Lead Article

The 5 Ws of a gluten challenge for gluten-related disorders

Karla A. Bascuñán, Leda Roncoroni, Federica Branchi, Luisa Doneda, Alice Scricciolo, Francesca Ferretti, Magdalena Araya, and Luca Elli

Gluten-related disorders (GRDs) are gradually emerging as epidemiologically relevant diseases, with a global prevalence estimated to be approximately 5% in the population. Conditions related to gluten ingestion include celiac disease (CD), wheat allergy (WA), and nonceliac gluten sensitivity (NCGS). Although mediated by different pathogenic pathways, these 3 conditions share similar clinical manifestations and can present a difficult differential diagnosis. The gluten challenge (GC) is an important diagnostic tool for GRDs, but there is great variability in regards to deciding which patients should be challenged, what amount of gluten should be used, what the GC duration should be, when and where the GC should occur, and, sometimes, why to conduct a GC. This review summarizes the current knowledge about the desirable characteristics of GCs in the 3 main GRDs following a 5 Ws approach—that is, the 5 main journalistic questions: who, what, when, where, why. The answers will help to determine the correct use of the GC in diagnosing GRDs.

INTRODUCTION

Consumption of cereals has been crucial to the progress of humankind, with wheat still the most consumed type of grain worldwide.[1] Gluten is a mixture of seed storage proteins. Approximately 80% of proteins in wheat are contained in gluten, with equivalent fractions found in rye and barley. In spite of its relevance in the diets of different populations, gluten has been identified as an environmental factor that triggers various health disorders.[2] The prevalence and clinical relevance in daily practice of the wide spectrum of gluten-related disorders (GRDs) have greatly increased in the last 20 years.[3,4] Although the causes of these increases are largely unknown, the increases are mostly attributed to changes in dietary habits in many countries, especially those experiencing the progressive Westernization of the human diet, which includes consumption of refined cereals.[3,5,6] Gluten-related disorders are triggered by the oral ingestion of gluten and are classified according to their main pathological mechanism[7]: autoimmune, in celiac disease (CD), dermatitis herpetiformis, and gluten ataxia[8–10]; allergic, in wheat allergy (WA)[11]; and nonautoimmune/nonallergic in nonceliac gluten sensitivity (NCGS).[12,13]

Gluten is one of the most abundant and widely distributed food components, and it can be found in wheat, rye, barley, oats, bulgur, and hybrids of such grains, such as kamut and triticale.[14,15] Wheat, which is the world's primary source of food, providing up to 50% of the total caloric intake in both developed and developing countries, is also the main source of the

Affiliation: *K.A. Bascuñán* is with the Department of Nutrition, School of Medicine, University of Chile, Santiago, Chile. *L. Roncoroni, F. Branchi*, *A. Scricciolo, F. Ferretti*, and *L. Elli* are with the Center for Prevention and Diagnosis of Celiac Disease, Gastroenterology and Endoscopy Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Università degli Studi di Milano, Milan, Italy. *L. Roncoroni* and *L. Doneda* are with the Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milano, Italy. *F. Branchi* is with the Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy. *M. Araya* is with the Institute of Nutrition and Food Technology, INTA, University of Chile, Santiago, Chile.

Correspondence: *E. Luca*, Center for Prevention and Diagnosis of Celiac Disease, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via F. Sforza 28, 20122 Milan, Italy. E-mail dottorlucaelli@gmail.com.

Key words: celiac disease, gluten challenge, gluten-related disorders, nonceliac gluten sensitivity, wheat allergy.

doi: 10.1093/nutrit/nux068

dietary intake of gluten.[16] A gluten-free diet (GFD) is the main treatment for GRD,[17,18] but the actual number of people following some GFD is considerably larger than solely the population with GRDs because many people consider this type of diet healthy and follow it without any medical justification.[19] In the GRD scenario, the gluten challenge (GC) represents an important tool because it supports diagnosis and helps to identify potentially new biomarkers that can be used to stratify patients, especially in NCGS.[20]

This review focuses on the GC and its use in the nutritional and gastroenterological diagnostic flowcharts of GRDs, following a 5 Ws approach—that is, who, what, when, where, and why.

GLUTEN-RELATED GASTROINTESTINAL DISORDERS

Celiac disease

Celiac disease is an autoimmune condition triggered by gluten. It affects approximately 1.5% of the population.[21,22] It is characterized by an inappropriate T-cell–mediated response that causes inflammatory injury to the small bowel in genetically predisposed individuals who carry the haplotype human leukocyte antigen (HLA) DQ2 and/or DQ8.[23] The diagnosis of CD is based on the presence of serological autoantibodies (mainly antitissue transglutaminase and/or antiendomysium immunoglobulin A [IgA]) and typical changes (atrophy) in duodenal histology.[24,25] Recently, novel pediatric guidelines suggested the possibility of avoiding duodenal biopsy in children with both antitransglutaminase and antiendomysium positivity who carry the genetic susceptibility.[26] Treatment consists of a strict lifelong GFD with the withdrawal of all products, both natural and processed, containing gluten.[17]

