will also prevent pathologists from suggesting a test that has already been performed.

Summary and Recommendations

- Practitioners should obtain at least 4 specimens from the distal (postbulbar) duodenum and 2 specimens from the duodenal bulb when performing biopsies for the assessment of celiac disease.
- Specimen quality may be improved by obtaining 1 specimen per pass of the biopsy forceps.
- Practitioners considering a diagnosis of celiac disease should provide the pathologist with available information relevant to the diagnosis, including signs and symptoms, endoscopic findings, medications, patient and family history, current adherence to a gluten-free diet, and serological or genetic test results.

HISTOLOGIC ORIENTATION OF SMALL BOWEL BIOPSY SAMPLES

When the concept of sampling the small bowel to investigate causes of malabsorption first came into practice, large biopsies were initially obtained during open surgery, and subsequently via intraluminal suction devices that allowed for sampling of the jejunum or, less often, the duodenum.[41,42] These biopsies were painstakingly oriented in the laboratory for optimal sectioning. With the advent of modern fiber optic and video endoscopy, suction biopsies gave way to smaller, visually targeted pinch biopsies. Initial attempts to orient pinch biopsies using the dissecting microscope were quickly abandoned due to technical complexity and in the face of increasing biopsy volumes. An additional challenge to proper orientation stemmed from the fact that the site of biopsy changed from jejunal to duodenal mucosa, with potentially shorter villi and prominent Brunner glands.

While the importance of proper orientation of small bowel biopsies for histologic interpretation is recognized, few studies address the practice of orienting gastrointestinal biopsy tissue during processing. The only study, to our knowledge, comparing architectural assessment in oriented versus randomly embedded small bowel specimens noted that a significant number of the oriented specimens were placed on the solid substrate upside down, leading the authors to conclude that attempting to orient biopsies is not helpful and may even introduce a false impression of flattened villi.[43]

Most small bowel mucosal biopsies are currently processed without orientation in the endoscopy or pathology laboratory. The percent of biopsies that are poorly oriented varies according to individual laboratory practices, but has been reported to range from 10% to 54%.[40,44] Efforts are made in some centers to place small intestinal tissue on edge in order to increase the likelihood that cross-sections will be obtained perpendicular to the villous crypt interface. This entails skill and painstaking effort in placing fixed biopsy tissues on edge in paraffin during the embedding process. Poor orientation of samples at microscopy can be mitigated by clinicians obtaining the recommended number of samples and by pathologists performing serial sections through tissue blocks; a practice that is not only encouraged, but that often provides adequate visualization to assess villous architecture in poorly oriented samples. While the study referenced above demonstrated that tangentially oriented samples can result in an erroneous assessment of the degree of villous shortening, we are not aware of additional data supporting the theory that the lack of more effective biopsy orientation techniques leads to misdiagnosis with respect to evaluation for celiac disease.[43] Current textbooks discussing the pathology of the gastrointestinal tract are either neutral on this topic or eschew the need for gastrointestinal biopsy orientation during processing.[45,46]

An important caveat to this discussion is the observation that intraepithelial lymphocyte density depends on location in the villus; lymphocytes are normally denser along the sides and bases of villi, with few intraepithelial lymphocytes at the villus tips (see the Evaluation intraepithelial lymphocytes section). Intraepithelial lymphocyte assessment must be limited to areas where orientation can be reasonably assured.

Summary and Recommendations

- There has been an evolution in the manner of obtaining and processing small bowel biopsies from original suction techniques to highly advanced endoscopic instruments that produce smaller samples.
- There are virtually no data comparing diagnostic accuracy or patient outcome between small bowel biopsies that are oriented in the laboratory versus those that are randomly embedded.
- While placing biopsies on edge in laboratories may improve orientation on microscopic slides, there are insufficient data to suggest that special efforts at tissue

orientation are required for the diagnosis of celiac disease.
- Adequate numbers of biopsies, along with the appropriate use of serial sectioning typically result in a sufficient number of well-oriented villus crypt units to accurately determine architecture in the majority of cases.

EVALUATION OF INTRAEPITHELIAL LYMPHOCYTES IN ARCHITECTURALLY NORMAL DUODENAL BIOPSY SAMPLES

Accepted norms regarding the number and distribution of intraepithelial lymphocytes in architecturally normal small intestinal biopsy samples have changed over the past several decades. Data from jejunal capsule biopsy studies predating the endoscopic era indicated that up to 40 intraepithelial lymphocytes per 100 enterocytes were present normally in the small intestine.[47] More recent investigations, however, describe fewer intraepithelial lymphocytes in normal duodenal mucosa.[48] The authors of a study of 20 healthy adult patients found an average of 11 intraepithelial lymphocytes/100 enterocytes (range: 2 to 26, SD: 6.8), leading them to conclude that 25 (mean+2 SD) per 100 enterocytes represents the upper limit of normal.[49] Another group reported similar results in which the authors counted at least 300 epithelial cells in duodenal biopsy samples from healthy individuals and found an average of 11 lymphocytes per 100 enterocytes.[50] Most investigators now consider 25 intraepithelial lymphocytes per 100 enterocytes to represent the upper limit of normal in duodenal biopsies, although lymphocytes are not evenly distributed over the surfaces of the villi.[51] Rather, they are more numerous at the bases of villi and along their lateral aspects compared with the tips, an observation that has been termed the normal "decrescendo" pattern of intraepithelial lymphocytosis.[52] Given that intraepithelial lymphocyte density does depend on location in the villus, assessment for intraepithelial lymphocytosis must be performed only in areas where proper orientation can be reasonably assured.

