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Non-celiac gluten sensitivity
-A real disease?-

Inês Cristina Ferreira Fernandes
N.º 12907
fernandes.ines@campus.ul.pt

Orientador: Dr. Manuel Martins Alves

Professor responsável da Unidade: Professor Doutor José Velosa

Clínica Universitária de Gastroenterologia do Hospital de Santa Maria
(Centro Hospitalar Lisboa Norte, EPE)
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Abstract

The past years have seen an increase in the use of a gluten-free diet (GFD) outside a diagnosis of coeliac disease (CD) or wheat allergy (WA). This trend has led to the identification of a new clinical entity termed non-celiac gluten sensitivity (NCGS), which has clinical features that overlap with those two previously mentioned. The onset of symptoms in patients with NCGS can occur within hours or days of gluten ingestion and the prevalence of NCGS is estimated to round 6%. The pathophysiologic process is thought to be through an innate immune mechanism, whereas CD and WA are autoimmune- and allergen-mediated, respectively. However, NCGS is not without its controversies and uncertainties, in particular pertaining to whether it is gluten or non-gluten components of the grain evoking symptoms; evidence suggests that dietary triggers other than gluten, such as FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols), as well as amylase-trypsin inhibitors may be implicated. Moreover, emerging evidence suggests that NCGS can be associated with other gastrointestinal pathologies such as irritable bowel syndrome (IBS).

Currently, no specific laboratory biomarkers are available to diagnose NCGS. Exclusion of CD and WA is necessary in the evaluation of a patient suspected to have NCGS, followed by an elimination diet of gluten-containing foods to evaluating whether health improves and a monitored open challenge to document the recurrence of gastrointestinal and/or extraintestinal symptoms associated with the reintroduction of gluten. However, one should bear in mind that most patients suspected to have NCGS have already initiated a GFD at the time of an evaluation.

Additional research studies as well as novel techniques that might help diagnose NCGS in the future are essential to promote a better understanding of NCGS and to identify a biomarker to facilitate diagnosis and patient selection for proper management.

Abbreviations and Acronyms

GFD: gluten-free diet; CD: coeliac/celiac disease; WA: wheat allergy; NCGS: non-celiac gluten sensitivity; FODMAP/FODMAPs: fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; IBS: irritable bowel syndrome, GS: gluten sensitivity; HLA: human leukocyte antigen.

Resumo

Nos últimos anos, têm-se observado uma grande adesão a uma dieta sem glúten, em situações que vão para além da doença celíaca ou alergia ao trigo. Esta tendência tem levado ao reconhecimento de uma nova patologia, denominada sensibilidade ao glúten não celíaca, que apresenta características clínicas que se sobrepõem às duas outras condições. A manifestação de sintomas nos doentes com sensibilidade ao glúten não celíaca, pode surgir dentro de horas ou dias após a ingestão do glúten e, estima-se que a prevalência da doença ronde os 6%. Pensa-se que a sua fisiopatologia se deve a um mecanismo de imunidade inata, enquanto que na doença celíaca e na alergia ao trigo, o mecanismo responsável é autoimune e mediado por alérgeno, respetivamente. No entanto, a sensibilidade ao glúten não celíaca apresenta várias controvérsias e incertezas, nomeadamente sobre se é o glúten ou outros elementos dos cereais que provocam a sintomatologia. Diversas evidências sugerem que existem outros fatores responsáveis além do glúten, nomeadamente os FODMAPs (Oligosacarídeos, Dissacarídeos, Monosacarídeos e Polióis Fermentáveis) e os inibidores amilase-tripsina. Além disso, estudos recentes sugerem que a sensibilidade ao glúten não celíaca pode estar associada a outras patologias gastrointestinais como o síndrome do intestino irritável.

Atualmente, não há biomarcadores laboratoriais específicos disponíveis para diagnosticar a doença. Na avaliação de um doente com suspeita de sensibilidade ao glúten não celíaca, é imperativo excluir a doença celíaca e a alergia ao trigo, assim como instituir uma dieta com a eliminação dos alimentos que contêm glúten, para se avaliar se existe alguma melhoria clínica e, posteriormente, fazer a reintrodução do glúten, para documentar a eventual recorrência de sintomas gastrointestinais e extra-intestinais. No entanto, é importante salientar que, na altura da avaliação, a maioria dos doentes com suspeita da doença já terá iniciado previamente uma dieta sem glúten.

Estudos de investigação adicionais, assim como a aposta em novas técnicas de diagnóstico da doença, são aspetos cruciais a desenvolver no futuro. A identificação de biomarcadores para um diagnóstico definitivo e rigoroso e uma melhor compreensão da doença, são elementos fundamentais para otimizar os cuidados aos doentes.

