Serologic testing in celiac disease

Practical guide for clinicians

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Abstract

Objective To address the questions and challenges commonly faced by primary care physicians when ordering serologic tests for celiac disease (CD) and provide practical clinical tips to help in the interpretation of test results.

Sources of information MEDLINE was searched from 2000 to 2015 for English-language guidelines on the diagnosis and management of CD published by professional gastroenterological organizations.

Main message To screen patients for CD, measurement of the immunoglobulin A (IgA) tissue transglutaminase antibody is the preferred test. Total serum IgA level should be measured to exclude selective IgA deficiency and to avoid false-negative test results. Patients with positive serologic test results should be referred to a gastroenterologist for endoscopic small intestinal biopsies to confirm the diagnosis. Testing for human leukocyte antigens DQ2 and DQ8 can help exclude the diagnosis. A gluten-free diet should not be started before confirming the diagnosis of CD.

Conclusion Serologic testing is very useful for screening patients with suspected CD. Early diagnosis is essential to prevent complications of CD.

Case

A boy aged 2 years and 4 months with a positive immunoglobulin A (IgA) tissue transglutaminase (tTG) antibody test result was referred to the pediatric gastroenterology clinic. He had been screened because his maternal first cousin was recently diagnosed with celiac disease (CD). He had no abdominal pain, bloating, vomiting, or diarrhea. His stools were occasionally hard and painful to pass. He had no fatigue, loss of appetite, or weight loss. There was no other family history of CD or autoimmune disorders. Physical examination findings were unremarkable with normal growth parameters. The IgA tTG antibody level was elevated at greater than 250 U/mL (normal level is <15 U/mL); the IgA tTG antibody measurement had been obtained because constipation can be a symptom of CD and there was a positive family history of CD. Hemoglobin, albumin, and thyroid-stimulating hormone levels were normal. Results of the endoscopic small intestinal biopsy, which was performed while the child was on a regular, gluten-containing diet, were completely normal. The IgA tTG antibody was repeated 3 months after the first measurement and the results were normal at less than 0.5 U/mL. Test results for human leukocyte antigens (HLA) DQ2 and DQ8 were also negative. The boy remained asymptomatic on a regular diet.

Celiac disease is a chronic gastrointestinal disorder in which ingestion of gluten—a protein found in wheat, rye, and barley—leads to villous atrophy of the small intestine by an immune-mediated mechanism in genetically susceptible individuals.[1-3] This can lead to a variety of intestinal and extraintestinal symptoms, along with a deficiency of macronutrients and micronutrients.

The diagnosis of CD is confirmed by endoscopic small intestinal biopsy. Patients must be on a regular, gluten-containing

EDITOR'S KEY POINTS

- Celiac disease (CD) is one of the most common gastrointestinal disorders and many cases remain undiagnosed. Because CD can present in a variety of ways, it is important to consider CD in the differential diagnosis and to have the appropriate knowledge about ordering serologic tests and interpreting their results.
- Serologic tests are for screening purposes and do not confirm the diagnosis of CD. Patients with positive serologic test results require endoscopic small intestinal biopsies to confirm the diagnosis of CD. It is imperative that a gluten-free diet not be started before the biopsy is performed because such a diet will lead to healing of the small intestinal mucosa and complicate the interpretation of the biopsy results.
- Because CD is a chronic disorder with substantial lifelong implications, all attempts should be made to confirm the diagnosis.

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diet at the time of the biopsy. It is also important that the biopsy is performed and processed properly, and that it is interpreted by an expert pathologist to avoid false-negative findings. Celiac disease can be effectively treated with a strict gluten-free diet (GFD). Untreated CD can lead to complications including nutritional deficiencies (eg, anemia), osteoporosis, and growth failure, as well as possible development of other autoimmune disorders and malignancy.[2,3]

The development of serologic tests has been one of the greatest advancements in CD. Highly sensitive and specific antibody tests are now available to screen individuals, especially those who are at high risk or are minimally symptomatic. However, challenges remain regarding who should be screened, how to screen, and how to properly interpret the serologic test results. The correct interpretation of results is essential for accurate diagnosis and subsequent treatment.