Duodenal biopsy samples from virtually all patients with celiac disease show an increase in surface epithelial infiltration by mature T lymphocytes upon exposure to gluten.[53] Gluten exposure in susceptible patients is generally associated with at least 30 intraepithelial lymphocytes per 100 duodenal enterocytes and more than 40/100 enterocytes in most cases; *Helicobacter pylori* infection, peptic duodenitis, medication-related injury, viral enteritis, and other disorders in the differential diagnosis are usually associated with a lesser degree of lymphocytosis. For this reason, some authors may advocate a higher threshold for a diagnosis of celiac disease. However, data from multi-institutional studies indicate that a requirement for 40 intraepithelial lymphocytes per 100 enterocytes detects celiac disease with only 80% sensitivity, compared with 100% sensitivity when 25 lymphocytes per 100 enterocytes are present.[53] Celiac disease may also be patchy in treatment naïve patients, and intraepithelial lymphocytes may decrease in number following gluten withdrawal. One study found that mean intraepithelial lymphocyte counts fell from 61/100 enterocytes to 38 in duodenal biopsy samples following gluten withdrawal.[54] An additional study evaluated duodenal mucosal biopsy samples from 28 patients with celiac disease, including four treated with gluten withdrawal. In this study a mean of 42 (range: 26 to 58) intraepithelial lymphocytes per 100 enterocytes was found in untreated patients compared with 29 (range: 25 to 36) among those with celiac disease who adhered to a gluten-free diet.[55]

In architecturally normal mucosa, intraepithelial lymphocytosis can be evaluated by a variety of methods. Some investigators count intraepithelial lymphocytes along the entire length of the villus, whereas others assess both number and distribution of lymphocytes within the epithelium.[56,57] A study that counted intraepithelial lymphocytes in the tips of 5 randomly selected, well-oriented villi found that a mean >12 intraepithelial lymphocytes per 20 enterocytes was a sensitive marker of gluten sensitive enteropathy.[52] The villus tip counting method was subsequently validated in 2 additional studies.[56,57] In the largest study, a mean of 6 intraepithelial lymphocytes per 20 villus tip enterocytes was present in 49 patients with normal villous architecture and positive antiendomysial antibodies, compared with 3 or fewer intraepithelial lymphocytes in control biopsies.[57] The counts obtained using the villus tip technique correlate well with the previously cited studies suggesting that >29 lymphocytes per 100 epithelial cells is abnormal. Others have compared the density of intraepithelial lymphocytes in the villus tips to that at their bases by counting intraepithelial lymphocytes per 100 enterocytes in both locations, then expressing the relationship as a tip-to-base ratio. Normal ratios are generally ≤ 1.5, whereas values >2 are suggestive of celiac disease. In 1 study, 88% of celiac disease samples showed a tip-to-base ratio of >1.7 compared with only 13% of controls without celiac disease.[55] It is likely that counting fewer enterocytes adequately identifies intraepithelial lymphocytosis: high-concordance was found when assessing intraepithelial lymphocytes among 50 enterocytes compared with 100 enterocytes in 1 study (Fig. 2).[51]

Immunohistochemical stains directed against CD3 can enhance detection of intraepithelial lymphocytes and have been recommended for clinical use by several investigators, most of whom counted the number of $CD3^+$ cells per 20 enterocytes in 3 well-oriented villi. Immunohistochemical stains generally detect a greater number of intraepithelial T lymphocytes than may be apparent in sections stained with hematoxylin and eosin; thus, the threshold for pathologic intraepithelial lymphocytosis is slightly higher (30 positive cells/100 enterocytes) when immunohistochemistry is used.[50,55,58] Unfortunately, immunostains can highlight lamina propria T lymphocytes near the basement membrane of the villus, thereby masking the decrescendo pattern of lymphocyte distribution and leading to the erroneous impression of intraepithelial

