The pathologist’s role in the management of coeliac disease

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Coeliac disease

- coeliac disease (CD) is a gluten-sensitive enteropathy characterised by villous atrophy, which is reversed by gluten withdrawal

- genetically susceptible individuals (99% HLA DQ2 or DQ8)

- classical presentation: steatorrhoea, weight loss or other signs of nutrient or vitamin deficiency

- iron deficiency (anaemia) is the commonest presenting feature in the UK

- coeliac disease may be clinically occult and may not be detected until late adulthood

- a strict GFD diet reduces the risk of malignancy to that of the general population

TABLE 1. Causes of iron deficiency anaemia with prevalence as percentage of total\(^a\)

<table>
<thead>
<tr>
<th>Occult GI Blood Loss</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Common</td>
<td></td>
</tr>
<tr>
<td>• Aspirin/NSAID use</td>
<td>10–15%</td>
</tr>
<tr>
<td>• Colonic carcinoma</td>
<td>5–10%</td>
</tr>
<tr>
<td>• Gastric carcinoma</td>
<td>5%</td>
</tr>
<tr>
<td>• Benign gastric ulceration</td>
<td>5%</td>
</tr>
<tr>
<td>• Angiodysplasia</td>
<td>5%</td>
</tr>
<tr>
<td>Uncommon</td>
<td></td>
</tr>
<tr>
<td>• Oesophagitis</td>
<td>2–4%</td>
</tr>
<tr>
<td>• Oesophageal carcinoma</td>
<td>1–2%</td>
</tr>
<tr>
<td>• Gastric antral vascular ectasia</td>
<td>1–2%</td>
</tr>
<tr>
<td>• Small bowel tumours</td>
<td>1–2%</td>
</tr>
<tr>
<td>• Ampullary carcinoma</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>• Ancylomasta duodenale</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Malabsorption</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td></td>
</tr>
<tr>
<td>• Coeliac disease</td>
<td>4–6%</td>
</tr>
<tr>
<td>• Gastrectomy</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>• H. pylori colonisation</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Uncommon</td>
<td></td>
</tr>
<tr>
<td>• Gut resection</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>• Bacterial overgrowth</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-GI Blood loss</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td></td>
</tr>
<tr>
<td>• Menstruation</td>
<td>20–30%</td>
</tr>
<tr>
<td>• Blood donation</td>
<td>5%</td>
</tr>
<tr>
<td>Uncommon</td>
<td></td>
</tr>
<tr>
<td>• Haematuria</td>
<td>1%</td>
</tr>
<tr>
<td>• Epistaxis</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

\(^a\)Figures may not add up to 100% due to overlap of causes.
Management of iron deficiency anaemia

BSG guidelines, 2005
Coeliac disease epidemiology

- prevalence of the disease increases as you go north and west in Europe
- very common in the Irish and in those of Irish descent.
- about 1% of population
- genetic gradients, largely determined by the advance of agriculture and historical patterns of cereal ingestion in Europe
Pathology and the diagnosis of coeliac disease

- coeliac disease is NOT a histological diagnosis

- BUT histology is (still) the gold standard and is required to support other features
  - anti-gliadin antibodies
  - anti-endomysial antibodies (EMA)
  - anti-tissue transglutaminase antibodies (TTG)
  - HLA-DQ2 and/or DQ8
  - response to gluten exclusion
  - gluten challenge

- your report should only say ‘in keeping with untreated coeliac disease’ as the disease has many histopathological mimics
## Serology for coeliac disease

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>tissue transglutaminase (TTG)</td>
<td>90-95%</td>
<td>50-95%</td>
<td>IgA Good screen for CD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Depends on lab and titre cut-off</td>
<td></td>
</tr>
<tr>
<td>anti-endomysial antibody (EMA)</td>
<td>90-95%</td>
<td>95%</td>
<td>IgA but really a crude TTG – needs very high titre</td>
</tr>
<tr>
<td>anti-gliadin antibody</td>
<td>90% if IgA</td>
<td>80%</td>
<td>Depends on whether IgG or IgA measured</td>
</tr>
</tbody>
</table>

*Lock et al, 2004*  
*Many thanks to Dr Joe Unsworth*
Serology for coeliac disease

- screen by TTG

- if positive, do EMA

- if positive, proceed to duodenal biopsy

- in UK, often duodenal biopsy is initial step (either that or we don’t get told the results of serology!!)

