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Position Paper

Nomenclature and diagnosis of gluten-related disorders: A position statement by the Italian Association of Hospital Gastroenterologists and Endoscopists (AIGO)

Luca Elli[a,*], Danilo Villalta[b], Leda Roncoroni[c], Donatella Barisani[d], Stefano Ferrero[e,f], Nicoletta Pellegrini[g], Maria Teresa Bardella[a], Flavio Valiante[h], Carolina Tomba[i], Antonio Carroccio[j,k], Massimo Bellini[l], Marco Soncini[i], Renato Cannizzaro[m], Gioacchino Leandro[n]

[a] Center for the Prevention and Diagnosis of Celiac Disease, Fondazione IRCCS Ca' Granda—Ospedale Maggiore Policlinico, Milano, Italy
[b] Allergology and Clinical Immunology Unit, AO "Santa Maria degli Angeli", via Montereale 24, 33170 Pordenone, Italy
[c] Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milano, Italy
[d] Dipartimento di Medicina e Chirurgia (school of Medicine and Surgery) Università Milano Bicocca, Monza, Italy
[e] Pathology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy
[f] Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy
[g] Department of Food Science, University of Parma, Parma, Italy
[h] Gastroenterology and Digestive Endoscopy Unit, Santa Maria del Prato Hospital, Feltre, Italy
[i] Gastroenterology Unit ASST – Santi Paolo e Carlo – Milano, Italy
[j] DiBiMIS, University of Palermo, Palermo, Italy
[k] Internal Medicine, Giovanni Paolo II Hospital, Sciacca, Italy
[l] Gastrointestinal Unit, Department of Gastroenterology, University of Pisa, Pisa, Italy
[m] Gastroenterology, CRO National Cancer Institute, via Franco Gallini 2, 33081, Aviano, Italy
[n] Gastroenterology, IRCCS De Bellis Hospital, Castellana Grotte, Italy

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ABSTRACT

Background: "Gluten-related disorders" is a term that encompasses different diseases induced by the ingestion of gluten-containing food. Because of their incidence the scientific community has been intensively studying them.
Aim: To support gastroenterologists with a correct nomenclature and diagnostic approach to gluten-related disorders in adulthood.
Methods: The Italian Association of Hospital Gastroenterologists and Endoscopists (AIGO) commissioned a panel of experts to prepare a position statement clarifying the nomenclature and diagnosis of gluten-related disorders, focusing on those of gastroenterological interest. Each member was assigned a task and levels of evidence/recommendation have been proposed.
Results: The panel identified celiac disease, wheat allergy and non-celiac gluten sensitivity as the gluten-related disorders of gastroenterological interest. Celiac disease has an autoimmune nature, wheat allergy is IgE-mediated while the pathogenesis of non-celiac gluten sensitivity is still unknown as is the case of non-IgE mediated allergy. Diagnosis should start with the serological screening for celiac disease and wheat allergy. In case of normal values, the response to a gluten-free diet should be evaluated and a confirmatory blind food challenge carried out.
Conclusions: Gluten-related disorders are clinically heterogeneous. Patients should be carefully managed and specific protocols applied for a correct differential diagnosis in gastroenterological setting.

* Corresponding author at: Center for the Prevention and Diagnosis of Celiac Disease, Fondazione IRCCS Ca' Granda—Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122 Milano, Italy.
E-mail addresses: lucelli@yahoo.com (L. Elli), danilo.villalta@aas5.sanita.fvg.it (D. Villalta), leda.roncoroni@tiscali.it (L. Roncoroni), Donatella.barisani@unimib.it (D. Barisani), stefano.ferrero@unimi.it (S. Ferrero), nicoletta.pellegrini@unipr.it (N. Pellegrini), flavio.valiante@gmail.com (F. Valiante), tomba.carolina@gmail.com (C. Tomba), acarroccio@hotmail.com (A. Carroccio), mbellini@med.unipi.it (M. Bellini), rcannizzato@cro.it (R. Cannizzaro), leandro@media.it (G. Leandro).

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1. Introduction

In spite of the importance of wheat in the human diet throughout history, the interaction between its components (gliadin, gluten, amylase trypsin inhibitor etc.) and the human body triggers an increasing variety of symptoms, syndromes, allergic reactions, autoimmune diseases [1]. The first description of a patient affected by a disorder related to gluten ingestion (celiac disease, CD) has been ascribed to Areteus of Cappadocia, who in the 2nd century AD reported a case of chronic diarrhea and malabsorption [2]. This scenario appears in contrast with the wide diffusion of the genetic background susceptible to immune reactions against gluten, the HLA DQ2 and/or DQ8 haplotypes, which are essential for the development of CD [3]. The continuous worldwide diffusion of diseases and syndromes recognizing gluten or other wheat components as the dominant environmental factors, has induced the scientific community to study the mechanisms underpinning the intestinal and systemic damage following their ingestion and to group them into the umbrella definition gluten-related disorders (GRD) [4,5]. GRD are divided on the basis of their pathomechanism: autoimmune, celiac disease [6]; allergic, wheat allergy (WA, IgE or non-IgE mediated) and unknown as in the case of non-celiac gluten sensitivity (NCGS) (Fig. 1) [5]. Taken together, these disorders can affect at least 3% of the population and probably an even greater proportion of the patients attending gastroenterological outpatient services [1,7]. For these reasons the Italian Association of Hospital Gastroenterologists and Endoscopists (AIGO) considered it important and necessary to develop a position statement on the nomenclature and diagnosis of GRD in adults, to clarify the clinical issues that gastroenterologists and endoscopists usually face with during their clinical practice.

