Non-celiac gluten sensitivity and
Gastrointestinal symptoms

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Abstract

Background: Non-celiac gluten sensitivity (NCGS) is a condition, in which the patient suffers gastrointestinal symptoms when consuming gluten, without having celiac disease or wheat allergy. A precise definition of NCGS does not yet exist, and the mechanism is unclear. However, the patient experience symptom relief on gluten free diet (GFD). Elimination of dietary gluten, followed by a standardized gluten challenge may confirm the condition.

Aim: To evaluate a standardized open gluten challenge in patients with suspected non-celiac gluten sensitivity at the gastroenterology unit at Oslo University Hospital, Rikshospitalet.

Material and methods: Forty one patients (34 females), mean age 41 years (range 17-75), mean BMI 23 kg/m² with suspected NCGS were investigated from 2009 to 2014. The patients were examined with serology (anti-tissue transglutaminase, total Ig A levels) and/or gastrointestinal biopsy (HLA DQ2/DQ8 haplotype), to exclude celiac disease as a cause of the patients’ symptoms. The patients underwent a 3-day open challenge with four slices of gluten-containing white bread per day. The endpoints were gastrointestinal symptoms and general health complaints reported using the Gastrointestinal Symptom Rating-Scale (GSRS), Subjective Health Complaints (SHC) and visual analogue scale (VAS), all self-administrated questionnaires.

Results: Of the forty one patients, thirty patients were clinically evaluated to meet the criteria for the ICD-10 K52.2 diagnosis “Allergic and dietetic gastroenteritis and colitis” after completing the gluten challenge. Of the patients that got the diagnosis, 16 patients were HLA DQ2/DQ8 positive, 9 patients were HLA DQ2/DQ8 negative. One patient was HLA DQ2/DQ8 positive, but did not get the diagnosis. For the group with positive test, all patients experienced a significant exacerbation (p<0.001) of symptoms in response to gluten except three patients, reported using GSRS, SHC and VAS. The difference between symptoms in their normal situation to provocation was significantly greater in the group that tested positive (∆GSRS=34.3, ∆SHC=17.1, ∆abdominal pain=56.1, ∆bloating=57.4, ∆wellbeing=55.3), compared with the group with negative test (∆GSRS=9, ∆SHC=6, abdominal pain=12.1, ∆bloating=26.6, ∆wellbeing=16.7). Symptomatic responses to gluten did not significantly differ in those expressing HLA DQ2/DQ8, compared with those who did not.

Conclusion: Seventy three per cent of the patients were evaluated to meet the criteria for the ICD-10 K52.2 diagnosis “Allergic and dietetic gastroenteritis and colitis” after completing the gluten challenge. The group that got the diagnosis reported more symptoms during challenge and more change in complaints from baseline to challenge compared with the group that did not get the diagnosis. Limitations of this study are the open challenge where the amount of gluten was not standardized, and the lack of a placebo control group. A better understanding of NCGS as a condition will require blinded, placebo-controlled challenge protocols.

Introduction

Today we are acquainted with three clinical forms of gluten intolerance, in which wheat is central in either the underlying disease or in causing the symptoms. The clinical conditions are non-celiac gluten sensitivity, celiac disease and wheat allergy. Non-celiac gluten sensitivity (NCGS) is a syndrome, in which the patient suffers gastrointestinal symptoms when consuming gluten, without having celiac disease. A precise definition of NCGS does not yet exist, and the mechanism is unclear. Currently NCGS is understood as a condition
associated with various symptoms when consuming food containing wheat, rye and barley (1). In contrast, celiac disease (CD) is defined as an enteropathy characterized by mucosal inflammation, villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis, which occur upon exposure to dietary wheat gluten and which improves after withdrawal of gluten from the diet (2, 3). It is well known that CD is an autoimmune disorder that is triggered by an environment agent, which is the gliadin component of gluten, in genetically predisposed individuals (close association with HLA-DQ2 and/or DQ8 gene loci) (4). However, it also seems like patients with NCGS experience better health on gluten free diet (GFD), even though they do not have the diagnostic histological and serological signs of CD (2). Wheat allergy has been classified as an Ig E mediated food hypersensitivity reaction, affecting the skin, GI-tract or respiratory tract (5, 6). Patients are therefore considered to have NCGS if the gastrointestinal symptoms markedly improve on a GFD and CD and wheat allergy has been excluded (5).

The overall prevalence of NCGS in the general population is still unknown, mainly because many patients are currently self-diagnosed and start a GFD without medical advice or consultation. However, new data claims the prevalence of NCGS to be as high as 6% in the American population. The prevalence of CD is estimated at ~1% (2, 8). This means that the majority of people on a GFD do not have CD; patients with self-reported NCGS therefor appear to outnumber those with CD.

Former studies have consistently shown that wheat is one of the most common factors inducing GI-symptoms (5). This is though a complex area, because a lot of people think that benefits of a gluten free diet or wheat free diet equate to a problem caused by gluten (5). Today there is no clear evidence indicating that gluten is the responsible agent alone. Whether gluten is the sole or main trigger contributing gastrointestinal symptoms in NCGS patients has never been directly assessed and still remains to find out (1, 9).

