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A clinical laboratory study of a non-classical case of celiac disease: how to anticipate the diagnosis

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ABSTRACT

Celiac disease (CD) is a systemic autoimmune pathological condition caused by the intake of gluten in genetically predisposed individuals. Despite its wide prevalence, it remains an underdiagnosed disease since a large percentage of individuals who suffer from the condition do not have the classic symptoms described for the disease.

We present the case of a 43-year-old man with severe iron deficiency and asthenia. We found high levels of anti-transglutaminase and anti-endomysium antibodies, a severe intraepithelial lymphocytosis, 3A Marsh-Oberhuber classification upon gastroscopy and the presence of HLA-DQ2 and HLA-DQ8 heterodimers.

The patient was diagnosed with CD and was placed on a gluten-free diet. After 19 months, an improvement in biomarkers of CD and other biochemical parameters was observed.

A delay in the diagnosis of CD can produce nutritional deficiencies, such as iron deficiency which may not improve even with oral iron treatment. In similar clinical presentation, the laboratory can advance a diagnosis of CD.

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INTRODUCTION

Celiac disease (CD) is immune-mediated and a highly prevalent chronic enteropathy caused by the ingestion of gluten in genetically susceptible individuals. It can be present at any age, with a peak onset of adult CD between the age of 40-60 years. Celiac disease is known to be underdiagnosed because of the heterogeneous presentation of clinical signs and symptoms. Steatorrhea, weight loss, anemia, hypo-proteinemia and electrolyte imbalance are known classic symptoms and usually trigger diagnostic work-up, but patients with less common presentations are often not screened for CD. The incidence of CD varies geographically, and appears to be increasing over time in several regions of the world. Its prevalence in Europe is 1% in both children and adults. Despite the growing recognition of CD, many cases remain undiagnosed. A gluten-free diet (GFD) is currently the only effective treatment for CD [1-3].

CLINICAL-DIAGNOSTIC CASE

We present the case of a 43 years old male with a 3-month history of asthenia, with no diarrhea or abdominal pain. No familial or personal history of interest were presented by the patient.

The first analysis showed a marked deficiency in serum ferritin and iron, as well as low hemoglobin (Hb) concentration, red blood cell (RBC) count and vitamin B12. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were within the reference range. No other alterations in hematological or biochemical parameters were found.

Possible causes of iron deficiency were ruled out and oral iron was prescribed to the patient. Intramuscular B12 vitamin treatment was prescribed too. Two months later a follow-up blood test was performed, where it was observed that ferritin levels were still at levels of <8 µg/L. After ruling out poor compliance with treatment, the study of CD biomarkers was initiated. The diagnostic work-up was according to the guidelines of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [7]. We determined immunoglobulin A (IgA) by nephelometry (Immage analyzer, Beckman-Coulter), and transglutaminase antibodies IgA (IgA tTg) (Elia Celikey IgA with Phadia-250 analyzer, Thermo Fisher) and IgA antibodies against endomysium (monkey esophagus sections) (EmA) (BioSystems) using indirect immunofluorescence (IIF). The results of the different analyses over time are shown in Table 1. The results of the EmA examination are illustrated in Figure 1.

For the gastroscopy examination, the patient was instructed not to be on a gluten restricted diet. During the examination, portions of the esophagus, stomach and duodenum were studied, the report enumerated a mucosa with normal characteristics, without apparent signs of atrophy, with vascularization and normal appearing villi. Three biopsies of the second duodenal portion and two fragments of the bulb were performed. Hematoxylin-eosin (HE) staining of the biopsy specimens showed severe intraepithelial lymphocytosis (> 30 intraepithelial lymphocytes per 100 enterocytes), crypt hyperplasia and mild villous atrophy (Figure 2). The patient was classified as having grade 3A CD according to the modified Marsh-Oberhuber histologic classification [7].

HLA DQ determination was also performed. Histocompatibility antigens were studied by sequence-specific oligonucleotide (SSO) PCR technique of HLA-DQA1 and HLA-DQB1 loci with Luminex technology.

The results were: HLA-DQA1 locus: DQA1 * 03, DQA1 * 05 and HLA-DQB1: DQB1 * 02 and DQB1 * 03. These results confirmed the presence of the CD risk factor, i.e., heterodimers DQ2 and DQ8.

DISCUSSION

According to population screening studies, the true prevalence of CD is greatly underestimated [3]. The reason for the underestimation could be that only a small portion of people affected by CD show the classical signs of the disease, while the majority have the asymptomatic form. Thus, the variability of clinical symptoms of this disease makes its diagnosis difficult [4].

