

Celiac disease diagnosis and gluten-free food analytical control

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Abstract

Celiac disease (CD) is an autoimmune enteropathy, characterized by an inappropriate T-cell-mediated immune response to the ingestion of certain dietary cereal proteins in genetically susceptible individuals. This disorder presents environmental, genetic, and immunological components. CD presents a prevalence of up to 1% in populations of European ancestry, yet a high percentage of cases remain underdiagnosed. The diagnosis and treatment should be made early since untreated disease causes growth retardation and atypical symptoms, like infertility or neurological disorders. The diagnostic criteria for CD, which requires endoscopy with small bowel biopsy, have been changing over the last few decades, especially due to the advent of serological tests with higher sensitivity and specificity. The use of serological markers can be very useful to rule out clinical suspicious cases and also to help monitor the patients, after adherence to a gluten-free diet. Since the current treatment consists of a life-long gluten-free diet, which leads to significant clinical and histological improvement, the standardization of an assay to assess in an unequivocal way gluten in gluten-free foodstuff is of major importance.

Keywords: Celiac disease · Autoimmune · Transglutaminase · Gliadin

Abbreviations

| | |
|-------|------------------------------------|
| AGA | anti-gliadin antibodies |
| CD | celiac disease |
| EIs | electrochemical immunosensors |
| EMA | endomysial antibodies |
| ELISA | enzyme-linked immunosorbent assay |
| Ig | immunoglobulin |
| ROC | receiver operating characteristics |
| tTG | tissue transglutaminase |

Introduction

Celiac disease (CD) is a disorder of the small intestine caused by an inappropriate immune response to wheat gluten and similar proteins of barley and rye in genetically susceptible individuals. CD can be also referred to as celiac sprue, nontropical sprue, gluten-sensitive enteropathy, or idiopathic steatorrhea [1]. The classic presentation, with malabsorption, was first described by Samuel Gee in 1888 [2], but the relation between the disease and wheat was not reported until the late 1940s by Willem Karel Dicke, who

observed that the ingestion of certain cereal grains was harmful to children with celiac disease [2, 3]; later, John W Pauley described the associated histologic changes in the intestine [4].

CD is found mainly in Caucasians, occurring in 1 per 130–300 individuals in the western European population [5–7]. Although CD is one of the most common immune-mediated disorders, there still remains a considerable prevalence of undetected cases [8].

It is more appropriate to consider CD as a multisystem disorder, rather than mainly gastrointestinal. A considerable number of conditions are associated with CD, namely osteoporosis, malignancy, and infertility. It is also associated with other autoimmune disorders, such as dermatitis herpetiformis, type 1 diabetes, or autoimmune thyroiditis, and with neurological and genetic disorders [4, 9–12].

Clinical presentation

Celiac disease can be diagnosed at any age; however, it presents most commonly in early childhood (between 9 and 24 months) or in the third or fourth decade of life [13–16]. As occurs in other autoimmune disorders, CD is more common in females than in males at a ratio of 3 to 1 [17]. Although, it is a disorder that primarily affects the small bowel, the symptoms can range from classic gastrointestinal symptoms, such as diarrhea and abdominal distension, which are more common in infants and young children, to nonspecific gastrointestinal symptoms and extraintestinal manifestations, typical of older age groups; moreover, some patients are asymptomatic [8, 18]. The concept of the “celiac iceberg” has been used to emphasize the clinical variability of celiac disease and that many cases are still undiagnosed [10].

The clinical presentation of CD is, therefore, very heterogeneous, ranging from an asymptomatic or silent stage to a clinically overt or symptomatic form [19]. The asymptomatic forms are characterized by extraintestinal symptoms, but with typical histological changes and positive serology. The classic or symptomatic form presents typical gastrointestinal symptoms, histological changes, and positive serology.

The term “latent” CD characterizes the subjects with genetic predisposition to develop CD. They do not have a flat mucosa, despite a gluten-containing diet, but probably will develop clinically overt CD later in life [20–22]. These patients usually present increased intraepithelial lymphocytes (IELs) and positive serology

for endomysial antibodies (EMA) and tissue transglutaminase (tTG) antibodies with HLA-DQ2 or DQ8 predisposing genotype [23–25].

Finally, the term “refractory” CD refers to patients who do not respond to a gluten-free diet or who previously

responded but later became nonresponsive, presenting severe villous atrophy despite maintenance of a strict gluten-free diet [26].