2. Methods

In March 2015 AIGO commissioned an experts' panel, composed of nutritionists, gastroenterologists, allergologists and biologists, to prepare a position statement on the nomenclature and diagnosis of GRD. A specific task was assigned to each member of the group on the basis of his/her expertise. In particular, LR and NP wrote the section about gluten and gluten-free diet (GFD) and supervised the manuscript from a nutritional point of view; DB described the genetic aspects; SF dealt with duodenal histology; MTB and Le organized the section on CD and FV, RC and CT the NCGS part; DV covered wheat allergy; AC summarized the connections between GRD and functional symptoms; MB and GL dealt with the possible use of symptomatic scores especially in NCGS. LE supervised the manuscript. Each panel member carried out a comprehensive PubMed research for English-written articles, without time limits, using appropriate MeSH terms. Regular conference calls and web-based exchanges were scheduled.

Whenever possible, the levels of evidence and recommendations were defined for each part of the statement following the GRADE system [8,9]. Briefly, the quality of the evidence is graded from high to low. "High" quality evidence is assigned when further research is unlikely to change the authors' confidence in the estimate of effect. "Moderate" quality evidence means that further studies would be likely to have an effect on the confidence of the estimate. Finally, "Low" quality evidence indicates that further research probably has an important impact on the confidence in the estimate of the effect and would change the estimate. About recommendations, a "Strong" recommendation is assigned in case of benefits clearly outweighing the negatives and the result of no action while "Conditional" is used when some uncertainty remains.

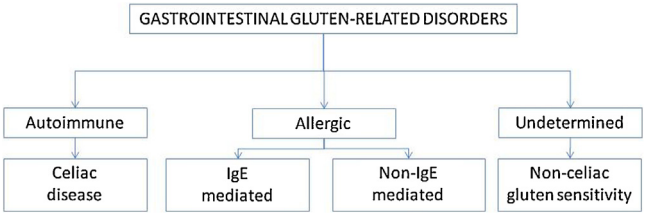

Fig. 1. A proposed scheme for the diagnosis of gluten-related disorders.

3. Gluten

Wheat (and other gluten-containing cereals) is one of the most important crops in the world and its dough can be processed into a variety of foodstuffs, notably bread, other baked products and pasta. The grain proteins determine the viscoelastic properties of dough, in particular, the storage protein that form a network in the dough called gluten [10]. Gluten is composed by a mixture of monomeric gliadins and polymeric glutenin subunits (in equal amounts) and represents approximately the 80% of storage proteins in wheat. Storage proteins can be subdivided on the basis of their alcohol solubility; prolamins, which make most of the proteic content in wheat, have been defined on the basis of their solubility in alcohol–water mixtures, typically 60–70% (v/v) ethanol [11]. The prolamins present in rye, barley and oats are named secalin, hordein and avenin, respectively. In wheat, these groups of monomeric prolamins are known as gliadins, which are usually subtyped following their electrophoretic mobility in α, γ (sulphur rich and usually harmful for CD) and ω (sulphur poor) [12]. Wheat prolamins are the major storage proteins present in the starchy endosperm cells of the grain, where they are synthetized and deposited via the secretory systems. Gliadin consists of about 300 aminoacids and is characterized by a high content of prolin and glutamine, and a low content of lysine and methionine [11]. These characteristics are responsible for the immunologic properties of gliadins and its immunodominant oligopeptide 33-mer. 33-mer is resistant to the intestinal enzymatic digestion and, after tissue transglutaminase (tTG) deamidation, it binds the CD specific HLA DQ, efficiently activating the T cells (adaptative immune response) [13]. Another gliadin peptide, the 13-mer is responsible for innate immunity and cooperates with 33-mer in CD pathogenesis [14]. Obscure is the gluten stimulatory mechanism in NCGS, although its cytotoxic effects could have a role [15].

Gluten has an important palatability [12]. Individual cells of wheat flour contain complex networks of gluten proteins, brought together during dough mixing. Nowadays, changes occuring during dough mixing are not exactly known; certainly, an increase in dough stiffness occurs as result of an increase and optimization of protein-to-protein interactions within the gluten network. This optimization is due to the formation of disulphide bonds while mixing in air; the presence of oxygen and nitrogen results in different effects on the sulphydryl and disulphide contents of dough [10,16].

Gluten is present in: durum wheat (*Triticum durum*), common wheat or bread wheat (*Triticum aestivum*), used for bread and fresh pasta and in bakery products, rye (*Secale cereale*), barley (*Hordeum vulgare*), the three species of spelt (einkorn, *Triticun monococcum*, emmer, *Triticum dicoccum Schrank* and spelta, *Triticum spelta*), khorasan wheat (*Triticum turanicum,* Kamut®), triticale (*Triticosecale Wittmack*), a hybrid of rye and common wheat. Other gluten-containing wheat derivates are bulgur and seitan [10].

