Interpreting tests for coeliac disease

Tips, pitfalls and updates

Jason A Tye-Din

This article is the second in a series on pathology testing. Articles in this series aim to provide information about emerging laboratory tests that general practitioners (GPs) may encounter.

Background
Coeliac disease is one of most prevalent autoimmune illnesses encountered in general practice, and GPs have a central role in its diagnosis and follow-up. Key challenges are improving its poor rate of detection, distinguishing it from ‘gluten sensitivity’, and monitoring and optimising treatment to enhance long-term outcomes.

Objective
The objective of this article is to review the evidence-based use of serology, histology and genetic testing in the diagnosis and follow-up care of adults and children with coeliac disease.

Discussion
Recognition and testing of at-risk patients are keys to expediting the diagnosis of coeliac disease. Knowing when and how to use serology, histology, human leukocyte antigen typing and gluten challenge will increase the accuracy of both diagnosis and disease monitoring.

COELIAC DISEASE is an immune illness, triggered by dietary gluten, that causes a broad range of gastrointestinal and extra-intestinal manifestations. Untreated disease reduces quality of life, increases healthcare use and is associated with substantial morbidity. Mortality is increased because of lymphoproliferative malignancy, sepsis and refractory disease. As 1.5% of Australians have coeliac disease, it is one of the most common autoimmune illnesses that general practitioners (GPs) will encounter. However, its broad and often subtle presentation makes detection challenging, and means 80% of Australians with coeliac disease remain undetected. As expeditious diagnosis and treatment with a strict, lifelong gluten-free diet (GFD) minimises long-term complications, application of the appropriate tests to ensure accurate diagnosis and follow-up is crucial.

When to test for coeliac disease

Symptoms and clinical features that identify patients who might benefit from testing are shown in Table 1. ‘Classical’ symptoms caused by intestinal inflammation, such as diarrhoea and weight loss, are frequent, but the ‘non-classical’, extra-intestinal manifestations are even more common. These non-classical features include lethargy, headaches, osteoporosis, iron deficiency, transaminase elevation, infertility, other autoimmune disease and dermatitis herpetiformis. A positive family history of coeliac disease carries the strongest predictive value for the disease.

Tips and pitfalls
- Coeliac disease can develop at any age. The median age of diagnosis is 40 years, but do not discount coeliac disease in the young and elderly.
- Coeliac disease affects both sexes, with a modest female predominance. Men with coeliac disease are often overlooked.
- Coeliac disease is a global disorder that

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- Coeliac disease can develop at any age. The median age of diagnosis is 40 years, but do not discount coeliac disease in the young and elderly.
- Coeliac disease affects both sexes, with a modest female predominance. Men with coeliac disease are often overlooked.
- Coeliac disease is a global disorder that
is common in Western populations, North Africa, the Middle East, India and Pakistan. Reports from Asian countries, such as China, are on the rise. Coeliac disease should not be excluded on the basis of a patient’s ethnicity or appearance.

- Clinical heterogeneity is substantial. Some patients have minimal or no obvious symptoms, or only extra-intestinal issues. One-third of patients with coeliac disease are overweight or obese at diagnosis.10 Reports from Asian populations, such as China, are on the rise. Coeliac disease should not be excluded on the basis of a patient’s ethnicity or appearance.

Coeliac serology

Currently, serologic testing for coeliac disease consists of the transglutaminase (tTG) and deamidated gliadin peptide (DGP) antibody tests. In practice, both tests have >85% sensitivity and >90% specificity.12 The DGP assay has replaced the whole-protein anti-gliadin antibody (AGA) assay because of improved specificity; however, many labs will report the DGP result as the ‘anti-gliadin antibody’. The anti-endomysial antibody (EMA) test measures tTG antibodies, but is labour-intensive, user-dependent and less widely performed. Testing approaches are shown in Table 2.

