Abstract
Celiac disease (CD) is found in genetically predisposed individuals, and characterized by intolerance to gluten ingestion, contained in cereals such as barley, rye, wheat and malt. Clinical manifestations vary from asymptomatic patients to severe forms of malabsorption syndromes, which may involve multiple systems and increase the risk of some neoplasias. Diagnosis of CD often requires a high degree of suspicion. There is not a single test for the diagnosis, which is reached after a combination of clinical and laboratory data. The first step may be a serum test, such as the antibodies anti-tissue transglutaminase, or antiendomisio. If serum result is positive, duodenal biopsy is necessary for diagnostic confirmation. IgA deficiency, which occurs in 3% of patients with DC, may lead to false-negatives because serology is based on IgA antibodies. Another cause of false-negative tests is diet restriction of gluten; therefore diagnostic investigation must be carried out during a diet containing gluten. The screening for CD in asymptomatic individuals is not indicated.

Key words: Celiac disease. Diagnosis. Adult

Introduction
Celiac disease (CD) is characterized by intolerance to gluten ingestion, which is contained in cereals such as barley, rye, hay, and malt, happening in genetically predisposed individuals and presents an inflammatory process that involves the small intestine mucosa, leading to atrophy of intestinal villi, malabsorption and a variety of clinical manifestations. Gluten proteins are relatively resistant to digestive enzymes, resulting in peptide derivatives that may lead to immunogenic response in CD patients.

CD's clinical manifestations may involve the gastrointestinal tract, as well as skin, liver, nervous system, reproductive system, bones, and endocrine system. Herpetiform dermatitis occurs in 10 to 20% of the patients and is a pathognomonic manifestation.

Until recently, CD diagnosis was recognized only in patients who presented typical clinical manifestations or high degree of suspicion. Diagnosis is generally performed in children with malabsorption syndrome. After the appearance of high accuracy serum tests and bigger attention by the physicians to atypical manifestations, CD's prevalence increased, as well as its diagnosis outside the field of pediatrics. Estimated prevalence in general population is around 1:100.

CD's clinical manifestations may vary, as described in Table 1. There is an important genetic predisposition in patients with CD, characterized by HLA-DQ2 and HLA-DQ8 surface markers. Gluten interacts with HLA markers, provoking an abnormal immune response of the mucosa and tissue injury.

A review has showed that the relation of patients with diagnosed and not diagnosed CD may be 1:7. A study indicates that over 36% CD patients had had a previous diagnosis of IBS (irritable bowel syndrome). If not treated, celiac disease has a high morbimortality. Anemia, infertility, osteoporosis, and cancer, mainly intestinal lymphoma, are among the risks of complication in patients not treated.

CD diagnostic investigation must be performed before the introduction of treatment, which is gluten-free diet, for it may negatively alter the serum tests' results and improve histology.

CD diagnosis is not always easy to be performed. In around 10% of cases, there is difficulty in diagnosis due to conflicting serology, histology, and clinical findings. CD's diagnosis must be considered in every patient presenting chronic diarrhea, abdominal distention, flatulence, iron-deficiency anemia, early onset osteoporosis, elevated transaminases, first and second degree relatives of the patients with CD, IBS, hypocalcaemia, as well as in face of folic acid and liposoluble vitamins. Besides that, CD is associated with various diseases such as type 1 diabetes mellitus, hypo and hyperthyroidism, Sjogren syndrome, primary biliary cirrhosis, autoimmune hepatitis, autism, depression, epilepsy, cerebellar ataxia, infertility, late puberty, selective IgA deficiency.
deficiency, Turner syndrome, Down syndrome and peripheral neuropathy.4,6,10-15

There is no justification in literature, at the moment, for population screening for CD diagnosis.

**SEROLOGY**

Markers used are the antibodies antiendomisio (EMA) and anti-tissue transglutaminase (anti-tTG), for being sensitive and specific for early CD's diagnosis.4 Several studies have evidenced a high correlation among their results, thus it is not necessary to research both of them.

The research of antigliadin antibody (AGA) performance is not comparable to test mentioned above and is disused.

Serum tests are responsible for recognizing that CD is not rare.9

Positive serum test suggests CD diagnosis, but duodenal biopsy is still gold standard.9

Positive serology may become negative after 6-12 months from the gluten-free diet introduction.

Serum markers sensitivity is related to the extent of histological damage in CD, both at diagnosis and in the follow up to gluten-free diet adherence. Serum tests sensitivity will be high when there is the presence of total villous atrophy and its progressive decrease, as histological findings are less altered. Thus, negative serology does not exclude CD diagnosis.

Serum tests may be used to evaluate the patient’s adherence to gluten-free diet. Antibodies become negative after 3-12 months of diet.16

**Anti-tissue transglutaminase (anti-tTG IgA)**

The antigen against which antiendomisio antibodies are directed is the transglutaminase enzyme. Anti-tTG IgA is the antibody against tissue transglutaminase (the enzyme responsible for deamination of gliadin the lamina propria).