4. Gluten-related disorders

4.1. Celiac disease

CD is a common T-cell mediated autoimmune disorder, which primarily affects the small bowel and is triggered, in genetically

Table 1
Diagnostic tests for gluten-related disorders.

GRD	Type of test			
	Serology	Histology	Skin test	Blind food challenge
CD	tTGA IgA screening test tTGA IgG and DGP IgG in case of IgA deficiency HLA typing in doubtful cases	Duodenal histology is mandatory	Not used	Not used
WA	sIgE screening test MA potentially useful but only a few allergens are available	Not used	Reliable but with low sensitivity	Gold standard (potentially dangerous)
Non-IgE WA	IgG is not supported by the scientific societies	Not used	Not used	Gold standard
NCGS	Unavailable	Not used	Not used	Gold standard

CD, celiac disease; GRD, gluten-related disorders; MA, molecular-based allergy diagnostics; WA, wheat allergy.

predisposed subjects, by gluten ingestion. A strict GFD usually normalizes the intestinal damage and clinical picture. The prevalence of CD is estimated from 0.5% to 1% of the worldwide population [1,17]. The overall prevalence of CD may be higher in northern Europe [18] or in specific "at risk" groups as first-degree relatives of patients with CD [19], patients with type-1 diabetes mellitus [20], Hashimoto's thyroiditis [21], genetic disorders (Down's syndrome and Turner's syndrome) [22], IgA deficiency [9].

The symptoms are various and different clinical pictures can be present, making CD largely under-diagnosed [23]. Clinical findings range from severe malabsorption syndrome to a mild or monosymptomatic form characterized by mild gastrointestinal symptoms such as dyspepsia, constipation, diarrhea, epigastric pain or even just extra-intestinal signs such as iron deficiency anemia or asthenia. CD can even occur without symptoms (silent form) [24–26].

The classic onset can be characterized by diarrhea (less frequently steatorrhea), weight loss or growth deficit during pediatric age, and symptoms sometimes related to liposoluble vitamins (A, D, E, K) malabsorption or electrolyte disorders [27].

About extra-intestinal manifestations, different systems biological processes can be involved [28] such as: hemopoietic process, bone mineralization, muscular function, nervous system, endocrine homeostasis and fertility [29].

In adults, CD diagnosis is performed by referring to different parameters: duodenal histology, serologic antibodies (anti tissue transglutaminase, tTG, anti endomisium, EmA, anti gliadin, AGA and anti deamidated gliadin, DGP), genetics, clinical picture and GFD response [30].

CD screening is performed during a gluten-containing diet and usually involves tTG IgA antibodies and total IgA dosages to exclude any IgA deficiency. tTG IgA antibodies are highly efficient: 98% sensitivity, 98% specificity, 72% positive predictive value and 99% negative predictive value; they are usually considered sufficient to rule CD out if negative or to suggest duodenal histology when positive [30]. In case of IgA deficiency, tTG IgG can be dosed but duodenal histology should always be considered. AGA should not be considered in CD diagnosis during adulthood [25]. When dealing with malabsorption symptoms or in the presence of a CD risk >5% (first-degree relatives, Down's syndrome or type-I diabetes), also in the absence of circulating tTG IgA, the exclusion of a duodenal atrophy must be considered due to the possibility of a seronegative CD, present in 5–22% of these cases (Table 1) [9].

Differently from childhood, duodenal histology remains pivotal in the CD diagnostic flowchart in adults. Histology is required to evaluate mucosal damage during gluten intake. Adequate histological samples should be obtained during endoscopy from the second duodenal portion and bulb. At least 4 oriented biopsies from the second part of the duodenum and 1 or 2 from the bulb are mandatory [31].

A correct orientation of samples can be obtained by positioning the fragments on cellulose acetate filters. These filters allow the technicians, with a 90° rotation, to paraffin-embed the combined biopsy-filter for correct evaluation by the pathologist [32].

CD duodenal damage is characterized by intraepithelial lymphocytosis, villous atrophy and crypt hyperplasia [24,33]. Histological evaluation for CD should take into account intraepithelial T lymphocytes (IEL) rate. The normal count has been variably cited in the literature, however a value <25 IEL/100 enterocytes is considered normal while a value >30 IEL/100 enterocytes represents a pathological "lymphocytosis" [24]. Although debated [34], immunohistochemical studies including CD3 immunostaining can highlight IEL infiltration.

Other minor histological parameters to evaluate are: decreased enterocyte height, intracytoplasmic enterocyte vacuolation with reduction or absence of brush border [33].

Moreover, novel findings suggest the presence of deposits of anti tTG antibodies, detectable in duodenal samples by means of immunofluorescence; these deposits seem useful in case of uncertain diagnosis without evident histologic alterations [35].

At diagnosis the severity of the damage is usually evaluated according to the Marsh–Oberhuber [36] or Corazza–Villanacci classification [37]. However, the pitfalls of duodenal damage in CD remain intra-epithelial lymphocythosis, crypt hyperplasia and villous atrophy [24].

