Carol Rees Parrish, M.S., R.D., Series Editor

Non-Celiac Gluten Sensitivity Where are We Now in 2015?

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Non-celiac gluten sensitivity (NCGS) is a term that is used to describe individuals who are not affected by celiac disease or wheat allergy yet who have intestinal and/or extraintestinal symptoms related to gluten ingestion with improvement in symptoms upon gluten withdrawal. The prevalence of this condition remains unknown. It is believed that NCGS represents a heterogenous group with different subgroups potentially characterized by different pathogenesis, clinical history, and clinical course. There also appears to be an overlap between NCGS and irritable bowel syndrome (IBS). Hence, there is a need for strict diagnostic criteria for NCGS. The lack of validated biomarkers remains a significant limitation in research studies on NCGS.

INTRODUCTION

The most common diseases caused by ingestion of wheat are autoimmune-mediated conditions such as celiac disease (CD) and IgE-mediated allergic reactions or wheat allergy (WA).1 CD affects roughly 1% of the general population. It is now increasingly clear that, besides CD and WA, an undefined percentage of the general population considers themselves to be suffering from problems due to wheat and/or gluten ingestion, relying largely on self-diagnosis. These individuals are generally considered to have gluten sensitivity (GS). An overlap between irritable bowel syndrome and GS has long been suspected and requires strict diagnostic criteria. Currently, the lack of biomarkers is a major limitation, and there remain many unresolved questions regarding GS. In this paper, we will discuss the current advances in our understanding of non-celiac gluten sensitivity (NCGS) including definition, epidemiology, clinical characteristics, diagnostic criteria and management.

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Definition

Recent publications show that there is great interest in defining gluten-related disorders (See Figure 1). This term encompasses all conditions related to the ingestion

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of gluten-containing food. Included within this category is *celiac disease* (CD), a chronic, small intestinal immune-mediated enteropathy triggered by exposure to dietary gluten in genetically predisposed individuals characterized by specific autoantibodies against tissue transglutaminase 2 (anti-TG2), endomysium (EMA) and/or deamidated gliadin peptide (DGP).[2] *Wheat allergy* (WA) is another gluten-related disorder that is defined as an adverse immunologic reaction to wheat proteins characterized by the production of wheat specific IgE antibodies that play a key role in disease pathogenesis. Cases of non-IgE-mediated wheat allergy also exist and can be confused with gluten sensitivity. Examples of WA include wheat-dependent, exercise-induced asthma (WDEIA), occupational asthma (baker's asthma), rhinitis, and contact urticaria.[1]

In 2011, an international panel of experts met in London and reached consensus on a definition of *non-celiac gluten sensitivity* (NCGS). They defined NCGS as a "non-allergic and non-autoimmune condition in which the consumption of gluten can lead to symptoms similar to those seen in CD".[3] The consensus statement elaborated that symptoms in NCGS are triggered by gluten ingestion in the absence of celiac-specific antibodies (tissue transglutaminase [tTG], endomysium [EMA] and/or deamidated gliadin peptide [DGP]) and absence of enteropathy although an increased density of CD3+ intraepithelial lymphocytes (IELs) can be detected. Patients with NCGS have variable human leukocyte antigen (HLA) status and variable presence of IgG anti-gliadin (first generation) antibodies.[3] NCGS is further characterized by resolution of symptoms with withdrawal of gluten and relapse of symptoms with gluten exposure. The clinical symptoms of NCGS can overlap with those of CD and WA. As our knowledge of NCGS continues to increase, this definition may require further modification in the future.

