



Persistence of Nonceliac Wheat Sensitivity, Based on Long-term Follow-up

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We investigated how many patients with a diagnosis of nonceliac wheat sensitivity (NCWS) still experienced wheat sensitivity after a median follow-up time of 99 months. We collected data from 200 participants from a previous study of NCWS, performed between July and December 2016 in Italy; 148 of these individuals were still on a strict wheat-free diet. In total, 175 patients (88%) improved (had fewer symptoms) after a diagnosis of NCWS; 145 of 148 patients who adhered strictly to a gluten-free diet (98%) had reduced symptoms, compared with 30 of 52 patients who did not adhere to a gluten-free diet (58%) ($P < .0001$). Of the 22 patients who repeated the double-blind, placebo-controlled challenge, 20 reacted to wheat. We conclude that NCWS is a persistent condition. [Clinicaltrials.gov](#) registration number: NCT02823522.

Keywords: Food Allergy; GFD; Outcome; IBS.

Nonceliac gluten/wheat sensitivity (NCG/WS) recently has been included among the gluten-related diseases.^{1,2} Monitoring the patient during the elimination diet and the re-introduction of wheat by a double-blind, placebo-controlled (DBPC) challenge has been suggested as the ideal diagnostic method.³ Many aspects of NCG/WS still remain unclear and it is unknown whether it is a long-life condition.

In the year 2012 we published a retrospective study of 276 patients with a marked immunologic condition (intraepithelial lymphocyte count $>25/100$ epithelial cells in the duodenal mucosa), and irritable bowel syndrome (IBS)-like symptoms, who had been diagnosed with NCG/WS by DBPC challenge during a 10-year period (2001–2011).⁴

The present prospective study aimed to investigate the persistence of NCG/WS in that cohort, and 200 patients (19 men, 181 women; mean age, 40 ± 13 y) were accepted for inclusion 65–72 months (median, 99 mo) after NCG/WS diagnosis (for details see the [Supplementary Materials and Methods](#) section).

Continuing adherence to a wheat-free diet and the severity of the IBS-like symptoms were evaluated via structured questionnaires ([Supplementary Materials and Methods](#)).

Furthermore, 22 consenting patients still on a wheat-free diet (21 women; mean age \pm SD, 35.4 ± 9.3 y) were randomized to undergo a repeat DBPC wheat challenge (see the [Supplementary Materials and Methods](#) section for more detail).

All authors had access to the study data and reviewed and approved the final manuscript.

Materials and Methods

See [Supplementary Materials and Methods](#).

Results

Compliance With the Wheat-Free Diet

Figure 1A shows that 148 (74%) patients still were following a wheat-free diet (score, 3 or 4). More specifically, 21 patients (10%) strictly avoided wheat, but consumed other gluten-containing foods, including barley and rye (score, 3), and 127 patients (64%) were on a strict gluten-free diet (score, 4). The patient groups with scores of both 3 and 4 reported that involuntary consumption of wheat-containing foods caused the recurrence of intestinal and extra-intestinal symptoms listed in the [Supplementary Materials and Methods](#) section ([Supplementary Table 1](#)). Only 42 patients (21%) did not adhere to the wheat-free diet at all (score, 0–1), and 10 others adhered to the diet but not strictly (score, 2). Among these, 15 patients had resumed a wheat-containing diet without any problems, 14 patients had received a diagnosis other than nonceliac wheat sensitivity (NCWS) and consequently were consuming wheat again, 11 patients confirmed that wheat triggered symptoms but they ate it by choice, and 12 patients ate wheat because they could not afford gluten-free products but reported IBS-like symptoms after consuming it.

Abbreviations used in this paper: DBPC, double-blind, placebo controlled; GAI, Global Assessment of Improvement; GFD, gluten-free diet; IBS, irritable bowel syndrome; NCG/WS, nonceliac gluten/wheat sensitivity; NCWS, nonceliac wheat sensitivity.

