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REVIEW ARTICLE

The role of serology in the diagnosis of coeliac disease

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ABSTRACT

Serology has significantly revolutionized the knowledge of celiac disease (CD), leading to the identification of unsuspected patients in at-risk CD groups, thereby increasing the number of CD diagnoses compared to the pre-screening era. Several markers for CD with a progressive diagnostic accuracy have been identified over the years, but only three of them, i.e. anti-tissue transglutaminase (anti-tTG), anti-endomysial (EmA) and anti-deamidated gliadin antibodies (DGP) are currently assessed in the daily clinical practice. A thorough review of the literature identified 44 original studies published between 1998 to 2022 for a total of 5098 pediatric and adult CD patients (without selective IgA deficiency) and 11930 disease controls. The results highlighted that anti-tTG IgA exhibited a higher sensitivity for CD (93.4%) than EmA IgA (92.8%), DGP IgG (81.8%) and DGP IgA (83.8%). The specificity of EmA IgA (99%) resulted to be higher than those of anti-tTG IgA (95.8%), DGP IgG (96.4%) and DGP IgA (92.1%). In patients with selective IgA deficiency, a condition closely related to CD, serological screening should include one of the three antibodies of IgG class, since anti-tTG, DGP and EmA have a very similar diagnostic accuracy in this clinical setting. According to age, there are two main diagnostic strategies for CD detection. In children, the revised ESPGHAN 2020 guidelines established that CD could be diagnosed in both symptomatic and asymptomatic children by high anti-tTG IgA titers (>10 times the cut-off) and EmA positivity with no need to obtain duodenal biopsy and HLA typing. In adult patients, although high tTG IgA titers (confirmed by EmA IgA positivity) correlate with villous atrophy, an intestinal biopsy is still considered mandatory for confirming CD diagnosis. Currently, a case finding approach in at-risk groups is preferred to mass screening for CD detection.

Keywords: Celiac disease serology, Anti-tissue transglutaminase antibodies, Anti-endomysial antibodies, Anti-deamidated gliadin antibodies, Case finding, Mass screening.

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Introduction

Over the last 40 years serology has become increasingly relevant to celiac disease (CD) diagnosis (1, 2). Before mid-1980s, CD could be diagnosed only via clinical suspicion and duodenal biopsy. Although intestinal biopsy showing the typical picture of flat mucosa is still regarded as the 'gold standard' for CD diagnosis, antibody markers have radically changed the celiac world by identifying unsuspected at-risk groups of patients with a vast range of clinical manifestations otherwise undiagnosed without the antibody support. CD serology can be split into two periods, i.e. the old and the modern era (2, 3). Early generation assays displayed a poor predictive value for CD with low specificity and sensitivity. The first serologic test, developed in the early 1970s, was the R1 anti-reticulin (R1-ARA) (4). R1-ARA of IgA class, detected by indirect immunofluorescence, have been observed in untreated CD with a prevalence varying from 36% to 78%. Because of their low sensitivity, R1-ARA IgA have proved of limited value in CD diagnosis. Their specificity, however, was high, although only rare 'false positive' cases have been described in other gastrointestinal and autoimmune disorders. The other marker of the old CD era was the anti-gliadin antibody (AGA), discovered in the early 1980s (5). AGA of IgA class performed better than IgG ones, but, despite a

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fairly high sensitivity (from 80 to 90%), they showed a very low specificity for CD with a high number of false positive cases in both disease and healthy controls (up to 29% in some studies) (3). Due to their very low diagnostic accuracy both R1-ARA and AGA are no longer used to detect CD; they have been replaced by tests of the modern era of CD serology, identified between mid-80s and the beginning of the third millennium.

