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Testing for Antireticulin Antibodies in Patients with Celiac Disease Is Obsolete: a Review of Recommendations for Serologic Screening and the Literature

Sarada L. Nandiwada, Anne E. Tebo

Department of Pathology, University of Utah, Salt Lake City, Utah, USA; ARUP Laboratories, Salt Lake City, Utah, USA

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 lumenal 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 lumenal 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 autoantibodies 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

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Address correspondence to Anne E. Tebo, anne.tebo@path.utah.edu.

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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 biopsy in individuals with elevated CD-specific antibodies. HLA typing and biopsy specimen evaluations are not the focus of this minireview and will not be discussed.

CELIAC DISEASE SEROLOGIC TESTS AND RECOMMENDATIONS FOR SCREENING

The detection and quantitation of specific antibodies in appropriate clinical specimens are usually the first steps in the evaluation of CD. The most widely described serologic tests for CD include antireticulin antibodies (ARA), antigliadin antibodies (AGA), and EMA, tTG, and DGP antibodies. The ARA test was first introduced as a diagnostic test for CD in 1977 and is routinely detected by indirect immunofluorescence assay (IFA) on rat tissue (23). These antibodies (IgG or IgA) are directed against the reticular fibers of endomysium, a layer of connective tissue which sheathes smooth muscle fibers. Five different patterns (R1 to R5) are associated with ARAs; however, only the R1 type is associated with CD and dermatitis herpetiformis. To be considered positive, the characteristic R1 type staining pattern should be seen on three rodent tissues, namely, the liver, kidney, and stomach. There are several drawbacks to the ARA test, including multifaceted procedure, poor sensitivity due to the rodent substrate, and the inherent subjectivity associated with IFA-based testing (24, 25).

Testing for antigliadin antibodies by enzyme-linked immunosorbent assay (ELISA) was developed in the early 1980s (26). Although still available for diagnosis, AGA immunoassays demonstrate variable clinical performance, particularly in adults, and are not recommended in screening patients with symptoms and/or at risk for CD (4, 21, 22). Subsequent to the development of AGA immunoassays, 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 AGA 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 tTG 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 tTG 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

TABLE 1 Diagnostic performance of CD serologic tests[a]

Test and group[b]	Sensitivity (%) (range)	Specificity (%) (range)	No. of studies
AGA IgA			
Adults	66.5 (31–100)	94 (87–92)	6
Children	95.8 (90–100)	94.5 (86–100)	4
AGA IgG			
Adults	69 (46–95)	92 (87–98)	6
Children	95 (91–100)	88.7 (67–100)	3
ARA IgA			
Adults	71.2 (41–92)	98.8 (95–100)	5
Children	72.6 (29–100)	99.6 (98–100)	5
EMA IgA			
Adults	93 (89–100)	98.8 (95–100)	5
Children	100 (100)	100 (100)	3

[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.

TABLE 2 Diagnostic accuracy of CD-specific serologic tests[a]

Test[b]	Sensitivity (%) (range)	Specificity (%) (range)	PPV[c] (%)	NPV[c] (%)	No. of studies
AGA IgA	85 (57–100)	90 (47–94)	18	99	5
AGA IgG	85 (42–100)	80 (50–94)	31	99	5
EMA IgA	95 (86–100)	99 (97–100)	83	99	6
tTG IgA	98 (78–100)	98 (90–100)	72	99	7
tTG IgG	70 (45–95)	95 (94–100)	42	99	7
DGP IgA	88 (74–100)	95 (90–99)	44	99	7
DGP IgG	80 (63–95)	98 (90–99)	68	99	7

[a] Adapted from the review by Leffler and Schuppan (41) with permission from Macmillan Publishers Ltd., based on the studies conducted from 1999 to 2010.
[b] Abbreviations: AGA, antigliadin antibodies; DGP, deamidated gliadin peptide; EMA, endomysial antibodies; IgA, immunoglobulin A; IgG, immunoglobulin G; tTG, tissue transglutaminase.
[c] The positive predictive value (PPV) and negative predictive value (NPV) both had a pretest probability of 5%.

