Chapter 8

The Role of Serology in Celiac Disease Screening, Diagnosis and Follow-up

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Abstract

Serological markers are an essential part of the diagnostic workup for celiac disease (CD). Diverse clinical forms can be detected at any age in genetically susceptible individuals who have gluten in their diet.

Quantitative, automated IgA-class anti-tissue transglutaminase antibody testing is the recommended serological marker for CD detection, replacing the classical antiendomysial antibody test determined by indirect immunofluorescence assay. Anti-deaminated gliadin peptide antibodies improve the specificity of anti-gliadin antibodies, but lack the diagnostic efficacy of anti-transglutaminase antibodies.

Anti-transglutaminase antibodies should be determined in patients with clinical suspicion of CD, in CD-risk groups and in patients with CD-associated diseases.

Laboratories carrying out these assays must meet the following criteria: 1) Participation in quality control programs; 2) Use of appropriate reference values; 3) Quantitative monitoring of gluten-free diets; 4) Results comparable with other commercial tests, in view of the lack of a calibration standard.

The choice of a commercial test should take into account the type of antigen(s) calibrator, level of accuracy, linearity and detection limits, and any interferences.

In clinical practice, we do not recommend the use of mixed tests to screen for antibodies and isotypes, due to their contrasting significance and kinetics. Similarly, we advise against rapid immunochromatographic tests, to avoid diagnostic confusion. CD markers can also be determined using rapid point of care tests. For the time being, clinicians should be aware that these tests are expensive and they carry the risk of patients starting a GFD themselves, which then makes it harder to confirm the diagnosis.

Finally, it should be noted that anti-transglutaminase antibodies are only of limited use in adults and patients with partial villous atrophy on biopsy.

1. Introduction

A diagnosis of celiac disease (CD) is reached through an overall assessment of serology, clinical symptoms, intestinal biopsy studies, risk factors and genetic predisposition. Specific autoantibodies (serological markers) play an essential role in the diagnostic workup. These markers are requested increasingly not only by pediatricians and gastroenterologists, but also by endocrinologists, hematologists, rheumatologists, neurologists and other medical specialists.

Unlike other organ-specific autoimmune diseases, CD is unique in that it has an identified trigger (gluten), characteristic inflammatory bowel lesion staging that is a reversible, and excellent diagnostic serological markers. It can appear at any age in genetically susceptible individuals. Positive HLA-DQ2 and/or HLA-DQ8 gluten intake are necessary, but not sufficient for the clinical expression of the disease. Other key aspects are also involved that have yet to be understood, such as molecular mechanisms that control the immune response; mechanisms related to the degree of clinical severity; and the natural history of asymptomatic, untreated CD.

Serological markers are useful for detecting and monitoring CD, but intestinal histology and the response to the gluten-free diet (GFD) establishes the diagnosis. An intestinal biopsy should be requested when there is clinical suspicion and/or positive serology or even when serological tests are negative in the presence of symptoms suggestive of the disease. The diagnosis is confirmed when there is a clinical, serological and/or histological response to a gluten-free diet (GFD) and it is reinforced by the presence of genetic susceptibility markers HLA-DQ2 or HLA-DQ8.

Serological markers have played a major role in highlighting the heterogeneity of clinical presentations and they have been used to conduct prevalence studies in the general population. They have also made it possible to identify risk populations and CD-associated diseases.

2. History of Serological Markers

Celiac disease was discovered in the 1950s when the Dutch pediatrician, Dicke,[1] identified a connection between "intractable diarrhea" and the presence of wheat flour in children's diet. Years later it was found that gluten was the antigen that triggered the disease and that intestinal atrophy was reversible.

In 1970, the *European Society of Paediatric Gastroenterology and Nutrition* (ESPGAN) published the first diagnostic criteria,[2] consisting of at least three intestinal biopsies: the first at baseline, the second on a GFD and the third after a gluten challenge test.