Serological CD markers of the modern era: sensitivity and specificity based on the literature review

The long way of the modern era of serological markers for CD diagnosis include several markers (Table 1), but only three of them are routinely searched in the daily clinical practice, i.e. anti-endomysial (EmA) of IgA class, anti-tissue transglutaminase (anti-tTG) of IgA class and anti-deamidated gliadin antibodies (DGP) of both IgG and IgA class (2, 3). EmA IgA assay was identified in 1983 and it marked the beginning of the modern CD serology. This represented a significant step forward for CD screening for the higher sensitivity and specificity compared with the old tests (6). EmA is assayed by indirect immunofluorescence on monkey oesophagus or human umbilical cord and, although this technique is quite standardized, it is highly subjective being characterized by a high inter- and intra-observer variability. Nevertheless, in centers with significant laboratory experience, EmA still remains the test with the highest positive predictive value for CD diagnosis. In 1997, tTG was identified as the coeliac autoantigen (7). This cornerstone discovery allowed for the development of an ELISA test that overcame the difficulties related to the interpretation of EmA immunofluorescent pattern (8). The early tTG measurements were limited by significant false-positive results due to impurities of the guinea pig liver tTG used as substrate in the commercially available assays. The human recombinant tTG as antigen improved the diagnostic accuracy of this ELISA test. Nevertheless, it is noteworthy to underline that there are a lot of commercially available ELISA kits for anti-tTG produced by different manufacturers and not all perform well, as demonstrated by a number of studies showing a high variability in accuracy (9, 10). At the beginning of the new millennium DGP have been introduced in the serological coeliac scenario (11). Deamidation of gliadin peptides is induced by tTG by replacing a molecule of glutamine with glutamic acid. This process plays a relevant role in CD pathogenesis, since deamidated gliadin peptides are specifically recognized by gliadin T lymphocytes, thereby enhancing the antibody response in CD patients. The result is the production of antibodies to DGP that display a higher diagnostic accuracy for CD than antibodies to native gliadin, but lower than those of anti-tTG and EmA (12).

DGP IgG can be useful for detecting CD in the first infancy since at this age they may precede the appearance of the other serological markers (13).

To establish the diagnostic accuracy of the 3 routinely assessed CD markers, i.e. anti-tTG, EmA and DGP, a literature search was carried out using PubMed in the last 25 years. Forty-four studies, published from 1998 to nowadays, yielding a total of 5098 pediatric and adult CD patients (without selective IgA deficiency) and 11930 disease controls, were considered (8, 10, 12, 14-54).

Table 1. The long way of the modern era of celiac disease serological markers. The table summarizes the many serological tests of the modern era for CD diagnosis, reporting the year of discovery of each immunological markers. Only 3 of these markers are routinely assessed in the daily clinical practice, i.e. anti-tissue transglutaminase (anti-tTG), anti-endomysial (EmA) and anti-deamidated gliadin antibodies.

Serological tests for celiac disease	Year of discovery
Anti-endomysial antibodies (EmA)	1983
Anti-jejunal antibodies (JAB)	1990
Anti-tissue transglutaminase antibodies(anti-tTG)	1997
Anti-actin antibodies (AAA)	2000
Anti-deamidated gliadin antibodies (DGP)	2001
Anti-epidermal transglutaminase antibodies (anti-TG3)	2005
Anti-neuronal transglutaminase antibodies (anti-TG6)	2008
Anti-deamidated-transglutaminase complex antibodies (DGP-tTG)	2019

Table 2. Sensitivity (%) and specificity (%) of serological tests for coeliac disease: a review of the literature. Sensitivity and specificity of IgA anti tissue transglutaminase (anti-tTG), IgA anti endomysial (EmA) and IgG and IgA deamidated gliadin antibodies (DGP) for coeliac disease (CD) diagnosis have been reassessed by means of a review of the last 25-year literature. Forty-four studies have been taken into consideration for Twenty-six studies fulfilled the required criteria for a total of 5098 pediatric and adult CD patients (without selective IgA deficiency) and 11930 disease controls.

