AP&T Alimentary Pharmacology and Therapeutics

# Randomised clinical study: gluten challenge induces symptom recurrence in only a minority of patients who meet clinical criteria for non-coeliac gluten sensitivity

B. Zanini*, R. Baschè*, A. Ferraresi*, C. Ricci*, F. Lanzarotto*, M. Marullo*, V. Villanacci†, A. Hidalgo‡ & A. Lanzini*

*Department of Gastroenterology, University and Spedali Civili of Brescia, Brescia, Italy.
†Department of Pathology, University and Spedali Civili of Brescia, Brescia, Italy.
‡Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy.

**Correspondence to:**
Prof. A. Lanzini, Gastroenterology Unit, University and Spedali Civili, Piazzale Spedali Civili 1, Brescia 25100, Italy.
E-mail: alberto.lanzini@unibs.it

**Publication data**
Submitted 24 March 2015
First decision 2 April 2015
Resubmitted 28 July 2015
Accepted 28 July 2015

*This article was accepted for publication after full peer-review.*

## SUMMARY

### Background
It is unknown whether symptoms in non-coeliac patients (non-CD) meeting clinical diagnostic criteria for noncoeliac gluten sensitivity (NCGS) are specifically triggered by gluten.

### Aim
To assess gluten sensitivity in patients diagnosed with NCGS.

### Methods
We studied 35 non-CD subjects (31 females) that were on a gluten-free diet (GFD), in a double-blind challenge study. Participants were randomised to receive either gluten-containing flour or gluten-free flour for 10 days, followed by a 2-week washout period and were then crossed over. The main outcome measure was their ability to identify which flour contained gluten. Secondary outcome measures were based upon Gastrointestinal Symptoms Rating Scale (GSRS) scores.

### Results
The gluten-containing flour was correctly identified by 12 participants (34%), who were classified as having NCGS. Their mean GSRS dimension scores were significantly higher following gluten challenge compared to baseline. The scores were: pain, 1.7 ± 0.8 vs. 2.6 ± 1.0; reflux, 1.6 ± 0.5 vs. 2.2 ± 0.9; indigestion, 1.9 ± 0.7 vs. 3.2 ± 1.1; diarrhoea, 1.6 ± 0.7 vs. 2.9 ± 1.5 and constipation, 1.9 ± 0.9 vs. 2.9 ± 1.3. Seventeen participants (49%) erroneously considered the gluten-free flour to contain gluten. Their mean GSRS dimension scores were significantly higher following gluten-free flour challenge compared to baseline. The scores were: pain, 1.6 ± 0.9 vs. 3.0 ± 0.9; reflux, 1.4 ± 0.5 vs. 2.3 ± 1.1; indigestion, 2.0 ± 1.1 vs. 3.7 ± 1.1; diarrhoea, 1.6 ± 0.7 vs. 3.0 ± 1.2 and constipation, 1.6 ± 0.9 vs. 2.6 ± 1.3. The other six participants (17%) were unable to distinguish between the flours.

### Conclusion
Double-blind gluten challenge induces symptom recurrence in just one-third of patients fulfilling the clinical diagnostic criteria for non-coeliac gluten sensitivity.




doi:10.1111/apt.13372

## INTRODUCTION

The perception that gluten causes gastrointestinal and extra gastrointestinal symptoms in patients who do not have coeliac disease (CD), and are not allergic to wheat, is an increasing clinical problem.[1] It is thought that there are now more people taking a gluten-free diet (GFD) following a self-diagnosis of gluten intolerance than there are with CD.[2] This has been called non-coeliac gluten sensitivity (NCGS) in an international consensus statement.[3] However, it is purely a clinical diagnosis as, in contrast to CD, it has no biomarker or characteristic intestinal biopsy findings.[4] Currently, its existence remains controversial, and if it does exist, its cause and prevalence are unknown.[5] The name NCGS implies that gluten is the trigger for the reported symptoms. However, other components in cereal could be responsible.

Three studies have recently explored the role of gluten in causing NCGS.[6–8] A randomised, placebo controlled, cross-over trial in Italian patients with self-diagnosed gluten sensitivity, showed a small, but statistically significant, deterioration in reported symptoms when they were challenged with gluten in comparison to when they were challenged with placebo.[6] A double-blind, placebo controlled parallel group study from Australia, found evidence of a specific effect of gluten.[7] However, this was not confirmed by a subsequent controlled dietary study with a cross-over design, undertaken by the same group. This found evidence that people who avoid gluten-containing food are more likely to be sensitive to poorly absorbable carbohydrates, which are more abundant in gluten-containing than in gluten-free cereals, than to gluten itself.[8] These carbohydrates include oligosaccharides, fructans and galacto-oligosaccharides.[9] They have been termed Fermentable Oligo- Di- Mono- saccharides And Polyols (FODMAPs), and they are capable of triggering symptoms in patients with inflammatory bowel disease (IBS); gluten-containing diets, but low in FODMAPs, reduce symptoms in IBS.[9–11] This suggests that the benefits of a GFD in some patients diagnosed with NCGS, may not be a consequence of the elimination of gluten, but rather may be due to their reduced intake of FODMAPs.

The aim of our study was to assess the prevalence of NCGS in a cohort of patients without CD or wheat allergy and fulfilling the currently accepted clinical criteria for NCGS[4] who were taking a GFD on their own initiative. Their responses to challenges with gluten-containing vs. gluten-free flour were assessed in a double-blind manner, using a cross-over design.