The set: consumption of wheat in the world

Consumption of wheat has increased exponentially over the last half-century in the Western world, becoming a more desirable grain than rice in large populations in China and India.[1]

10,000 years ago, with the worldwide spread of the Mediterranean diet, the native human diet of fruits, vegetables, fish and meat was for the first time introduced to a higher exposure to grain and gluten-containing foods.[2] Nowadays, it is estimated that the mean daily gluten intake rounds 20 g and even higher in some Mediterranean countries.[3, 4]

However, there has been a mistrustful spreading perception among the general public that eating food with wheat or other gluten-laden grains is harmful[5] and may lead to weight gain, obesity, depression, anxiety, arthritis and autism.[6] Media and general public interest in this topic is overwhelming. This is evident by the number of Google to Pubmed hits (~5000:1) for gluten-free diet (GFD).[5]

Given the recent increase of the gluten-free market worldwide,[7] an increasingly larger proportion of the population, mainly in Europe, Australia, New Zealand and the United States[2] is now either avoiding dietary wheat foods that contain gluten or eliminating gluten entirely from their diets.[8] Every major change in our diet carries with it the possibility of unforeseen risks. Wheat avoidance can have a huge impact on nutritional intake (without appropriate dietary advice) and quality of life, and studies investigating these are of the utmost urgency.[9]

There is a clear need of “separating the wheat from the chaff”.[7]

What is gluten?

‘Gluten’ is a complicated term by itself. It refers to the main protein complex of wheat and other cereals, including barley, rye and spelt.[10]

It also refers to the glue-ish mass with well-known baking properties: it is often added to improve product characteristics[4] as it provides viscosity and elasticity, forming a dough from its washing starch granules (which is named vital gluten) and helping food products to rise and keep their shape and chewy texture.[1] Depending on the thoroughness of washing, the dry solid has 75–85% protein and 5–10% lipids, being the remainder starch and non-starch carbohydrates.[11]

Gluten contains hundreds of proteins and protein components and they have been conventionally divided according to their functions into soluble gliadins (that contain monomeric proteins which provide viscosity and extensibility of the dough system) and insoluble glutenins (containing aggregated proteins which contribute to its strength and elasticity).[12]

Amongst its other uses, gluten can also be used in cosmetics, hair products, and other dermatological preparations.[13]

Development of gluten-related pathology

Many factors might have contributed to the development of gluten-related pathology[9] such as variations in individual diets with regard to a higher intake of gluten-containing foods. The amount and types of wheat consumed, along with the higher gluten quantity in bread and bakery products, due to the reduced time of dough fermentation, is something to be conscious about, although there is no clear evidence of an increase in the gluten content of wheat during the 20th century.[1]

Nowadays, most of the wheat products we consume are made from modern wheat varieties bred after the ‘‘Green Revolution” (in the 1970s) and the introduction of dwarf genes led to the development of shorter straw lines.[14]

The development of new types of wheat with high amounts of toxic gluten peptides was triggered by the mechanization of farming, agronomic practices (such as nitrogen fertilization) and the growing industrial use of pesticides,[15] having led to substantial gains in productivity and technological quality.[16]

The variation in dough rheology and bread-making performance among wheat varieties is largely determined by its protein content differences,[17] depending on specific gliadins and high molecular weight glutenin subunits.[18] Any systematic increase in the protein content of wheat might also play a role in non-celiac gluten sensitivity (NCGS), as the average slice of bread weighs approximately 40 g and contains about 2.4 g of protein, 1.8 g of which would be gluten.[1]

Highly sensitive diagnostic tools for gluten-induced disorders have also gradually improved over time[9], allowing for the identification of new cases and one cannot also rule out the process by which the immune system switches from tolerance of wheat gluten protein to intolerance, describing this increasingly frequent presentation to primary care and gastroenterologists practitioners.[19]

Gluten-related pathology and the appearance of NCGS

The term ‘gluten-related disorders’ is complicated and refers to the suggested umbrella term for all diseases triggered by gluten.[20] The development of adverse reactions against gluten, is supposed to be concomitant with the very moment humans started its ingestion[21] although in the last 10 years it has become clear that there are distinguished clinical conditions related to gluten consumption.[22]

Each gluten-related disorder exhibits a unique pathophysiological response to gluten ingestion, though they may maintain considerable overlap in their clinical presentation.[2] Among them are: wheat allergy (WA), an autoimmune form that includes coeliac disease (CD), dermatitis herpetiformis, gluten-related ataxia and peripheral neuropathy[9] and NCGS, which is possibly immune-mediated and currently the most common.[22]

Hemmings, Ellis and Linaker incorrectly used the term NCGS for the first time in 1978, in WA patients that presented abdominal pain and diarrhea, which remitted after gluten withdrawal.[24] Only in 1980 was this entity considered a

syndrome[25] after several cases in which non-CD or WA patients' symptoms dramatically disappeared after gluten withdrawal from diet.

