

Diagnostic Challenges in Celiac Disease and the Role of the Tissue Transglutaminase–Neo-Epitope

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Abstract The diagnosis of celiac disease (CD) remains a clinical challenge based on the incomplete specificity and sensitivity rates of current non-invasive tests. Furthermore, histological assessments fail to identify all overt cases and, in particular, do not manifest pathognomonic alterations in silent cases. Accordingly, the majority of CD cases are diagnosed with great delay. Recent research into the pathogenesis of CD, allowed us to identify a neo-antigen that appears to be the most promising serological tool for the detection of anti-tissue transglutaminase as well as anti-gliadin antibodies.

Keywords Celiac disease · Tissue transglutaminase · Anti-gliadin antibodies

The constellation of celiac disease

Celiac disease (CD) is a very common chronic bowel condition recognizing an autoimmune pathogenesis leading to different degrees of malabsorption. Its common hallmark

is the histological demonstration of villous atrophy during small intestine evaluation. Until quite recently, CD has been considered as an allergic condition caused by gliadin, the alcohol soluble part of gluten (a glycosylated storage protein of cereals like wheat, rye, and barley). CD is as well characterized by the effectiveness of a strict gluten-free diet as the only treatment currently available. Earlier observations that serum autoantibodies are highly specific for CD soon prompted the assumption that the disease is ultimately secondary to autoimmune mechanisms and was then followed by the development of multiple non-invasive markers for disease [1]. Among autoimmune diseases, CD manifests several peculiar features, including a quite balanced sex ratio [2] and the highest concordance rate between monozygotic twins [3, 4] while other observations in antibody-mediated autoimmune diseases [5, 6] may hold the key to future developments in the treatment of CD.

The molecular mechanisms of CD are illustrated in Fig. 1. Gliadin as a glutamine- and proline-rich protein (35/15%) is only partially digested in the digestive tract comprising peptides which are resistant to digestion [7]. Such digestion-resistant peptides can thus be modified by tissue transglutaminase (tTG) in two alternative ways [8–10] that include deamidation (in which a glutamine residue is cleaved into a glutamate residue and an ammonium ion) and transamidation (in which a glutamine residue of the gliadin peptide and a lysine residue of an acceptor protein are being cross-linked). *In vitro* studies have demonstrated that transamidation is the major reaction (75% vs. 25% for deamidation) [9] thus increasing the antigenicity of the gliadin peptides. Cross-linking also occurs outside the active site of tTG and results in permanently and covalently linked deamidated gliadin peptide/tTG complexes [11, 12] which are found in small intestine biopsies of patients with CD [13]. It remains poorly understood how the autoanti-

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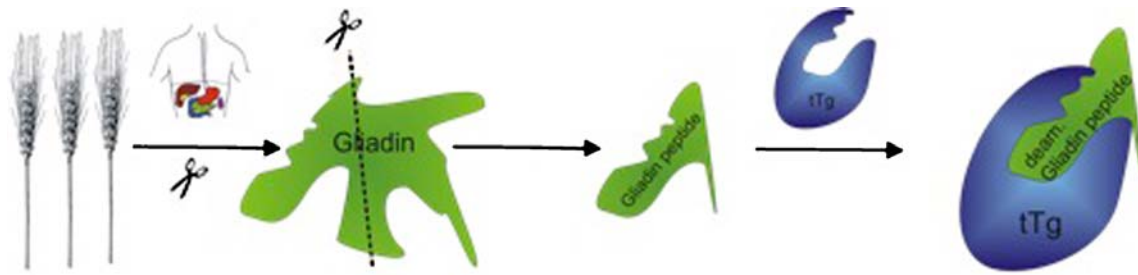


Fig. 1 The gliadin processing pathway. Cereal products are digested in the intestine and in this process gliadin is cleaved into peptides which react in the subsequent steps with tissue transglutaminase to form covalently cross-linked complexes

bodies to tTg develop [14–16] although it has been postulated that the formation of the complex of gliadin peptides and tTG is a hint supporting epitope spreading from gliadin to tTg.