Celiac disease affects 1% of the population, making it one of the most common gastrointestinal disorders. Many cases remain undiagnosed owing to a lack of awareness by health care professionals or because patients present with nonclassical extraintestinal symptoms. Celiac disease has been called a "clinical chameleon" because it can present in a variety of ways. Family physicians are at the forefront of delivering health care to the public; it is important to consider CD in the differential diagnosis and to have the appropriate knowledge about ordering serologic screening tests and interpreting their results.

This article reviews the currently available serologic tests for CD and identifies the various indications and pitfalls in ordering tests and interpreting test results in clinical practice. The use of endoscopic small intestinal biopsy and HLA testing in the diagnosis of CD is also addressed.

Sources of information

MEDLINE was searched from 2000 to 2015 for English-language guidelines on the diagnosis and management of CD published by professional gastroenterological organizations. If more than 1 set of guidelines were developed by the organization, the most recent guidelines were selected.

Four North American and 2 European guidelines were identified.[4-9] Of these, 2 were directed specifically to the pediatric population.[4,5]

These guidelines discuss the diagnosis and management of CD and also address the role of serologic testing in great detail. However, they are intended mainly for gastroenterologists, making it challenging for primary care physicians to interpret and apply them in a busy clinical practice. Answers to common clinical questions relevant to serologic testing for CD in primary care based on these published guidelines are summarized below.

Main message

Who should be screened for CD? Because CD can present with mild or atypical symptoms, a high index of suspicion is required. **Box 1** lists the clinical situations in which screening should be considered.[4-9]

Delays in the diagnosis of CD are common.[10-12] In 2 large surveys from Canada, the mean time from symptom onset to diagnosis of CD in adults was approximately 12 years.[11,12] In a multicentre study involving primary care physicians and an active case-finding strategy using serologic testing in adults, a 32-fold to 44-fold increase in the diagnostic rate was achieved.[13]

What is the clinical spectrum of CD? Celiac disease has a broad clinical spectrum with 4 patterns recognized.[14] In classical CD, the patient presents with features of malabsorption such as diarrhea, steatorrhea, and weight loss or growth failure.

In nonclassical CD, signs and symptoms of malabsorption are absent and patients might have other

Box 1. Clinical indications to screen for CD

Indications to screen for CD include the following:

- Abdominal pain or bloating
- Abdominal distension
- Autoimmune liver disease
- Autoimmune thyroid disease
- Chronic diarrhea
- Chronic fatigue
- Dental enamel defects
- Dermatitis herpetiformis
- Down syndrome
- First-degree relatives with CD
- Idiopathic elevation of transaminases
- Iron deficiency anemia
- Irritable bowel syndrome
- Osteopenia or osteoporosis
- Peripheral neuropathy
- Recurrent aphthous stomatitis
- Selective IgA deficiency
- Unexplained weight loss
- Turner syndrome
- Type 1 diabetes

Additional clinical features in children include the following:

- Anorexia
- Chronic constipation
- Delayed puberty
- Growth failure
- Irritability
- Recurrent vomiting
- Short stature

CD—celiac disease, IgA—immunoglobulin A.
Data from Hill et al,[4] Husby et al,[5] American Gastroenterological Association Institute,[6] Rostom et al,[7] Rubio-Tapia et al,[8] and Ludvigsson et al.[9]

intestinal or extraintestinal symptoms. In both classical and nonclassical CD, the serologic test results are abnormal and villous atrophy is present.

Subclinical CD is when the disease is below the threshold of clinical detection without symptoms or signs sufficient to trigger CD testing in routine clinical practice. Some of these patients might be screened because they have a high risk of developing CD. These patients will have abnormal serologic test results, as well as villous atrophy.

In potential CD, previously called *latent CD,* the patient has an abnormal antibody test result but a normal intestinal mucosa on histology. Several of these individuals will develop the intestinal lesion over time, thus requiring careful monitoring and follow-up.

What are the various serologic tests available to screen for CD? The following are available serologic tests to screen for CD.

Antigliadin antibody: This was the first serologic test, developed in the 1980s. Owing to its relatively low sensitivity and specificity, it should not be used to screen for CD.

Antireticulin antibody: This was the second serologic test that was developed and it was in use for a short period of time. Because there are more sensitive tests available, it should not be used for screening purposes.