The symptoms associated with NCGS are mainly subjective, including abdominal pain, diarrhea, constipation, nausea, vomiting, headache, “brain fog”, tingling and/or numbness in hands and feet, fatigue and musculoskeletal pain. Other, more severe conditions, linked to neurology and psychiatry, have also been reported (1). It is interesting to see how earlier studies support that removal of gluten from the diet in NCGS patients does not lead to reduction of symptoms (5, 10). In fact, in many cases a GFD has resolved their symptoms, but in other cases, the patients continue their diet although the symptoms remain uncontrolled. It is therefore reasonable to think that besides gluten, other components of wheat may be responsible for the symptoms reported by NCGS patients (8).

Besides non-celiac gluten sensitivity, celiac disease and wheat allergy, irritable bowel syndrome (IBS) is also a clinical condition in which wheat has a pathogenic role in causing the symptoms. IBS is characterized by abdominal pain, bloating, wind, distension and altered bowel habit, but without abnormal pathology (5). The condition is by many considered to be a functional gastrointestinal disorder, in the sense that the clinical presentation cannot be explained by laboratory testing or biopsy testing. However, the Rome criteria is used to define IBS, as “recurrent abdominal pain or discomfort at least three days per month in the last three months with two or more of the following: Improvement with defecation, onset associated with a change in frequency of stool, or onset associated with a change in form (appearance) of stool.” (11).

An overlap between the irritable bowel syndrome (IBS) and NCGS has also been suspected, requiring even more strict diagnostic criteria defining NCGS (1, 8). It has been detected that gluten withdrawal may improve patient symptoms in IBS (8, 9). However, how specific the effect of gluten withdrawal from the diet of patients with IBS is, still remains to be investigated. Besides gluten, wheat, and wheat derivatives contain other constituents that could play a role in triggering symptoms in IBS patients, e.g. amylase-trypsin inhibitors.
(ATIs) and fructans. In a study conducted by Biesiekirski et al. (10), 37 patients with IBS/self-reported NCGS were investigated by a double-blind crossover trial. Patients were randomly assigned to a period of reduced low-fermentable, poorly-absorbed, short-chain carbohydrates (fermentable oligo-, di-, and mono-saccharides and polyols = FODMAPs) diet, and then introduced to either a gluten diet or whey proteins challenge. During reduced FODMAP, all participants consistently experienced reduced gastrointestinal symptoms. Simultaneously, symptoms increased with diets including gluten or whey proteins. FODMAPS list includes fructans, galactans, fructose, and polyols that are found in several foodstuffs, including wheat, vegetables, and milk derivatives. The outcome of the study opens the possibility that the alleviation of symptoms with GFD in patients with IBS is an unspecific consequence of decreased FODMAPs intake - provided that wheat is one of the possible sources of FODMAPs. It is important to note that FODMAPs cannot wholly and exclusively explain the symptoms experienced by NCGS subjects. The reason is that these patients experienced an amelioration of symptoms while on a GFD despite continued ingestion of FODMAPs from other sources. It is possible to discern that some IBS cases may be entirely caused by FODMAPs, and hence cannot be classified as NCGS patients (8). With the absence of objective clinical diagnostic criteria and the lack of specific biomarkers, it becomes hard to define NCGS without using only clinical terms. The definitive diagnosis of NCGS can so far only be made through gluten challenge (1). As a result of this approach, it is hard to detect which component(s) of wheat that is contributing to gastrointestinal symptoms in NCGS patients.

The aim of this study is to evaluate a standardized open gluten challenge in patients with suspected non-celiac gluten sensitivity at the gastroenterology unit at Oslo University Hospital, Rikshospitalet.

Methods

Patients
We describe patients with suspected non-celiac gluten sensitivity (NCGS) that was referred to the outpatient clinic at Oslo University Hospital from November 2009 to June 2014. The patients were examined with serology and/or gastrointestinal biopsy, to exclude celiac disease as a cause of the patients’ symptoms. Serologic testing included testing for Ig A antibodies to tissue transglutaminase (tTG). We also examined if the patients were HLA-DQ2 or HLA-DQ8 positive or negative. The inclusion criteria therefore encompassed negative serology (anti-tissue transglutaminase, total Ig A levels), the absence of HLA-DQ2 or HLA-DQ8 haplotype or a normal duodenal biopsy with preserved villous anatomy (12). Patients with clinical defined wheat allergy were not included.

Gluten challenge
The patients were challenged with four slices of gluten-containing white bread per day for 3 days, 7 days or 14 days. The patients were instructed to eat four slices of gluten-containing white bread per day; preferably pre sliced sandwich bread. The amount of gluten in the white bread used in the challenge varied from 14000-181400 mg/kg within the same brand. Four slices of bread (120 g) contained 1,7 g gluten or 21,7 g gluten. This means that the amount of gluten for challenge was not standardized.
**Measures**

The endpoints were gastrointestinal symptoms and general health complaints recorded by self-administered questionnaires. All measures were recorded for 3 days before gluten challenge, 3 days during challenge, and 3 days after the gluten challenge. Two patients completed the challenge over 7 days, ten persons completed it over 14 days, and one over 17 days.