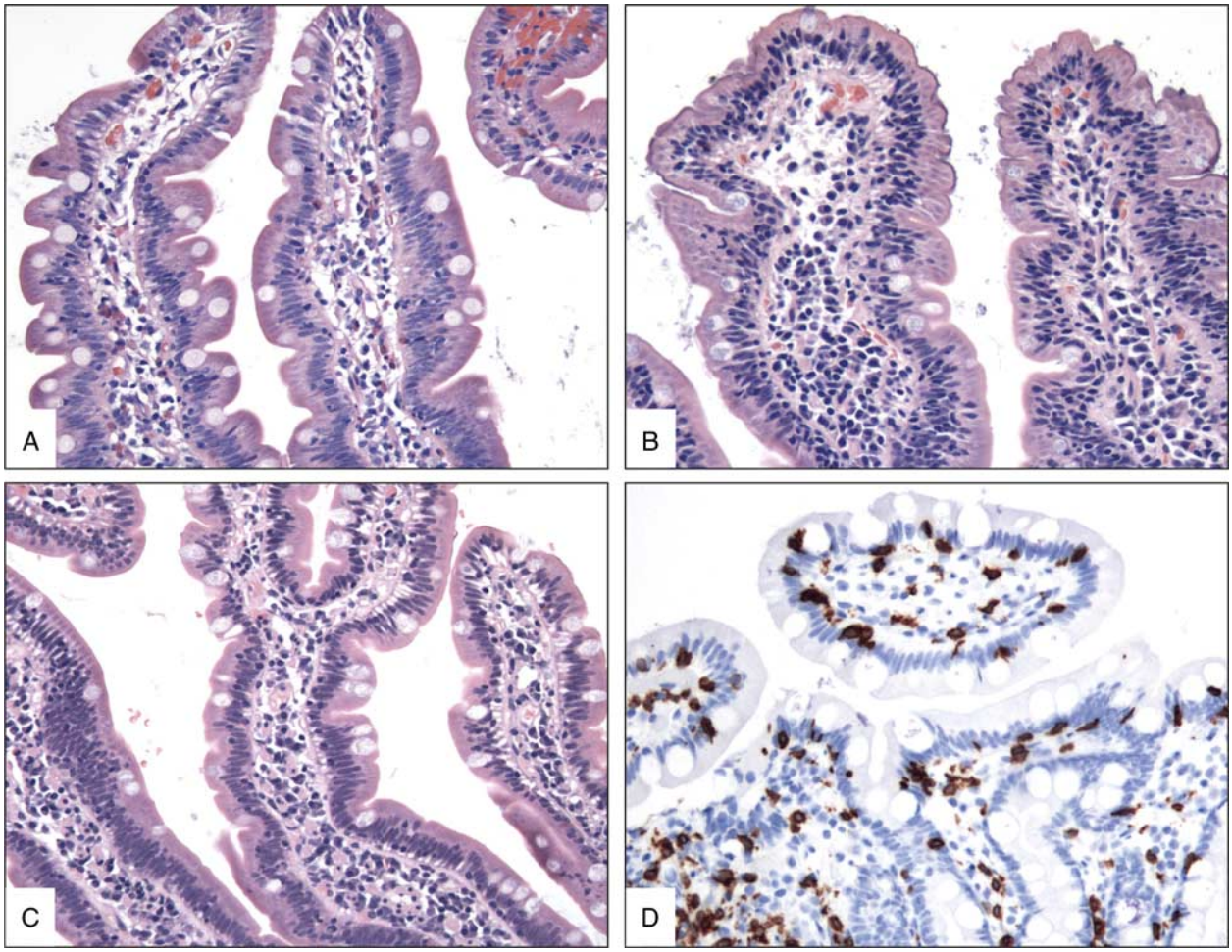

FIGURE 2. A, Scattered intraepithelial cells are normally present along the lateral aspects of villi and decrease in number at the villous tips. B, Intraepithelial lymphocytes are increased when they number > 25 per 100 enterocytes and are evenly dispersed across the entire villous surface, or more numerous in the tips. C, Some duodenal biopsy samples display mildly increased intraepithelial lymphocytes; they may be counted across the villous tip or over the entire surface of the villus. D, Immunostains for CD3 demonstrate T cells in the lamina propria near the basement membrane, leading to an overestimation of the number of intraepithelial lymphocytes.

lymphocytosis. This error is less likely in hematoxylin and eosin–stained sections where the interface between the epithelium and lamina propria is more visible. Most importantly, immunostains do not improve detection of celiac disease when it is not already suspected. One study prospectively examined 200 duodenal biopsy samples from patients undergoing clinical evaluation for potential celiac disease, none of which showed villous abnormalities or increased intraepithelial lymphocytes by routine evaluation. It was found that, although CD3 immunostains detected slightly more numerous intraepithelial lymphocytes than were evident in hematoxylin and eosin–stained sections, the difference was not clinically relevant, as the means for both groups were well within the range of normal (3.2 and 2.1/20 enterocytes, respectively).[59] Indeed, there are no data to suggest that any immunostains for T-lymphocyte markers, including stains for anti-TCR gamma receptor, improve detection of celiac disease compared with routine histologic evaluation.[60] For all of these reasons, routine use of T-cell markers in the evaluation for celiac disease is not recommended.[38]

Intraepithelial lymphocytosis is a sensitive marker of celiac disease, but this pattern of inflammation is quite common and can be seen in a spectrum of disorders; recent data suggest that nearly 7% of duodenal biopsy samples show increased intraepithelial lymphocytes with normal villous architecture.[48] Limited intraepithelial lymphocytosis in the duodenal bulb is a common manifestation of *H. pylori* infection, or may represent a reaction to luminal substances such as medications and foods, whereas the differential diagnosis of more extensive lymphocytosis is broad.[61–63] In 1 study investigating the clinical features of 43 patients with intraepithelial lymphocytosis, normal villous architecture and no history of celiac disease, only 10% of patients proved to have celiac disease. The remainder had an underlying

immunoregulatory disorder (14%), infection (2%), history of NSAID use (14%), or no identifiable association.[63] The differential diagnosis of intraepithelial lymphocytosis is discussed in a subsequent section.

Summary and Recommendations

- Occasional intraepithelial lymphocytes (up to 25/100 enterocytes) are present in duodenal biopsy samples in patients who do not have celiac disease; they tend to be more numerous along the lateral aspects of villi and decrease toward the villous tips (decrescendo pattern).
- Virtually all patients with celiac disease and duodenal architectural abnormalities have increased numbers of intraepithelial lymphocytes in excess of 40/100 enterocytes.
- Patients with celiac disease and normal villous architecture show intraepithelial lymphocytes that are evenly distributed over the entire villous (>25/100 enterocytes) or are more numerous in the villous tips (>6/20 enterocytes); assessment in either location detects gluten sensitivity in most patients and may prompt additional serologic studies if they have not already been carried out.
- Immunohistochemical stains for T-lymphocyte markers do not improve detection of celiac disease in cases that are not suspected after evaluation of hematoxylin and eosin–stained sections. There are no data to support the "up front" ordering of immunohistochemical stains to detect gluten sensitivity.
- Intraepithelial lymphocytosis, with or without villous blunting, is a sensitive but not specific histologic marker of celiac disease; the differential diagnosis includes a variety of immune-mediated, infectious, and medication-related injuries that should be clinically and histologically excluded (Appendix 1).

VILLOUS REMODELING, CRYPT HYPERPLASIA, AND THE VILLOUS TO CRYPT RATIO

The villus height to crypt depth ratio is normally 3:1 in the duodenum, which is less than that in distal small bowel.[64] However, there are several situations in which tissue artifacts or normal variation result in the false impression of villous blunting in the duodenum. In the duodenal bulb, the presence of Brunner gland nodules, gastric heterotopia, and lymphoid aggregates typically distort overlying villi. Prominent intraepithelial lymphocytes may also be seen in the epithelium over lymphoid aggregates. Thus, pathologists should restrict their evaluation of villous architecture to well-oriented mucosa away from lymphoid aggregates and nodules of Brunner glands.

