Evolution of Celiac Disease Testing

The laboratory is challenged to provide guidance on test ordering and interpretation while ensuring accurate performance and appropriate test utilization

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Celiac Disease Testing

Celiac disease (CD) is an immune-mediated inflammatory process elicited by gluten that occurs in genetically susceptible individuals. The inflammatory response is triggered when partially digested gluten peptides (especially gliadin) reach the intestinal mucosa. These peptides are a substrate for tissue transglutaminase (TTG), which acts to deamidate gliadin, forming deamidated gliadin peptide (DGP). Both DGP and DGP-TTG complexes can be immunogenic, resulting in the production of antibodies against TTG and DGP, local inflammation, and damage to the intestinal mucosa causing malabsorption. This can lead to signs and symptoms such as anemia, failure to thrive, weight loss, diarrhea and bloating. HLA molecules encoded by the DQ2 and DQ8 haplotypes are associated with this immune process, forming part of the genetic basis of CD.

Traditionally, CD diagnosis requires duodenal biopsy to characterize the degree of mucosal damage. Histological changes seen in CD include villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes. Classification of damage severity is outlined by the Marsh Criteria, which remains the gold standard for CD diagnosis. Histological analysis is not without its limitations, however (Table). Biopsy procedures are invasive, so other means of identifying CD patients have been sought. Serological tests, which detect antibodies produced during the mucosal immune reaction, provide a less invasive alternative.

Serological Testing

Early indirect immunofluorescence methods detected CD-specific antibodies in a semi-quantitative fashion. The anti-endomysial antibody test (EMA) remains the most sensitive and specific of these. A positive test is defined by the presence of staining restricted to smooth muscle fibers, and titers can be reported for semi-quantitative results. This assay can be challenging due to scarcity of tissue substrates, subjective interpretation and method heterogeneity between labs. Other immunofluorescence-based markers such as reticulin and gliadin antibodies have been shown to have inferior sensitivity and specificity to EMA, and should not be used for CD testing. The antigenic target of EMAs was later discovered to be TTG; DGP was also found to be a target of antibodies in CD patients. This led to the production of quantitative immunoassays for both anti-TTG and anti-DGP antibodies. Solid-phase immunoassays using immobilized TTG or DGP are commonly used. Following incubation with a patient’s serum, anti-TTG or anti-DGP antibodies are detected using labeled anti-human, class-specific detection antibodies. Radiobinding assays have also been described. These assay formats allow for the use of a calibration curve and reporting in concentration units. A positive result is defined at a specific cut-off. However, because assay reagents vary by manufacturer, laboratories and clinicians need to be aware that cut-offs and results are not interchangeable.

Other Testing Considerations

Several other important considerations must be made when using serological testing in CD. First, mucosal immune responses typically involve IgA-class antibodies; therefore, serological testing commonly targets IgA class antibodies. However, IgA deficiency occurs more frequently in CD patients compared to population estimates (2.5% versus 0.25%), and IgA-based serological tests may not provide informative results in these patients. IgA quantitation can help interpret...
and prompt the use of IgG-based tests when necessary.\textsuperscript{23-25} The use of immunosuppressants can decrease immunoglobulin levels, also impairing result interpretation. Therefore, medication status of patients must be considered.\textsuperscript{1} Finally, some studies have suggested that not all serological markers perform equally in young children, so alternative testing strategies may be required in some patient groups.\textsuperscript{26,27}

The nature of the immune response in CD should also be considered. Since antibodies are produced in response to gluten exposure, false-negative results may occur if a patient is on a gluten-free diet (GFD). However, since symptoms and antibodies remit upon removal of gluten, serological tests are useful for monitoring adherence to a GFD.\textsuperscript{1,28} A portion of CD risk is heritable and related to HLA molecules, specifically those encoded by DQ2 and DQ8 haplotypes.\textsuperscript{4,29,30} While these are thought to be required for the development of CD, their presence is not sufficient for diagnosis. Absence of HLA DQ2 or DQ8-associated alleles is thought to rule out CD, making HLA typing useful due to its high negative predictive value.\textsuperscript{1,4,28} In some situations, a positive HLA result may also aid in patient management decisions.\textsuperscript{1}