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0016-5085/\$36.00

<http://dx.doi.org/10.1053/j.gastro.2017.03.034>

EDITOR'S NOTES
BACKGROUND AND CONTEXT
Non-celiac wheat sensitivity (NCWS) has been recently included among the gluten-related diseases, however, many aspects of NCG/WS remain unclear and it is unknown whether it is a long-life condition.
NEW FINDINGS
After a follow-up time of 8 years, 148 of the 200 NCWS patients were still on a strict wheat-free diet. The percentage of patients who had improved was significantly higher in those who still fully complied with the diet. Double-Blind-Placebo-Controlled challenge confirmed NCWS in 90% of the patients.
LIMITATIONS
This Study included patients who had a marked immunologic component that can suggest an allergic condition. Thus, NCWS can be considered a persistent condition only in the presence of similar patient characteristics.
IMPACT
NCWS can be a persistent condition and, in these cases, a long-term adherence to a wheat-free diet determines a significant improvement in the patients' symptoms.

IBS Symptoms

Figure 1B shows the "well-being score" according to the IBS Global Assessment of Improvement (GAI). In total, 175 patients (88%) improved after a NCG/WS diagnosis. The frequency of patients who slightly, moderately or substantially improved (IBS-GAI respondent) after a NCWS diagnosis was significantly higher in the subjects who still strictly adhered to the gluten-free diet (GFD) than in those who did not: 145 of 148 patients (scores E-G, 98%) vs 30 of 52 patients (scores A-D, 58%) (chi-square for Mantel-Haenszel, 36.7; $P < .0001$) (Figure 2).

Variables Associated With Following the GFD

Adherence to the diet was associated only with female sex ($P = .03$, Fisher's exact test) and histology findings of eosinophil infiltrate in the intestinal mucosa, evaluated at diagnosis ($P < .0001$, Fisher's exact test; odds ratio, 5.8; 2.7–12.3). No difference in diet adherence was observed for HLA status.

Results of the DBPC Wheat Rechallenge

The 22 patients randomized to the DBPC wheat rechallenge study were all on a strict gluten-free diet and asymptomatic before entering the study. Symptoms reappeared during the DBPC wheat challenge after a median time of 2 days (range, 3 h to 8 days). Only 2 patients reacted to the placebo. The score on the wheat-containing diet was significantly higher than on the placebo both at the end of the first and second weeks into the DBPC challenge ($P < .0001$, Mann-Whitney). Including the 2 patients who also reacted to the placebo, 20 of the 22 (91%) patients were confirmed to be suffering from NCG/WS.

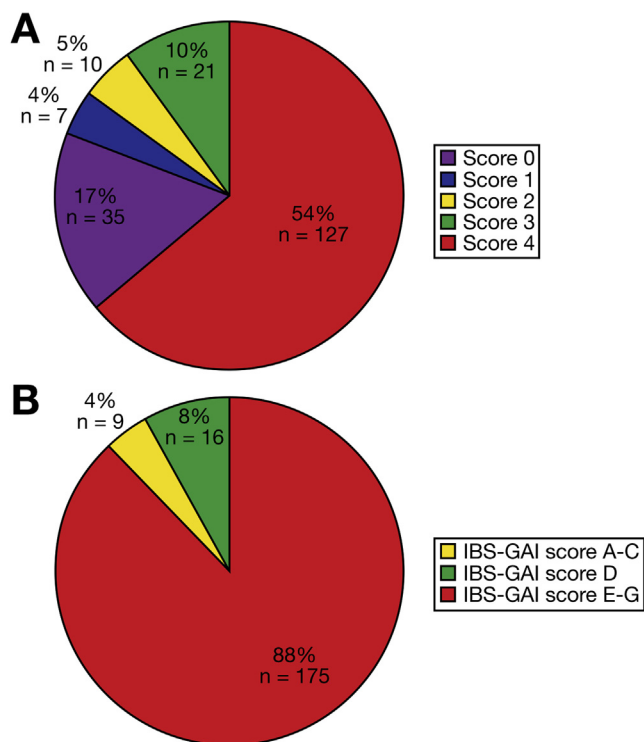


Figure 1. (A) Level of adherence to a wheat-free diet in patients diagnosed with NCWS, after a 65- to 172-month follow-up period. The percentage of the patients is shown. Adherence was scored according to a modified version of Biagi et al¹² (see the [Supplementary Materials and Methods](#) section), as follows: score of 0 indicates that they consume wheat regularly or taste it so often that they cannot remember when and how many times that has happened; score of 1 indicates that they consume wheat rarely, the patient consumes wheat only occasionally; they can remember when and how many times that has happened; score of 2 indicates that they avoid wheat but when they eat out they do not inform the person who is cooking for them about their disease or do not check the labels on packaged food; score of 3 indicates that they avoid wheat regularly, even at social events; however, they eat packaged food even if it is not guaranteed by the Celiac Association, and eat other nonwheat, gluten-containing foods; and score of 4 indicates that they avoid wheat and gluten regularly, even at social events, and eat packaged food only if it is guaranteed by the Celiac Association. (B) A well-being score was recorded for the study patients according to the IBS-GAI. The percentage of the patients is shown. We asked patients the following, "Compared with the way you felt before the NCWS diagnosis and the consequent wheat-free diet, have your IBS symptoms been: worse (IBS-GAI score, A-C), unchanged (IBS-GAI score, D), or improved (IBS-GAI score, E-G)?"