- duodenal biopsy is the gold standard and things may go awry if CD is not so confirmed
Duodenal biopsy for ‘malabsorption’

- coeliac disease/gluten enteropathy/gluten sensitivity
- giardiasis
- other infections +/- immunodeficiency
- Whipple’s disease
- chronic inflammatory bowel disease
- other rare causes of malabsorption (especially in children)
- food allergy
- bacterial overgrowth
- ‘mechanical’ causes of villous atrophy and inflammation
- auto-immune enteropathy
Can villous atrophy be diagnosed endoscopically and/or macroscopically?
Current biopsy recommendations in the UK

Biopsy of D2 or beyond remains the gold standard for the diagnosis of coeliac disease as serological tests are neither 100% specific nor sensitive. There are national and international recommendations which indicate that four “good-sized” biopsies are taken from D2 or beyond as the histopathological changes of coeliac disease can be strikingly focal.

‘Histopathology/Cytopathology of limited or no clinical value’
How to biopsy the duodenum – traditional teaching

- four good-sized biopsies from the second or third part of the duodenum
- reflecting variability of changes seen in coeliac disease and other disorders
- the fact that confounding features can be seen (esp HP/peptic duodenitis) in bulb/D1
Duodenal biopsy for coeliac disease - new concepts

- 665 children & adolescents with proven CD
- multiple duodenal biopsies (1 D1, 3-4 more distally)
- ALL CD patients had CD type changes in D1
- 16 had normal biopsies more distally
- 20 had patchy lesions more distally
- no D1 lesions in 348 non-CD subjects
- recommend 2 biopsies from bulb and 2 more distally, especially in children

Duodenal biopsy for coeliac disease - new concepts

• villous atrophy may be present only in the duodenal bulb

• the optimal assessment of patients in whom coeliac disease is suspected (with positive serology) and those with established coeliac disease requires one or more duodenal bulb biopsy in addition to distal duodenal biopsies


• the disease is notably patchy and the 9 or 12 o’clock position in the duodenal bulb may be the optimal biopsy site

An audit of duodenal biopsies

- Gloucester, UK audit 2007 – four 3mm biopsies was the gold standard
  - 415 duodenal biopsies
  - biopsy numbers: mean 2.51 (range 1-6);
  - total biopsy ‘volume’: mean 7.49 (range 2-24);
  - ‘mean’ biopsy size 2.99
  - only 35% fulfilled national recommendations

- Let’s make our lives easier by ensuring adequate number, size and site of duodenal biopsies
Normal small intestinal mucosa

- villous height: crypt depth: $3:1$
- subepithelial collagen plate: $<10\mu$
- IELs /100 epithelial cells: $<25$
Normal small intestinal mucosa

- number of intra-epithelial lymphocytes
- formerly 40 per 100 epithelial cells
- then 30 per 100 epithelial cells
- now 25 per 100 epithelial cells in the UK
Gloucestershire Cellular Pathology Laboratory
Normal decrescendo pattern of IELs
Tip-heavy lymphocytosis


Goldstein NS. Histopathology 2004; 44: 199-205

Villous tip count method: > 6 per 20 enterocytes in 5 villi

Duodenal biopsy artefacts

distortion

poor orientation

Gloucestershire Cellular Pathology Laboratory
Small intestine: duodenal biopsy

Gloucestershire Cellular Pathology Laboratory
An algorithm for the diagnosis of coeliac disease