Table 1 Results of the 6 serial analyses performed in our laboratory [a, b]

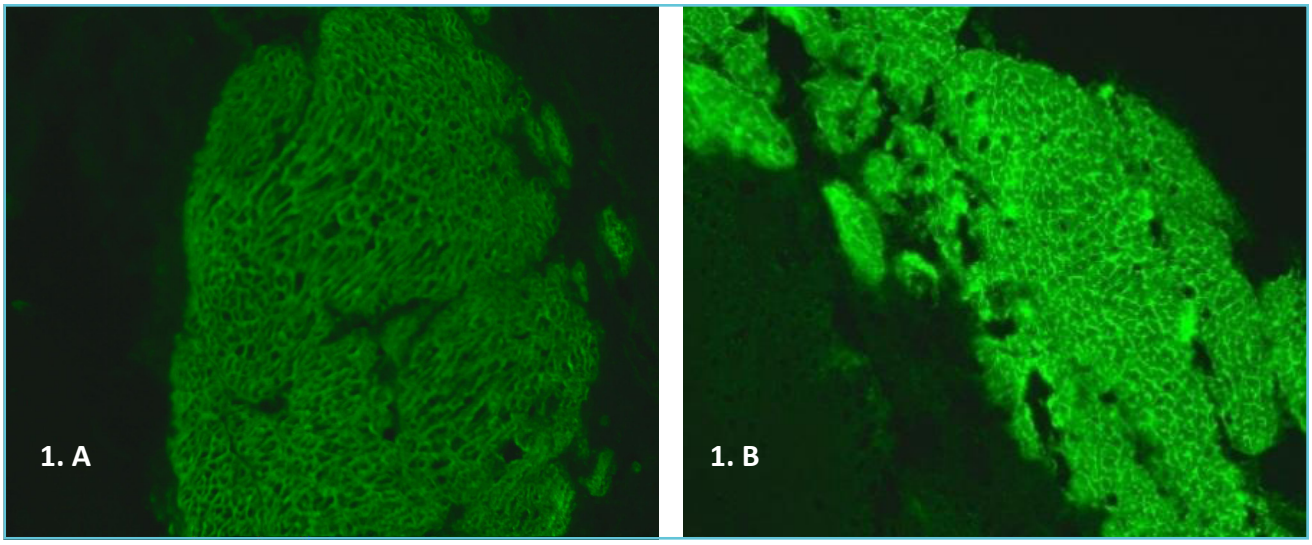
Laboratory tests (normal range)	Nov 19, 2019	Jan 31, 2020	Feb 21, 2020	Dec 30, 2020	Jun 11, 2021	Sep 27, 2021
Haemoglobin (13,5-18 g/dL)	13,6	13,1	14,4	16	15,3	15,6
Red blood cells (4.7-6.0 x10*12/L)	3,81	3,93	4,35	4,61	4,58	4,59
Mean Corpuscular Volume (MCV) (78-100 fL)	103	98	98	97,7	97,1	98,1
Mean Corpuscular Haemoglobin (MCH) (27-31 pg)	35,8	33,2	33,2	34,7	33,5	34
Mean corpuscular haemoglobin concentration (MCHC) (32-36 g/dL)	34,7	33,7	33,9	35,5	34,5	34,7
Red cell distribution wide (RDW) (11,5-14 %)	15,7	13,8	13,6	13,4	13,1	13,4
Ferritin (20.0-250.0 µg/L)	8	<8	<8	102	141	141
Iron (70.0-180.0 µg/dL)	49	60	70	56	105	90
Transferrin saturation index (25-50 %)	NP	16,7	18,1	16,2	34,8	29,5
B12 Vitamin (180.0-914.0 pg/mL)	105	117	121	329	237	216

Aspartate Aminotranferase (5.0-35.0 U/L)	NP	61	49	37	28	27
Inmunoglobulin IgG (800.0-1400.0 mg/dL)	NP	NP	1320	NP	NP	NP
Inmunoglobulin IgA (100.0-300.0 mg/dL)	NP	NP	398	NP	NP	NP
Transglutaminase antibodies IgA (<7 U/mL)	NP	NP	**186**	19	9,7	6,6
IgA antibodies endomysium (EmA)	NP	NP	**POSITIVE**	NP	NP	NP

[a] *The first (November 19, 2019) and second (January 31, 2020) tests showed severe iron deficiency, vitamin B12 values below the population reference values, and elevated AST, with normal values of MCV and MCH. The second analysis was performed after supplementation with oral iron and intramuscular vitamin B12 after 2 months. The third (February 21, 2020) examination was requested in the following month, where the requisition was extended to the serological study of celiac disease (CD) biomarkers, the result of which confirmed the diagnosis. The last three tests (December 30, 2020; June 11, 2021 and September 27, 2021) were performed after the diagnosis of CD and gluten-free diet, where a decrease in transglutaminase IgA antibody titers can be observed until normalization, as well as AST, vitamin B12, ferritin, serum iron, erythrocytes and hemoglobin.*