The Gastrointestinal Symptom Rating Scale-IBS (GSRS-IBS) is a validated symptom scale developed for patients with IBS (13). It includes symptoms related to irritable bowel syndrome and is an expansion of the reliable Gastrointestinal Symptom Rating Scale, GSRS, which has been widely used in patients with celiac disease. It is a disease-specific symptom questionnaire where the aim is to detect which symptoms that are the most aggravating, and to monitor how patients respond to intervention. A cut-off for the presence of IBS has not been established. GSRS-IBS consists of 13 items, categorised into five clusters of symptoms; abdominal pain, bloating, diarrhoea, constipation and satiety. It measures symptoms during the past 7 days with a 7-point Likert Scale (*no discomfort to very severe discomfort*), were higher score denotes more symptoms. Minimum score for GSRS is 13. Brottveit et al. adjusted the GSRS-IBS before use in an earlier study on patients with possible gluten intolerance, to reflect the last 3 days instead of the last 7 days (2). This modification was also used on our study sample.

General health problems were registered by completion of the Subjective Health Complaints Inventory (SHC) at the same time as the GSRS-IBS. The questionnaire intends to record subjective health complaints, regardless of any particular diagnosis (14). The original form consists of 29 questions concerning severity and duration of subjective somatic and psychological complaints experienced during the last 30 days. The severity of each complaint is graded = *none*, 1 = *some*, 2 = *much*, 3 = *severe*. The complaints are categorized into five categories: *Musculoskeletal pain, pseudoneurology, gastrointestinal problems, allergy and flu* (14). The sum score ranges from 0-87, and is calculated by adding the degree of severity for each question in each category. The version of SHC that was used in this study has also been modified by Brottveit et al. in terms of duration of reported complaints, in the same way as for GSRS-IBS. The participants were asked to record subjective health complaints experienced during the last 3 days instead of 30 days as in standard SHC.

During challenge the patients were also asked to scale the presence of “abdominal pain”, “bloating” and “wellbeing” using the visual analogue scale (VAS). Visual analogue scale is a psychometric response scale where respondents specify their level of agreement to a statement by marking on a line the position that best represents their perception of the current state between two end-points. The end-point to the left was “No abdominal pain”, and the end-point to the right was “Severe abdominal pain”. An identical diagram was designed for bloating and wellbeing, using a 100 mm analogue line. The VAS score is then determined by measuring in millimetres from the left end of the line to the point indicated by the patient (15).

For statistical analysis of the results reported by GSRS, SHC and VAS in different periods, we used paired t-test. For testing difference in symptom score between groups, we used two independent sample t-tests. Where groups’ size has been small in comparing groups, non-parametric test was used.
Results

This study population consisted of 41 patients, 34 women (83%) and 7 men (17%). The mean age was 41 years of age (range 17-75). Of the patients tested for the presence of HLA DQ2 or DQ8 (n=30), 17 patients (41%) were HLA DQ2/DQ8 positive. Eleven patients were not genomically tested for HLA DQ2/DQ8 status. (Table 1)

Table 1 Study Subject Characteristics (n=41)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>34</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
</tr>
<tr>
<td>HLA DQ2/DQ8 positive (n=30)</td>
<td>17</td>
</tr>
<tr>
<td>Age (mean years, range)</td>
<td>41 (17-75)</td>
</tr>
<tr>
<td>BMI (mean kg/m^2, range n=25)</td>
<td>23 (15-34)</td>
</tr>
<tr>
<td>Duration of GFD (mean months, range n=27)</td>
<td>26 (0.5-95)</td>
</tr>
</tbody>
</table>

The duration of GFD among the participants varied considerably. Some of the patients were on a gluten free diet on their own initiative long before (months-years) consulting a physician, while others started their GFD only weeks before the challenge, as a part of this study. The mean duration of time on a GFD before challenge was 26 months (range 0.5-95, n=27), which means that all patients were on a GFD for minimum 14 days of duration before challenge. The mean duration of time on the scheduled gluten challenge was 6.4 days. Twenty-three patients completed the challenge over 3 days, two patients completed the challenge over 7 days, ten persons completed it over 14 days, and one over 17 days. Of the 41 glutenchallenged patients, 38 (93%) consumed the prescribed amount of gluten containing bread (mean amount of bread was 4.1 slices per day) and completed the study as per protocol. One patient did not meet for challenge because of lack of motivation. Two patients ceased the study diet prematurely because of intolerable symptoms.

