Villous Blunting in Small Bowel Biopsies: Would the Real Celiac Disease Please Stand Up?

Rhonda K. Yantiss, M.D.
Professor of Pathology and Laboratory Medicine
Department of Pathology and Laboratory Medicine
Weill Cornell Medicine, New York, New York, USA

Challenges to Duodenal Biopsy Interpretation

- Clinical issues
  - Not enough, if any, clinical information
    - Rule out sprue, abdominal pain
- Technical issues
  - Poor tissue orientation
  - Small biopsy or too few tissue fragments
- Interpretive issues
  - Difficulty understanding spectrum of normal
  - Extensive differential diagnosis for non-specific findings

Who Gets a Duodenal Biopsy?

- Diarrhea
  - With or without malabsorption
- Iron deficiency anemia
- GI hemorrhage
- Immunodeficiency
  - HIV, bone marrow/solid organ transplant, others
- Inflammatory bowel disease
- Anyone getting upper endoscopy
  - Reflux, gastritis, H. pylori, pain, dyspepsia
Where Do We Biopsy...and How Many Samples Do We Need?

• Most studies suggest at least 4 samples
  • 5 is probably better with 1 of the duodenal bulb
• 10% of patients with celiac disease have involvement of bulb only
• 10% of patients with celiac disease have normal duodenal bulb biopsies and diagnostic changes more distally
• Probably best to biopsy bulb and more distal duodenum, and submit samples in different containers


Adequate Understanding of Clinical Context

• Close communication and working relationship with clinicians
• Knowledge of serologic study results
• Ideally receive endoscopy reports with images
• Background clinical information
  • Requisition doesn’t hold all the information
  • A phone call may be the best ancillary test

37-year-old female with candidal esophagitis and nodular duodenum
Intraepithelial lymphocytes
Chronic duodenitis with partial villous shortening and increased intraepithelial lymphocytes

Note: The findings are compatible with a diagnosis of celiac disease in the appropriate clinical setting. Other diagnostic considerations include immune-mediated disorders, infection, and a medication-related injury.
At which time the gastroenterologist said, “Well you know the patient has HIV and AIDS. I thought you would guess that when I told you that she had candidal esophagitis” (which, by the way, he didn’t biopsy).

Tip: Suboptimal tissue preservation can lead to suboptimal diagnoses

Adequate Tissue Sample

- Three to four intact well-oriented villi in a row
- Avoid stripped villi
- Poorly oriented villi may look blunted
- Orient samples on mesh or filter
- Must include samples distal to duodenal bulb
- Peptic injury
Normal villous: crypt ratio is 3:5:1
easily identifiable goblet cells and Paneth cells
one or two mitotic figures per crypt

Normal duodenum
- Lymphocytes
- Plasma cells
- Macrophages
- Eosinophils
  - Acceptable as long as they are not in epithelium
- Neutrophils
  - Acceptable as long as they are not in epithelium

Absorptive cells and goblet cells in villi and crypts
- Up to 20-25 lymphocytes per 100 epithelial cells
  - More numerous on sides of villi with relative sparing of tips
Jejunum similar to duodenum, but villi are slightly broader at tips.

Ileal villi tend to be taller, goblet cells more numerous.

Villi blunted overlying lymphoid aggregates.
Intraepithelial inflammation over lymphoid aggregates is also normal.

Stretched fragments can simulate loss of villi.

Stripped villi look flat.
Duodenal Bulb Biopsies

- Prone to difficult interpretation
- Brunner gland hyperplasia
- Heterotopias
- Peptic injury is common (up to 80%)

Villi are broad and short overlying Brunner glands
Gastric heterotopia

Note intraepithelial lymphocytes over aggregate

Peptic duodenitis: Gastric mucous cell metaplasia

Intraepithelial lymphocytes

Peptic duodenitis: Neutrophils may be numerous

Foveolar cell metaplasia
Challenges to Biopsy Interpretation

Summary of Issues

• Artifacts simulate enteritis and villous abnormalities
• Small bowel histology is site dependent
• Villous morphology varies across populations
  • Pediatric biopsies show more crypt crowding and more goblet cells
  • Villous height varies with geography: shorter in tropics
• Brunner glands, gastric heterotopias, and lymphoid follicles cause villous blunting
• Be careful interpreting changes limited to duodenal bulb-they often don’t mean much