This test is performed by the ELISA method and uses as subtract the guinea pig protein – first generation (90% sensitivity and 95.3% specificity), cells derived from human erythrocytes (95.1 sensitivity and 98.3 specificity) or human recombinant – second generation.9 Some diseases may interfere in the results, leading to false-positives, such as chronic hepatic disease, heart failure, arthritis, diabetes mellitus, and intestinal inflammatory disease. This interference has been decreased with newer tests.9

Separately, it is the most efficient serum test for detecting CD. It may be performed with a small blood sample collected from the finger.

It was recently demonstrated that tTG-Abs RIA may be detected in human saliva, which can prevent the need of collecting blood, making the CD diagnosis easier, especially in children. The research on anti-tTG IgA is highly sensitive to CD diagnosis and for the follow up of patients under gluten-free diet.19

**Antiendomisio IgA (EMA)**

EMA IgA antibodies bind endomisio, the conjunctive tissue enveloping the smooth muscle, producing a characteristic pattern. It is detected by indirect immunofluorescence. It is a method that requires more time if compared to the ELISA method, besides being operator-dependent.9 For its performance, monkey esophagus (EMA IgA 97.4% sensitivity and 99.6% specificity) or human umbilical cord (EMA IgA 90.2 sensitivity and 99.6% specificity) as subtracts for performing the test.9

It is recognized that the presence of EMA is a predictor of progression in direction of villous atrophy.17,18

**Antigliadin antibodies (AGA IgA)**

This is the oldest marker and is determined by the ELISA method. Reference values are not constant among laboratories. Its efficacy is difficult to define, for available data in literature are heterogeneous and do not permit comparison. Its specificity is approximately 90%, and the sensitivity is around 85%-90%, presenting low positive predictive value.9 There are other tests with higher diagnostic performance.

**IgA selective deficiency**

IgA deficiency is the most common human immunodeficiency and it is 10-15 times more common in CD patients. However, IgA dosage must be performed only if there is high suspicion of its deficiency. Approximately 3% of CD patients have this deficiency.

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**Table 1 – Celiac disease's clinical manifestations**

| Classic form: | Symptomatic intestinal malabsorption. Chronic diarrhea, abdominal pain, abdominal distention, weight loss, and flatulence may occur. |
| Atypical form: | Absence or few gastrointestinal symptoms, presence of atypical symptoms, such as anemia due to iron deficiency, osteoporosis or osteopenia, infertility, low stature. It is the most common presentation. |
| Silent form: | Occasional diagnosis, histological or serological, in asymptomatic individuals. |
| Latent form: | There are 2 forms: 1 – Patients with previous CD diagnosis who responded to gluten-free diet and presented a normal histology or only intraepithelial lymphocytes increase. 2 – Individuals with normal intestinal mucosa, under diet including gluten, who will subsequently develop CD. |
| Refractory form: | Patients with CD who do not respond to gluten-free diet. |
which may produce false-negative in serum tests EMA, anti-tTG IgA and AGA IgA, all of them based on IgA. In patients with IgA selective deficiency serology can be performed with IgG, both EMA IgG and tTGA IgG have excellent sensitivity (close to 100%) and specificity. Nevertheless, IgG-based tests have lower sensitivity and specificity in relation to those based on IgA, those with normal IgA levels. Thus, if serology (EMA IgA or tTGA IgA) is negative in patients with high CD suspicion, seric IgA must be dosed.

If CD suspicion is high, with persistently negative tests, individuals must perform typing for HLA and, if positive, they must perform duodenal biopsy or alternatively perform biopsy directly.

**HLA typing**

It is the first step for investigating relatives of CD patients. HLA typing excludes one third of 1st degree relatives and identifies individuals for evaluation with biopsy. It is also the clinical exam indicated if the individual presents negative serology and refuses to undergo biopsy. HLA allele DQ2 is identified in 90%-95% of celiac patients, and HLA DQ8, in most of the others. Therefore, their absence has a negative predictive value next to 100%.4,9

HLA typing is useful also to exclude the disease in patients who, unwittingly, are already undergoing gluten-free diet or individuals in which diagnosis is not clear.

**Duodenal biopsy**

CD diagnosis and lifelong gluten-free diet introduction must not be firmly without compatible histological findings, regardless of the results of serological tests. However, it is also not advised to affirm a diagnosis based only on the histological diagnosis, because the disease does not compromise uniformly intestine, and alterations are not observed exclusively in CD. In spite of these problems, intestinal biopsy is considered ‘gold standard’ diagnosis.4

Patients who present persistently positive serology and negative biopsy probably have latent CD.

The proper number of biopsy fragments from the duodenal second portion or the more distal part is between 4 and 6. A recent study demonstrated that four biopsies may be sufficient for CD diagnosis in 100% of cases.21,22 Mucous alterations have an irregular pattern, well demonstrated in magnification, mainly associated with chromoendoscopy, Brunner glands and peptic alterations may hinder histological exam, if biopsies are too proximal.