Symptom score differed significantly before, during and after challenge for both GSRS-IBS and SHC. Before challenge (day -2 to 0), the population reported a mean score of gastrointestinal symptoms (GSRS-IBS) to be 17.7 (n=38, SD 10.2). During the challenge, GSRS-IBS mean score increased to 47.0 (n=31, SD 14.3), and decreased to 34.9 (n=37, SD 12.4) after provocation. Minimum score for GSRS is 13. Mean score of Subjective Health Complaints (SHC) was 10.4 (n=37, SD 9.3) before, 25.2 (n=29, SD 11.1) during and 18.5 (n=36, SD 11.25) after challenge.

Changes in symptoms from baseline (before) to challenge (during) and back to GFD (after) were also significantly greater for abdominal pain, bloating and total wellbeing, reported by Visual Analogue Scale (VAS, n=26). Mean score for abdominal pain was 10.1 mm (n=26, SD 13.2) before challenge, 57.7 mm (n=25, SD 26.7) during challenge and 38.4 mm (n=25, SD 23.4) after challenge. Mean score for bloating was 7.5 mm (n=26, SD 8.3) before, 59.0 mm (n=25, SD 26.2) during and 33.8 mm (n=25, SD 24.2) after challenge. Mean score for total wellbeing was 13.4 mm (n=26, SD 15.1) before, 61.5 (n=25, SD 27.0) mm during and 49.8 mm (n=25, SD 26.7) after challenge. Mean stool frequency per day was 1.6 before (n=28), 2.2 during (n=29) and 2.0 after challenge (n=29).
Table 2 Mean scores for Gastrointestinal Symptom Rating-Scale IBS (GSRS-IBS), Subjective Health Complaints (SHC) and VAS before, during and after gluten challenge.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Before*</th>
<th>During*</th>
<th>After*</th>
<th>p-value¹</th>
<th>p-value²</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSRS</td>
<td>17.7 (n=38)</td>
<td>47.0 (n=31)</td>
<td>34.9 (n=37)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SHC</td>
<td>10.4 (n=37)</td>
<td>25.2 (n=29)</td>
<td>18.5 (n=36)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>10.1 (n=26)</td>
<td>57.7 (n=25)</td>
<td>38.4 (n=25)</td>
<td>&lt;0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>Bloating</td>
<td>7.5 (n=26)</td>
<td>59.0 (n=25)</td>
<td>33.8 (n=25)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Wellbeing</td>
<td>13.4 (n=26)</td>
<td>61.5 (n=25)</td>
<td>49.8 (n=25)</td>
<td>&lt;0.001</td>
<td>0.021</td>
</tr>
</tbody>
</table>

¹ Mean of 3, 7 or 14 days recording. ** Before vs. during, during vs. after, paired t-test.

During challenge the most frequently reported complaints with score 3 were bloating, abdominal pain, tiredness, constipation, headache and sleeping problems. Tiredness, bloating, abdominal pain, depression, headache, sleeping problems, diarrhea and musculoskeletal pain were the dominating symptoms of score 2 during challenge. Fewer and less severe symptoms were reported before challenge. Most frequent were musculoskeletal symptoms and headache with score 2, and bloating, obstipation, diarrhea, tiredness, musculoskeletal symptoms, depression, flu and allergy with score 3, reported by one or two patients.

All patients experienced an exacerbation of symptoms in response to gluten except three patients. Yet, there was a high degree of variation in symptoms at baseline, and in the level of symptoms reported during challenge (Figure 1).

Figure 1

a)  

![GSRS Graph](image)

b)  

![SHC Graph](image)
Figure 1 Mean symptom score before, during and after challenge measured by a) GSRS-IBS, b) SHC and c) Visual Analogue Scale (VAS).

Table 3 shows the symptom response to gluten in HLA DQ2/DQ8 positive compared to HLA DQ2/DQ8 negative patients. There was no significant difference between the groups. There was no significant difference in symptom change from baseline to end of challenge grouped by HLA DQ2/DQ8 typing (Figure 2).

Table 3 Mean score in level of symptoms in patients expressing HLA DQ2/DQ8, and in patients who are HLA DQ2/DQ8 negative.

<table>
<thead>
<tr>
<th>Measure</th>
<th>HLA DQ2/DQ8 positive* n=17</th>
<th>HLA DQ2/DQ8 negative* n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSRS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>15.2 (n=17)</td>
<td>13.7 (n=13)</td>
</tr>
<tr>
<td>During</td>
<td>48.6 (n=14)</td>
<td>42.0 (n=9)</td>
</tr>
<tr>
<td>After</td>
<td>35.3 (n=16)</td>
<td>33.1 (n=13)</td>
</tr>
<tr>
<td>SHC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>8.2 (n=17)</td>
<td>9.7 (n=12)</td>
</tr>
<tr>
<td>During</td>
<td>26.4 (n=13)</td>
<td>20.8 (n=9)</td>
</tr>
<tr>
<td>After</td>
<td>17.6 (n=16)</td>
<td>18.8 (n=12)</td>
</tr>
<tr>
<td>Wellbeing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>12.3 (n=11)</td>
<td>5.6 (n=8)</td>
</tr>
<tr>
<td>During</td>
<td>61.0 (n=11)</td>
<td>62.1 (n=8)</td>
</tr>
<tr>
<td>After</td>
<td>38.4 (n=11)</td>
<td>53.0 (n=8)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>6.6 (n=11)</td>
<td>5.1 (n=8)</td>
</tr>
<tr>
<td>During</td>
<td>56.3 (n=11)</td>
<td>58.9 (n=8)</td>
</tr>
<tr>
<td>After</td>
<td>34.4 (n=11)</td>
<td>43.6 (n=8)</td>
</tr>
<tr>
<td>Bloating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>6.9 (n=11)</td>
<td>5.3 (n=8)</td>
</tr>
<tr>
<td>During</td>
<td>60.2 (n=11)</td>
<td>61.8 (n=8)</td>
</tr>
<tr>
<td>After</td>
<td>29.2 (n=11)</td>
<td>38.2 (n=8)</td>
</tr>
</tbody>
</table>