The severity of symptoms is not necessarily proportional to the severity of the mucosal lesions. In fact, currently, there are more subjects with asymptomatic or mild celiac disease than with the classic symptoms of severe malabsorption [3, 21].

Genetics and pathogenesis

The disease is genetically determined, affecting 10% of first-degree relatives and 75% of the monozygotic twins being both affected. The human leucocyte antigen HLA-DQ2 is presented in 90–95% of CD patients and the remaining 5–10% of patients are HLA-DQ8 positive [18]. Although the possession of the HLA proteins is necessary, it is not sufficient for CD development, since about 30% of the healthy population possess them [27–29].

Gluten is a complex mixture of wheat storage proteins that can be alcohol-soluble fractions, the gliadins, and alcohol-insoluble fractions, the glutenins [28]. Based on their differential N-terminal sequence, size, and electrophoretic mobility [30] gliadins are subdivided into α , β , γ , and ω -gliadins. Glutenins consist of low molecular weight (LMW) and high molecular weight (HMW) glutenins [31]. Gliadins are also called prolamins due to their high content of the amino acids proline and glutamine. It is generally accepted that prolamins are the major triggering factors in CD [32]. Gliadins have analogous proteins that are present in barley (hordeins), rye (secalins), and oats (avenins). Recent studies failed to identify the toxic amino acid sequence in oats [33], which is considered toxic in only a minority of patients with CD [18].

The mechanism underlying CD pathogenesis can be explained by the ingestion of the alcohol-soluble protein components of wheat, barley, and rye. These gluten peptides are resistant to digestion by gastric and pancreatic enzymes due to their high content in proline, reaching the epithelial cell membrane and passing into the cytosol [34]. The deamidation of these proline-rich gluten peptides is mediated by tTG enzyme [35] creating epitopes with increased immunostimulatory potential. The deamidated epitopes are then presented, in association with the human leucocyte antigens DQ2 and DQ8 of antigen-presenting cells, to CD4⁺ T cells expressing α/β T cell receptor [36]. These T cells become activated and express proinflammatory cytokines [37] that, in turn, promote the release of matrix metalloproteinases which cause epithelial cell damage

leading to the development of the flat mucosa, typical of CD [37]. The resulting tissue injury leads to further release of tTG [29].

The in vitro study by Lu Shan and colleagues [38] reported a highly stable 33-mer peptide, rich in proline and glutamine, which has been isolated from gliadin and is thought to contain the toxic sequence. This 33-amino-acid peptide has been reported to have immunodominant characteristics, being resistant to degradation by all gastric, pancreatic, and intestinal brush border membrane proteases in the human intestine, and is readily available for T cell recognition and activation.

Antibody tests cannot replace histological studies of bowel biopsies; however, they are very important as a

Diagnosis and management

The diagnosis of CD is complicated by the diversity of clinical manifestations that are related to the age at onset and symptomatology.

Biopsy

Diagnostic criteria for CD in both children and adults are still based on the guidelines proposed in 1990 by the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) [39]. Small bowel biopsy has been the standard diagnostic test for CD during the last 30 years. The biopsy is performed during an upper endoscopy and should only take place during a normal gluten-containing diet, when villous atrophy and crypt hyperplasia can be detected. Multiple samples are taken from the second or third part of the duodenum. Inadequate sampling and patchy villous atrophy can lead to an incorrect diagnosis. Moreover, it should also be considered that isolated marked villous flattening and IELs can be found in other diseases [40].

Recently, there has been an increase in atypical forms of CD, including cases without significant gastrointestinal symptoms, and cases presenting symptoms and complications of CD before the development of villous atrophy [41, 42].

Biological markers

Recent guidelines from the ESPGAN [39], and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) [43] have suggested an algorithm for diagnosis (Fig. 1), in which the serological tests appear as the first test to clear clinical suspicion of celiac disease in patients presenting characteristic symptoms or in those who belong to a risk group. These patients at risk are those with celiac disease-associated disorders, such as endocrine, neurological, liver, genetic, and autoimmune diseases; first- and second-degree relatives of celiac patients are also at risk.

screening tool, for early detection of CD cases [1]. Serological tests can also be employed in the follow-up and management of CD. Indeed, according to the ESPGAN criteria, when the diagnosis of CD is established by clinical, analytical, and histologic studies, repeated endoscopy with duodenal biopsy is not necessary if the patient's condition improves after introducing a gluten-free diet; the results of repeated endoscopy could be rather confusing, since normalization of the histology may take up to 8 years [44].