## MATERIALS AND METHODS

This was a prospective, randomised, double-blind, placebo controlled, cross-over, challenge study undertaken in patients referred to our CD Clinic, who were on GFD because of symptoms they experienced when taking a diet which contained gluten. It was undertaken in an out-patient setting. The study protocol was approved by our local Ethics Committee. Written informed consent was obtained for all participants. This trial is registered at: Clinical Trials. Gov. Number, NCT 001827566.

We identified patients with a clinical diagnosis of NCGS seen in our clinic between 2008 and 2013 by reviewing their medical records. They were contacted by telephone or by letter. Their potential eligibility to participate in the study was assessed, using the following criteria: (i) strict adherence to a self-prescribed GFD over a period of at least 6 months, because of gastrointestinal and extra gastrointestinal symptoms perceived to be a consequence of dietary gluten; (ii) asymptomatic or mildly symptomatic [a score of <4 on the Gastrointestinal Symptoms Rating Scale (GSRS)[12]] while on the GFD, with recurrence of symptoms on any accidental exposure to gluten; (iii) exclusion of CD prior to starting the GFD as demonstrated by negative serum anti-tissue-transglutaminase (t-TG) and/or endomysial antibodies, and normal villous structure on duodenal biopsies (Marsh stage 0, 1 or 2); and (iv) exclusion of IgE mediated wheat allergy.

Patients fulfilling these criteria and who were willing to consider participating in the challenge study were seen in clinic. The following information was collected from them and from their medical records: demographic data; clinical data and the results of previous relevant investigations, including human leucocyte antigen (HLA) typing, serological testing and duodenal biopsy findings.

All patients that were willing to participate had their t-TG antibody level checked or rechecked. They completed a GSRS questionnaire, and were evaluated by the study dietician for adherence to their GFD, given further advice on adherence, and instructions on how to keep a 7-day dietary diary. They were reviewed 3 months later. Their adherence to their GFD was re-assessed by the dietician, their t-TG antibody levels were rechecked and they completed a further GSRS questionnaire. This visit was considered the baseline visit. Those who were still willing to participate and were eligible to do so were randomised with regards to the order in which they would receive a gluten or nongluten challenge. The randomisation was performed by computer and the study investigators were blinded to the randomisation results.

The challenges comprised gluten-containing and gluten-free flours. These were dispensed in sealed sachets labelled A and B. The participants and the investigators were blind to the contents of the sachets. Previously, we had established that 20 patients with CD were all unable to distinguish the flours by sight. Each sachet contained 10 g of flour, and instructions were given to the participants to sprinkle the contents of the sachet over pasta or soup; one sachet to be consumed each day for 10 consecutive days. This was followed by a 14-day washout period, and then by a further 10-day challenge, when participants were crossed over to receive the other flour. If patients experienced symptoms they judged to be severe, they were told to stop taking the challenge flour, wait until their symptoms had subsided, and if they were on their first challenge, to enter the washout period and take the next challenge as per the protocol.

Instructions were given to return unused sachets, to check compliance with the study protocol. During the study, participants kept 7-day dietary diaries. The information from these was entered into a data base (Microdiet, Downlee Systems Ltd, Bankhall Chapel-en-le-Frith High Peak, UK). This returns information on energy and macro- and micro nutrient intake. Adherence to the GFD was assessed by the study dietician, using a Likert scale, graded 1 (no digression from the diet) to 4 (no adherence to the diet), as previously described.[13]

At the end of each challenge phase, participants were asked, 'According to your symptoms, do you think that gluten was in the sachets labelled A, or in those labelled B'? They also completed a GSRS questionnaire and a Visual Analog Fatigue Scale (VAFS). The GSRS questionnaire has 15 questions concerning gastrointestinal symptoms experienced over the preceding week. Each is scored on a Likert scale, graded 1 (no discomfort at all) to 7 (very severe discomfort). These can then be grouped into five dimensions: abdominal pain; reflux; indigestion; diarrhoea and constipation. During the challenge periods, participants kept a daily diary of any symptoms they experienced.

The primary outcome measure of the study was the participants' ability to correctly identify the contents of sachets A and B based on symptom recurrence. Those who were able to were classified as having NCGS. Secondary outcome measures were determined by analysing the answers given to the GSRS questionnaires, the scores on the VAFS and changes in the t-Tg IgA and the antigliadin antibodies (AGA) IgA: IgG serum levels. Analyses were also undertaken according to HLA type and the Marsh stage of the duodenal biopsies.

### Gluten-containing and gluten-free flours

The gluten-containing and gluten-free flours were obtained commercially and comprised respectively: vital wheat gluten Amygluten (Tereos Syral, Marckolsheim, France, containing 78.9 g crude protein, 10.4 g starch, 2.1 g maltose, 0.8 g fructans, 0.2 g sucrose, 0.1 g glucose and 0.08 g fructose, per 100 g product); and Sfornagusto (BiAglut, Latina, Italy, containing 76.7 g starch, 6.8 g lactose, 0.16 g fructans, 6.6 g glucose, 0.04 g sucrose, 4 g protein, 5.5 g fibre and 0.2 g lipids, per 100 g product). The latter product is prepared from maize and potato and, apart from containing FODMAPs, its macronutrient composition is similar to that of the gluten-containing flour commonly used for bread-making.

