The Clinical Significance of Duodenal Lymphocytosis With Normal Villus Architecture

Suntrea T. G. Hammer, MD; Joel K. Greenson, MD

• Context.—The finding of increased intraepithelial lymphocytes with normal villous architecture (Marsh I lesion) is seen in up to 3% of duodenal biopsies. The differential diagnosis includes a wide range of possibilities, including celiac disease, bacterial overgrowth, nonsteroidal antiinflammatory drug damage, reaction to Helicobacter pylori infection, tropical sprue, and chronic inflammatory bowel disease.

Objectives.—To highlight the histologic features of the Marsh I lesion, review the diseases and conditions associated with that finding, and to provide pathologists with a rationale and a template for how to identify and report such cases.

The full significance of intraepithelial lymphocytosis in duodenal biopsies remains an enigma to both pathologists and clinicians. Intraepithelial lymphocytes (IELs) can be identified in normal duodenal mucosa as part of the normal host-defense system. However, a number of symptomatic patients biopsied each year show more IELs, which have been described in association with a number of disease conditions, most notably, celiac disease. Separating celiac disease from other diseases associated with increased IELs is difficult.

Celiac disease affects approximately 1% of the US population. Clinically, the disease leads to malabsorption and nutritional deficiencies. Prolonged involvement of the small intestine by celiac disease can lead to the development of enteropathy-associated T-cell lymphoma and other malignancies.1 The changes of increased IELs with normal villous architecture may represent early celiac disease. Recognition of this by pathologists and clinicians may lead to earlier therapeutic intervention, which would hopefully prevent the long-term complications of celiac disease. These histologic findings are common and occur in 1% to 3% of duodenal biopsies.2 Here, we discuss the relevance of these histologic changes, the ability to distinguish those associated with celiac disease from other diseases, and the general approach to signing out these cases.

Data Sources.—A review of the literature regarding the histologic features and clinical associations of Marsh I lesions.

Conclusions.—Marsh I lesions are a nonspecific finding associated with a number of disease conditions. Historically, between 9% and 40% of cases have been shown to represent celiac disease. Current data do not suggest histologic features to differentiate between diseases associated with this histologic change.

HISTOLOGY OF THE SMALL INTESTINE

Small intestinal mucosa has the same general architecture throughout. The mucosa is made up of villi, which extend above the surface mucosa, and the crypts of Lieberkühn, which extend below the surface. The normal small intestinal mucosa has a villous to crypt ratio of around 3:1 in mucosal biopsy samples.3 When assessing the villous to crypt ratio, several histologic changes must be taken into consideration. Blunting and distortion of villi may be observed in the duodenal bulb, secondary to expanded Brunner glands. Similar villous changes can be seen in the terminal ileum from Peyer patches. Additionally, poor orientation can affect the appearance of the ratio, so care must be taken to evaluate properly oriented sections.

The villous epithelium is composed primarily of absorptive cells with occasionally interspersed goblet cells. Between the epithelial cells is a normal population of CD3+ IELs, which are normally present at a level of 4 IELs per 20 epithelial (EP) cells, or 20 IELs/100 EP cells.5,6 The level of lymphocytosis may be increased in regions overlying intestinal lymphoid aggregates, areas that should be avoided when performing lymphocyte counts.

MARSH-OBERHUBER CLASSIFICATION SCHEME

The histologic changes seen in duodenal and jejunal biopsies of celiac disease are within a spectrum of inflammation levels and architectural distortions that often occur over time.7,8 In the modified Marsh grading system, there are 5 main categories of severity from 0 to 4.9 Type 0 represents normal small intestinal mucosa with less than 40 IELs/100 EP. Type 1, or the Marsh I lesion, has normal villous architecture with increased IELs (>40 IEL/100 EP cells) (Figure). Type 2 lesions also have a normal villous architecture with increased IELs; however, type 2 also has crypt hyperplasia. The type 3 lesions (destructive lesions) all

Accepted for publication May 5, 2013.
From the Department of Pathology, University of Michigan Health System, Ann Arbor.
The authors have no relevant financial interest in the products or companies described in this article.
Presented in part at the New Frontiers in Pathology: An Update for Practicing Pathologists meeting: Homestead Resort; August 3–5, 2012; Glen Arbor, Michigan.
Reprints: Suntrea T. G. Hammer, MD, Department of Pathology, University of Michigan Health System, 1301 E Catherine St, 4211 MS1, Ann Arbor, MI 48109 (e-mail: sgoudeau@med.umich.edu).