Based on the aforementioned histological changes, CD diagnosis should always be correlated with clinical and serological findings in order to exclude other causes of villous atrophy (Giardia lamblia, Cryptosporidium, Microsporidium, cytomegalovirus, tropherima whippeli, lymphoma, Crohn's disease, common variable immune deficiency and olmesartan enteropathy) [38].

The recurrence/persistence of malabsorptive symptoms in spite of a strict adherence to GFD for at least 6–12 months and the persistence of villous atrophy (in the absence of other causes), suggests refractory CD (RCD). In such a case, further analysis is required to differentiate RCD between type I (at low risk of malignant evolution) and type II (at high risk of enteropathy-associated T-cell lymphoma, EATL): duodenal TCRγ rearrangement in order to reveal any monoclonal pattern and cytofluyorimetry in order to detect any aberrant duodenal lymphocytes (CD103+, CD3−, CD4−, CD8−, CD3ε+) [39].

About 30% of treated CD patients retain duodenal alterations in spite of the absence of RCD [40]. Although a second duodenal biopsy is not routinely suggested in case of good response to the GFD treatment, in case of unresponsiveness duodenal histology could be considered [9,41]. This scenario suggests the need to evaluate histologic modifications during GFD by comparing histology before and after GFD. Recently, a new method (Elli–Ferrero's method), based upon the Marsh–Oberhuber score, has been proposed to evaluate the trend of the duodenal injury [40]. In this latter method, a calculation of the area covered by each Marsh grade (before and after GFD) has been proposed to describe the mucosal response to gluten withdrawal, instead of a simple description of the worst lesion observed at histological examination.

4.1.1. Celiac disease genetics

Different MHC and non-MHC genes concur in the predisposition to CD. The Major Histocompatibility Complex (HLA) region contains a large number of genes with immunological functions; they are important predisposing factors for CD. The association between CD and the heterodimer DQ2 is well known. HLA-DQ molecules are formed by alpha and beta subunits, encoded by HLA-DQA1 and HLA-DQB1 genes, respectively. About 90% of patients with CD carry the heterodimer DQ2.5 (DQA1*0501 – DQB1*0201). Most of the remaining patients carry the heterodimer DQ8 (DQA1*0301 – DQB1*0302), whereas the few patients lacking DQ2.5 or DQ8 have one of the two alleles that encode the subunits of DQ2.5. In this case they carry the heterodimer DQ2.2 (DQA1*0201 and DQB1*0202) or DQ7.5 (with the allele DQA1*0505 which encodes a polypeptide that is identical, after processing, to that generated by the allele DQA1*0501) [42,43].

Notably, 30% of Caucasians carry the heterodimer DQ2; thus, HLA typing can be useful to exclude CD (negative predictive value close to 99%). For this reason, there must be other predisposing alleles involved in the development of CD. In the last ten years there has been a great effort aiming to identify additional predisposing loci, mainly using the Genome-Wide Association Study (GWAS) approach [44]. Up to now these studies have identified five more alleles within the MHC region and 40 non-HLA loci (with 58 variants in total) localized on various chromosomes. Taken together, these polymorphisms account for about 50% of the genetic predisposition to celiac disease. It is interesting to note that most of these genes are involved in the immune response, and that polymorphisms in the same genes have been identified in other autoimmune disorders, such as rheumathoid artritis or Crohn's disease [42].

Clinically, HLA typing can be employed when there is doubt or in those patients that have already started GFD (Table 1) while the identification of the heterodimers DQ2 and/or DQ8 can be a useful screening tool in families with an affected subject to exclude subjects from follow-up. However, the use of HLA typing for the screening of at-risk groups should be interpreted with caution.

4.1.2. Celiac disease recommendations

- tTGA IgA is the first-line screening to detect CD in the general population (strong recommendation, high level of evidence).
- Duodenal histology is mandatory in those adults with positive tTGA and in at-risk patients independently of serology. At least 4 biopsies in second part of duodenum and 1–2 in the bulb must be taken (strong recommendation, high level of evidence).
- The Marsh–Oberhuber classification should be used to establish the presence or not of any duodenal atrophy at diagnosis; during follow-up the use of new scores (such as Elli–Ferrero's) should be considered (conditional recommendation, high level of evidence).
- The absence of HLA type-II DQ2 and/or DQ8 excludes the presence or development of CD (strong recommendation, high level of evidence).
- Genetic testing should be considered in those cases where there is doubt and unresponsiveness to the diet (conditional recommendation, moderate level of evidence).

4.2. Wheat allergy

Wheat allergy (WA) is an immune-mediated adverse reaction to the proteins contained in wheat. Depending on the route of allergen exposure, WA is classified into occupational asthma (baker's asthma) and rhinitis; food allergy (FA), affecting the skin, the gastrointestinal tract or the respiratory tract; wheat-dependent exercise-induced anaphylaxis (WDEIA) and contact urticaria. In all these disorders the immunoglobulin E (IgE) antibodies are pivotal [45].

In adults FA to ingested wheat is infrequent: the most common variant in adults is WDEIA, where the symptoms result from the combination of causative food intake and physical exercise (as well as non-steroidal anti-inflammatory drugs or alcohol) [46]. Patients with WDEIA display a range of clinical symptoms, from generalized urticaria, dyspnea, gastrointestinal symptoms to severe anaphylaxis when they perform physical exercise within 3–4 h from wheat consumption. In some cases symptoms can also occur when wheat is consumed immediately after exercise. The most important grain protein associated with WDEIA is ω5-gliadin, even if other gliadins, nsLTP and other grain proteins may be the elicitors [47].