Study	UCD	Controls	Age groups	Anti-tTG IgA		EmA IgA		DGP IgG		DGP IgA	
				Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Dieterich W et al., 1998*[8]	106	114	adults	98.1	94.7	98.1	100	NT	NT	NT	NT
Sulkanen S et al., 1998*[15]	136	207	children	94.9	93.7	92.6	99.5	NT	NT	NT	NT
Biagi F et al., 1999*[16]	39	61	adults	94.9	91.8	100	100	NT	NT	NT	NT
Troncone R et al., 1999*[17]	48	63	children	92.0	98.0	87.5	98.4	NT	NT	NT	NT
Lock RJ et al., 1999*[181]	27	65	adults	85.2	96.9	100	100	NT	NT	NT	NT
Sardy M et al., 1999*[19]	55	53	children/adults	98.2	98.1	100	98.2	NT	NT	NT	NT
Vitoria JC et al., 1999*[20]	27	33	children	100	94.1	100	100	NT	NT	NT	NT
Stern M et al., 2000*[21]	103	149	children/adults	98.1	96.3	93.2	99.3	NT	NT	NT	NT
Sblattero D et al., 2000°[22]	65	170	children/adults	98.5	95.9	92,3	100	NT	NT	NT	NT
Biagi F et al., 2001*[23]	52	56	adults	98.2	84.6	94.6	100	NT	NT	NT	NT
Fabiani E et al., 2001°[24]	387	432	children/adults	91.5	94.9	94.3	100	NT	NT	NT	NT
Leon F et al., 2001°[25]	86	152	children	98.8	99.3	98.8	98.7	NT	NT	NT	NT
Bardella MT et al., 2001*[26]	40	110	adults	100	98.2	100	97.3	NT	NT	NT	NT
Dahele AV et al., 2001*[27]	114	65	adults	80.7	96.9	86.8	100	NT	NT	NT	NT
Dickey W et al., 2001*[28]	73	58	children/adults	75.3	98.3	80.6	96.6	NT	NT	NT	NT
Bonamico M et al., 2001°[29]	62	56	children	100	100	95.2	98.2	NT	NT	NT	NT
Carroccio A et al., 2002*[30]	24	183	adults	100	91.8	100	100	NT	NT	NT	NT
Burgin-Wolff A et al., 2002°[31]	157	208	children/adults	96.2	99.4	96.6	100	NT	NT	NT	NT
Tesei N et al., 2003°[32]	250	176	children	91.0	96.0	86.0	100	NT	NT	NT	NT
Llorente MJ et al., 2004°[33]	60	64	children/adults	98.4	97.0	97.7	95.0	NT	NT	NT	NT
Hill ID et al., 2005°[14]	75	1554	adults	92.0	98.9	91.8	98.9	NT	NT	NT	NT
Baudon JJ et al., 2004°[34]	30	109	children	93.3	97.4	90.0	100.0	NT	NT	NT	NT
Collin P et al., 2005°[35]	126	105	children/adults	94.0	99.0	89.0	98.0	NT	NT	NT	NT
Sugai E et al., 2006°[36]	92	113	adults	95.0	95.6	NT	NT	96.7	100	94.6	93.8
Bazzigaluppi E et al., 2006°[37]	143	64	children	99.3	95.3	97.9	96.9	NT	NT	NT	NT
Agardh D., 2007°[38]	119	57	children	97.0	96.0	NT	NT	95.0	86.0	91.0	91.0
Kaukinen K et al., 2007°[39]	44	46	children/adults	88.6	97.8	79.5	100	90.9	97.8	NT	NT
Ankelo A et al., 2007°[40]	87	81	children/adults	90.0	90.0	NT	NT	75.0	98.0	92.0	90.0
Niveloni S et al., 2007°[12]	60	81	adults	95.0	97.5	NT	NT	96.7	100	98.3	93.8
Hopper AD et al., 2008°[41]	77	1923	adults	90.9	90.9	85.7	98.6	NT	NT	NT	NT
Volta U et al., 2008°[42]	128	134	children/adults	96.8	91.0	93.7	100	84.4	98.5	83.6	90.3
Rashtak S et al., 2008°[43]	92	126	adults	78.0	98.0	NT	NT	65.0	98.0	74.0	95.0
Korponay-Szabo IR, 2008°[44]	74	65	children/adults	100	98.5	100	100	100	98.5	NT	NT
Prause C et al., 2009°[45]	142	160	children	95.1	98.1	NT	NT	95.1	94.4	87.3	93.1
Basso D et al., 2009°[46]	161	129	children	92.5	97.6	NT	NT	80.1	96.9	80.7	92.9
Volta U et al., 2010°[47]	48	96	children/adults	93.7	96.6	91.6	100	82.3	98.9	84.3	79.8
Dahle C et al., 2010°[48]	79	97	adults	76.0	95.0	61.0	100	87.0	96.0	87.0	96.0
Sakly W et al., 2012°[49]	103	194	children/adults	96.1	100	96.1	100	94.2	95.4	97.0	90.7
Borroni G et al., 2013°[50]	62	41	adults	97.2	97.6	100	100	NT	NT	NT	NT
Srinivas M et al., 2014°[51]	88	664	adults	84.0	96.0	83.0	99.0	NT	NT	NT	NT
Wolf J et al., 2017°[52]	529	345	children	97.1	89.3	95.8	94.0	76.9	94.2	NT	NT
Ermarth A et al., 2017°[53]	517	3038	children	90.0	90.0	NT	NT	13.0	93.0	29.0	93.0
Gulseren YD et al., 2018°[54]	21	61	adults	100	98.4	100	100	76.2	97.7	90.4	98.4
Singh P et al., 2020°[10]	290	172	adults	86.3	95.6	NT	NT	NT	NT	NT	NT
Total cases	5098	11930	total	93.4	95.8	92.8	99.0	81.8	96.4	83.8	92.1

