Celiac disease (CD), also known as gluten sensitive enteropathy, is a gluten induced inflammatory disease that affects the small intestine. The T-cell derived inflammatory reaction results in a variety of histological abnormalities of the small intestine, ranging from increased intraepithelial lymphocytes to partial and total villous atrophy with mucosal crypt hyperplasia.\textsuperscript{1-3} Strong antibody response (immunoglobulin [Ig] A, IgG, IgM) is also generated against gluten (particularly against the $\alpha$-gliadin part of gluten) and connective tissues. These antibodies are not believed to play a part in the pathogenesis of CD, however, they do play an important role in the diagnosis and monitoring of patients with CD.\textsuperscript{2,4} Celiac disease initially affects the proximal part of the small intestine; however, as the disease progress, the entire small intestine can become affected. Consequently, the clinical manifestations associated with CD depend on the degree of inflammation and the extent of the involved small intestine. The clinical presentation also depends on the age of the patient.\textsuperscript{2,3,6} Pediatric patients with CD commonly present with chronic diarrhea with, or without, malabsorption, abdominal bloating and pain and failure to thrive. In contrast, adult patients more often present with a variety of extra-intestinal clinical manifestations including lethargy, anemia, osteoporosis and osteomalacia (Table 1).\textsuperscript{1,2,6-8} Most clinicians still associate CD with gastro-intestinal clinical manifestations, notably chronic diarrhea, mal-absorption and steatorrhea and therefore many patients with non specific symptoms, or extra-intestinal manifestations, go undiagnosed. Moreover, the old perception by many physicians of the expensive and invasive investigatory procedures associated with the investigation of CD patients, aside from lack of awareness of the protean manifestations of the disease, further contributes to delay in the diagnosis of the disease.\textsuperscript{1,3} Celiac disease was once thought to be a rare disease limited to the Caucasian population. However, with increased awareness by physicians of the different manifestations of CD, combined with the availability of non-invasive serological investigatory procedures, have resulted in increased and early diagnosis of CD.\textsuperscript{1,2,8,9} It is
well documented now that the prevalence of CD is high worldwide and ranges between 1:266 and 1:1000. However, full awareness of the protein manifestations, and the serological tests available for screening, of the disease may not have spread widely amongst all physicians. Consequently, CD is still believed to be under diagnosed worldwide. The true prevalence of CD has been estimated to be in the order of 1:200. In addition, it is anticipated that with increased awareness of the disease, a parallel increase in the use of serological diagnostic tests would occur, and because of the different sensitivities and specificities of these various tests, over/under interpretation of results could lead to over/under referral of patients to the gastroenterologist.

In the present review, we have introduced CD and then discussed in detail the different serological tests available in the Clinical Immunology laboratory for screening, diagnosing and monitoring patients with CD, with the hope of raising awareness of CD amongst clinicians as well as helping them to select the right test and interpret results more effectively.

**Reticulin antibodies.** Anti-reticulin antibodies (ARA, IgA) are detected by indirect immunofluorescence (IIF) method using rodent substrates (rat/mouse kidneys). Of the 5 different IF-patterns that can be recognized (R1-R5), only the R1-pattern is associated with CD. The other 4 patterns have been attributed to heterophile antibodies, since they can not be detected on monkey tissues, the other alternative substrate. The IIF R1-pattern is characterized by peri-glomerular and peri-tubular fluorescence staining. The antigen recognized by the ARA has been suggested recently to be the tissue transglutaminase (tTG). The latter enzyme is also believed to be the same antigen recognized by the anti-endomysial antibodies. The specificity of ARA for CD is very low (Table 2), as the antibodies can be detected in patients with inflammatory bowel disease and in normal elderly people. Although, IgA ARA are regarded as more specific for CD than IgG ARA, the specificity is still very much lower than that associated with the endomysial antibodies (EMA) and, therefore, the ARA test has been discontinued in favor of the more sensitive and specific EMA-test.

**Anti-gliadin antibodies (AGA).** Anti-gliadin antibodies are antibodies produced against the alcohol-extractable α-gliadin components of gluten. Anti-gliadin antibody-test, employing ELISA or Immunocap (IC) assays, measures both IgG and IgA antibodies. The specificity of AGA for CD is very low (Table 2) as the antibodies can be found in patients with other intestinal diseases including cow’s milk intolerance and infectious mal-absorption. Immunoglobulin A AGA are more specific for CD than IgG AGA, while IgG AGA occurring alone have no particular diagnostic significance as they can be found in a range of inflammatory and infectious bowel conditions. However, in IgA deficient patients, IgG can have the same clinical significance as the IgA antibodies. Since AGA-test is based on ELISA and IC methods and, therefore, can be automated, the test has been used in the past as the preferred test to screen patients for CD. However, because of the low sensitivity and specificity of the test, it has been replaced by the new more sensitive and specific tissue transglutaminase (tTG)-test (ELISA-method). In some laboratories, the AGA-test is still used as an adjunct test for screening IgA deficient, and pediatric, patients for CD. However, this is also likely to change with the introduction of the IgG based tTG assays, which have better sensitivities and specificities for CD.