Case

• 60-year-old man with unexplained, severe diarrhea
• Requisition
  • Rule out IBD, microscopic colitis, celiac disease, amyloid, Whipple disease, etc.
• Endoscopic findings
  • Mild congestion of colonic and gastric mucosae
  • Nodular duodenal mucosa with some scalloping of folds throughout
• 60-year-old man with unexplained, severe diarrhea
• Requisition
  • Rule out IBD, microscopic colitis, celiac disease, amyloid, Whipple disease, etc.
• Endoscopic findings
  • Mild congestion of colonic and gastric mucosae
  • Nodular duodenal mucosa with some scalloping of folds throughout
Scalloped mucosal folds

Duodenum is flat and blue
Duodenal biopsy: Increased lamina propria plasma cells with blunt villi.

Antral biopsy: Chronic gastritis, H. pylori-negative.
Colon shows chronic colitis; crypt architecture mostly preserved.

Summary of Findings

- Diffuse villous shortening with chronic inflammation in jejunum and duodenum
- Chronic, focally active pan-gastritis with deep inflammation
- Chronic, focally active colitis without crypt architectural distortion
Differential Diagnosis
Villous Abnormalities with Lymphocytosis

- Peptic injury (mostly confined to duodenal bulb)
- Celiac disease
- Protein intolerance (non-gluten)
- Food allergies (cereals, eggs, milk, etc)
- Autoimmune enteropathy
- Common variable immunodeficiency
- Inflammatory bowel disease
- Eosinophilic gastroenteritis
- Bacterial overgrowth
- Tropical sprue
- Infection (viruses, coccidians, protozoa)
- Targeted biological agents (often immunomodulators for cancer)
- Olmesartan (and related agents)

Celiac Disease

- Genetic factors
  - Familial clustering
  - 70% concordance between identical twins
  - HLA associations (DQ2, DQ8) in almost all patients
    - Corollary: celiac disease unlikely in patients without one of these HLA types
- Environmental factors
  - Dietary gluten
  - Probably triggered by viral exposure (adenovirus type 12)
  - Emerging evidence for reovirus as a stimulus

Serologic Markers of Celiac Disease

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-gliadin</td>
<td>31-100%</td>
<td>85-100%</td>
</tr>
<tr>
<td>Anti-reticulin</td>
<td>42-100%</td>
<td>95-100%</td>
</tr>
<tr>
<td>Anti-endomysium</td>
<td>60-100%</td>
<td>95-100%</td>
</tr>
<tr>
<td>Anti-tissue transglutaminase</td>
<td>85-100%</td>
<td>92-97%</td>
</tr>
</tbody>
</table>

- Serologic tests need to be done in patients who are not on gluten restriction
- Sensitivities for all markers decrease among patients with mild/no symptoms
- None are entirely specific for celiac disease; can be spuriously elevated in other immune-mediated disorders
Celiac disease: Complete villous shortening with crypt hyperplasia

Celiac disease: Partial villous shortening

Celiac disease: Normal villous architecture with increased intraepithelial lymphocytes
Intraepithelial lymphocytes are universally present when villi are shortened.

Tip: If there aren't lots of IELs, then villous abnormalities aren't due to celiac disease.

Neutrophils may be seen when mucosa is completely flat.

IELs decrease from base to surface.

IELs evenly dispersed over villus.

IELs decrease from base to surface.
Increased Intraepithelial Lymphocytes

- Diffuse (or tip-predominant) distribution
- More than 20-25 IELs/100 enterocytes
- >12 IELs/20 enterocytes in tip of villus
- >8 CD3+/CD8+ cells per 20 enterocytes in villous tip

Do I Need Immunostains to Detect IELs?

- No
- Only >25 IELs/100 enterocytes pathologic
- No value in detecting fewer IELs
- Overestimate number
  - Lymphocytes are numerous in lamina propria
  - Those near basement membrane appear "intraepithelial" with tangential sectioning
Extra-duodenal inflammation: Lymphocytic gastritis

Extraduodenal lymphocytosis: Increased intraepithelial lymphocytes in ileum

Lymphocytic colitis

Colonic lymphocytosis

Extra-duodenal inflammation: lymphocytic colitis or colonic lymphocytosis
Recurrent Symptoms After Initial Response to Gluten Withdrawal

- Exposure to gluten (intentional or not)
- Other gastrointestinal lymphocytosis (e.g. lymphocytic colitis)
- Collagenous sprue
- Refractory celiac disease
- Development of ulcerating disease or lymphoma
Refractory Celiac Disease

