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.[1] Untreated disease reduces quality of life, increases healthcare use and is associated with substantial morbidity.[2–4] Mortality is increased because of lymphoproliferative malignancy, sepsis and refractory disease.[3] 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.[5] As expeditious diagnosis and treatment with a strict, lifelong gluten-free diet (GFD) minimises long-term complications,[3] application of the appropriate tests to ensure accurate diagnosis and follow-up is crucial.

Making the diagnosis

In clinical practice, suspected patients are generally screened with coeliac disease serology. In patients with positive coeliac disease serology, the diagnosis is confirmed by the presence of characteristic small intestinal mucosal changes. The key diagnostic features are:

- intestinal histology showing raised intraepithelial lymphocytes (>25 per 100 enterocytes), crypt hyperplasia and villous atrophy (Figure 1)
- disease remission confirmed by symptom resolution, normalised coeliac disease serology and, most reliably and importantly, mucosal healing following treatment with a GFD.[3,6]

Correlation of histology and serology with clinical history is important. Coeliac disease can be present despite negative coeliac disease serology, but this is uncommon and excluding other causes of villous atrophy (see below) is important.

Testing at-risk individuals is strongly recommended to detect cases before substantial morbidity develops.[3,6] An active case-finding approach can improve detection of coeliac disease by more than 40-fold,[7] but this only works when doctors are mindful of the disease. Approximately 30 at-risk individuals need to be tested to find a positive case of coeliac disease.[7] There is insufficient evidence to support population screening.[8] Figure 2 provides an outline of a recommended diagnostic pathway.

When to test for coeliac disease

Symptoms and clinical features that identify patients who might benefit from testing are shown in Table 1.[9] '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.[5]
- Coeliac disease is a global disorder that

is common in Western populations, North Africa, the Middle East, India and Pakistan.[10] 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.[11]

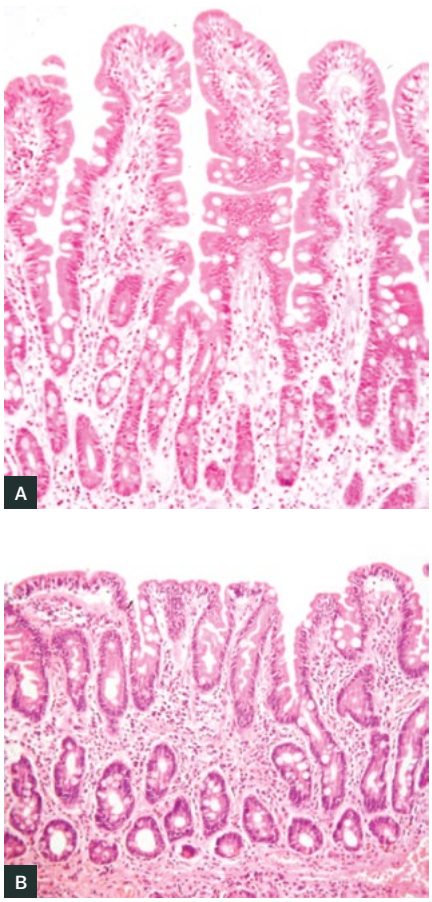

Figure 1. Healthy small intestine, compared with villous atrophy in coeliac disease.
A. Normal small intestinal mucosa in adequately treated coeliac disease
B. Untreated coeliac disease showing the classic triad of infiltration of the epithelium with lymphocytes, crypt hyperplasia and villous atrophy
Magnification ×100, haematoxylin and eosin stain.