Epidemiology and Natural History of NCGS

The overall prevalence of NCGS in the general population is currently unknown largely because patients often self-diagnose and place themselves on a GFD without medical consultation. Anecdotal observations indicate that the prevalence ranges from 0.5% to 6% but this is based on studies with heterogenous study design and inconsistent definitions of the disease. In a large study of 5896 patients evaluated at the University of Maryland between 2004-2010, 347 patients fulfilled diagnostic

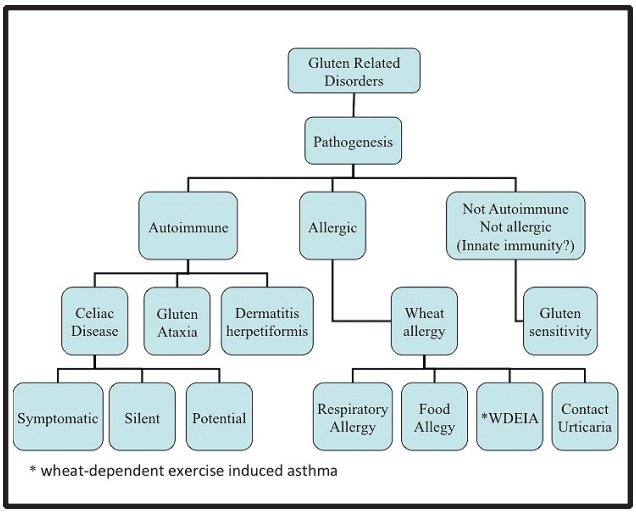

Figure 1. Gluten-Related Disorders

criteria for NCGS leading to a prevalence of nearly 6%.[1,4] Furthermore, data from the National Health and Nutrition Examination Survey (NHANES) for 2009-2010 reported a possible prevalence of NCGS of 0.55% in the general U.S. population.[5] Given the reported overlap between IBS and NCGS, epidemiologic studies on IBS can shed some light, albeit indirectly, on the frequency of NCGS. In one highly selected series of adults with IBS, the frequency of NCGS was reported to be 28% based on a double-blind, placebo-controlled gluten challenge.[6] Furthermore, in a large study by Caroccio et al, 276 out of 920 (30%) of subjects with IBS-like symptoms based on Rome II criteria reported wheat sensitivity or multiple food hypersensitivities.[7] It is estimated that the prevalence of NCGS in the general population is likely higher than that of CD (1%). The prevalence of NCGS in children is still unknown. Although risk factors for NCGS have not yet been identified, this disorder appears to be more common in females, with a male-to-female ratio of about 1:3, and in young/middle aged adults.

Due to a lack of longitudinal data and prospective studies on the natural history of NCGS, it is unclear if NCGS predisposes to any long-term complications. In the current literature, there are no reports of major complications such as intestinal lymphoma, gastrointestinal (GI) malignancies or associated autoimmune illness as observed in CD.

Pathogenesis

The pathophysiology of NCGS remains largely undetermined. A study by Sapone et al. has found that NCGS subjects have normal intestinal permeability

Table 1. Clinical Characteristics of Non-Celiac Gluten Sensitivity (NCGS) vs. Celiac Disease (CD) Subjects

Symptoms	CD	NCGS
Intestinal	Chronic diarrhea Abdominal pain Weight fluctuation Weakness Smelly, fatty stools	Diarrhea Abdominal pain Weight loss Gas
Extra-intestinal	Bone or joint pain Osteoporosis Behavioral changes Tingling, numbness in legs Muscle cramps Infertility Recurrent miscarriage Delayed growth Thyroiditis Tooth discoloration Unexplained anemia	Bone or joint pain Leg numbness Muscle cramps Glossitis Behavioral changes Foggy mind Headache Dermatitis Anemia

compared to CD patients, intact level of protein expression that comprise intestinal epithelial tight junctions and a significant reduction in T-regulatory cell markers compared to controls and CD patients.[4] Moreover, NCGS patients have an increase in the α and β classes of intraepithelial lymphocytes (IELs) with no increase in adaptive immunity-related gut mucosal gene expression. These findings suggest an important role of the intestinal innate immune system in the pathogenesis of NCGS without an adaptive immune response.[8] Unlike duodenal mucosa from CD patients exposed to gliadin *in-vitro*, intestinal mucosa from NCGS patients do not express markers of inflammation. Newer techniques such as examination of basophil activation in response to gluten or wheat stimulation might suggest alternative pathogenic mechanisms for NCGS.