IgA-class antigliadin antibodies (IgA-AGA) were described at the beginning of the 1980s.[3] IgA-AGA was the first serological marker available for CD, which meant that patients could be screened pre-biopsy, and other clinical forms of CD could be detected in addition to the classical presentation of diarrhea with abdominal distension. IgA-AGAs are not CD-specific. They are antibodies to gluten components in the diet, and probably reflect increased intestinal permeability, because they are also found in other gut diseases.

The sensitivity and specificity[4] of the IgA-AGA test is in the range of 70-80%. In clinical practice, lack of sensitivity (the risk of false negatives) causes more harm than the lack of specificity. The IgA-AGA test gave false negative results in 10 out of 31 CD cases among first-degree relatives of patients with CD,[5] and in 4 out of 15 CD cases in a cohort of patients with Down syndrome,[6] all of who had asymptomatic CD. This supports the strong association between IgA-AGA and digestive symptoms.

The technical advantages of using IgA-AGA markers in quantitative immunoassays has meant that they have been widely implemented in CD serological studies and in clinical practice. In fact, they are supplied commercially and form part of the serological marker panels for CD used in some laboratories.

In patients with selective IgA deficiency, IgG markers are determined instead, although IgG-class AGA markers have low specificity and are very common in the general population.

Antiendomysial antibodies (EmA) were identified[7] through their association with dermatitis herpetiformis. The high sensitivity and specificity of IgA-EmA markers (above 95%) marked a turning point in serological CD detection. Epidemiological studies that ensued showed high prevalence of CD in the general population, and diversity of its clinical forms. The 1970s triple biopsy diagnostic protocol was revised and simplified by ESPGAN in the 1990s[8] and reduced to a single initial biopsy followed by clinical improvement.

Indirect immunofluorescence (IIF) is used to determine IgA-EmA markers and other antibodies when the antigen is unknown. The immunological reaction takes place on a slide with fixed tissue slices containing the antigen. The most commonly used tissue was monkey distal esophagus. Human umbilical cord (HUC), jejunum (AJA anti-jejunal antibodies) and rat kidney (ARA, anti-reticulin antibodies) have also been used. IIF is a qualitative or semiquantitative technique using progressive serum dilutions. It can entail manual or semi-automated processing, and requires well-trained, experienced observers to interpret the patterns under a fluorescent microscope. Monkey distal esophagus slides have a high financial and ecological cost. A reasonable alternative is to use commercially available or in-house-prepared human umbilical cord, although the fluorescent pattern is harder to visualize on umbilical cord tissue.

In patients with IgA deficiency, IgG-class EmA markers are used, and their microscope image usually shows nonspecific fluorescence. This makes it harder to interpret an IgG pattern than an IgA pattern.

Anti-transglutaminase antibodies (IgA-tTG) appeared in 1997, when Dieterich[9] identified tissue transglutaminase (tTG) as the autoantigen recognized by EmA.

tTG is an enzyme protein that modifies the indigestible peptides in dietary gluten in the gut lamina propria, so that they are recognized by HLA-DQ2 molecules and are presented to CD4+ T cells, triggering an inflammatory humoral response, with the production of specific autoantibodies in patients with CD.

With the tTG antigen now available, EmA can be determined as tTG using quantitative, automatable immunoassay techniques, thus solving the technical limitations of IIF.

Quantitative determination of IgA-tTG makes it easier to monitor GFD serology and detects low autoantibody concentrations that cannot be detected using IIF. This is useful in the adult population, as explained later in this chapter. Isolated cases of falsely elevated IgA-tTG levels have been reported in patients with acute or serious diseases,[10,11] as well as small elevations of IgA-tTG, unrelated to dietary gluten, in patients with autoimmune diseases,[12] but they were considered to be nonspecific and possibly attributable to impurities in the tTG antigen.

In 2005, scientific societies[13] recommended IgA-tTG and/or IgA-EmA (with whole IgA) for serum CD detection; this was the first time that the use of IgA-AGA was discouraged.