Nonceliac gluten sensitivity

An increasing percentage of the general population avoids gluten ingestion to improve nonspecific intestinal and extraintestinal symptoms.[4] Nonceliac gluten sensitivity is considered a new clinical entity characterized by the appearance, after the ingestion of gluten-containing foods, of intestinal (abdominal distention, bloating, abdominal pain) and extraintestinal (fatigue, anxiety, depressive symptoms, and other) signs and symptoms in individuals with proved negative screening for CD and WA.[3,27] Nonceliac gluten sensitivity diagnosis is difficult because of the absence of biomarkers and is often uncertain, making the very existence of NCGS debated.[28] Complete or partial clinical remission after gluten withdrawal from the diet remains the starting point of the NCGS diagnostic roadmap.[29–31] Symptoms have been shown to disappear shortly after the start of a GFD and to relapse within hours or days after the dietary reintroduction of gluten.[32] Nonceliac gluten sensitivity diagnosis is based upon the exclusion of other compatible diseases (mainly CD and WA) and the GC result; the only treatment available to date is a GFD.[20] The clinical course, complications, and prognosis of NCGS are unknown, and how strict the GFD should be is also under discussion.[33]

Wheat allergy

Wheat allergy is an adverse immune reaction to wheat proteins. It is typically characterized by a T-helper type 2 (Th2) lymphocytic inflammation with predominant Th2 cytokines expression (interleukin 4, interleukin 13, interleukin 5). Th2 inflammation induces B cells to produce specific immunoglobulin E (IgE) antibodies or, in some cases, can lead to chronic cellular inflammation characterized by the presence of T cells and eosinophils, as in eosinophilic esophagitis or enteritis (sometimes associated with WA). These latter forms are not well understood, and the presence of non-IgE–mediated food allergy represents a challenging clinical issue.[11] In adults, WA can lead to serious reactions, including anaphylaxis and death.[34] In children, WA frequently occurs in patients with a multiple food allergy. In contrast with CD, which includes autoimmune phenomena, WA is IgE mediated and clinically characterized by itching; swelling of the mouth, eyes, nose, and trachea; skin rash; wheezing; gastrointestinal symptoms, such as bloating, cramps, diarrhea; and potentially life-threatening anaphylaxis.[35] Wheat allergy diagnosis is supported by skin prick and blood IgE tests and is sometimes based on a double-blind, placebo-controlled challenge. However, the interpretation of results may be difficult, and some confounding factors associated with the diagnostic techniques used should be taken into consideration.[2] Treatment of WA entails the complete avoidance of wheat. In parallel, educating patients, caregivers, and physicians about anaphylaxis and its treatment is an essential component of WA management.[11] A growing body of evidence shows that specific oral tolerance induction may be an option for the treatment of food allergy patients in the future.[36]

FOOD CHALLENGE

There are different types of oral food challenge: open, single-blind, double-blind, and placebo-controlled. Challenges are not standardized procedures, but, in general, they involve administration of increasing doses of the food under investigation at defined time intervals.[37]

Food challenge tests support the diagnosis of food allergy/hypersensitivity and/or can provide evidence of the disappearance of a food reaction that was previously

present. Furthermore, in case of an unclear clinical history, food challenges can verify the clinical relevance of serological IgE against specific allergenic food. They also raise the opportunity of educating patients and/or their caregivers on the characteristics of the clinical condition, alerting them about the symptoms that may be expected if any accidental ingestion of the specific food occurs and how to respond to it.[38,39]

The choice of which oral food challenge type to apply is not standardized and should be based on the specific patient's characteristics. In general, if a patient presents objective signs, an open oral food challenge can be executed, taking into account that this type of challenge presents the highest bias interference; on the other hand, a negative open oral food challenge reasonably excludes the presence of a specific food reaction. Moreover, open food challenges are simple and can be carried out in an office setting, which makes them a first-line choice.[40]

In case of subjective symptoms, a single- or double-blind food challenge is preferable to reduce bias. The use of a placebo control should be left to the physician's judgment; in such a case, it is extremely important to ensure that the placebo and the tested food are indistinguishable in taste, odor, and appearance. The double-blind, placebo-controlled food challenge remains the most rigorous test.[41]

In blind food challenges, the vehicles and the methodology used to mask food are very important. The use of capsules guarantees blindness by hiding odor, color, texture, and taste; however, this method may be limited by the use of dehydrated food, the reduction in the number of analyzable foods, the difficulty of swallowing capsules, especially in a pediatric setting; and, sometimes, the need to use a high number of capsules. Mixing the tested food with other ingredients to prepare muffins or bars can be an alternative, although blindness is not guaranteed and the use of other foods and/or additives can turn out to be a confounder.[37]

Gluten challenge type protocols can be different in case of CD, WA, or NCGS. In CD and WA, signs and biomarkers can be objectively evaluated, making an open challenge feasible in most cases. In NCGS, because the symptoms reported by the patient are subjective, a single- or double-blind, placebo-controlled GC is preferred. The GC should be conducted using bars with and bars without gluten (8 g), with each type of bar administered for 1 week with a 1-week washout between the 2 treatments. An attempt to standardize GCs is represented by the Salerno criteria.[20]

THE 5 Ws OF GLUTEN CHALLENGE

Gluten challenge is one option for diagnosing GRDs. However, GCs can be conducted in varying ways among countries and, within the same country, at different levels of medical service. In addition, the GFD requirements of each of the 3 conditions discussed herein may be different. In the following sections, the GC will be analyzed following the 5 Ws approach.

