A New Testing Algorithm for the Diagnosis of Celiac Disease

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ABSTRACT

The clinical investigation of patients with potential Celiac disease currently encompasses a large differential diagnosis including food allergies, medications, bacterial, viral and parasitic infection, AIDS, and Crohn's disease. The large diagnostic selection, coupled with a vast array of serological markers as well the self-treatment of patients makes diagnosing Celiac disease difficult. A newer marker, anti-deamidated gliadin peptide, should be paired with both the total serum IgA and anti-tissue transglutaminase IgA and IgG for screening symptomatic patients on a gluten diet for best diagnosis. Those patients that test positive by deamidated gliadin peptide and/or anti-tissue transglutaminase should then be confirmed with HLA typing and/or biopsy and started on a gluten-free diet sooner, reducing the destructive effects gluten can do on the intestine.

ABBREVIATIONS: CD - celiac disease, GFD - gluten free diet, AGA - anti-gliadin antibodies, TTG - anti-tissue transglutaminase antibodies, EMA - anti-endomysial antibodies, ARA - anti-reticulin antibodies, DGP - anti-deamidated gliadin peptide antibodies, IEL - intraepithelial lymphocytes, GALT - gut-associated lymphoid tissue, EATL - enteropathy-associated T-cell lymphoma.

INDEX TERMS: Algorithm, Autoantibodies, Celiac Disease, Gastroenterology, Genetic Testing, Gliadin, IgA Deficiency, Intestinal Mucosa, T-Lymphocytes, Transglutaminases

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INTRODUCTION

Celiac disease (CD) can be a devastating immunological disease that encompasses multiple signs and symptoms and where the only current treatment regimen is absolute adherence to a gluten-free diet (GFD). With a large differential diagnosis and several serological markers to use, the diagnosis of CD can be confusing not only to the patient but to the healthcare provider as well. The genetic predisposition for CD causes an autoimmune-mediated destruction of the small intestine villi followed by malnourishment, gastrointestinal distress and, if left undiagnosed and/or untreated, the possibility of the failure to thrive and even lymphoma. Current testing algorithms mainly include a combination of anti-gliadin autoantibodies (AGA), anti-tissue transglutaminase antibodies (TTG), anti-endomysial antibodies (EMA) and possibly anti-reticulin antibodies (ARA). However, failure to test for overall IgA status can lead to false-negative results as many patients with a genetic predisposition to CD actually have an IgA deficiency as well.[1] The newer anti-deamidated gliadin peptide, DGP, should also be added to the testing algorithm in place of the current AGA, EMA and ARA for more accurate test results. This article will look at the current prevalence and pathophysiology and will be followed by a new and easier testing algorithm for both patients and healthcare providers.

Prevalence

Celiac disease, also known as non-tropical sprue, is an autoimmune enteropathy that affects as many as 1 in 141 individuals in the United States and this rate could be on the rise due to the number of undiagnosed individuals with symptoms of CD.[2] There is racial and ethnic variation with non-Hispanic whites holding the highest incidence rate of 1% indicating at least 1.7

million in this population have CD, compared to the national average of .71%.[2] Risk factors of CD include, but are not limited to, other autoimmune-mediated diseases such as diabetes and thyroiditis, genetic factors like Down's syndrome, Turner's syndrome and IgA deficiency or a first-degree relative with these conditions or a diagnosis of CD.[3] Patients with IgA deficiency are 10-15 times more prone to CD than those without the deficiency.[1]

Presentation

There are four main presentations of CD that have been determined by a task force of physicians from seven different countries: classic CD, non-classic CD, subclinical and potential CD and can be seen in Table 1 below.[1,3,4] Nutritional deficiency is a common sign in the pediatric population, which can cause growth and psychomotor delays, rickets, and other hematological symptoms.[3] Infants have presented with severe diarrhea, dehydration, lethargy, and marked abdominal distention, which left untreated or undiagnosed can cause severe malnutrition.[3]

Non-classic CD does not manifest with typical gastrointestinal distress and may account for as much as 70% of CD cases.[3] Most of the symptoms seen in non-classical CD are due to the nutritional deficiency that ensues from malabsorption in the small intestines, as seen in the majority of pediatric cases, or in anemia in the teenage and young adult population.[3] In subclinical CD, many patients already suffer from another autoimmune disease such as diabetes type 1, thyroiditis, psoriasis, or genetic conditions such as Downs Syndrome, Turners Syndrome, and IgA deficiency.[3]