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.[3,6]

Gastroscopy and small bowel biopsies

Histological evaluation of biopsies from the small intestine is the cornerstone of coeliac disease diagnosis.[3,6] 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).[3,6] 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[9]

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

- Persistent unexplained abdominal or gastrointestinal symptoms
- Faltering 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

Reproduced with permission from the National Institute for Health and Care Excellence. Coeliac disease: Recognition, assessment and management. London: NICE, 2015. Available at www.nice.org.uk/guidance/ng20

when the susceptibility genotypes are absent (likelihood of coeliac disease <1%). A positive HLA test does not diagnose coeliac disease, but indicates that further investigation may be warranted. Genotyping is widely available through commercial labs in Australia with a request for '*HLA-DQ2/8* genotyping'. It is performed on a blood sample, but can be done on a buccal scrape through some collection centres (patients can check in advance). HLA typing reports can be difficult to interpret, so Australasian guidelines have been developed to simplify and standardise reporting.[16]

Tips and pitfalls

- HLA typing is expensive (Medicare Benefits Schedule item number 71151; $118.85), so it is important to use it prudently (Table 3).[16]
- HLA typing is a 'once only' test as a person's genotype does not change.
- HLA typing results are not adversely affected by a GFD.
- Most patients with HLA susceptibility for coeliac disease will not have the disease or ever develop it.

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.[17] 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).[18]

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%.[19,20] 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.[3,6] As it is increasingly recognised that many 'asymptomatic' patients with coeliac disease have