Clinical Characteristics of NCGS

The clinical symptoms of NCGS are elicited soon after gluten exposure, improve or disappear with gluten withdrawal and reappear following gluten challenge, usually within hours or days. While this finding could be attributed to a placebo/nocebo effect, the 2011 study by Biesiekierski et al. argues for the existence of a true NCGS disorder. In a double-blind randomized, placebo-controlled study design, the authors found that IBS-like symptoms of NCGS were significantly higher in the gluten-treated group (68%) than subjects treated with placebo (40%).[6]

Studies suggest that the clinical presentation of NCGS follows an IBS-like picture characterized by abdominal pain, bloating, bowel irregularity (diarrhea and/or constipation) and systemic manifestations including "brain fog", headache, joint and muscle pain, fatigue, depression, leg or arm numbness, dermatitis (eczema or skin rash) and anemia.[1,4,9] In one study of IBS patients, the two most common extraintestinal manifestations with gluten challenge were "foggy mind" (42%) and fatigue (36%).[9] Currently, data are lacking on the actual prevalence and type of intestinal and extraintestinal symptoms in patients with NCGS. Unlike CD, NCGS patients do not have an increased prevalence of autoimmune illness. In one group of 78 NCGS patients, none had type I diabetes mellitus and only one patient (1.3%) had autoimmune thyroiditis. This is compared to 5% and 19% prevalence for these autoimmune comorbidities, respectively, in a study of 80 patients with CD.[9] With regards to psychiatric comorbidities, a recent study found no significant difference between patients with CD and NCGS in

Table 2. Laboratory Criteria for Non-Celiac Gluten Sensitivity (NCGS)

Diagnostic Test	NCGS	CD	WA
Celiac Disease Serology			
Anti-tissue transglutaminase	Negative	Positive	Negative
Anti-endomysial antibody	Negative	Positive	Negative
Anti-deamidated gliadin peptide	Negative	Positive	Negative
Anti-gliadin (IgG) antibody	Positive (~56%)	Positive	Negative
Duodenal Histology	Negative (Marsh 0-1)	Positive	Negative
Other Histologic Findings	Activated circulating Basophils Eosinophilic infiltration in small intestine, colon		
HLA Haplotypes (DQ2 and DQ8)	Absent/Present (50%)	Present	Absent
IgE-based Assays	Negative	Negative	Positive

terms of anxiety, depression and quality of life indices.[10] Overall, the role of NCGS in neuropsychiatric conditions (i.e. schizophrenia, autism spectrum disorders) remains a controversial and highly debated topic. However, NCGS patients reported more abdominal and non-abdominal symptoms after gluten exposure than CD patients (see Table 1).

In a recent retrospective review of IBS-like patients who underwent a double-blind placebo-controlled wheat challenge, nearly 25% of the patients were identified with NCGS. The study showed that a history of food allergy in infancy, coexistent atopic disease, multiple food intolerances, weight loss and anemia were more common in the NCGS group compared to the IBS controls.[7] Therefore, it may be useful for physicians to enquire about these conditions in patients with IBS type symptoms to gauge the potential utility of a trial of gluten restriction.

NCGS and IBS

The relationship between NCGS and IBS is complex, and IBS-like symptoms are common in patients with NCGS. Vasquez et al. showed that gluten ingestion can elicit GI symptoms in non-CD patients, specifically, patients with diarrhea-predominant IBS (IBS-D).[11] The IBS-D patients, particularly those with the HLA-DQ2 and/or DQ8 genotypes, had more frequent bowel movements per day on a gluten-containing diet, and this diet was associated with higher small intestinal permeability. This finding gave some insight into the role of the GFD in improving GI symptoms in IBS patients.

However, the exact role of gluten withdrawal in mitigating symptoms requires further investigation. In addition to gluten, it has been shown that wheat and wheat derivatives contain components such as amylase-trypsin inhibitors (ATIs) that can trigger symptoms in IBS patients. Another potential trigger for symptoms are the highly fermentable and osmotic, poorly-absorbed, short-chain carbohydrates (fermentable oligo-, di- and monosaccharide and polyols), also called FODMAPs which include fructans, galactans, fructose, lactose and polyols found in wheat, certain fruits, vegetables and milk as well as their derivatives.[3] There is ongoing debate on the contribution of each of these diet components to symptoms experienced by patients with NCGS and IBS. In a placebo-controlled cross-over re-challenge study in 37 subjects with self-reported NCGS/IBS, subjects were randomly assigned to a reduced FODMAPs diet and then challenged with gluten or whey protein.[12]

