Reactivity to dietary gluten: new insights into differential diagnosis among gluten-related gastrointestinal disorders

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ABSTRACT
The ingestion of dietary gluten sometimes may trigger allergic, autoimmune or nonallergic and nonautoimmune response. The typical gluten-related allergic disorder is the wheat allergy (WA). Celiac disease (CD) is a well-known gluten-related autoimmune condition. The clinical expression of a gluten-related nonallergic and nonautoimmune response is nonceliac gluten sensitivity (NCGS), an emerging condition whose framework is yet unclear and whose diagnosis is suggested only by demonstration of gluten-dependency in patient’s symptoms after exclusion of WA and CD. This review discusses the current tools to identify patients suffering from WA, CD, and NCGS, as well as the most recent insights in the differential diagnosis among these gluten-related gastrointestinal disorders.

Introduction
Wheat allergy (WA) and celiac disease (CD) are 2 distinct immunologically-mediated diseases associated with the ingestion of proteins from wheat and some related cereals. Both conditions usually recede after a gluten-free diet (GFD). Currently, the spectrum of gluten-related disorders includes also nonceliac gluten sensitivity (NCGS).1 This review discusses the current tools to identify patients suffering from WA, CD, and NCGS, as well as the most recent insights in differential diagnosis among these gluten-related gastrointestinal disorders.

Wheat allergy
In WA children, wheat ingestion elicits typical immunglobulin E (IgE)-mediated reactions of immediate onset, including urticaria, angioedema, bronchial obstruction, nausea, and abdominal pain, or systemic anaphylaxis in severe cases. Late manifestations appear about 24 h after wheat ingestion and include gastrointestinal symptoms and exacerbation of atopic dermatitis. In adults, allergy to ingested wheat seems to be infrequent, with a prevalence of about 0.1%, and may lead to exacerbation of atopic dermatitis or gastrointestinal symptoms. As the diagnostic performances of wheat-specific skin prick tests and in vitro IgE assays have been shown unsatisfactory, often the diagnosis of WA is almost exclusively based on the results of oral wheat challenges.2 On the other hand, to define the involvement of circulating basophils in allergic reactions, it has been recently proposed a flow cytometric test able to investigate the allergen-induced activations of basophilic granulocytes. The basophil activation test (BAT) evaluates the percentage of basophils expressing 1 or more activation markers (e.g., CD63 and CD203c) after in vitro whole blood stimulation, in order to address a hypersensitivity reaction to a specific allergen.3-5 However, BAT has still not been currently applied in studies including allergenic extracts from wheat or gluten.6,7

Celiac disease
CD is a gluten-related immunological disorder different from WA. It is a chronic inflammatory, autoimmune disorder, and one of the most common gastrointestinal and systemic diseases worldwide, with a prevalence of about 1%.8,9 This condition is triggered by the ingestion of gluten-containing foods in genetically susceptible individuals carrying HLA-DQ2 and/or -DQ8 alleles (90%-95% of CD patients).10 The gold standard for its diagnosis is still based on the finding of villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis on histological examination of duodenal biopsies. On the other hand,
the presence of only intraepithelial lymphocytes is often used, perhaps erroneously, to discriminate whether or not patients really present CD.11,12

Recent literature emphasizes that an adequate number of duodenal biopsies should always be taken in order to improve the accuracy of CD diagnosis,13 since the CD-specific histological lesions often may have a discontinuous distribution along the small bowel, showing a pattern called “patchy atrophy”. In newly diagnosed CD, some variability of histological lesions can even be found within the same duodenal biopsy, in which areas of apparently normal mucosa with increased intraepithelial lymphocyte (IEL) number often coexist with sites of villous atrophy.14-16

Nowadays, it is general opinion that serum antibodies play a supportive role in CD diagnosis. In fact, the presence of circulating autoantibodies against tissue transglutaminase (anti-tTG) and endomysium (EMA) is also necessary to make a proper diagnosis of CD.17 Present guidelines are generally in agreement as to which serum test(s) is best. Immunoglobulin A anti-tTG antibodies are recommended as the most reliable and cost-effective serum test for CD, even if an increase of their levels can also occur in other pathological conditions involving tissue damage, such as arthritic diseases, inflammatory bowel disease (IBD), and cardiovascular disorders.18-20 Recent studies even suggest that duodenal biopsies may not be necessary when anti-tTG serum levels are extremely high. In fact, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) recommends this option for individuals with typical symptoms of CD and serum anti-tTG >10 times the upper limit of normal (ULN) together with a positive serum EMA and presence of a HLA-DQ2 or -DQ8 allele.21 Although anti-tTG serum test has a specificity ranging between 90% and 95%, serum EMA are often required for diagnostic confirmation, because of their high specificity approaching 100%.22,23 However, the interpretation of EMA is operator-dependent and this can seriously affect the CD diagnosis if data are not contextualized. Anti-gliadin antibodies (AGA) are considered unreliable and no longer recommended for routine screening purposes even if a new enzyme immunoassay using deamidated gliadin peptides (DGP) as antigen has proved to be very sensitive and specific for CD diagnosis.24,25