Clinical Significance of Marsh I—Hammer & Greenson

1216 Arch Pathol Lab Med—Vol 137, September 2013
show some level of a villous blunting and are further subdivided into types 3a, 3b, and 3c. Type 3a lesions have mild villous blunting, type 3b lesions have marked villous blunting, and type 3c lesions have a flat mucosal surface. These type 3 lesions also have increased IELs as well as crypt hyperplasia. Type 4 lesions (hypoplastic lesions) tend to occur in children and are characterized by flattened mucosa with normal crypt height and normal levels of IELs.

The Marsh I lesion is the earliest recognizable, histologic abnormality in patients with celiac disease. It is also the most difficult to recognize. The histologic changes include an increase in lamina propria cellularity, primarily plasmacytic, with an appreciable increase in villous IELs. Although histologic evaluation for diagnosis tends to be subjective, there are defined levels of lymphocytosis that are considered normal, which varies based on the histologic preparation. The reference range for IELs set by Marsh and Oberhuber of 40 IELs/100 EP cells was based on hematoxylin-eosin (H&E) 7-μm-thick sections. Veress et al studied H&E-stained, 3-μm-thick sections and found the mean number of IELs plus 3 SD to be 20 IELs/100 ep. Other authors have found similar numbers. Hayat et al reported an upper level of the reference range to be 25 IELs/100 EP cells (mean [2 SD]) in 4-μm-thick, H&E sections. Jarvinen et al found a level of 4.2 IELs/20 EP cells at the villus tips to distinguish between patients with probable celiac disease and controls, which included patients with dyspepsia and nonceliac intestinal disease. In our own data, we found that patients without celiac disease had lower levels of IELs (range, 0–4.3 IELs/20 EP cells at the villus tips; mean [SD] = 1.9 [1.1]) than did patients with confirmed celiac disease (range, 5–17.5 IELs/20 EP cells at the villus tips; mean [SD] = 9.6 [2.3]). Kakar et al found that patients with gluten-sensitive enteropathy had increased levels of IELs; however, they were not able to demonstrate significant elevations compared with patients with immune disorders or nonsteroidal antiinflammatory drug use.

Different methods for counting IELs have been proposed. Oberhuber et al supports the use of traditional H&E staining for quantification. Other authors have explored the use of immunohistochemistry for CD3 to quantify the number of intraepithelial T cells. In general, CD3 staining identifies a slightly greater number of IELs, and correspondingly, the upper limit of the IEL reference range is slightly greater when immunohistochemistry is used. For example, Veress et al reported an upper reference range of 25 IELs/100 EP cells when using CD3 staining, compared with 20 IELs/100 EP cells by H&E analysis. Nasseri-Moghaddam et al found a higher level of lymphocytosis with 35 IELs/100 EP cells using immunohistochemistry compared with an H&E level of 34 IELs/100 EP cells in 4-μm-thick sections. We caution against using CD3 stains in ambiguous cases unless the pathologist is used to reviewing such stains in normal small bowel because the apparent increase in T cells from easy identification with chromagen may increase the IELs reported.

Specificity of the Marsh I Lesion

Although the Marsh I lesion has been recognized as an early manifestation of celiac disease, it lacks specificity. Previous studies have found that patients with Marsh I lesions have a prevalence of celiac disease ranging from 9% to 40%. Several diseases have been associated with duodenal biopsies showing normal villous architecture and increased IELs. These findings are summarized in the Table.

