Celiac disease diagnosis and gluten-free food analytical control

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Abstract

Celiac disease (CD) is an autoimmune entero- pathy, 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 com- ponents. 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 immunosensores
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 herpeti- forms, 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 gastrointesti- nal 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 lympho- cytes (IELs) and positive serology for endomysial anti- bodies (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 electropho- resis 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 pathogenis 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 proinflamma- tory 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.

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, Hepatol-ogy 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 symp-toms 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 autoim- mune diseases; first- and second-degree relatives of celiac patients are also at risk.

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

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].

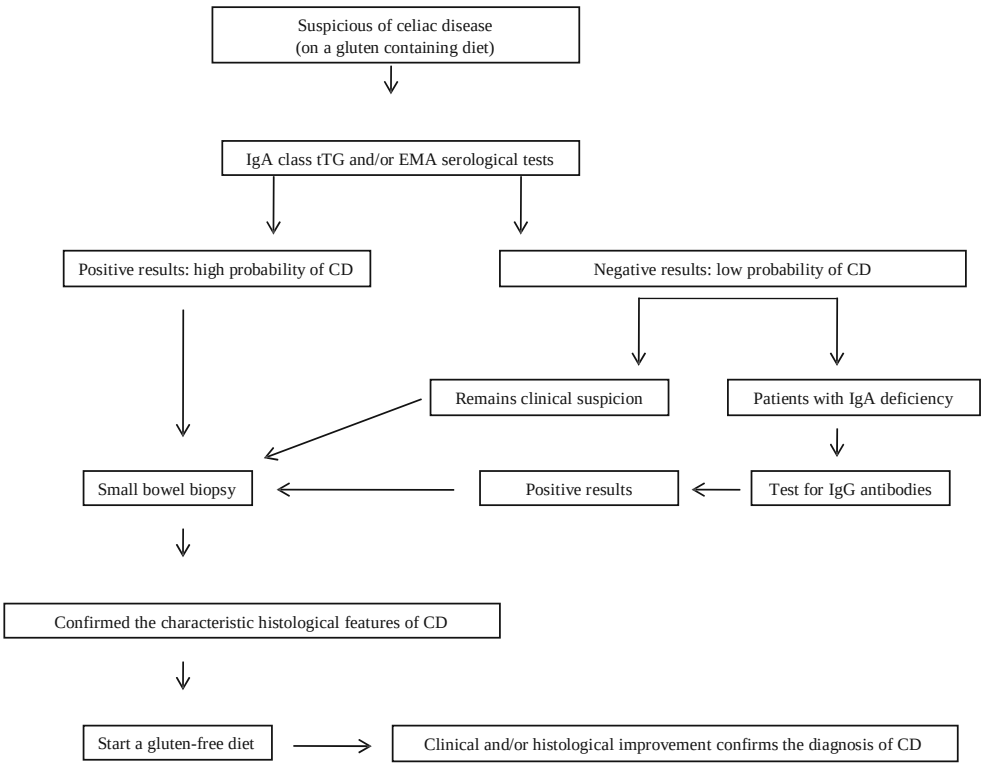
The serologic tests use highly specific antibody–antigen interactions and are fundamental to identify the gluten intolerance and to monitor the response of the CD patients to a gluten-free diet [45]. There are two types of serologic tests. One detects the antibodies against the antigen gliadin (i.e., anti-gliadin antibodies (AGA)), in which the immu- noglobulin 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 extracellulary, 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 for 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 sensitiv- ities (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 sensitiv- ity 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 Revise Standard (2000) are 20 ppm for foodstuffs naturally gluten-free and

200 ppm for foodstuffs rendered gluten-free [86]. Never- theless, 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 quanti- tative 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]. Piknova 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 immuno-polymerase 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 microquantifi- cation 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 Capparrelli 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 under10 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 measure- ments 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-phenylalaline-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].

