Serum Markers in the Clinical Management of Celiac Disease

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Key Words

Celiac disease · Serology · Tissue transglutaminase · Deamidated gliadin peptide · Endomysial antibody · Biomarker · Intestinal fatty acid-binding protein

Abstract

The advent of highly reliable noninvasive celiac diagnostic tests has transformed the field of celiac disease, from diagnosis, to evaluation of epidemiology, to clinical and translational research. Serologic tests in their modern forms are highly sensitive and specific for diagnosis, allowing for consideration of avoidance of diagnostic intestinal biopsy in some settings. On the other hand, as predictors of intestinal damage and for use in monitoring disease activity, currently available noninvasive tests have been disappointing. Serologic tests, while a measure of disease activity, do not correlate well with histology or symptomatology, and it is unclear if they predict long-term risk. Additionally, while the many clinically available tests have improved accessibility, they can have widely different cutoff levels and overall performance, making the comparison of levels in individual patients over time and across populations quite difficult. In the future, we can expect to see improvement in the currently available serologic tests including tissue transglutaminase and deamidated gliadin peptide with expansion of the dynamic range of the tests, and the celiac care community should push for a standardization of assays that would simplify research and patient care. Additionally, current serolog-

ic tests are measures of the adaptive immune response in celiac disease but do not directly measure intestinal inflammation. Promising work on intestinal fatty acid-binding protein and other assays which directly measure intestinal damage may complement traditional serologic tests and further improve our ability to noninvasively diagnose and monitor celiac disease. The coming years hold promise for the continuing evolution of serum-based tests in celiac disease with the possibility of substantial improvement of patient care and clinical research.

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Introduction

Celiac disease (CD) is a common immune-mediated enteropathy triggered by the intake of dietary gluten in wheat, barley and rye, in genetically susceptible individuals [1]. Gluten peptides in the intestine trigger T-helper 1-mediated immune responses resulting in epithelial inflammation, an increased number of intraepithelial lymphocytes, intestinal villous atrophy, autoantibody production, and malabsorptive symptoms. Traditionally, the diagnosis of CD relies on visualizing the typical lesion via small intestinal biopsy. Early diagnosis and treatment of CD can prevent severe complications. A gluten-free diet (GFD) generally leads to improvement in intestinal mucosal damage, clinical symptoms and CD autoantibody titers.

The widespread availability of highly accurate serologic testing for CD has been a fundamental driver of advances in clinical care and research in this field [2]. The serologic tests for CD are more accurate than most other antibody-based tests for immune-based disorders. In the early 1960s, the gliadin component of wheat was found to be the pathologic trigger for CD. Autoantibodies (AGA) against this protein were subsequently the first to be used as a screening tool for the disease [3]. Since that time, serologic testing advanced from an adjunctive aid in diagnosis to an integral component of diagnosis, management and clinical research. Highly sensitive and specific tests including tissue transglutaminase (tTG), endomysial (EMA) and deamidated gliadin peptide (DGP) autoantibodies have been identified optimizing diagnostics and screening studies. Indeed, for all individuals in whom CD is being considered, serologic blood testing should be the initial step in evaluation [4–6]. With improvement of serologic antibody tests and highly accurate assays, we are potentially reaching the time where a diagnosis of CD can be made without intestinal biopsies, at least in select well-defined situations [5].

Despite these advances and the overall laudable test performance of EMA, tTG and DGP for CD diagnosis, current testing still is subject to a number of important limitations that are important for both clinicians and researchers to recognize. First, one of the most practical issues currently faced by clinicians is the diversity of available testing platforms, many of which have different cutoff levels, dynamic ranges and overall test performance. This issue, which has gone largely unaddressed, can be a major impediment to both patient care and research when values are not comparable between providers or between studies. In addition, it has been noted that the excellent test performance shown in validation studies of various serologic tests may not accurately reflect test performance in clinical practice and more diverse populations.

Furthermore, monitoring disease activity in treated CD patients remains a challenge. Although the CD antibody tests show a high accuracy for selecting patients needing a diagnostic biopsy, these tests do not seem to be reliable after diagnosis as the autoantibody titers do not correlate well with histological findings or symptoms in CD patients on a GFD. To optimize the noninvasive diagnostic algorithm and improve monitoring of disease activity, different tests for CD activity have been studied. Two promising markers are intestinal fatty acid-binding protein (I-FABP), a marker reflecting enterocyte damage, and citrulline (CIT), a marker for functional enterocyte mass, and will be discussed in this review.