Pathophysiology

Celiac disease is an autoimmune, gastrointestinal disorder caused by the destruction of the small intestine mucosa leading to malabsorption and gastrointestinal distress.[5] Proline- and glutamine-rich residues compose the protein subunits of gliadins and glutenins which make up the wheat, rye and barley glutens that are the main culprits of the disease.[6] $CD4^+$ αβ T cells, coded for by the HLA-DQ2 variant 2.5 and HLA-DQ8 genes, become sensitized to these gluten proteins after translation modification deamidation by transglutaminase 2 and secrete an increased amount of interferon-γ (INFγ).[6,7] Transglutaminase is an enzyme responsible for transamination, or cross-linking, and deamination of glutamine residues, such as gluten, and is the primary autoantigen seen in CD.[7] This sensitization allows T cell infiltration of mucosa causing damage, villous atrophy, crypt hyperplasia and eventual activation of B cells which produce IgA antibodies to gliadin, endomysium and transglutaminase.[6,7] In addi-

Table 1. Clinical forms of Celiac Disease with respective serological and biopsy results. Adapted from Clinical Presentation of Celiac Disease Masks Therapeutic Perspectives of Celiac Disease, Pharmaceutica Analytica Acta, 2013 and The Olso Definitions for Celiac Disease and Related Terms. Gut, 2013.

	Symptoms	Serology Results	Biopsy Results
Classic CD	Pediatric: chronic diarrhea, vomiting, anorexia, abdominal pain and distention, decreased weight gain, weight loss, nutritional deficiency; Adults: constipation, nausea, loss of appetite, intermittent diarrhea	Positive	Positive
Non-classic CD	Intestinal manifestations: mild gastrointestinal distress; Extra-intestinal manifestations: anemia, height and weight deficiency, failure to thrive, delayed puberty, dermatitis herpetiformis, dental enamel hypoplasia, iron-deficiency anemia resistant to oral therapy, low bone mineral density, liver dysfunction, neurologic disorders of depression, anxiety, autism, peripheral neuropathy, cerebral ataxia, epilepsy and migraines	Positive	Positive
Subclinical CD	Rare, usually healthy patients	Positive	Positive
Potential CD	Constipation, nausea, loss of appetite, intermittent diarrhea	Negative	Negative

tion, $CD8^+$ αβ T cells and γδ T cells, also called intraepithelial lymphocytes (IEL), are activated by gluten as the body responds to the gluten onslaught. The IELs act as natural killer cells destroying the self's mucosa and with CD demonstrate memory and effector receptors against intestinal epithelial cells.[5] Both types of IEL in patients that are programed prior to being enlisted from the GALT to the small intestines.[5] These receptors and memory functions indicate that after a GFD is stopped, symptoms and intestinal damage will resume.[5]

Plasma cells secrete IgA class 2 antibodies to gliadin, endomysium and transglutaminase.[7] The endomysium is a connective tissue component where only a small portion of autoantibodies are targeted against.[7] B cells can act as antigen-presenting cells to T cells, further exacerbating the autoimmune response to gluten as well as acting as effector cells by direct damage to small intestinal mucosa, preventing angiogenesis and interfering with epithelial cell differentiation.[7]

Undiagnosed or untreated CD can cause severe intestinal damage and lead to other autoimmune diseases such as diabetes or intestinal lymphoma.[1,5] The IELs associated with CD have been shown to transform into enteropathy-associated T-cell lymphoma (EATL) which has a poor prognosis.[5] EATL has been found in those with poor adherence to a GFD, HLA-DQ2 homozygous state, and those with a late diagnosis of CD, all of which cause an extreme intensity of classic CD symptoms and tumor growth.[8] The 5-year survival in some patient populations with EATL is as low as 8%.[8]

Current Laboratory Tests

Current laboratory testing includes measurements of antibodies to AGA IgA and IgG, TTG IgA and IgG, ARA IgA, EMA IgA by immunoassay and/or immunofluorescence and testing levels of total IgA. Genetic testing for HLA-DQ2.5 and HLA-DQ8 haplotypes are seen as a necessary second step in CD.[9]