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 prolamin 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 recog- nition 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

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

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 semiquan- titative analytical information using an immobilized immu- nological 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 immu- nosensor 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 promis- ing, 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 immunoasorbent 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 per- formance 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 anti- bodies 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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References

1. Holtmeier W, Caspary WF (2006) Celiac disease. Orphanet J Rare Dis 1:1–3
2. Goddard CJR, Gillett HR (2006) Complications of celiac disease: are all patients are at risk? Postgrad Med J 82 (973):705–712
3. Abdulkarim AS, Murray JA (2003) Review article: the diagnosis of coeliac disease. Aliment Pharmacol Ther 17(8):987–995
4. Briani C, Samaroo D, Alaedini A (2008) Celiac disease: from gluten to autoimmunity. Autoimmun Rev 7(8):644–650
5. Ascher H, Krantz I, Kristiansson B (1991) Increasing incidence of coeliac disease in Sweden. Arch Dis Child 66(5):608–611
6. Catassi C, Fabiani E, Rätsch IM, Coppa GV, Giorgi PL, Pierdomenico R, Alessandrini S, Iwanejko G, Domenici R, Mei E, Miano A, Marani M, Bottaro G, Spina M, Dotti M, Montanelli A, Barbato M, Viola F, Lazzari R, Vallini M, Guariso G, Plebani M, Cataldo F, Traverso G, Ventura A et al (1996) The coeliac iceberg in Italy. A multicentre antigliadin antibodies screening for coeliac disease in school-age subjects. Acta Paediatr Suppl 412:29–35
7. Kolho KL, Färkkilä MA, Savilahti E (1998) Undiagnosed coeliac disease is common in Finnish adults. Scand J Gastro- enterol 33:1280–1283
8. van Heel DA, West J (2006) Recent advances in coeliac disease. Gut 55(7):1037–1046
9. Freeman HJ (2008) Adult celiac disease in the elderly. World J Gastroenterol 14(45):6911–6914
10. Leeds JS, Hopper AD, Sanders DS (2008) Coeliac disease. Br Med Bull 88(1):157–170
11. Plot L, Amital H (2009) Infectious associations of celiac disease. Autoimmun Rev 8(4):316–319
12. Torres MI, López Casado MA, Ríos A (2007) New aspects in celiac disease. World J Gastroenterol 13(8):1156–1161
13. AGA (2001) American Gastroenterological Association medical position statement: celiac sprue. Gastroenterology 120(6):1522– 1525
14. Ciclitira PJ, King AL, Fraser JS (2001) AGA technical review on celiac sprue. American Gastroenterological Association. Gastro-enterology 120(6):1526–1540
15. Fasano A, Catassi C (2001) Current approaches to diagnosis and treatment of celiac disease: an evolving sprectrum. Gastroenter- ology 120(3):636–651
16. Farrell RJ, Kelly CP N (2002) Celiac sprue. N Engl J Med 346 (3):180–188
17. Green PHR, Stavropoulos SN, Panagi SG, Goldstein SL, Mcmahon DJ, Absan H, Neugut AI (2001) Characteristics of adult celiac disease in the USA: results of a national survey. Am J Gastroenterol 96(1):126–131
18. Hill PG, McMillan SA (2006) Anti-tissue transglutaminase antibodies and their role in the investigation of coeliac disease. Ann Clin Biochem 43(Pt2):105–117
19. Bottaro G, Cataldo F, Rotolo N, Spina M, Corazza GR (1999) The clinical pattern of subclinical/silent celiac disease: an analysis on 1026 consecutive cases. Am J Gastroenterol 94 (3):691–696
20. Mäki M, Holm K, Koskimies S, Hällström O, Visakorpi JK (1990) Normal small bowel biopsy followed by coeliac disease. Arch Dis Child 65(10):1137–1141
21. Collin P, Kaukinen K, Mäki M (1999) Clinical features of celiac disease today. Dig Dis 17(2):100–106
22. Holmes GK (2001) Potential and latent coeliac disease. Eur J Gastroenterol Hepatol 13(9):1057–1060
23. Mäki M, Holm K, Collin P, Savilahti E (1991) Increase in gamma/ delta T cell receptor bearing lymphocytes in normal small bowel mucosa in latent coeliac disease. Gut 32(11):1412–1414
24. Kim CY, Quarsten H, Bergseng E, Khosla C, Sollid LM (2004) Structural basis for HLA-DQ2-mediated presentation of gluten epitopes in celiac disease. Proc Natl Acad Sci USA 101 (12):4175–4179
25. Louka AS, Sollid LM (2003) HLA in coeliac disease: unravel- ling the complex genetics of a complex disorder. Tissue Antigens 61(2):105–117
26. Schuppan D, Dennis MD, Kelly CP (2005) Celiac disease: epidemiology, pathogenesis, diagnosis, and nutritional manage- ment. Nutr Clin Care 8(2):54–69
27. van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M, Wapenaar MC, Barnardo MC, Bethel G, Holmes GK, Feighery C, Jewell D, Kelleher D, Kumar P, Travis S, Walters JR, Sanders DS, Howdle P, Swift J, Playford RJ, McLaren WM, Mearin ML, Mulder CJ, McManus R, McGinnis R, Cardon LR, Deloukas P, Wijmenga C (2007) A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. Nat Genet 39(7):827–829
28. McGough N, Cummings JH (2005) Coeliac disease: a diverse clinical syndrome caused by intolerance of wheat, barley and rye. Proc Nutr Soc 64(4):434–450
29. Green PH, Cellier C (2007) Celiac disease. N Engl J Med 357 (17):1731–1743
30. Molberg Ø, Solheim FN, Jensen T, Lundin KE, Arentz-Hansen H, Anderson OD, Kjersti UA, Sollid LM (2003) Intestinal T-cell responses to high-molecular-weight glutenins in celiac disease. Gastroenterology 125(2):337–344
31. Koning F, Gilissen L, Wijmenga C (2005) Gluten: a two-edged sword. Immunopathogenesis of celiac disease. Springer Semin Immunopathol 27(2):217–232
32. van de Kamer JH, Weijers HA, Dicke WK (1953) Coeliac disease. IV. An investigation into the injurious constituents of wheat in connection with their action on patients with coeliac disease. Acta Paediatr 42(3):223–231