### Serological assays and duodenal biopsies

We measured t-TG antibodies using an enzyme-linked immunosorbent assay which utilised a human recombinant t-Tg antigen known as Eu t-Tg® (Eurospital, Trieste, Italy). Antigliadin antibodies were measured using Antigliatest® (Eurospital). HLA typing was carried out using commercial kits (Innogenetics, Ghent, Belgium and Dynal Biotech Ltd., Bromborough, UK).

Duodenal biopsies which had been undertaken previously were reviewed by one of the authors (VV), blind to the patient's details, and classified by him according to the Marsh stage.

### Statistical analysis

Categorical variables were expressed as numbers and proportions. Continuous variables were expressed as means ± s.d. and tested for normal distribution using the D'Agostino and Pearson omnibus normality test. Scores on the GSRS questionnaire were expressed as the mean values of the items grouped in each dimension.[14, 15] Differences between continuous variables were assessed using *t*-tests and Wilcoxon tests for paired and unpaired samples respectively. Differences between categorical variables were tested using the $\chi^2$ test or Fisher's exact test, as appropriate. Statistical significance was determined by $P < 0.05$.

Statistical analysis was carried out using the GraphPad Prism 5 statistical package (GraphPad Software, San Diego, CA, USA).

## RESULTS

We identified 112 potential candidates from their case records for inclusion in the study. Of these, 77 agreed to be reviewed in clinic, and 53 of these were assessed as eligible to participate in the study, and agreed to do so.

However, 18 potential candidates were subsequently excluded because they had not fully adhered to their GFD in the 3-month period prior to baseline, or because of doubts regarding the diagnosis of NCGS. The remaining 35 patients (31 females) were randomised. (Figure 1 and Table S1). Their mean age was 41 ± 2 years. All reported having experienced gastrointestinal symptoms when eating gluten-containing diet; 30 also reported having experienced extra gastrointestinal symptoms.

Fourteen participants were HLA DQ2/8 positive, 16 were negative, and information on HLA type was unavailable in five patients. All 35 had had duodenal biopsies. Of these 15 were classified as Marsh stage 0, 12 as Marsh stage 1, and 5 as Marsh stage 2. In three cases, the Marsh stage could not be determined. None of the biopsies showed eosinophilic infiltration. In cases in which duodenal biopsy was classified as Marsh stages 1 & 2, investigations for *Helicobacter pylori* infection and for other causes of intraepithelial lymphocytosis had not been systematically undertaken.

There were no significant difference in the clinical characteristic of patients and their investigation findings (including the Marsh stage), except that patients who were HLA DQ2/8 positive were significantly older and had higher body mass indexes when compared to those who were HLA DQ2/8 negative (Table S2).

Prior to commencing the challenge phase of the study, no participant had tested positive for IgA or IgG AGA, or for IgA t-TG. All achieved Likert scores of 1 (indicating no digression from their GFD). This was consistent with the data from their 7-day dietary diaries.

## Gluten recognition

All randomised patients completed the challenge phase of the study, although one patient stopped taking the sachets of the first phase which he was randomised to, 2 days after starting the challenge, because of nausea and vomiting. On the primary outcome measure, 12 participants (34%) correctly identified gluten as causing symptom