Who to challenge

Celiac disease. It is widely agreed that a food challenge should be considered for patients already on a GFD who carry the genetic susceptibility to CD (ie, the HLA DQ2 and/or DQ8) but did not undergo a complete and correct CD screening while on a gluten-containing diet.[2] The clinical response of such patients to GC should be carefully evaluated, and successively they should be prepared for serological tests (antitissue transglutaminase IgA) and duodenal biopsy if necessary.[42] Similarly, a GC should be used for those patients on a GFD but with uncertain diagnosis due to the absence of efficient biomarkers in the past or without a correct diagnostic algorithm. It has been demonstrated that CD misdiagnosis is potentially around 20%.[43]

Nonceliac gluten sensitivity. Although double- or single-blind, placebo-controlled GCs comprise the most widely accepted standard for diagnosing NCGS, there is a clear need for the standardization of a diagnostic protocol for the GC besides the Salerno criteria.[20,29,44,45] Theoretically, all nonallergic and nonceliac patients with functional gastrointestinal disorders are potentially affected by NCGS; as a consequence, a large portion of gastroenterological patients should undergo a GC. However, as indicated in the Salerno criteria, evaluating the symptomatic response to a GFD as a first step is mandatory. The symptomatic response to a GFD is usually substantial, but it is influenced by a high rate of placebo effect.[44,46] Consequently, a GC is recommended for those patients reporting symptomatic relief after the withdrawal of gluten from the diet. A clear clinical picture of NCGS patients is difficult to draw because of the variety of symptoms (intestinal and extraintestinal) frequently reported by the patients after the ingestion of gluten-containing food.[47,48] Additionally, standardized evaluation of clinical responses to a GFD and blind GC and a cut-off value for differentiating between those who are and those who are not sensitive to gluten have not been widely adopted.

Wheat allergy. Oral food challenge is considered the reference standard in case of equivocal situations.[37,40,49] Usually food allergies can be detected by means of serological IgE, skin prick tests, and molecular-based allergy tests, which have demonstrated good diagnostic performance.[7] However, in WA, a double- or single-blind,

placebo-controlled challenge or an open food challenge may be considered diagnostic if the challenge elicits objective symptoms in agreement with the patient's medical history and supporting laboratory tests.[50] It should be noted that patients with a previous history of anaphylactic reactions must be carefully managed; a food challenge is potentially dangerous to such patients and should not be performed.[44]

What to use for the challenge

Whether to challenge with wheat, gliadin, gluten, or other wheat proteins is still a matter of debate. Wheat farming dates back to the onset of agriculture, when wheat spontaneously appeared in the fertile crescent of southeastern Asia (ie, modern-day Turkey, Palestine, Lebanon, and northern Iraq). Primitive wild cereals included rye too, and they remain widely consumed to this very day.[51] Wheat has experienced numerous genetic modifications; some authors postulate that the current hexaploid type of wheat is more antigenic than the former diploid version, but the evidence does not support this view.[52]

Gluten represents 75%–85% of the proteins contained in wheat. It is a mixture of seed storage proteins rich in prolamines due to the significant amount of glutamine and proline residues present in primary structures.[53] Gluten proteins have no relevant biological role and, given their low content of essential amino acids, their nutritional value is quite low. Gluten is widely found in processed food products because of its ability to retain air in the protein matrix and thus facilitate culinary processing.[54] Of gluten's 2 main components, glutenin is further divided based on the molecular weights,[55] whereas gliadin is the alcohol-soluble component (30 kDa),[56] which can be divided into 4 fractions: α, β, γ, and ω. Of these, α-gliadin has been hown to have the strongest toxic effect for individuals with CD.[57–59] At the lower peptide level, it has been shown that the 13-mer and 33-mer α-gliadin motifs exert a cytotoxic effect on the intestinal epithelial cells and also activate gut-derived T-cell lines in patients with CD.[10,60] This explains why 33-mer gluten fractions rather than entire wheat or gluten proteins are often used for challenging.

The pros and cons of using gluten, gliadin, wheat, or their fractions for a GC differ depending on the condition. In CD, challenges have used bread[61] (real-life situation), gliadin,[62] or gluten[63] ("toxic") fractions in different dosages and times, making results difficult to compare. Using wheat and not fractions is vital in WA, because patients may be sensitized to any fraction or subfraction depending on the presence of primary or conformational epitopes. Although the number of studies reporting on food (wheat) challenges is on the increase, the actual amount of data to date is insufficient to conclude the debate about what makes the best protocol to carry out challenges, both for WA and for NCGS.[64]

Celiac disease. Although gluten or gliadin appears to be the best choice for a food challenge for CD, available evidence about safe amounts of peptides that celiac patients may ingest without damaging their small intestinal mucosa is still controversial. Moreover, variability of symptomatic, serological, and histological responses in patients greatly differs.[65] All of this information is crucial for setting the gluten dosage to use for the oral challenge. In regard to any long-term consequences, Kaukinen et al.[66] showed that patients with CD who consumed an average of 34 mg of gluten per day over a 8-year period had not developed any mucosal histological abnormality, but patients with a gluten intake of 1–2 g per week did have villous alterations. In a short-term study, Ciclitira et al.[67] showed that 1.2–2.4 mg of gliadin added to gluten-free bread consumed throughout the day for 1 week induced histological mucosal changes. However, these data were not confirmed by another study that used the same amount of gliadin (administered as gluten-free bread for 6 wk); this study showed no histological mucosal changes.[66] In children, the challenge seems feasible with small amounts of peptide; Catassi et al.[62] administered 100 or 500 mg of gliadin daily over 4 weeks to children with CD and showed that those who had received 100 mg had minimal changes in the intestinal mucosa, whereas those who had received 500 mg showed pronounced damage. Successively, Catassi et al.[63] performed a prospective, randomized, double-blind, placebo-controlled study on adults with CD randomly divided into 3 groups: those that received 10 mg of purified gluten in capsules, those that received 50 mg of purified gluten in capsules, and those that received placebo, all over a 3-month period. This study showed that a substantial proportion of patients treated with small amounts of gluten showed morphological alterations in the small intestine (altered villous height/crypt depth ratio). The authors concluded that prolonged daily intake of 50 mg of gluten causes architectural damage to the intestinal mucosa of treated patients with CD.[61,63]

In a more recent and specifically designed study, Leffler et al.[61] studied 20 patients with biopsy-proven CD. Those patients underwent a prestudy period of 14 days on a GFD followed by a 14-day challenge and a final visit at day 28. The participants received a randomly assigned dose of 3 or 7.5 g of gluten per day (given as 2 slices of bread). The authors found a substantial reduction in the villous height/crypt depth ratio and

increased intestinal epithelial lymphocytes from baseline to day 14 of the GC. Antibody titers had increased slightly from baseline to day 14 but had increased markedly by day 28. Gastrointestinal symptoms had increased by day 3 and returned to baseline levels by day 28 without differences between the 2 gluten doses.