Several classification schemes evaluating villous architecture exist; perhaps the most widely utilized being the Oberhuber-Marsh system. This scale describes 4 stages of abnormality: normal villous architecture with a normal distribution of intraepithelial lymphocytes, normal villous architecture with increased intraepithelial lymphocytes, intraepithelial lymphocytosis associated with mild, moderate, or complete villous blunting (villus loss with crypt hyperplasia), and hypoplastic (atrophic) (Table 1).[65] Subsequent classification schemes also catalogue cases according to the degree of villous blunting (Table 1).[64,66,67] The Corazza classification scheme condensed the histologic changes into 3 categories, A = normal villous architecture, B1 = shortened but detectable villi, and B2 = complete loss of villi.[67] One study comparing the reproducibility of the Corazza classification scheme to that of the Oberhuber-Marsh system among 6 pathologists found fair agreement among the participants when using the Oberhuber-Marsh classification ($k = 0.35$) compared with good agreement when the Corazza classification ($k = 0.55$) was used.[67] Ensari proposed a 3-tiered classification scheme identical to that of Corazza, although he provided numerical labels (grades 1, 2, and 3) instead of A, B1, and B2 for categories.[64]

TABLE 1. Celiac Disease Classification Schemes

Scheme	Grade	Villi	Crypts	IELs
Marsh	0	Normal	Normal	Normal
	1	Normal	Normal	Increased
	2	Normal	Hyperplastic	Increased
	3	Flat	Hyperplastic	Increased
	4	Flat	Atrophic	Normal
Oberhuber-Marsh	0	Normal	Normal	Normal
	1	Normal	Normal	Increased
	2	Normal	Hyperplastic	Increased
	3A	Mild blunting	Hyperplastic	Increased
	3B	Marked blunting	Hyperplastic	Increased
	3C	Flat	Hyperplastic	Increased
	4	Flat	Atrophic	Normal
Corazza	A	Normal	Normal	Increased
	B1	Mild to marked blunting	Hyperplastic	Increased
	B2	Flat	Hyperplastic	Increased
Ensari	1	Normal	Normal	Increased
	2	Mild to marked blunting	Hyperplastic	Increased
	3	Flat	Hyperplastic	Increased

IELs indicates intraepithelial lymphocytes.

Despite the existence of various classification methods, there is evidence that measuring the length of villi and crypts (and calculating their ratios) has no clinical relevance in celiac disease, as the severity of symptoms is unrelated to the degree of mucosal damage.[68,69] The use of quantitative histologic techniques to measure villus/crypt length ratios is largely relegated to clinical trials and, in some centers, to the evaluation of the response to a gluten-free diet.

In addition, Marsh and colleagues recently challenged the concept of subdividing the Marsh III category (3A, 3B, 3C) in celiac disease with evidence from a scanning electron microscopy study of duodenal biopsies. The observations suggest that the histologic appearance of mild, moderate, and severe blunting may represent an artifact of alternating surface openings surrounded by raised collars of mucosa in flattened or regenerating mucosa; the latter of which may be misinterpreted to represent blunted villi.[70] These intriguing

findings may benefit from further study and discussion prior to consideration of changing routine practice standards.

Some patients with celiac disease undergo endoscopic procedures with mucosal biopsy after gluten withdrawal is initiated. In this situation, pathologists may be asked to compare the pretreatment and posttreatment tissue samples, and comment on improvement in villous morphology as well as the relative number of intraepithelial lymphocytes. This comparison should be made in the best-oriented areas of each specimen.

In conclusion, pathologists should include a comment regarding villous morphology whenever they encounter duodenal biopsy samples from patients in whom there is a clinical suspicion of celiac disease. Semiquantitative assessment of the degree of villous blunting can be performed using one of the proposed classification schemes outlined in Table 1, or pathologists may simply state that the villi are normal, are of reduced height or are flat (Fig. 3). That the gastroenterologist and pathologist use agreed upon terminology is more important than the classification system used.

Summary and Recommendations

- Care must be taken to avoid over interpreting villous abnormalities in areas of Brunner gland hyperplasia or lymphoglandular complexes.
- Pathology reports should mention semi-quantitatively the degree of villous blunting and should compare the villous architecture with existing previous biopsies if clinically indicated in patients with suspected or proven celiac disease. Emerging data may result in a deemphasis on subclassifying degrees of villous blunting.
- A named classification system score may be included in pathology reports if it is understood by, and enhances communication with, clinicians. No specific system is endorsed as superior (Appendix 1).

CONDITIONS WITH PATHOLOGIC CHANGES THAT CAN OVERLAP WITH CELIAC DISEASE

As mentioned, numerous conditions are associated with increased intraepithelial lymphocytes, with or without villous blunting (Table 2). A full description of the differential features of these entities falls outside the scope of this document. The following discussion highlights the most common confounders and new associations that should be considered. It should be noted that in up to 34% of patients undergoing upper endoscopy with the finding of increased intraepithelial lymphocytes (>30/100 epithelial cells) and normal villous architecture, no cause or association is identified.[48] In addition to the specific entities discussed in this section, a variety of infections, including those due to Giardia, Cryptosporidium, Cyclospora, and HIV, should be kept in mind. Table 2 contains an expanded differential diagnosis, with distinguishing features. See Figure 4 for selected examples.