The submerged burden of CD

From a clinical standpoint, CD is often associated with diabetes mellitus, autoimmune-thyroiditis, and osteoporosis [17]. Patients with any one of these conditions have a higher prevalence of CD compared to age-matched controls. More importantly, these disorders can be effectively cured once the diagnosis of CD-associated malabsorption is established. There is evidence that a gluten-free diet leads to the improvement of all associated conditions, as is well represented in the case of patients with CD and type 1 diabetes [18]. Similarly, bone density also significantly increases following a strict dietary regimen, with better results compared to classical treatments of osteoporosis [19]. A careful hypothesis-driven histological assessment remains the cornerstone for CD diagnosis, yet the development of new serum markers, namely autoantibodies, appear as a promising alternative which needs to be rigorously tested in the future. This article will illustrate the current knowledge in the field of CD diagnosis as well as the newer tools that are being proposed to overcome the current limitations.

The epidemiology of CD remains poorly understood and recent population-based studies in healthy subjects report prevalence rates as high as 1% [16]. The typical celiac patient has a strikingly high probability (approximately six out of seven) to go undetected or to be diagnosed with CD only later in life. Indeed, it has been estimated that the diagnostic delay for celiac disease ranges from 5 to 11 years, particularly if no classical symptom (such as diarrhea, fatigue, bone loss, or anemia) is present. As a result of this peculiar epidemiology, the picture of an iceberg is commonly used to describe the prevalence and clinical manifestations of CD. In this scenario (Fig. 2), the emerged

tip represents symptomatic cases with pathognomonic mucosal lesions while the submerged parts illustrate the silent or latent cases where the latter do not manifest typical histological changes. For these reasons, the diagnostic tools to be used to screen asymptomatic subjects or confirm suspect cases are of paramount importance in clinical practice, particularly when first-degree relatives are to be tested [20]. The ideal serum test, in particular, should allow the identification of 100% of cases with maximum specificity and prove to have a similar or better effectiveness compared to small bowel histology in the diagnosis of CD.

Where do undiagnosed patients fall out of the current diagnostic picture?

Currently, we have three major tools to diagnose CD, namely small intestine histology, serum antibodies, and human leukocyte antigen (HLA) genotyping for DQ2 and DQ8 [21]. Since endoscopy is invasive and genetic testing is expensive, the development of new serum tests allowing

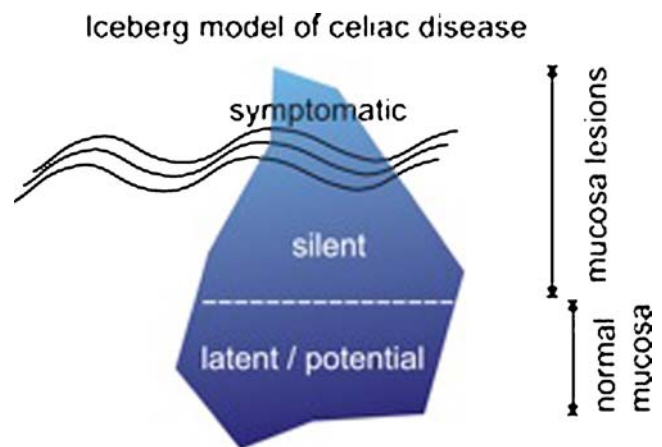
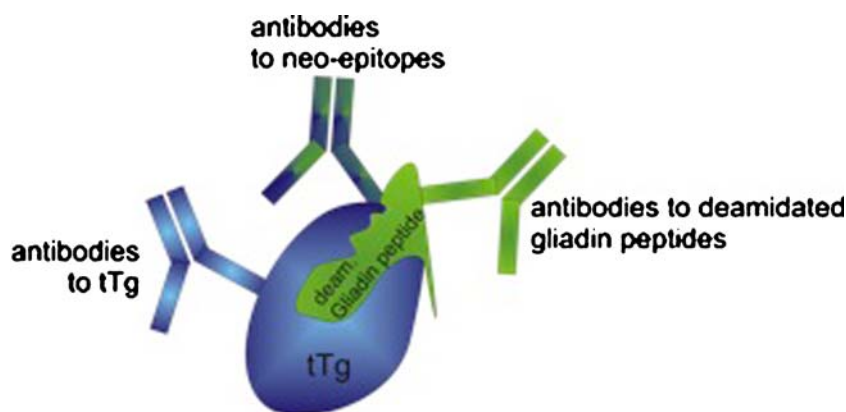