The serologic tests use highly specific antibody-antigen interactions and are fundamental to identify the gluten intolerance and to monitor the response of the CD patients to a gluten-free diet [45]. There are two types of serologic tests. One detects the antibodies against the antigen gliadin (i.e., anti-gliadin antibodies (AGA)), in which the immunoglobulin A (IgA) isotype is considered to be the most specific [46]; and the other, the autoantibody test, which detects IgA antibodies to tissue transglutaminase (IgA-tTG antibodies) and IgA endomysial antibodies (IgA-EMA) [18] that are usually present in serum during the active phase of CD [47]. However, some patients are IgA deficient and, therefore, in that case the detection of the pathology by the serological tests is jeopardized. Selective IgA deficiency affects about 2–5% of patients diagnosed with CD [48]. In these cases, the determination of the IgG class of antibodies against gliadin (IgG-AGA), endomysium (IgG-EMA), and tTG (IgG-tTG antibodies) has been suggested as an alternative [47].

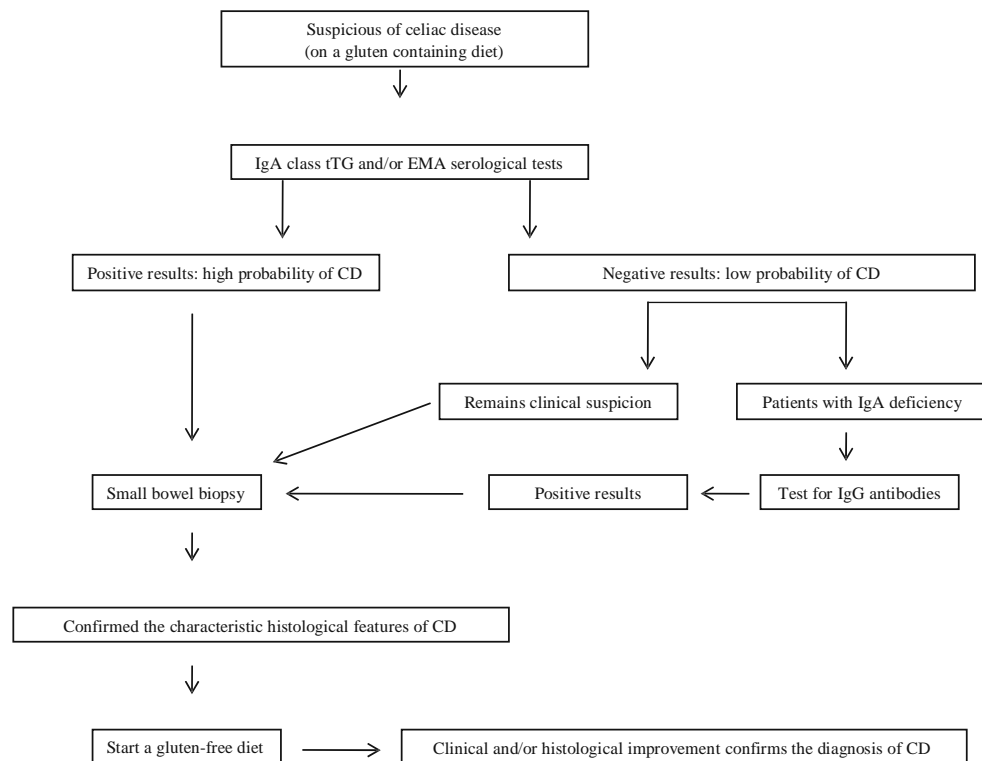
Since their description in 1958, AGA have been used as the serological markers for CD [28]. Both IgA and IgG AGA are present in the sera of patients with CD, although they lack specificity, as gliadin may cross the normal gut mucosa, being present in 5–10% of healthy population.

Endomysium is a connective tissue protein found in the collagenous matrix of human and monkey tissue [3]. EMA association with CD was first described in 1984 [49] and its detection rapidly became the serological test of choice, due to its specificity of almost 100%. The use of human umbilical cord as substrate has been proposed as a valid alternative to the monkey esophagus [50–52].