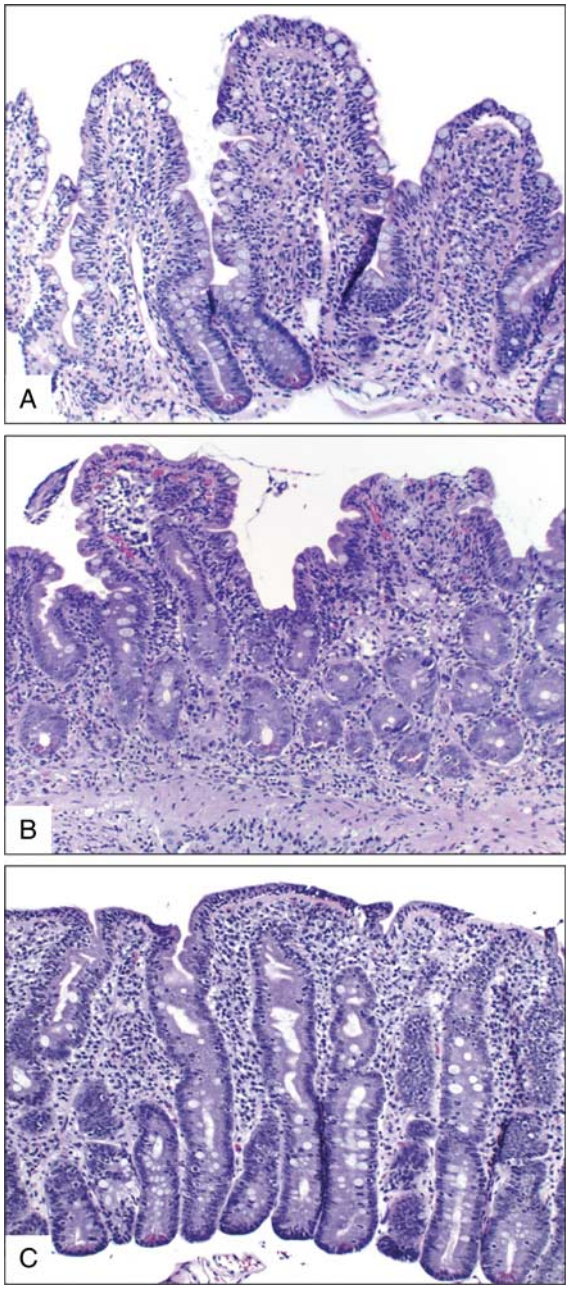

FIGURE 3. Gradations of villous blunting to be documented in pathology reports. Regardless of the scoring system used, duodenal biopsies with architectural abnormalities are typically reported as showing mild (A), moderate (B), or severe (C) villous blunting. Alternatively, blunting can be reported in a 2-tier system: partial or complete villous blunting (Table 1 and Appendix 1). Note that villous blunting is accompanied by crypt hyperplasia, such that the overall width of the mucosa is usually unchanged. In future, architectural assessments may be of less importance as the degree of mucosal damage does not correlate with symptoms. Comparisons between biopsies from the same patient should be made when clinically relevant to document changes in architecture and intraepithelial lymphocytes over time.

TABLE 2. Differential Diagnosis of Celiac Disease

Disease	Increased IEL's	Villous Blunting	Distinguishing Pathologic Features	Distinguishing Clinical Features
Peptic duodenitis	No	Yes, variable	Neutrophils, erosions, changes usually confined to bulb; gastric surface metaplasia common, but may be physiological	No specific clinical symptoms in peptic duodenitis
Helicobacter pylori gastritis	Yes	Rare, if present mild	Fewer IELs than in CD. Blunting almost never present	May need to do serology to exclude celiac disease
NSAID injury	Yes	Patchy	Patchy involvement, erosions, neutrophils	History of NSAID use; lack of typical celiac symptoms/serology
Tropical sprue	Yes	Yes, moderate	Changes extend to ileum. Usually not severe blunting	Patient demographics, and travel history
Bacterial overgrowth	Yes	Sometimes	Most biopsies normal in this setting, but no distinguishing features when abnormal	Condition predisposing to intestinal stasis
Soy and cow's milk protein intolerance	Sometimes	Yes	Colitis and enteritis, including ileum, prominent eosinophils	Usually children with feeding intolerance
Crohn disease	Sometimes	If present, not usually diffuse	Patchy involvement, erosions, ulcers, crypt branching, granulomas (rare)	Usually occurring in setting of known Crohn disease with distal intestine involvement
UC-associated duodenitis	Not usually	Sometimes	Diffuse lamina propria expansion with basal plasmacytosis, IEL's not usually increased	Usually discovered in setting of known UC
ARB injury (Olmesartan and others)	Yes	Yes	No distinguishing features, may show collagenous sprue	History of ARB use; must have high index of suspicion
Immune modulatory drugs (including checkpoint inhibitors)	Rarely	Yes	Mixed inflammation, with neutrophils, apoptosis, and occasionally crypt branching. Involves upper and lower GI tract	Usually easily distinguished by clinical setting
CVID	Yes	Sometimes	Absence of mucosal plasma cells, giardiasis, BNLH	History of chronic infections
Autoimmune enteropathy	Not usually	Yes, variable	Neutrophils, crypt apoptosis, decreased goblet and Paneth cells, involves entire small bowel, stomach and colon, usually no increase in IEL's	Often infants, syndromic, gut epithelial autoantibodies
Refractory CD	Often	Yes	Thin mucosa, basal plasmacytosis, collagenous sprue; histology may be indistinguishable from untreated responsive celiac disease. In some patients, loss of CD8 and surface CD3 antigens in IEL's on IHC	Refractory clinical course after initial response to GFD or never responded to GFD
Immunoproliferative disease of the small intestine	Not in surface, lymphoepithelial lesions in crypts	Yes	Broadened villi with diffuse plasma cell infiltrates and deep mucosal centrocyte lymphocytes with lymphoepithelial lesions. Alpha-one heavy chain without light chain expression on IHC	Mediterranean populations, response to antibiotics in early stages

ARB indicates angiotensin II receptor blocker; BNLH, benign nodular lymphoid hyperplasia; CD, celiac disease; CVID, common variable immunodeficiency; GFD, gluten-free diet; GI, gastrointestinal; IEL, intraepithelial lymphocytes; IHC, immunohistochemistry; RCD, refractory celiac disease; UC, ulcerative colitis.