Anti-deaminated gliadin peptide antibodies (IgA-DGP) have been developed in order to improve the efficiency of classical AGA using modified gliadin peptides as an antigen, which emulate gluten peptides in the gut lamina propria. The IgA-DGP test was found to be more effective than AGA at distinguishing patients with CD from controls in a pediatric population.[14] These observations have led to growing expectations[15] with regard to the usefulness of this new serological marker.

In 2008, the consensus document[16] issued by the *Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition* did not include the IgA-DGP test in the protocol for CD serological detection, recommending that its specificity needed further study.

Children younger than two years with clinically suspected CD may have negative IgA-tTG but positive IgA-DGP. Analyzing the natural history of IgA-tTG and IgA-DGP in infants,[17] it was observed that IgA-DGP and IgA-tTG markers have different kinetics: IgA-DGPs appear before IgA-tTGs and disappear sooner when a GFD is introduced.

In infants, the sensitivity of IgA-DGP contrasts with its lack of specificity. Thus, IgA-DGP disappeared spontaneously[18] in most children under two years taking gluten who had clinical suspicion of CD and negative IgA-tTG. In these cases, intestinal biopsy was performed due to clinical suspicion rather than serology results. Another study,[19] however, surprisingly found that the diagnostic performance of IgG-DGP and IgA-tTG was comparable.

In the same context, one meta-analysis[20] of 11 studies with a total of 937 patients and 1328 controls published between 1998 and 2008, found greater discriminative power and diagnostic efficiency in IgA-tTG versus IgA-DGP.

3. Criteria for Choosing a Commercial Reagent

There is a wide range of commercial tests to determine serological markers in CD. Tests can be grouped by antibody (AGA, EmA, tTG, DGP, mixed DGP/tTG, etc.) isotype (IgA, IgG, IgA/IgG) or technique (IIF, ELISA, fluorescent immunoassay, chemiluminescence assay, etc.)

The choice of test to be used in the clinics should follow international recommendations and take into account the recent evidence-based literature. Laboratories should choose the most effective test for the context (adults, children, CD detection only, detection and follow-up). Cost should be reasonable, but should not be the only factor in the decision.

Laboratories should be involved in clinicians' decisions to modify or improve CD serology protocols. There must be a good reason for switching tests or adding a new one because this requires an adaptation period and new reference values.

Serum antibody levels are determined by quantitative and qualitative immunoassay techniques, which may be manual or automated. Analyzers are usually connected to an online system. These techniques include ELISA (enzyme-linked immunosorbent assay) with spectrophotometric reading of the final result, adapting signal amplification systems using fluorescence (e.g. fluorescent immunoassay in tTG EIiA™ by Phadia) or luminescence (e.g., chemiluminescence in tTG BioflashR) to increase sensitivity.

The following technical aspects should be taken into account when choosing a test:

- The nature of the antigen(s) in the immunoassay and calibrator type (2, 3 or 6 points).
- The manufacturer's cut-off values.
- Grey or inconclusive zones, if any.
- Sensitivity and specificity in terms of histology and in the general population.
- Imprecision at different concentrations (within-run and between-run %CV), particularly at low concentrations.
- Linearity and detection limits, and any interferences or other limitations.

Pack size (e.g., 50 or 500 test pack) and reagent stability should match workload, because a laboratory must be able to offer a reasonable response time at a reasonable cost. If the test requires calibration for each analytical run, samples should be grouped to reduce calibration costs. However, if the calibration is stored in the analyzer memory, individual samples can be determined at no additional cost. Quality controls must be performed for each analytical run.