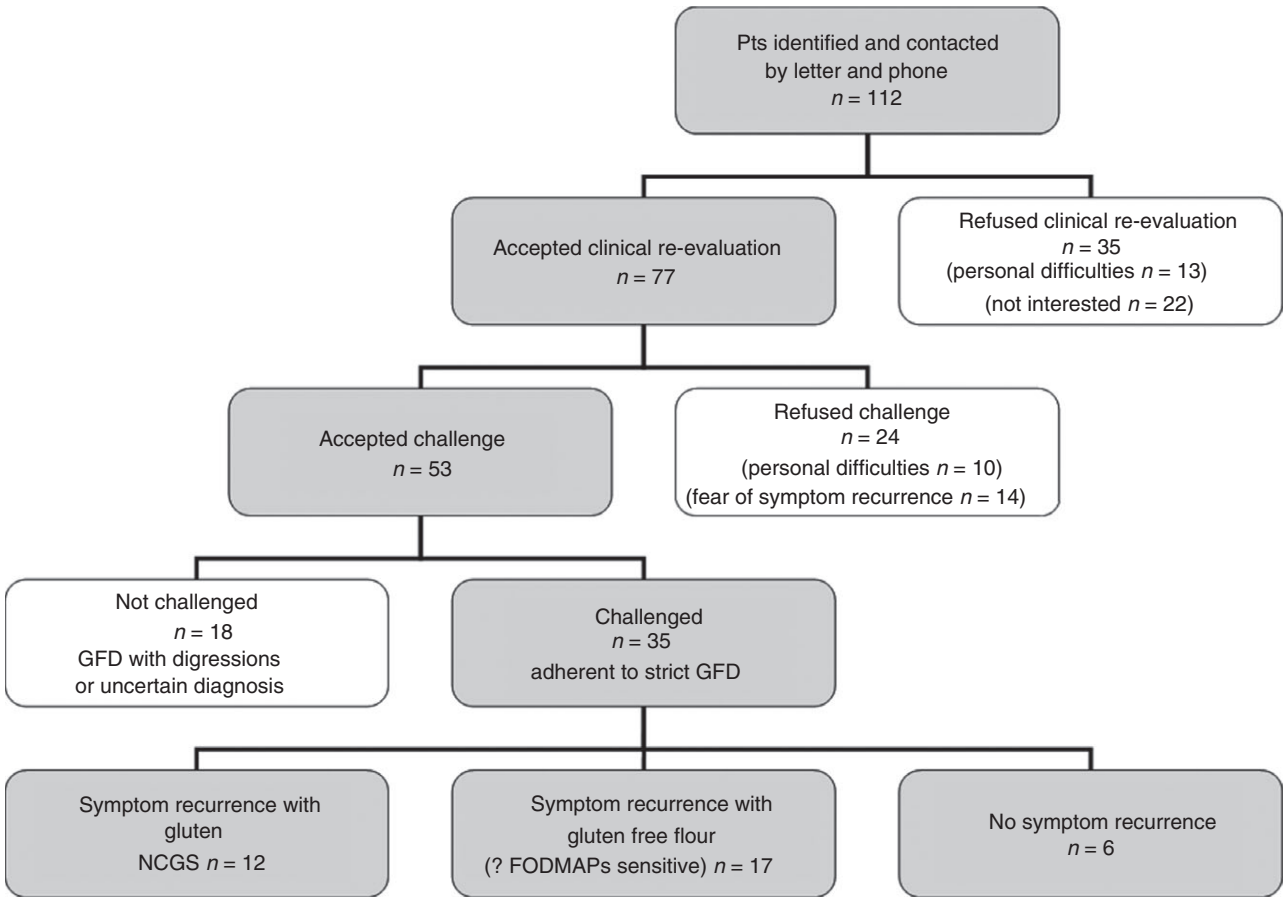


**Figure 1 |** Study flow chart. Thirty-five of 112 patients with a clinical diagnosis of noncoeliac gluten sensitivity (NCGS) were challenged with gluten-containing and gluten-free flour in a double-blind cross-over study.

recurrence and were classified as having NCGS, 17 (49%) identified gluten-free flour and six (17%) reported no symptom recurrence during challenge (Figure 1).

The symptoms recorded in the diaries of those classified as having NCGS were: fatigue ($n$ = 3 participants); nausea and vomiting ($n$ = 2); diarrhoea ($n$ = 2); abdominal bloating ($n$ = 2); regurgitation ($n$ = 1); constipation ($n$ = 1) and headache ($n$ = 1). The severity of these symptoms was scored as ≥4, and the maximum intensity occurred within 4 days of being challenged with gluten-containing flour in four participants and on days 6 and 9 in two participants. One participant stopped taking the initial sachet was randomised to, and was subsequently found to be taking gluten-containing flour.

The symptoms recorded in the diaries of those reporting symptom recurrence with gluten-free flour were: abdominal bloating, distension and pain (10 participants); tiredness (four participants); diarrhoea (two participants) and nausea (one participant). These symptoms had reached their maximum intensity within 5 days of the challenge.

The order of the challenge did not affect the ability of those classified as having NCGS to correctly recognise the gluten-containing flour; six participants correctly recognised the gluten-containing flour during the first challenge, and six during the second challenge. Similarly, of those reporting symptom recurrence with gluten-free flour, six participants recognised the gluten-free flour during the first challenge and 11 during the second challenge ($P$ = 0.6798).

The outcome of the challenges was not affected by HLA type or the Marsh stage on duodenal biopsy, either when analysed separately or in combination (Table 1). In particular, three of the eight patients with HLA DQ2/8 and Marsh I-II were sensitive to gluten compared with two of the five with Marsh 0 ($P$ = 0.3878).

## GSRS and VAFS scores

The mean GSRS dimension scores were higher when compared to baseline following challenge with the gluten-containing flour in the participants classified as having NCGS (Figure 2 and Table S3). The scores (baseline vs. after gluten-containing flour challenge) were: pain, 1.7 ± 0.8 vs. 2.6 ± 1.0 ($P$ = 0.005); reflux, 1.6 ± 0.5 vs. 2.2 ± 0.9 ($P$ = 0.019); indigestion, 1.9 ± 0.7 vs. 3.2 ± 1.1 ($P$ < 0.001); diarrhoea, 1.6 ± 0.7 vs. 2.9 ± 1.5 ($P$ = 0.014); and constipation, 1.9 ± 0.9 vs. 2.9 ± 1.3 ($P$ = 0.033). The mean GSRS dimension scores were not significantly different following challenge with the gluten-free flour when compared to baseline.

The mean GSRS dimension scores were significantly higher compared to baseline following challenge with gluten-free flour in participants reporting symptom recurrence with gluten-free flour (Figure 3 and Table S3). The scores were: (baseline vs. after gluten-free flour challenge): pain, 1.6 ± 0.9 vs. 3.0 ± 0.9 ($P$ = 0.005); reflux, 1.4 ± 0.5 vs. 2.3 ± 1.1 ($P$ = 0.006); indigestion, 2.0 ± 1.1 vs. 3.7 ± 1.1 ($P$ < 0.002); diarrhoea, 1.6 ± 0.7 vs. 3.0 ± 1.2 ($P$ = 0.001) and constipation, 1.6 ± 0.9 vs. 2.6 ± 1.3 ($P$ < 0.001). The mean GSRS dimension scores were not significantly different following challenge with the gluten-containing flour when compared to baseline (Table S3).