In 2010, Sapone et al., described the clinical and diagnostic features of this condition and a First Expert Meeting was held in London in 2011, to develop a consensus on new nomenclature and classification of gluten-related disorders. Since then, many names have been suggested for this disorder, such as gluten sensitivity (GS), gluten hypersensitivity or non-celiac gluten intolerance.[26] The "labeling" of this disorder has been a matter of massive debate among the panel experts, reflecting the poor knowledge of the pathophysiology of this condition,[7] and only after a Second Expert Meeting on GS, held in Munich in 2012, was its current name, NCGS, provisionally defined in order to avoid confusion with similar conditions.[7, 8] Nonetheless, its definition still requires refinement in the future.[27]

NCGS apparently involves neither the allergic nor autoimmune mechanisms nor a specific genetic background. It can be diagnosed in those patients with a reaction to gluten, with a confirmed absence of allergic or autoimmune mechanisms after appropriate laboratory testing such as celiac-specific antibodies and classical celiac villous atrophy lesions in the duodenal mucosa.[28] It may be caused by improper immune responses, intolerance to poorly digestible and fermentable substances in wheat, or a combination of these.[10]

Wheat allergy

Amongst gluten-related pathology, WA is the least common of them all, affecting about 1% if the population and being less prevalent than CD.[8] It is defined as an acute anaphylactic condition,[10] an adverse immunological reaction (immunoglobulin E- and non-immunoglobulin E-mediated) to gluten and other proteins found in wheat.[1] It is the cross-linking of immunoglobulin E by repeat sequences in gluten peptides (for example, serine-glutamine-glutamine -glutamine-(glutamine-)proline-proline-phenylalanine) that triggers the release of chemical mediators, such as histamine, from basophils and mast cells[12] and diagnosis is made by skin prick test and invitro immunoglobulin E assays. Depending on the route of allergen exposure and the underlying immunological mechanisms, it clinically manifests as either classic food allergy (affecting the skin, gastrointestinal and respiratory tract), wheat-dependent exercise-induced anaphylaxis, occupational asthma (so-called baker's asthma) and rhinitis, and contact urticaria.[5, 8, 23]

Coeliac disease

CD is an autoimmune inflammatory disorder of the small intestinal mucosa,[10] with a strong genetic basis, positively correlated with specific HLA (human leukocyte antigen)-DQ gene pairs and potentially triggered by other environmental factors.[29] It affects roughly 1% of the general population[30]

and it has specific serologic markers, comprising immunoglobulin A antibodies to the enzyme tissue transglutaminase (IgA tTG2) or immunoglobulin A and immunoglobulin G deamidated gliadin peptide antibodies.[10, 31] Endomysial antibody testing (IgA endomysial) also seems to have a high predictive value for the future development of CD.[29, 32, 33]

Its pathophysiology stars once ingested gluten is degraded into relatively proline-rich fragments, binding to disease predisposing HLA molecules, that bound to specific CD4 T cells,[34] leading to intestinal villous atrophy and increased intestinal permeability. Gastrointestinal symptoms include those associated with malabsorption in the small intestine of the affected individual, such as chronic diarrhoea, bloating, abdominal distension, chronic constipation and weight loss.[35]

Prevalence of non-celiac gluten sensitivity

Its frequency in the general population is still uncertain and difficult to estimate, as many patients are currently self-diagnosed and start a GFD without any medical advice or consultation.[36] Although there is evidence of NCGS cases in adults since the 20th century, the first cases in children were only described in 2012.[37]

Epidemiological data has been generated to help establish the magnitude of the problem and according to studies that cover approximately the last half of the 20th century,[1] it seems clear that in recent years it has rapidly increased in adults[3] and in children.[37] From a 2010 report with a possible prevalence of NCGS of 0.55%,[30] it is now estimated that the real prevalence can be as high as 6%,[10] affecting more females (male-to-female ratio of 1:3) and young/middle age adults, in the absence of identified risk factors.[30, 38, 39] However, when explored in a selected setting of patients affected by irritable bowel syndrome (IBS), the prevalence, as proved through a double-blind placebo-controlled challenge, reached 28%.[23]

Pathophysiology of non-celiac gluten sensitivity

Antigen-presenting cells

NCGS has many grey areas concerning its pathophysiology and it remains largely undetermined, but it is recognized that gluten and its related peptides, namely undigested or partly digested gliadin, can stimulate dendritic cells and induce an innate immune response.[12]

This enhanced innate immune response is immediate and fast, including both cellular and humoral components, affecting a wide range of human cell functions.

Reduced intestinal permeability

However, unlike CD there seems to be no enhanced intestinal permeability and levels of tight junction protein levels between epithelial cells remain intact.[30] A significant reduction in T-regulatory cell markers has also been

found in NCGS compared to CD patients, which can be interpreted as a reduced activation of adaptive immunity.[9, 21]

Furthermore, NCGS subjects who go through a lactulose/mannitol absorption test display a reduced intestinal permeability, which goes in line with the hypothetical reduced permeability of the intestinal barrier in NCGS.[9] This hypothesis has already been questioned by Biesekierski et al., who despite showing that patients with NCGS truly develop symptoms when eating gluten,[20] did not find any significant difference in the intestinal barrier function of two randomly treated groups of NCGS patients (one challenged by gluten, the other by placebo) using the dual sugar absorption test.[36] Controversially, Vazquez-Roque et al. have documented increased intestinal permeability in a subgroup of HLA-DQ2/DQ81 NCGS patients with IBS, diarrhea and GS.[9]

Adaptive immunity markers

Also, several studies have confirmed the central role of adaptive immunity in the development of CD by showing systemic and mucosal expression of cytokines associated with Th1 and Th17 responses, contrasting with NCGS patients where adaptive immunity markers such as IL-17A, IL-6, interferon-c, IL-17 and IL-21 have not been increased in intestinal biopsies. Moreover, NCGS patients have an increase in the α and β classes of intraepithelial lymphocytes with no increase in adaptive immunity-related gut mucosal gene expression.[21]