- 5% of patients with celiac disease
- Type I
  - Non-clonal intraepithelial lymphocytes (CD3+/CD8+)
  - Better prognosis, but can progress to Type II
- Type II
  - Clonal intraepithelial lymphocytes
    - Aberrant phenotype (CD3+/CD8-)
    - T-cell gene rearrangements
    - Loss of staining for T-cell αβ receptor
    - 50% mortality

Celiac Disease

Summary of Features

- Intraepithelial lymphocytosis always present
- Variable villous abnormalities, crypt hyperplasia
- Lymphocytes and plasma cells predominate in lamina propria
- Eosinophils and neutrophils present in small numbers
- Neutrophilic/eosinophilic cryptitis is not prominent (especially distal to bulb)
- May be associated with generalized gastrointestinal lymphocytosis

Autoimmune Enteropathy

- Severe protracted diarrhea that may result in hospitalization (very unusual for celiac disease)
  - No response to gluten withdrawal
  - Extra-intestinal immune-mediated diseases
  - Reported associations with thymoma
  - Autoantibodies to enterocytes, goblet cells, and others (e.g. ANA)
  - More common in young children, especially males
  - Sporadic
  - Immunodysregulation, polyendocrinopathy and enteropathy, X-linked (IPEX) syndrome
  - APECED syndrome (mucocutaneous candidiasis, ectodermal dystrophy)
Diffuse, full-thickness plasma cell-rich inflammation with complete villous shortening

Autoimmune enteropathy

Intraepithelial lymphocytes not striking, especially in surface

Decreased goblet cells

Autoimmune enteropathy associated with variable villous abnormalities

Decreased goblet cells

More intraepithelial lymphocytes in crypts than surface; more inflammation in deep mucosa
Chromogranin immunostain confirms loss of endocrine cells.

Tip: Think about autoimmune enteropathy if you see crypt abscesses and apoptosis.

Decreased goblet cells and Paneth cells.

Chronic gastritis with deep inflammation and gland destruction.
Histologic Features

- **Celiac disease**
  - Surface more than crypt injury
  - IELs uniformly present in surface and crypts
  - Granulocytes are infrequent
  - Crypt abscesses rare
  - Apoptosis uncommon
  - Duodenum and proximal jejunum most severely affected

- **Autoimmune enteropathy**
  - Severe crypt injury with relative sparing of surface
  - IELs more prominent at crypt bases
  - Neutrophils readily identified
  - Crypt abscesses frequent
  - Apoptotic crypt cells common
  - Stomach and colon often affected

Common Variable Immunodeficiency

- Immunodeficiency resulting from failed plasma cell maturation
  - Absent, or decreased plasma cells
  - Plasma cells present, but non-functional
- Symptoms of malabsorption
- Chronic giardiasis and CMV
- Often affects entire gut
Common variable immunodeficiency: Complete villus shortening, crypt hyperplasia

Diffuse lamina propria inflammation

Common variable immunodeficiency

Neutrophilic abscesses

No plasma cells

Apoptosis

Cellular lamina propria with decreased, or absent, plasma cells

Crypt abscesses

Apoptosis
Common variable immunodeficiency: Normal villous architecture
Intraepithelial lymphocytes
Nearly normal cellularity of lamina propria, but no plasma cells

Common variable immunodeficiency: Variable villous abnormality
Oops! Here is a viral inclusion

Intraepithelial inflammation more prominent in crypts than surface
Lymphoid hyperplasia of the duodenum

Tip: Look for plasma cells when you see duodenal lymphoid follicles and/or Giardia.
Terminal ileum
Pyloric metaplasia

Common variable immunodeficiency mimics Crohn disease

Terminal ileum
Ulcers
No plasma cells

Patchy inflammation simulates Crohn disease
Sigmoid colon biopsy
Cryptitis with increased lamina propria cellularity, but few, if any, plasma cells

Common variable immunodeficiency
Crohn disease
Pericryptal granulomatous inflammation simulates Crohn disease

More plasma cells

Plasma cell

Common variable immunodeficiency: Prominent apoptosis is characteristic

Crypt cell apoptosis is not a striking feature of inflammatory bowel disease
A 32-year-old woman was diagnosed with ulcerative colitis. The histologic features of ulcerative colitis include diffuse lamina propria inflammation with cryptitis and basal inflammation. Infiltrate does not contain plasma cells.