Tips and pitfalls

- Positive coeliac disease serology in isolation is insufficient for the diagnosis of coeliac disease.
- The higher the titre of serology, the greater the positive predictive value for coeliac disease.13
- Coeliac disease serology has a false negative rate of 10–15%.14 Check if your patient is on a GFD or taking immunosuppressants.
- The tTG normal range varies by manufacturer as there is no international standard. Comparing titres is not possible if different labs or tTG assays are used.
- In patients with risk factors for coeliac disease, negative coeliac disease serology has lower negative predictive value, so further work-up should be considered.15
- Point-of-care tests to detect coeliac disease antibodies have not been validated in primary practice, so cannot currently be recommended.11
- Patients with persistently positive coeliac disease serology but normal small intestinal histology may have ‘potential’ (or ‘latent’) coeliac disease, and follow-up is recommended.36

Gastroscopy and small bowel biopsies

Histological evaluation of biopsies from the small intestine is the cornerstone of coeliac disease diagnosis.36 Gastroscopy is typically performed with intravenous sedation, is simple and safe, and takes as little as 10 minutes. As coeliac disease causes patchy involvement of the proximal small intestine, multiple biopsies are recommended (eg two from the first and four from the second part of the duodenum).36 Endoscopic changes of coeliac disease, such as mucosal scalloping, are occasionally seen, but diagnosis rests on the microscopic appearance.

Tips and pitfalls

- Villous atrophy is suggestive but not pathognomonic of coeliac disease. Other causes to consider, especially if coeliac disease serology is negative, include Giardia, common variable immunodeficiency, Crohn’s disease, tropical sprue, autoimmune enteropathy, cow’s milk protein intolerance and some medications (eg olmesartan).
- A GFD or immunosuppression can obscure changes of villous atrophy.
- Correct biopsy processing and interpretation by a skilled pathologist is vital. When there is diagnostic uncertainty, review of the pathology can be informative.

Human leukocyte antigen DQ2/8 genotyping

The strong association between coeliac disease and specific human leukocyte antigen (HLA) genes makes HLA genotyping a useful tool in specific situations (Table 3).16 The main susceptibility genes are HLA-DQ2 (specifically HLA-DQ2.5 and HLA-DQ2.2) and HLA-DQ8, which are collectively seen in almost all (99%) patients with coeliac disease, compared with 40–50% of the Australian community.5 Although these genes, especially HLA-DQ2.5, impart substantial relative risk for coeliac disease, the absolute risk is low, and most patients with one or more of these genes will not develop coeliac disease. HLA-DQ7 (composed of half the DQ2.5 allele, DQA1*05) may impart a very low risk for coeliac disease but this remains unclear.16

The main benefit of HLA typing is its ability to exclude coeliac disease diagnosis.
Table 1. When to test for coeliac disease

Offer serological testing for coeliac disease to people with any of the following:

- Persistent unexplained abdominal or gastrointestinal symptoms
- Falttering growth
- Prolonged fatigue
- Unexpected weight loss
- Severe or persistent mouth ulcers
- Unexplained iron, vitamin B12 or folate deficiency
- Type 1 diabetes, at diagnosis
- Autoimmune thyroid disease, at diagnosis
- Irritable bowel syndrome (in adults)
- First-degree relatives of people with coeliac disease

Consider serological testing for coeliac disease in people with any of the following:

- Metabolic bone disorder (reduced bone mineral density or osteomalacia)
- Unexplained neurological symptoms (particularly peripheral neuropathy or ataxia)
- Unexplained subfertility or recurrent miscarriage
- Persistently raised liver enzymes with unknown cause
- Dental enamel defects
- Down syndrome
- Turner syndrome

Gluten-sensitive or wheat-sensitive patients

Many Australians adopt a GFD without assessment for coeliac disease. This poses a diagnostic dilemma as coeliac disease serology and intestinal histology can become falsely negative if the patient has been on a GFD for more than a few months. Many Australians remove gluten from their diet because they feel it helps improve gastrointestinal or other symptoms. For these people, a definitive diagnosis is desirable as:

- a formal diagnosis of coeliac disease will ensure strict treatment and follow-up of a serious medical illness
- many people who self-report ‘gluten sensitivity’ are not actually sensitive to gluten. These patients, instead of excluding gluten, may benefit more from excluding other symptom-inducing wheat components, such as fermentable carbohydrates (FODMAPs). There are two diagnostic approaches – option 1 may be appropriate if the patient is unwilling to undertake the gluten challenge; however, option 2 is definitive.