These studies suggest an important role of the intestinal innate immune system in the pathogenesis of NCGS without an adaptive immune response.[30]

HLA-DQ2 and -DQ8

Unlike duodenal mucosa from CD patients exposed to gliadin *in-vitro*, intestinal mucosa from NCGS patients do not express markers of inflammation. New insights to explain the pathogenic mechanisms of NCGS have been provided by HLA-DQ8 transgenic mice sensitized to gliadin hypothesizing that neuromuscular dysfunction and gut microbiota could have a role in gluten-induced symptoms.[9] Genetic predisposition to CD has also been suggested as a factor for NCGS where patients carrying the HLA-DQ2 allele, but without villous atrophy on duodenal biopsy, have been shown to symptomatically improve on a GFD.[40]

Amylase–trypsin inhibitors

Furthermore, recent studies have shown that gluten and its related proteins are not the only triggers of NCGS and that other molecules also contained in wheat likely play a relevant role in causing this syndrome, namely amylase–trypsin inhibitors.[41] Another component in wheat, wheat lectin agglutinin, has also shown increased intestinal permeability and potential activation of the immune system, but needs further clinical studies to establish the its pathogenic role in humans.[40]

Opioid-like activity of gluten

Others studies have also shown that another factor that could play a role in NCGS development could be the opioid-like activity of gluten. Gluten proteins can

mimic some of the effects of opiates by altering the intestinal transit time in healthy volunteers in a naloxone-reversible manner.[9]

Multiple food hypersensitivities and FODMAPs

Many patients with NCGS display multiple food hypersensitivities and some authors believe that this could in part be related to the physiological effects of FODMAPs: Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols.[40]

A diet rich in poorly absorbed and fermentable short-chain carbohydrates induces intestinal lumen distension (osmotic activity of liquid and gas occurs due to their small molecular size) and rapid fermentation in the colon, leading to functional and unspecific gastrointestinal symptoms (especially bloating).[21, 40]

Therefore, NCGS might be confused with FODMAP sensitivity, and gluten might be only a cofactor (or a confounder) when looking for the origin of gastrointestinal symptoms.[21, 42, 43] In a study, many self-perceived NCGS patients still had significant symptoms despite a GFD and there was significant improvement of gastrointestinal symptoms by following a low FODMAP diet for two weeks.[40] In fact, the literature suggests that FODMAPs and not gluten *per se* are the triggers of gastrointestinal symptoms in patients that fit most of the proposed NCGS definitions. Interestingly, wheat, rye, and barley are food sources of FODMAPs and should be avoided in FODMAP sensitive individuals.[33]

However, functional gastrointestinal symptoms in NCGS as well as other disorders, could also be partly related to food additives, such as glutamates, benzoates, sulfites and nitrates, which are added to commercial products to improve flavour, colour and for preservation. In general, the stronger the flavor of the food, the higher the chemical content will be. Food chemicals add strong afferent stimuli to the enteric nervous system and food-induced modifications can target the brain through the microbiota-brain-gut axis.[42] When patients display visceral hypersensitivity, normal physiological stimulation by such chemicals may result in exaggerated effector responses leading to luminal distension. Consequently, a low-FODMAP diet can be a realistic and efficacious attempt for improving gastrointestinal symptoms in NCGS patients.[9, 40]

Nocebo effect

Finally, it must be emphasized that in some circumstances NCGS is an imaginary ailment caused by the nocebo effect (the adverse reaction experienced by a patient who receives such a therapy) of gluten ingestion. This possibility in patients with a self-diagnosis of food hypersensitivity has clearly been established by double-blind trials. The placebo effect of the elimination diet is generally regarded as superior to that of drug treatment.[9]

A real disease?

Symptoms alone cannot reliably differentiate WA and CD from NCGS, as there is often substantial overlap in symptoms between conditions,[31] hence the need

to perform serological and histological objective tests as well as HLA-DQ typing.[44]

Before 2000, only two papers reported of the possible existence of NCGS. In 1980, in a double-blind crossover trial, Cooper et al. reported six of eight GS cases in women complaining of abdominal pain, bloating and diarrhea, without any serological or histological evidence of CD.[25, 26] Later in 2000, Kaukinen et al. reported that 63% of 94 adults, that did not satisfy the diagnostic criteria for CD and WA, complained of abdominal symptoms after gluten ingestion and benefited from a GFD.[45]

In 2008, another study showed that after gluten withdrawal, gastrointestinal symptoms improved in 64.7% of CD patients and 75.0% of non-CD patients, followed by clinical exacerbation in 71.4% of CD patients and 54.2% of non-CD patients with gluten re-introduction.[46]

