Testing for Antireticulin Antibodies in Patients with Celiac Disease Is Obsolete: a Review of Recommendations for Serologic Screening and the Literature

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Celiac disease (CD) is an autoimmune disorder that occurs in genetically susceptible individuals of all ages and is triggered by immune response to gluten and related proteins. The disease is characterized by the presence of HLA-DQ2 and/or -DQ8 haplotypes, diverse clinical manifestations, gluten-sensitive enteropathy, and production of several autoantibodies of which endomysial, tissue transglutaminase, and deamidated gliadin peptide antibodies are considered specific. Although antireticulin antibodies (ARA) have historically been used in the evaluation of CD, these assays lack optimal sensitivities and specificities for routine diagnostic use. This minireview highlights the advances in CD-specific serologic testing and the rationale for eliminating ARA from CD evaluation consistent with recommendations for diagnosis.

Celiac disease (CD) is an autoimmune disorder elicited by gluten and related proteins in genetically susceptible individuals of all ages. It is characterized by the presence of diverse clinical symptoms, CD-specific antibodies, the presence of HLA-DQ2 and/or -DQ8 molecules, and gastrointestinal tissue damage (1–5). While the presence of HLA-DQ2 and/or -DQ8 haplotypes constitutes a genetic risk for CD, several non-HLA genes, especially interleukin-21 (IL-21), IL-2, and KIAA1109 gene clusters, have been reported (6, 7). Furthermore, the availability of sensitive and more-specific serologic tests such as the tissue transglutaminase (tTG), endomysial antibody (EMA), and more recently the deamidated gliadin peptide (DGP) antibody assays permits the efficient screening of symptomatic and nonsymptomatic patients at risk for CD. The combination of serologic and molecular genetic diagnostic tools has significantly increased our current knowledge of the clinical spectrum of CD as well as its epidemiology. Based on current literature, the estimated ratio of diagnosed to undiagnosed cases varies between 1:5 to 1:8 with most individuals presenting with atypical clinical manifestations of disease (8, 9). Overall, CD appears to be more common in individuals of northern European origin; in this population, it is estimated to affect approximately 1 to 2%. Recent epidemiological studies show that CD also occurs in other parts of the world. Based on current trends, the frequency of CD may increase as these developing countries adopt gluten-rich diets (1, 10, 11).

PATHOGENESIS OF CELIAC DISEASE

CD is one of the better-understood autoimmune diseases with key features of its immunopathogenesis and underlying genetics described (1, 2, 12, 13). It is thought to be initiated in genetically predisposed individuals by the ingestion of gluten and related proteins found in grains such as wheat, rye, and barley. The events leading to CD are thought to include luminal and early mucosal events, activation of the innate and adaptive immune systems, as well as intestinal tissue damage (12–15). In the early stages of CD, ingested gluten (gliadin and glutenin are the major protein components of gluten) is digested by luminal and brush-border enzymes into amino acids and α-gliadin peptides that are resistant to further degradation. Partially digested α-gliadin peptides are able to cross the epithelial cells and enter the lamina propria where they are cross-linked and deamidated by tTG to produce DGP. Induction of CD4 T-cell-specific responses is thought to be initiated by DGP bound with high affinity to HLA-DQ2/DQ8 molecules expressed on the surfaces of antigen-presenting cells (APCs). Activated CD4 T cells, in addition to providing help to B cells in eliciting antibody-specific responses produce proinflammatory cytokines such as gamma interferon (IFN-γ), IL-15, and IL-17. Gliadin is also thought to stimulate the innate immune system directly through the upregulation of IL-15 in the intestinal epithelial cells. IL-15 is widely recognized to activate intraepithelial lymphocytes (IEL) as well as upregulate MIC-A, a stress molecule on enterocytes and the NKG2D receptor, promoting lymphocyte-mediated cytotoxicity of enterocytes. Additionally, CD4 T cells that are activated by IL-15- and IFN-α-secreting dendritic cells (DCs), produce IL-21, which in turn induces stromal cells to produce matrix metalloproteinases (MMPs). Thus, inflammatory cytokines (as described above), apoptotic proteins (granzyme B and perforin), and cytotoxic proteins (metalloproteinases) are thought to be responsible for damage to intestinal tissue seen in patients proven to have CD by biopsy specimens (14–18).

Some models propose that the tTG-gliadin complexes themselves are immunogenic, resulting in the production of autoimmune antibodies against tTG (5). Presentation of DGP by APCs requires HLA-DQ2 or -DQ8 molecules. These HLA types are expressed in nearly all patients with CD and contribute to the genetic component of CD pathophysiology (1, 4, 19).