Tissue transglutaminase (tTG) is a calcium-dependent enzyme expressed both intra- and extracellularly, and is implicated in physiologic processes like extracellular matrix (ECM) formation, cell adhesion, and apoptosis [53]. tTG serves as a cross-linker of different ECM proteins, resulting in the formation of an ϵ -(γ -glutamyl)-lysine bond. Gliadin is the preferred substrate for tTG [3]. It was suggested that tTG has the ability to cross-link itself to gluten leading to antibody formation [54]. In

1997 tTG was identified as the autoantigen recognized by the EMA [55] and was identified as the main autoantigen in CD.

Fig. 1 Proposed approach for the evaluation of patients with suspected celiac disease [39, 43]



Sensitivity and specificity of serological markers

The most sensitive and specific serologic markers of celiac disease are the IgA EMA and the tTG IgA antibodies [48]. While two different types of tests are used to detect these autoantibodies, they detect antibodies to the same antigen, the tTG [56]. IgA anti-tTG antibody and IgA anti-EMA tests have sensitivities higher than 90% and specificities higher than 95% and, therefore, the serological tests to detect those antibodies are recommended for initial screening [57]. In contrast, IgA AGA has a sensitivity of about 80% and a specificity ranging from 80 to 90%. For routine diagnosis, the determination of gliadin antibodies in serum is no longer recommended, since they are less sensitive and specific than EMA and tTG antibody tests [43, 58].

IgG anti-EMA and anti-tTG antibodies, in spite of having a specificity rounding 95%, present poor sensitivities (around 40%) and for this reason these antibodies have been used less frequently as serological markers [57]. However, because IgA deficiency has an increased prevalence among celiac patients [29], care should be taken in interpreting the results of IgA antibody tests. In the case of IgA deficiency, measurement of IgG anti-EMA/tTG and IgG anti-gliadin antibodies should be performed. Positive results for IgA anti-EMA/tTG antibodies, or IgG anti-EMA/tTG antibodies and AGA in the case of IgA deficiency should be followed by intestinal biopsy. A biopsy might also be recommended in cases of negative serology, when

there is a high clinical suspicion.

In positive serological cases, followed by a negative biopsy, it is important to consider HLA typing, since the absence of both HLA-DQ2 and/or HLA-DQ8 alleles has a very high negative predictive value, helping to rule out the disease in cases of equivocal biopsy results [3, 4, 56].

It is established that the most widely used methodology for CD clinical serological diagnostic purposes is an enzyme-linked immunosorbent assay (ELISA). Several high-quality commercial kits are available to detect IgA antibodies to tTG and endomysial IgA antibodies, which appear to have equivalent diagnostic sensitivity and specificity. The advantages of tTG testing is that the ELISA test eliminates the disadvantages associated with the use of EMA, namely the higher cost, time-consuming protocol which is unsuitable for testing large numbers of samples, the use of monkey esophagus (or human umbilical cord), and the subjective interpretation of the immunofluorescence analysis [15]. Several studies have compared the analytical and clinical utility of commercially available anti-transglutaminase ELISAs assays [59–73], and found that the use of tTG as antigen for CD diagnosis presents an adequate sensitivity and specificity. Therefore, major efforts have been concentrated on developing a tTG-based ELISA, using either the commercially available guinea pig tTG or human recombinant tTG.

The first-generation assays for tTG antibodies detection used guinea pig liver tTG as the antigen. Second-generation kits using purified human tTG or human recombinant tTG were developed and introduced in routine practice. Several studies compared the first- and second-generation kits and

concluded that the human antigens improve assay sensitivity and selectivity [63, 74–76]. A third generation of kits using tTG–gliadin peptide complexes as the antigen has also been proposed; however, it seems to have no advantage over human recombinant antigen kits, especially regarding specificity [77, 78].

Several second-generation assays are commercially available, and were introduced in routine practice of clinical laboratories. These assays use either recombinant human tTG or purified human tTG as antigen.

Van Meensel [79] and colleagues evaluated 10 different commercially available second-generation IgA anti-tTG ELISA kits, and showed that most of these assays presented excellent performance, with good linear ranges. According to the optimal receiver operating characteristics (ROC) curve cutoff employed, the sensitivity values ranged from 91 to 97% and the specificity ranged between 96 and 100%. Since the areas under the ROC curve did not differ significantly, the results from using the kits could be compared; however, there is some variability between these immunosorbent assays which needs to be solved in order to reach higher homogeneity.