Also, unlike histology, there are virtually no data on the impact of serologic test results of long-term prognosis. Fortunately, work is ongoing to address these limitations, and the coming years hold promise for the continuing evolution of serum-based tests in CD with the possibility of substantial improvement of patient care and clinical research. The aim of this review is to evaluate the currently available serum markers and their accuracy in clinical practice in patients with a suspicion of CD, and their use for monitoring disease activity in CD patients on a GFD. Next to the traditional (gliadin, reticulin, actin) and modern tests (tTG, EMA and DGP), two novel promising markers for disease activity (I-FABP and CIT) will be discussed.

Traditional Autoantibody Tests: Anti-Gliadin, Anti-Reticulin and Anti-Actin

Anti-Reticulin and Anti-Actin

Anti-reticulin antibodies (ARA) were first described as a possible predictive test for CD in the early 1970s [7]. While IgA antibodies to reticulin connective tissue did see some use, primarily in pediatric CD evaluation and appeared to have test performance equal to or better than AGA [8], ARA was largely supplanted first by AGA for technical reasons and later on by EMA and tTG which had markedly better test performance, as described below.

Around the same time as ARA were described, anti-actin antibodies (AAA) were found to be present in a number of autoimmune and neoplastic conditions [9]. AAA has been evaluated on multiple occasions as a supplemental serologic test in CD. While AAA may have a higher predictive value for severe intestinal damage than other serologic tests, the relatively low sensitivity has made the clinical value of this test uncertain and AAA have never gained widespread use in CD [10, 11].

Anti-Gliadin

About one decade later, in the 1980s, the native anti-gliadin antibody (AGA) test was developed for CD [12]. This test for both IgA and IgG antibodies was relatively easy to manufacture and perform (enzyme-linked immunosorbent assay, ELISA). It was a major step forward as they were the first tests to allow some degree of noninvasive risk stratification, especially important in what was, at that time, a primarily pediatric disease, and quickly became the standard serologic test.

There is marked heterogeneity in results of studies evaluating the sensitivity and specificity of AGA tests, as

a results of assay variants and cutoffs provided, a common problem for all CD diagnostic tests [13–15]. The average sensitivity and specificity of the best tests for both IgA and IgG AGA is between 80 and 90%, with lower specificities generally seen in adults compared with children [2, 13]. In recent years, AGA-IgA tests have been replaced by more accurate serological assays, such as tTG, EMA and DGP.

One historic advantage of AGA had been the availability of a moderately accurate IgG-based test. While IgA is the major component of the gluten-autoantibody interaction in the small intestinal lumen, IgG represents a longer-term immune response. As selective IgA deficiency occurs in up to 2% of patients with CD, the need for a non-IgA-based test is apparent [16, 17]. IgG AGA testing remained the standard diagnostic test for CD in individuals with selective IgA deficiency through the early 2000s. However, in the past few years, IgG-tTG and particularly IgG-DGP assays have been developed that are superior to IgG-AGA for this population [18–21].

Recently, AGA has been suggested as a marker of non-celiac gluten sensitivity (i.e. those with negative antibodies to tTG and/or EMA and normal intestinal histology) [22]. However, at this point, there are no clear data that support the use of AGA testing in any clinical setting where more modern tests are available.

Newer Tests: EMA, tTG and DGP

Endomysium

Introduction of the EMA test in the mid-1980s was a significant step forward from AGA testing. Although heterogeneous, the sensitivity of EMA-IgA is generally reported to be above 90% with a specificity up to 100% [13, 23, 24]. Because of the close to 100% specific association with CD, EMA testing has generally been used as a confirmatory test for tTG. However, approximately 10% of CD patients remain negative for EMA. The test has been suggested to be less sensitive in patients with milder small intestinal lesions (Marsh 3A) [25, 26] and among CD children under 2 years of age [27]. Nevertheless, different studies suggest that EMA appears in the serum before development of villous atrophy, making it a marker of early or ‘potential’ CD [28, 29]. EMA is detected by an indirect immunofluorescence method (monkey esophagus or human umbilical cord tissue as antigen). A consequent disadvantage of this method is that the results are labor-intensive and operator-dependent, increasing the costs and diminishing the objectivity of the test [2].