Abbreviations: UCD: untreated coeliac disease; anti-tTG: anti tissue transglutaminase; EmA: anti endomysial antibodies; DGP: deamidated gliadin peptide antibodies (DGP), NT: not tested.

The results highlighted that anti-tTG IgA exhibited a higher sensitivity for CD (93.4%) than EmA IgA (92.8%), DGP IgG (81.8%) and DGP IgA (83.8%). The specificity of IgA EmA (99%) resulted to be higher than those of anti-tTG IgA (95.8%), DGP IgG (96.4%) and DGP IgA (92.1%) (Table 2).

Besides the standardized ELISA tests for anti-tTG IgA, a tTG-based point of care, i.e. finger-stick test, has been introduced in the clinical practice. The advantage of this assay is the easy interpretation (positive or negative) and a rapid result (available in a few minutes) (55). Even though some studies reported a very high diagnostic accuracy for this test, its predictive value is definitely lower than that obtained by ELISA (56).

A recent paper identified a new serological marker directed against the tTG-DGP complex showing very promising results for CD, but these data need to be confirmed from other studies (57).

The high levels of sensitivity and specificity found in the literature for serological CD markers (EmA, anti-tTG and DGP) can be partly influenced by the fact that in the majority of the published studies only patients with positive serological markers underwent duodenal biopsy to confirm the diagnosis of CD (3, 58). Indeed, by performing intestinal biopsy only in patients with seropositivity, the diagnostic accuracy of immunological markers for CD is overestimated since from 2 to 8% of CD patients are seronegative (59, 60).

Age-related diagnostic strategies

Consistent data have clearly demonstrated that high anti-tTG IgA titers strongly correlate with a severe CD-related small-intestinal atrophy (61). Hence, since 2012, ESPGHAN CD guidelines have indicated that CD diagnosis can be established without duodenal biopsy in symptomatic children and adolescents with anti-tTG IgA > 10 times the upper normal limit (UNL), confirmed by EmA IgA positivity in presence of CD genetic predisposition (62). Moreover, in 2020, the revised ESPGHAN guidelines attributed a relevant role to serology pointing out that anti-tTG IgA > 10 times UNL, and confirmed by EmA IgA positivity, were sufficient to diagnose CD in a child or an adolescent without duodenal biopsy even in the absence of symptoms and genetic assessment (63). Although large multicenter European studies have confirmed a high diagnostic accuracy of the ESPGHAN CD guidelines, it is noteworthy to underline that these diagnostic criteria have not been accepted in the USA because of the poor reproducibility of most commercially available anti-tTG kits (64). Some studies aimed at detecting CD in the adult population using the ESPGHAN guidelines yielded encouraging results (65, 66), however several lines of evidence demonstrated that small intestinal biopsy still represents the cornerstone for adult CD diagnosis. The main reasons for maintaining endoscopy with histologic evaluation in adults are many and can be listed as follows: a) useful not only for a definitive confirmation of CD, but also for identifying co-morbidities such as lymphocytic or autoimmune gastritis, peptic ulcer and Helicobacter pylori infection; b) the possibility to disclose the simultaneous occurrence of small bowel adenocarcinoma or enteropathy-associated T-cell lymphoma (EATL); c) the availability of the histological picture at diagnosis is crucial for the management of CD follow-up in non-responsive or refractory CD cases; d) duodenal biopsy in adults is particularly important to persuade asymptomatic patients that the diagnosis is true and that it is mandatory to follow a strict GFD; e) anti-tTG (even at a very high titer) in adults may yield false positive results particularly in patients with autoimmune disorders such as type 1 diabetes mellitus, Hashimoto thyroiditis and connective tissue disorders.