Option 1: HLA-DQ2/8 genotyping

The absence of HLA susceptibility means that coeliac disease is unlikely and further investigations can focus on other diagnoses. The presence of HLA susceptibility genes is not diagnostic of coeliac disease, so option 2 is required.

Option 2: Gluten challenge, then testing

The amount and duration of gluten required to consistently trigger diagnostic changes of coeliac disease appears highly variable and more research is required. Approximately 3–6 g of gluten consumed daily for two weeks will cause intestinal changes of coeliac disease in 50–70% of affected adults, with the development of positive serology after four weeks in 10–55%. To optimise the diagnostic yield, patients should be encouraged to return to consuming 3–6 g or more of gluten each day for, ideally, six or more weeks. This daily amount of gluten can be found in two to four slices of wheat bread, two to four Weet-Bix or 0.5–1 cup of cooked pasta.

Tips and pitfalls

- Symptomatic relapse with a gluten challenge is common, but has poor predictive value for coeliac disease. Gluten challenge is informative only if accompanied by objective testing.
- The tolerability of a gluten challenge may be improved by commencing with a small amount of gluten and slowly increasing over subsequent days, and consuming it in divided doses over the course of the day (eg breakfast and lunch). Fermented breads with lower FODMAP content (that still contain gluten) are commercially available and may also improve challenge tolerability.

Family screening

The risk of coeliac disease in patients who have an affected family member with the disease is 10%, but increases up to 20% if multiple family members are affected. Screening patients with a family history of coeliac disease is important and strongly indicated when there are suggestive symptoms or signs. As it is increasingly recognised that many ‘asymptomatic’ patients with coeliac disease have
underlying nutrient deficiencies, reduced bone density, or have symptom improvement following a GFD (indicating they were never asymptomatic), this means all relatives, irrespective of symptom status, should be considered for screening. First-degree relatives should be screened, and if there are several affected family members second-degree relatives should also be tested.

Family screening using HLA DQ2/8 genotyping with coeliac disease serology is more informative than serology alone. A relative without HLA susceptibility does not require monitoring for coeliac disease. If HLA susceptibility is present but coeliac disease serology is normal, the individual is at risk for future development of the disease. Repeat coeliac disease serology would be recommended if they develop suggestive symptoms. If asymptomatic, some experts recommend screening every two to three years during childhood to avoid the detrimental effects of unrecognised coeliac disease on growth and bone health.

### Tips and pitfalls
- Screening children with a family history of coeliac disease can be delayed until the age of four years if they are well and symptom-free.
- Remind your patients with coeliac disease that their relatives are at increased risk of the disease and should be considered for testing.

### Paediatric testing

Although similar to adults, there are additional considerations when assessing children for coeliac disease. New European guidelines, based on evidence that high-titre tTG is strongly predictive of coeliac disease in children, suggest
small intestinal biopsies can be avoided if children meet the following criteria:\textsuperscript{19} • characteristic symptoms of coeliac disease • tTG-IgA levels $>10\times$ upper limit of normal • a positive endomysial antibody (EMA) on a different blood sample • positive HLA susceptibility for coeliac disease.

The utility of this approach in Australia is uncertain because of the limited availability of the EMA test, as well as intra-lab variation and lack of standardisation of the tTG assay. Further validation is warranted. The decision to make a non-biopsy diagnosis should only be made with specialist paediatric input.

**Tips and pitfalls**
- The tTG assay has lower sensitivity in children under three years of age. Ensure DGP-IgG testing is performed alongside tTG-IgA to overcome this issue.
- Children with coeliac disease may present with more ‘classical’ complaints than adults,\textsuperscript{22} but be mindful of extra-intestinal issues. Anxiety, depression, aggressive behaviour and sleep problems can be a presenting feature.\textsuperscript{23}

**Clinical follow-up**

Confirming successful treatment of coeliac disease with the GFD is important as the risk of complications is higher in patients not achieving mucosal remission.\textsuperscript{3} Yearly follow-up to review medical and dietary progress is recommended.\textsuperscript{3} A repeat gastroscopy may be considered in adults after two years of starting a GFD to assess for mucosal healing.\textsuperscript{3} Coeliac disease serology is frequently used as a surrogate marker of intestinal healing. In adults, values correlate poorly with the state of the intestinal mucosa.\textsuperscript{24} A trend for normalisation (titres generally normalise on a GFD in 12 months), and persistently positive titres suggest ongoing gluten exposure. In children, resolving tTG titres correlate better with mucosal healing.\textsuperscript{25} A child who improves clinically and normalises their serology on a GFD does not require follow-up endoscopy. Coeliac disease serology cannot detect small dietary indiscretions, and intestinal biopsies are recommended when disease activity needs to be accurately assessed.