Morphologic Findings Commonly Present in the Duodenal Bulb (So Called "Peptic Duodenitis")

The term "peptic duodenitis" has been used to refer to a variety of findings commonly seen in bulb mucosa, some of which (eg, gastric surface metaplasia and prominent mucosal Brunner glands) may represent normal variants at the gastro-duodenal junction, as opposed to pathologic processes. Whether "peptic duodenitis" exists as a stand-alone entity or is a cofactor in the duodenal injury caused by *H. pylori* or certain medications is a question beyond the scope of this treatise.

The changes typically described as "peptic" in origin are described here, as they are common and should not be used as evidence of celiac disease. So called "peptic duodenitis" is typically characterized by the presence of varying degrees of increased plasma cell infiltration, neutrophils in the lamina propria or epithelium (or both), and reactive epithelial changes, including villous blunting.[71–74] As mentioned, gastric surface metaplasia and prominent mucosal Brunner glands are not an absolute criterion for "peptic duodenitis," as they may be found in biopsies without inflammation, perhaps representing an adaptive response to chronic acid exposure.[73] In severe cases with marked neutrophilic infiltrates, the epithelium shows mucin depletion, syncytial growth pattern, and more marked reactive epithelial changes, including nuclear hyperchromasia and increased mitoses.[71,73,74] Increased intraepithelial lympho-

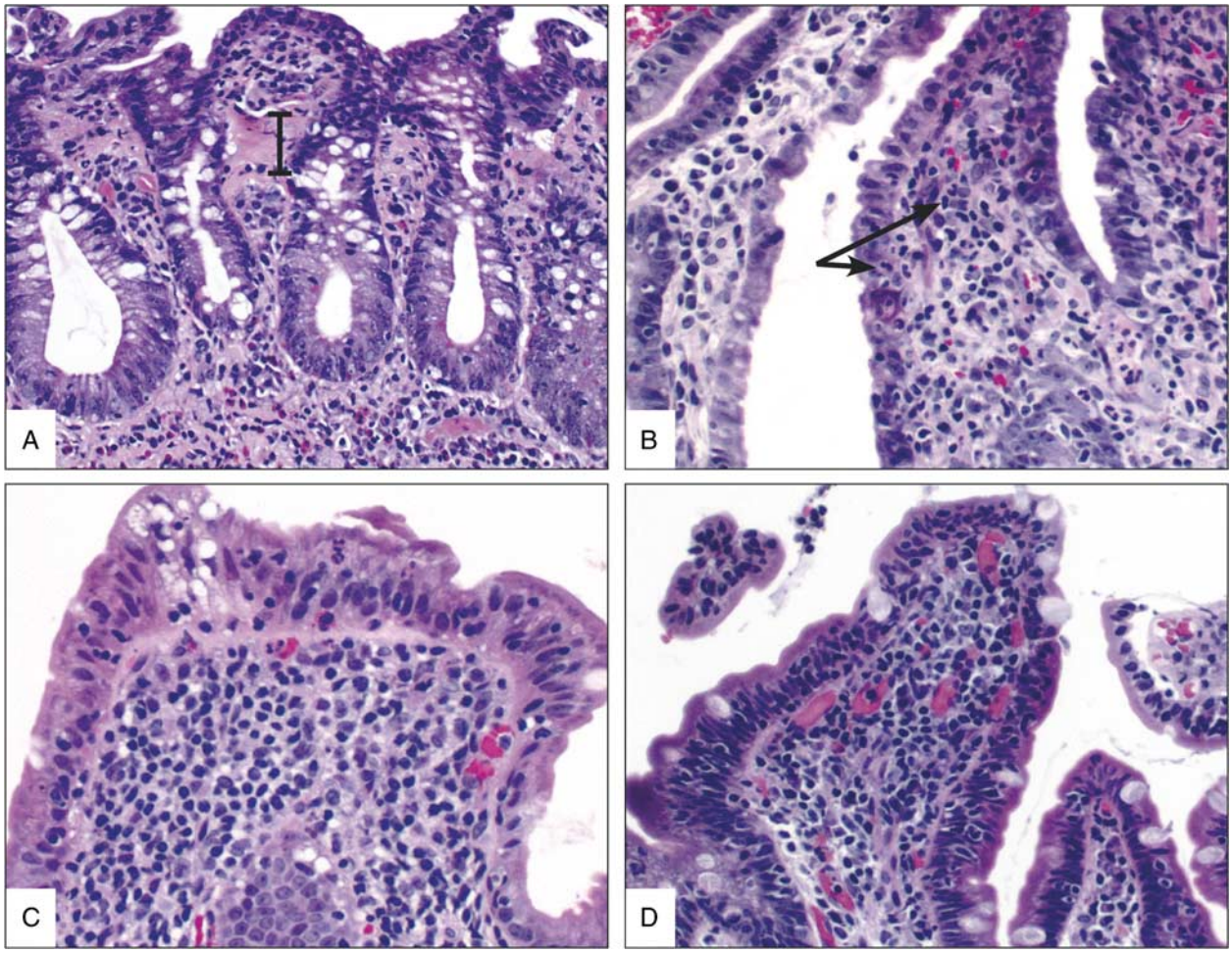

FIGURE 4. A, Collagenous sprue is one of several histologic findings associated with a refractory course in celiac disease. In this patient who failed a gluten-free diet, the mucosa is flat, with severe reactive surface and crypt epithelium, and shows a thick band of collagen extending from just beneath the basement membrane into the lamina propria (bar). B, In this example of Ipilimumab-associated enterocolitis mild villous blunting was noted at low power. At high power, the epithelium and lamina propria are infiltrated by neutrophils (arrows) and intraepithelial lymphocytes are focally increased. Marked reactive epithelial changes and mucin depletion are seen. C, Patients with common variable immunodeficiency may develop villous blunting and increased intraepithelial lymphocytes/neutrophils, among other findings. Note the lack of plasma cells in the lamina propria, which instead contains small lymphocytes. D, This small bowel biopsy from a Haitian patient with chronic diarrhea shows minimal villous blunting, increased intraepithelial lymphocytes and increased lamina propria cellularity, characteristic of tropical sprue.

cytes, erosions and ulceration are more likely to be associated with *H. pylori* gastritis (see below).[75]

Important for the distinction from celiac disease, villi may appear blunted in "peptic duodenitis"; however, increased intraepithelial lymphocytes are not seen. Conversely, neutrophils may be present in duodenal biopsies from both adults and children with celiac disease.[76] As acute inflammation and mild blunting in the duodenal bulb are common, they may occur in patients who also have celiac disease. Correlation with distal duodenal biopsy findings, and serological data should be pursued if a diagnosis of celiac disease is suspected clinically.