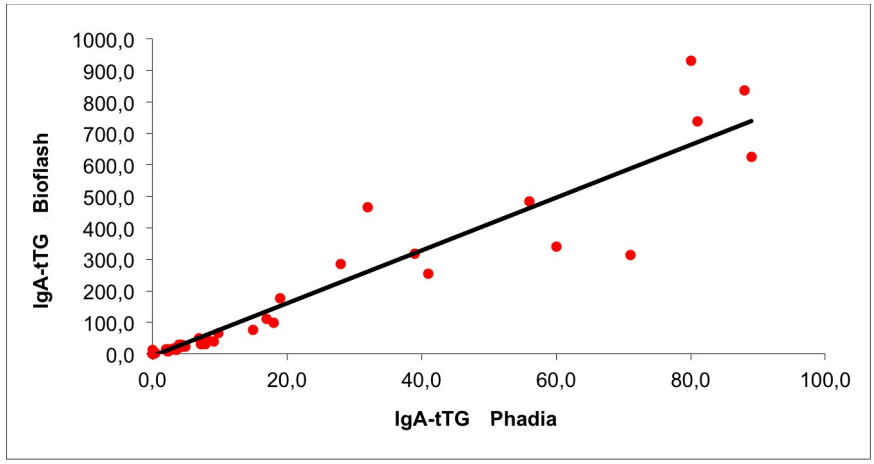

Figure 1. IgA-tTG: Fluorescent immunoassay vs. chemiluminescence, range 0-80 U/ml

Before introducing a new test, studies must be conducted on imprecision (within-run and between-run %CV) and reproducibility of results using another reference test for the same antibody. In this respect, the lack of a universal calibration standard is a major drawback, because each manufacturer defines its own arbitrary standards.

Figure 1 shows IgA-tTG results in 70 samples from patients with active CD or on a GFD, using two automated next-generation commercial tests, a fluorescent immunoassay (EliA™ by Phadia) and a chemiluminescence assay (Bioflash®). The Passing-Bablock test to analyze reproducibility of results shows that there is no constant error between tests [intercept: -0.15 (-0.45 to 0.12)] but there is a proportional error [slope: 6.190 (5.537 to 7.010)]. The results obtained using the two techniques show good correlation (r = 0.952), despite the proportional difference, which is explained by the differences between the manufacturers' calibrators.

Figure 2 shows the same comparison but in the grey zone (between 2 and 10 U/ml IgA-tTG in the fluorescent immunoassay taking Phadia as the reference; n=27). In this range, the regression line for the tests is [tTG Bioflash] = 5.2846 [tTG Phadia] - 0.4173. This gives a grey zone using the chemiluminescence assay of approximately 10-60 U/ml [Passing-Bablock: slope 6.000 (4.923 to 7.527); intercept -3.40 (-8.53 to 0.85)]. In clinical practice, it is important for the laboratory to identify this grey zone clearly, and to make sure that the analytical imprecision for this range is adequate.

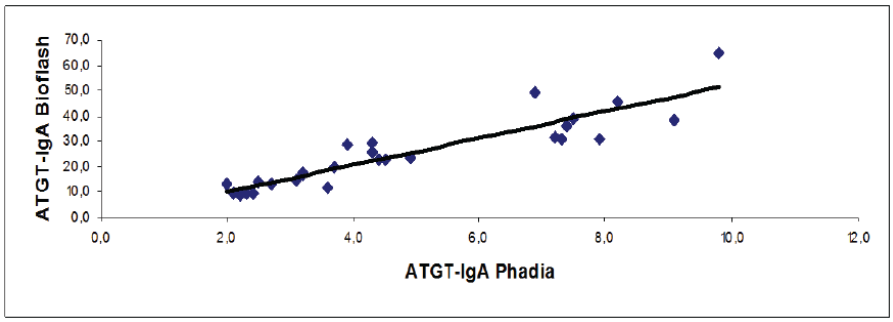

Figure 2. IgA-tTG: Fluorescent immunoassay vs. chemiluminescence, range 0-10 U/ml

In order to compare the sensitivity (SENS) and specificity (SPEC) of different representative serology strategies to detect CD, we used samples that were specifically selected from our experience of false positive (SENS) and false positive (SPEC) results. We tested 23 serum samples with high IgA-tTG in patients with asymptomatic CD diagnosed by intestinal biopsy (SENS) and 22 serum samples from children aged 1 to 2 years with diarrhea that had resolved with conventional treatment (SPEC).