Current guidelines recommend concomitant measurement of total IgA serum levels to identify selective IgA deficiency (SlgAD).26,27 In SlgAD patients, IgG-based tests (EMA, anti-tTG, and/or anti-DGP) have been suggested.28-30

However, in some cases, the diagnosis is not straightforward and represents a challenge for clinicians. Potential dilemmas include patients with positive serology but normal histology, negative serology but abnormal histology, failure to respond to a GFD or response to a GFD without evidence of CD. In recent years, development of new assays and modifications of diagnostic algorithms for CD have even called into question the crucial role traditionally played by duodenal histology.21

Nonceliac gluten sensitivity Recently, NCGS has been described as a distinct gluten-related clinical entity, with an estimated prevalence of 6%. It is characterized by gastrointestinal symptoms such as abdominal pain, bloating, bowel habit abnormalities (either diarrhea or constipation), and extraintestinal manifestations such as “foggy mind”, headache, fatigue, joint and muscle pain, leg or arm numbness, dermatitis (eczema or skin rash), depression, and anemia. All these manifestations usually occur soon after gluten ingestion, disappear with gluten withdrawal and relapse in short time following a gluten challenge, within hours or few days.

In clinical practice, it has become relevant to differentiate CD from NCGS, in light of the increasing prevalence of both these gluten-related disorders and their relevant dietary, medical, social, and economic implications.1 NCGS is lacking of sensitive and specific diagnostic tools, and its possible pathogenetic mechanisms are yet to be known. The absence of CD-specific serum antibodies and villous atrophy (with or without HLA-DQ2 and/or -DQ8 alleles) currently identifies NCGS, although first-generation AGA test frequently presents positive results.31 Anyway, the use of double-blinded placebo-controlled gluten challenge should be recommended in patients suspected to be affected by NCGS.

Though there is a clear cause-effect relationship between gluten ingestion and symptoms reported by NCGS patients, pathogenetic mechanisms are not yet known.

The hypothesis that, in gluten-sensitive patients, a favorable response to a GFD as well as the exacerbation of symptoms after a gluten-containing diet are due actually to gluten, could be a trap. Importance could be given to other wheat-related food constituents able to cause gastrointestinal symptoms. The reluctance to acknowledge other components of wheat, such as nongluten proteins, fructans, galacto-oligosaccharides, and other short-chain carbohydrates (fermentable oligo-, di-, monosaccharides and polyols; FODMAPs), as other potential pathogenetic factors often hamper the correct interpretation of clinical observations.32 Therefore, is it questionable whether these patients are recognizable as irritable bowel syndrome (IBS)-like patients?24 For many years, IBS and CD have been considered 2 completely separate entities: CD as a gluten-related condition and IBS without relation with gluten intake. The IBS and CD symptoms may be, however, indistinguishable, especially when diarrhea, bloating, or abdominal pain predominate. In the last decade, several studies have further shown that the boundary between CD and IBS is not always so clear. Consistently, some patients who have been identified as IBS, suffer actually from CD. In addition, it seems that there is another group of patients who, without having CD,
suffer from gluten intolerance causing them digestive symptoms similar to those of IBS. Since gluten sensitivity is defined as the spectrum of clinical and functional abnormalities that respond to a GFD, it is mandatory to establish in which patients this diet will be beneficial as well as when this is not justified.

In light of all these findings, it is evident the needs to make a correct diagnosis among gluten-related disorders to avoid unnecessary and repeated over time diagnostic tests. It is, therefore, necessary to use the most sensitive and specific diagnostic tests and strategies.

