



## Mutatis mutandis: Are we diagnosing too many people with non-celiac gluten sensitivity? Multiple case report

Raffaele Borghini<sup>1</sup>, Giuseppe Donato<sup>2</sup>, Marco Di Tola<sup>1</sup>, Claudia Isonne<sup>1</sup>, Antonio Picarelli<sup>1</sup>

<sup>1</sup>Department of Internal Medicine and Medical Specialties, Sapienza University - Policlinico Umberto I, Rome, Italy

<sup>2</sup>Department of Clinical Medicine, Sapienza University- Policlinico Umberto I, Rome, Italy

### ABSTRACT

We report three patients presenting with gluten-related signs and symptoms. Since villous height/crypt depth ratio, intraepithelial lymphocyte count, and serum antibody tests were not diagnostic for celiac disease (CD), a diagnosis of non-celiac gluten sensitivity (NCGS) was suggested. On the other hand, antibodies suggestive for CD surprisingly showed positive results in the duodenal biopsy organ culture of all three cases. The reported cases suggest the precious potential role that organ culture systems may play in differentiating CD from NCGS. This method should be recommended when gluten-related disorders are suspected in order to reduce the inappropriate diagnosis of NCGS.

**Keywords:** Case finding, celiac disease, celiac serology, celiac testing, non-celiac gluten sensitivity, organ culture

### INTRODUCTION

Nowadays, it has become relevant in clinical practice to differentiate between celiac disease (CD) and non-celiac gluten sensitivity (NCGS), in light of the increasing prevalence of both of these gluten-related disorders and their relevant dietary, medical, social, and economic implications (1).

Celiac disease is a multisystemic chronic autoimmune inflammatory disorder triggered by the ingestion of gluten, and its prevalence is about 1:100. It develops in genetically susceptible subjects who present with Human Leucocyte Antigen (HLA) DQ2 (95%) or HLA DQ8 (5%), and it is generally characterized by intestinal manifestations, such as diarrhea, abdominal pain, and swelling. On the other hand, extraintestinal signs and symptoms (e.g., iron deficiency anemia, foggy mind, fatigue, skin itch, headache, arthralgia) as well as silent forms are frequent, too. Its diagnosis is based on histological examination of the second part of the duodenum, showing villous atrophy, crypt hyperplasia, and inflammation of the lamina propria. Positive

serological IgA and IgG anti-endomysium (EMA), anti-transglutaminase (anti-Ttg), and anti-deamidated gliadin peptide (AGA DGP) antibody results can support the diagnosis of CD (2). Recent literature has also shown the precious role of duodenal biopsy organ culture system when villous height/crypt depth ratio, intraepithelial lymphocyte count, or serum antibody tests are not clearly diagnostic: EMA- and anti-tTG-positive results in culture biopsy supernatants of the second part of the duodenum may be helpful in making a CD diagnosis (3-5). CD recedes after a gluten-free diet is started.

Recently, a gluten-related clinical picture similar to CD has been described as NCGS. It has an estimated prevalence of 6%, but today, diagnostic markers for NCGS are not available: only serological AGA IgG shows positive results (56.4%) (6). Moreover, HLA DQ2 and DQ8 haplotypes are present in only 50% of NCGS patients, and the histological examination usually does not show specific alterations (7). Despite this negative clinical pattern, NCGS patients respond to a gluten-free diet.

**Address for Correspondence:** Antonio Picarelli, Department of Internal Medicine and Medical Specialties, Sapienza University - Policlinico Umberto I, Rome, Italy

E-mail: antonio.picarelli@uniroma1.it

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For a correct differential diagnosis among gluten-related disorders, wheat allergy must be considered, too (prevalence 0.1%). Once the diagnosis is made with paper radioimmunosorbent test (PRIST) and radioallergosorbent test (RAST), it recedes after a gluten-free diet is started (8).

As previously mentioned, unfortunately, there are no sensitive and specific markers for NCGS today. Moreover, histology often does not give an unequivocal answer, despite it being considered the gold standard for the diagnosis of CD (9).

In this frequent condition of blurred boundaries between CD and NCGS, recent literature (3) has already shown that organ culture of duodenal biopsy could be a useful method for problem-solving and decision-making.

The reported cases stress the role of organ culture in celiac patients, although serological and histological features are not clearly diagnostic for CD, and clinical data could hastily suggest a "trendy" diagnosis of NCGS.