*HLADQ2/DQ8 positive vs. negative, two independent sample t-test, non significant
Figure 2

a) GSRS

b) SHC

c) Wellbeing
Figure 2 Changes in level of symptoms from baseline to end of challenge in patients expressing HLA DQ2/DQ8 and in patients who are HLA DQ2/DQ8 negative, reported by a) GSRS, b) SHC and c) VAS. No significant changes between groups were shown (Mann Whitney two independent sample test).

Of the 41 patients included in our study, 30 patients (73%) were clinically evaluated to meet the criteria for the ICD-10 K52.2 diagnosis “Allergic and dietetic gastroenteritis and colitis” (16) after completing the gluten challenge. Eight patients (19.5%) had negative test of gluten challenge. Three patients did not complete the study. Of the 30 patients that met the diagnosis criteria for K52.2, 16 patients were HLA DQ2/DQ8 positive, while 9 patients were HLA DQ2/DQ8 negative (Table 4). Five patients of those who were not tested for HLA DQ2/DQ8 status got the diagnosis.

Table 4 Patients fulfilling the K52.2 diagnosis criteria “Allergic and dietetic gastroenteritis and colitis” and their HLA DQ2/DQ8 status.

<table>
<thead>
<tr>
<th>HLA-genotype</th>
<th>K52.2 (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA DQ2/DQ8 positive (n=17)</td>
<td>16</td>
</tr>
<tr>
<td>HLA DQ2/DQ8 negative (n=13)</td>
<td>9</td>
</tr>
<tr>
<td>Not HLA DQ2/DQ8 tested (n=11)</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 5 gives an overview of mean symptom score before, during and after challenge grouped by diagnosis K52.2. Symptoms during challenge in both groups were statistically tested and found significantly different for GSRS (p=0.004) and bloating (p=0.038). Figure 3 shows the change in symptom score grouped by diagnosis K52.2. The change was significant for GSRS (p=0.02), abdominal pain (p=0.04) and bloating (p=0.04).
Table 5 An overview over mean score in level of symptoms in patients that were clinically evaluated to meet the criteria for the diagnosis “Allergic and dietetic gastroenteritis and colitis” (K52.2), and the patients that did not.

<table>
<thead>
<tr>
<th>Measure*</th>
<th>K52.2 fulfilled n=30</th>
<th>K52.2 not fulfilled n=11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GSRS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>16.2 (n=30)</td>
<td>23.5 (n=8)</td>
</tr>
<tr>
<td>During</td>
<td><strong>50.5 (n=25)</strong></td>
<td>32.5 (n=6)</td>
</tr>
<tr>
<td>After</td>
<td>35.3 (n=29)</td>
<td>33.8 (n=8)</td>
</tr>
<tr>
<td><strong>SHC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>8.9 (n=29)</td>
<td>16.0 (n=8)</td>
</tr>
<tr>
<td>During</td>
<td>26.0 (n=23)</td>
<td>22.0 (n=6)</td>
</tr>
<tr>
<td>After</td>
<td>16.9 (n=29)</td>
<td>25.3 (n=7)</td>
</tr>
<tr>
<td><strong>Wellbeing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td><strong>10.8 (n=20)</strong></td>
<td>26.5 (n=5)</td>
</tr>
<tr>
<td>During</td>
<td>66.1 (n=20)</td>
<td>43.2 (n=5)</td>
</tr>
<tr>
<td>After</td>
<td>50.2 (n=20)</td>
<td>48.0 (n=5)</td>
</tr>
<tr>
<td><strong>Abdominal pain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>6.7 (n=20)</td>
<td>25.5 (n=5)</td>
</tr>
<tr>
<td>During</td>
<td>62.8 (n=20)</td>
<td>37.6 (n=5)</td>
</tr>
<tr>
<td>After</td>
<td>39.1 (n=20)</td>
<td>35.7 (n=5)</td>
</tr>
<tr>
<td><strong>Bloating</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>7.0 (n=20)</td>
<td>11.0 (n=5)</td>
</tr>
<tr>
<td>During</td>
<td><strong>64.4 (n=20)</strong></td>
<td>37.6 (n=5)</td>
</tr>
<tr>
<td>After</td>
<td>34.3 (n=20)</td>
<td>31.6 (n=5)</td>
</tr>
</tbody>
</table>

*Symptoms before, during and after tested by two independent sample t-test. Number in bold means significantly different.