Gastroenterologists and allergologists at the time, without evidence to explain the symptoms, did not treat these patients and advised them to continue integrating gluten into their diet, as gluten was not thought to be the cause of their condition. In most cases, patients were regarded as suffering from mental disorders and were frequently referred to psychiatrists. Even nowadays, there is a tendency by some to attribute NCGS presentation to placebo effect or somatization, particularly as the diagnosis is based on subjective self-reporting by patients.[39, 47] Many patients are deeply influenced by the fact that GFD is the latest diet craze embraced by many celebrities and there is a possibility of displaying an imaginary syndrome with a subjective sensation of improvement due to the placebo effect of gluten withdrawal.[5, 9] The self-reported prevalence of NCGS was 13% in a United Kingdom population questionnaire-based survey, with less than 1% subjects having a medical diagnosis of the condition.[23]

There has also been presented an association with fibromyalgia and diarrhea-predominant IBS.[12] However, patients' symptoms significantly improved when similar dietary changes were made[38], with no difference regarding neuropsychiatric symptoms[48, 49] such as personality traits, anxiety and depression, therefore suggesting NCGS to be a credible physical diagnosis.[42, 47, 50]

Placebo effect induced by gluten withdrawal cannot be unambiguously excluded[51] although studies with double-blind placebo-controlled challenge trials already showed recurrence and a significant worsening of clinical symptoms in gluten vs. the placebo group.[27, 36]

Although NCGS cannot be definitively diagnosed at this time in the absence of objective specific markers and cases remain highly presumptive[9], a diagnosis of NCGS should be considered when patients[41] experience symptoms upon exposure to foods containing gluten, that remit after gluten exclusion from the diet.[9]

Signs and symptoms

The majority of symptoms experienced by these patients are subjective[5] and may resemble those associated with CD and WA.

Gastrointestinal symptoms include abdominal pain, bloating/abdominal distension, flatulence, nausea, diarrhea, but there is a prevalence of extraintestinal symptoms. It is common to find general symptoms such as headaches, migraines, bone or joint pain, muscle cramps, tingling and/or numbness in the legs, arms or fingers, brain fog, chronic fatigue and weight loss; dermatological symptoms such as eczema, erythema or rashes; haematological symptoms like anemia; dental signs such as chronic ulcerative stomatitis; behavioural disturbances such as mood changes, depression or hyperactivity.[5, 8, 12, 52]

Several studies suggest a relationship between NCGS and neuropsychiatric disorders, particularly autism and schizophrenia[9, 53, 54], reporting patients the development of severe neurological symptoms like hallucinations and cerebellar ataxia, although these lack strong evidence.[55, 56]

It is yet to be proven if and how gluten has direct causal effects on extra-intestinal symptoms in patients without CD.[40]