**Endomysial antibodies (EMA).** Endomysial antibodies are antibodies directed against the connective tissue (the endomysium) that surrounds smooth muscles. The antibodies are detected by an IIF-technique using tissues from the distal part of monkey esophagus and human umbilical cord; as substrates. The EMA-test is highly sensitive and specific for CD (Table 1), and positive results are invariably associated with CD. Indeed, the EMA-test has been shown to be more sensitive for the detection of CD than the biopsy (generally regarded as the gold standard test for the diagnosis of the disease) and led some clinicians to suggest that the EMA-test should be used in place of a biopsy; particularly in infant patients. The low sensitivities and specificities reportedly associated with the EMA-test are due to a combination of technical problems (such as use of low and high dilutions; 1:2-1:25) and poor result interpretation (such as reading smooth muscle staining patterns as endomysial patterns). Therefore, with good experience in technical performance and result interpretation, the sensitivity and specificity of the EMA-test should approach 100%, and thus would be the ideal test to use to screen patients for CD.

**Tissue transglutaminase antibodies.** Tissue transglutaminase (tTG) enzyme has been recently identified as the autoantigen that is recognized by the EMA. Since its discovery, tTG based ELISA-assays have been developed for screening patients for CD. Initial results showed that ELISA-assays, employing guinea pig (GP) liver extract as a source of the TG, were highly sensitive, but less specific, for the detection of CD. The low specificity was attributed to contaminating GP
Diagnosis of celiac disease … Aziz & Polson

Table 1 - Clinical manifestations associated with celiac disease.

<table>
<thead>
<tr>
<th>Gastrointestinal manifestations</th>
<th>Extra-gastrointestinal manifestations</th>
<th>Autoimmune diseases associated with celiac disease</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic diarrhea (osmotic and secretary)</td>
<td>Lethargy</td>
<td>Insulin dependent diabetes</td>
<td>Immunoglobulin A deficiency</td>
</tr>
<tr>
<td>Steatorrhea</td>
<td>Anemia (folate- and Fe-deficiency)</td>
<td>Primary biliary cirrhosis</td>
<td>Infertility</td>
</tr>
<tr>
<td>Abdominal distension and pain</td>
<td>Hypocalcemia</td>
<td>Thyroid disease</td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>Osteoporosis</td>
<td>Sjogren’s syndrome</td>
<td></td>
</tr>
<tr>
<td>Flatulence</td>
<td>Osteomalacia</td>
<td>Systemic lupus erythematosus</td>
<td></td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>Arthralgia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophageal reflux</td>
<td>Psychological (depression and anxiety)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>Neurological (epilepsy, dementia, seizures)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatitis and aphthous ulcers</td>
<td>Anorexia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glossitis</td>
<td>Failure to gain weight/thrive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteropathy associated T-cell lymphoma</td>
<td>Weight loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raised liver transaminases</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gastrointestinal manifestations:
- Chronic diarrhea (osmotic and secretory)
- Steatorrhea
- Abdominal distension and pain
- Constipation
- Flatulence
- Dyspepsia
- Esophageal reflux
- Vomiting
- Stomatitis and aphthous ulcers
- Glossitis
- Enteropathy associated T-cell lymphoma
- Raised liver transaminases

Extra-gastrointestinal manifestations:
- Lethargy
- Anemia (folate- and Fe-deficiency)
- Hypocalcemia
- Osteoporosis
- Osteomalacia
- Arthralgia
- Psychological (depression and anxiety)
- Neurological (epilepsy, dementia, seizures)
- Anorexia
- Failure to gain weight/thrive
- Weight loss

Autoimmune diseases associated with celiac disease:
- Insulin dependent diabetes
- Primary biliary cirrhosis
- Thyroid disease
- Sjogren’s syndrome
- Systemic lupus erythematosus

Others:
- Immunoglobulin A deficiency
- Infertility

Table 2 - Serological tests used to screen for celiac disease.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-reticulin antibodies</td>
<td>25-92</td>
<td>59-100</td>
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<tr>
<td>Anti-gliadin antibodies</td>
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<td></td>
</tr>
<tr>
<td>IgA</td>
<td>31-100</td>
<td>85-100</td>
</tr>
<tr>
<td>IgG</td>
<td>46-100</td>
<td>67-100</td>
</tr>
<tr>
<td>Endomysial antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>96-100</td>
<td>99-100</td>
</tr>
<tr>
<td>GP-tTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>96-100</td>
<td>88-96</td>
</tr>
<tr>
<td>Human-tTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>96-100</td>
<td>96-100</td>
</tr>
</tbody>
</table>

Data obtained from references: 2, 13, 17, 23, 24, 26. GP - guinea pig, tTG - tissue transglutaminase. Ig - immunoglobulin.