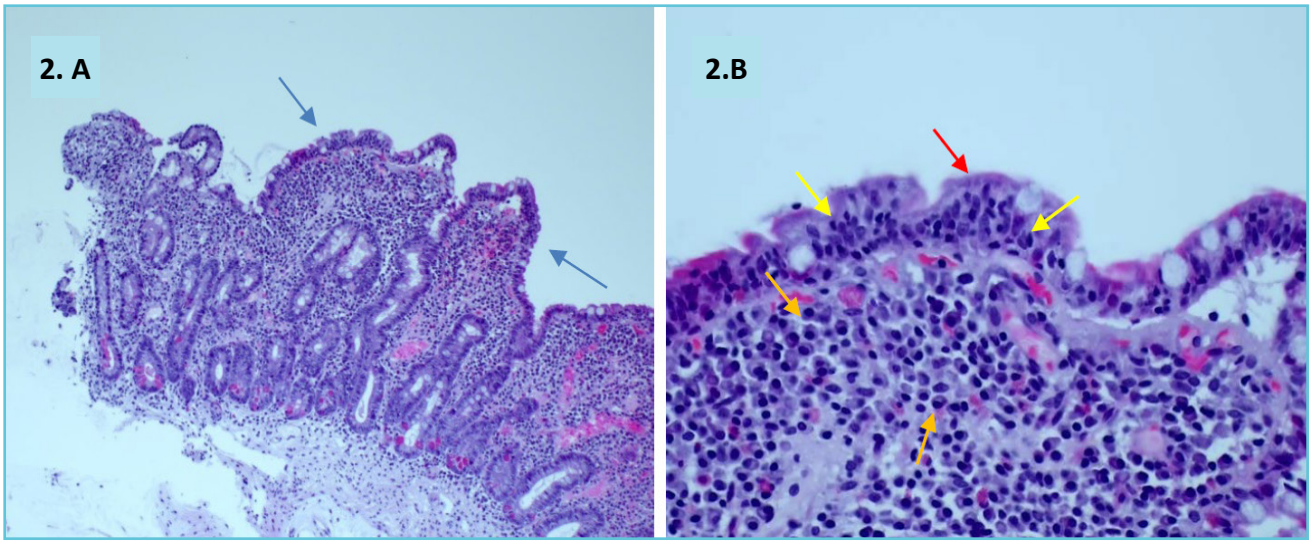
[b] *NP: not performed.*

Figure 1 **Figure 1.A (Positive control) and 1.B (patient's serum) show the results of the IIF study under fluorescence microscopy***



* *The images were obtained after a 1/20 dilution of the patient's serum and were observed at 400. In both, we can see the network-like fluorescence labeling of the layer surrounding the smooth muscle fibers of the monkey esophagus with the large fluorescence emission of **1.B** image compared to that produced in the positive control used in the technique, depicted in Figure **1.A**.*

Figure 2 **Low magnification image (100X) of the duodenum's second portion shows mucosa and submucosa***



** An increased number of intraepithelial lymphocytes and mild villous atrophy (blue arrows) (HE) can be seen (**Fig. 2.A**). Higher magnification image (200X) showing the damaged surface of the epithelium (red arrow), with numerous intraepithelial lymphocytes (yellow arrows) and an increase in plasma cells in the lamina propria (orange arrows) (HE) (**Fig. 2.B**).*

A delay in the diagnosis of CD may account for cases of adult patients manifesting severe nutritional deficiencies and iron malabsorption anemia, which is the most common extraintestinal subclinical manifestation in CD, even in patients who do not have duodenal villous atrophy [5]. Oral iron therapy may be ineffective, leading to chronic iron deficiency anemia [6, 7].

Such is the importance of iron deficiency anemia in these cases that the British Society of Gastroenterology guidelines recommend a CD work-up in every patient with iron deficiency anemia [8].

In our case, the extraintestinal manifestations (asthenia) and several altered biomarkers in the analytical tests (iron deficiency, low vitamin B12 and elevated liver enzyme levels), were the prelude to suspect possible CD [9]. In addition, the patient did not respond to oral iron therapy before the diagnostic work-up for CD.

The importance of early diagnosis is illustrated by reduction in the risk of morbidity and mortality due to complications associated with CD, such as bone abnormalities (osteopenia and osteoporosis), liver damage, anemias, neurological manifestations (neuropathies and headaches, among others), other autoimmune diseases and malignancies [10]. The prevalence of these complications is related to age and duration of gluten exposure [11].

Our patient did not present with clinically evident symptoms of CD, nonetheless, we considered initiating the study of serological markers for CD, based on similar cases in the literature [12].

In addition to the above, the examination of the alleles encoding CD risk molecules helped determine the patient's HLA status and initiate tests in first-degree relatives [13].

In conclusion, the determination of the serological markers studied by the laboratory were

particularly useful in the diagnosis of our patient [14, 15].

An early diagnosis can avoid serious complications and we recommend screening for CD in patients with findings similar to our case.

LEARNING POINTS

- CD is underdiagnosed because it presents a wide spectrum of associated symptomatology (classical and non-classical symptoms).
- The clinical laboratory can perform serological determinations of CD biomarkers to advance the diagnosis of the disease.
- In cases of patients with abnormally low ferritin levels, without an apparent cause who do not respond to oral iron therapy, it is recommended to rule out the presence of CD.
- A delay in the diagnosis of CD can lead to severe nutritional deficiencies and chronic iron deficiency anemia.

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