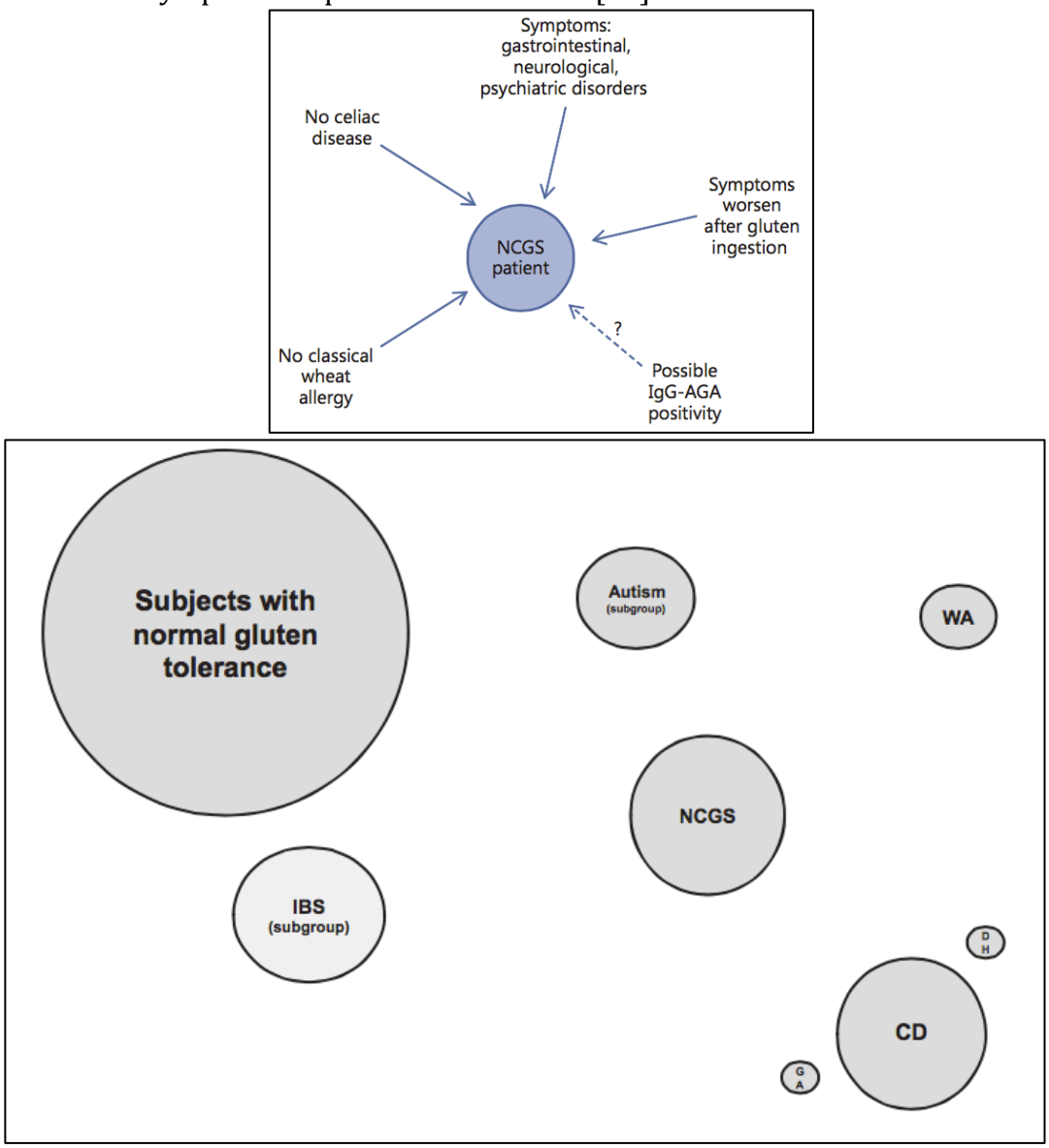

Figure 1 - Key characteristics of patients with NCGS and the astronomical system of gluten-related disorders. Beyond the autoimmune planet of CD, and related satellites, i.e. dermatitis herpetiformis (DH) and gluten ataxia (GA), other identified planets include WA, NCGS, and subgroups of patients affected with autism and IBS **[57]**

Diagnostic criteria & management

There have been proposed several algorithms for the differential diagnosis of gluten-related disorders, including WA, CD and NCGS. Based on a combination of clinical, biological, genetic and histological data, it is possible to differentiate these three conditions.[12]

To this moment, there are no specific laboratory biomarkers for NCGS, which is still a major limitation of clinical studies, making it difficult to differentiate NCGS from other gluten related disorders. Therefore, it is essentially a diagnosis of exclusion[22] and the diagnosis relies on the accurate assessment of clinical features along with the exclusion of WA and CD[9] followed by an open challenge to evaluate whether health improves with the elimination or reduction of gluten from the patient's diet.

Patients suspected of suffering from a gluten-related disorder should preliminarily undergo a full clinical and laboratory evaluation to exclude both WA and CD, using appropriate tests performed under a gluten-containing diet.[42] If a patient with true CD has been following a GFD, they may have reversals in pathologic and laboratory findings, making it harder to confirm a diagnosis.[28] In fact, the main bias is caused by the absence of a clear diagnostic flowchart for NCGS as all the studies simply enrolled patients clinically responding to a period of gluten-free dieting without excluding the placebo effect.[40]

		Pathogenic mechanisms	Morbidity	Genetic background	Antibodies in serum	Atrophy of duodenal villi and histology	Symptoms	Symptoms onset and severity	Time of GFD duration	Dietary exclusion	Mortality
Gluten Related Disorders	**Coeliac Disease**	Autoimmune: disturbances in the acquired immune response to gluten depend on the combination of HLA-DQ2 and HLA-DQ8	1%	In 95%: HLA-DQ2 or HLA-DQ8	tTG, EMA, DGP, AGA primarily in the IgA class, less frequently in the IgG class	Present. Marsh I-III (III more prevalent)	Intestinal and extra-intestinal	Delayed. On a spectrum: mild to severe	Lifelong	All gluten containing products must be avoided	Increased
	Wheat allergy	Allergic: IgE-dependent reactions, prevalent Th2 combination in the immune response to wheat allergens	1%	In 100%: atopy	IgE for wheat and IgE for u5-gliadin (in anaphylaxis) In 25%: IgG-AGA	May be present. Marsh 0,I		Immediate. On a spectrum: mild to severe	The average of 6 years, individual; lifelong in anaphylaxis. In WA only wheat is eliminated from the diet	All wheat-based products must be avoided	Increased
	Non-celiac gluten sensitivity	Not autoimmune, not allergic: unknown, probably the disturbances in the primary immune response to gluten	Possibly 6%	In 50%: HLA-DQ2 or HLA-DQ8	In 50%: IgG-AGA	Absent. Marsh 0,I,II		Delayed. Usually mild	Unknown	May be able to tolerate gluten in small amounts	Unknown

Table 1 - Comparison between gluten related disorders. tTG (tissue transglutaminase), EMA (endomysium antibodies), DGP (deamidated gliadin peptide), AGA (antigliadin antibodies), IgA (immunoglobulin A), IgG (immunoglobulin G), IgE (immunoglobulin E).