Figure 3

a)
b) SHC

![Graph showing SHC scores before, during, and after with K52.2 fulfilled and not fulfilled.]

- Total score: K52.2 fulfilled vs K52.2 not fulfilled
- p-value = 0.36

c) Wellbeing

![Graph showing Wellbeing scores before, during, and after with K52.2 fulfilled and not fulfilled.]

- Total score: K52.2 fulfilled vs K52.2 not fulfilled
- p-value = 0.08

Abdominal pain

![Graph showing Abdominal pain scores before, during, and after with K52.2 fulfilled and not fulfilled.]

- Total score: K52.2 fulfilled vs K52.2 not fulfilled
- p-value = 0.04
Figure 3 Changes in level of symptoms in patients that were clinically evaluated to meet the criteria for the diagnosis “Allergic and dietetic gastroenteritis and colitis” (K52.2), and the patients that did not, reported by a) GSRS, b) SHC and c) VAS. Statistics: Mann Whitney two independent sample test.

Discussion

Description of the study sample from table 1
Previous studies on NCGS as a syndrome show that there is a high correlation with female gender and adult age (2, 17, 18). Biesiekierski et al. (17) report a population with a mean age of 43.5 years and 88% were female, and Volta et al. (18) describe patients with a mean age of 38 years and 84% female. Brottveit et al. (2) report a study sample with mean age 40.5, where 68% were women. This is consistent with our result, with a mean age of 41 years and 83% of our patients were women. Forty one per cent of our patients were HLA DQ2/DQ8 positive, even though eleven patients never got tested for HLA DQ2/DQ8 status. Compared with Volta et al. and their results, HLA DQ2/DQ8 positivity was only found in 25% and 8% of the patients. An important finding of our study is therefore that our results confirm that suspected NCGS seems to be much more frequent in females than in males, and that the condition can have its onset at any age, but is much more frequent in adulthood.

Significant changes in symptoms from baseline
Symptom score differed significantly before, during and after challenge for GSRS-IBS and SHC, and for abdominal pain, bloating and total wellbeing reported by VAS. Although subjectively reported, these findings support the existence of NCGS as a diagnosis.

No difference between HLA positive and HLA negative patients
HLA typing is particularly useful in distinguishing between NCGS and CD, as negativity for HLA DQ2/DQ8 has a great negative predictive value for CD (1). However, it cannot to a similar extent predict whether a person would or would not have a symptomatic response to gluten when CD is already excluded. Our results show that the symptomatic response to gluten did not differ in those expressing HLA DQ2/DQ8 compared with those who were HLA DQ2/DQ8 negative. Our result correlates with the findings of Volta et al. (18), where no correlation was found between NCGS and positivity for HLA DQ2/DQ8, found in 25% and 8% of the patients. This is in contrast to the results of previous studies, which show that subjects with positive HLA genes are more likely to respond to a GFD compared with subjects with negative genes (19, 20). On the other hand, in general, around 40% of the population is positive for HLA genes (21). This number is similar to our result, where 41% of
the patients were HLA DQ2/DQ8 positive. HLA typing therefore may not give us specific information about how the patient will respond to a gluten free diet. At the same time, we must also consider that this finding might be attributed to selection bias, as the patients in our group come for second-opinion visits.

**73% with a positive test**

Seventy three per cent of our patients were clinically evaluated to meet the criteria for the ICD-10 K52.2 diagnosis “Allergic and dietetic gastroenteritis and colitis”. This is a selected group, so the number may not be representative for a general population. Biesiekierski et al. (17) found that only 28% fulfilled the criteria of NCGS. The remaining had inadequate exclusion of CD (62%), had uncontrolled symptoms despite gluten restriction (24%), or were not following a GFD (27%). Volta and colleagues (18) evaluated 12,255 patients in their study, but only 3.19% was identified with suspected NCGS. Our 73% represent the proportion with positive test in a group where NCGS is already suspected.

**Patients fulfilling the diagnosis criteria**

A key issue is to try to discriminate into two separate groups the patients that met the criteria for the ICD-10 K52.2 diagnosis “Allergic and dietetic gastroenteritis and colitis”, and the patients who did not. Our result show that there is a significant difference (p<0.05) between the patients that fulfilled the diagnosis criteria and the patients who did not when evaluating gastrointestinal symptoms reported using GSRS, and bloating and abdominal pain reported using VAS. Gastrointestinal symptoms differed most between the two groups (p=0.02). This means that the group that got the diagnosis reported more symptoms during challenge and more change in complaints from baseline to challenge compared with the group that did not get the diagnosis. As we know, gastrointestinal symptoms are one of the main symptom categories in NCGS when ingesting wheat. The level of subjective health complaints (SHC) and the patients’ wellbeing did not significantly differ between the two groups (p>0.05) (Figure 3).