**Histologic Features**

- **Celiac disease**
  - Surface more than crypt injury
  - IELs uniformly present in surface and crypt
  - Numerous lamina propria plasma cells
  - Granulocytes are infrequent
  - Crypt abscesses rare
  - Apoptosis uncommon
  - Duodenum and proximal jejunum most severely affected

- **Common variable immunodeficiency**
  - Crypt more than surface injury
  - IELs more prominent in crypts
  - Lamina propria plasma cells are decreased or absent
  - Neutrophils readily identified
  - Crypt abscesses frequent
  - Apoptotic crypt cells common
  - Stomach and colon often affected
  - CMV and/or Giardia
Crohn Disease

- Simulates celiac disease
  - Increased intraepithelial lymphocytes
  - Villous blunting
- Other features typical of Crohn disease
  - Ulcers
  - Active enteritis
  - Granulomata and giant cells
  - Metaplasia
- Most patients with duodenal disease also have ileal and colonic involvement

Tip: Ulcers are not characteristic of celiac disease

Neutrophils often more prominent than intraepithelial lymphocytes.
Eosinophilic Gastroenteritis

- 75% of patients have peripheral eosinophilia
- Negative tissue transglutaminase
- No response to gluten withdrawal
- Infiltration of one, or more segments of the GI tract, pancreas, or biliary tree
- Villous abnormalities, increased intraepithelial lymphocytes

Tip: Celiac disease doesn’t feature lots of eosinophils, especially in the epithelium

Courtesy of Dr. Laura Lamps, University of Michigan
Differential Diagnosis

**Villous Abnormalities with Lymphocytosis**

- Peptic injury (mostly confined to duodenal bulb)
- Celiac disease
- Protein intolerance (non-gluten)
- Food allergies (cereals, eggs, milk, etc)
- Autoimmune enteropathy
- Common variable immunodeficiency
- Inflammatory bowel disease
- Eosinophilic gastroenteritis
- Bacterial overgrowth
- Tropical sprue
- Infection (viruses, coccidians, protozoa)
- Targeted biological agents (often immunomodulators for cancer)
- Olmesartan (and related agents)

Stasis Syndrome

**Bacterial Overgrowth**

- Post-surgical
  - Blind loops and pouches
  - Entero-enterostomy
  - Afferent loops
  - Fistulae
  - Adhesions and partial obstructions
- Pseudo-obstruction and dysmotility
  - Small bowel diverticulosis
  - Crohn disease
  - Scleroderma

Bacterial overgrowth: Irregular villous abnormalities
Tropical Sprue

- Chronic malabsorption after episode of infectious diarrhea
- Most common in tropical regions
- Bacterial overgrowth with B12 and folate deficiency
- Responds to antibiotic therapy and vitamin supplements
- Variable biopsy changes
  - Intraepithelial lymphocytosis
  - Mild villous abnormalities

Tropical sprue generally causes duodenal lymphocytosis
Tropical sprue: More severe abnormalities in distal small bowel (ileum)

Adenovirus enteritis

Disorganized nuclei
Adenovirus enteritis

Tufted epithelium is a clue for adenovirus

Differential Diagnosis

- Peptic injury/H. pylori gastritis
- Bacterial overgrowth
- Tropical sprue
- Infection (viral, coccidian, protozoa)
- Celiac disease
- Protein intolerance (non-gluten)
- Food allergies (cereals, eggs, milk, etc)
- Common variable immunodeficiency
- Autoimmune enteropathy
- Inflammatory bowel disease
- Eosinophilic gastroenteritis
- Idelalisib
- Olmesartan and related agents
Intraepithelial Lymphocytosis
HIV Infection/AIDS

- Giardiasis
- Cryptosporidiosis
- Cystoisosporiasis
- Microsporidiosis
- Cyclosporiasis

Giardia lamblia associated with minimal inflammation and no villous abnormalities.

Giardia lamblia have teardrop or "falling leaves" appearance.