Testing for serum immunoglobulin E antibodies to gluten and wheat fractions as well as skin-prick tests should rule out WA, whereas CD must be excluded by the absence of specific serological tests, such as immunoglobulin A tTGA, immunoglobulin A EmA and immunoglobulin G deamidated gliadin peptide antibodies. Furthermore, a duodenal biopsy is highly recommended in suspicious patients when on a gluten-containing diet, in order to rule out a CD diagnosis, because in 1–2% of the total cases of CD, the celiac serology is negative.[58]

Compared with CD patients, around 60% of NCGS patients have a normal histology of duodenal mucosa with a much lower number of intraepithelial lymphocytes, there is not an increase of T-cell receptor γ/δ intraepithelial lymphocytes, and class II MHC haplotype HLA-DQ2 and/or HLA-DQ8 are present in only about 50% of patients (versus being present in almost all CD patients).[12]

The only serological marker found in patients with NCGS is the first-generation antibody to gliadin (AGA) and studies report an AGA immunoglobulin G prevalence of 56% in NCGS patients and a much lower prevalence of 8% regarding AGA immunoglobulin A.[9, 59, 60]

However, it cannot be considered a reliable marker as AGA immunoglobulin G disappeared in most NCGS patients within 6 months of initiating a GFD and remained positive in about half of CD patients after gluten withdrawal. A hypothesis that immunological memory might be active in CD but not in NCGS has been suggested so far.[9]

The marker is also not specific for the condition, as they can be found in many others, such as autoimmune liver diseases, IBS, connective tissue disorders and even blood donors.[9]

Recently, a NCGS Diagnostic Protocol has been suggested. Its aim is to assess the clinical response to an elimination diet of gluten-containing foods, the GFD, evaluating whether health improves with the elimination or reduction of gluten from the patient's diet, and to measure the effect of reintroducing gluten after a period of treatment with the GFD for a potential management.[47]

The diet can only be started in the patient who is on a normal, gluten-containing diet for at least six weeks, although a simplified/shortened diagnostic procedure may be adopted in patients that are already on the GFD when first seen at a specialty clinic.

Baseline symptoms are established through a modified version of the Gastrointestinal Symptom Rating Scale (GSRS), which also includes extra-intestinal NCGS manifestations. The patient identifies one to three main symptoms that will be quantitatively assessed using a Numerical Rating Scale (NRS) with a score ranging from 1 (mild) to 10 (severe), at weeks -2, -1 and 0.

At time 0 a strictly gluten-free baseline diet is started after detailed explanation (preferably by a dietitian). Although an improvement of the symptoms is expected shortly, a prolonged observation is needed to properly investigate the causal relationship, particularly for fluctuating symptoms (e.g., headache).

At least six weeks of verified GFD data should be recorded (from week 0 to 6), identifying one to three main symptoms weekly. Responders are patients who

fulfill the response criteria (>30% reduction of one to three main symptoms or at least 1 symptom with no worsening of others) for at least 50% of the observation time (i.e., at least three of six weekly evaluations).

Although a symptomatic response to a GFD alone does not differentiate NCGS and CD, nor should it be used to diagnose CD, [28] this symptom improvement or cessation, as well as their reoccurrence attributable to the absence or presence of dietary gluten, is highly suggestive of gluten-related disorders, namely NCGS.[9]

The diagnosis of NCGS is excluded in subjects failing to show symptomatic improvement after six weeks of GFD and in these patients it should be investigated other possible causes of IBS-like symptoms, such as intolerance to FODMAPs or small bowel bacterial overgrowth.

After a GFD, an amount of 8 grams (with pro-inflammatory factor amylase-trypsin inhibitors of at least 0.3 g) should be used for the challenge, daily, in the form of a muesli bar, bread, muffin or any other cooked baking good, homogeneously distributed with gluten. The placebo vehicle must be completely gluten-free and both must be undistinguishable in look, texture and taste, FODMAPs free and balanced in fibers, carbohydrate, fat and possibly protein content.

The gluten challenge includes a one-week challenge followed by a one-week washout of strict GFD and by the crossover to the second one-week challenge. The duration of the challenge period may occasionally be longer than a week in patients showing fluctuating symptoms.

The same questionnaire is self-administered and filled, daily, during the first seven-day challenge (or less if symptoms prevent completion of seven days), the washout period, and the second seven-day challenge (or less). During the challenge, the patient will identify and report one to three main symptoms. A variation of at least 30% between the gluten and the placebo challenge discriminates a positive from a negative result, although the threshold is somewhat arbitrary and needs scientific validation. Patients showing a negative gluten challenge should also be investigated for other possible causes.

No further intervention is required if symptoms improve although re-challenging the patient with a trial of gluten-containing foods in the future is advised,[51] as there is always the possibility of a placebo effect.[9, 61] Given the difficulties of maintaining a GFD, not only because gluten-free foods cost more than 3.5 times the price of gluten-containing foods with no proven increase in nutritional value, but also because gluten-free baked goods are still scarce in most stores and have to be carefully prepared at home or by specialized bakers,[28] it may be reasonable to first make general dietary improvements (more fresh fruits, vegetables, whole grains, and non-fried low-fat sources of protein, avoiding processed snack foods), before instituting a permanent GFD.