Gluten-free food control

Two guidelines concerning the management of CD were recently published: “Recommendations of NASPGHAN” [43] and “National Institutes of Health (NIH) consensus development conference statement on celiac disease” [80]. After a positive diagnosis for CD, the only treatment available, so far, is a lifelong strict adherence to a gluten-free diet, which will permit the recovery of the intestinal mucosa [18]. However, a diet completely free of gluten would be difficult, if not impossible, to maintain.

Gluten is a common component in the human diet, and its exclusion presents a big challenge for celiac disease patients. Gluten plays a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesivity, viscosity, and elasticity to dough [81]. After sugar, it is perhaps the second most widespread food component in Western civilization [82]. Since about 10% of gluten seems to be made up of potentially toxic gliadin peptides [83] it is extremely important to evaluate the purity of gluten-free products to ensure a safe diet for celiac patients. To certify gluten-free products, the use of highly sensitive assays is mandatory. The European Union, World Health Organization, and Codex Alimentarius require reliable measurement of the wheat prolamins, gliadins, rather than all wheat-derived

proteins [84]. There is still no general agreement on the analytical method to measure gluten in ingredients and food products [85], although the official limits described in the Codex Draft Revised Standard (2000) are 20 ppm for foodstuffs naturally gluten-free and

200 ppm for foodstuffs rendered gluten-free [86]. Nevertheless, to measure gluten traces in food, immunochemical methods are usually chosen to determine gliadins [87–92]. Besides the quality control of gluten-containing products, it is important to assess gluten in foodstuff that can be contaminated with native or heated proteins from wheat, barley, and rye.

In recent years several analytical possibilities for the detection of the wheat protein component gliadin in food products have been exploited, such as the polymerase chain reaction (PCR) technology, mass spectrometry, or high-performance liquid chromatography (HPLC). PCR allows the amplification of a specific DNA fragment, flanked by two oligonucleotides that act as primers in the amplification reaction carried out by DNA polymerase. The amplified product is visualized by staining with a fluorescent dye or by Southern blotting after a gel electrophoresis. The amplification of gluten-specific DNA fragments by PCR has been reported [93, 94]. Normally, PCR results are only qualitative; however, by incorporating internal standards, the results provide semiquantitative measurements. By employing real-time PCR (rt-PCR) highly accurate quantitative results can be obtained. A quantitative competitive PCR system has been constructed, evaluated, and compared with ELISA, obtaining a good correlation of the results between the two methods [95]. In this study a wheat-, barley-, and rye-specific WBR11/WBR13 primer pair was used. These primers were also used in a quantitative competitive PCR system to detect gluten traces in flours and “gluten-free” bakery products [96]. Píknova and colleagues achieved detection limits of 200 mg/kg of wheat in flour using real-time PCR [97]. Henterich and colleagues performed a rt-iPCR (real-time immunopolymerase chain reaction (iPCR)) for the detection of the cereal protein gluten, gliadin. By using iPCR a detection limit of 16 mg gliadin/100 g food was achieved [98].

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry allows the detection of large proteins from highly complex protein mixtures such as those present in gluten prolamins [99]. The high resolution and sensitivity of this technique allow the elucidation of protonated molecular masses of most of the gliadin, hordein, secalin, and avenin components. Méndez and colleagues [100] concluded that the analysis of gliadin-containing foods by this technique allows the immediate identification of the characteristic gliadin mass pattern, consequently permitting easy identification of gliadins in such samples with a detection sensitivity of 50–100 ng total protein loaded. In another study a nonimmunological

alternative to quantify gluten gliadins in food samples was presented [101]. The procedure allowed the microquantification of gluten in processed and unprocessed gluten-containing food samples below toxic levels for CD patients,

with a linear response in the 0.4–10 mg per 100 g range and a detection sensitivity similar to that of ELISA systems. A new protocol for determining small amounts of gliadins in foods that contain relatively large amounts of other prolamin proteins from maize and/or rice was also described [102]. This strategy combines a two-step procedure of extraction (60% aqueous ethanol followed by 1 M acetic acid) with subsequent MALDI-TOF analysis to corroborate the presence of these ethanol-soluble wheat prolamin fractions. HPLC allows the separation and qualitative and quantitative determination of compounds of analytical interest. A widely used HPLC technique is reversed-phase high performance liquid chromatography (RP-HPLC). In reversed-phase systems, stationary phase is slightly polar or nonpolar, while mobile phase has stronger polarity. An RP-HPLC system has already been described for the separation and quantitative determination of wheat prolamins in food [103, 104]. Also a flow cytometry (FCM) method for the quantitative determination of picogram levels of gliadin was developed [105]. FCM is a high-throughput technique that is able to analyze large numbers of cells individually using light-scattering and fluorescence measurements [106]. In the work by Capparello and colleagues [105], rat antibodies against a 16-residue peptide of gliadin, common to the α , β , γ , and ω -gliadins, were used. A detection limit under 10 pg/mL was achieved.