33. Vader LW, De Ru A, van der Wal Y, Kooy YM, Benckhuijsen W, Mearin ML, Drijfhout JW, van Veelen P, Koning F (2002) Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. J Exp Med 195(5):643–649
34. Stepniak D, Koning F (2006) Celiac disease—sandwiched between innate and adaptive immunity. Hum Immunol 67 (6):460–468
35. Skovbjerg H, Hansen GH, Niels-Christiansen LL, Anthonsen D, Ascher H, Midhagen G, Hallert C, Norén O, Sjöström H (2004) Intestinal tissue transglutaminase in coeliac disease of children and adults: ultrastructural localization and variation in expression. Scand J Gastroenterol 39(12):1219–1227
36. Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM (1993) Gliadin-specific, HLA-DQ (alpha 1*0501, beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. J Exp Med 178 (1):187–196
37. Mowat AM (2003) Coeliac disease—a meeting point for genetics, immunology, and protein chemistry. Lancet 361 (9365):1290–1292
38. Shan L, Molberg Ø, Parrot I, Hausch F, Filiz F, Gray GM, Sollid LM, Khosla C (2002) Structural basis for gluten intolerance in celiac sprue. Science 297(5590):2275–2279
39. Revised criteria for diagnosis of celiac disease (1990) Report of working group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child 65(8):909–911
40. Goldstein NS (2004) Non-gluten sensitivity-related small bowel villous flattening with increased intraepithelial lymphocytes: not all that flattens is celiac sprue. Am J Clin Pathol 121(4):546–550
41. Kaukinen K, Mäki M, Partanen J, Sievänen H, Collin P (2001) Celiac disease without villous atrophy: revision of criteria called for. Dig Dis Sci 46(4):879–887
42. Paparo F, Petrone E, Tosco A, Maglio M, Borrelli M, Salvati VM, Miele E, Greco L, Auricchio S, Troncone R (2005) Clinical, HLA, and small bowel immunohistochemical features of children with positive serum antiendomysium antibodies and architecturally normal small intestinal mucosa. Am J Gastroenterol 100(10):2294–2298
43. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, Hoffenberg EJ, Horvath K, Murray JA, Pivor M, Seidman EG, Society NA, North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (2005) Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastro- enterol Nutr 40(1):1–19
44. Collin P, Mäki M, Kaukinen K (2004) Complete small intestine mucosal recovery is obtainable in the treatment of celiac disease. Gastrointest Endosc 59(1):158–159
45. Kaukinen K, Collin P, Laurila K, Kaartinen T, Partanen MM (2007) Resurrection of gliadin antibodies in coeliac disease. Deamidated gliadin peptide antibody test provides additional diagnostic benefit. Scand J Gastroenterol 42(12):1428–1433
46. Troncone R, Ferguson A (1991) Anti-gliadin antibodies. J Pediatr Gastroenterol Nutr 12(2):150–158
47. Dahlbom I, Olsson M, Forooz NK, Sjöholm AG, Truedsson L, Hansson T (2005) Immunoglobulin G (IgG) anti-tissue transglutaminase antibodies used as markers for IgA-deficient celiac disease patients. Clin Diagn Lab Immunol 12(2):254–258
48. Cataldo F, Marino V, Bottaro G, Greco P, Ventura A (1997) Celiac disease and selective immunoglobulin A deficiency. J Pediatric 131(2):306–308
49. Chorzelski TP, Beutner EH, Sulej J, Tchorzewska H, Jablonska S, Kumar V, Kapuscinska A (1984) IgA anti-endomysium antibody. A new immunological marker of dermatitis herpeti- formis and coeliac disease. Br J Dermatol 111(4):395–402