1. anti-TTG IgA antibody serology as an initial screen

2. anti-EMA IgA antibody serology as a further test in those who are anti-TTG-positive

3. duodenal biopsy in those who are anti-TTG and anti-EMA positive
Coeliac disease, serology and histopathology

• the changes of coeliac disease may be evident on duodenal biopsy when serology is negative: the changes tend to be less severe

• pathological features of CD return to normal more slowly than serology, following introduction of GFD, especially in adults

• in biopsies, patients with CD may show active inflammation (neutrophil polymorphs): this does not exclude coeliac disease
The morphological spectrum of coeliac disease

To many clinicians and to many pathologists, this, and only this, is untreated coeliac disease
The morphological spectrum of coeliac disease

Marsh recognised five types of mucosal lesion:

Type 0  pre-infiltrative lesion: normal histological appearances

Type 1  infiltrative lesion: intra-epithelial lymphocytosis with normal villous and crypt architecture

Type 2  crypt hyperplastic lesion: intra-epithelial lymphocytosis with normal villous architecture but with crypt hyperplasia

Marsh MN, 1992
The morphological spectrum of coeliac disease

Marsh recognised five types of mucosal lesion:

Type 3 destructive lesion:
villous atrophy (partial/complete) with intra-epithelial lymphocytosis and crypt hyperplasia.

Type 4 hypoplastic lesion:
total villous atrophy and crypt hypoplasia (+/- collagenous sprue)

Marsh MN, 1992
Gluten-Sensitive Enteropathy (Celiac Disease)

Controversies in Diagnosis and Classification

Arzu Ensari, MD, PhD

Table 2. Classification Schemes For Pathologic Evaluation of Gluten-Sensitive Enteropathy

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Type 1</td>
<td>Type 1</td>
<td>Grade A</td>
<td>Type 1</td>
</tr>
<tr>
<td>Type 2</td>
<td>Type 2</td>
<td>Grade A</td>
<td>Type 1</td>
</tr>
<tr>
<td>Type 3</td>
<td>Type 3A</td>
<td>Grade B1</td>
<td>Type 2</td>
</tr>
<tr>
<td></td>
<td>Type 3B</td>
<td>Grade B1</td>
<td>Type 2</td>
</tr>
<tr>
<td></td>
<td>Type 3C</td>
<td>Grade B2</td>
<td>Type 3</td>
</tr>
<tr>
<td>Type 4</td>
<td>Type 4</td>
<td>obsolete</td>
<td>obsolete</td>
</tr>
</tbody>
</table>
The variation in the changes of coeliac disease
Marsh type 1 lesion – normal villi but raised intra-epithelial lymphocytic infiltrate (well above 25 per 100 epithelial cells)
Marsh type 3 lesion – destructive lesion: villous atrophy (partial/complete) with intra-epithelial lymphocytosis and crypt hyperplasia
The pathology of coeliac disease

• variable & severity (Marsh type) does not necessarily correlate with clinical features & serology

• what is a normal IEL count? It may not be less than 25 per 100 epithelial cells in countries with high rates of enteric infection, etc

• pathology not always consistent between & within biopsy sets

• requires correlation with clinical data (if we get any....) and serology (if we get these results: in our audit we got these in 6% of cases)

• biopsies from patients with well-treated coeliac disease may be normal. Review the index diagnostic biopsy if there is any doubt regarding the diagnosis
The pathology of coeliac disease

• never has CONTEXT been more important as many conditions mimic many of the five Marsh types of CD and several can mimic classical CD:

  ▪ other food intolerances, especially in children
  ▪ auto-immune enteropathy
  ▪ tropical sprue
  ▪ post-infectious gastroenteritis
  ▪ stasis syndrome
  ▪ kwashiorkor

• reports should read ‘consistent with a diagnosis of..’
Duodenal intra-epithelial lymphocytosis with normal villous architecture (lymphocytic duodenosis)

- by definition greater than 25 IELs per 100 ECs (in Western Europe)
- 1.3% and 2.2% of proximal small intestinal biopsies had this finding in two studies
Duodenal biopsies in a large general hospital in the UK