## CASE PRESENTATION

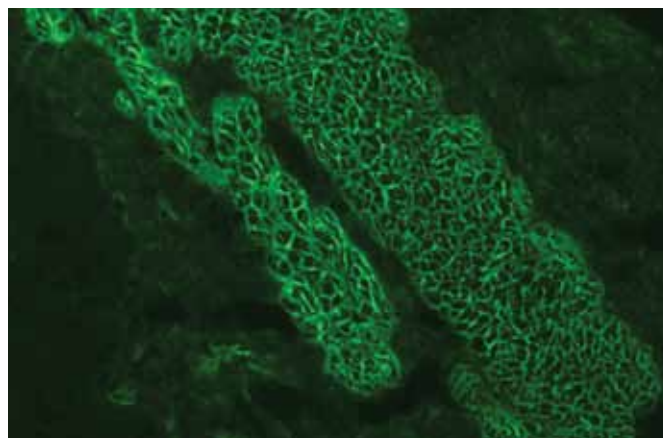
Valid and appropriate informed consent was obtained in all cases reported.

### Case 1

A 27-year-old woman on a gluten-containing diet presented with diarrhea, abdominal pain, and swelling for the last 6 months, reporting a gluten dependence of her symptoms. She had already undergone hydrogen breath test, PRIST, and RAST for wheat, barley, gluten, and rye, obtaining negative results. On the basis of clinical and anamnestic data, once selective IgA deficiency was excluded, she underwent serological screening for CD (EMA IgA and IgG, anti-tTG IgA and IgG, AGA DGP IgA and IgG, AGA IgA and IgG) with negative results, whereas she was positive for HLA DQ2. She underwent esophagogastroduodenoscopy (EGDS) with no macroscopic alterations found. Histology of the second part of the duodenum showed a picture compatible with Marsh-Oberhuber class 1 (>40 intraepithelial lymphocytes per 100 enterocytes but no villous atrophy). EMA IgA was also searched in undiluted cultures of the second part of duodenal biopsy supernatants by indirect immunofluorescence analysis on cryostat sections of monkey esophagus, and doubtful EMA results were identified (Figure 1). Moreover, anti-tTG IgA was measured in culture supernatants diluted 1:5 by enzyme-linked immunosorbent assay (ELISA) on microtiter plate wells coated with recombinant human tTG. Culture supernatant results were expressed in  $A_{450nm}$ , and an antibody level of 0.300 was used as a cutoff value to identify anti-tTG-positive results: in this case, the anti-tTG level was 0.700. All symptoms progressively receded after a gluten-free diet was started.

### Case 2

A 32-year-old woman on a gluten-containing diet complained of abdominal swelling since she was young and had iron de-



**Figure 1.** Biopsy organ culture of the second part of duodenum showing EMA IgA positive results (indirect immunofluorescence analysis on cryostat sections of monkey esophagus).

ficiency anemia refractory to oral iron supplementation (red blood cells 3,850,000/mm<sup>3</sup>, hemoglobin 10.5 g/dL, hematocrit 35%, mean cell volume MCV 72 fL, iron 56 mcg/dL, ferritin 5 ng/mL). Lactose breath test was negative. She had already excluded possible gynecological causes of anemia and never presented with rectal bleeding. Repeated fecal occult blood tests as well as urea breath tests showed negative results. She also underwent a pancolonoscopy because of a colorectal cancer family history, with no alterations found. Moreover, she recently noticed worsening of swelling related to the ingestion of gluten. PRIST and RAST for wheat, barley, gluten, and rye had negative results. Once selective IgA deficiency was excluded, she underwent serological screening for CD, showing doubtful EMA IgA results and borderline anti-tTG IgA levels (11 U/mL, with normal values <10 U/mL). AGA IgA and IgG were clearly positive. She tested positive for HLA DQ2, too. She underwent EGDS, presenting no macroscopic alterations. Histology of the second part of the duodenum showed a picture compatible with Marsh-Oberhuber class 1 (>40 intraepithelial lymphocytes per 100 enterocytes but no villous atrophy) and no gastric abnormalities. The second part of the duodenal biopsy specimen organ culture showed EMA IgA- and anti-tTG IgA-positive results (anti-tTG IgA 1.100, with 0.300 used as cutoff value) (Figure 1). The abdominal swelling progressively receded after a gluten-free diet was started, and iron deficiency anemia recovered (red blood cells 4,800,000/mm<sup>3</sup>, hemoglobin 12.2 g/dL, hematocrit 40%, MCV 84 fL, sideremia 120 mcg/dL, iron 56 mcg/dL, ferritin 24 ng/mL).