Ludwig. Subsequent use of human derived (purified and recombinant) tTG enhanced the specificity of the ELISA assays for CD. However, the specificity has remained below that of the EMA-test; with most of the false positive results (occasionally with values equal to those obtained from CD patients) originating from patients with inflammatory bowel disease, chronic liver- and lymphoproliferative diseases. In comparison with the AGA-test, the tTG-test is more sensitive and specific for CD. Consequently, increasing number of laboratories are now using the tTG-test as their initial screening test for CD, and positive results are confirmed by the EMA-test. Immunoglobulin G-based tTG-test, which is more sensitive and specific for CD than the IgG-based AGA-test, can be employed to screen for CD in IgA-deficient patients (IgA deficient patients and pediatric patients <2 years). Finally, since the sensitivity and the specificity of tTG-ELISA-assays vary from one manufacturer to another, laboratories intending to introduce this new test would need to evaluate a number of ELISA kits prior to selecting the most optimal kit.

Anti-enterocyte antibodies and anti-intestinal goblet cell antibodies (AEAs). Anti-enterocyte antibodies are associated with autoimmune enteropathy (AE), a variant form of CD. The disease is associated with a protracted diarrhea, mucosal atrophy and crypt hyperplasia, lack of intraepithelial lymphocyte infiltrate and resistance to gluten free diet. A variant form of AE (characterized, additionally, by collagen band and goblet cell depletion of the small intestine) is associated with antibodies to the intestinal goblet cells (IGA). Intestinal goblet cells are specific for the AE disease (IGA are not detected in other diseases of the gastrointestinal system including Crohn Disease, ulcerative colitis, peptic ulcer and lymphocytic colitis). Testing for AE associated antibodies is performed by an indirect immunofluorescence method using rat small intestine tissues as substrate. Testing should be considered in pediatric patients with chronic diarrhea, and in patients with resistant CD.

Pattern of testing for CD. The old criteria for the diagnosis of CD required 3 biopsies, one showing the characteristic intestinal histology, a second one showing normalization of intestinal morphology following gluten free diet, and a third biopsy showing deterioration of the intestinal mucosa following reintroduction of gluten. In the newly revised European criteria for the diagnosis of CD, biopsies required for diagnosis of the disease has been reduced to one, and serological markers can now provide the information previously obtained from the other 2 subsequent biopsies (namely reduction and eventually disappearance of CD associated antibodies following gluten free diet,
Diagnosis of celiac disease … Aziz & Polson

**Figure 1** - Depicts a possible flow chart for testing for celiac disease (CD) using a 2 stage testing system. It is not clear whether all patients with IgA-deficiency (IgAD) and autoimmune diseases (AD) should be screened for CD. Equally, there are a lot of controversies regarding screening patients with iron deficiency anemia, osteomalacia and osteoporosis. Presence of strong reticulin/endomysial staining on tissue block (rat stomach/liver/kidney), used for screening for autoimmune disease, would also need to be further tested for CD. DH - dermatitis herpetiformis, tTG - tissue transglutaminase, EMA - endomysial antibodies.

and their re-appearance following gluten rechallenge).1-3,28 Unlike biopsy, serological tests are non-invasive and cheap and have allowed screening of large number of patients resulting in increased, and early, diagnosis of patients with CD. Moreover, serological tests can play a major role in the diagnosis of patients with CD in situations when the intestinal lesion is equivocal and the disease is patchy. In addition, since serological tests are cheap, sensitive and non-invasive, they can be used for monitoring CD patient response to, and compliance with, gluten free diet.1,28 It is possible that serological testing could eventually obviate the need for a biopsy altogether. Since the prevalence of CD is very high amongst the general population, and since untreated disease is associated with high morbidity and mortality, serological tests used for screening patients for CD should be sensitive, specific and cheap. From the above discussion, the test that meets all these criteria is the EMA-test. With good experience in performing the EMA-assay, and reading the IIF-patterns, the EMA-test is both sensitive and specific for CD, and work out reasonably cheap compared to the other tests.9 The EMA-test, therefore, can be used to screen patients for, as well as to monitor patients with CD. Pediatric (2< years) and IgA-deficient patients can be screened and monitored using IgG based EMA-test.