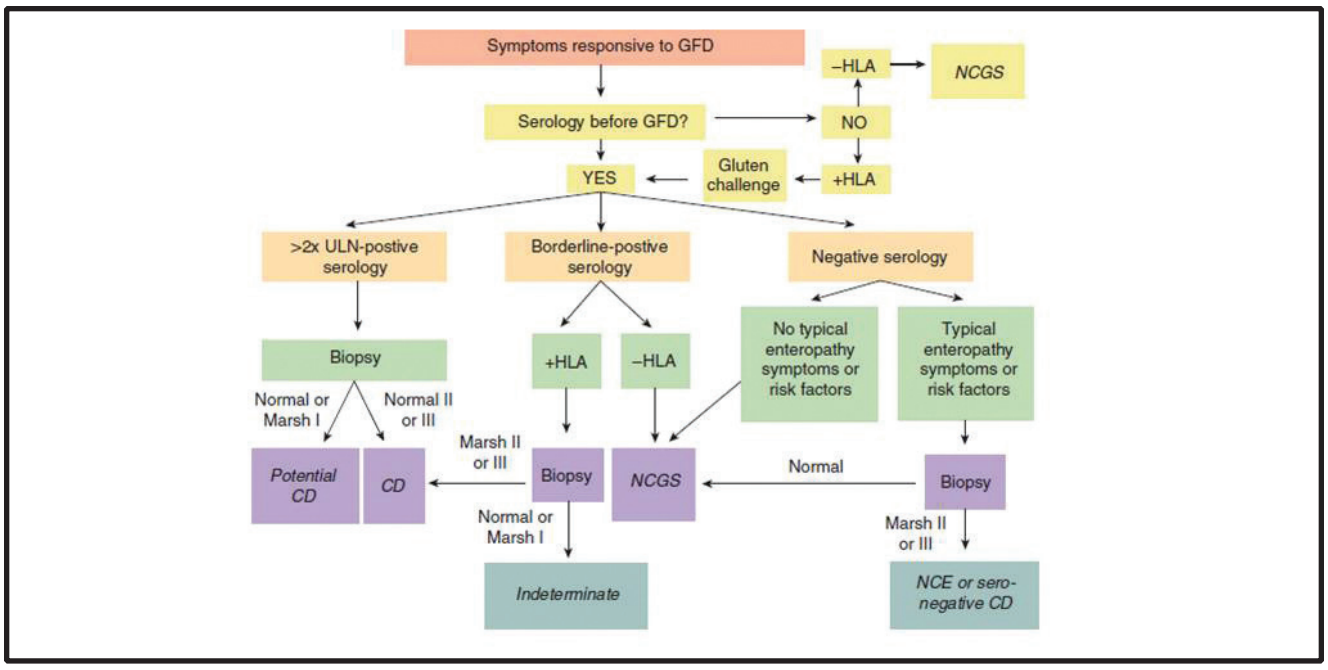

Figure 2. Diagnostic Algorithm to Differentiate Non-Celiac Gluten Sensitivity (NCGS) from Celiac Disease and Wheat Allergy (WA)

All 37 subjects had improvement in their GI symptoms on the reduced FODMAPs diet with significant worsening of their symptoms when challenged with gluten or whey protein. It is important to note that the symptoms experienced by the NCGS patients cannot be attributed solely to FODMAPs since they often experienced resolution of symptoms with a GFD alone while still consuming FODMAPs from other sources such as legumes. However, this finding raises the possibility that some cases of IBS may, in fact, be due largely to FODMAPs and should not be classified as having NCGS. Therefore, there is a great need to identify and validate specific biomarkers that will play an important role in further defining NCGS as a clinical condition and clarify its prevalence in at-risk groups and the general population.

Laboratory Evaluation in NCGS

No specific biomarker has been identified for NCGS. However, trends in laboratory evaluation including serology, HLA genotyping and histology have been noted in patients meeting diagnostic criteria for NCGS.