50. Kárpáti S, Bürgin-Wolff A, Krieg T, Meurer M, Stolz W, Braun-Falco O (1990) Binding to human jejunum of serum IgA antibody from children with celiac disease. Lancet 336 (8727):1335–1338
51. Ladinser B, Rossipal E, Pittschieler K (1994) Endomysium antibodies in coeliac disease: an improved method. Gut 35 (6):776–778
52. Volta U, Molinaro N, De Franceschi L, Fratangelo D, Bianchi FB (1995) IgA anti-endomysial antibodies on human unbilical cord tissue for celiac disease screening. Dig Dis Sci 40(9):1902–1905
53. Lorand L, Graham RM (2003) Transglutaminases: crosslinking enzymes with pleiotropic functions. Nat Rev Mol Cell Biol 4 (2):140–156
54. Sollid LM, Molberg Ø, McAdam S, Lundin KE (1997) Autoantibodies in coeliac disease: tissue transglutaminase—guilt by association? Gut 41(6):851–852
55. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D (1997) Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 3(7):797–801
56. Alaedini A, Green PH (2005) Narrative review: celiac disease: understanding a complex autoimmune disorder. Ann Intern Med 142(4):289–298
57. Rostom A, Murray JA, Kagnoff MF (2006) American Gastro-enterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology 131(6):1981–2002
58. Schuppan D (2000) Current concepts of celiac disease patho- genesis. Gastroenterology 119(1):234–242
59. Sulkanen S, Halttunen T, Laurila K, Kolho KL, Korponay-Szabó IR, Sarnesto A, Savilahti E, Collin P, Mäki M (1998) Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology 115(6):1322– 1328
60. Fasano A (1999) Tissue transglutaminase: the holy grail for the diagnosis of celiac disease, at last? J Pediatr 134(2):134–135
61. Sárdy M, Odenthal U, Kárpáti S, Paulsson M, Smyth N (1999) Recombinant human tissue transglutaminase ELISA for the diagnosis of gluten-sensitive enteropathy. Clin Chem 45 (12):2142–2149
62. Troncone R, Maurano F, Rossi M, Micillo M, Greco L, Auricchio R, Salerno G, Salvatore F, Sacchetti L (1999) IgA antibodies to tissue transglutaminase: an effective diagnostic test for celiac disease. J Pediatr 134(2):166–171
63. Sblattero D, Berti I, Trevisiol C, Marzari R, Tommasini A, Bradbury A, Fasano A, Ventura A, Not T (2000) Human recombinant tissue transglutaminase ELISA: an innovative diagnostic assay for celiac disease. Am J Gastroenterol 95(5):1253–1257
64. Sugai E, Selvaggio G, Vazquez H, Viola M, Mazure R, Pizarro B, Smecuol E, Flores D, Pedreira S, Mauriño E, Gomez JC, Bai JC (2000) Tissue transglutaminase antibodies in celiac disease: assessment of a commercial kit. Am J Gastroenterol 95(9):2318– 2322
65. Fabiani E, Catassi C, International working group (2001) The serum IgA class anti-tissue transglutaminase antibodies in the diagnosis and follow up of coeliac disease. Results of an international multi-centre study. International Working Group on Eu-tTG. Eur J Gastroenterol Hepatol 13(6):659–665
66. Carroccio A, Vitale G, Di Prima L, Chifari N, Napoli S, La Russa C, Gulotta G, Averna MR, Montalto G, Mansueto S, Notarbartolo A (2002) Comparison of anti-transglutaminase ELISAs and an anti- endomysial antibody assay in the diagnosis of celiac disease: a prospective study. Clin Chem 48(9):1546–1550
67. Hansson T, Dahlbom I, Rogberg S, Dannaeus A, Hopfl P, Gut H, Kraaz W, Klareskog L (2002) Recombinant human tissue transglutaminase for diagnosis and follow-up of childhood coeliac disease. Pediatr Res 51(6):700–705