**Table 1 |** Number of patients with symptom recurrence with gluten flour, with gluten-free flour and with no symptom recurrence during double blind challenge. Patients are classified according to HLA genotype and Marsh stage of duodenal histology while on a gluten containing diet. Information on both HLA genotype and Marsh stage was available in 28 patients

| | Symptom recurrence | | | |
|---|---|---|---|---|
| | Gluten flour n (%) | Gluten-free flour n (%) | Absent n (%) | $P$ |
| HLA* | | | | |
| DQ2/8 | 6 (43) | 7 (50) | 1 (7) | 0.4137 |
| Not DQ2/8 | 5 (31) | 7 (44) | 4 (25) | |
| Marsh† | | | | |
| 0 | 3 (20) | 9 (20) | 3 (60) | 0.6258 |
| 1–2 | 6 (35) | 8 (47) | 3 (18) | |
| HLA DQ2/8+ Marsh | | | | |
| Marsh 0 | 2 (40) | 2 (40) | 1 (20) | 0.3878 |
| Marsh 1–2 | 3 (37) | 5 (63) | 0 (0) | |
| HLA not DQ2/8+ Marsh | | | | |
| Marsh 0 | 1 (14) | 5 (71) | 1 (14) | 0.1986 |
| Marsh 1–2 | 3 (37) | 2 (24) | 3 (37) | |

* Information not available in five patients.

† Distinction of Marsh 0–2 not available in three patients.

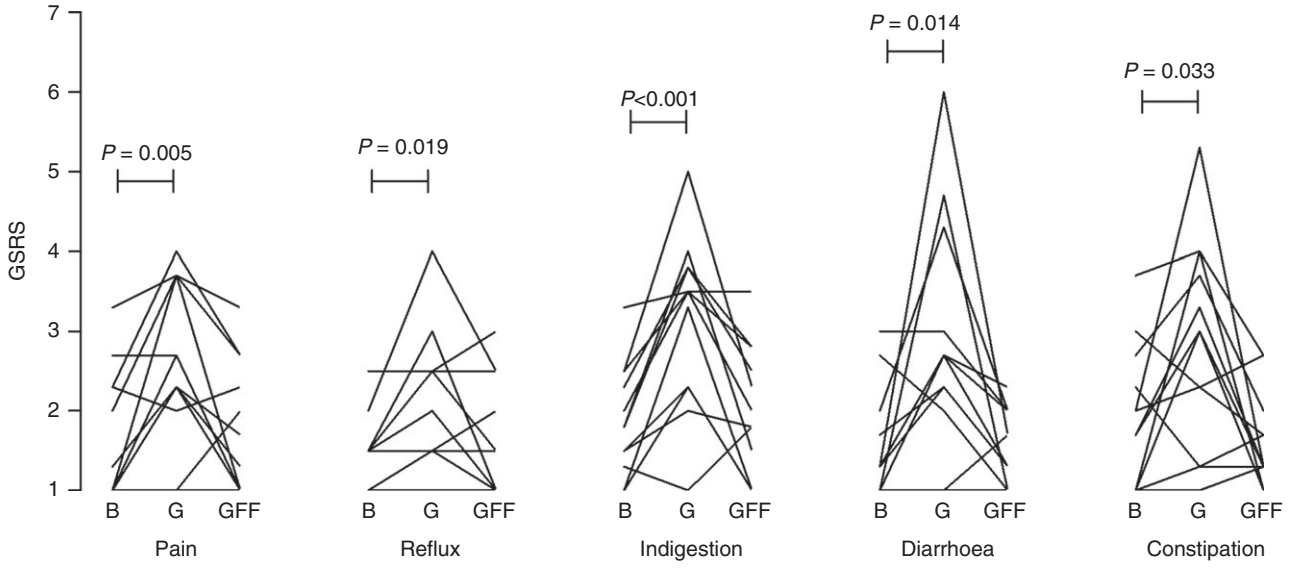


**Figure 2 |** Gastrointestinal Symptoms Rating Scale (GSRS) dimension scores at baseline (B) and following the blinded challenge with gluten-containing flour (G) and gluten-free flour (GFF) in patients who correctly identified the gluten-containing flour.