CLINICAL INDICATIONS AND DIAGNOSTIC RECOMMENDATIONS FOR CELIAC DISEASE

Timely and accurate diagnosis of CD is important to avoid negative health outcomes, particularly in children. Untreated CD can
lead to decreased nutrient absorption and malnutrition. Patients with CD are also at increased risk for other autoimmune diseases and other conditions such as non-Hodgkin’s lymphoma (1, 4, 20). To prevent diagnostic delays, guidelines for the diagnosis of CD recommend testing based on the presence of symptoms and/or risk factors for disease (1, 4, 21, 22). Symptoms associated with CD in children and adolescents include the following: chronic or intermittent diarrhea; failure to thrive (FTT); weight loss; stunted growth; delayed puberty; amenorrhea; iron deficiency; anemia; nausea; vomiting; chronic abdominal pain, cramping, or distension; chronic constipation; chronic fatigue; recurrent aphthous stomatitis (mouth ulcers); dermatitis herpetiformis-like rash; fracture with inadequate trauma/osteopenia/osteoporosis; and abnormal liver biochemistry. Individuals with type 1 diabetes mellitus (T1DM), Down syndrome, autoimmune thyroid disease, Turner syndrome, Williams syndrome, selective immunoglobulin A (IgA) deficiency, and autoimmune liver disease and first-degree relatives with CD are also considered to be at increased risk for disease and should be evaluated.

Different groups have developed guidelines for the diagnosis of CD, with the most recent recommendations published in 2012 by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (4). Among the most current sources of recommendations are the 2009 United Kingdom National Institute for Health and Clinical Excellence (NICE) guidelines and the 2005 North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) (21, 22). The testing algorithms proposed by different societies endorse the use of a number of diagnostic tools, albeit in different orders or combinations depending on the presence of symptoms or risk for disease (4, 21, 22). Diagnostic tools include (i) serologic testing for specific antibodies, (ii) histological analysis of small bowel biopsy specimens, and (iii) recommendation for HLA typing (DQ2 and DQ8) in populations that are at risk as well as an alternative to specimens, and (iii) recommendation for HLA typing (DQ2 and DQ8). In 1977, antigliadin antibodies (AGA) and antigliadin antibodies (AGA) and EMAs were reported in patients with dermatitis herpetiformis and CD (27). EMA is detected by IFA using monkey esophageal or human umbilical cord tissue, and results are reported in titers. Major limitations of this test include the inherent subjectivity of IFA, expertise needed for interpretation, and cost. Despite these challenges, the EMA antibody assay continues to be a mainstay in the diagnosis of CD due to its excellent predictive value for disease. Serologic evaluation for AGA, ARA, and EMA IgA antibodies became a part of the CD diagnostic scheme for the first time in 1990 (28). Following the 1990 ESPGHAN guidelines, comparison of ARA to AGA and EMA IgA tests found ARA testing to be a less reliable screening tool, since only 65% of CD patients were positive (25). Similarly, the clinical relevance of the ARA test became questionable, as a plethora of false-positive results occurred in low-risk patients, as well as patients with non-CD-related gastrointestinal problems (29, 30).

The timely identification of TG as the target antigen of the EMA by Dieterich et al. (31) in 1997 with subsequent development of anti-tTG immunoassays is believed to have radically changed how screening for CD is performed. Anti-tTG IgA immunoassays are generally more sensitive but less specific than the EMA immunoassays (32). Furthermore, the diagnostic performance of anti-tTG IgA tests has been reported to be dependent on the assay principle, including the source of TG antigen (human recombinant or purified tTG of human and nonhuman origin) (32–36). Despite this, the anti-tTG immunoassays are easier to perform, less subjective, and more amenable to automation than EMA immunoassays. Thus, anti-tTG tests are more likely to be offered as first-line screening assays in the evaluation of CD.

In 1999, Quarsten and colleagues made the seminal observation that tTG is responsible for the processing events (deamidation of gliadin) leading to the preferential presentation of gliadin peptide by the HLA-DQ2 molecule (37). Following the confirmation of their report by several others, measurement of anti-DGP antibodies by ELISA was developed and reported in CD patients (38). The anti-DGP antibody test provides a more specific marker for disease compared to its predecessor, the antigliadin antibody assay. Although the anti-DGP antibody test is not currently recommended for routine screening, it may be useful for patients in whom suspicion of CD is high, but anti-tTG and/or EMA is not detected (39, 40).