In patients with IgE-mediated wheat FA the clinical cross-reactivity to multiple cereal grains rarely occurs; therefore, the elimination of all grains from the diet in these patients is not recommended and may be nutritionally harmful [48].

The diagnosis of WA is classically based on the clinical picture, skin prick tests (SPTs), in-vitro specific IgE (sIgE) assays and functional assays. SPTs and sIgE in-vitro assays represent the first-level tests, although they are affected by a low predictive value. sIgE in-vitro assays are more sensitive (75–80%) than SPT but less specific (60%), mainly because of the cross-reactivity with grass pollens. Molecular-based allergy (MA) diagnostics can overcome the low accuracy of sIgE in-vitro assays using wheat flour extracts. Many allergenic proteins are involved in wheat and the latest update of the WHO/IUIS Allergen Nomenclature Database describes 21 different well-classified wheat allergens. Unfortunately, until now only ω-5 gliadin (Tri a 19), nsLTP (Tri a 14) and gliadin mixtures are available in the ImmunoCAP™ assay, whereas the ATI (Tri a aA/TI) is available only in the microarray ISAC™ assay. The sIgE to ω-5 gliadin assay is highly reliable and now widely used to identify patients with WDEIA. However, the test is estimated to miss approximately 20% of the cases. In Table 2 molecular allergens involved in wheat food allergy are reported. If SPT and the sIgE assays with flour extracts or molecular allergens are inconclusive, then functional assays are required. They are considered the gold standard for the diagnosis but are accompanied by a risk of severe induced reactions and are impractical in busy practice settings. Functional tests include the double-blind placebo-controlled food challenge (DBPCFC) and the open oral food challenge (Table 1) [49].

4.2.1. The strange case of non-IgE food allergy

Food allergy has been defined as "an adverse health effect arising from a specific immune response that reproducibly occurs on exposure to a given food" [50]. Theoretically, this definition includes all types of immune-mediated reactions, including those caused by the innate immune system. Although one can suppose that food antigen-specific IgG can cause adverse reactions via type-II or type-III hypersensitivity, the position papers from the European and American allergy societies strongly advise against testing for food antigen-specific IgG in the diagnosis of food allergy [51]. On the other hand, type-IV hypersensitivity reactions are involved in some well-established clinical entities in infants for whom wheat may to represent one of the offending foods [52].

The existence of non-IgE mediated food allergies in adults suffering of chronic gastrointestinal symptoms is a much debated question. Recently, some authors have showed that a subset of patients with irritable bowel syndrome (IBS) are affected by multiple food hypersensitivities, wheat being the most frequently involved food [53]. However, we are still at the beginning of understanding the complex immunological mechanisms of non-IgE mediated food allergies and further studies are necessary to confirm the results accomplished to date. Interestingly, some studies have highlighted that a certain food protein (ATI) can induce

Table 2
Molecular allergens involved in wheat food allergy.

Allergen	Biochemical name	FA	WDEIA
Tri a 14	Non-specific lipid tranfer protein	Yes	
Tri a 18	Agglutinin isolectin 1	Yes	
Tri a 19	Omega-5 gliadin	Yes	Yes
Tri a 20	Gamma gliadin	Yes	Yes
Tri a 25	Thioredoxin	Yes	
Tri a 26	High molecular weight glutenin	Yes	Yes
Tri a 36	Low molecular weight glutenin	Yes	
Tri a 37	Alpha purothionin	Yes	
Tri a 40	Chloroform/metanol-soluble (CM) 17 protein [alpha amylase inhibitor]	Yes	
Tri a 41	Mitochondrial ubiquitine ligase acticator of NFKB	Yes	
Tri a 42	Hypotetical protein fron cDNA	Yes	
Tri a 43	Hypotetical protein fron cDNA	Yes	
Tri a 44	Endosperm transfer cell specific PR60 precursor	Yes	
Tri a 45	Elongation factor 1 (EIF 1)	Yes	

FA, immediate wheat food allergy; WDEIA, wheat dependent exercise induced anaphylaxis.

inflammation via the direct activation of the innate immune system [54].

The first step in the diagnosis of non-IgE mediated FA is undertaking an elimination diet. If the resolution of symptoms is achieved, the second step is represented by a DBPCFC (Table 1) [52].

4.2.2. *Wheat allergy recommendations*

- SPTs and sIgE assays represent the first-level diagnostics for WA. However, the test results alone should not be considered diagnostic for FA; MA diagnostics may be considered, but until now only a few wheat allergens are available (strong recommendation, moderate level of evidence).
- Performing an oral food challenge should be considered for the conclusive diagnosis of wheat FA (strong recommendation, high level of evidence).

4.3. *Non-celiac gluten sensitivity*

CD and WA do not explain all the GRD spectrum and most of the patients reporting symptoms after gluten ingestion (in the absence of CD and WA) used to be previously diagnosed with IBS. Nowadays, they are suspected to have NCGS, a "syndrome characterized by intestinal and extra-intestinal symptoms related to the ingestion of gluten-containing food, in subjects that are not affected by CD or WA" [5,7].