H. pylori-associated Duodenal Inflammation

It has now been established that *H. pylori* gastritis is associated with increased intraepithelial lymphocytes in the proximal duodenum.[62,77,78] The histopathology can be indistinguishable from mild celiac disease (increased intraepithelial lymphocytes) with normal villous architecture), such that *H. pylori*-associated duodenitis must be considered in all patients with increased intraepithelial lymphocytes and normal villous architecture. Rare reports of mild and even moderate/severe villous blunting due to *H. pylori* infection exist in nonwestern populations.[79] Importantly, the distribution of duodenal intraepithelial lymphocytes associated with *H. pylori* gastritis does not conform to any specific pattern (villus dominant or crypt dominant distribution), and the number of surface intraepithelial lymphocytes tends to be smaller in *H. pylori*-associated duodenitis.[62,78] Intraepithelial lymphocytes can also be increased beyond the bulb in patients with *H. pylori* infection, again highlighting the need for

clinical correlation to avoid over-diagnosing celiac disease. This possibility is readily addressed by taking gastric biopsies from antral and oxyntic mucosa to evaluate both for *Helicobacter* species and chemical gastropathy.

Medications

In addition to NSAIDs, a number of less commonly used medications, including recently approved antineoplastic agents, are associated with small intestinal injury patterns that include inflammation and villous blunting. Distinguishing drug injury from celiac disease is usually straightforward, in conjunction with clinical history.

NSAID Injury

A variety of pathologic findings are associated with NSAID use, the most mild of which is superficial erosions with neutrophilic and plasmacytic infiltrates.[80–82] These may be multiple and can progress to form ulcers associated with hemorrhage or strictures.[83,84] While colonic intraepithelial lymphocytosis due to NSAIDs is common (lymphocytic/collagenous colitis pattern), marked increases in intraepithelial lymphocytes and diffuse villous blunting are not typical of upper gastrointestinal tract NSAID injury. However, studies clearly document the association of NSAID use with focal increases in intraepithelial lymphocytes and mild villous abnormalities.[48] In that situation, correlation with serology results may be necessary, depending on the degree of clinical suspicion for celiac disease. Distinction from celiac disease may be facilitated by the presence of neutrophils and erosions in NSAID injury, although these changes may rarely occur in celiac disease.[76]

Olmesartan

In 2012 a severe sprue-like disorder with the development of villous blunting, inflammation, collagenous sprue and microscopic colitis was reported in 22 patients taking the angiotensin II antagonist, olmesartan.[85] All tests for celiac disease were negative and patients recovered fully, with resolution of pathologic changes in mucosal biopsies following cessation of the drug. Duodenal biopsy samples from all 22 patients demonstrated total or partial villous shortening with mononuclear cell-rich mucosal inflammation. Seven patients had collagenous sprue, and samples from 14 patients showed intraepithelial lymphocytosis indistinguishable from celiac disease.[85] Recognition of mucosal injury associated with olmesartan, and other angiotensin II receptor blockers requires a high index of suspicion, and is facilitated by negative serological tests for celiac disease.

Immunomodulatory and Other Antineoplastic Drugs (Checkpoint Inhibitors; Kinase Inhibitors)

Several new antineoplastic therapies (ipilimumab, pembrolizumab, nivolumab) aimed at activating the immune system to achieve tumor cell death have the adverse consequence of inciting inflammatory reactions that affect multiple organ systems, including the gastrointestinal tract.[86,87] A growing body of literature describing pathologic features of medication-related injury discuss a variety of inflammatory changes, including "IBD-like" chronic inflammation with crypt architectural distortion, as well as neutrophilic infiltration, ischemic changes, and villous blunting in the duodenum and terminal ileum. Most of these agents elicit some degree of intraepithelial lymphocytosis accompanied by crypt epithelial cell apoptosis and variable neutrophilic cryptitis (Fig. 4). Drug withdrawal usually results in resolution of symptoms and inflammatory changes, although both may persist, requiring anti-inflammatory therapy. Idelalisib, a kinase inhibitor employed in the treatment of hematologic malignancies, induces apoptosis and has been associated with both colitis and enteritis, with villous blunting.[88,89] While it is important for pathologists to be aware of these new drug-associated reactions, confusion with celiac disease is unlikely, with awareness of the clinical context. One report describes the development of celiac disease, with response to a gluten-free diet, following ipilimumab therapy, raising the possibility that immunomodulatory therapy could serve as a trigger to unmask celiac disease.[90]

In addition to these emerging therapies, anti-inflammatory medications, such as methotrexate, azathioprine, and mycophenolate mofetil have rarely been associated with severe villous blunting, without intraepithelial lymphocytosis.[91–93]

Summary and Recommendations

Increased intraepithelial lymphocytes with or without villous blunting is an injury pattern with many causes (Table 2).

- In order to avoid the over or under-diagnosis of celiac disease pathologists and clinicians should be aware of the differential diagnostic considerations for inflammatory changes seen in duodenal biopsies.
- The correlation of histologic findings in duodenal biopsies with patient demographics, symptoms, medication use, evidence of *H. pylori* infection, and laboratory data, especially serological and genetic tests for celiac disease is required for correct diagnosis (Appendix 1).