Discussion

Several studies have confirmed an immunologic activation in NCG/WS,⁵⁻⁷ however, it is not known whether this is a definitive condition.^{8,9}

Our data show that approximately 74% of 200 patients had remained on a strict wheat-free diet for a median time of >8 years after a NCG/WS diagnosis, and they all reported that involuntary wheat consumption regularly caused symptoms. Only 42 patients had abandoned the wheat-free

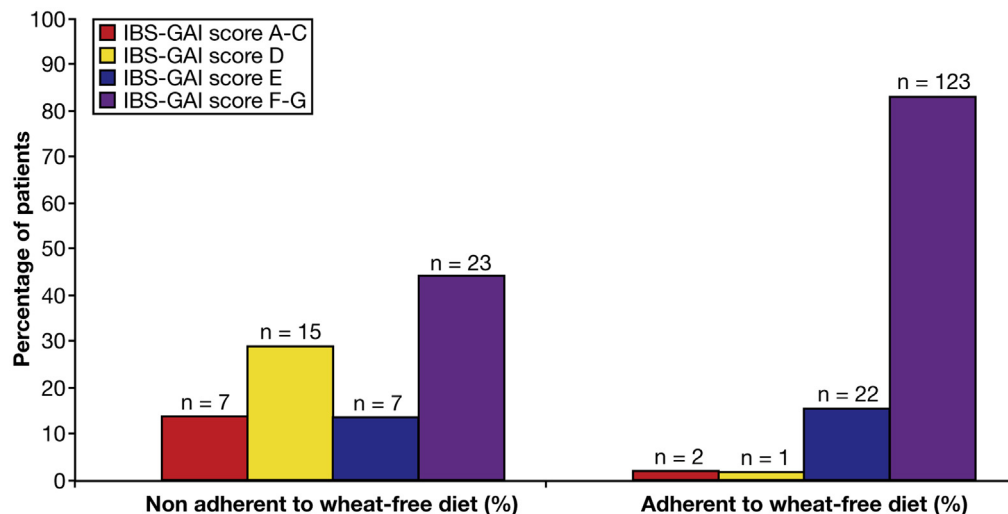


Figure 2. Relationship between Biagi et al¹² and IBS-GAI score in NCWS patients. The number and percentage of the patients are shown.

diet, although 23 of these patients confirmed they were sure of their wheat sensitivity because they had symptoms after consuming wheat. In total, NCWS persisted in 171 of 200 patients (85%).

Ninety percent of the patients who maintained a wheat-free diet had a statistically significant improvement in their IBS symptoms. These results underline the importance of correctly identifying NCWS patients because the elimination diet definitively resolved their symptoms, a result that no medical treatment achieves in IBS patients.

The real persistence of NCWS also was shown by the results of the DBPC wheat challenge, repeated approximately 8 years after the first diagnostic challenge in a small group of patients.

Our findings must be considered with caution. We studied a cohort of patients who had a marked immunologic component, which can suggest an allergic condition.¹⁰ It could be hypothesized that these patients were suffering from a non-IgE-mediated food allergy, a hypothesis that may be consistent with the confocal endoscopy images of the duodenal mucosa during provocative tests in IBS patients.¹¹ Furthermore, the study was underpowered to determine if male sex was associated with adherence to the GFD. Thus, NCWS can be considered a persistent condition only in the presence of similar patient characteristics.

In conclusion, our study shows that NCWS can be a persistent condition.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at <http://dx.doi.org/10.1053/j.gastro.2017.03.034>.

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Received January 12, 2017. Accepted March 23, 2017.

Reprint requests

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Acknowledgments

The authors wish to thank native English speaker Carole Greenall for revising the text.