Mass screening or case finding: which serological strategy should be used?

The iceberg of CD is still submerged and there is an open debate in order to choose the best serological strategy for favoring the implementation of CD diagnoses (1, 67). Researchers are questioning whether it is better to promote a serological mass screening in the general population or it is preferable to realize a case finding approach in the at-risk groups. On the one hand, CD fulfills the World Health Organization criteria for mass screening, since this food intolerance is a common disorder with available highly predictive non-invasive tests and with highly effective therapy (i.e. GFD); on the other hand, mass screening would identify a large number of asymptomatic people with very low antibody titers and with mild intestinal lesions for whom GFD not only would not add any advantage, but could affect both patients' quality of life and

psychological stability. For this reason, most CD scientists do not support a mass screening policy, while recommending a case finding strategy in at-risk CD groups. Many conditions are at a relatively increased risk for CD, including 1st degree relatives of CD patients, iron- and folic acid-deficiency anemia, aphthous stomatitis, dental enamel defects, short stature, cryptogenic hypertransaminasemia, unexplained / early osteoporosis, infertility, recurrent miscarriages, late menarche, early menopause, fibromyalgia and irritable bowel syndrome. Moreover, many autoimmune disorders, such as dermatitis herpetiformis, psoriasis, alopecia areata, Hashimoto thyroiditis, Graves' disease, type 1 diabetes mellitus, Sjögren syndrome, autoimmune liver diseases (autoimmune hepatitis, primary biliary cholangitis, sclerosing cholangitis), neurological disorders (cerebellar ataxia, peripheral neuropathy, cryptogenic epilepsy) as well as some chromosomal disorders (Down's, Turner's and Williams' syndromes) should be screened by serology for the possible association with CD (1, 68). The case finding approach allows for the identification of large majority of symptomatic patients with severe small intestinal lesions who will significantly benefit from GFD. Moreover, CD patients identified by case finding display a better compliance with the diet than those detected by mass screening (Figure 1).

Serological protocol in patient at high and low risk for CD: what does it change?

Different diagnostic algorithms have been proposed in various clinical settings. In patients with malabsorption syndrome (i.e., diarrhea / steatorrhea, weight loss and systemic impairment) in whom a high CD prevalence is expected (once selective IgA deficiency has been ruled out), testing for anti-tTG IgA or EmA IgA is mandatory (in this clinical setting both antibodies display a very similar sensitivity) (2). Because of seronegative CD (found in 2-8% of the total CD diagnoses) (59, 60), all patients at a high CD-risk should undergo duodenal biopsy regardless of anti-tTG / EmA results. Three possible scenarios can be identified: a) cases with positive anti-tTG IgA and / or EmA IgA with a more or less severe villous atrophy

