Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Diagnosis of Celiac Disease

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Slide 1:
Title slide

Slide 2:
Celiac disease is a chronic inflammatory condition that primarily affects the small intestine. It is classified as an autoimmune disease due to its association with the presence of autoreactive lymphocytes and the production of autoantibodies. It is caused by an inflammatory response mounted by the patient's own immune system against dietary gluten. However, this inflammatory response only occurs in individuals with a specific genetic susceptibility. The inflammatory response associated with celiac disease ultimately results in damage and atrophy of the villae within the small intestine. It is this inflammation and villous atrophy that ultimately lead to the clinical symptoms generally associated with celiac disease.

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Patients with celiac disease may present with constitutional symptoms, or intestinal symptoms, or a combination of both. Pediatric patients may present with a failure to thrive or developmental delays. They may also have evidence of malnutrition, including iron-deficient anemia and vitamin deficiency. In adults, patients with longer-standing disease may show symptoms of malnutrition, including weight loss, anemia and osteoporosis. Patients may also complain of fatigue and generalized arthralgias. With regard to intestinal symptoms, both pediatric and adult patients may report episodes of diarrhea, vomiting, and/or abdominal pain. However, it is estimated that less than half of patients with celiac disease actually present to their physician with intestinal involvement. In many patients, the symptoms of celiac disease are nonspecific, often making for a challenging diagnosis.

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The systemic and intestinal symptoms associated with celiac disease are also reflected in some of the general lab testing performed for these patients. Many of the laboratory abnormalities, such as iron-deficient anemia and mineral and vitamin deficiencies, probably reflect the malabsorption present in these patients. In some cases, patients with celiac disease may present with elevated transaminase concentrations, likely reflecting an element of liver dysfunction. Hypoproteinemia, hypoalbuminemia, and/or hypogammaglobulinemia may also be present. This may reflect both loss of protein through the gastrointestinal tract and low protein production due to liver dysfunction.

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Celiac disease is associated with a variety of co-morbid conditions. Patients with celiac disease may have evidence of immunologic abnormalities, specifically a selective IgA deficiency. This type of IgA deficiency occurs significantly more frequently in patients with celiac disease as compared to the general population, although the prevalence is still only on the order of 3% to 4%. However, IgA deficiency is important to recognize because, as we will discuss later, many specific antibody tests used to diagnose celiac disease detect antibodies of the IgA isotype. Celiac disease is also associated with several other autoimmune endocrine diseases, such as type I diabetes. This association is quite significant, and in some practices, newly diagnosed type I diabetics are screened for possible celiac disease. Celiac disease may also be found in individuals with autoimmune liver and certain systemic rheumatic diseases. Lastly, the dermatologic disorder dermatitis herpetiformis occurs frequently in patients with celiac disease. In fact, this disorder is associated with one of the same autoantibodies implicated in the pathogenesis of celiac disease.

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Patients with celiac disease do have a 2-fold increase in mortality. This excess risk is generally most evident during the first 3 years after diagnosis and is frequently associated with malignancy, such as lymphoma. There is a relationship between an increased risk for mortality and a delay in diagnosis, more severe clinical presentation, and lack of compliance with treatment. The treatment for celiac disease is a life-long maintenance of a gluten-free diet. This treatment is most successful when patients have guidance from a nutritionist with experience in celiac disease management. Approximately 70% to 80% of patients will have a good response to a gluten-free diet, with resolution of many of their clinical symptoms. Patients who do not have a good initial response to this diet alone may benefit from a course of corticosteroid treatment, followed by maintenance of the gluten-free diet. The goal with treatment is to remove the initiator of the immune response and to down-regulate the inflammation.

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For celiac disease to develop, the proper environmental exposure must occur in an individual with genetic susceptibility. The genetic component of celiac disease had been inferred from observations that the disease occurred in families, with family members of individuals with a confirmed diagnosis of celiac disease being at greater risk of being affected themselves. Ultimately, specific alleles of the human leukocyte antigen, or HLA, complex were demonstrated to be responsible for much of the genetic susceptibility. The two specific alleles associated with celiac disease are HLA-DQ2 and HLA-DQ8. HLA-DQ2 and HLA-DQ8 are found in virtually all patients with celiac disease.

The environmental component that initiates the inflammatory response associated with celiac disease is exposure to protein from wheat, barley, or rye, collectively known as gluten. Gluten is responsible for the elastic properties associated with grain proteins. Gluten has high proline and glutamine content, which makes it relatively resistant to proteolytic degradation within the gastrointestinal tract. It is this property, in part, that makes this protein so immunogenic.

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The diagnosis of celiac disease relies on both laboratory evaluation and biopsy findings. A presumptive diagnosis can be established if a patient has positive serology, which I will expand on in a moment, and an intestinal biopsy that demonstrates villous atrophy. In the upper figure, you see a biopsy of a normal small intestine, with intact villae. In the bottom figure, you see the villous atrophy and inflammatory response that occurs in celiac disease. Once a presumptive diagnosis has been established, the patient will be started on a gluten-free diet. Once gluten has been successfully removed from the diet, the patient should begin to see resolution of their clinical symptoms, which is often accompanied by conversion to a negative serology and reconstitution of the villae in the small intestine. Using these criteria, a definitive diagnosis of celiac disease can be established.