In 2005, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition issued guidelines for the diagnosis of CD with recommendations for using EMA and/or tTG antibodies in serologic screening. These were the first recommendations that did not include ARA and AGA testing in the routine evaluation for CD (21). Subsequently, other guidelines have been published in the United Kingdom (31) and by ESPGHAN (4). None of the three guidelines endorse the use of ARA and/or AGA tests in screening for CD. Due to their high sensitivities and lack of subjectivity, anti-tTG IgA is the recommended first-line serologic screening tool for identifying individuals at risk for CD in all three recent guidelines (4, 21, 22). The EMA IgA by IFA may also be used to screen for CD; however, its limited availability, subjectivity, and cost are usually a deterrent in routine diagnostic evaluation. Of all three guidelines, only the ESPGHAN guideline provides crucial recommendations regarding the use of anti-tTG IgA.
levels in the interpretation and prediction of CD. It is also impor-
tant to note that, the use of IgA-specific tests is restricted in the
case of selective IgA deficiency, which occurs more commonly
in patients with CD. Thus, the failure to identify IgA deficiency
correctly can result in premature cessation of CD workup, leading
to delayed diagnosis of disease. The inclusion of serum IgA deter-
mination as part of CD testing algorithms is a strategy to identify
patients who require IgG-based serologic testing.

TESTING FOR ANTIRETICULIN ANTIBODIES IS OBSOLETE IN
THE EVALUATION OF CELIAC DISEASE

Testing for ARA and/or AGA is no longer advocated for screening
individuals who have CD symptoms or are at risk for CD (4, 21,
22). While testing for AGA has largely been replaced by the more-
specific anti-DGP antibody assays, ARA tests are requested by
quite a number of clinicians in the routine evaluation for CD. To
determine the relevance of ARA in present-day CD diagnostic
practice, we searched PubMed databases from 1990 to 2012 for
reviews and peer-reviewed articles in English. We found an exten-
sive quantity of published literature on the clinical significance of
CD tests in primary care settings which would be reflective of
their actual diagnostic performances. Two reviewers independ-
ently conducted data extraction and quality assessment using the
Quality Assessment of Diagnostic Accuracy Studies (QUADAS)
tool, recommended by the Cochrane Collaboration. Studies were
eligible for inclusion if they met the following criteria. (i) The
study population consisted of adults, and the prevalence rate of
disease of 5% for their analysis (Table 2). Their evaluation re-
vealed a wide range in the sensitivities and specificities for AGA
tests compared to EMA, tTG IgA, DGP IgG, and IgA assays. Un-
like the tTG IgA assay, the diagnostic value of the tTG IgA assay
had variable sensitivities with the EMA assay showing the overall
best performance characteristics. In the last review we chose to
discuss, van der Windt and colleagues (32) evaluated the diagno-
sic significances of AGA, EMA, and tTG tests in primary care set-
tings based on populations with a similar prevalence or spectrum
disease (Table 3). This study searched MEDLINE (beginning in
January 1966) and EMBASE (beginning in January 1947) through
December 2009. The authors emphasized the importance of evalu-
ating CD tests in primary care settings which would be reflective
of their actual diagnostic performances. Two reviewers indepen-
dently conducted data extraction and quality assessment using the
Quality Assessment of Diagnostic Accuracy Studies (QUADAS)
tool, recommended by the Cochrane Collaboration. Studies were
eligible for inclusion if they met the following criteria. (i) The
study population consisted of adults, and the prevalence rate of
gastrointestinal symptoms was 50% or greater. (ii) Diagnostic
studies used a cohort design, as well as nested case control designs
in which consecutive cases of CD were compared with the appro-
priate controls. Similar to the second review, these analyses also
conveyed that AGA and tTG IgG tests are not dependable due to
the variability in their sensitivities.