The antigens used in the tests were:

1.- Recombinant human tTG (reference test);

2.- Native gliadin

3.- Synthetic Gliadin Peptide

4.- Purified erythrocyte tTG and DGP mixture (Screen)

5.- DGP-bound recombinant human tTG

6 and 7.- GAF3X peptide obtained by emulating DGP

The test results for each group (see Table 1) show that AGA and DGP are indeed less sensitive than tTG for detecting asymptomatic CD. They are also less specific than tTG in the specially selected group of children with diarrhea resolved with conventional treatment.

	Marsh III asymptomatic CD n = 23		Children aged 1-2 yeas with diarrhea n = 22	
	Positive	False Negative	False Positive	Negative
IgA-tTG	n=23	-	-	n=22
IgA-AGA	n=12	n=11	n=3	n=19
IgA-DGP	n=17	n=6	n=1	n=21
Screen IgA/IgG tTG/DGP	n=23	-	n=1	n=21
Anti-neo IgA-tTG bound DGP	n=23	-	n=2	n=20
IgA-anti-GAF3X	n=19	n=4	n=1	n=21
IgG-anti-GAF3X	n=17	n=6	n=2	n=20

Table 1. Results of different serology strategies to detect CD in selected cases

Mixed tests (used in screening) with more than one antigen (tTG and DGP) and polyvalent conjugates simultaneously detect IgA and IgG class DGP and tTG. They have good sensitivity for CD detection but they are not useful as a baseline for GFD serological monitoring, because the antibodies and isotypes have different kinetics. Likewise, caution should be exercised with some commercial tTG tests in which the tTG antigen has been "enriched" with PGD in order to increase sensitivity, as they can give false positive results due to the lack of specificity of the DGP.

CD markers can also be determined using rapid point of care (POC) tests. Pharmacies can make up these immunochromatographic tests individually to detect IgA and/or IgG tTG and/or DGP using a rapid fingerprick method. They are available as a self-testing kit and provide a quick, initial result for CD that can be carried out at the clinic or at home. The biggest disadvantage is that the test should always be followed up with a conventional analysis. A positive result needs to be confirmed using the classical diagnostic workup and a negative result also needs to be investigated if clinical suspicion persists. Furthermore, these tests are expensive and they carry the risk of patients starting a GFD themselves, which then makes it harder to confirm the diagnosis.

4. Recommendations for the Use of Serological Markers

Individuals of any age should undergo CD serological screening if they have any unexplained signs or symptoms summarized in Table 2, taken from the Working Group document on "Early Diagnosis of Celiac Disease",[21] published by the Spanish Ministry of Health and Consumer Affairs in 2008.

Age group	Symptoms	Signs
Children	Chronic Diarrhea Abdominal pain Vomiting Anorexia Apathy Moodiness	Malnutrition Abdominal bloating Failure to thrive Muscular Hypotrophy Iron deficiency Hipoproteinemia
Pre-teens and teenagers	Oligosymptomatic Abdominal pain Diarrhea-constipation Pubertal developmental delay Menstrual alterations Headaches Arthralgia	Low stature Iron deficiency Mouth ulcers Muscular weakness Osteopenia Skin and teeth alterations
Adults	Unspecific digestive symptoms: Dyspepsia Diarrhea Constipation Vomiting Weight loss Osteomuscular symptoms Infertility, repeated abortions Neurological alterations: Paresthesias Tetany Ataxia Epilepsy Psychiatric alterations: Depression Irritability Asthenia	Malnutrition Iron deficiency Hypoalbuminemia Coagulation alterations Vitamin deficiencies Hypertransaminasemia Peripheral neuropathy Myopathy Hyposplenism Mouth ulcers Osteoporosis and osteopenia

Based on the Working Group document on "Early diagnosis of celiac disease." Spanish Ministry of Health and Consumer Affairs. April 2008.

Table 2. Signs and symptoms of celiac disease

Individuals consider to belong to risk populations should also be screened. Risk populations consist of persons with a higher prevalence of CD than the general population, which stands at about 1%. Risk groups of note[22] are first-degree relatives (10-20%) of CD patients, patients with CD-associated diseases[22] such as type 1 diabetes mellitus (T1DM) (2-12%), Down syndrome (5-12%), autoimmune thyroid disease (up to 7%), Turner syndrome (2-5%), Williams syndrome (up to 9%), selective IgA deficiency (SigAD) (2-8%) and patients with autoimmune liver disease (12-13%).