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As I stated in the last slide, laboratory testing plays a key role in establishing a diagnosis of celiac disease. The laboratory testing can be divided into serologic testing and HLA typing. The serologic evaluation generally includes measuring total IgA concentrations and specific autoantibodies, namely tissue transglutaminase, or TTG, antibodies; deamidated gliadin antibodies; and endomysial antibodies, or EMA. One important consideration to keep in mind regarding these specific antibodies is that, in patients with celiac disease, their titers may decrease with proper adherence to a gluten-free diet. The other laboratory test available for celiac disease is HLA typing. In the context of celiac disease, HLA typing focuses only on determining if either the DQ2 or DQ8 are present. In the next few slides, I will cover the specific utilities of these various tests.

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In most cases, evaluation for celiac disease will include measurement of total IgA concentrations. The specific autoantibodies used to diagnose celiac disease may be either the IgA or IgG isotype. In general, these two isotypes have similar specificity, whether for TTG or deamidated gliadin. However, the IgA isotypes usually have much better sensitivity in comparison to the IgG isotypes. Therefore, the IgA isotypes are considered the optimal isotype when evaluating for the presence of TTG or deamidated gliadin antibodies. However, this would obviously be a problem in patients who are IgA deficient. For these individuals, testing for the IgG isotype is the only alternative. So, the utility of total IgA testing is to identify individuals who are IgA deficient and in whom specific autoantibodies of the IgG isotype should be tested.

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After assessing for total IgA, the next step would be to test for the specific antibodies associated with celiac disease. EMA, one of the first antibodies found to be useful for the diagnosis of celiac disease, is so-named because it detects an antigen in the endomysium, which is the connective tissue that surrounds smooth muscle fibers. It was subsequently determined that the antigen target of the EMA was tissue transglutaminase. It was this discovery that led to development of immunoassays specific for TTG. TTG is an enzyme that is capable of deamidating glutamine to glutamic acid. The testing methodology for EMA and anti-TTG antibodies is substantially different. EMAs are detected by an immunofluorescent assay using a substrate that includes smooth muscle, such as monkey esophagus, while anti-TTG antibodies are detected using plate-based enzyme immunoassays. Testing is generally available for TTG-IgA and TTG-IgG antibodies, although EMA assays are generally available only for the IgA isotype.

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The other specific serology useful for the diagnosis of celiac disease are antibodies against gliadin. When wheat or barley is ingested, the gluten protein is digested into smaller peptides about 30 to 35 amino acids in length. The resulting peptides can be divided into ethanol-soluble and ethanol-insoluble fractions. The ethanol-soluble fraction is referred to as gliadin. The first immunoassays developed tested for antibodies against unmodified, or native, gliadin. However, these assays were inferior to the TTG antibody and EMA assays and were not recommended. The newest generation of gliadin antibody assays uses a novel form of this antigen, specifically deamidated gliadin. These assays detect antibodies against gliadin that has undergone enzymatic deamidation by tissue transglutaminase. These newer assays specific for deamidated gliadin are very similar to the TTG antibody assays in both sensitivity and specificity. Given the difference in performance between unmodified and deamidated gliadin assays, it is critical that laboratories clearly specify on the patient report which test is being used, as this will have a significant impact on the interpretation of the result.

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Lastly, separate from the serology testing is the HLA typing. The HLA-DQ molecules are composed of an alpha and a beta chain. Identification of both chains is required in order to determine if either the DQ2 or DQ8 alleles are present. In general, this type of testing is performed using PCR amplification of the alpha and beta chains, followed by allele-specific identification. For HLA-DQ2, DQA1*05xx in conjunction with DQB1*0201 or *0202 are considered permissive for celiac disease, even if an individual is heterozygous. In contrast, DQA1*0201 with DQB1*0202 must be present as a homozygote in order to be considered permissive for celiac disease. If only one copy of DQA1*0201 with DQB1*0202 is found, the results are considered to be equivocal. For HLA-DQ8, a single copy of DQA1*03xx with DQB1*0302 would be considered to be permissive for celiac disease. The HLA-DQ2 allele is found in approximately 90% to 95% of individuals with celiac disease; the remaining 5% to 10% possess the HLA-DQ8. This testing is definitely not required to establish a diagnosis of celiac disease. Although the absence of these alleles virtually excludes celiac disease as a diagnosis, the presence of either allele is not diagnostic for the disease. In other words, the HLA alleles are necessary, but not sufficient, for celiac disease to present in a given individual.

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To summarize the testing available for celiac disease, quantitative assessment of total IgA is useful in identifying individuals with IgA deficiency. For this sub-group of individuals, testing for specific antibodies of the IgA isotype is of little use. The purpose of the TTG and deamidated gliadin antibody tests is to identify individuals with suspected celiac disease. These are the individuals in whom a small intestinal biopsy is most warranted. One caveat to this testing strategy is that TTG and deamidated gliadin antibody titers generally decrease when a patient has been following a gluten-free diet. For the HLA typing, although a positive result does not confirm a diagnosis of celiac disease, a negative result, meaning that both DQ2 and DQ8 alleles are absent, essentially rules out the diagnosis. This may be of use in individuals with equivocal serologic and/or biopsy results or for whom there is a family history of celiac disease.