Non-celiac gluten sensitivity in individuals represents a challenge in current clinical practice. The lack of standardized methods in symptom scoring and specific biomarkers makes it challenging to understand the NCGS as an independent entity and its pathogenic mechanisms. At present, the condition is best understood as a result of disadvantageous immune responses, intolerance to poorly digestible and fermentable components in the wheat, or a combination of these two aspects (22). Patients are therefore considered to have NCGS if gastrointestinal symptoms markedly improve on a gluten free diet, and CD and wheat allergy has been excluded (5).

Based on the current definition of NCGS, it is anticipated that exposure to gluten will lead to specific triggering of symptoms. One of the main questions is still by what mechanism symptoms is induced when ingesting gluten. At this point, we are not able to discriminate which components of the wheat that are responsible for the development of symptoms during gluten challenge. There is no clear evidence indicating that gluten proteins are the sole or main trigger molecules in NCGS. Especially the role of fructans and non-gluten proteins as symptom triggers must be further investigated. The induction of symptoms in this study might therefore not be a gluten specific phenomenon, but a wheat-specific phenomenon. We may not be closer to detect which component(s) of wheat that is contributing to gastrointestinal symptoms in NCGS patients, but our results show that all patients experienced an exacerbation of symptoms when digesting gluten-containing white bread, except in three patients (n = 41).
Limitations

The limitations of this study include the low number of patients participating in our gluten challenge. A low number of patients may not be representative for the general population having gastrointestinal symptoms when ingesting wheat-containing food. In addition, the study is not blinded and does not have a control group. Therefore, it is no basis for actual comparison. This could also lead to an “expectation bias” among the NCGS patients.

As part of the examinations, both celiac disease and wheat allergy has been investigated and excluded. However, some patients may have other food intolerances and/or allergies.

Self-administrated questionnaires were used as the main instrument for investigating gastrointestinal symptoms and general health complaints before, during and after gluten challenge. This kind of approach requires that the patient is well informed and understand exactly how to use the questionnaires. On the other hand, the method is highly based on the patient’s subjective experience of the symptoms, which is highly individual and susceptible to biased interpretations. This might have an impact on how the individual patient report symptoms before, during and after gluten challenge. Especially VAS (Visual Analogue Scale) has methodical weaknesses. It is particularly useful for comparing the change and development of symptoms for the same patient, but might be imprecise in comparing groups. Using VAS as a symptom scale also requires that the patients have the same perception of the intensity of the symptoms from 0-100, which is highly subjective and individual. Extra intestinal symptoms are poorly explored by the diagnostic method used in this study.

The amount of gluten has not been standardized. This can affect our results, because the amount of gluten consumed during challenge might have varied from 1.7 g to 21.7 g gluten per day (consuming four slices of bread). A 3-day challenge for the majority of the patients may be too short for capturing all gluten related symptoms, although there is evidence of a rapid gluten response that occurs during the first week of challenge (9). A longer time frame of gluten challenge would possibly have captured any delayed responses to gluten. There is an additional issue in that the basis of comparison may not be optimal as some patients in our group actually consumed gluten containing bread for seven and fourteen days, instead of the majority of patients, who were challenged for three days only.

Conclusion

The results in this study support the existence of NCGS. Patients with a diagnosis report more symptoms compared with those not fulfilling the diagnosis criteria. The dominating symptom category reported during gluten challenge is gastrointestinal symptoms. However, we have not been able to distinguish which component in wheat that is responsible for the symptoms, or specify by what mechanism the symptoms are induced. Future research and a better understanding of NCGS will require blinded, placebo-controlled challenge protocols.

Acknowledgements

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Declaration of interest: The author reports no conflict of interest. The author alone is responsible for the content and writing of the paper.

References


Les dette først:

Undersøkelsen inneholder spørsmål om hvordan du har følt deg og hvordan du har hatt det DE 3 SISTE DAGER. Sett kryss (X) ved det alternativet som passer best på deg og din situasjon.

Dato: ____________

1. Har du i løpet av de siste tre dagene vært plaget av MAGESMERTER?

   ☐ Ingen plager i det hele tatt
   ☐ Ubetydelige plager
   ☐ Milde plager
   ☐ Moderate plager
   ☐ Ganske alvorlige plager
   ☐ Alvorlige plager
   ☐ Meget alvorlige plager

2. Har du i løpet av den siste tre dagene vært plaget av SMERTER ELLER UBEHAG I MAGEN SOM GIR SEG NÅR DU HAR HATT AVFØRING?

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3. Har du i løpet av den siste tre dagene vært plaget av OPPBLÅSTHET?

☐ Ingen plager i det hele tatt
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☐ Ganske alvorlige plager
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☐ Meget alvorlige plager

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☐ Ingen plager i det hele tatt
☐ Ubetydelige plager
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☐ Ganske alvorlige plager
☐ Alvorlige plager
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5. Har du i løpet av den siste tre dagene vært plaget av FORSTOPPELSE (problemer med å tømme tarmen)?

☐ Ingen plager i det hele tatt
☐ Ubetydelige plager
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6. Har du i løpet av den siste tre dagene vært plaget av DIARÉ (hyppig avføring)?