levels in the interpretation and prediction of CD. It is also important to note that, the use of IgA-specific tests is restricted in the context 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 determination 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 extensive quantity of published literature on the clinical significance of tTG, EMA, and DGP antibodies in CD. However, very few studies discussed how ARA testing fares compared to the more contemporary serologic assays. We carefully examined meta-analyses and systemic reviews on the diagnostic performances of CD tests and selected three to discuss in this minireview. The first, a review by Maki (25) evaluated ARA, AGA, and EMA assays by pooling specific diagnostic studies published between 1971 and 1994 (Table 1). Based on these analyses, ARA testing had high specificities (greater than 96%) in both children and adults; however, a major setback was its inconsistent sensitivities: 29 to 100% in children and 41 to 92% in adults. Compared to AGA and ARA tests, the EMA IgA assay had the best overall clinical performance in both pediatric and adult populations.

The second, a recent meta-analysis by Leffler and Schuppan in 2010 (41) assessed the diagnostic accuracies of AGA, EMA, and tTG and DGP antibodies by analyzing results from several publications between 1999 and 2010. To determine the positive and negative predictive values, the authors used a pretest probability of disease of 5% for their analysis (Table 2). Their evaluation revealed a wide range in the sensitivities and specificities for AGA tests compared to EMA, tTG IgA, DGP IgG, and IgA assays. Unlike the tTG IgA assay, the diagnostic value of the tTG IgG 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 diagnostic significances of AGA, EMA, and tTG tests in primary care settings based on populations with a similar prevalence or spectrum of 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 evaluating CD tests in primary care settings which would be reflective of their actual diagnostic performances. Two reviewers independently 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 appropriate 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 DGP antibody tests are good at predicting the absence of CD; however, disparities were observed in their sensitivities, especially for AGA (IgA/IgG) and anti-tTG IgG tests (20, 25, 41). The AGA tests also tended to exhibit discrepancies in specificities (47 to 94% for IgA and 50 to 94% for IgG isotypes) compared to the DGP assays (90 to 100% for IgA and 94 to 100% for IgG; Table 2). Overall, the sensitivities between the DGP and AGA assays appear to be comparable but less than either the anti-tTG IgA or EMA IgA test. Early clinical studies suggested that EMA and AGA tests are useful in very young children (Table 1); however, more-recent data indicate that anti-tTG or DGP IgA immunoassay is similar and in some cases more accurate than the AGA immunoassay (29,

TABLE 3 Diagnostic performance of CD-specific serologic assays in adult patients[a]

Test[b]	Sensitivity (%) (range)	Specificity (%) (range)	Positive LHR[c] (range)	Negative LHR (range)	No. of studies
AGA IgA	0.65 (0.46–0.87)	0.91 (0.70–0.98)	17.9 (2.59–41.9)	0.38 (0.14–0.55)	6
AGA IgG	0.62 (0.25–0.93)	0.90 (0.80–0.97)	10.1 (4.67–17.8)	0.41 (0.08–0.76)	5
me-EMA IgA	0.91 (0.74–1.0)	0.99 (0.97–1.00)	149 (26.3–495)	0.12 (0.05–0.28)	8
hr-tTG IgA	0.90 (0.80–1.00)	0.96 (0.91–0.99)	48 (9.99–109.9)	0.108 (0.02–0.21)	7
tTG IgG	0.72 (0.27–1.00)	0.88 (0.77–0.95)	8.48 (8.8–12.7)	0.31 (0.06–0.74)	3

[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 endomysial antibodies; hr-tTG, human recombinant tissue transglutaminase; IgA, immunoglobulin A; IgG, immunoglobulin G.
[c] LHR, likelihood ratio.

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 IgG 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 represent the cornerstone for identifying patients with CD and/or at risk for disease. The use of ARA testing deviates from current recommendations for serologic screening. There are very few 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.

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