The EMA and ARA assays are used less frequently due to the fact that the EMA is an immunofluorescence test which is costly, time consuming and subjective while the ARA test has limited availability. AGA and TTG are enzyme-linked immunosorbent assays (ELISA) and are highly specific and sensitive when used together in patients with current symptoms.[9] Testing with only the IgA versions of the AGA and TTG tests is not recommended as there can be false-negative results due to the high incidence of IgA deficiency, which is why a total IgA should always be performed. Gliadin antibodies were once thought to be accurate assays for CD, however, in the last several years the sensitivity and specificity were found to be much less than previously thought, giving rise to inaccurate results that do not correlate with other serological testing or symptoms of CD.[10]

HLA typing for the DQ2.5 and DQ8 variants is highly sensitive and a negative result all but rules out CD.[4] However, the HLA typing should not be performed alone because a positive result without other assay results or current symptoms cannot be used to diagnose CD since as much as 30% of the general population have the DQ2.5 and DQ8 HLA genotype.[11]

The biopsy of the small intestine is still considered the gold standard for diagnosis of CD, despite the advances in serological and genetic testing.[9] Upper gastrointestinal endoscopy is the method of choice for obtaining biopsies and should include a minimum of 4 biopsies with two from the distal duodenum and two from the bulbus, however, the American Gastroenterology Association recommends at least six biopsies.[11]

Newer Testing Options

One biochemical marker that is being used more often is the DGP. This marker is both more sensitive and more specific than the gliadin antibodies for detecting patients with CD.[10] The DGP assay is an ELISA test with deamidated gliadin peptide-coated wells and horseradish-peroxidase-labeled goat anti-human IgA or IgG as the conjugate.[10] The DGP IgA+IgG assay is similar except it tests for both IgA and IgG at the same time using tetramethylbenzidine as the conjugate for spectrophotometric readings.[10] Several studies suggest the current gliadin assays are obsolete for use in CD diagnosis and prognosis as the sensitivity and specificity for gliadin-IgA is only 52% and 71% and 57% and 47% for gliadin-IgG, respectively.[10,13] Deamidation of the gliadin proteins by tissue transglutaminase increases the binding of anti-gliadin antibodies giving a sensitivity and specificity of 74% and 95% for IgA and 65% and 98% for IgG, respectively.[10] Another study

found both the sensitivity and specificity to be 95% for IgA, and recommend the use of the DGP assay for better prognosis once the patient initiates the GFD to monitor persistent villi damage as opposed to TTG.[13]

Another marker being studied more is the anti-actin antibody (AAA) IgA, an antibody whose levels correlate with intestinal damage.[14] This assay is an immunufluorescent serological assay that in one study by Schirru et al identified 20 more patients with severe small intestinal lesions known as a Marsh 3 lesion, a hyperplastic lesion with villous atrophy.[12,13] The sensitivity and specificity in this study were 60% and 100%, respectively.[14] The authors also suggest using the AAA for symptomatic individuals with TTG values that do not correlate with CD, however, results need to be interpreted carefully due to the subjectivity of the test.[14]

Diagnosis

Patients presenting with classic or non-classic symptoms of CD should be tested for serum levels of total IgA, DGP IgA and IgG and TTG IgA and IgG. If the serum IgA is normal and the DGP and TTG tests are positive, HLA testing for DQ2.5 and DQ8 should be performed and if positive, small intestine biopsy should follow to identify the typical lesions of CD. However, if the above serological tests are all positive, biopsy may not be necessary since the serological tests are highly sensitive and specific for patients with CD when used with the HLA typing.[9] Catassi and Fasano suggest that 4 out of the 5 following criteria should be met before a diagnosis of CD is given; symptoms, seropositivity, HLA-DQ2.5 and/or DQ8 positive, biopsy positive and symptom-free after GFD initiation.[9] If HLA typing is not performed, at least 3 of the 4 should be positive, one of which should be the biopsy, before a diagnosis of CD is given.[9]

In individuals with IgA deficiency, the clinical utility of DGP, TTG and EMA IgA assays are decreased due to false-negative results and the IgG results should be interpreted in light of current symptoms, biopsy results, HLA typing and symptom reversal with a GFD. All patients should be tested for all antibodies and HLA testing at the same time to minimize the stress on the patient of repeat venipuncture and delayed results. Many laboratories now offer serological tests as part of a reflex panel; if the total serum IgA is decreased, the laboratory will automatically reflex to perform DGP and/or TTG IgG tests. Serum EMA testing is falling out of use due to the subjectivity of the assay, cost and