Serological CD screening is not an urgent procedure in clinical laboratories. CD has a slow onset and it also resolves gradually. Laboratories can therefore return results in a reasonable period ranging from 1 to 7 days, depending on clinics' logistics and clinicians' expectations. Results should be assessed in a clinical-dietary-historical context and the head of the laboratory should

add comments or contact the clinician, if required. Likewise, the head of the laboratory should play an active part in selecting the most appropriate markers and most appropriate patients for serological testing, in close coordination with the referring clinician. Positive tTG cases should be recorded in a database for future use. According to the latest recommendations issued by ESPGHAN,[22] IgA-tTG and/or IgA-EmA (if total serum IgA is normal) are the markers of choice for CD, while IgA-DGP markers are recommended as an additional test in children younger than two years of age with negative IgA-tTG and suspected CD. Children who are diagnosed before the age of two are candidates for a gluten challenge test, in view of the lack of knowledge of the natural course of gluten intolerance at this early disease stage.

A good clinical laboratory[22] should:

1.- Participate in internal and external quality control programs.

2.- Use tests that are validated against an EmA reference standard or histology, with >95% agreement.

3.- Use tests with a manufacturer-defined cut-off or ULN (upper limit of normal) that has been adapted according to personal experience or in view of the population studied.

4.- Express results in figures and specify immunoglobulin class. Classification as "positive" or "negative" is not sufficient because it does not provide a baseline value for GFD serological monitoring.

5.- Specify the immunoglobulin class and cut-off dilution in EmA reports, indicating whether the result is positive or negative, along with the dilution.

6.- Flag negative IgA-tTG results to avoid misinterpreting cases such as patients with IgA deficiency, children younger than two years, patients on a gluten-poor or gluten-free diet (because a few weeks of a GFD will confound a negative result), and patients receiving immunosuppressive therapy.

Depending on the test, different figures and units of measurements are used for CD serological markers. Reproducibility studies are useful for verifying whether results from such tests are comparable. Results can be expressed as multiples of the upper limit of normal (ULN) or with their respective cut-off values.

Active collaboration between clinic and laboratory undoubtedly improves the quality of care. In this respect, it is useful to set up and maintain a database with demographic, clinical, serological, genetic, histological and family data of patients with high IgA-tTG levels as an initial inclusion criterion. Mining this database may help to further our knowledge of CD and improve care protocols.

When laboratories receive a request for "CD serological markers" they should be able to offer the most appropriate screening tests available. They should not resort to a general panel of antibodies and isotypes that does not provide evidence-based additional information and is simply a burden on human and economic resources.

Serology tests precede histology in all CD diagnostic algorithms. In the latest recommendations issued by ESPGHAN,[22] the main change is the possibility of diagnosing CD without resorting to intestinal biopsy in children with compatible symptoms, genetic susceptibility and IgA-tTG serum levels >10 times the cut-off value or ULN.

However, this protocol has been subject to considerable debate,[23,24] and its opponents suggest that an initial biopsy serves as a reference for the baseline lesion, should the disease develop in an unexpected way, and that it detects discrepancies between serology and histology results. Technical disadvantages include lack of result reproducibility studies on commercial tests, lack of a universal calibration standard (as mentioned above) and the fact that the positive predictive value (PPV) of IgA-tTG tests depends on CD prevalence in the population studied.

5. The Relationship between Serology and Histology

Although the level of specific antibodies in blood is generally understood to reflect the degree of intestinal histological lesion, serology and histology results do not always match.