The medical literature has greatly helped to improve the capacity to manage CD. However, because of the enormous differences in gluten sensitivity among patients with CD, there are relevant questions still awaiting clarification as to what the best option for GC is for this condition. More research is needed to establish which protein should be used; at what dosage it should be used; how long it should take to elicit a detectable clinical, serological, or histological response; and how long the symptoms should take to remit after gluten suspension. In the authors' opinion, gluten can be the best choice for oral food challenge in a CD scenario in light of the possibility that some patients could have an immunological response toward nongliadin epitopes.[68]

Nonceliac gluten sensitivity. Different studies have assessed GCs in subjects with NCGS. Biesiekierski et al.[29] conducted a double-blind, placebo-controlled challenge among symptomatically controlled patients on a GFD. The participants continued on a GFD throughout the study and received either gluten (16 g/d) or placebo in the form of 2 bread slices plus 1 muffin per day for up to 6 weeks. Thirteen of the 19 patients in the gluten group reported that symptoms were not adequately controlled compared with 6 of the 15 patients in the placebo group. Within 1 week, subjects in the gluten group substantially worsened (assessed by a visual analog scale) with regard to all symptoms, including pain, bloating, satisfaction with stool consistency, and tiredness. The authors concluded that gluten is indeed a trigger of gut symptoms and tiredness. No evidence for intestinal inflammation or damage or latent CD was found to offer a mechanistic explanation for symptom deterioration caused by gluten.

Carroccio et al.[69] focused on wheat sensitivity (considered as a new clinical entity) to define clinical, serological, and histological markers. In their study, 70 patients suffering wheat sensitivity and 206 with hypersensitivity associated with multiple foods underwent a double-blind, placebo-controlled trial that consisted of a regular diet with a minimum of 30 g/day of wheat for 2–4 weeks, then an elimination diet, and finally a challenge of wheat reintroduction. The patients with wheat sensitivity had a high frequency of anemia, weight loss, self-reported intolerance to wheat, and coexistence of atopy and food allergy in childhood compared with the control group. The score of the visual analog scale for each symptom was substantially higher than at baseline (on the wheat-free diet) after the first week on a wheat-containing diet and increased further at the end of the second week. A remarkable histological characteristic was eosinophil infiltration into the duodenal and colonic mucosa.[69] Elli et al.[45] challenged patients with functional gastrointestinal disorders with 5.6 g of gluten capsules per day; 14% of patients responded positively to the challenge, reporting an increase of symptoms while on blind intake of gluten. Similarly, Di Sabatino et al.[70] used 4.37 g of gluten capsules with similar findings, whereas other authors[46] used gluten-containing or gluten-free flours.

To date, the mechanisms by which food components may be responsible for gastrointestinal and extraintestinal symptoms in NCGS are not clear. Besides gluten, wheat amylase trypsin inhibitors (ATIs) may be also involved.[71–73] The discussion of potential challenge protocols for NCGS is ongoing. In general, the open challenges undertaken by patients frequently make use of actual foods, whereas the medically controlled challenges tend to add gluten to a GFD so that the amount of gluten ingested is controlled. Future studies will have to define the best algorithm for a GC in NCGS, although the use of a controlled quantity of gluten containing a known dose of ATIs seems a feasible approach.

Wheat allergy. As for any food allergy, WA diagnosis is based on an oral food challenge whenever possible. Skin tests and both total and specific serum IgE levels are also helpful. Skin tests and serological IgE (sIgE) have low sensitivity and low predictive value, partly explained by the fact that the commercial kits do not include water/salt-soluble wheat proteins and therefore lack the allergens contained in the insoluble gluten fraction.[74] However, the absence of specific IgE in certain cases—for example, WA—indicates that the patient is probably not allergic to wheat because the specificity of this sensitization test is very high.[38] Evidence suggests that specific wheal size and the ratio of an allergen-induced wheal to a histamine-induced wheal diameter (skin index) is useful to diagnose some allergies and predict outgrowth.[75] In a study of 83 Japanese patients who, in the 2 years prior to the study, had showed allergic reaction to noodles, a low-dosage challenge proved useful to allergy management, shifting the group from complete avoidance to partial wheat intake.[76]

Although oral food challenge is considered the reference standard to diagnose food allergy,[11,44] there are no standardized protocols for oral food challenge, especially with regard to the use of wheat or gluten. The use of orally administered wheat solutions at incremental doses to a total amount of 10 g of wheat with a

30-minute interval between each dose can be considered an efficient protocol.[77] The protocol is stopped when a reaction is observed.[78]