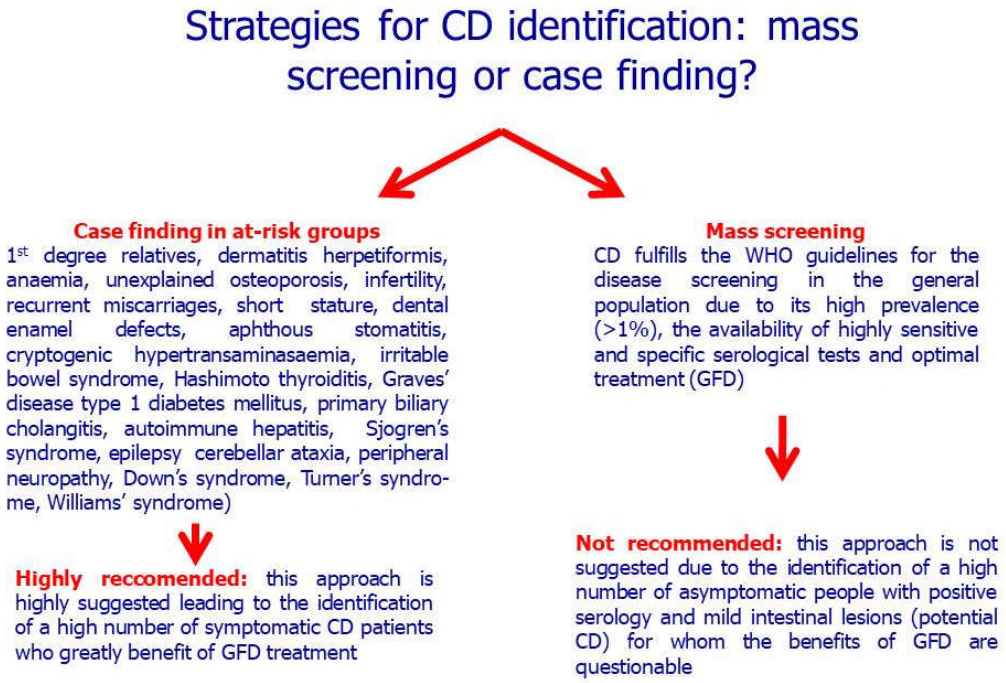

Figure 1. Case finding strategy vs. mass screening. Which one is the recommended approach for increasing the number of coeliac disease diagnoses? The former should be preferred to the latter since it allows for the identification of a high number of symptomatic patients who greatly benefit of gluten free diet (GFD) therapy, whereas mass screening would favor the detection of many asymptomatic patients with positive serology but with only minimal lesions of small intestinal mucosa (asymptomatic potential CD) with more negative than positive effects induced by GFD.

confirming CD diagnosis; b) cases with negative serology and with normal small intestinal mucosa, for whom CD can be excluded; c) cases with negative serology and with villous atrophy in whom HLA typing is necessary. In those cases testing positive for HLA-DQ2 and/or -DQ8, a provisional CD diagnosis can be made waiting for its confirmation by clinical and histological improvement after 1-year GFD. In those patients testing negative for DQ2 and DQ8, CD can be ruled out and other non-gluten-dependent villous atrophy should be sought (Figure 2).

In the clinical setting of at-risk CD groups that are characterized by a low-medium CD prevalence, the diagnostic algorithm is based on anti-tTG IgA and total serum IgA. Patients with normal serum IgA and positivity for anti-tTG IgA at a titer > 2 UNL should be investigated by duodenal biopsy which will confirm the diagnosis of frank CD or a potential CD when villous atrophy or minimal lesions/ normal small intestinal mucosa are found, respectively (69). In those cases with positivity for anti-tTG IgA at a low titer (<2 UNL), which is frequently an expression of a false positive result (2), EmA IgA should be screened. Only those patients with EmA IgA positivity should undergo duodenal biopsy with the two previously exposed scenarios (frank CD and PCD). Cases that resulted negative for EmA are recruited in a follow-up program by means of anti-tTG IgA (Figure 3).

Both high- and low-medium risk CD patients with a selective IgA deficiency (serum IgA < 7 mg/dl) should be tested by anti-tTG IgG or, alternatively, by DGP IgG or EmA IgG; since the 3 antibodies deserve a very