### Case 3

A 42-year-old male outpatient on a gluten-containing diet has been complaining of diarrhea, abdominal swelling, and foggy mind for almost 2 years. Symptoms seemed to be triggered or accentuated by the ingestion of gluten-containing food. He had already undergone hydrogen breath test, PRIST, and RAST for wheat, barley, gluten, and rye, obtaining negative results. Once selective IgA deficiency was excluded, he underwent serological screening for CD, showing doubtful EMA IgA results and border-

line anti-tTG IgA levels (9 U/mL, with normal values <10 U/mL). AGA IgG was clearly positive. He tested positive for HLA DQ2, too. He underwent EGDS, presenting no macroscopic alterations. Histology of the second part of the duodenum showed a picture compatible with Marsh-Oberhuber class 1 (>40 intraepithelial lymphocytes per 100 enterocytes but no villous atrophy) and no gastric abnormalities. Biopsy organ culture of the second part of the duodenum showed EMA IgA and anti-tTG IgA antibody-positive results (anti-tTG 0.900, with 0.300 used as cutoff value) (Figure 1). Diarrhea, abdominal swelling, and foggy mind progressively receded after a gluten-free diet was started.

## DISCUSSION

Nowadays, the number of patients affected by gluten-related disorders is increasing, as well as the necessity of a correct diagnosis and appropriate treatment. However, it is not always so easy to recognize and differentiate CD from NCGS and vice versa, although there is no difference in treatment. Currently, the gold standard diagnosis of CD is by the demonstration of villous atrophy on duodenal biopsies, with serological EMA and anti-tTG playing a supportive role (10). In particular, EMA IgA and anti-tTG IgA have high sensitivity (respectively, 95% and 98%) and specificity (respectively, 99% and 98%) (11).

But, the real controversy arises when histology is not diagnostic for CD and CD serology is negative or borderline, although clinical data are clearly gluten-related. On these premises, a diagnosis of NCGS could be suggested and CD excluded. Notwithstanding, is it still possible to suspect and prove CD instead of NCGS in this unclear context?

This atypical condition occurred in the three cases reported: all patients were on a gluten-containing diet and complained of gluten-related signs and symptoms, suggesting a diagnosis compatible with CD or NCGS. On the other hand, an opportune differential diagnosis with other gastrointestinal functional disorders was made. Lactose intolerance was investigated, in order to exclude a possible common cause of diarrhea, abdominal pain, and swelling. Wheat allergy was excluded, too, in all three cases reported. Moreover, iron deficiency anemia was studied in case 2, excluding inadequate intake of iron, gastrointestinal and extra-intestinal bleeding, and gastric disorders (gastritis, *Helicobacter pylori* infection).

The suspicion of CD and HLA DQ2-positive results suggested the performance of EGDS with duodenal biopsy. The resulting histological picture showed the presence of increased intraepithelial lymphocytes but no sign of villous atrophy (Marsh-Oberhuber class 1). As far as serological data are concerned, EMA and anti-tTG did not play a supportive role for a CD diagnosis, since they showed negative results in case 1 and doubtful/borderline results in cases 2 and 3. AGA IgA-positive results in cases 2 and 3, as well as AGA IgG-positive results in case 3, were not sufficient for a correct CD diagnosis because of their low sensitivity and specificity (11). On the other hand, the lit-

erature describes AGA IgG-positive results as present in more than 50% of NCGS cases (6).

Recent work already showed the useful role of duodenal biopsy organ culture when villous height/crypt depth ratio, intraepithelial lymphocyte count, or serum antibody tests are not diagnostic for CD (3). The reported cases confirm the crucial role that this method could play in the differential diagnosis between CD and the still relatively unknown NCGS. In particular, all three cases did not show a typical pattern of CD from a histological and serological point of view, and they would have been considered NCGS, since the signs and symptoms progressively disappeared after starting a correct gluten-free diet.

In our opinion, the relevant data consisted of clear positive results for EMA and anti-tTG IgA of the second part of the duodenal biopsy cultures obtained in cases 2 and 3, who presented doubtful serological EMA and anti-tTG IgA results. Despite a totally negative serology for CD, case 1 showed EMA and anti-tTG IgA-positive results in cultured supernatants, orienting towards CD diagnosis.

In conclusion, duodenal biopsy organ culture can tip the balance in favor of CD, avoiding the overestimation of NCGS.

**Ethics Committee Approval:** Ethics committee approval was received for this study.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - A.P., R.B.; Design - R.B.; Supervision - A.P.; Resource - R.B., C.I.; Materials - R.B., C.I.; Data Collection&/or Processing - R.B., C.I.; Analysis&/or Interpretation - G.D., M.D.T.; Literature Search - R.B., A.P.; Writing - R.B.

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