**Conclusion**

Coeliac disease is highly prevalent in general practice. Good patient care depends on knowing when and how to test for it and how to monitor progress. Awareness of the strengths and limitations of each testing approach is vital for optimal diagnosis and follow-up.

**Key points**
- Recognition and testing at-risk patients are keys to expediting coeliac disease diagnosis.
- Coeliac disease serology and histology are not accurate in people following a GFD. Ask about diet when testing.
- Positive coeliac disease serology does not diagnose coeliac disease in isolation. The diagnosis depends on showing the characteristic intestinal changes and improvement on the GFD.
- Diagnosing coeliac disease without intestinal biopsies has been considered for children, but is contentious and requires specialist input.
- HLA genotyping can exclude a coeliac disease diagnosis, but has poor positive predictive value. A positive result does not diagnose coeliac disease.
- Distinguishing coeliac disease from ‘gluten sensitivity’ has important implications for the patient and their family’s medical care.
- Do not forget family screening given the insidious nature and adverse outcomes of undiagnosed coeliac disease.\textsuperscript{26,27}

**Table 2. How to test for coeliac disease**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Confirm your patient is consuming a normal, gluten-containing diet</td>
</tr>
<tr>
<td>2.</td>
<td>Request coeliac serology as follows:</td>
</tr>
<tr>
<td><strong>Option 1</strong>:</td>
<td>Transglutaminase-IgA (tTG-IgA) + Deamidated gliadin peptide-IgG (DGP-IgG)</td>
</tr>
<tr>
<td></td>
<td>Medicare Benefits Schedule (MBS) item number 71164, double antibody test ($39.90) is the preferred* one-step approach.</td>
</tr>
<tr>
<td>Or</td>
<td>Transglutaminase-IgA (tTG-IgA) + Total IgA level† – If the IgA level is low, perform the deamidated gliadin peptide-IgG (DGP-IgG). MBS item number 71163, single antibody test ($24.75).</td>
</tr>
<tr>
<td>3.</td>
<td>If tTG-IgA and/or DGP-IgG is positive, irrespective of titre, refer for confirmatory small intestinal biopsy</td>
</tr>
</tbody>
</table>

*Option 1 overcomes the need to assess the total IgA level by performing DGP-IgG, which is not adversely affected by IgA deficiency. Further, DGP-IgG enhances the pick-up of coeliac disease by 15% compared to tTG-IgA alone.\textsuperscript{25} Positive tTG-IgA and DGP-IgG together provides greater predictive value for coeliac disease than either alone.\textsuperscript{24} Total IgA level detects the 3% of people with coeliac disease with selective IgA deficiency that can cause false negative results.

**References**

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Competing interests: JT-D is a co-inventor of patents pertaining to the use of gluten peptides in diagnostic applications and therapeutics, and non-toxic gluten for the management of coeliac disease. He is a shareholder of Nexpep Pty Ltd and a scientific advisor to ImmusanT Inc, companies developing novel diagnostic and therapeutic approaches for coeliac disease. Provenance and peer review: Commissioned, externally peer reviewed.
Box 1. Clinical scenarios when HLA DQ2/8 genotyping can be useful

- When coeliac disease serology and/or small bowel examination is inconclusive or equivocal
- When there has been failure to improve on a gluten-free diet
- When a person has commenced a gluten-free diet prior to assessment by serology or small bowel examination and are unwilling or unable to undertake a gluten challenge prior to investigation
- In patients clinically assessed to be at higher risk of coeliac disease in order to exclude those where further testing for coeliac disease is not required