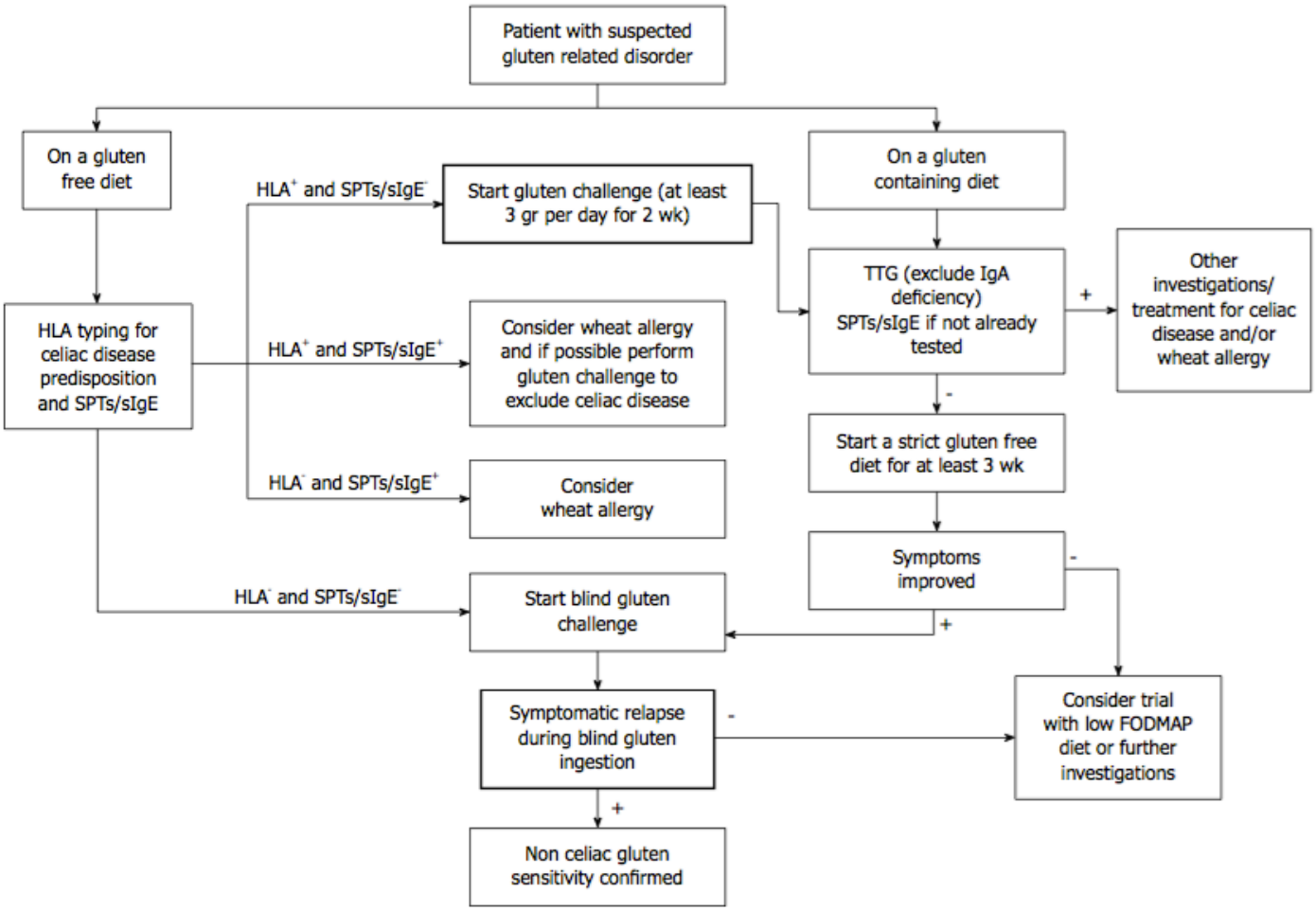

Figure 2 - Diagnostic chart in a suspected gluten related disorder. HLA (Human leukocyte antigen), SPTs/sIgE (skin prick tests/specific immunoglobulin E) **[23]**

Conclusion

NCGS is a 're-discovered' syndrome of gluten intolerance. Until recently, it has been thought to be typical of CD and WA,[8] and symptomatically similar to IBS[5] but it is in fact a distinct clinical entity characterized by intestinal and extra-intestinal symptoms related to the ingestion of gluten-containing food.[40]

Knowledge of the pathogenesis, epidemiology, and natural history of NCGS is still quite rudimentary and questions remain unanswered, including whether the gluten-mediated effect is an all-or-none or a dose-related phenomenon and what part of the gluten is responsible. High quality, well-controlled research in human trials is difficult to carry out and is fraught with its own hurdles as the self-reported NCGS patients are heterogeneous in their range of reported symptoms, clinical histories and characteristics. Randomised trials on NCGS in children are also lacking[8] and there is a strong clinical need for biomarkers in the diagnostic work-up of NCGS.[33]

Gluten avoidance can have an impact on nutritional intake and quality of life and the differentiation of gluten-related pathologies is crucial to advise patients regarding the importance of ongoing disease monitoring, a required GFD, and for counselling and testing of family members.[31]

Important considerations for the future include well-designed dietary trials investigating NCGS include high order effects in cross-over designs, large nocebo effects, patient selection and clear entry criteria, methods of gluten challenge, properties of what is used as the placebo use, ensure successful blinding, well-defined endpoints, control of confounding dietary factors and trying to ensure that the protocol and provision of food is well received by participants.

Providing these the next several years will provide a stronger quality of evidence and exciting key pieces to understand this NCGS puzzle.[40]

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Conflict of interest

There are no conflicts of interest to declare.

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