The sensitivity of IgA-tTG/EmA tests is lower in patients with partial villous atrophy. In fact, it has been observed that IgA-tTG/EmA markers may be negative in 60% of patients with Marsh 3a lesions.[25,26] Furthermore, negative IgA-tTG/EmA tests in patients taking a GFD do not necessarily imply histological recovery of villous architecture, especially in adults.[27,28]

Serological response to GFD varies from one patient to another. According to the ESPGHAN, a 12-month GFD is required to achieve a negative result for CD-specific antibodies, but this period can range from 3 months to 3 or 4 years, depending on the patient's gluten sensitivity. Likewise, the length of time required for a gluten challenge test (about 15 g/day in children) to achieve a positive serological result varies greatly. Blood tests should be taken every 3-6 months if there is no clear clinical response. Generally, a positive serological result is sufficient to confirm the diagnosis.

In clinical practice, dietary non-adherence can be detected by serology findings of some degree of IgA-tTG elevation in CD patients on a GFD with several previous successive negative serological controls. This is a common finding in adolescents and can be attributed to deliberate or accidental non-adherence to their GFD, which they may or may not be aware of. EmA levels determined using IIF assays are less sensitive to these minor serological variations. IgA-DGP testing is recommended[29] to detect dietary non-adherence, but its lack of specificity has a negative impact on the cost-benefit ratio.

6. Serological Markers in Adult CD

Adulthood-onset CD can occur in genetically susceptible individuals taking dietary gluten. In these cases, elevated IgA-tTG findings can confirm clinical suspicion before the histological diagnosis is reached. In some cases, late disease onset may be triggered by pregnancy, infections, trauma or stress, and it can also occur in elderly patients.

Furthermore, CD in adults may go undiagnosed due to the heterogeneity of its signs and symptoms. Serological markers have poor sensitivity because villous atrophy may be partial. An interesting study[30] of consecutive CD diagnoses across all ages showed that CD in adults has

attenuated clinical symptoms, serology and intestinal histological lesions compared with CD in children. This means that it takes longer to reach a definitive diagnosis in adults than in children.

While a low-grade intestinal lesion is associated with mild clinical symptoms, a case-finding study in relatives of patients with CD[31] proved that clinical symptoms (anemia, abdominal pain or distension or bone density alterations) are as important in Marsh stage 1 as they are in Marsh stage 3. This study proposed performing an intestinal biopsy in all DQ2-positive relatives of patients with CD, regardless of serological results. The study showed that if a conventional serological screening protocol had been followed (IgA-tTG and/or IgA-EmA with the standard cut-off value), there would have been a 15.6% detection rate of cases with Marsh 1 lesions and 84.6% detection rate of cases with Marsh 3 lesions. This confirms the low sensitivity of IgA-tTG markers in adult patients with CD and low-grade intestinal lesions, in whom IgA-tTG elevations may be below the established cut-off value.

In view of these results and with the aim of increasing IgA-tTG sensitivity in the adult population, a new lower cut-off value was sought, based on the IgA-tTG level below which 98% of the general adult population belong.[32] This resulted in a cut-off value for adults four times lower than the cut-off value for children.

Applying this new cut-off value for adults in a CD case-finding study in the working population,[32] it was found that IgA-tTG had 89% sensitivity and IgA-EmA had 11% sensitivity among patients with Marsh 1 lesions, while both markers had 100% sensitivity for patients with Marsh 3 lesions.

Marsh 1 lesions are not only found in CD; they can also occur in other diseases such *Helicobacter pylori* infection, parasite infestation and other enteropathies. The suitability of the GFD in patients with Marsh 1 lesions in the absence of clinical symptoms is controversial.[33] In these cases, flow cytometry studies in gut mucosa are particularly relevant because they can detect patterns that are compatible with CD, regardless of histological changes.

7. Serological Markers in At-Risk Populations

Serological screening to detect asymptomatic CD in at-risk populations can be optimized by identifying DQ2 positive cases beforehand. The high negative predictive value of DQ2 (NPV > 99%) makes it almost impossible to diagnose CD in DQ2-negative individuals.