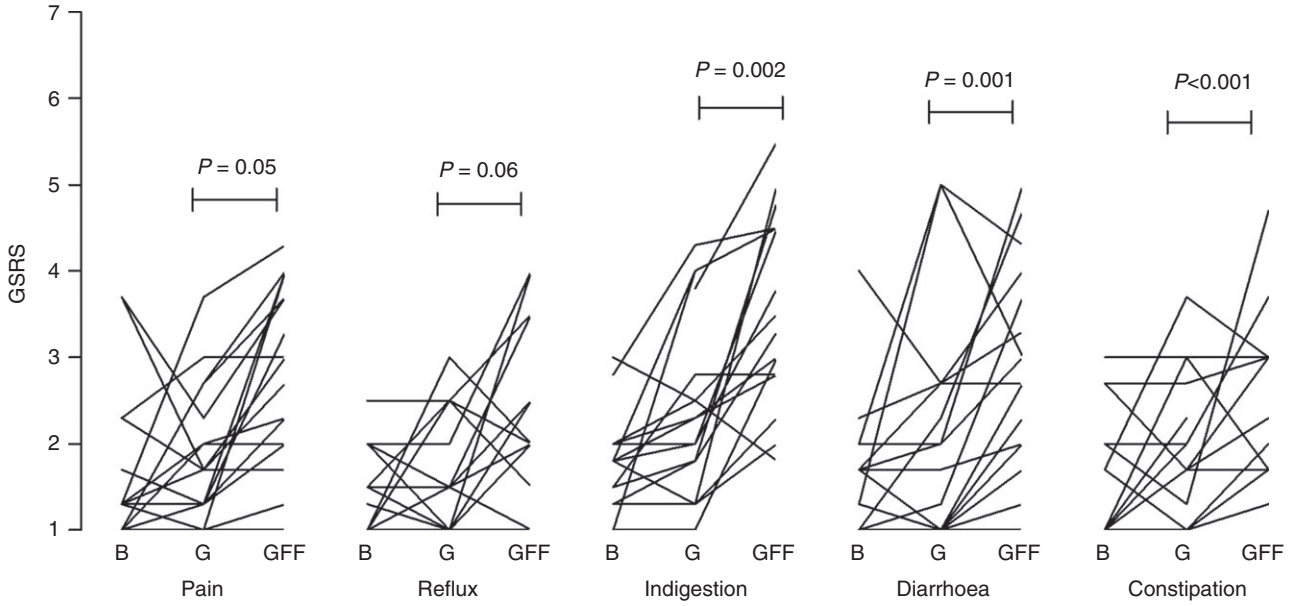


**Figure 3 |** Gastrointestinal Symptoms Rating Scale (GSRS) dimension scores at baseline (B) and following the blinded challenge with gluten-containing flour (G) and gluten-free flour (GFF) in patients who incorrectly identified the gluten-containing flour.

The mean GSRS dimension scores in those reporting no symptom recurrence were not significantly different following challenge with either gluten-containing flour or gluten-free flour compared to baseline (Table S3).

VAFS scores increased significantly following challenge with gluten-containing flour compared to baseline in participants classified as having NCGS (3.4 ± 1.2 vs. 4.9 ± 1.9; $P = 0.024$) and following challenge with gluten-free flour in patients classified as sensitive to

gluten-free flour (2.4 ± 1.5 vs. 5.6 ± 3.0; $P < 0.001$; Table S3).

## DISCUSSION

We found evidence from our study to support a diagnosis of NCGS in only a third of patients who met internationally recognised criteria for its diagnosis.[3, 4] That these patients have a sensitivity to gluten is supported by: them correctly identifying the flour containing gluten from that which did not; their report of symptoms after being challenged with gluten; and their increased GSRS scores following challenge with gluten, but not when challenged by gluten-free flour.

However, two-thirds of the participants were unable to correctly identify the flour containing gluten. Indeed, almost half of the participants erroneously identified the gluten-free flour as being the gluten-containing flour. These patients recorded symptoms, and their GSRS scores increased, after the gluten-free flour challenge, but not after the gluten-containing flour challenge. The explanation for this is unclear. However, they may be sensitive to a component of the flour, besides gluten. A role for undigested starch is possible.[16] Another possibility, is sensitivity to FODMAPs. The gluten-free flour used in the study contained FODMAPs, mainly because it included lactose and maize starch. Maize starch has been described as a 'red flag' for FODMAPs (Low FODMAP diet app, Monash University; available on the AppStore), which are well-recognised triggers of symptoms in IBS.[17] The possibility that these participants were sensitive to FODMAPs is supported by the results of a further study, so far only reported in abstract form, in which we found that a subgroup of those studied here with gluten-free flour sensitivity (but not those diagnosed with NCGS), improved when on a low FODMAP diet.[18]

Just under a fifth of the participants in the study, were unable to distinguish between the two flours, did not record any worsening of symptoms with either challenge, and did not have any significant changes in GSRS scores after either challenge. These findings suggest that the symptoms they experience when exposed to gluten-containing food in normal life, is related to a psychological anticipation of intolerance. This appears to be a nocebo effect. Emotional factors and commercial pressure may be important, even in patients with low levels of somatisation.[19]

We found that sensitivity to gluten-containing and gluten-free flours were independent of the HLA DQ2/8 or intestinal biopsy findings classified by Marsh stage, either separately or in combination. Indeed, there were fewer participants with gluten sensitivity who were both HLA DQ2/8 positive and had biopsies classified as Marsh stages 1 or 2, than in those without an HLA predisposition to CD. Given the small numbers, caution is required when interpreting this data. However, it is supportive of the contention that NCGS and CD are distinct conditions. At least one previous study included only those with intestinal biopsies classified as Marsh stage 0.[8] We included those whose biopsies were classified as Marsh stages 0, 1 and 2. We did so, because Marsh stages 1 and 2 reflect common, nonspecific histological findings, which are often transient, and are not associated with any specific functional gastrointestinal disorder,[20] or with the severity of gastrointestinal symptoms.[6]

Our findings should be considered alongside the three other gluten challenge studies in patients with cereal intolerance reported to date. The results are conflicting. The same Australian group first reported a controlled parallel group study, which gave support to the concept that gluten acted as a specific trigger for symptoms.[7] However, they then reported a controlled cross-over study and found that only 8% of participants reacted specifically to gluten[8]; there appeared to be a strong nocebo effect. A separate group from Italy found evidence of worsening of gastrointestinal and extra gastrointestinal symptoms following gluten challenge when compared with placebo, but although statistically significant, the clinical significance was felt to be irrelevant, when the intensity of the symptoms caused by the gluten challenge were compared to those reported by the participants when eating a gluten-containing diet.[6] The authors concluded that the study did not provide 'crucial evidence' for the existence of NCGS.