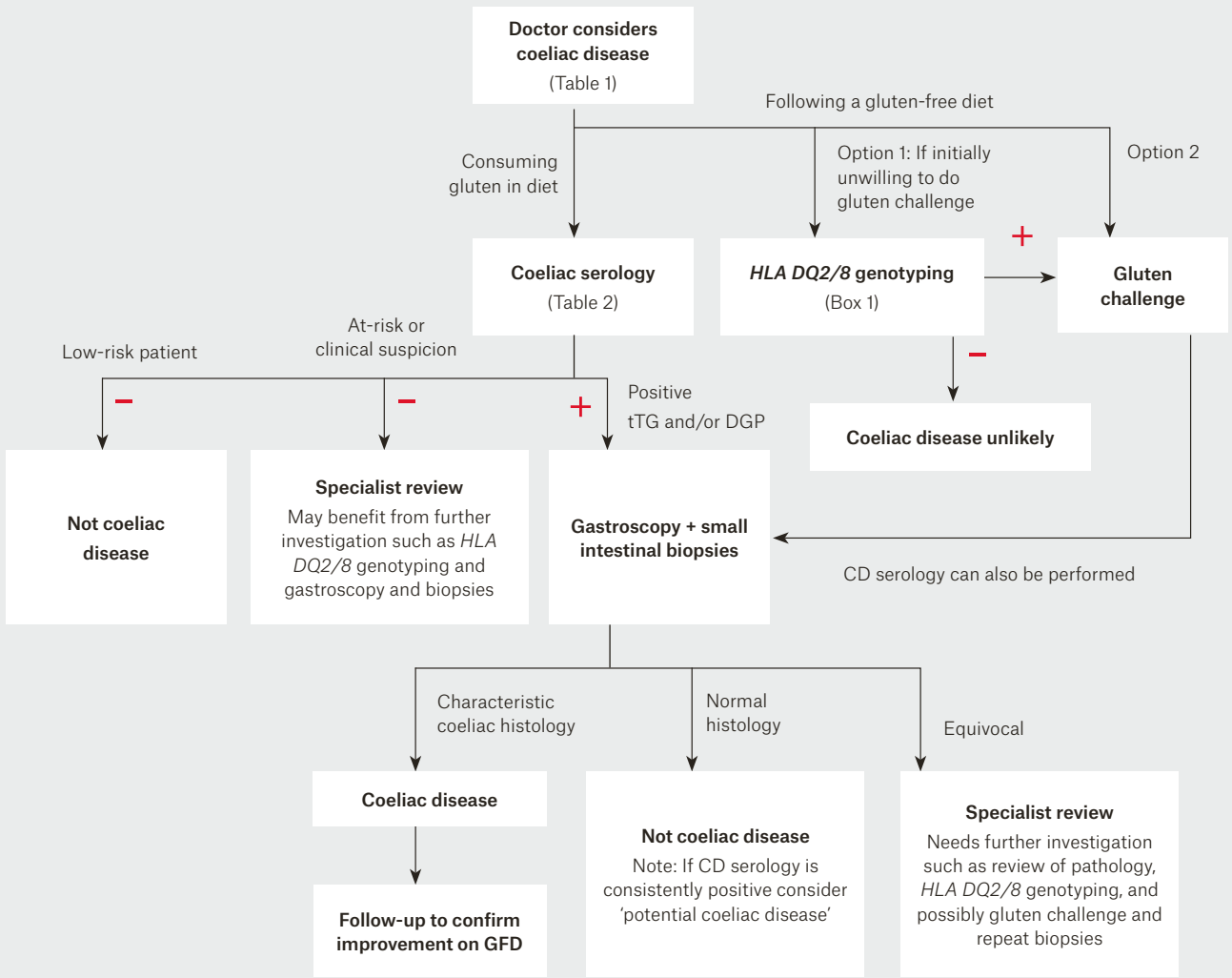

Figure 2. Recommended diagnostic pathway for coeliac disease
CD, coeliac disease; DGP, deamidated gliadin peptide antibody; tTG, tissue transglutaminase antibody

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.[21] 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.[16] 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.[13]

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.[22] 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:[13]

- characteristic symptoms of coeliac disease
- tTG-IgA levels >10× 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.

Table 2. How to test for coeliac disease

1. Confirm your patient is consuming a normal, gluten-containing diet
2. Request coeliac serology as follows: **Option 1:** Transglutaminase-IgA (tTG-IgA) + Deamidated gliadin peptide-IgG (DGP-IgG) – Medicare Benefits Schedule (MBS) item number 71164, double antibody test ($39.90) is the preferred* one-step approach. Or **Option 2:** 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).
3. If tTG-IgA and/or DGP-IgG is positive, irrespective of titre, refer for confirmatory small intestinal biopsy

**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.[25] Positive tTG-IgA and DGP-IgG together provides greater predictive value for coeliac disease than either alone.[26] †Total IgA level detects the 3% of people with coeliac disease with selective IgA deficiency that can cause false negative results.*

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,[22] but be mindful of extra-intestinal issues. Anxiety, depression, aggressive behaviour and sleep problems can be a presenting feature.[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.[3] Yearly follow-up to review medical and dietary progress is recommended.[9] A repeat gastroscopy may be considered in adults after two years of starting a GFD to assess for mucosal healing.[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.[24] A trend for normalisation is reassuring (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.[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.[26,27]

Authors

Jason A Tye-Din MBBS, FRACP, PhD, Consultant Gastroenterologist and Laboratory Head, Immunology Division, The Walter and Eliza Hall Institute Parkville, and Gastroenterology Department, The Royal Melbourne Hospital, Parkville, Vic. tyedin@wehi.edu.au

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.

References

1. Hardy MY, Tye-Din JA. Coeliac disease: A unique model for investigating broken tolerance in autoimmunity. Clin Transl Immunology 2016;5(11):e112.
2. Biagi F, Corazza GR. Mortality in celiac disease. Nat Rev Gastroenterol Hepatol 2010;7(3):158–62.
3. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of Gastroenterology. ACG clinical guidelines: Diagnosis and management of celiac disease. Am J Gastroenterol 2013;108(5):656–76.
4. Mogul D, Nakamura Y, Seo J, Blauvelt B, Bridges JF. The unknown burden and cost of celiac disease in the U.S. Expert Rev Pharmacoecon Outcomes Res 2017;17(2):181–88.

Box 1. Clinical scenarios when *HLA DQ2/8* genotyping can be useful[16]