When and for how long to challenge

Celiac disease. In the past, a GC was mandatory for celiac patients to demonstrate their clinical and histological remission after a GFD and, later, a third biopsy would have to demonstrate histological damage relapse after gluten reintroduction.[79] However, since highly sensitive autoantibodies have become available (mainly antitransglutaminase autoantibodies), challenges are considered only for special situations. At present, use of a GC in cases of CD is infrequent and limited to those cases with no initial firm diagnosis and for those patients that demand it to confirm their lifelong diagnosis. A GC should not be carried out for just a short period of time (few days) because severe symptoms can appear after gluten has been administered to celiac patients for 6–8 weeks (at 10 g of gluten/d).[25] Many patients are reluctant to partake in this scheme of gluten reintroduction, which requires sustained gluten ingestion for several weeks, because of the evident risk of developing discomfort. Many recent and ongoing trials of novel celiac therapies rely on a GC for the initial assessment of efficacy, but an accurate study design is not feasible without substantial data on the effects of a GC on currently available endpoints. In fact, there is no standardized GC protocol that shows a linear correlation between gluten intake and development of duodenal histological damage or the increase of serological autoantibodies (antitissue transglutaminase IgA). This makes it difficult to establish the real benefit of any eventual drug. A better understanding of the kinetics of serological and histologic changes that can occur during GC is clearly needed. One of the latest studies[61] evaluating the time of exposure (at a given amount of gluten) necessary to induce detectable histological and serological changes has shown that a challenge for 14 days, using 3 gram of gluten, is enough to observe histological changes (ie, increase of intraepithelial lymphocytes and reduction of the villous height/crypt depth ratio) in 90% of patients with CD. This new proposal of challenging with a low-dosage gluten over a 14-day period has shown high adherence and tolerability.[61]

Nonceliac gluten sensitivity. It is not yet known whether NCGS is a transient, recurrent, or permanent condition, and there are no data establishing the benefits or drawbacks of receiving treatment over longer or shorter periods. At present, some specialists strongly recommend periodic evaluations with a GC—for example, every 6–12 months—especially with pediatric populations.[20] In practice, however, many patients on a GFD are reluctant to reintroduce gluten because they prefer to avoid the possibility of reappearance of symptoms. On the other hand, many patients undergo frequent self-administered challenges because they want to return to their customary diet. There is an urgent need for guidelines on how to best monitor patients with NCGS.[3,73] The majority of authors have used exposure periods between 7[45,70] and 10[46] days for a GC, usually with a washout interval. Following the Salerno criteria, gluten should be blindly administered for 1 week with a 1-week interval between the placebo and gluten administration.[20]

Wheat allergy. As for any food allergy, WA challenges are required in 2 situations: at diagnosis and to decide on the outgrowth of the allergy and the return to a full diet. When a double-blind oral challenge is not feasible and a single-blind oral challenge is undertaken, special attention must be paid to the effective disguise of the test food administered in order to hide its consistency, smell, color, and flavor. This helps with assessment of symptoms, especially chronic or subjective ones, as patients will not know whether they are receiving active food or placebo.[80] In WA, the oral challenge can be shorter than those used for NCGS and CD. Increasing doses of wheat can be administered with 30-minute intervals between doses until the onset of symptoms.[78]

Where to challenge

There is no clear evidence about the setting in which a GC should ideally be conducted for CD, NCGS, or WA. One can hypothesize that challenges should be performed in tertiary centers. More important is that GCs be performed in centers where a formal protocol is applied and specialist medical personnel (ie, gastroenterologist, allergologist, and nutritionist) are available. This is most relevant in the case of WA, especially for those patients that may develop severe acute reactions or anaphylaxis.[76]

Why to challenge

Celiac disease. A GC is relevant for all 3 conditions included herein because it is the means to secure a firm diagnosis. With CD, there is a longer history of research, and, in general, a GC is well accepted by pediatric patients and their families.[79] A GC is especially necessary when an initial diagnosis is in doubt, either because the patient was on a GFD without adequate diagnostic studies or because the initial clinical condition was severe enough to make the biopsy procedure too risky for the patient. For patients suspected to have potential CD (positive autoantibodies and normal duodenal histology, a specialist should decide whether to perform a GC.

Table 1 **Gluten challenge characteristics in celiac disease, nonceliac gluten sensitivity, and wheat allergy following the 5 Ws approach**

Consideration	Celiac disease	Nonceliac gluten sensitivity	Wheat allergy
Who	Patients on a gluten-free diet with HLA DQ2 and/or DQ8 with no previous celiac disease screening Patients on a gluten-free diet with an uncertain celiac disease diagnosis in the past	Patients without celiac disease or wheat allergy, responsive to a gluten-free regimen	Doubtful cases reporting symptoms after food ingestion but without specific skin or serological tests
What	From 3 to 7.5 g of gluten daily (2 slices of bread) for at least 14 d The challenge can be open	8 g of gluten using bars as the vehicle. If possible, the bars should include a known amylase tripsyn inhibitor content. The challenge should be single- or double-blind placebo-controlled with crossover	There is no indication about a standardized protocol The challenge should start with low doses of wheat, and it can be an open challenge for an initial evaluation
When (How long)	Challenge should take at least 14 d and can be prolonged if necessary	After at least 3 wk on a gluten-free diet the patients are blindly administered gluten for at least 1 wk, with 1 wk of washout between crossover	The challenge should be quick (hours) looking for rapid immunoglobulin E–mediated symptoms
Where	Gastroenterological or nutritional units	Gastroenterological or nutritional units	In allergological settings with particular attention to anaphylactic reactions
Why	To verify the diagnosis through the presence of gluten-dependent duodenal histological damage compatible with celiac disease	To reduce the bias among patients responsive to the gluten withdrawal from their diet and to facilitate the discovery of biomarkers or the development of clinical score systems	To verify diagnosis, assess the allergy trigger, and educate toward the management of allergic symptoms

Nonceliac gluten sensitivity. For NCGS, it is necessary to strictly follow a formal algorithm during a diagnostic GC.[20] Although there is no consensus on the challenge protocol yet, it is important to avoid placebo effects. Indeed, many patients may feel better on a GFD simply because they expect to. Instead, a "nocebo" effect may lead patients to feel worse when they are exposed to gluten again.