Despite the efforts in developing new analytical strategies for gluten control in foodstuffs, the most used method of measurement of gliadin still relies on the ELISA-like techniques. Thus, two commercial immunoassays are currently available to assess gluten content of gluten-free foods. The Association of Analytical Communities endorses the method originally developed by Skerritt and Hill [107], the sandwich ω -gliadin ELISA, which uses a monoclonal antibody to the heat-stable ω -gliadin fraction. Since the ω -gliadin fraction is not denatured when heated for cooking or processing, this assay can be used to assess gluten content of foods containing both native and heated protein. One major drawback of this assay is that measurements of this subfraction with the extrapolation to total gliadin have theoretical errors of –44 to +80% [108]. Moreover it is unable to accurately detect and quantify barley prolamins and cannot accurately quantify hydrolyzed gluten [109].

The other test is the R5 ELISA [110] that has been proposed as the standard method for gluten analysis in gluten-free foods by the Codex Committee on Methods of Analysis and Sampling [111], promoted by the Codex Committee on Nutrition and Foods for Special Dietary Uses [112]. This test uses the R5 monoclonal antibody

directed to the potentially celiac toxic epitope QQPFP (glutamine-glutamine-proline-phenylalanine-proline) present

in wheat, rye, and barley prolamins. This immunoassay is able to quantify native and heated gluten although it seems to overestimate barley hordein [113] and to be unable to accurately quantify hydrolyzed gluten [109].

Yet, the most commonly used biosensor strategy relies on electrochemical sensors. New electrochemical immunosensors (EIs), which employ cost-effective, user-friendly, and

New strategies for celiac disease diagnosis and gluten-free food analytical control

Currently, biosensor development is widespread in many fields and a considerable effort is being focused on the development of even more rapid, sensitive, high sample throughput, and, especially, on-site analytical strategies that can be applied in point-of-care analysis. Two kinds of sensors are found in the literature: optical and electrochemical.

Optical biosensors

De Stefano and colleagues [114] proposed the development of a porous silicon-based (PSi) optical biosensor for the detection of trace amounts of gliadin using a recombinant glutamine-binding protein (GlnBP) from *Escherichia coli* as a molecular probe. The solutions containing the molecular probe and the analyte, peptic-tryptic (PT)- gliadin, were directly spotted on the sensor surface. GlnBP was covalently linked to the surface of the PSi surface via a functionalization process. The proposed optical protein microsensor with a PSi-based transducer sensor allows a sensitive, fast, and easily readable optical response; moreover, it is able to work under reducing conditions, which solves some problems related to prolamins extraction. The results showed that about 45% of the spotted proteins had selectively bound the respective peptide. A fiber-optic biosensor for the detection of anti-gliadin antibodies was also developed [115]. This biosensor was developed by coating a tapered optical fiber by immobilization of gliadin using the electrostatic self-assembly (ESA) method which allows the construction of nanometric-scale recognition surfaces on the fiber-optic, allowing real-time monitoring of the sensor behavior. Gliadin antigens were successfully immobilized onto the surfaces of tapered optical fibers using the ESA method which has been proved to be an efficient immobilization strategy. The biosensors were tested by using antibodies conjugated with and without peroxidase. A high sensitivity sensor was obtained, with fast response times as compared with standard ELISA tests.

Electrochemical immunosensors

highly sensitive analytical transduction devices, have appeared as new exciting alternatives to the conventional immunochemical tests which are based on indirect detection compromising real-time analysis. Electrochemical immunosensors are a self-contained integrated device that is capable of providing specific quantitative or semiquantitative analytical information using an immobilized immunological recognition element (for detecting a target analyte by structural complementarity) and an electrochemical based-transducer which converts the biological interaction into a measurable signal [116].