- 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

5. Anderson RP, Henry MJ, Taylor R, et al. A novel serogenetic approach determines the community prevalence of celiac disease and informs improved diagnostic pathways. BMC Med 2013;11:188.
6. Ludvigsson JF, Bai JC, Biagi F, et al. Diagnosis and management of adult coeliac disease: Guidelines from the British Society of Gastroenterology. Gut 2014;63(8):1210–28.
7. Catassi C, Kryszak D, Louis-Jacques O, et al. Detection of celiac disease in primary care: A multicenter case-finding study in North America. Am J Gastroenterol 2007;102(7):1454–60.
8. US Preventive Services Task Force, Bibbins-Domingo K, Grossman DC, et al. Screening for celiac disease: US Preventive Services Task Force recommendation statement. JAMA 2017;317(12):1252–57.
9. National Institute for Health and Care Excellence. Coeliac disease: Recognition, assessment and management. UK: NICE, 2015.
10. Bai JC, Ciacci C. World Gastroenterology Organisation global guidelines: Celiac disease February 2017. J Clin Gastroenterol 2017;51(9):755–68.
11. Newnham ED, Shepherd SJ, Strauss BJ, Hosking P, Gibson PR. Adherence to the gluten-free diet can achieve the therapeutic goals in almost all patients with coeliac disease: A 5-year longitudinal study from diagnosis. J Gastroenterol Hepatol 2016;31(2):342–49.
12. Reeves GE, Squance ML, Duggan AE, et al. Diagnostic accuracy of coeliac serological tests: A prospective study. Eur J Gastroenterol Hepatol 2006;18(5):493–501.
13. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012;54(1):136–60.
14. Hill ID. What are the sensitivity and specificity of serologic tests for celiac disease? Do sensitivity and specificity vary in different populations? Gastroenterology 2005;128(4 Suppl 1):S25–32.
15. Hopper AD, Cross SS, Hurlstone DP, et al. Pre-endoscopy serological testing for coeliac disease: Evaluation of a clinical decision tool. BMJ 2007;334(7596):729.
16. Tye-Din JA, Cameron DJ, Daveson AJ, et al. Appropriate clinical use of human leukocyte antigen typing for coeliac disease: An Australasian perspective. Intern Med J 2015;45(4):441–50.
17. Golley S, Corsini N, Topping D, Morell M, Mohr P. Motivations for avoiding wheat consumption in Australia: Results from a population survey. Public Health Nutr 2015;18(3):490–99.
18. Biesiekierski JR, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. Gastroenterology 2013;145(2):320–28; e1–3.
19. Leffler D, Schuppan D, Pallav K, et al. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. Gut 2013;62(7):996–1004.
20. Sarna VK, Skodje GI, Reims HM, et al. HLA-DQ: Gluten tetramer test in blood gives better detection of coeliac patients than biopsy after 14-day gluten challenge. Gut 2017;doi: 10.1136/gutjnl-2017-314461.
21. Kurppa K, Paavola A, Collin P, et al. Benefits of a gluten-free diet for asymptomatic patients with serologic markers of celiac disease. Gastroenterology 2014;147(3):610–17; e1.
22. Steele R; CRF. Diagnosis and management of coeliac disease in children. Postgrad Med J 2011;87(1023):19–25.
23. Smith LB, Lynch KF, Kurppa K, et al. Psychological manifestations of celiac disease autoimmunity in young children. Pediatrics 2017;139(3).
24. Silvester JA, Kurada S, Szwajcer A, Kelly CP, Leffler-DA, Duerksen DR. Tests for serum transglutaminase and endomysial antibodies do not detect most patients with celiac disease and persistent villous atrophy on gluten-free diets: A meta-analysis. Gastroenterology 2017;153(3):689–701.
25. Bannister EG, Cameron DJ, Ng J, et al. Can celiac serology alone be used as a marker of duodenal mucosal recovery in children with celiac disease on a gluten-free diet? Am J Gastroenterol 2014;109(9):1478–83.
26. Hoerter NA, Shannahan SE, Suarez J, et al. Diagnostic yield of isolated deamidated gliadin peptide antibody elevation for celiac disease. Dig Dis Sci 2017;62(5):1272–76.
27. Vermeersch P, Geboes K, Mariën G, Hoffman I, Hiele M, Bossuyt X. Diagnostic performance of IgG anti-deamidated gliadin peptide antibody assays is comparable to IgA anti-tTG in celiac disease. Clin Chim Acta 2010;411(13–14):931–35.

correspondence **ajgp@racgp.org.au**