Antiendomysial antibody: The antiendomysial antibody is a highly specific and sensitive test for CD. Initially, tissue from monkey esophagus and later human umbilical cord were used as substrates, making the test fairly expensive. Furthermore, the assay requires the laboratory technician to assess for immunofluorescence, leading to potential interobserver variability in interpretation of the test results.

Anti-tTG antibody: In 1997, it was discovered that the antigen against which endomysial antibodies were being formed was the enzyme tTG. With recombinant technology, human tTG became available for commercial use and the cost of testing decreased considerably. Most hospital laboratories now measure the tTG antibody instead of the antiendomysial antibody.

Anti-deamidated gliadin peptide (DGP) antibody: This is the latest generation serologic test. It does not offer any considerable advantage over measurement of the tTG antibody as the primary screening test; however, the immunoglobulin G (IgG)–based DGP antibody is slightly more sensitive than the IgG-based tTG antibody and should be considered the test of choice in patients with selective IgA deficiency.

Table 1 provides a summary of the sensitivity and specificity of the various serological tests.[15] Overall, the IgA-based tests are more sensitive and are preferred for screening.

Which serologic tests should be ordered to screen for CD? The IgA tTG antibody is the preferred test for screening patients of all ages. It is cost effective to order just this test, as opposed to ordering a panel of several tests.

The sensitivity of serologic tests is lower in children younger than 2 years of age. Therefore, testing with the DGP antibody (IgA and IgG), along with the IgA tTG antibody, is recommended in this age group.

Tissue transglutaminase antibody testing is currently available to physicians on the provincial diagnostic laboratory checklist in all provinces except Ontario. For outpatient cases in Ontario, individuals have to pay for the test.

An over-the-counter home self-testing kit for CD has been available in pharmacies across the country for the past few years.[16] Using a pinprick to obtain a tiny blood sample from the fingertip, the home test identifies the tTG antibody present in the blood of individuals with CD. While this can be used as a point-of-care test, caution should be exercised in making a diagnosis based solely on a positive test result. There is concern that individuals might begin to diagnose themselves with CD and start a GFD without confirmation of diagnosis and nutritional counseling by a dietitian.

Should total serum IgA levels be obtained while ordering the screening test? Patients with CD are 5 to 10 times more likely to have selective IgA deficiency compared with the general population.[8] As the currently recommended tests are IgA based, a patient with IgA deficiency might have a false-negative test result. Therefore, serum IgA levels should be included in the initial screening. In patients with mildly low total IgA levels, the IgA-based tests still remain valid. Some hospital laboratories will automatically test for total IgA levels when tTG antibody testing is requested.

What test should be ordered in a patient with selective IgA deficiency? When the total serum IgA level is less than 0.2 g/L, an IgG-based screening test should be performed. Measurement of IgG DGP antibodies is the recommended test; however, other IgG-based tests such as IgG tTG antibody testing can also be done. Clinicians should check with their local laboratories about the availability of these tests.

If the serologic test results are negative, could the patient still have CD? It is important to note that a negative serologic test result does not rule out CD. **Box 2** lists factors that might contribute to false-negative test results.

Results of serologic tests tend to gradually become negative while on a GFD. How quickly this happens can vary from several weeks to months. Individuals with negative test results might have reduced the amount of gluten in their diet or have eliminated it altogether when they go see their family physicians. Therefore, it is imperative that a detailed dietary history be obtained to

Table 1. Serologic tests for celiac disease

ANTIGEN	ANTIBODY TYPE	TEST	SENSITIVITY, % (RANGE)	SPECIFICITY, % (RANGE)
Gliadin	IgA	ELISA	85 (57-100)	90 (47-94)
	IgG	ELISA	80 (42-100)	80 (50-94)
Endomysium	IgA	IFA	95 (86-100)	99 (97-100)
	IgG	IFA	80 (70-90)	97 (95-100)
Tissue transglutaminase	IgA	ELISA	98 (78-100)	98 (90-100)
	IgG	ELISA	70 (45-95)	95 (94-100)
Deamidated gliadin peptide	IgA	ELISA	88 (74-100)	90 (80-95)
	IgG	ELISA	80 (70-95)	98 (95-100)

ELISA—enzyme-linked immunosorbent assay, IgA—immunoglobulin A, IgG—immunoglobulin G, IFA—immunofluorescence assay.
Data from Leffler and Schuppan.[15]

ensure that the patient is consuming a regular, gluten-containing diet. If not, this diet should be resumed before ordering the serologic test.