Tissue Transglutaminase

The identification of tTG as autoantigen in the late 1990s enabled the development of an easy to perform ELISA-based test with recombinant tTG. tTG is responsible for the deamidation of gluten-derived gliadin peptides, resulting in DGP with greatly enhanced immunoreactivity [30, 31]. Gluten in this altered confirmation binds with high affinity to HLA DQ2 or HLA DQ8 molecules on antigen-presenting cells which present the deamidated gluten and bound tTG to helper T cells triggering production of antibodies to tTG and DGP [32–35]. Enteropathy is caused by cell-mediated cytotoxicity triggered also by the binding of deamidated gluten to HLA molecules on antigen-presenting cells but resulting in CD8-mediated inflammation with infiltration of lymphocytes into the mucosa along with villous destruction. (The pathogenic significance of the autoantibody response in CD patients remains unclear; however, celiac antibodies have been postulated to increase intestinal permeability, activate monocytes, disturb angiogenesis, inhibit epithelial cell differentiation or induce cell proliferation.) Since they were described, tTG quickly became the standard test used in CD diagnosis, as this method is more reproducible and objective compared with EMA. The best tests for tTG IgA have shown both sensitivity and specificity of 95% and above [2, 13, 14, 36]. The performance of commercial ELISA assays for tTG however may vary depending on the quality of the antigen, and discrepancies between different kits are also present in the cutoff values. The concordance rate of tTG and EMA test is very high, but based on its high sensitivity, lower costs and ease of interpretation, tTG is suggested as the first screening test [37, 38]. Nevertheless, in most studies, the specificity of EMA tests is higher, suggesting that this test can be performed as confirmatory in tTG-positive individuals [38, 39]. Furthermore, as discussed above, the accuracy of all serologic tests depends on disease prevalence and may be lower in clinical settings compared with highly enriched validation cohorts.

Deamidated Gliadin Peptides

Most recently, drawing off the known pathophysiology of CD, antibodies directed to DGP have been introduced into the spectrum of serologic tests for CD. DGP reflects the CD B-cell epitopes more specific than the native nonamidated peptides targeted by the traditional AGA [20], and consequently has a higher sensitivity and specificity. Although DGP-IgA was shown to be nearly as sensitive and specific as tTG-IgA [40, 41], recent studies revealed that tTG performs significantly better [36, 42]. The sensitivity and specificity for DGP-IgA have gener-

ally been shown to be around 85 and 95%, respectively [2, 36, 43]. Nevertheless, while IgG-based tTG tests in IgA-deficient patients have disappointing sensitivity, DGP-IgG tests display high specificity for CD (99%, similar to EMA) [20, 40, 42–44]. DGP-IgG allows for the identification of all CD cases in IgA-deficient patients with a high sensitivity in young children, proposing a new protocol for CD that uses only two tests.

The revolution in antibody tests has substantially improved the diagnostic algorithm for CD. Further improvement can be expected by rapid point of care tests that can be performed at the outpatient clinic, instead of requiring trained personnel and centralized laboratories. Point of care tTG-IgA tests have been developed, but reports on their accuracy are contradictory, and these tests need further exploration before clinical implementation [39, 45].

Despite the major improvements in serologic testing, no antibody test shows an absolute sensitivity and specificity for CD. In the majority of patients, the diagnosis of CD still relies on the demonstration of the characteristic small intestinal changes by duodenal biopsy. The best serologic approach for case finding might be the combined detection of a panel of antibodies. Recently, the European Society for Pediatric Gastroenterology Hepatology and Nutrition introduced a revised guideline for CD in children based on the optimization of antibody assays. According to this guideline, children can be diagnosed with CD without a duodenal biopsy when presenting with symptoms, a strongly elevated tTG-IgA (>10 cutoff value), confirmed by a positive EMA-IgA test on a separate occasion, and positive genetic testing (HLA-DQ2 and/or HLA-DQ8) [5]. Such a strategy seems premature for CD in adults, and a substantial proportion of children with suspicion of CD do not fulfill all four diagnostic criteria. Thus, currently a diagnostic biopsy remains necessary for most patients.