All three reviews demonstrated that AGA, EMA, and tTG and
generated data indicate that anti-tTG or DGP IgA immunoassay is similar
and in some cases more accurate than the AGA immunoassay (29),

<table>
<thead>
<tr>
<th>Test and group</th>
<th>Sensitivity (%) (range)</th>
<th>Specificity (%) (range)</th>
<th>No. of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA IgA</td>
<td>Adults: 66.5 (31–100)</td>
<td>94 (87–92)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Children: 95.8 (90–100)</td>
<td>94.5 (86–100)</td>
<td>4</td>
</tr>
<tr>
<td>AGA IgG</td>
<td>Adults: 69 (46–95)</td>
<td>92 (87–98)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Children: 95 (91–100)</td>
<td>88.7 (67–100)</td>
<td>3</td>
</tr>
<tr>
<td>ARA IgA</td>
<td>Adults: 71.2 (41–92)</td>
<td>98.8 (95–100)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Children: 72.6 (29–100)</td>
<td>99.6 (98–100)</td>
<td>5</td>
</tr>
<tr>
<td>EMA IgA</td>
<td>Adults: 93 (89–100)</td>
<td>98.8 (95–100)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Children: 100 (100)</td>
<td>100 (100)</td>
<td>3</td>
</tr>
</tbody>
</table>

a Adapted and modified from Tables 1 and 3 in the review by Maki (25) with
permission from Elsevier. AGA test results are based on studies conducted from 1983 to
1994; ARA test results are based on studies conducted from 1971 to 1994, and EMA test
results are based on studies conducted from 1974 to 1994.

b Abbreviations: AGA, antigliadin antibodies; EMA, endomysial antibodies; IgA,
immunoglobulin A; IgG, immunoglobulin G.
30, 39, 40, 42, 43). In one of the most recent studies examining the significance of AGA, EMA, and ARA in genetically at-risk children for CD from birth, AGA IgA appeared 3 months earlier than anti-tTG, with anti-tTG, EMA, and ARA emerging concurrently (44). Unfortunately, anti-DGP antibodies were not evaluated in this study cohort. Overall, compared to all available serologic tests for CD, the EMA IgA has the highest positive predictive value and best positive likelihood ratio for disease irrespective of age (11, 22).

CONCLUSION
Celiac disease is a complex, systemic disease affecting the growth, development, and quality of life of a significant proportion of the population. Detection of anti-tTG and/or EMA antibodies represents the cornerstone for identifying patients with CD and/or at risk for the disease. The use of ARA testing deviates from current recommendations for serologic screening. There are very recent clinical investigations comparing the diagnostic significance of ARA to contemporary serologic tests for CD. Based on the results from these limited studies and their performance in past investigations, their use in current practice is unwarranted. In addition, our current knowledge of CD-specific serologic testing and the immunobiology of disease leads us to conclude that ARA testing is no longer useful in the routine evaluation of both patients with CD symptoms and individuals at risk for CD.

REFERENCES

### TABLE 3 Diagnostic performance of CD-specific serologic assays in adult patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%) (range)</th>
<th>Specificity (%) (range)</th>
<th>Positive LHR (range)</th>
<th>Negative LHR (range)</th>
<th>No. of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA IgA</td>
<td>0.65 (0.46–0.87)</td>
<td>0.91 (0.70–0.98)</td>
<td>17.9 (2.59–41.9)</td>
<td>0.38 (0.14–0.55)</td>
<td>6</td>
</tr>
<tr>
<td>AGA IgG</td>
<td>0.62 (0.25–0.93)</td>
<td>0.90 (0.80–0.97)</td>
<td>10.1 (4.67–17.8)</td>
<td>0.41 (0.08–0.76)</td>
<td>5</td>
</tr>
<tr>
<td>me-EMA IgA</td>
<td>0.91 (0.74–1.00)</td>
<td>0.99 (0.97–1.00)</td>
<td>149 (26.3–495)</td>
<td>0.12 (0.05–0.28)</td>
<td>8</td>
</tr>
<tr>
<td>hr-tTG IgA</td>
<td>0.90 (0.80–1.00)</td>
<td>0.96 (0.91–0.99)</td>
<td>48 (9.99–109.9)</td>
<td>0.108 (0.02–0.21)</td>
<td>7</td>
</tr>
<tr>
<td>tTG IgG</td>
<td>0.72 (0.27–1.00)</td>
<td>0.88 (0.77–0.95)</td>
<td>8.48 (8.8–12.7)</td>
<td>0.31 (0.06–0.74)</td>
<td>3</td>
</tr>
</tbody>
</table>

a Adapted and modified from Table 4 in the review by van der Windt et al. (32) with permission of the publisher (Copyright © 2010, American Medical Association. All rights reserved). Results are based on the search conducted in MEDLINE (beginning in January 1966) and EMBASE (beginning in January 1947) through December 2009. b Abbreviations: AGA, antigliadin antibodies; me-EMA, monkey esophagus endomyosal antibodies; hr-tTG, human recombinant tissue transglutaminase; IgG, immunoglobulin G; IgA, immunoglobulin A; LHR, likelihood ratio.