Methodological issues may, at least in part, explain these contrasting findings. There are marked methodological differences between our study and the other studies that may at least partly explain the different results. The Australian cross-over study used as its primary outcome measure, a global assessment of wellbeing by the participants.[8] The Italian study's primary outcome measure was based upon scoring by the participants of their gastrointestinal symptoms.[6] Our primary outcome measure was the participants' ability to identify the gluten challenge and distinguish it from the nongluten challenge. The significance of this is that it allowed those participants with NCGS to be identified. Following this, the symptoms they experienced on gluten challenge could be analysed. Worsening of symptoms with gluten challenge in those patients satisfying the diagnostic criteria for NCGS, is evident in both the other studies.[6, 8]

A separate methodological consideration is the length of time participants had been taking GFD prior to the entering the studies. In the Australian cross-over study this was only 6 weeks, in contrast to our study, in which it was 6 months; the shorter period may be associated with a larger placebo effect. Information on the duration of the pre-study GFD in the Italian study was not provided.

All the participants in the Australian cross-over study had had intestinal biopsies classified as Marsh stage 0.[8] In contrast, nearly half of our participants had biopsies classified as Marsh stages 1 and 2. Our reasoning for including these participants has already been discussed. We feel that it is supported by others.[6, 21–23] However, it means that our study population was, in at least one possibly significant respect, different from the Australian one.

Finally, one other difference between the results of our study and the others, is that they found that the order in which the challenge was given affected the intensity of the reported symptoms irrespectively of the nature of the challenger. We did not find such an effect.

A limitation of our study, and indeed all studies of NCGS, is that it is not possible to exclude the possibility that some of those diagnosed with NCGS, may be in an early 'latent' stage of CD. Half of our participants were HLA DQ2/8 positive. Most had low levels of t-TG antibodies, suggesting that some of them may have had a low intake of gluten, favouring latency of CD. Caution is appropriate when diagnosing NCGS in subjects with a genetic susceptibility to CD (as evidenced by HLA type) and Marsh stage 1 and 2 intestinal biopsy findings. According to a recent systematic review, 20% of patients with suspected NCGS who are HLA DQ2/8 positive, whose intestinal biopsy is classified as Marsh stage 1, and who have negative CD serology, may come within the spectrum now recognised as CD.[5]

Notwithstanding the above, our study design was a particular strength. The study used a double-blind, controlled challenge methodology, which despite having recognised pitfalls,[24] is considered to be the gold standard for diagnosing adverse reactions to food.[25] Moreover our methodology fulfils the diagnostic protocol for the confirmation of NCGS, as recommended by the recent consensus 'The Salerno Experts' Criteria'.[26]

In conclusion, our study has shown that gluten challenge leads to a recurrence of symptoms in only a third of patients fulfilling the recognised diagnostic criteria for the clinical diagnosis of NCGS. Consequently, NCGS is likely to be the correct diagnosis in only a minority of those who do not have CD, but whom themselves choose to follow a GFD. They are outnumbered by those sensitive to other components, such as FODMAPs, in cereal. The distinction between these patient groups is important clinically, as only patients with NCGS need to adhere strictly to a GFD. Meanwhile, the effectiveness of a low FODMAP diet in cereal intolerant patients, who are not gluten sensitive, deserves further investigation.[27]

## AUTHORSHIP

*Guarantor of the article*: A. Lanzini.

*Author contributions*: BZ jointly had the original idea for the study, and was involved in its design, the acquisition and interpretation of data, and in the drafting of the manuscript. RB assisted in the acquisition and interpretation of data. CR assisted in the acquisition and interpretation of data. FL assisted in the acquisition and interpretation of data. MM provided dietetic support. AH provided technical support. AL jointly had the original idea of the study, and was involved in the interpretation of data and revision of the manuscript.

All authors approved the final version of the manuscript.

## ACKNOWLEDGEMENT

*Declaration of personal and funding interests*: None.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Anthropometric characteristics, gastrointestinal and extra-gastrointestinal symptoms, duodenal histology according to Marsh classification, anti-tissue-tranglutaminase antibodies and HLA genotype of patients when on a gluten containing diet.

**Table S2.** Clinical characteristics of patients prior to self prescription of gluten-free diet and outcome of double bind challenge categorized according to HLA (upper panel) and Marsh classification of duodenal biopsies (lower panel).

**Table S3.** Scores of Gastrointestinal Symptoms Rating Scales (GSRS) and Visual Analogue Fatigue Scale (VAFS) during double blind challenge.