Recently, two EIs for the detection of celiac disease toxic gliadin in foodstuffs were reported. Nassef and colleagues [117] developed an electrochemical immunosensing strategy for the detection of toxic gliadin using an antibody, coined CDC5, which was raised against the putative immunodominant celiac disease toxic epitope of α -gliadin, 56–75. For anchoring the captured antibody, two different surfaces, based on a gold electrode modified with acidic self-assembled monolayers (SAMs), were proposed. A good performance regarding sensitivity, specificity, and reproducibility was obtained. When applied to real sample analysis an excellent performance correlation was achieved when compared with ELISA as well as considerable decrease in the time to perform the assay. In order to improve sensitivity, the use of antibody fragments instead of whole antibodies was also exploited. A new electrochemical immunosensor based on the spontaneous adsorption of anti-gliadin Fab fragments (CDC5-Fab) on gold surfaces was developed [118]. CDC5-Fab forms a stable monolayer on gold after 15 min, which has long-term stability (2 months), when stored at 4 °C with more than 90% of antigen recognition ability. By using amperometry to evaluate the ability of Fab-modified electrodes to detect gliadin a limit of detection of 3.29 ng/mL was achieved. This Fab immunosensor has been shown to be highly sensitive, rapid, and simple and to have a short assay time.

Regarding clinical diagnosis of CD using EIs, some advances are also being achieved. Balkenhohl and Lisdat developed impedimetric immunosensors for the detection of antibodies directed against gliadin [119] and for the detection of autoantibodies against transglutaminase [120] in human serum. The immunosensors were based on the immobilization of gliadin and transglutaminase onto disposable screen-printed gold electrodes which were covered with a polyelectrolyte layer of poly(sodium 4-styrenesulfonic acid). Although the results suggest a lower precision, as compared with ELISAs, an acceptable sensitivity was achieved, which makes the developed sensors reliable

and promising methodologies for the analysis of anti-transglutaminase and anti-gliadin antibodies in human serum. On the other hand, Pividori and colleagues

proposed an amperometric electrochemical immunosensor based on the physical adsorption of tTG from guinea pig liver onto graphite–epoxy composite (GEC) electrodes [121]. For 10 positive and 10 negative processed serum samples a sensitivity of 70% and a specificity of 100% were achieved, as compared with the commercial ELISA method. The developed sensor appears as a promising alternative to the conventional ELISA assays, as it is a simple, low cost, and point-of-care analytical method. The authors also proposed the evaluation of the benefits of transferring the developed methodology to disposable screen-printed electrodes.

Although the immunosensor technology seems promising, some limitations still remain, such as long-term stability, surface effects, and interferences resulting from complex sample matrices.

Conclusions

The knowledge of celiac disease has grown in the last two decades and there has been a sharp increase in the number of newly diagnosed individuals. The different clinical presentations of CD can complicate the diagnosis and, therefore, delay the treatment of the disease. The advances in the efficacy of serological antibody testing potentiate the possibility of future accurate screening programmes in the community, working as a first-line method to clarify clinically suspicious cases in an underdiagnosed stage and also to manage the follow-up of this multifactorial disease. Patients with a low to moderate probability of presenting the disease should be submitted to blood studies rather than to small bowel biopsy. Several commercially available enzyme-linked immunoassorbent assay kits can be employed in CD screening. Among them, those using the serological markers IgA anti-tTG antibodies present the highest diagnostic accuracy. Although the overall diagnostic performance of the tests is good and similar for the different assays, greater standardization is required.

After a positive diagnosis, the implementation of a gluten-free diet is the only treatment available for CD. Two immunological methods for gluten food analysis are commercially available, and both use monoclonal antibodies toward gluten proteins. Further studies are necessary to develop an analytical method that can discriminate and quantify the celiac-toxic polypeptides in food ingredients and processed foods.

At the moment, ELISA assays are the recommended approach to the diagnosis of CD. Regarding gluten assessment in food products, ELISA R5 was provisionally endorsed by Codex Alimentarius, although there is not a

standard methodology that receives universal agreement. There are several problems regarding the pretreatment of

the food products which starts immediately with the extraction process.

The need for a standardized methodology to perform an unequivocal clinical diagnosis of CD as well as to determine quantitatively the gluten content in food products with the gluten-free label still remains.

Future perspectives

Possible areas of future study may be directed toward new immunosensing strategies that combine the high specificity of traditional immunochemical methods with miniaturized systems that allow development of a point-of-care test for CD clinical diagnosis and gluten-free food quality control. Nevertheless, the reference methods need to present better international agreements, in order to achieve a higher standardization for the different immunoassays.

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