Although NCGS is considered common, little is known about its prevalence. Published experiences show a prevalence of 0.6% in primary care centers [55] and 6% in third-level ones [5]. In the UK the self-reported NCGS population prevalence has been estimated at 13% [56].

NCGS is more frequent in 30–40 years old females (F:M ratio 5:1) [57]. Symptoms usually appear within a few hours or days following the ingestion of gluten-containing products: they quickly disappear after gluten withdrawal. Symptoms of NCGS are both intestinal and extra-intestinal. Among the gastrointestinal symptoms, abdominal pain and bloating, diarrhea, dyspepsia and oral aphthosis are frequent. The most frequent (30–40%) extra-intestinal symptoms are: foggy mind, mental confusion after gluten consumption, paresthesia, anxiety, depression skin disorders and headaches [57].

The suspicion of NCGS (frequently reported by patients), should comply with a diagnostic process aimed at firstly ruling CD and WA out. As first-line investigations serological tests for CD and WA should achieve this goal. However, in clinical practice patients seek medical evaluation while already avoiding gluten. HLA typing can exclude CD when GFD is ongoing in case of absence of HLA DQ2/DQ8. If genetic testing is unavailable or inconclusive, a gluten challenge should be planned. In such cases, a two-week period of resumed gluten consumption is considered adequate before CD testing [58].

A clinical history of malabsorptive symptoms, nutrient deficiencies and autoimmune comorbidities has been described to be less frequent in gluten-sensitive subjects than in CD [59]. The presence of such clinical features or a family history of CD in subjects with negative CD serology suggest the execution of duodenal histology to rule out the presence of seronegative CD [25].

Notwithstanding, an overlap between clinical findings may occur as anemia and weight loss have been reported in NCGS patients too; however, since alterations of intestinal permeability and/or absorption have not been proved in these cases [60,61], they seem to be due to patient-driven restrictions of the diet.

Serological biomarkers are not available for NCGS, since the determination of celiac-related antibodies is not sensitive nor specific to NCGS [62]. However, in selected cohorts of NCGS-suspected patients, the serological presence of IgG class AGA has been described in more than half of the investigated subjects [63].

In view of the current lack of serological or histological biomarkers for NCGS, the 3rd International Experts' Meeting on GRD in Salerno, stated that the diagnosis of NCGS should be based upon a standardized gluten DBPCFC recording symptoms with visual analogical scales (VASs) (Table 1) [64]. Accordingly, the diagnostic work-up should start with a six weeks long GFD, symptomatic improvement being monitored through periodic VAS. In case of GFD responsiveness, patients would access to the second step of the protocol in which the confirmation of diagnosis comes from a DBPCFC. The proposed gluten consumption is 8 g per day (i.e., the usual gluten content of Western diets), for a one-week period. Gluten is administered through FODMAPs-free bars with a standardized dose of ATI. Symptoms variations are assessed at the end of each treatment week in order to classify the patient's response.

4.3.1. *The NCGS–IBS connection, the role of other environmental factors and use of symptomatic scores*

The aetiology of IBS, a very common syndrome, is not entirely clear and the long-term inadequacy of the current drug treatment lead patients to seek a variety of alternative remedies, especially of a dietary nature [65]: in fact, about 50% of them attribute their symptoms to adverse food reactions [66]. From this point of view, an important role can be played by wheat as highlighted in the cornerstone article by Verdu et al. in 2009 [67]. These authors referred to "gluten-sensitivity" as a condition of some morphological, immunological, or functional disorder that responds to gluten exclusion. They also quoted that some scientific articles had been published in the previous years which linked GS to IBS [68].

Recent studies have demonstrated that GFD decreases the severity of IBS symptoms with normalization achieved in 50% of all cases;

stool passing frequency decreased and 37% of the patients reported formed stools [68]. In particular, Wahnschaffe et al. [68] showed an association between the effectiveness of the GFD and the presence of the HLA-DQ2 haplotype; in fact, after GFD, the stool passing frequency and gastrointestinal symptom score returned to normal values in 60% of IBS-D patients who were positive and in 12% who were negative for HLA-DQ2.

The first prospective gluten DBPCFC of IBS patients with self-reported GS included 34 patients administered with snacks containing 16-g gluten: it demonstrated a symptomatic worsening in the gluten group compared to placebo [60]. Patients were significantly worse with gluten as to overall symptoms, abdominal pain, bloating, stool dissatisfaction and tiredness.

Carroccio et al.[69] reviewed the clinical records of 920 IBS patients who undertook an elimination diet and wheat DBPCFC with cross-over: 30% of those patients reacted to the wheat challenge reporting abdominal pain, bloating and altered stool consistency. Prospectively, Elli et al.[70] and Di Sabatino et al.[71] examined patients with functional gastrointestinal symptoms by gluten DBPCFC and individuated a subgroup of gluten-responding patients.

The NCGS–IBS relationship can be seen as part of multiple food hypersensitivity, as shown by Fritscher-Ravens et al. via confocal laser endomicroscopy [53]. In line with this study, Vazquez-Roque et al. [72] have demonstrated that gluten-eating IBS has an increase of daily bowel movements associated with the increased permeability of the small bowel.