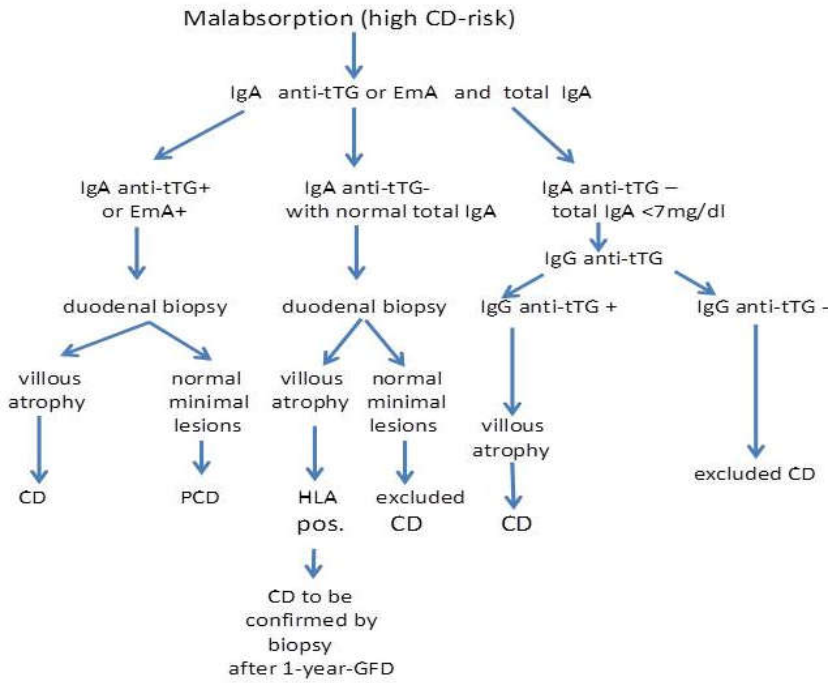

Figure 2. Serological testing for celiac disease (CD) in patients with malabsorption at a high CD-risk. IgA anti tissue transglutaminase (anti-tTG) or IgA anti-endomysial antibodies should be detected since in this clinical setting the predictive value of the two antibodies is very similar). Total serum IgA must be sought in order to rule out IgA deficiency. Three scenarios are possible: a) cases with normal serum IgA and antibody positivity will undergo duodenal biopsy confirming CD diagnosis in presence of villous atrophy or potential CD in presence of normal mucosa or minimal lesions; b) keeping in mind the possibility of seronegative CD, also cases with normal serum IgA and negativity of anti-tTG or EmA will undergo duodenal biopsy: if the biopsy is normal or with minimal lesions the diagnosis of CD can be excluded, whereas the finding of villous atrophy will suggest suspected seronegative CD to be confirmed by HLA positivity and by clinical and histological improvement after 1-year of GFD; c) if selective IgA deficiency is present, anti-tTG of IgG instead of IgA class must be detected. Cases with antibody positivity will be assessed by intestinal biopsy confirming CD diagnosis in presence of villous atrophy, whereas antibody negativity will exclude CD diagnosis (only in selected cases intestinal biopsy will be performed). Besides anti-tTG IgG, also DGP IgG and EmA IgG have the same diagnostic accuracy for detecting CD in patients with IgA deficiency. Abbreviations: CD, celiac disease; PCD, potential celiac di anti-tTG, anti-transglutaminase antibodies; EmA, anti-endomysial antibodies; IgA, immunoglobulin A; IgG, immunoglobulin G; GFD, gluten-free diet.

similar diagnostic accuracy for identifying CD in selective IgA deficiency (70, 71). As reported in Figures 2 and 3, patients with selective IgA deficiency should be tested positive for IgG antibodies must be assessed by duodenal biopsy which will confirm CD diagnosis in presence of villous atrophy, whereas a negative result for IgG antibodies is already considered enough for ruling out CD (only in cases with a severe malabsorption syndrome a decision for intestinal biopsy should be taken because of a possible seronegative CD, usually rarer in IgA deficiency patients than in patients with normal levels of serum IgA.