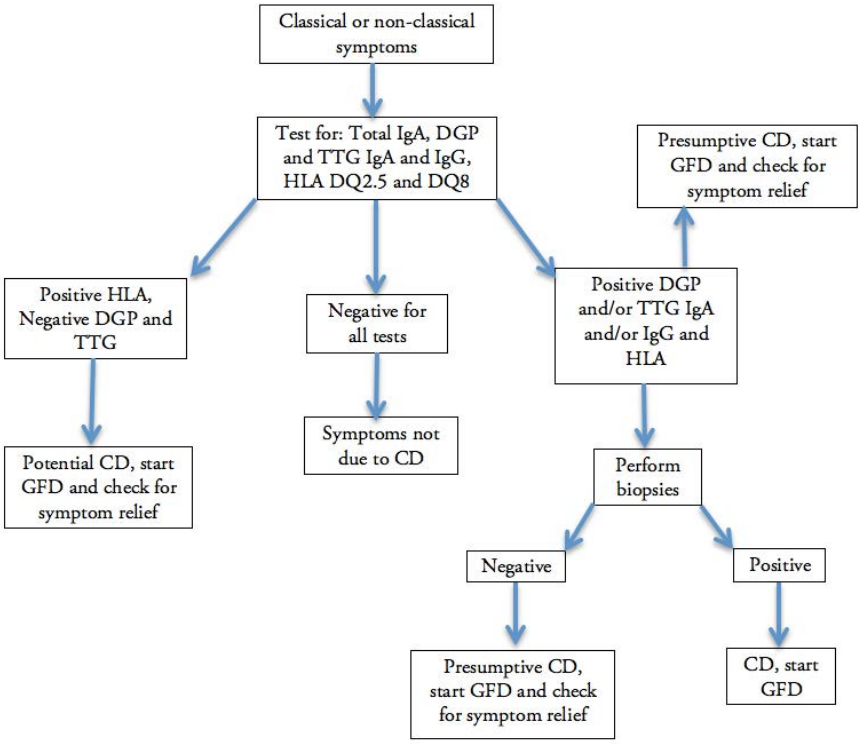

Figure 1. A new testing algorithm for diagnosing CD uses serological, genetic and endoscopy results in conjunction with current signs and symptoms. Including all serological (total IgA, DGP and TTG both IgA and IgG) and genetic markers (HLA DQ2.5 and DQ8) at once is both more efficient and easier for the patient.

the limited utility since DGP, TTG and HLA are all highly specific. Figure 1 demonstrates a new testing algorithm for diagnosing CD.

Diagnostic and Testing Considerations

All patients with chronic gastrointestinal distress should be tested for CD and those with a history of another autoimmune disorder or a family member with CD should also be tested with the above algorithm if symptoms arise. CD should be included in the differential diagnosis of those with chronic gastrointestinal issues although screening for CD is not necessary on a population level due to the low incidence of the disease, which is under 1% nationally.[2]

The difficulty in diagnosing CD can be due to self-treated patients on a GFD and the high proportion of patients with IgA deficiency. If patients have already started a GFD before serologic testing occurs, the results will be inaccurate as antibodies levels fall in correlation to the length of the GFD, which may cause false-negative results.[1,5] Patients need to be on a gluten-containing diet before serologic testing for accurate results; however, if a patient cannot tolerate restarting a gluten diet, HLA testing should be performed first to confirm a genetic predisposition of CD. As described above, IgA deficiency in patients suspected of having CD can also cause issues for a correct diagnosis. If a clinician fails to test for total serum IgA, the remaining serological tests for CD are moot as they will be falsely negative. This can lead to a potential misdiagnosis where a patient may undergo unnecessary testing or unwarranted stress and intestinal damage.

CONCLUSION

Celiac disease is not a highly prevalent disease, however, the differential diagnosis for CD is varied and can include many different etiologies from chronic food allergies and organism infections, to medications and Crohn's disease.[12] Proper inclusion of CD into the differential diagnosis of patients with chronic gastrointestinal distress will increase the diagnosis and the prognosis of these patients. Correctly assessing the patient's immune state by including the total serum IgA assay coupled with the DGP assay to screen symptomatic patients on a gluten-containing diet will decrease false-negative tests and correctly identify those patients that require HLA typing or intestinal biopsy. Including the HLA typing in the first round of serological testing can help reduce unnecessary biopsies and properly identify those at risk for CD. Patients that do subsequently test positive for CD can then be put on a GFD sooner, reducing the intestinal damage and malnutrition that ensues

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