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☐ Ganske alvorlige plager
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7. Har du i løpet av den siste tre dagene vært plaget av LØS AVFØRING?

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☐ Meget alvorlige plager

8. Har du i løpet av de siste tre dagene vært plaget av HARD AVFØRING?

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☐ Ganske alvorlige plager
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9. Har du i løpet av den siste tre dagene vært plaget av TVINGENDE AVFØRINGSBEHOV (plutselig behov for å gå på toalettet for å tømme tarmen)?

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10. Har du i løpet av de siste tre dagene vært plaget av en FØLELSE AV UFULLSTENDIG TØMMING AV TARMEN ETTER AVFØRING?

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11. Har du i løpet av den siste tre dagene vært plaget av at du FØLER DEG METT LIKE ETTER AT DU HAR BEGYNT PÅ ET MÅLTID?

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12. Har du i løpet av den siste tre dagene vært plaget av at du FØLER DEG METT SELV LENGE ETTER AT DU ER FERDIG MED Å SPISE?

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13. Har du i løpet av den siste tre dagene vært plaget av at MAGEN ER SYNLIG OPPBLÅST?

☐ Ingen plager i det hele tatt
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☐ Moderate plager
☐ Ganske alvorlige plager
☐ Alvorlige plager
☐ Meget alvorlige plager

KONTROLLER AT ALLE SPØRSMÅLENE ER BESVART!

TAKK FOR DIN MEDVIRKNING.
**SHC – Subjective Health Complaints Inventory**

### Nedenfor nevnes noen alminnelige helseproblemer (sett ring rundt tallet som passer)

<table>
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<td>29. Nedtrykt, depresjon</td>
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</table>

**Tilleggsfråstill dersom du har angitt flere helseproblemer:**
Hvilket av disse problemene har vært mest plagsomt for deg siste 3 dager?.................................
(skriv navnet på helseproblemet)
VAS – Visual Analogue Scale

DAGBOK

Navn: ____________________________

Denne dagboken fylles ut i 3 dager FØR provokasjon, i 3 dager UNDER provokasjon og i 3 dager ETTER provokasjon (Dag -2, Dag -1 osv)
Sett en strek på skalaen etter det som passer best med symptomene dine (F.eks.: "Føler meg bra __________I________________________ Føler meg dårlig")
Husk å føre på datoer.

FØR PROVOKASJON

Spis glutenfritt som vanlig.

**Dag -2**

**Dato:** _________________
Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luftsmerter ____________________________ Mye luftsmerter

Hvor mange avføringer hadde du?____

**Dag -1**

Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luftsmerter ____________________________ Mye luftsmerter

Hvor mange avføringer hadde du?____

**Dag 0**

Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luftsmerter ____________________________ Mye luftsmerter

Hvor mange avføringer hadde du?____

Fylle ut skjema merket GSRS-IBS og SHC, og krysse av for symptomene slik de har vært de tre dagene før provokasjonen starter (dag -2 til dag 0).
UNDER PROVOKASJON

Du skal spise 4 skiver "Fint Sandwichbrød" fra Bakers daglig i 3 dager. Ellers følger du ditt vanlige, glutenfrie kosthold.

**Dag 1**
Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luft-smerter ____________________________ Mye luftsmerter

Hvor mange avføringer hadde du?____
Hvor mange skiver "Fint Sandwichbrød" spiste du?____

**Dag 2**
Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luft-smerter ____________________________ Mye luftsmerter

Hvor mange avføringer hadde du?____
Hvor mange skiver "Fint Sandwichbrød" spiste du?____

**Dag 3**
Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luft-smerter ____________________________ Mye luftsmerter

Hvor mange avføringer hadde du?____
Hvor mange skiver "Fint Sandwichbrød" spiste du?____

Fylle ut skjema merket GSRS-IBS og SHC, og krysse av for symptomene slik de har vært de tre dagene provokasjonen varer (dag 1 til dag 3).
ETTER PROVOKASJON

Dag 4 – Dag 4-5-6 skal du kun spise gluten fri mat, hvis ikke annet er avtalt.

Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luft- smerter ____________________________ Mye luftsmeneter

Hvor mange avføringer hadde du?___

Dag 5

Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luft- smerter ____________________________ Mye luftsmeneter

Hvor mange avføringer hadde du?___

Dag 6

Føler meg bra ____________________________ Føler meg dårlig
Lite vondt i magen ____________________________ Mye vondt i magen
Lite luft- smerter ____________________________ Mye luftsmeneter

Hvor mange avføringer hadde du?___

Fylle ut skjema merket GSRS-IBS og SHC, og krysse av for symptomene slik de har vært de tre dagene etter provokasjonen.

Ferdig utfylte skjemaer sendes til:

Ieva Toleikyte
Klinisk ernæringsfysiolig
Seksjon for klinisk ernæring
Oslo Universitetssykehus HF
Rikshospitalet
Postboks 4950 Nydalen
0424 OSLO

Tlf: 230 71 909