## REFERENCES


1. Verdu EF, Armstrong D, Murray JA. Between celiac disease and irritable bowel syndrome: the "no man's land" of gluten sensitivity. *Am J Gastroenterol* 2009; **104**: 1587–94.
2. Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. The prevalence of celiac disease in the United States. *Am J Gastroenterol* 2012; **107**: 1538–44.
3. Ludvigsson JF, Leffler DA, Bai JC, *et al.* The Oslo definitions for coeliac disease and related terms. *Gut* 2013; **62**: 43–52.
4. Sapone A, Bai JC, Ciacci C, *et al.* Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med* 2012; **10**: 13.
5. Molina-Infante J, Santolaria S, Sanders DS, Fernandez-Banares F. Systematic review: noncoeliac gluten sensitivity. *Aliment Pharmacol Ther* 2015; **41**: 807–20.
6. Di Sabatino A, Volta U, Salvatore C, *et al.* Small amounts of gluten in subjects with suspected nonceliac gluten sensitivity: a randomized, double-blind, placebo-controlled, cross-over trial. *Clin Gastroenterol Hepatol* 2015; doi: 10.1016/j.cgh.2015.01.029 [Epub ahead of print].
7. Biesiekierski JR, Newnham ED, Irving PM, *et al.* Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial. *Am J Gastroenterol* 2011; **106**: 508–14.
8. Biesiekierski JR, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. *Gastroenterology* 2013; **145**: 320–8.
9. Biesiekierski JR, Rosella O, Rose R, *et al.* Quantification of fructans, galacto-oligosacharides and other short-chain carbohydrates in processed grains and cereals. *J Hum Nutr Diet* 2011; **24**: 154–76.
10. Gibson PR, Newnham E, Barrett JS, Shepherd SJ, Muir JG. Review article: fructose malabsorption and the bigger picture. *Aliment Pharmacol Ther* 2007; **25**: 349–63.
11. Shepherd SJ, Lomer MC, Gibson PR. Short-chain carbohydrates and functional gastrointestinal disorders. *Am J Gastroenterol* 2013; **108**: 707–17.
12. Svedlund J, Sjodin I, Dotevall G. GSRS–a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci* 1988; **33**: 129–34.
13. Zanini B, Lanzarotto F, Mora A, *et al.* Five year time course of celiac disease serology during gluten free diet: results of a community based "CD-Watch" program. *Dig Liver Dis* 2010; **42**: 865–70.
14. Dong R, Guo ZY. Gastrointestinal symptoms in patients undergoing peritoneal dialysis: multivariate analysis of correlated factors. *World J Gastroenterol* 2010; **16**: 2812–7.
15. Zanini B, Ricci C, Bandera F, *et al.* Incidence of post-infectious irritable bowel syndrome and functional intestinal disorders following a water-borne viral gastroenteritis outbreak. *Am J Gastroenterol* 2012; **107**: 891–9.
16. Levitt MD. Malabsorption of starch: a normal phenomenon. *Gastroenterology* 1983; **85**: 769–70.
17. Tuck CJ, Muir JG, Barrett JS, Gibson PR. Fermentable oligosaccharides, disaccharides, monosaccharides and polyols: role in irritable bowel syndrome. *Expert Rev Gastroenterol Hepatol* 2014; **8**: 819–34.
18. Zanini B, Baschè R, Ferraresi A, Ricci C, Lanzarotto F, Lanzini A. Non celiac gluten sensitivity (NCGS) is outnumbered by FODMAPs sensitivity in patients spontaneously adhering to gluten free diet (GFD): a two stage double blind prospective study. *Gastroenterology* 2014; **146**: S–348.
19. Brottveit M, Vandvik PO, Wojniusz S, Lovik A, Lundin KE, Boye B. Absence of somatization in non-coeliac gluten sensitivity. *Scand J Gastroenterol* 2012; **47**: 770–7.
20. Zanini B, Lanzarotto F, Villanacci V, Carabellese N, Ricci C, Lanzini A. Clinical expression of lymphocytic duodenosis in "mild enteropathy" celiac disease and in functional gastrointestinal syndromes. *Scand J Gastroenterol* 2014; **49**: 794–800.
21. Sapone A, Lammers KM, Casolaro V, *et al.* Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Med* 2011; **9**: 23.
22. Volta U, Tovoli F, Cicola R, *et al.* Serological tests in gluten sensitivity (nonceliac gluten intolerance). *J Clin Gastroenterol* 2012; **46**: 680–5.
23. Carroccio A, Mansueto P, Iacono G, *et al.* Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: exploring a new clinical entity. *Am J Gastroenterol* 2012; **107**: 1898–906.
24. Asero R, Fernandez-Rivas M, Knulst AC, Bruijnzeel-Koomen CA. Double-blind, placebo-controlled food challenge in adults in everyday clinical practice: a reappraisal of their limitations and real indications. *Curr Opin Allergy Clin Immunol* 2009; **9**: 379–85.
25. Sampson HA, Sicherer SH, Birnbaum AH. AGA technical review on the evaluation of food allergy in gastrointestinal disorders. American Gastroenterological Association. *Gastroenterology* 2001; **120**: 1026–40.
26. Catassi C, Elli L, Bonaz B, *et al.* Diagnosis of non-celiac gluten sensitivity (NCGS): the Salerno Experts' Criteria. *Nutrients* 2015; **7**: 4966–77.
27. Rao SS, Yu S, Fedewa A. Systematic review: dietary fibre and FODMAP-restricted diet in the management of constipation and irritable bowel syndrome. *Aliment Pharmacol Ther* 2015; **41**: 1256–70.