CONCLUSIONS

The diagnosis of celiac disease requires close cooperation between clinical, endoscopic, and laboratory practices. Optimizing the accuracy of diagnosis requires recognition of those crucial elements, including appropriate biopsy sampling strategies, and awareness of the differential diagnosis for increased intraepithelial lymphocytes with or without villous blunting. Clinicians must be aware that there are limitations to the histologic component of the diagnosis and should consider alternative diagnoses requiring clinical information that may not be available to the pathologist. Pathologists must be cognizant of the histologic differential diagnosis of celiac disease, and recognize the limited role of special testing, such as immunohistochemical stains, which are rarely required for the diagnosis. Informed dialogue between the specialties is crucial. This review is intended to help clinicians and pathologists with the finer points of diagnosis, suggest how different situations that include an intraepithelial lymphocytosis should be reported, and facilitate that cross-disciplinary cooperation.

ACKNOWLEDGMENTS

The authors wish to thank Drs Robert Riddell, TT Wu, Ivor Hill, and Anthony DiMarino for serving as expert reviewers of the manuscript.

Appendix 1*

Example Templates for Pathology Reports and Associated Clinical Considerations:**

1. Increased intraepithelial lymphocytes without villous blunting*:**

Duodenum, Biopsy:

> Duodenal mucosa with normal villous architecture and a patchy/diffuse (circle one) increase in intraepithelial lymphocytes, see note.

Note: Increased intraepithelial lymphocytes in the setting of normal villous architecture can be seen in patients with symptomatic or asymptomatic celiac disease. Other associations include *Helicobacter pylori* gastritis, medications (especially NSAIDs and olmesartan and related angiotensin II receptor blockers), infections and immune-mediated disorders. Correlation with celiac disease associated serologic and/or genetic tests may be considered.

Notes for clinicians:

Increased Intraepithelial Lymphocytes without villous blunting:

It is important that the clinician not over-react to this finding, nor jump to the conclusion that this represents celiac disease. The great majority of patients with this type of histology do not have celiac disease. In this situation, it is suggested that celiac serological testing be performed. If celiac-specific serology is positive and the patient has symptoms consistent with celiac disease, then a trial of a gluten-free diet is reasonable. However, if the celiac serology is positive, and the patient lacks symptoms, then the initial conclusion is that of potential celiac disease. This can occur, for example, when a family is screened for celiac disease. Patients in this category are at risk for developing celiac disease in the future and should undergo careful follow-up over time.

If celiac serology is negative, other specific associations with this biopsy finding should be considered (Table 2). The most common of these are *H. pylori* gastritis, drugs (such as NSAIDS and sartan-related agents) and small intestinal bacterial overgrowth. Obtaining a gastric biopsy at the time of the initial duodenal biopsy is helpful to evaluate for the presence of *H. pylori*-associated gastritis. An equally important consideration in the setting of negative celiac serology is the possibility that the patient may have been on a low gluten diet at the time of biopsy, a history that should be sought.

If all of these potential causes are ruled out and no other explanation can be found for the finding of increased duodenal intraepithelial lymphocytes, it may be reasonable to undertake HLA testing for celiac susceptibility genes. If HLA testing is negative, then celiac disease is effectively excluded. If the HLA is permissive for celiac disease then a trial of gluten exclusion may be tried for symptom benefit, though the likelihood of celiac disease is not high. Finally, it should be acknowledged that no specific association may be found for the histologic changes in some patients.

2. Increased intraepithelial lymphocytes with villous blunting:

Duodenum, Biopsy:

> Duodenal mucosa with partial/complete (circle one) villous blunting and increased intraepithelial lymphocytes, see note.

Note: The findings suggest celiac disease in the appropriate clinical setting. Other associated conditions include medication injury (especially olmesartan and related angiotensin II receptor blockers), infections and immune-mediated disorders. Correlation with celiac disease associated serological and/or genetic studies is suggested.

Notes for clinicians:

Increased intraepithelial lymphocytes with villous blunting:

If celiac-specific serology is positive in this circumstance, a presumptive diagnosis of celiac disease can be made, and a gluten-free diet initiated with a follow-up examination. If these patients are seronegative and are on a gluten-containing diet, other causes for these findings should be evaluated (Table 2). If no other cause is apparent, HLA susceptibility testing for celiac disease should be undertaken and, if positive, treatment with a gluten-free diet is the next diagnostic test. If the HLA type is negative, celiac disease is ruled out.

In the rare circumstance in which villous blunting *without* increased intraepithelial lymphocytosis is reported, celiac serology should be performed despite the high probability that it will be negative. In that case, other sources of enteritis should be sought, including drug injuries, autoimmune enteropathy and other associations, (Table 2). A final consideration for the situation of villous blunting without intraepithelial lymphocytosis is the patient with a history of treated celiac disease, in which case follow-up is determined by symptoms.

*Appendix 1 summarizes practical suggestions for evaluating patients and samples when a diagnosis of celiac disease is suspected. These comments represent the collective opinions of the authors. They should not be considered absolute rules for practice, but rather examples of how reporting and clinical follow-up may be approached.

**Named classification systems to describe villous blunting may be used if understood by both clinician and pathologist. Otherwise, the descriptive terms embedded in these systems may be used to communicate that villi are normal, are of reduced height, or are flat.

***In the setting of normal villous architecture, it is useful to distinguish between a focal or patchy increase in intraepithelial lymphocytes and a diffuse increase across all villi in the biopsy fragments. The former is less likely to

represent untreated celiac disease in patients on a gluten-containing diet. This distinction gives clinicians additional, nuanced information as they interpret the report and consider the need for further testing. Providing exact numbers of intraepithelial lymphocytes in the pathology report is not necessary in our opinion.

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