However, 64% of first-degree relatives of patients with CD, 57% of patients with T1DM, 29% of patients with Down syndrome and 25% of the general population are DQ2 positive.[34] In consequence, in a DQ2-positive patient with Down syndrome (6% CD prevalence), the probability of positive serology is 1:5, and in a DQ2-positive individual in the general population (1% CD prevalence), the probability is 1:25. Therefore, DQ2 is useful to select candidates for serological surveillance in at-risk populations. In these cases, there are no recommendations regarding the frequency of performing serological screening in asymptomatic individuals. In the best scenario, these individuals undergo annual serological testing.

7.1. IgA-tTG in patients with T1DM

A case-finding strategy to detect asymptomatic CD in the T1DM population consists of IgA-tTG testing when diabetes is diagnosed, and annual serological monitoring thereafter. With this strategy,[35] CD was diagnosed in 6.4% (13 out of 202) patients who had newly-diagnosed T1DM, during 6 consecutive years of systematic screening. According to the same study, CD has a strong association with early-onset T1DM and there is no preferential order of appearance between the two diseases. This is corroborated by asymptomatic CD being detected by serology at the time of the diabetes diagnosis in half the cases, whereas it was detected during serological monitoring during the next three years in the other half of cases. Inconclusive or weak positive CD serology at the onset of diabetes should be interpreted with caution, and developments should be monitored.

7.2. IgA-tTG in patients with IgA Deficiency

CD is associated with selective IgA deficiency. Serum IgA deficiency (serum IgA < 10 mg/L) is the most common of primary immunodeficiency diseases and it affects 0.2% of the general population. It is usually asymptomatic.

If selective IgA deficiency is found by chance, IgG-class tTG markers should be systematically tested. Following this strategy,[36] 6.6% (22/330) of children with absolute, partial or transient IgA deficiency (IgA < 50 mg/L) were diagnosed with CD.

Analyzing the serology findings in this population,[37] it was found that IgA deficiency was absolute in 70% of cases and that only class IgG tTG antibodies were detected. The remaining 30%, however, accounted for partial or transient IgA deficiency, and tTG antibodies of IgA and IgG classes were detected in 80% of cases.

Since IgG antibodies have a longer half-life than IgA antibodies, they take longer to disappear in GFD monitoring. Thus, IgG-tTG tests are still positive after 2 to 11 years of GFD, while IgA-tTG tests are negative after 1 to 4 years of GFD in 100% of cases.

8. Serological Markers in the General Population

CD is a common disease. It may remain undiagnosed because of the heterogeneity of clinical presentations. It has excellent diagnostic serological markers. It has effective treatment without side effects and lack of treatment is associated with negative effects.

In view of the above, CD appears to be an ideal disease to study in the general population.[38,39] However, there are drawbacks to conducting a massive study of CD in the general population such as lack of knowledge of the natural history of asymptomatic, untreated CD; lack of motivation for asymptomatic patients to adhere to a GFD; CD onset at any age, which would require repeated serological screening; and lack of cost-benefit studies.

For the same reasons, it is not recommended to add IgA-tTG screening to routine tests in healthy general population groups, such as medical check-ups at work, blood donors and pre-operative

checklists for minor surgery. However, if there is an accidental finding of microcytic anemia or a slight elevation of ALT levels in blood[40] in routine tests, then systematic IgA-tTG determination would facilitate asymptomatic CD detection. Unexplained anemia or elevated transaminases are known extradigestive manifestations of asymptomatic CD.

It is accepted that CD prevalence in the general population is approximately 1:100. However, it is still widely held that CD is more prevalent in children. Epidemiological studies conducted in different age groups in the Spanish population show that prevalence ranges from 1:118 in children younger than 3 years[41] to 1:220 in primary school children[42], and 1:389 in the general population[43] with a mean age of 35 years. This apparent decreasing prevalence as age increases has recently been confirmed in an epidemiological study[44] of 4230 persons aged 1 to 90 years in the general population in Catalonia. The results from this study show that CD is 5 times more common in children than in adults, and that this increase is largely due to prevalence in the youngest children. This age-related fall in prevalence is hard to explain in view of the fact that CD is a permanent, non-resolving disease. The hypothesis of spontaneous progress to a latent state can only be investigated through longitudinal natural history studies.

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