**Helicobacter pylori** infection is one of the more common associations seen with Marsh I lesions. Memeo et al evaluated 50 consecutive, paired gastric antral and duodenal biopsies to evaluate the level of duodenal lymphocytosis in patients with gastric infections of *H pylori*. The levels of duodenal lymphocytosis were significantly greater than it was in noninfected controls and overlapped with the reported levels seen in celiac disease (range, 3–45 IELs/100 EP cells). Monzon et al reported similar findings. The association has been further solidified by the response of the lymphocytosis to treatment in these patients. Nahon et al reported a significant decrease in the level of intraepithelial lymphocytosis 2 months after the eradication of *H pylori* infection. These data strongly support *H pylori* as a cause of Marsh I lesions.

Other infectious conditions have also been described as associated with increased IELs. A number of studies have identified associations with giardiasis, small-intestine bacterial overgrowth, and other infective enteralities.

Tropical sprue has also been associated with Marsh I lesions. Marsh et al describe increased levels of IELs in patients with tropical sprue. They noted that the lymphocytosis tended to predominate within the crypt epithelium in contradistinction to celiac disease. Usually, there is also some degree of villous blunting.

The most commonly reported drug association seen with Marsh I lesions is with nonsteroidal antiinflammatory drugs. Kakar et al reported that association in approximately 14% of his study patients with increased IELs. In our experience, 8 of our 100 patients (8%) with Marsh I lesions had nonsteroidal antiinflammatory drug use as the only known clinical association. Brown et al reported similar findings in 15% of patients and in 20% of patients using a proton-pump inhibitor.

Inflammatory bowel disease has also been associated with Marsh I lesions. Kakar et al reported a statistically significant rate of association of about 12%, which were all cases of Crohn disease. Memeo et al also reported a case of Crohn disease in a patient with increased IELs.
although that was not statistically significant. Similarly, we found 8 cases of associated inflammatory bowel disease (including both Crohn disease and ulcerative colitis cases).31 Several immunologic disorders are associated with Marsh I lesions, including Hashimoto thyroiditis, Grave disease, rheumatoid arthritis, psoriasis, multiple sclerosis, diabetes mellitus, systemic lupus erythematous, graft versus host disease, chronic variable immunodeficiency syndrome, and immunoglobulin (Ig) A deficiency.24–26

THE MARSH I LESION: DISTINGUISHING CELIAC DISEASE FROM OTHER ASSOCIATED DISEASES

Few studies have evaluated specific histologic changes that can reliably separate the early changes of celiac disease from other associated conditions. Patients with celiac disease have classically been described as having a tip lymphocytosis. Many studies examining this feature have not been able to establish this pattern as a distinguishing factor between celiac and nonceliac-associated Marsh I lesions.2,27 Goldstein et al28 found a diffuse villous pattern of lymphocytosis to be supportive of a celiac association in Marsh I lesions. This was statistically significant, although there was overlap between patients with celiac disease and other disease-associated groups. Interestingly, Memeo et al16 noted an equal tendency toward basal and diffuse villous lymphocytosis in patients with H pylori infection, further complicating the picture. Most studies do not recognize a statistically significant difference in the level of lymphocytosis between patients with celiac disease and patients with nonceliac-associated Marsh I lesions either.2,27 However, Mino et al27 was able to find a statistically significant difference in the tip to crypt IEL ratio between gluten-associated and nongluten-associated increases in IELs with higher ratios being present in patients with celiac disease. Another study, performed by Meijer et al,30 also found the disease to be patchy but found a good concordance rate between duodenal and jejunal biopsies (94% agreement), which is concordant with other research.31,32 Furthermore, he found that the remaining 6% of biopsies, although they showed different grades, would not have significantly affected clinical management. Lesions of higher severity could be found limited to either the duodenum or the jejunum. Severity of lesions also varies within different parts of the duodenum. Evans et al33 found a difference in severity between bulb biopsies and biopsies taken from the second part of the duodenum in cases of both confirmed celiac disease and newly diagnosed celiac disease. That study advocated sampling the duodenal bulb in addition to other areas of the small intestine. Discrepancies in histologic severity can exist between different intestinal sites within the same patient.