68. Martini S, Mengozzi G, Aimo G, Giorda L, Pagni R, Guidetti CS (2002) Comparative evaluation of serologic tests for celiac disease diagnosis and follow-up. Clin Chem 48(6 Pt 1):960–963
69. Wolters V, Vooijs-Moulaert AF, Burger H, Brooimans R, De Schryver J, Rijkers G, Houwen R (2002) Human tissue transglutaminase enzyme linked immunosorbent assay outperforms both the guinea pig based tissue transglutaminase assay and anti-endomysium antibodies when screening for coeliac disease. Eur J Pediatr 161(5):284–287
70. Johnston SD, McMillan SA, Collins JS, Tham TC, McDougall NI, Murphy P (2003) A comparison of antibodies to tissue transglutaminase with conventional serological tests in the diagnosis of coeliac disease. Eur J Gastroenterol Hepatol 15(9):1001–1004
71. Korponay-Szabó IR, Dahlbom I, Laurila K, Koskinen S, Woolley N, Partanen J, Kovács JB, Mäki M, Hansson T (2003) Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. Gut 52(11):1567–1571
72. Tesei N, Sugai E, Vázquez H, Smecuol E, Niveloni S, Mazure R, Moreno ML, Gomez JC, Mauriño E, Bai JC (2003) Antibodies to human recombinant tissue transglutaminase may detect coeliac disease patients undiagnosed by endomysial antibodies. Aliment Pharmacol Ther 17(11):1415–1423
73. Hill PG, Forsyth JM, Semeraro D, Holmes GKT (2004) IgA antibodies to human tissue transglutaminase: audit of routine practice confirms high diagnostic accuracy. Scand J Gastroenterol 39(11):1078–1082
74. Lampasona V, Bazzigaluppi E, Barera G, Bonifacio E (1998) Tissue transglutaminase and combined screening for coeliac disease and type 1 diabetes-associated autoantibodies. Lancet 352(9135):1192–1193
75. Blackweel PJ, Hill PG, Holmes GKT (2002) Autoantibodies to human tissue transglutaminase: superior predictors of coeliac disease. Scand J Gastroenterol 37(11):1282–1285
76. Perticarari S, Znidarcic C, Granzotto M, Parco S (2003) Human recombinant tissue-transglutaminase antibodies: evaluation of a commercial kit and comparison of methods using guinea pig antigen. Minerva Pediatr 55(3):277–282
77. Wong RCW, Wilson RJ, Steele RH, Radford-Smith G, Adelstein S (2002) A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits. J Clin Pathol 55(7):488–494
78. Villalta D, Crovato M, Stella S, Tonutti E, Tozzoli R, Bizzaro N (2005) False positive reactions for IgA and IgG anti-tissue transglutaminase antibodies in liver cirrhosis are common and method-dependent. Clin Chim Acta 356(1–2):102–109
79. Van Meensel B, Hiele M, Hoffman I, Vermeire S, Rutgeerts P, Geboes K, Bossuyt X (2004) Diagnostic accuracy of ten second-generation (human) tissue transglutaminase antibody assays in celiac disease. Clin Chem 50(11):2125–2135
80. NIH (2004) National Institutes of Health consensus development conference statement on celiac disease, 28–30 June, 2004. Gastroenterology 128(4 Suppl 1):S1–9
81. Wieser H (2007) Chemistry of gluten proteins. Food Microb 24 (2):115–119
82. Morón B, Bethune MT, Comino I, Manyani H, Ferragud M, López MC, Cebolla A, Khosla C, Sousa C (2008) Toward the assessment of food toxicity for celiac patients: characterization of monoclonal antibodies to a main immunogenic gluten peptide. PLoS ONE 3(5):e2294
83. Khosla C, Gray GM, Sollid LM (2005) Putative efficacy and dosage of prolyl endopeptidase for digesting and detoxifying gliadin peptides. Gastroenterology 129(4):1362–1363
84. Codex Alimentarius (2003) Draft revised standards for gluten-free foods, report of 25th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses, November 2003