Seronegative CD is rare but can occur. If the concern remains that the patient might have CD but the serologic test results are negative, a referral should be made for small intestinal biopsy. Alternatively, HLA testing can be performed; if the patient's results are negative for HLA-DQ2 and HLA-DQ8, CD is not present and an alternate diagnosis should be considered. The role of HLA testing is discussed in detail later.

In a patient with positive serologic test results, is a small intestinal biopsy really necessary or can the GFD be started? Serologic tests are for screening purposes and do not confirm the diagnosis of CD, as illustrated by the case at the beginning of this article. Celiac disease is not a trivial disorder and the diagnosis should be taken seriously. It is a lifelong condition that requires a strict GFD with all its challenges of cost, complexity, and social restrictions.[17] The patient needs to be followed regularly on a long-term basis for monitoring of complications of CD and development of other autoimmune disorders.

Patients with positive serologic test results should be referred to a gastroenterologist for endoscopic small intestinal biopsies to confirm the diagnosis of CD. Endoscopic small intestinal biopsies are safe and carry minimal morbidity. However, timely availability of endoscopy remains a problem that requires attention. It is important that a GFD not be started before the biopsy is performed because such a diet will lead to healing of the small intestinal mucosa and complicate the interpretation of the biopsy results.

Dermatitis herpetiformis is "CD of the skin." Patients with this condition present with a chronic, severely itchy, blistering rash. A skin biopsy can confirm the diagnosis and small intestinal biopsy is not required because most of these patients will have villous atrophy.

The European Society for Paediatric Gastroenterology, Hepatology and Nutrition proposed a nonbiopsy approach to the diagnosis of CD in children.[5] These guidelines state that before a small intestinal biopsy can be avoided, a symptomatic patient must fulfil the following criteria:

- have a positive tTG antibody test result more than 10 times the upper limit of normal,
- have a positive endomysial antibody test result, and
- have positive results for HLA-DQ2 or HLA-DQ8 testing.

If the patient meets all 3 criteria, the family might be offered the opportunity to decline a biopsy because CD is highly likely.

It is important to note that just having a very high level of tTG antibodies is not enough to make a diagnosis of CD. Also, according to the diagnostic algorithm proposed in the guidelines, a patient with a positive tTG antibody test result should be referred by the primary care physician to a pediatric gastroenterologist for further assessment and workup.

False-positive results in serologic tests are uncommon but can occur. The case of the little boy described previously is an example. Because CD is a chronic disorder with substantial lifelong implications, all attempts should be made to confirm the diagnosis. It is bad to miss a diagnosis of CD, but it is also harmful to diagnose it in someone who does not have it.

What is the role of HLA testing in CD? Celiac disease has a strong genetic component. The most important

Box 2. Factors that might contribute to false-negative serologic test results in celiac disease

Factors include the following:

- Age < 2 y
- Possible laboratory error
- Reduction or elimination of gluten from the diet
- Selective IgA deficiency
- Use of corticosteroids or immunomodulator drugs

IgA—immunoglobulin A.

genetic risk factor for the development of CD is the presence of HLA-DQ2 (encoded by alleles A1*05 and B1*02) and HLA-DQ8 (encoded by alleles A1*03 and B1*0302) heterodimers. Almost all patients with CD will have one of these HLA types (HLA-DQ2 in about 95% of people with CD and HLA-DQ8 in 5% of people with CD).[8] Absence of these carries a negative predictive value of more than 99% and essentially excludes CD as a diagnosis. However, HLA typing is not performed routinely to establish a diagnosis of CD; this is because approximately 30% of the white population carries HLA-DQ2 or HLA-DQ8 and only about 4% of these individuals will develop CD.[7] Individuals who carry the homozygous HLA-DQ2 have the highest risk (up to 40%) of developing CD.[3] Individuals who carry heterozygous HLA-DQ2 and HLA-DQ8 are also at high risk.