The Diagnostic Line

Patients undergo duodenal biopsies to identify a number of treatable conditions, ranging from microbial disease to autoimmune diseases. These disease categories, although very different in etiology, often have overlapping clinical symptoms. One percent to 3% of patients undergoing duodenal biopsy are found to have a Marsh I lesion, leaving both pathologists and clinicians alike to ponder the significance.3,34 The association between Marsh I lesions and celiac disease is well established. Wahab et al35 reported on a clinical study in which symptomatic patients with biopsy–demonstrated Marsh I lesions underwent trials of gluten challenge and withdrawal, followed by subsequent biopsies. The study was able to demonstrate histologic changes in 37% of patients undergoing gluten diet modification. Despite that knowledge, multiple studies have attempted without success to identify histologic features to differentiate celiac disease from other associated disease states. The specificity of the Marsh I lesion remains low. Most authors agree that the association between celiac disease and Marsh I lesions is strong enough that it needs to be mentioned in the pathology report. At our institution, these histologic changes are reported as “increased intraepithelial lymphocytes with normal villous architecture” and includes an accompanying comment mentioning the most commonly associated diseases. In our experience, these include celiac disease, H pylori gastritis, nonsteroidal antiinflammatory drug use, bacterial overgrowth, tropical sprue, and certain autoimmune diseases. In most cases, a clinical association is never identified. However, this diagnostic approach provides enough information to allow

---

**Table 1. Disease Associations**

<table>
<thead>
<tr>
<th>Disease Associations</th>
<th>Kakar et al,2 2003, No. (%), n = 43</th>
<th>Mahadeva et al,4 2002, No. (%), n = 14</th>
<th>Hammer et al,11 2010, No. (%), n = 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celiac disease</td>
<td>4 (9)</td>
<td>3 (21)</td>
<td>18 (18)</td>
</tr>
<tr>
<td>Tropical sprue</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Bacterial overgrowth</td>
<td>2 (5)</td>
<td>0 (0)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>NSAID use</td>
<td>6 (14)</td>
<td>0 (0)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>5 (12)</td>
<td>0 (0)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>Autoimmune conditions</td>
<td>6 (14)</td>
<td>0 (0)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Infection</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>3 (7)</td>
<td>3 (21)</td>
<td>26 (26)</td>
</tr>
<tr>
<td>Irritable bowel syndrome</td>
<td>4 (9)</td>
<td>2 (14)</td>
<td>20 (20)</td>
</tr>
<tr>
<td>Other</td>
<td>12 (28)</td>
<td>6 (43)</td>
<td>4 (4)</td>
</tr>
</tbody>
</table>

Abbreviation: NSAID, nonsteroidal antiinflammatory drugs.
the gastrointestinal to provide further work for the patient and to avoid missing potential cases of celiac disease. There are other important factors. A diagnosis of celiac disease involves not only histologic evaluation but also clinical signs and symptoms, serologic testing, and the clinical response to diet modification. Immunoglobulin A deficiency can often accompany a diagnosis of celiac disease. In patients with IgA deficiency, results from several of the serologic tests used to diagnose celiac disease may be negative, putting a higher emphasis on histologic findings. Notably, the current American Gastroenterological Association guidelines currently support tissue transglutaminase IgA antibodies as first-line serologic testing in patients with suspected celiac disease. Antibody results can also be lower or negative in patients with less-destructive histologic lesions. When dealing with a Marsh I lesion, negative or low-level antibody titer findings do not exclude a diagnosis of celiac disease.

In summary, duodenal lymphocytosis with normal villous architecture can be seen in a variety of conditions. It is important for the pathologist to recognize this relatively subtle diagnosis because it can have important implications for the patient.

References