85. Hischenhuber C, Crevel R, Jarry B, Mäki M, Moneret-Vautrin DA, Romano A, Troncone R, Ward R (2006) Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. Aliment Pharmacol Ther 23(5):559–575
86. Bermudo Redondo MC, Griffin PB, Garzon Ransanz M, Ellis HJ, Ciclitira PJ, O'Sullivan CK (2005) Monoclonal antibody-based competitive assay for the sensitive detection of coeliac disease toxic prolamins. Anal Chim Acta 551(1–2):105–114
87. Denery-Papini S, Samson MF, Autran JC (2000) Antipeptide antibodies directed against omega-gliadins for the detection of sequences from bread and durum wheats. Food Agric Immunol 12(1):67–75
88. Nicolas Y, Denery-Papini S, Martinant JP, Popineau Y (2000) Suitability of a competitive ELISA using anti-peptide antibodies for determination of the gliadin content of wheat flour: comparison with biochemical methods. Food Agric Immunol 12(1):53–65
89. Horwitz W (2000) Official method 991.19. Official methods of analysis of AOAC International, 17th edn. AOAC, Gaithersburg, p 15
90. Meyer J, Karst U (2001) Enzyme-linked immunosorbent assays based on peroxidase labels and enzyme-amplified lanthanide luminescence detection. Analyst 126(2):175–178
91. Rumbo M, Chirdo FG, Fossati CA, Añón MC (2001) Analysis of the effects of heat treatment on gliadin immunochemical quantification using a panel of anti-prolamin antibodies. J Agric Food Chem 49(12):5719–5726
92. Prabhasankar P, Sai-Manohar R (2002) Development of enzyme- linked immunosorbent assay for evaluation of chapati-making quality of wheat varieties. J Agric Food Chem 50(25):7455–7460
93. Allmann M, Candrian U, Hofelein C, Luthy J (1993) Polymerase chain-reaction (PCR)—a possible alternative to immunochemical methods assuring safety and quality of food - detection of wheat contamination in non-wheat food-products. Z Lebensm Unters Forsch 196(3):248–251
94. Köppel E, Stadler M, Luthy J, Hubner P (1998) Detection of wheat contamination in oats by polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). Z Lebensm Unters Forsch 206(6):399–403
95. Dahinden I, von Büren M, Lüthy J (2001) A quantitative competitive PCR system to detect contamination of wheat, barley or rye in gluten-free food for coeliac patients. Eur Food Res Technol 212(2):228–233
96. Olexová L, Dovicovicová L, Svec M, Siekel P, Kuchta T (2006) Detection of gluten-containing cereals in flours and “gluten-free” bakery products by polymerase chain reaction. Food Control 17 (3):234–237
97. Piknova L, Brezna B, Kuchta T (2008) Detection of gluten-containing cereals in food by 5’-nuclease real-time polymerase chain reaction. J Food Nutr Res 47(3):114–119
98. Henterich N, Osman AA, Méndez E, Mothes T (2003) Assay of gliadin by real-time immunopolymerase chain reaction. Nahrung 47(5):345–348
99. Méndez E, Valdés I, Camafeita E (2000) Methods in molecular biology. Chapman, New Jersey
100. Méndez E, Camafeita E, SanSebastian J, Valle I, Solis J, MayerPosner FJ, Suckau D, Marfisi C, Soriano F (1995) Direct identification of wheat gliadins and related cereal prolamins by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. J Mass Spectrom S123-S128
101. Camafeita E, Solis J, Alfonso P, Lopez JA, Sorell L, Mendez E (1993) Selective identification by matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry of different types of gluten in foods made with cereal mixtures. Z Lebensm Unters Forsch 196(3):248–251
102. Hernando A, Valdes I, Méndez H (2003) New strategy for the determination of gliadins in maize- or rice-based foods matrix-