Unassociated with substantial inflammation and easily overlooked.
### Features of Enteric “Coccidians”

<table>
<thead>
<tr>
<th>Feature</th>
<th>Cryptosporidia</th>
<th>Cystoisospora</th>
<th>Microsporidia</th>
<th>Cyclospora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (µm)</td>
<td>2-5</td>
<td>15-20 (largest)</td>
<td>2-3 (smallest)</td>
<td>2-3 (smallest)</td>
</tr>
<tr>
<td>Location</td>
<td>Apical surface</td>
<td>Epithelium</td>
<td>Lumenal aspect of epithelial cells</td>
<td>Lumenal aspect of epithelial cells</td>
</tr>
<tr>
<td>Morphologic Features</td>
<td>“Blue beads on a string” at surface</td>
<td>Large, ellipsoid, banana-shaped</td>
<td>Multiple tiny round organisms in supranuclear vacuole</td>
<td>Multiple stages of life cycle</td>
</tr>
<tr>
<td>Ancillary Stains</td>
<td>Giemsa, Gram</td>
<td>Giemsa, Gram, PAS</td>
<td>Modified trichrome, Giemsa, Gram, PAS, Warthin-Starry</td>
<td>Acid fast</td>
</tr>
</tbody>
</table>

- Self-limited disease in immunocompetent patients, but more severe in immunocompromised
- Cryptosporidia and Microsporidia can disseminate
- Microsporidia are now classified as fungi
- Cryptosporidia are still parasites, but gregarines, not coccidia

### Lymphocytosis in Patients with HIV/AIDS

**Take Home Points**

- *De novo* immune-mediated disease is rare
- Be very careful with new diagnoses of celiac disease, ulcerative colitis, and Crohn disease
- Enteric coccidians usually associated with mild inflammatory changes and readily overlooked
  - Apoptosis, nuclear disarray in surface, vacuoles in superficial cytoplasm
  - Cryptosporidia most common and associated with greatest degree of lymphocytosis
  - Cystoisospora are largest, simulate degenerated nuclei
  - Microsporidia simulate goblet cells
Differential Diagnosis

Villous Abnormalities with Lymphocytosis

- Peptic injury (mostly confined to duodenal bulb)
- Celiac disease
- Protein intolerance (non-gluten)
- Food allergies (cereals, eggs, milk, etc.)
- Autoimmune enteropathy
- Common variable immunodeficiency
- Inflammatory bowel disease
- Eosinophilic gastroenteritis
- Tropical sprue
- Infection (viruses, coccidian, protozoa)
- Targeted biologic agents (often immunomodulators for cancer)
- Olmesartan (and related agents)

Medications/Treatment-Related Injury

- NSAIDs
- Graft versus host disease
- Mycophenolate
- Chemotherapy/Radiation
- Targeted biologic agents (-ib, -ab)
  - Idelalisib (inhibits PI3Kδ-mediated signaling)
  - Ipilimumab (inhibits CTLA-4-mediated immune tolerance)
  - Pembrolizumab (inhibits PD-L1-mediated immune tolerance downstream to CTLA-4)
- Olmesartan (Benicar) and related compounds
Idelalisib-related injury

Completely flat mucosa

Intraepithelial lymphocytes

Apoptotic crypt cells
Olmesartan Enteropathy

- Angiotensin II receptor antagonist (anti-hypertensive)
- Severe chronic diarrhea; may require hospitalization
- Serologic studies generally normal
- Mimic of celiac disease and autoimmune enteropathy
  - May affect stomach and colon as well
  - Culprit in collagenous “-itis” throughout GI tract

Photograph courtesy of Dr. Laura Lamps, University of Michigan

Sprue-like lesion
Olmesartan-induced colonic injury mimics IBD

Collagen deposits

Apoptosis

Olmesartan-induced gastropathy
Tip: Think about olmesartan when you see collagenous "itis" anywhere in GI tract

Back to the Case

- Multifocal involvement of GI tract
- Diffuse inflammation with neutrophils and numerous apoptotic epithelial cells
- Surface intraepithelial lymphocytosis minimal
- HLA haplotypes don’t fit for celiac disease
- Plenty of plasma cells
- Goblet cells and Paneth cells present
- Taking olmesartan

Olmesartan-Induced Enteropathy
The Flat, Blue Duodenal Biopsy Sample

**Take Home Points**

- The first, and most reliable, change of celiac disease is intraepithelial lymphocytosis
- Most patients with negative serologies and no response to gluten withdrawal do not have celiac disease
- Disorders of altered immunity show similar features
  - Loss of goblet cells, Paneth cells, endocrine cells, and apoptosis suggest autoimmune enteropathy
  - Common variable immunodeficiency shows decreased or absent plasma cells
- Medications can mimic immune-mediated disorders—get history in all adult patients
  - Apoptosis, neutrophils, and lymphocytes are clues