Other serum markers are warranted to prevent the diagnostic biopsies in all children and in adult patients. Research has been underway for many years for a noninvasive test that cannot only screen individuals at risk for CD, but also correlate with the severity of the intestinal lesions and monitor the histological response to a GFD. Potential markers are discussed below.

Experimental Tests: I-FABP and CIT

Citrulline

CIT is a nonprotein amino acid, and is a metabolic intermediate in the urea cycle. CIT is almost exclusively present in the enterocytes of the small intestine [46–48].

Consequently, circulating CIT levels have been proposed to be a potential marker of small intestinal function and mass. Recently, CIT levels have been shown to be a reliable marker of small intestinal damage in other disease associated with villous atrophy and enterocyte alterations, including HIV and tropical enteropathy [49, 50].

Publications on the value of CIT testing in CD are conflicting. A few studies showed that CIT levels are reduced in children and adults with untreated CD compared with healthy controls [51–53]. Most of these studies showed an inverse correlation between fasting plasma CIT level and the extent of villous atrophy [51, 52]. However, significant overlap was present between CD patients and controls [52, 53]. Furthermore, others found that fasting plasma CIT levels were unable to reflect a decreased intestinal energy absorption capacity in patients with enterocyte damage [54]. This may be due to a milder degree of intestinal disease [54], suggesting that significant intestinal damage must be present for CIT levels to decrease.

Initiation of a GFD in studies with decreased CIT levels at diagnosis led to an increase in CIT levels [51–53], depending on the histological responsiveness [51], and has been shown to normalize CIT levels to the levels of controls after more than 2 years of GFD [52].

As an alternative to fasting CIT levels, a CIT generation test (CGT) has been provided to assess enterocyte function [55]. Loading the enterocyte with orally administered glutamine, the natural precursor of CIT, may increase CIT output, reflecting the functional capacity of the enterocyte. This study revealed that the generation of CIT was delayed in patients with CD. This test might offer a new possibility to assess the functional capacity of enterocytes in CD. For now however, the clinical utility of CIT measurement is questionable in CD and needs further evaluation.

Intestinal Fatty Acid-Binding Protein

Measurement of circulating endogenous enterocyte proteins has been shown to be useful to estimate enterocyte damage. Fatty acid-binding protein comprises a class of low-molecular-weight (14–15 kDa) cytosolic proteins found in high concentrations in tissues involved in the uptake and consumption of fatty acids [56]. Three types of FABP are present in the gut: intestinal FABP (I-FABP), liver FABP (L-FABP) and ileal bile acid-binding protein (I-BABP). I-FABP is exclusively present in the gut, as is I-BABP which is exclusively present in the ileum, while L-FABP is also present in the liver and kidneys. I-FABP is primarily limited to mature enterocytes of the small intestine, with a maximal expression in the jejunum, while

the expression in the colon is low [57]. Since I-FABPs are small water-soluble proteins, they are easily released into the circulation upon enterocyte damage and rapidly cleared through the kidneys (half-life of 11 min). I-FABP levels can be measured sensitively using an ELISA. It circulates in low amounts in the bloodstream of healthy individuals, likely reflecting the physiological turnover rate of enterocytes. Outside of CD, elevated circulating FABP levels have been reported in patients with intestinal ischemia, necrotizing enterocolitis and the early phase of sepsis [58–60].

Recent studies have revealed that serum I-FABP may be a sensitive marker for enterocyte damage in CD. These studies showed that I-FABP levels are significantly elevated in untreated CD adults and children at diagnosis as compared with control subjects [57, 61, 62]. Interestingly, serum I-FABP levels correlated with the severity of the histological lesions. These studies showed a sensitivity of 80 and 82%, and a specificity of 87 and 90% in adults and children, respectively, for CD, suggesting that serum I-FABP might be a useful additional marker in the diagnostic algorithm of CD [61, 62]. Patients with positive CD-specific markers and an elevated serum I-FABP level seem to suffer from CD, suggesting that a diagnostic biopsy can be omitted in these patients. Nevertheless, in patients with elevated CD, autoantibody titers and a normal serum I-FABP level, a duodenal biopsy would remain necessary.