Conclusion

Nowadays serological markers are a powerful tool for the diagnosis of CD patients and their predictive value for gluten sensitive enteropathy is so high that they reached the duodenal biopsy as the gold standard for this food intolerance. The reproducibility and the reliability of immunological markers is very often higher than that of histology without the frequent processing mistakes of duodenal biopsies (lack of orientation and sampling errors) (72). In line with this statement, it has been proposed that the revised ESPGHAN criteria, already very effective for identifying CD in children and adolescents, might be extended to highly symptomatic (malabsorption, anemia, weight loss and unexpected osteoporosis) young adults (under the age of forty), leading to a gradual transformation of CD diagnostic criteria also in adulthood. This new approach has been already applied in two European countries such as Finland and the United Kingdom, as recently confirmed in the 19th International Celiac Disease Symposium (ICDS), held in Sorrento (Italy) in October 2021. The future of serology in the diagnostic workup of CD could further improve via new immunological tests such as the DGP-tTG complex antibodies which are expected to confirm an even higher predictability than currently used tests (60). To guarantee the perfect efficiency of serology, it is mandatory to use the correct diagnostic algorithm in the different clinical settings, characterized by a high- and low-risk CD subsets. The case finding approach

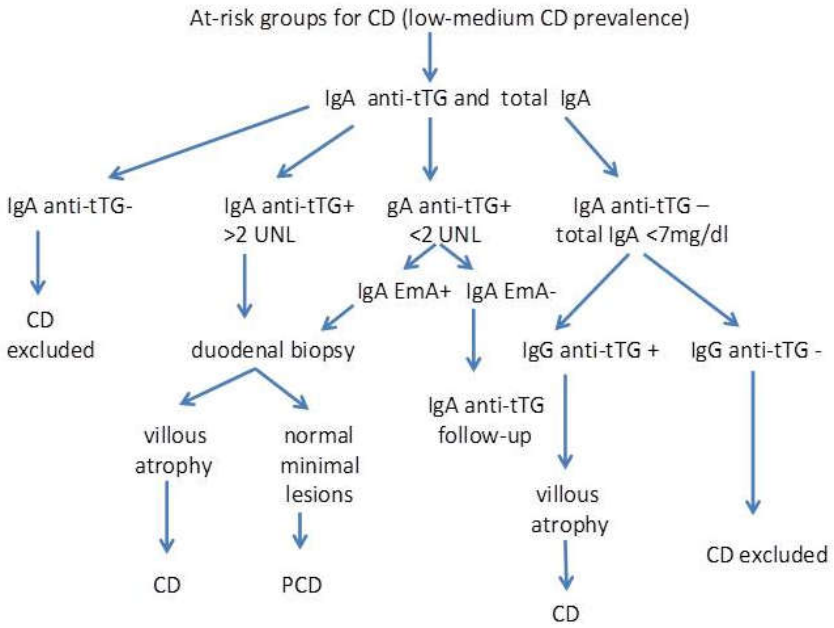

Figure 3. Serological testing for celiac disease (CD) in at-risk groups for celiac disease (low-medium expected CD prevalence. IgA anti tissue transglutaminase antibodies (anti-tTG) and total serum IgA are sought. In patients with normal serum IgA three possible scenarios can be present: a) negativity of anti-tTG IgA rule out CD ; b) cases positive for anti-tTG IgA >2 UNL will undergo duodenal biopsy confirming CD in presence of villous atrophy or potential CD in presence of normal mucosa or minimal lesions; c) cases positive for anti-tTG IgA < 2 UNL will be tested for EmA IgA; if the test is positive, a duodenal biopsy is required (thus with the two options previously illustrated); if negative, a follow-up with anti-tTG is recommended. In the case of IgA deficiency patients, the same diagnostic protocol, described in figure 2, is applied. Abbreviations: CD, celiac disease; PCD, potential celiac disease anti-tTG, anti-transglutaminase antibodies; EmA, anti-endomysial antibodies; IgA, immunoglobulin A; IgG, immunoglobulin G.

should be preferred to the mass screening to reveal the submerged iceberg of unrecognized celiac patients, thus avoiding the identification of many asymptomatic potential CD, whose management is still under evaluation. Some limitations of serology must be recognized. Many patients are tested for antibodies only after the partial or total elimination of gluten from the diet causing false negative results. Moreover, the policy to minimize the costs in medicine reduces the number of tests for CD diagnosis, e.g. EmA IgA evaluation, which is still the test with the highest specificity for CD. In conclusion, serological tests are mandatory in the diagnostic management of CD and their assessment, together with the finding of the typical histological lesion, strongly corroborates the certainty of CD diagnosis.

Conflict of interests

The authors declare that there is no conflict of interest.

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