Additionally, GCs can be used to help improve the discovery of new biomarkers or diagnostic score systems by allowing studies to enroll those with a definite hypersensitivity to gluten and not those undergoing placebo effects. Having analyzed patients with a GC-proven NCGS, Udhe et al.[81] recently described increases in soluble CD14, antilipopolysaccharide binding protein, antiflagellin antibodies, and fatty acid binding protein 2 as potential factors associated with NCGS. Unfortunately, a score system is not yet available for NCGS, at least to reduce the need for GC in clinical practice.

Wheat allergy. Patients with WA may be the most difficult to convince to accept an oral food challenge. The procedure is time-consuming, so, when symptoms are mild, patients often think it is not worth their effort. When symptoms are moderate to severe, the challenge is often rejected because the patients or their families consider it unacceptable to agree to a clearly uncomfortable experience. The main reasons to perform an oral food challenge in cases of WA are to corroborate a diagnosis and to educate the patient on how to best manage clinical symptoms.

CONCLUSION

A GC is a valuable medical tool for the 3 conditions reviewed herein. The actual dose of gluten (whether administered as gluten, gliadin, or wheat), the time needed to evaluate substantial changes, and the indicators demonstrating positivity/negativity beyond clinical responses require further study. It has been shown that GC is useful to confirm a diagnosis of CD. Although there are numerous studies evaluating different protocols, these studies are directed toward demonstrating disease relapse, not defining the best gluten challenge protocol. Guidelines should be developed to recommend a protocol or a range of acceptable algorithms. For NCGS, which has only recently begun to receive medical attention, a GC represents the best option to confirm a diagnosis after CD and WA have been ruled out. The need for guidelines for the use of a GC for NCGS diagnosis is clear. Finally, in regards to WA, although the available evidence derived from food allergies management is generally more abundant, the variability of the challenge protocols used is quite large. Table 1 summarizes the main points regarding use of a GC in CD, NCGS, and WA. Achieving consensus among specialists on challenge protocols seems a

reasonable way forward in advancing the management of these medical conditions.

Acknowledgments

Author contributions. K.A.B. and L.R. wrote the paragraphs on gluten and food challenge; F.B. and L.E. described the use of gluten challenge in celiac disease, and L.D. and M.A. illustrated its use in allergy. A.S. and F.F. dealt with nonceliac gluten sensitivity. Moreover, L.E. supervised the manuscript.

Funding. Research support for this study was provided by Fondazione IRCCS Ca' Granda and by grants from the Italian Ministry of Health and Lumbardy's Regional Government Authority (Ministero della Salute e Regione Lombardia call no. 2011-02348234).

Declaration of interest. L.E. is a member of the Dr Schaer Institute scientific board. L.E. and L.R. are inventors of a patented gluten challenge test for NCGS. The other authors have no relevant interests to declare.