assisted laser desorption/ionization time-of-flight mass spectrome- try: fractionation of gliadins from maize or rice prolamins by acidic treatment. J Mass Spectrom 38(8):862–871
103. Nicolas Y, Martinant JP, Denery-Papini S, Popineau Y (1998) Analysis of wheat storage proteins by exhaustive sequential extraction followed by RP-HPLC and nitrogen determination. J Sci Food Agric 77(1):96–102
104. Wieser H, Seilmeier W, Belitz HD (1994) Quantitative-determination of gliadin subgroups from different wheat cultivars. J Cereal Sci 19(2):149–155
105. Capparelli R, Ventimiglia I, Longobardo L, Iannelli D (2005) Quantification of gliadin levels to the picogram level by flow cytometry. Cytometry A 63A(2):108–113
106. Comas-Riu J, Rius N (2009) Flow cytometry applications in the food industry. J Ind Microbiol Biotech 36(8):999–1011
107. Skerritt J, Hill A (1991) Enzyme immunoassay for determination of gluten in foods: collaborative study. J Assoc Anal Chem 74 (2):257–264
108. Wieser H, Seilmeier W, Belitz H-D (1994) Quantitative determination of gliadin subgroups from different wheat culti- vars. J Cereal Sci 19(3):149–155
109. Thompson T, Méndez E (2008) Commercial assays to assess gluten content of gluten-free foods: why they are not created equal. J Am Diet Assoc 108(10):1682–1687
110. Valdés I, Garcia E, Llorente M, Méndez E (2003) Innovative approach to low-level gluten determination in foods using a novel sandwich enzyme-linked immunosorbent assay protocol. Eur J Gastroenterol Hepatol 15(5):465–474
111. Codex Alimentarius Commission (2005) Status of endorsement of methods of analysis and sampling. Codex Alimentarius ALINORM 05/28/23. Appendix III, Rome, pp 31–40
112. Codex Alimentarius Commission (2004) Draft revised strandard for gluten-free foods. Codex Alimentarius ALINORM 04/27/26. Appendix III, Rome, pp 42-43
113. Kanerva PM, Sontag-Strohm TS (2006) Problems in detecting prolamins contaminants in oat-based foods by commercial ELISA kits. 9th international gluten workshop, September 2006; p 33. Available via American Association of Cereal Chemists. www. aaccnet.org/meetings/glutenworkshop/pdfs/programbook.p df. Accessed 2 Nov 2009
114. De Stefano L, Rossi M, Staiano M, Mamone G, Parracino A, Rotiroti L, Rendina I, Rossi M, D'Auria S (2006) Glutamine-binding protein from escherichia coli specifically binds a wheat gliadin peptide allowing the design of a new porous silicon-based optical biosensor. J Proteome Res 5:1241–1245
115. Corres JM, Matias IR, Bravo J, Arregui FJ (2008) Tapered optical fiber biosensor for the detection of anti-gliadin anti- bodies. Sens Actuators B Chem 135:166–171
116. Rodríguez-Moraz S, López de Alda MJ, Barceló D (2006) Biosensors as useful tools for environmental analysis and monitoring. Anal Bioanal Chem 386(4):1025–1041
117. Nassef HM, Bermudo Redondo MC, Ciclitira PJ, Ellis HJ, Fragoso A, O'Sullivan CK (2008) Electrochemical immunosen- sor for detection of celiac disease toxic gliadin in foodstuff. Anal Chem 80(23):9265–9271
118. Nassef HM, Civit L, Fragoso A, O'Sullivan CK (2009) Ampero-metric immunosensor for detection of celiac disease toxic gliadin based on fab fragments. Anal Chem 81(13):5299–5307
119. Balkenhohl T, Lisdat F (2007) An impedimetric immunosensors for the detection of autoantibodies directed against gliadins. Analyst 132(4):314–322
120. Balkenhohl T, Lisdat F (2007) Screen-printed electrodes as impedimetric immunosensors for the detection of anti-transglutaminase antibodies in human sera. Anal Chim Acta 597(1):50–57
121. Pividori MI, Lermo A, Bonanni A, Alegret S, del Valle M (2009) Electrochemical immunosensor for the diagnosis of celiac disease. Anal Biochem 388(2):229–234