Human leukocyte antigen testing can be used when the biopsy results are equivocal or in individuals who self-treated with a GFD and did not have serologic testing done before commencing the diet. Human leukocyte antigen testing can also be used to rule out CD in a symptomatic patient with negative serologic test results and to minimize future testing in high-risk individuals such as family relatives. To contain cost, when ordering HLA testing through the hospital laboratory, one should specify allele testing for only HLA-DQ2 and HLA-DQ8 rather than full HLA typing.

If a patient has clinical features suggestive of CD but has already started a GFD by the time of the office visit and is now feeling better, what are the options? This is a common clinical scenario as patients commence a GFD on their own. Feeling better on a GFD does not imply CD. The tTG antibody titres tend to decrease on GFD and eventually normalize. The rapidity with which this happens is variable. If the patient has started the GFD within a month or so, it is reasonable to obtain a tTG antibody measurement. If the tTG antibody test result is positive, the patient likely has CD. In that case, if the patient is not willing to stop the GFD and go for a small intestinal biopsy, the GFD can be continued. Further confirmation of the diagnosis can be done by obtaining the HLA type as discussed above. If the patient agrees to a biopsy, gluten should be resumed in the diet for several weeks and a referral made to a gastroenterologist.

If the tTG antibody test result is negative for a patient on a GFD, CD cannot be ruled out. In such cases, HLA typing should be obtained. If the test results are negative for HLA-DQ2 and HLA-DQ8, the diagnosis of CD is confidently excluded and no further testing for CD is required.

Can serologic tests be used in follow-up of a patient with CD? The patient's symptoms should resolve and the serum antibody titres normalize after starting a GFD. The speed with which the antibody test results normalize can vary depending on the severity of the disease and initial titres. In most cases, the test results become negative after 6 to 12 months of a GFD. Persistently positive antibody titres strongly suggest ongoing gluten exposure, often due to dietary contamination with gluten. The tTG antibody levels should be measured on a yearly basis. It is important to note that the test is not robust enough to show positive results with small or infrequent exposures to gluten.

There is debate about the usefulness of a repeat small intestinal biopsy in CD. A follow-up biopsy is not indicated in children. In adult patients, some gastroenterologists routinely perform a follow-up biopsy to confirm resolution of intestinal inflammation while others do not.[9] However, lack of a clinical response to a strict GFD in a patient (nonresponsive CD) should prompt repeat biopsy and additional investigations.

It is important to note that patients with CD are at risk of developing other autoimmune disorders.[3] Autoimmune thyroid disease is the most common comorbid autoimmune problem present. Autoimmune hepatitis and type 1 diabetes might also occur. In addition to clinical assessment, follow-up of patients with CD should also include thyroid function testing and measurement of liver transaminase levels every 1 to 2 years.

How can CD be differentiated from nonceliac gluten sensitivity (NCGS)? Nonceliac gluten sensitivity is a recently described phenomenon in which a patient has intestinal or extraintestinal symptoms that improve on a GFD in the absence of CD.[2] Autoantibodies, such as the tTG antibody, are absent and there is no villous atrophy or risk of other autoimmune disorders. The pathogenesis of NCGS is not well understood. It is not clear if NCGS is a transient or permanent problem and whether it is related to the dose of gluten ingested. Currently, there are no biomarkers for NCGS and hence the diagnosis is established only by excluding CD by serology (and biopsy) findings and based on the patient's symptomatic response to withdrawal of gluten from the diet.

Conclusion

Serologic tests are very useful for screening patients with suspected CD. Early diagnosis is essential in preventing complications of CD. Measurement of IgA tTG antibodies is the preferred test for screening patients. Total serum IgA level should be measured to exclude selective IgA deficiency and to avoid false-negative test results. Patients with positive serologic test results should be referred to a gastroenterologist for endoscopic small intestinal biopsies to confirm the diagnosis. Testing for HLA-DQ2 and HLA-DQ8 can help exclude the diagnosis. A GFD should not be started before confirming the diagnosis of CD.

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Contributors
Both authors contributed to the literature review, analysis, and interpretation, and to preparing the manuscript for submission.

Competing interests
None declared

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