REFERENCES

1. Rosegrant MW, Leach N, Gerpacio RV. Alternative futures for world cereal and meat consumption. Proc Nutr Soc. 1999;58:219–234.
2. Elli L, Branchi F, Tomba C, et al. Diagnosis of gluten related disorders: celiac disease, wheat allergy and non-celiac gluten sensitivity. WJG. 2015;21:7110–7119.
3. Catassi C, Bai JC, Bonaz B, et al. Non-celiac gluten sensitivity: the new frontier of gluten related disorders. Nutrients. 2013;5:3839–3853.
4. Sapone A, Bai JC, Ciacci C, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med. 2012;10:13.
5. Mansueto P, Seidita A, D'Alcamo A, et al. Non-celiac gluten sensitivity: literature review. J Am Coll Nutr. 2014;33:39–54.
6. Sazzini M, De Fanti S, Cherubini A, et al. Ancient pathogen-driven adaptation triggers increased susceptibility to non-celiac wheat sensitivity in present-day European populations. Genes Nutr. 2016;11:15.
7. Elli L, Villalta D, Roncoroni L, et al. Nomenclature and diagnosis of gluten-related disorders: a position statement by the Italian Association of Hospital Gastroenterologists and Endoscopists (AIGO). Digest Liver Dis. 2017;49:138–146.
8. Hadjivassiliou M, Sanders DD, Aeschlimann DP. Gluten-related disorders: gluten ataxia. Dig Dis. 2015;33:264–268.
9. Jakes AD, Bradley S, Donlevy L. Dermatitis herpetiformis. BMJ. 2014;348:g2557.
10. Shan L, Molberg O, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. Science. 2002;297:2275–2279.
11. Cianferoni A. Wheat allergy: diagnosis and management. J Asthma Allergy. 2016;9:13–25.
12. Bardella MT, Elli L, Ferretti F. Non celiac gluten sensitivity. Curr Gastroenterol Rep. 2016;18:63.
13. Czaja-Bulsa G. Non coeliac gluten sensitivity—a new disease with gluten intolerance. Clin Nutr. 2015;34:189–194.
14. Gutierrez L. Impacts of El Nino-Southern Oscillation on the wheat market: a global dynamic analysis. PLoS One. 2017;12:e0179086.
15. Fric P, Gabrovska D, Nevoral J. Celiac disease, gluten-free diet, and oats. Nutr Rev. 2011;69:107–115.
16. Tovoli F, Masi C, Guidetti E et al. Clinical and diagnostic aspects of gluten related disorders. World J Clin Cases. 2015;3:275–284.
17. Bascunan KA, Vespa MC, Araya M. Celiac disease: understanding the gluten-free diet. Eur J Nutr. 2017;56:449–459.
18. Gaesser GA, Angadi SS. Navigating the gluten-free boom. JAAPA. 2015;28:1–7.
19. Aziz I, Lewis NR, Hadjivassiliou M, et al. A UK study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care. Eur J Gastroenterol Hepatol. 2014;26:33–39.
20. Catassi C, Elli L, Bonaz B, et al. Diagnosis of non-celiac gluten sensitivity (NCGS): the Salerno experts' criteria. Nutrients. 2015;7:4966–4977.
21. Ludvigsson JF, Card TR, Kaukinen K, et al. Screening for celiac disease in the general population and in high-risk groups. United European Gastroenterol J. 2015;3:106–120.
22. Buscarini E, Conte D, Cannizzaro R, et al. White paper of Italian Gastroenterology: delivery of services for digestive diseases in Italy: weaknesses and strengths. Dig Liver Dis. 2014;46:579–589.
23. Sollid LM, Iversen R, Steinsbo O, et al. Small bowel, celiac disease and adaptive immunity. Dig Dis. 2015;33:115–121.
24. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 1999;11:1185–1194.
25. Rostom A, Murray JA, Kagnoff MF. American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology 2006;131:1981–2002.
26. Husby S, Koletzko S, Korponay-Szabo IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54:136–160.
27. Elli L, Roncoroni L, Bardella MT. Non-celiac gluten sensitivity: time for sifting the grain. World J Gastroenterol. 2015;21:8221–8226.
28. Elli L. Where's the evidence for gluten sensitivity? BMJ. 2012;345:e7360.
29. Biesiekierski JR, Newnham ED, Irving PM, et al. Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial. Am J Gastroenterol. 2011;106: 508–514. quiz 515.
30. Troncone R, Jabri B. Coeliac disease and gluten sensitivity. J Intern Med. 2011;269:582–590.
31. Verdu EF, Armstrong D, Murray JA. Between celiac disease and irritable bowel syndrome: the "no man's land" of gluten sensitivity. Am J Gastroenterol. 2009;104:1587–1594.
32. Lundin KE. Non-celiac gluten sensitivity—why worry? BMC Med. 2014;12:86.
33. Pasha I, Saeed F, Sultan MT, et al. Wheat allergy and intolerence; recent updates and perspectives. Crit Rev Food Sci Nutr. 2016;56:13–24.
34. Pietzak M. Celiac disease, wheat allergy, and gluten sensitivity: when gluten free is not a fad. J Parenter Enteral Nutr. 2012;36:68S–75S.
35. Comberiati P, Cipriani F, Schwarz A, et al. Diagnosis and treatment of pediatric food allergy: an update. Ital J Pediatr. 2015;41:13.
36. Berin MC, Shreffler WG. Mechanisms underlying induction of tolerance to foods. Immunol Allergy Clin North Am. 2016;36:87–102.
37. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, et al. Work group report: oral food challenge testing. J Allergy Clin Immunol. 2009;123:S365–S383.
38. van Maaren MS, Dubois AE. Dutch guideline on food allergy. Neth J Med. 2016;74:376–382.
39. Ferretti F, Branchi F, Dell'Osso B, et al. Coping with celiac disease: how heavy is the burden for caregivers? Rev Esp Enferm Dig. 2017;109:250–255.
40. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, et al. Standardization of food challenges in patients with immediate reactions to foods—position paper from the European Academy of Allergology and Clinical Immunology. Allergy. 2004;59:690–697.
41. Bahna SL. Food challenge procedure: optimal choices for clinical practice. Allergy Asthma Proc. 2007;28:640–646.
42. Ludvigsson JF, Bai JC, Biagi F, et al. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. Gut. 2014;63:1210–1228.
43. Biagi F, Bianchi PI, Campanella J, et al. The impact of misdiagnosing celiac disease at a referral centre. Can J Gastroenterol. 2009;23:543–545.
44. Boyce JA, Assa'ad A, Burks AW, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. J Allergy Clin Immunol. 2010;126:1105–1118.
45. Elli L, Tomba C, Branchi F, et al. Evidence for the presence of non-celiac gluten sensitivity in patients with functional gastrointestinal symptoms: results from a multicenter randomized double-blind placebo-controlled gluten challenge. Nutrients. 2016;8:84.
46. Zanini B, Basche R, Ferraresi A, et al. Randomised clinical study: gluten challenge induces symptom recurrence in only a minority of patients who meet clinical criteria for non-coeliac gluten sensitivity. Aliment Pharmacol Ther. 2015;42:968–976.
47. Volta U, Bardella MT, Calabro A, et al. An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity. BMC Med. 2014;12:85.
48. Wahnschaffe U, Schulzke JD, Zeitz M, et al. Predictors of clinical response to gluten-free diet in patients diagnosed with diarrhea-predominant irritable bowel syndrome. *Clin* Gastroenterol Hepatol. 2007;5:844–850, quiz 769.
49. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. J Allergy Clin Immunol. 2012;130:1260–1274.
50. Lieberman JA, Sicherer SH. Diagnosis of food allergy: epicutaneous skin tests, in vitro tests, and oral food challenge. Curr Allergy Asthma Rep. 2011;11:58–64.
51. van den Broeck HC, van Herpen TW, Schuit C, et al. Removing celiac disease-related gluten proteins from bread wheat while retaining