\textbf{Guideline Highlights}

Clinical guidelines from a number of organizations have been recently updated to reflect advancements in our understanding of CD pathophysiology, biology and genetics. The lab needs to be aware of these updates to provide guideline-based services, optimize testing algorithms, and understand changes in ordering patterns. The reader is referred to the full guideline documents for complete information.\textsuperscript{1,5,14,28,31} The recent European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guideline update includes novel approaches to CD testing in children, with separate algorithms proposed for symptomatic patients and asymptomatic patients at high risk of CD (for example, those with affected first-degree relatives, other autoimmune conditions, or certain chromosomal and endocrine abnormalities).\textsuperscript{1} The former advocates for IgA-TTG testing first, along with ascertainment of IgA sufficiency. Very high TTG results may not need confirmation by biopsy, but can be substantiated by EMA and HLA testing.\textsuperscript{1} While prospective data is still needed, a few studies have yielded promising results for this approach.\textsuperscript{32,33} In asymptomatic high-risk children, HLA testing is suggested as the first-line test due to its high negative predictive value. TTG testing is then reserved for those who are HLA-positive, or have not had HLA typing performed. Confirmation of low-positive TTG results by EMA is recommended to reduce the number of false-positive results and unnecessary biopsies.\textsuperscript{1}

Guidelines from other agencies are applicable to a broader age group and may not distinguish between low and high-risk individuals. These include documents from the American College of Gastroenterology (ACG), North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHN), the World Gastroenterology Organization (WGO) and the UK National Institute for Health and Care Excellence (NICE).\textsuperscript{5,14,28,31} Most of these organizations recommend IgA-TTG as the first-line test and outline its use in symptomatic or high-risk individuals. The WGO suggests TTG and/or DGP, with the addition of EMA to increase specificity if screening in a general population.\textsuperscript{28} Biopsy remains the gold standard for diagnosis and is not omitted in any case by these organizations, with possible exceptions in resource-limited settings.\textsuperscript{28}

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Sidebar: Case for the Neo-epitope By Dr. Torsten Matthias

The so called neo-epitope is the complex of tissue transglutaminase (tTg) and gliadin peptides proven to form in vitro and in vivo.

Fleckenstein et al characterized the molecular structure of the covalent complexes between tissue transglutaminase and gliadin peptides. Ciccocioppo et al demonstrated that the deamidation and crosslinking of gliadin peptides by transglutaminases formed supramolecular complexes in normal and diseased duodenal mucosa and that the level of both molecules was increased in active celiac disease.

Several external studies proved the superior performance of ELISA assays using the neo-epitope as antigen to capture autoantibodies linked to celiac disease (CD) and dermatitis herpetiformis (DH). In 2006, Reeves published an Australian multicenter study in which the neo-epitope screening test performed best compared to 11 competitor assays. In 2008, Marcos demonstrated that the neo-epitope ELISA assay was a much better screening tool for CD in pediatric and adult samples compared with assays using pure tTg.

McMillan revealed in his study that high positive results for neo-epitope antibodies correlate with the severity of mucosal damage. Therefore, he stated that confirmatory small bowl biopsy might not be needed in patients with high levels of CD antibodies.

Jaskowski showed that neo-epitope IgA assays exhibited good sensitivity and specificity in pediatric population confirmed by biopsy. Moreover, this study proved its very good correlation with MARSH criteria also in pediatrics.

A year later, Tozzoli stated that the neo-epitope ELISA assay was able to identify CD patients who have been tested negative with conventional antibody assays. In the same year, Tonutti demonstrated that the neo-epitope IgA test became positive earlier than anti-tTg assays.

Two years later, Bizzaro confirmed that the neo-epitope ELISA assay had a very high sensitivity and that this method for measuring anti-complex antibodies could be used as a reliable test for screening in the general population or in at-risk groups. He also stated that one could hypothesize that these antibodies were present early in the natural course of CD and, therefore, had a predictive value for clinical and subclinical disease patients. In 2013, Lytton stated that the neo-epitope IgA autoantibodies represented a new and sensitive marker for DH.

These and further studies (comprising more than 50,000 samples) demonstrated the superior performance of the neo-epitope antigen as a highly sensitive and specific screening assay for both adults and pediatrics. Due to its very good correlation with MARSH criteria, biopsy could be potentially avoided in cases of high levels of neo-epitope antibodies. Studies also demonstrated that the neo-epitope IgA antibodies represented a sensitive marker for both CD and DH.

More importantly, it showed that antibodies to the neo-epitope could be positive up to 12 months earlier than anti-tTg antibodies. This shortens the time to diagnosis, which has an important impact on the health improvement status of the patients.

Dr. Matthias has nearly two decades of experience with R&D in the autoimmune field. AESKU, based in Wendelsheim, Germany, is a research-focused supplier of products and services for early detection diagnosis.

References (Sidebar)
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