913 duodenal biopsies

- anaemia 496 (55%)
- weight loss and/or malabsorption 179 (20%)
- dyspepsia 56 (6%)
- change in bowel habit 57 (6%)
- other 127 (13%)

- normal 776 (85%)
- in keeping with coeliac disease 63 (7%)
- ↑IEL with preservation of villous architecture 20 (2%)
- acute/chronic duodenitis 26 (3%)
- gastric heterotopia 6 (<1%)
- Whipple’s disease 1 (<1%)
- giardiasis 6 (<1%)
- other 12 (1%)

Williams JW, Shepherd NA: Gloucestershire audit, 2008
Lymphocytic duodenosis/intra-epithelial lymphocytosis with normal villous architecture/Marsh type 1 lesion

<table>
<thead>
<tr>
<th>study</th>
<th>how CD was diagnosed</th>
<th>how many were CD?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kakar et al, 2003</td>
<td>+ve EMA response to GFD</td>
<td>9%</td>
</tr>
<tr>
<td>Goldstein et al, 2001</td>
<td>progression to VA +/- +ve EMA</td>
<td>15%</td>
</tr>
<tr>
<td>Dilworth et al, 2005</td>
<td>+ve TTG, AGA</td>
<td>15%</td>
</tr>
<tr>
<td>Wahab et al, 2001</td>
<td>progression to VA response to GFD</td>
<td>30%</td>
</tr>
<tr>
<td>Mahadeva et al, 2002</td>
<td>+ve EMA or unexplained anaemia</td>
<td>40%</td>
</tr>
</tbody>
</table>
Associations/causes of intra-epithelial lymphocytosis with normal villous architecture (lymphocytic duodenosis)

- gluten sensitivity
  Marsh type 1 lesion
  patients on a gluten-free diet

- infection
  viral – especially norovirus
  parasites – giardiasis, cryptosporidiosis, cyclosporiasis
  HIV infection

Associations/causes of intra-epithelial lymphocytosis with normal villous architecture (lymphocytic duodenosis)

- asymptomatic 1st degree relatives of coeliac disease patients
  - 15% have subtotal villous atrophy (half with normal nutritional tests)
  - 25% have intra-epithelial lymphocytosis

- dermatitis herpetiformis
  - 40% have flat mucosa (half asymptomatic)
  - 40% have intra-epithelial lymphocytosis
Associations/causes of intra-epithelial lymphocytosis with normal villous architecture (lymphocytic duodenosis)

- HP
  can cause problems if biopsies are from the proximal duodenum
  IELs are unusual BUT possible cause in 25% of cases in one series
  
  Dilworth et al, 2005

- auto-immune disease
  Hashimoto’s thyroiditis, Graves’ disease, rheumatoid arthritis, SLE, type 1 diabetes, ankylosing spondylitis, autoimmune enteropathy

- non-gluten food hypersensitivity
  cow’s milk, soy products, fish, rice, chicken

- drugs
  especially NSAIDs, proton pump inhibitors & chemotherapeutic agents
Associations/causes of intra-epithelial lymphocytosis with normal villous architecture (lymphocytic duodenosis)

• hypogammaglobulinaemic sprue
  IgA deficiency, CVID

• irritable bowel syndrome

• inflammatory bowel disease
  both UC and CD, especially the latter (10% in one series – Dilworth et al, 2005)

• tropical sprue
Associations/causes of intra-epithelial lymphocytosis with normal villous architecture (lymphocytic duodenosis)

- bacterial overgrowth
- glomerulonephritis
  especially nephrotic syndrome
- idiopathic
  includes “diffuse lymphocytic gastro-enteropathy”
How many cases of intra-epithelial lymphocytosis with normal small intestinal villous architecture represent a ‘mild’ form of coeliac disease (Marsh type 1 lesion)?