Positive EMA-test would strongly suggest a diagnosis of CD, which can be confirmed by a biopsy. However, it must be remembered that, because of patchiness of mucosal abnormality in some patients with CD, a normal biopsy, in the presence of confirmed EMA positive results, would not exclude a diagnosis of CD. In such situations, biopsies from multiple sites would need be taken and close examination of the mucosa and intraepithelial lymphocyte (IEL) count would need to be carried out.29,32 If the histology is still normal, or equivocal, a trial of GFD may be considered to see if this will have a positive outcome on patient symptoms.24 Since gastroscopic biopsy is an invasive procedure for the patient, as well as being expensive, it may be advisable to confirm positive serological results on a second occasion before proceeding to a biopsy; particularly when the clinical suspicious for CD is low. An alternative to the above one-stage EMA-testing scheme would be a 2-stage testing system where patients can be screened for CD by the tTG-test (based on an ELISA assay which can be automated to accommodate large number of samples), and positive results confirmed by the EMA-test (Figure 1). Negative tTG-ELISA results, combined with low clinical suspicion for CD, can be taken as an exclusion of CD. Patients with high suspicious of CD would need endoscopic biopsy regardless of the serological results, whereas patients with positive tTG and EMA tests should have a small bowel biopsy regardless of the clinical picture. In patients who test positive by the tTG-test, but negative by the EMA-test, the decision to biopsy would depend on the clinical picture. For patients with low clinical suspicion for CD, it may be advisable to simply monitor the patient (such as repeat testing in 6-12 months).

The above serological tests can be used to monitor patient response to, and compliance with, GFD. The titres of IgA AGA and IgA EMA fall on
commencement of GFD. The rate of EMA fall and their disappearance is usually within a period of 6-12 months, but can take up to 31 months if the initial titre is high. Reduction of antibody titres usually precede clinical improvement. Disappearance of clinical symptoms can take between 2-15 weeks from the start of GFD, while normalization of the mucosal morphology can take between 11-14 months. In IgA deficient patients, IgG antibodies disappear within 8-10 months from commencement of GFD and IgG1-EMA, or IgG-AGA, test can be used to monitor patient’s dietary compliance.

Other laboratory tests used in the screening and monitoring of patients with CD. Measurement of serum immunoglobulins. Since the prevalence of IgA-deficiency is high in patients with CD, and since the serological tests used to screen for the disease are based on IgA, parallel measurement of serum Igs is essential. Immunoglobulin A-deficient patients should be screened for CD by an IgG based EMA, or tTG, assay.

Measurement of all 3 Igs (IgA, IgG, IgM) is preferable to measurement of only the IgA, since the results will reveal any other immunodeficiency disease (hypogammaglobulinemias), which would be among the differential diagnosis of gut associated enteropathies. Low serum IgM, and increased serum IgA, is a feature of CD, however, this has no proven diagnostic significance. Another feature associated with CD is splenic atrophy which develops in CD patients regardless of their response to gluten free diet. Since hyposplenic patients are susceptible to infection with encapsulated bacteria, regular checks for splenic atrophy (Heinz and Howell-Jolly bodies on blood film) in patients with CD need to be undertaken. Hyposplenic patients need to receive prophylactic antibiotics and have their Pneumococcal, and Hib immunity status (specific antibodies) checked and if deficient, vaccinated in order to maintain adequate antibody levels.

Finally, in patient with positive CD serology, and biopsy, who fails to respond to gluten free diet, testing for AEA and IGA should be considered. In addition, immuno-histological staining of gut mucosa for clonal T-cells (intracellular lymphocytes) should be performed in order to detect the pre-T-cell lymphoma (cryptic enteropathy-associated T Cell lymphoma), or if T cell lymphoma is suspected, B2-microglobulin should also be measured and monitored.

In conclusions, CD was once considered to be a rare disease and largely restricted to the Caucasian population. With the slow realization that the disease is very common and affects people worldwide, it is anticipated that increased utilization of serological tests for screening patients will follow. However, since there are many serological tests available, each with its own sensitivity and specificity, use of these tests could lead to a lot of confusion with regards to the diagnosis/exclusion of CD. We hope the present review will prove useful to physicians and help them to select the right tests and interpret the results more efficiently.

Acknowledgment. We would like to acknowledge the contribution made to this review by Dr. A. A Faizal (Department of Rheumatology).

References