technological properties: a study with Chinese spring deletion lines. BMC Plant Biol. 2009;9:41.
52. van den Broeck HC, de Jong HC, Salentijn EM, et al. Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: wheat breeding may have contributed to increased prevalence of celiac disease. Theor Appl Genet. 2010;121:1527–1539.
53. Balakireva AV, Zamyatnin AA. Properties of gluten intolerance: gluten structure, evolution, pathogenicity and detoxification capabilities. Nutrients. 2016;8:644–671.
54. Greco L. From the neolithic revolution to gluten intolerance: benefits and problems associated with the cultivation of wheat. J Pediatr Gastroenterol Nutr. 1997;24:S14–S16, discussion S16–17.
55. Ang S, Kogulanathan J, Morris GA, et al. Structure and heterogeneity of gliadin: a hydrodynamic evaluation. Eur Biophys J. 2010;39:255–261.
56. Frisoni M, Corazza GR, Lafiandra D, et al. Wheat deficient in gliadins: promising tool for treatment of coeliac disease. Gut. 1995;36:375–378.
57. Carroccio A, Di Prima L, Noto D, et al. Searching for wheat plants with low toxicity in celiac disease: between direct toxicity and immunologic activation. Dig Liver Dis. 2011;43:34–39.
58. De Re V, Caggiari L, Tabuso M, et al. The versatile role of gliadin peptides in celiac disease. Clin Biochem. 2013;46:552–560.
59. Spaenij-Dekking L, Kooy-Winkelaar Y, van Veelen P, et al. Natural variation in toxicity of wheat: potential for selection of nontoxic varieties for celiac disease patients. Gastroenterology. 2005;129:797–806.
60. Maiuri L, Troncone R, Mayer M, et al. In vitro activities of A-gliadin-related synthetic peptides: damaging effect on the atrophic coeliac mucosa and activation of mucosal immune response in the treated coeliac mucosa. Scand J Gastroenterol. 1996;31:247–253.
61. Leffler D, Schuppan D, Pallav K, et al. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. Gut. 2013;62:996–1004.
62. Catassi C, Rossini M, Ratsch IM, et al. Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study. Gut. 1993;34:1515–1519.
63. Catassi C, Fabiani E, Iacono G, et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am J Clin Nutr. 2007;85:160–166.
64. Schalk K, Lexhaller B, Koehler P, et al. Isolation and characterization of gluten protein types from wheat, rye, barley and oats for use as reference materials. PLoS One. 2017;12:e0172819.
65. Ciclitira PJ, Ellis HJ, Fagg NL. Evaluation of a gluten free product containing wheat gliadin in patients with coeliac disease. Br Med J (Clin Res Ed). 1984;289:83.
66. Kaukinen K, Collin P, Holm K, et al. Wheat starch-containing gluten-free flour products in the treatment of coeliac disease and dermatitis herpetiformis. A long-term follow-up study. Scand J Gastroenterol. 1999;34:163–169.
67. Ciclitira PJ, Cerio R, Ellis HJ, et al. Evaluation of a gliadin-containing gluten-free product in coeliac patients. Hum Nutr Clin Nutr. 1985;39:303–308.
68. Sollid LM, Qiao SW, Anderson RP, et al. Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. Immunogenetics. 2012;64:455–460.
69. Carroccio A, Mansueto P, Iacono G, et al. Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: exploring a new clinical entity. Am J Gastroenterol. 2012;107:1898–1906. quiz 1907.
70. Di Sabatino A, Volta U, Salvatore C, et al. Small amounts of gluten in subjects with suspected nonceliac gluten sensitivity: a randomized, double-blind, placebo-controlled, cross-over trial. Clin Gastroenterol Hepatol. 2015;13:1604–1612. e1603.
71. Biesiekierski JR, Peters SL, Newnham ED, et al. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. Gastroenterology. 2013;145:320–328. e321–e323.
72. Capannolo A, Viscido A, Barkad MA, et al. Non-celiac gluten sensitivity among patients perceiving gluten-related symptoms. Digestion. 2015;92:8–13.
73. Fasano A, Sapone A, Zevallos V, et al. Nonceliac gluten sensitivity. Gastroenterology. 2015;148:1195–1204.
74. Hischenhuber C, Crevel R, Jarry B, et al. Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. Aliment Pharmacol Ther. 2006;23:559–575.
75. Kido J, Hirata M, Ueno H, et al. Evaluation of the skin-prick test for predicting the outgrowth of cow's milk allergy. Allergy Rhinol (Providence). 2016;7:139–143.
76. Okada Y, Yanagida N, Sato S, et al. Better management of wheat allergy using a very low-dose food challenge: a retrospective study. Allergol Int. 2016;65:82–87.
77. Niggemann B, Wahn U, Sampson HA. Proposals for standardization of oral food challenge tests in infants and children. Pediatr Allergy Immunol. 1994;5:11–13.
78. Niggemann B, Sielaff B, Beyer K, et al. Outcome of double-blind, placebo-controlled food challenge tests in 107 children with atopic dermatitis. Clin Exp Allergy. 1999;29:91–96.
79. McNeish AS, Harms HK, Rey J, et al. The diagnosis of coeliac disease. A commentary on the current practices of members of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN). Arch Dis Child. 1979;54:783–786.
80. Gonzalez-Mancebo E, Alonso Diaz de Durana MD, Garcia Estringana Y, et al. Validation of recipes for double-blind placebo-controlled challenges with milk, egg white, and hazelnut. J Investig Allergol Clin Immunol. 2017;27:40–45.
81. Uhde M, Ajamian M, Caio G, et al. Intestinal cell damage and systemic immune activation in individuals reporting sensitivity to wheat in the absence of coeliac disease. Gut. 2016;65:1930–1937.