Antonio Carroccio designed the research study, collected and analyzed the data, wrote the paper, and is the guarantor of the article; Alberto D'Alcamo collected and analyzed the data and revised the manuscript; Giuseppe Iacono collected the data and revised the manuscript; Maurizio Soresi performed the statistical analysis and revised the manuscript; Rosario Iacobucci, Francesca Fayer, and Francesco La Blasca collected the data; Andrea Arini and Girolamo Geraci performed the endoscopy study; Francesca Cavataio collected the data and revised the manuscript; Ada M. Florena performed the histology studies; Pasquale Mansueto collected and analyzed the data and revised the manuscript; and all of the authors approved the final version of the manuscript.

Conflicts of interest

The authors disclose no conflicts.

Funding

The study was supported by an Italian Foundation for Celiac Disease grant for project 013/2014 (F.C.). The sponsor had no role in the study design, collection, analysis, or interpretation of the data.

Supplementary Materials and Methods

Patient Enrollment

Between July and December 2016, the patients included in the previous retrospective study¹ were contacted by telephone, mail, and e-mail, and invited to return to the respective clinics where they had been diagnosed initially as NCG/WS patients: the Department of Internal Medicine at the University Hospital of Palermo and the Department of Internal Medicine of Sciacca Hospital. The 22 patients included in the previous study who tested positive for anti-endomysium antibodies in the culture medium of the duodenal biopsies, even if the villi crypt ratio in the duodenal mucosa was normal, were excluded from the present study. Of the 254 remaining patients, 34 were not found and 20 refused to return to the clinics. In all, 200 patients (19 men, 181 women; mean age, 40 ± 13 years) previously diagnosed with NCG/WS by DBPC challenge were re-evaluated. These patients had been diagnosed with NCG/WS in our outpatient clinics in a period ranging between 65 and 172 months (median, 99 mo) before the present study.

Questionnaire to Verify Adherence to a Wheat-Free or Gluten-Free Diet

A structured questionnaire was administered to evaluate how many patients were still on a wheat-free or gluten-free diet. We used a simple score suggested by Biagi et al² based on 4 simple questions, modified to be applied to a wheat-free diet. The score ranges from 0 to 4, in which a score of 0 or 1 indicates that the patients are not on a wheat-free diet anymore; a score of 2 indicates that they are on a wheat-free diet but not strictly; a score of 3 indicates that they are on a strict wheat-free diet, but not a GFD; and a score of 4 indicates that they are on a strict GFD.

Questionnaires to Evaluate the Severity of IBS and the Effect of the Wheat-Free Diet

The same researchers (A.C., P.M., A.D., and G.I.) who initially evaluated the patients and reached the NCG/WS diagnosis administered a modified version of the IBS-GAI scale.³ According to the IBS-GAI, we asked patients: "Compared with the way you felt before the NCG/WS diagnosis, have your IBS symptoms been (A) substantially worse, (B) moderately worse, (C) slightly worse, (D) no change, (E) slightly improved, (F) moderately improved, or (G) substantially improved."

According to the IBS-GAI score, a respondent was defined as a patient whose symptoms were either slightly, moderately, or substantially improved compared with the period preceding the NCG/WS diagnosis.

We also evaluated the correlation between the level of adherence to the wheat-free diet with the following variables, as evaluated in the retrospective study, at the time of the NCG/WS diagnosis: age, sex, duration of the IBS symptoms, age at the NCG/WS diagnosis, presence of multiple food hypersensitivity, presence of the DQ2 and/or DQ8 HLA

haplotypes, presence of intraepithelial lymphocyte infiltration in the duodenal mucosa, presence of eosinophil infiltration in the duodenal or rectal mucosa, and associated atopic diseases. The methods used and the definitions of the earlier-described variables are described in the section on the *Definitions, Criteria, and Methods Adopted*.

Double-Blind Challenge Method

The double-blind challenge method was performed with the same method used in the retrospective study and previously described.¹ In brief, the DBPC challenge was performed with capsules coded as A or B, containing 15 g of wheat flour or xylose, respectively. Capsules A or B were given for 2 consecutive weeks and then after 1 week of washout the patients received the other capsules for another 2 weeks (cross-over design). During all phases of the study, including the challenge period, the severity of symptoms was recorded: the patients completed a 100-mm visual analog scale, with 0 representing no symptoms, which assessed overall symptoms and the specific symptoms each patient reported. The challenges were considered positive when clinical reactions occurred (increase in VAS score >30) for at least 2 consecutive days (onset of abdominal discomfort or pain, associated with a change in stool frequency and/or appearance), and if the same symptoms that had been presented initially reappeared after their disappearance on elimination diet.