- this depends on how coeliac disease is defined
- those five studies (and more) contain data
- range 9% to 40% (9%, 15%, 15%, 30%, 40%)
- it is likely that 9% is an underestimate and 40% is an overestimate - probably around 20-30%
Why should we detect the gluten sensitivity cases in patients with duodenal intra-epithelial lymphocytosis?

- even without villous atrophy, patients can develop significant nutritional deficiencies leading to anaemia (usually iron deficiency related) and osteoporosis at a young age
- risk of developing intestinal T cell lymphoma
- risk of patient or first degree relative developing other malignancies
Can the histopathologist suggest gluten sensitivity is the most likely diagnosis?

- a uniform pattern of intra-epithelial lymphocytosis in the villous tip is more suggestive of gluten sensitivity than the other causes.

- the loss of the ‘decrescendo sign’

- this is not absolute (25% of non-gluten sensitive cases also show this finding)

*Goldstein, 2004*
Collagenous sprue

- 12 cases (4 males), 41-84 yrs
- 4 typical coeliac disease in the past & 4 had positive coeliac serology
- seven had collagenous colitis and three collagenous gastritis
- clonal T cell population in 5/6 tested
- five improved on GFD + immunosuppression
- one resolved completely

Coeliac disease & enteropathy-associated T cell lymphoma

- refractory coeliac disease
- ulcerative jejunitis
- cavitation of mesenteric lymph nodes
- may arise ‘de novo’ or complicate coeliac disease
- most cases HLA-DQ2 & DQ8 coeliac-associated antibodies

Refractory coeliac disease

persistent malabsorptive symptoms and villous atrophy despite strict adherence to a gluten-free diet (GFD) for 6-12 months
Prevalence of refractory sprue in coeliac disease

- real prevalence of RCD is unknown but it is definitely rare

- 0.7% - 1.5% of patients with coeliac disease (non-referral population-based cohorts)

- more common in women

- most cases diagnosed after age 50

*West J. Celiac Disease and Its Complications: A Time Traveller’s Perspective.*  
*Gastroenterology 2009 136: 32-4*  
*Rubio-Tapia A, Murray JA. Classification and management of refractory coeliac disease.*  
*Gut 2010 59: 547-557*
Refractory coeliac disease: two types

- **type 1**
  - normal intra-epithelial cell immunophenotype
  - polyclonal T-cells
  - relatively benign course

- **type 2**
  - CD3+ CD8- and/or T cell monoclonality
  - associated with ulcerative jejunitis
  - aberrant IELs also found in stomach and colon
  - high risk of overt enteropathy-associated T cell lymphoma


Current & future guidelines

- four to six duodenal biopsies
- sampling both the bulb and the second part of duodenum
- may be helpful to indicate these sites separately by submitting in different specimen containers
- orientation of the fragments is not necessary as random processing of multiple fragments usually yields sufficient orientation in at least some fragments for adequate evaluation of villous atrophy

The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) proposed new guidelines for diagnosis of CD in children. It suggests that, in symptomatic paediatric patients, in whom the IgA-TG2 level exceeds $10 \times$ upper limit of normal, EMA antibodies are positive on a separately taken blood sample and HLA-DQ2 or -DQ8 are positive, then biopsies do not need to be performed to confirm the diagnosis of CD.


_Draft BSG guidelines on the ‘Management of coeliac disease’_
The pathologist’s role in the management of coeliac disease: take home messages

- Coeliac disease is common and a common cause of iron deficiency (anaemia).
- Ensure adequate numbers of biopsies from appropriate sites.
- Know your normal and artefacts.
- The pathology is only ‘in keeping with …..’
- Intra-epithelial lymphocytosis is the most useful feature of CD but its specificity decreases as the villi get longer.
- Small intestinal complications of coeliac disease are rare and immunohistochemistry (and/or molecular analysis) is only required to diagnose these.
- As other, less invasive, tests become more reliable, we may see a diminishing role for pathologists in the management of coeliac disease.