However, it should be noted that some studies have questioned the role of gluten in this scenario suggesting that fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) are possibly the actual environmental factor inducing gastrointestinal symptoms in these patients [62]. Biesiekierski et al. [62] performed a double-blind crossover trial in 37 subjects with suspected NCGS and IBS; the participants were randomly assigned to groups given a 2-week diet of reduced FODMAPs, and were then placed on high-gluten (16 g gluten/day), low-gluten (2 g gluten/day and 14 g whey protein/day), or control (16 g whey protein/day, used as placebo) diets for one week, followed by a wash-out period. In all the participants gastrointestinal symptoms significantly improved in the run-in period, during reduced FODMAP intake, but significantly worsened to a similar degree when their diets included gluten or placebo. These authors noted also a clear nocebo effect. The authors concluded that there was no evidence of specific or dose-dependent effects of gluten in patients with suspected NCGS being placed on diets low in FODMAPs [73]. A limitation of the above study was to exclude the subjects who had a HLA haplotype DQ2/DQ8 or who showed intra-epithelial inflammation in the duodenal mucosa (Marsh 1 lesion). As the previous studies had demonstrated that these two characteristics were very frequent (>50% of the cases) in NCGS subjects, it has been suggested that Biesiekierski et al. studied a different NCGS cohort and thus, their results cannot be compared with the previous findings [74].

Due to the NCGS–IBS overlap, in NCGS patients the assessment of symptom severity is of paramount importance in order to evaluate the clinical response to GFD and the effect of the gluten DBPCFC.

Because of the similarities between IBS and NCGS, some of the scores used to assess symptom severity and the impairment of quality of life in IBS patients, can also be suggested to assess the clinical burden of NCGS.

The IBS Symptom Severity Score may apply to assess the clinical severity of intestinal symptoms. It is a 5-item questionnaire generating a maximum score of 500. It is very easy to use and very fast to complete. It is considered reliable also in assessing any changes of the gut symptoms severity over time [75].

The IBS quality of life questionnaire allows practitioners to follow their patients longitudinally and can be used in clinical trials to evaluate possible QoL improvements due to specific therapies [76].

The Gastrointestinal Symptom Rating Scale, used to evaluate common symptoms of gastrointestinal disorders, has been specifically conceived by Catassi et al.: it evaluates lower and upper digestive symptoms and extra-intestinal NCGS manifestations by means of VAS. It is a self-administered questionnaire, which has been proposed for use in clinical practice and for research purposes, even if it still needs scientific validation [64].

In conclusion, the current data point to at least an overlap between IBS and NCGS; it is likely that about 25–30% of IBS patients, especially those with diarrhea-type IBS carrying the HLA haplotypes DQ2/DQ8, are with NCGS. A concurrent role of FODMAPs or other antigens can be considered and should be further evaluated.

4.3.2. NCGS recommendations

- The initial assessment in NCGS diagnosis must aim to exclude CD and WA. With regard to patients responding to GFD, DBPCFC is mandatory to confirm diagnosis as described in the Salerno criteria (strong recommendation, high level of evidence).

5. Withdrawal of gluten from the diet

The GFD is characterized by a combination of naturally occurring gluten-free (GF) foods, common GF cereals (e.g., rice and corn), pseudocereals (i.e., amaranth, quinoa and buckwheat), and minor cereals (e.g., millet and sorghum). Besides these foods, GFD is commonly supplemented with GF substitutes of bread, cookies, pasta and other cereal-based foods made by either ingredients that do not include gluten-containing cereals (e.g., wheat, rye, barley) or ingredients from cereals that have been specifically processed to remove gluten. Foods not allowed in any GFDt include: (a) all types of bread and food prepared with flour from all the varieties of wheat; (b) food that contains wheat, or derivates of gluten used as thickeners; (c) medicinal products that use gluten as a binder for pills or tablets [77].

Regarding oats, its use in GFD remains controversial as the contamination with prolamins of other cereals is frequent and some clinical and experimental studies support the view that a subgroup of celiac patients may be intolerant to pure oats [78]. Thus, the status of oats in the international GF Standard has been reassessed (Codex Alimentarius Commission, 2008). Yet, oats are kept in the category of gluten-containing cereals, but a footnote has been added which states: “oats can be tolerated by most but not all people who are intolerant to gluten. Therefore, the use of oats not contaminated with wheat, rye or barley in foods covered by the standard may be determined on national level”.

Several studies have shown that the dietary habits of celiac subjects did not differ basically from those of the general healthy population [79–81]. However, because the derivates of gluten-rich grains are important sources of nutrients in the general diet [82], their exclusion can potentially affect the nutritional adequacy of a celiac patient’s diet and in turn have a major effect on their nutritional status if such foods are not replaced with balanced alternatives. Although it remains difficult to date to draw conclusions about the nutritional adequacy of a GFD regimen because of conflicting study results, several studies have pointed out that celiac patients have a different intake of macro- and micro-nutrients as compared to healthy control subjects. Among the macronutrients, the major concern regards the higher intake of total and saturated fats recorded in the celiac patient’s diet than in that of healthy control subjects [83]. This has raised concerns also about the lipid content of commercial GF foods [84,85]. This imbalance in the daily fat intake may lead to overweight and obesity in celiac patients, especially children and adolescents [85,86].