CD Serology

Volta et al. investigated the CD serologic patterns in 78 patients with untreated NCGS. They found that 56.4% of the patients had elevated titers of "first generation" IgG-anti-gliadin antibody (AGA) compared to patients with untreated CD. The prevalence of IgG-AGA in NCGS was lower than that in CD patients (81.2%), but higher than in patients with connective tissue diseases (9%) or autoimmune liver disease (21.5%), and in healthy blood donors (2-8%). However, the prevalence of IgA-AGA in NCGS was low at 7.7%.[9] Of note, the three key CD antibodies, IgA-tTG, IgG-DGP and IgA-EMA, were negative in all patients with NCGS except for a single low titer IgG-DGP.

HLA Genotyping

The CD-predisposing HLA-DQ2 and HLA-DQ8 haplotypes are found in roughly 50% of NCGS patients compared to 95% in CD patients and 30% in the general population.[1]

Histologic Findings

Sapone et al. compared small intestinal biopsy findings from patients with NCGS, CD and controls. Patients with NCGS had normal to mildly inflamed mucosa categorized as Marsh 0 or 1, while partial or subtotal villous atrophy (Marsh 3) with crypt hyperplasia was seen in all CD patients.[4] In addition, the CD patients had increased intraepithelial lymphocytes (IELs) compared to controls. The level of CD3+ IELs in the NCGS patients was found to be in between that seen in CD patients and controls in the context of normal villous architecture. Other histologic findings that might be

specific to NCGS patients include an increased level of activated circulating basophils[7,13] and increased infiltration of eosinophils in the duodenum and/or ileum and colon.[7,14]

Diagnostic Approach to NCGS

As clinicians, it is important to suspect NCGS in a patient who presents with IBS-like symptoms such as abdominal pain, bloating, diarrhea and constipation as well as "foggy brain," fatigue, headaches, joint or muscle pain that appear to improve on a GFD. Since these symptoms can also be seen with CD and, to a lesser extent, with wheat allergy (WA), these conditions need to be excluded in order to make a diagnosis of NCGS (see Table 2). Kabbani et al. have proposed a diagnostic algorithm to help differentiate NCGS from CD and WA[15] (See Figure 2). The first step in the evaluation of a subject with symptoms responsive to a GFD is to check for the presence of celiac serologies (IgA-tTG and IgA/IgG DGP) on a gluten-containing diet. If the celiac serologies are negative and there is no IgA deficiency, a diagnosis of CD is unlikely, making NCGS a more likely diagnosis. Moreover, lack of symptoms of malabsorption (weight loss, diarrhea, nutrient deficiencies, iron deficiency anemia) and no CD risk factors (family history of CD, personal history of autoimmune illness) were found to further support a diagnosis of NCGS. WA allergy should similarly be evaluated for with IgE-based assays.

The authors found that incorporating a personal history of autoimmune illness, family history of celiac disease and nutrient deficiencies could help in the diagnostic model particularly in subjects with negative serology. Subjects with negative serology on a gluten-containing diet, no risk factors and no symptoms of enteropathy are highly likely to have NCGS and do not require further testing. Conversely, in a subject with negative serology but with typical symptoms of malabsorption or risk factors for CD, a biopsy is indicated. In a subject with borderline serology on a gluten-containing diet, the next step is HLA typing to determine whether a biopsy is indicated. A subject with borderline serology and negative HLA typing is considered to have NCGS. HLA typing is also useful to evaluate subjects suspected of NCGS or CD who self-start a GFD without a prior check of celiac serologies on a gluten-containing diet. Due to the high negative predictive value of the genetic assay, a diagnosis of CD can be effectively excluded with a negative finding. If HLA testing is negative in a subject without serology on a GFD whose symptoms are responsive to a GFD, the subject likely has NCGS and a gluten challenge would be unnecessary. However, if HLA testing is positive despite symptom resolution on a GFD, it is recommended that the subject undergo a gluten challenge followed by evaluation of celiac serologies. A gluten challenge is the monitored reintroduction of gluten containing food items usually over a two week period. The recommended daily gluten load is the equivalent of 1-2 slices of wheat bread.