Serum I-FABP levels may also be useful for monitoring disease activity after diagnosis. After introduction of the GFD, serum I-FABP levels decrease relatively fast. This is especially useful to confirm a diagnosis made without duodenal biopsy. In children, serum I-FABP levels have been shown to generally decrease and normalize within 6 months after initiation of gluten elimination [61]. This is faster than the decrease in tTG levels. However, in adult patients, levels appeared elevated compared with controls, even after up to 3–4 years of apparent compliance with the GFD [62]. This is in line with studies on histological healing in patients on a GFD, generally showing slow and incomplete mucosal healing in adult CD patients, while recovery in children seems to be faster and more complete [63, 64]. As postulated, a correlation has been found between serum I-FABP levels and the severity of the histological lesions in CD patients on a GFD [62]. Furthermore, a pilot study showed that I-FABP may be used as an early responsive marker during a gluten challenge procedure [unpubl. data]. Large international studies are underway to validate this marker for use in diagnosis, monitoring and research trials.

Limitations and Areas for Future Study

CD is a significant disorder with limited treatment adherence [65, 66] and adverse effects on quality of life [65, 67, 68] and nutritional [69] and metabolic indices [70, 71]. It is also associated with increased risk of malignancy and mortality [72–74]. For this reason, all major societies recommend routine follow-up of patients with CD [4–6, 75]. The goal of routine monitoring is to identify and treat coexisting disorders and manage active CD.

Thus, reliable but also easily applicable markers are needed. The modalities commonly used for CD monitoring are serologic tests and less often intestinal biopsy. While both tests are clearly supported as part of CD diagnosis, their use in monitoring is much less clear. Both small intestinal mucosal inflammation and serologic tests are reflections of different aspects of the adaptive immune response triggered by gluten in CD. When CD is highly active, as at diagnosis before the GFD is initiated, highly elevated serologic titers are correlated with intestinal damage, as both arms of adaptive immunity are fully activated [2, 76]. With initiation of the GFD however, this relationship becomes unstable. Data show that EMA, tTG and DGP titers should fall significantly in the first months after initiation of GFD and be normal or close to normal one year after diagnosis [77–79]. It is clear from gluten challenge studies that increases in antibody titers reflect increased disease activity and increased intestinal inflammation. However, elevation of CD-specific serology is delayed significantly from episodes of gluten exposure [80], and CD antibodies are poor predictors of dietary adherence with low levels of cross contamination or inadvertent gluten [81, 82]. CD serology lacks both sensitivity and specificity for ongoing enteropathy on a long-term GFD [83]. Furthermore, there are no studies that evaluate the relationship between normalization of serologic tests and risk of long-term complications. Although more research is necessary, promising experimental markers for enterocyte damage, such as I-FABP and CIT, may be more accurate for monitoring disease activity in CD patients on a GFD.

Conclusion

Serum markers for CD play an important role in CD management. In recent decades, the evolvement from detection of the disease trigger to several antibody tests has greatly improved the diagnostic algorithm, epidemiology and research, and will continue to do so.

In the upcoming years, further improvement and expansion of existing and new serological tests can be expected, with wider dynamic ranges, improved responsiveness and possibly higher sensitivity and specificity. Combination of different noninvasive markers may lead to diagnostic algorithms with greatly reduced need for diagnostic duodenal biopsies. Novel markers measuring direct mucosal damage, like I-FABP and CIT, instead of reflecting the immune response, may complement traditional serologic tests. These markers may also be more accurate predictors for monitoring disease activity after diagnosis, as the currently used antibody tests do not correlate well with histology or symptomatology. Once these novel tests are validated and clinically available, guidelines for CD diagnosis and monitoring may look very different from those available today.

At the same time, use of different antibody tests and cutoffs make comparison in individual patients over time and across populations difficult in both clinical and research situations. The range of competing tests is likely only to grow in the coming years. A centralized effort to

perform an open and robust comparison across testing platforms paired with detailed clinical and histologic data is feasible and could be completed with modest resources. These data would also be essential to the next major question of whether serologic titers, either at diagnosis or in follow-up, are of any prognostic significance in diagnosed patients. A number of studies have provided important data on the relationship between histology and long-term outcomes in CD. Clearly, the fact that we have only had serologic tests for a few decades has limited the conduct of these studies. However, sufficient time has elapsed to begin to make these studies possible.

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