Even though not all the studies reported a different fiber intake between celiac patients and the matched control group [79,80], it has been suggested that GFD is inadequate in terms of fiber content [81,87–89]. This has been attributed to a decreased consumption of grain products, exacerbated by the fact that many GF foods are made with starches or refined flours with low fiber content. However, in recent years, manufacturers have improved the fiber content of breads, flour mixes and other GF products, as hydrocolloids and gums having colloidal properties are used for replacing the gluten network and improving the technological properties of GF products [84].

Regarding micro-nutrients, lower levels of vitamins, such as folate, niacin, vitamin B12, vitamin D were described in celiac individuals than in control subjects [79,81,88]. Moreover, such a low intake has the consequence that, in many cases, celiac patients did not meet the recommended intake of these vitamins [80]. Such a low intake can partly be due to the low content of folates in starches and low-protein flours (e.g., corn and rice), commonly used as main components of GF products [84]. Similarly to vitamins, some studies reported lower intakes of several minerals in celiac patients than in the controls and inadequate intakes against the current recommendations. For instance, as compared to control subjects, the daily intake of iron was significantly lower in female and male celiac patients [80,89] and that of calcium was significantly lower in female celiac patients [81,88]. Lee et al. [90] suggested that the inclusion of alternative GF grains (e.g., oats and quinoa) can significantly increase the nutritional quality of GFD including the levels of iron and calcium.

5.1. Gluten-free diet recommendations

- GFD does not present side effects and is usually balanced and safe (strong recommendation, high level of evidence).
- Supplementation therapy is not routinely needed during GFD (strong recommendation, high level of evidence).

6. Conclusions

GRD currently pose as a challenging issue for gastroenterologists, especially when facing with patients with functional symptoms. In such a case the different options should be considered and a specific diagnostic roadmap established whenever possible. The starting point is to establish the dietetic status of the patient and, in particular, if he is eating gluten due to the high percentage of subjects following a GFD or a poor-gluten diet without a medical advise. Sometime this step could be difficult and thus the use of a specific alimentary questionnaire is suggested [91]. Once the dietetic status of the patient has been ascertained, clinician should follow two different roadmaps. For patients following a gluten containing diet the adoption of screening tests for CD (tTGA) and WA (sIgE) are mandatory. For patients following a GFD, and usually unwilling to reintroduce gluten, HLA typing could represents a first step to establish the CD risk. In case of absence of the CD genetic background and after the exclusion of WA, the patient could be blindly challenged with gluten in order to establish the presence of a NCGS. When facing with a CD compatible genetic background a low dose gluten challenge (at least 3 g of gluten per day for 2 weeks) should be administered in order to return to a “normal diet” status [58]. If all the preliminary tests for CD and WA result negative and symptoms improve after a correct GFD, NCGS can be suspected and appropriately looked for with a standardized double (or at least single) BPCFC. Nowadays, in absence of biomarkers, the distinction between NCGS and non-IgE allergy appears more academic than real.

If in case of positivity of one of the above mentioned tests a GRD is proven or highly suspected, an important clinical question is represented by those patients (many) without any tangible proof of a connection between their symptoms and gluten ingestion but reporting an important improvement of their clinical picture when following a GFD. These patients are usually difficult to face due the lack of answers to their questions; however, clinicians should follow them in order to avoid their appeals to unscientific tests and potentially dangerous diets without medical control.

A possible diagnostic flowchart for GRD is reported in Fig. 2.

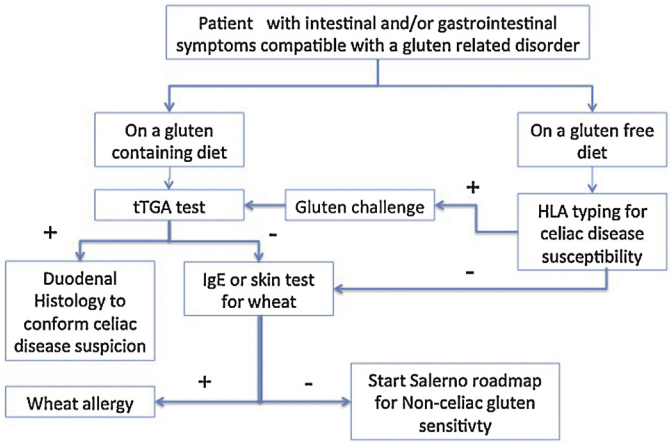

Fig. 2. A proposed flowchart for patients with suspected gluten-related disorder.

Conflict of interest

Danilo Villalta, Donatella Barisani, Flavio Valiante, Carolina Tomba, Antonio Carroccio, Massimo Bellini, Marco Soncini, Renato Cannizzaro and Gioacchino Leandro have no conflict of interest.

Luca Elli and Nicoletta Pellegrini are scientific board members of the Dr Schaer Institute.

Luca Elli, Maria Teresa Bardella, Leda Roncoroni and Carolina Tomba are the inventors of a commercially available diagnostic kit for NCGS.

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