Statistical Analysis

When data distribution was Gaussian, values were expressed as means ± SD and differences between the 2 groups were calculated using the Student *t* test. For parameters with non-Gaussian distribution, values were expressed as range and median, and differences between the 2 groups were calculated using the Mann-Whitney *U* test. The Mantel-Haenszel test was used to compare the response to the gluten-free diet (well-being score according to IBS-GAI) in NCG/WS patients who still adhered to the GFD or not. The chi-square test or the Fisher exact test was used to test the frequencies. The SPSS software package (version 16.0; Chicago, IL) was used for statistical analysis.

The study was approved by the Ethics Committee of the University Hospital of Palermo.

All patients provided informed consent to participate.

Definitions, Criteria, and Methods Adopted

Multiple food sensitivity. All patients underwent at least 4 weeks of elimination diet (exclusion of wheat, cow's milk and derivatives, egg, tomato, and chocolate). Then they underwent a DBPC wheat challenge and all the patients included in the present study reacted to the wheat challenge. All of these patients repeated the same elimination diet and after 4 weeks underwent a DBPC cow's milk protein challenge. Those patients who also reacted to cow's milk protein challenge were classified as suffering from multiple food sensitivities.

Presence of the DQ2 and/or DQ8 HLA haplotypes. Patients were typed for HLA-DQ phenotypes

by polymerase chain reaction using sequence-specific primers, with a DR and DQ sequence-specific primers kit (Unipath SpA, Milan, Italy), or a rapid method (DQ-CD Typing Plus; BioDiaGene, Palermo, Italy).

Presence of intraepithelial lymphocyte infiltration in the duodenal mucosa. The number of intraepithelial lymphocytes per 100 villous epithelial cells was assessed by immunohistochemical staining: CD3+ intraepithelial lymphocytes were stained with monoclonal antibody Leu-4. The upper limit of the reference interval in our laboratory was 25 intraepithelial lymphocytes per 100 epithelial cells. Consequently, duodenal histology lesions were classified according to Corazza and Villanacci: normal mucosa (villi to crypt ratio >3 and CD3+ intraepithelial intestinal lymphocytes <25/100 enterocytes) or grade A (villi to crypt ratio >3 and CD3+ intraepithelial intestinal lymphocytes >25/100 enterocytes).⁴

Presence of eosinophil infiltration in the duodenal or in the rectal mucosa. In the duodenum, the number of lamina propria eosinophils per high-power field ($\times 40$) was assessed in the mucosa; the upper limit of the reference interval in our laboratory, at the time of the retrospective study,¹ was 60 lamina propria eosinophils per 10 high-power fields. In the rectum, eosinophils were

counted at a high-power field ($\times 40$) in cross-sections of 50 crypts randomly selected from each slide and these cell numbers were expressed per 100 deep-crypt epithelial cells. The eosinophil count in the lamina propria was expressed as a percentage of eosinophils per 1000 lamina propria cells per section (5 sections per biopsy were examined). On the basis of the mean values ± 2 SD observed in the colon biopsy specimens of the control subjects, the upper limit of the reference interval in our laboratory at the time of the retrospective study was lamina propria eosinophils less than 9.

Associated atopic diseases. Atopic diseases were rhinitis, conjunctivitis, bronchial asthma, and atopic dermatitis. They were diagnosed according to standard criteria.

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Supplementary Table 1. Extraintestinal Symptoms That Disappeared on a Wheat-Free Diet and Recurred After Occasional and Involuntary Consumption of Wheat-Containing Food, Reported by the 148 NCWS Patients Who Still Were Following a Strict Wheat-Free Diet

Symptom	Number of cases
Lack of well-being	135 (91%)
Tiredness	102 (69%)
Foggy mind	68 (46%)
Menstrual alterations	54 (36%)
Anemia	46 (31%)
Weight increase	45 (30%)
Joint/muscle pain	35 (24%)
Headache	31 (21%)
Weight loss	30 (20%)
Anxiety	18 (12%)
Skin rash	16 (11%)
Recurrent cystitis	12 (8%)
Depression	10 (7%)