Fig. 2 The iceberg model of celiac disease. It is currently estimated that six out of seven patients remain undiagnosed, as illustrated by the part of the iceberg below the waterline representing latent, potential, and silent cases

Fig. 3 The complex of deamidated gliadin peptides cross-linked with tissue transglutaminase (tTG) can detect three different antibodies entities: antibodies to tTg, to deamidated gliadin peptides and to the neo-epitope



maximum specificity and sensitivity in less defined cases is the current priority for researchers worldwide. With the exception of subjects of pediatric ages, antibodies to tTg have recently become the gold standard for CD detection quickly making previously used tests such as anti-gliadin antibodies unjustified in clinical practice. The CD iceberg, however, is far from melting despite promising data from comparisons between different laboratory approaches [22] and different from other autoimmune conditions [23] seem to hold promising scenarios for future screening methods.

The recent development of a CD serum marker based on tTg covalently cross-linked to deamidated gliadin peptides (coined neo-epitope) takes into account all current research developments. In fact, the antigen used for detecting CD-associated antibodies is the purified cross-linked complex which is formed under physiological *in vivo* conditions. This antigen was designed to detect antibodies to tTg, modified gliadin peptides as well as the newly formed surface structure of the cross-linked complex, as illustrated in Fig. 3. Data supporting the use of this new assay (AESKULISA[®] CeliCheck New Generation) in CD diagnosis were recently independently obtained [24] in a longitudinal study on a total of 2,684 eligible subjects (with 254 fulfilling the study requirements). This study demonstrated a sensitivity of 92.31% and a specificity of 82.89% of the neo-epitope-based ELISA. Of note, these rates were the highest when compared to 21 other tTg ELISA kits from ten different manufacturers. Furthermore, anti-endomysial antibody tests performed with significantly lower sensitivity (68.42% for the IgA/IgG conjugate). Additional analyses are being performed to stratify the higher sensitivity and the early detection of all approaches utilizing the small intestine histology and, more importantly, longer follow-up times. Based on these data, a revised screening protocol has been proposed where the initial testing should be the simultaneous testing of IgA and IgG anti-tTg and a positive serological result should be followed by an endoscopy of the upper digestive tract and

histology. Indeed, we should also emphasize that discrepancies between serology and histological findings are not uncommon and HLA genotyping should be obtained in case of persistent clinical suspicion [24, 25]. This is a similar principle to data in other autoimmune diseases [26–30].

What the future holds for CD

With the high proportion of silent or latent CD cases, the known association of CD and other autoimmune diseases and the debilitating consequences of malabsorption, it is crucial to utilize the most sensitive tests for the screening of the general population. Using a serological approach for the screening which employs antigens mimicking the physiological antigens appears as a cost-efficient method and increases the sensitivity to higher standards. As is well illustrated in other autoimmune conditions [31], the diagnostic challenge posed by CD and, accordingly, its true epidemiology impact remain largely to be determined and accuracy issues to be solved. We submit that only large population-based studies having solid gold standard endpoints such as the longitudinal changes in mucosal histology will eventually lead to an answer to all open questions in the diagnosis of CD.

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