The pathologist must be familiarized with the spectrum of alterations compatible with CD, evaluate and describe lymphocytic infiltration, pattern of crypts, and villous atrophy. Classification is done using modified Marsh’s criteria and Oberhuber et al. Classification proposed by Marsh in 1992 is the most widely used until today. The patient’s symptom frequently correlates with the degree of tissue injury, according to what is described below.9

- **Marsh I:** infiltrative lesion, normal villous architecture and mucosa, IEL increase (>30-40 lymphocytes/enterocytes counted).
- **Marsh II:** hyperplasic lesion; similar to Marsh I, but it also presents crypt hyperplasia.
- **Marsh III:** destructive lesion, subdivided in IIIa – partial villous atrophy; IIIb – subtotal villous atrophy, and IIIc – total villous atrophy.

Intraepithelial lymphocytes (IEL) increase, with normal mucosa architecture may be observed in autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and Hashimoto’s thyroiditis, in patients using non-hormonal anti-inflammatories, in CD’s initial presentation and latent CD. An increase in LIE may also reflect a state of T cells activation triggered by gluten, immune disturbs, drugs, and infectious agents.

Patients with CD who present only IEL increase, with no alterations in the architecture of the mucosa, may be symptomatic and be under increased osteoporosis risk.

Studies suggesting that bulb biopsies seem to be adequate and this may be the only one to show villous atrophy.

In patients that have already initiated GFD, even before the confirmation biopsy, with high CD suspicion and negative serology, a test with a diet containing gluten may be performed, in this case for at least four weeks and, afterwards, biopsy. However, some patients are late responders and may take years to have their histology altered.

It must be very clear that GFD must be established only after a confirmed CD diagnosis.

Diagnosis may be difficult, because the serology may be negative, the disease may have an irregular histological behavior or the number or place of biopsies may not be adequate. Biopsies may have an adequate size, be well oriented with villi turned upside, on filter paper, enabling for crossing and non-tangent sections, for tangential cuts may lead to misinterpretations. The type of

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**Table 2 – Celiac disease differential diagnosis**

<table>
<thead>
<tr>
<th>Anorexia nervosa</th>
<th>Autoimmune enteropathy</th>
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<td>Bacterial overgrowth</td>
<td>Collagenous sprue</td>
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<td>Crohn’s disease</td>
<td>Giardiasis</td>
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<td>HIV enteropathy</td>
<td>Hepagammaglobulinemia</td>
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<td>Gastroenterite infectiosa</td>
<td>Intestinal lymphoma</td>
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<td>Radiation enteritis</td>
<td>SII</td>
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<td>Ischemic Enteritis</td>
<td>Lactose intolerance</td>
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<td>Common variable immunodeficiency</td>
<td>Soy protein intolerance</td>
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<td>Tropical sprue</td>
<td>Tuberculosis</td>
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<tr>
<td>Whipple’s disease</td>
<td>Zollinger-Ellison syndrome</td>
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<td>Eosinophilic gastroenteritis</td>
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clamp does not seem to be relevant.\textsuperscript{21,22} Mucosa inflammation and architecture alterations may be concealed using corticoids and immunosuppressive agents.

Duodenal mucosa inspection, during upper gastrointestinal endoscopy, is important and may reveal significant findings; the endoscopist must be attentive to findings related to villous atrophy, despite of low sensitivity of the tests. During endoscopy the following findings, suggesting CD, may be identified: thickened mucous folds, mosaic pattern, flat folds, smaller size and disappearance of maximum insulfated folds. Patients who undergo upper gastrointestinal endoscopy for weight reduction, anemia, diarrhea, and those with high CD risk (irritable bowel syndrome, inflammatory bowel disease, chronic hepatic disease, Down syndrome, several autoimmune diseases, mainly diabetes mellitus type 1) must take intestinal biopsy.

In Table 2 some diseases that are part of differential diagnosis are presented.\textsuperscript{1,33,34}

**Other exams – Endoscopic capsule**

Abnormalities in CD patients' mucosa without previous diagnosis may be observed through the endoscopic capsule exam, for investigating anemia and iron deficiency.\textsuperscript{34} In these cases serology and duodenal biopsies could probably eliminate the need of endoscopic capsule exam. Duodenum, evaluated by upper gastrointestinal endoscopy (UGE), may appear entirely normal, while in proximal and distal intestines classic findings of CD are discovered with endoscopic capsule.\textsuperscript{34}

**Other exams – Magnification endoscopy**

Recently an article was published demonstrating that high resolution UGE with magnification (with OBI – optimal band imaging) permits a clear visualization of duodenal villi pattern (sensitivity, specificity, positive and negative predictive value are 100\%). OBI system may play a role in optimizing UGE accuracy in CD.\textsuperscript{33}

**Discussion/Conclusion**

CD diagnosis is complex, especially in asymptomatic patients or those with atypical manifestations. Intestinal biopsy is needed for this diagnosis, even in face of a positive serology. However, histological findings are not specific, then the diagnosis can be established only after clinical correlation. Still today most patients with CD do not have this diagnosis, although, in the last years, prevalence has grown due to higher suspicion degree and higher accuracy of serum tests. The meaning of the great number of patients not diagnosed is not well established, as well as that of those patients who present only extraintestinal or non-classic symptoms.

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