Once CD and WA have been excluded clinically and by laboratory evaluation, a patient suspected of having NCGS should be asked to avoid a gluten-containing diet for at least 4-8 weeks. Gluten withdrawal is usually associated with significant improvement in symptoms within days. After the period of gluten withdrawal, a gluten challenge should be performed for confirmation of diagnosis. Since placebo effect from gluten withdrawal cannot be excluded entirely, a more ideal method for diagnosing NCGS is a double-blinded, placebo-controlled design, however this is unlikely to be feasible in most clinical practices.

Currently, research efforts are focusing on the use of an *ex-vivo* gluten challenge to distinguish patients with CD (treated and untreated) from NCGS, further classify NCGS and distinguish true NCGS from cases of mild CD without enteropathy. In the *ex-vivo* gluten challenge, cultured duodenal biopsies are exposed to gluten and maintained in various laboratory conditions to determine unique cytokine profile and histologic findings that can be used to classify different patient groups. This method would eliminate the need for a two week gluten challenge followed by an upper endoscopy with duodenal biopsies in patients already on a GFD in whom the diagnosis of CD is not clear. Patients frequently find the gluten challenge to be onerous and, in some cases, intolerable due to significant side effects from gluten exposure.

Management of NCGS

Successful treatment and management of NCGS is based on a multidisciplinary approach involving the primary care physician, gastroenterologist and nutritionist. It must be emphasized that dietary treatment should be implemented only after an appropriate diagnosis has been established. Patients with NCGS are advised to follow a diet with sufficiently reduced gluten content to manage and mitigate symptoms. Based on severity

of symptoms, some patients may choose to follow a gluten-free diet (GFD) indefinitely. Since gluten-free food products are often not fortified with necessary vitamins and minerals, it is important to evaluate a patient with NCGS for any vitamin and mineral deficiencies and manage them appropriately. NCGS patients are typically advised to start a multivitamin. If a patient has persistent symptoms despite a low gluten or GFD, there should be consideration for other associated conditions such as lactose intolerance and/or fructose malabsorption. These conditions can be evaluated for with breath testing and/or an empiric trial of a low FODMAP diet. It is important to also consider and exclude other conditions such as IBS and small intestinal bacterial overgrowth that can contribute to ongoing symptoms.

Since there is no biomarker for NCGS to monitor a patient's status, clinicians are left to rely on symptom resolution. Based on our current understanding of NCGS, there is no intestinal or extraintestinal damage with gluten exposure. Since it is not yet known whether NCGS is a transient or permanent condition, it is strongly recommended by experts such as Fasano et al. that patients undergo periodic re-evaluation with reintroduction of gluten (e.g. every 6-12 months), particularly in the pediatric population, in an effort to liberalize the diet where possible.[3] In clinical practice, however, many patients with symptom control on a low gluten or GFD are averse to intentional exposures to gluten. Currently, there are no guidelines on how best to monitor patients with NCGS.

Unanswered Questions and Future Research

The clinical spectrum of gluten-related disorders appears to be more heterogenous than previously appreciated. However, evidenced-based research in this area is lacking. Although NCGS is currently defined by gluten related symptoms in the absence of CD, this does not rule out the possibility that gluten could be "toxic" and have long-term clinical sequelae. A number of unanswered questions remain about NCGS that will dictate future research. What is the prevalence of NCGS both in the general population and in at-risk groups? What is/are its pathogenic mechanisms? Is the condition permanent or transient, and is the threshold of sensitivity the same or different for subjects and does it change over time in the same subject? Research on NCGS suggests that it may be a heterogenous condition comprised of several subgroups. There is a need for:

- Prospective, multi-center studies on the natural history of this condition.
- Biomarkers to properly diagnose and better define the different NCGS subgroups.
- Research on the potential pathogenic role of other wheat components besides gluten and ATI, namely, FODMAPs in NCGS.

It is also anticipated that the definition of NCGS will undergo further modification with the accumulation of more data. In the meantime, it is important to have a standardized definition for NCGS to assist in diagnosis and to improve study design for future research. ■

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