Organ culture system  In 1996, it has been demonstrated that EMA can be detected in culture media of duodenal biopsies from untreated CD patients, as well as from treated CD patients after in vitro exposure to peptic-tryptic digest of gliadin (PT-gliadin). Three years later, a first improvement in EMA determination has been achieved by using a synthetic peptide corresponding to 31–43 a.a. position of the α-gliadin (activator peptide), shown to be active at concentrations 20-fold lower than those necessary for the PT-gliadin. In 2001, the extension of the time of culture from 24 to 48 h, in concert with the replacement of the “on dish” standard method with a more easy and quick “in batch” new method, has led to a further improvement in EMA determination, as well as a substantial simplification of the entire organ culture system.

One year later, Carroccio et al. have started a series of clinical validation studies, highlighting the usefulness of the organ culture system in identifying CD also in seronegative patients. The slight differences in sensitivity among data achieved in the latter study and those obtained in previous investigations are attributable to indirect immunofluorescence analysis (IFA) used for EMA detection. In fact, this is a qualitative method leading to subjective interpretations. A multicenter study dated 2006 has shown a good correlation between the organ culture system and duodenal histology and, furthermore, has demonstrated that this new method can be useful to identify CD also in patients presenting a normal villous morphology. To further improve the performance of the organ culture system, this study has also pointed out that in vitro stimulation must be always performed, and that particular attention must be always paid to the biopsy sample size. In the same year, it has been demonstrated that measurement of anti-tTG in culture media increases the ability of the organ culture system to identify CD in seronegative patients. Since anti-tTG are measured by enzyme-linked immunosorbent assay, a quantitative and objective method, the use of these antibodies appears clearly helpful to standardize the organ culture system for diagnostic purposes. In 2008, Sántaolalla et al. have demonstrated that measurement of anti-tTG in culture media improves the ability of the organ culture system in identifying CD also in patients with an increased density of γ/δ+ IEL but normal villous morphol-

The use of the organ culture system has also allowed to demonstrate that the immune response to gluten involves not only the small bowel mucosa, but also that of other areas of the gastrointestinal tract. Specifically, EMA and anti-tTG antibodies can also be detected in culture supernatants of oral and colonic mucosa biopsy specimens, suggesting that these mucosal areas could be used as alternative and/or opportunistic sites in which adverse effects to gluten are reproducible.

Concluding remarks and future perspectives  Summarizing, WA is an IgE-mediated basophil degranulation that may be triggered by gluten fractions ingestion and other wheat proteins. Its diagnosis is suggested by wheat-specific skin prick tests and by in vitro IgE assays, while food challenge and BAT can be used as confirmatory test. CD is an autoimmune enteropathy triggered by wheat gluten ingestion and related prolamines in rye and barley. Its diagnosis is based on the presence of serum EMA, anti-tTG and anti-DGP antibodies, intestinal villous atrophy with increased IEL number. NCGS is a non-allergic and non-autoimmune disorder associated with gluten intake. Its diagnosis is suggested only by demonstration of gluten-dependency in patients’ symptoms after exclusion of WA and CD. Therefore, the need to make a correct diagnosis among these gluten-related disorders by using the most sensitive and specific diagnostic strategies and tests is evident. The organ culture system, has been recently proposed as an ancillary method to perform a correct CD diagnosis, mainly in cases without villous atrophy or in seronegative patients. The proofs that organ culture system sensitivity is higher than that of duodenal histology alone, confirm its inclusion among diagnostic procedures in differential diagnosis of gluten-related gastrointestinal disorders. Future studies aimed to confirm and extend this observations will be welcome.

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STRESzczenie
Spożycie pokarmów zawierających gluten może wywołać reakcje alergiczne, autoimmunologiczne lub niealergiczne i nieautoimmunologiczne. Typową alergiczną chorobą glutenozależną jest alergia na pszenicę (wheat allergy – WA). Dobrze znaną autoimmunologiczną chorobą glutenozależną jest celiakia (celiac disease – CD). Manifestacją kliniczną niealergicznej i nieautoimmunologicznej reakcji na gluten jest nieceliakalna nadwrażliwość na gluten (nonceliac gluten sensitivity – NCGS) – nowo wyodrębniony stan, którego patogeneza jest wciąż niejasna, a rozpoznanie sugeruje tylko wykazanie związku objawów z glutenem, po wykluczeniu WA i CD. W niniejszym przeglądzie omówiono dostępne metody rozpoznania WA, CD i NCGS, a także najnowsze poglądy na te maty diagnostyki różnicowej tych zaburzeń przewodu pokarmowego związanych z glutenem.