

## Diagnostic challenges in celiac disease

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### Abstract

Diagnosis of celiac disease in adults is currently based on serologic tests in combination with histopathological assessment of small intestinal biopsy specimens. High titers of celiac-specific antibodies in immunocompetent patients with villous atrophy in a good quality biopsy sample allow us to state a confident diagnosis. The relief of symptoms and histological improvement after embarking on a gluten free diet further support the initial diagnosis. However, in some cases, these conditions are not fulfilled, which requires a critical evaluation of laboratory and histopathology results and a consideration of other potential causes for the observed pathologies. To avoid diagnostic uncertainty, both biopsy and laboratory testing should be performed on a diet containing gluten. Immune deficiency, cross reaction of antibodies and possibilities of seronegative or latent celiac disease should be considered while evaluating serology results. Uneven distribution and variable intensity of histopathological changes in the small intestine along with multiple disorders presenting a similar specimen image may lead to invalid biopsy results. Additional laboratory testing and careful examination of a patient's history may deliver important data for a differential diagnosis and a more specific biopsy evaluation. Persistence or recurrence of symptoms, despite the ongoing treatment, requires a revision of the initial diagnosis, an evaluation of the gluten free diet and a search for concurrent disorders or complications.

**Key words:** celiac disease, villous atrophy, lymphocytic duodenitis, non-celiac enteropathy, non-responsive celiac disease

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## Introduction

Celiac disease (CD) is a chronic small intestinal immune-mediated enteropathy triggered by the exposure to dietary gluten in genetically predisposed individuals.<sup>1</sup> Diagnosis of CD is valid in patients who, while remaining on gluten containing diet, present positive serology and obvious celiac histopathology. These patients can readily initiate a gluten free diet (GFD). Strict adherence to a GFD in every case is important, as untreated CD may lead to severe complications, e.g. increased risk of malignancies, bone fractures or infertility.<sup>2</sup> However, in some cases, laboratory and histological findings are inconsistent with symptoms presented by patients and are insufficient for straightforward diagnosis. Different factors can influence serology or histopathological results reducing their sensitivity and specificity. A variety of other disorders with similar symptoms and histopathology can mimic CD and should be considered in the differential diagnosis. In these equivocal cases, a detailed investigation is required, using ancillary testing and different diagnostic approach.

## Serologic testing for celiac disease

The preferred single test for detecting CD is IgA anti-tissue transglutaminase antibody (TTG IgA), which is characterized by high sensitivity (93%) and specificity (95%).<sup>3,4</sup> Another commonly used test is IgA anti-endomysial antibody (EMA), which is the most specific of all assays.<sup>4</sup> However, the latter test is technically more difficult, as it requires an immunofluorescence technique, which is a qualitative method; therefore, it is observer dependent and less objective. This makes EMA less time and cost efficient compared to ELISA based TTG test.<sup>4</sup> Although these tests are both very effective, serology alone is not sufficient to confirm the diagnosis (at least in adult patients).<sup>4</sup>

### False positive results

False positive results can occur due to a cross reaction of antibodies in such conditions as enteric infection, chronic liver disease, congestive heart failure or hypergammaglobulinemia.<sup>5</sup>

### False negative results

The most important factor for reliable serology results is that the patient needs to be on gluten containing diet before testing, as being on a low-gluten diet is the main reason for false negative serology.<sup>5</sup> False negative results may be associated with IgA deficiency, which is more common in CD patients than in the general population, since 2–3% of the patients are affected.<sup>3</sup> To ensure all pa-

tients with IgA deficiency are properly tested for CD it is recommended to either measure serum IgA level in all patients or include both IgA and IgG based testing.<sup>3,5</sup> In IgA deficient patients, an IgG based test (anti-deamidated gliadin peptide [DGP] IgG antibodies or TTG IgG antibodies) should be performed.<sup>3</sup> The most effective combination of immunoassays is TTG IgA and DGP IgG.<sup>4</sup>

The antibody titers correspond with the degree of villous atrophy and in less destructive lesions are often low-level or negative.<sup>6–8</sup> Therefore, negative serology does not exclude diagnosis and if suspicion of CD is high, intestinal biopsy should be performed even if serology is negative.<sup>3</sup>

## Seronegative celiac disease

Seronegative celiac disease (SNCD) is defined by the absence of TTG antibodies in the presence of a positive histopathology and antigen (HLA) haplotype DQ2 and/or DQ8.<sup>8</sup> This condition may be caused by strong antigen-antibody affinity resulting in mucosal deposition of tissue transglutaminase (tTG)/anti-tTG immuno-complexes and lack of passage of antibodies to circulation.<sup>8</sup> Detection of these deposits in small intestinal biopsy strongly suggests CD.<sup>9</sup> Another possible explanation might be an incomplete maturation of plasma cells with a consequent failure of antibodies production.<sup>8</sup> Although SNCD is uncommon, it is responsible for 6–28%<sup>2,10</sup> of seronegative villous atrophy and is, therefore, one of the main reasons for this condition.<sup>10</sup>

## Latent or potential celiac disease

There are several reasons for positive serology without villous atrophy, a condition defined as latent or potential CD.<sup>1</sup> In these patients mild histological changes, such as increased intraepithelial lymphocytes (IELs) can be present.<sup>11</sup> Because lesion distribution in CD is often uneven, villous atrophy may be localized solely in duodenal bulb or distal parts of jejunum, thus missed by biopsy. Therefore, if this condition persists, repeated biopsy or capsule endoscopy might be necessary.<sup>2</sup>

## Histopathological changes in celiac disease

The small intestinal mucosa is made up of villi which extend above the surface mucosa and the crypts of Lieberkühn, which extends below the surface.<sup>6</sup> The normal villous to crypt ratio ranges from 3:1 to 5:1.<sup>12</sup> The villous epithelium is composed primarily of absorptive cells and goblet cells, with IELs between them. These are mainly CD3+ and CD8+ T lymphocytes, usually distributed in decrescendo-like pattern with higher count at the basis of the villi and decreasing towards the tip. The normal IELs

count is now considered as less than 25 per 100 enterocytes.<sup>13</sup> Plasma cells, lymphocytes and little amount of eosinophils and macrophages are usually present in the lamina propria.<sup>6</sup>

### An approach to biopsy

Small bowel biopsy is considered to be the gold standard for diagnosis of CD.<sup>5</sup> Because histopathological changes related to CD are patchy, multiple biopsies of the duodenum, including one or two biopsies of the bulb and at least 4 biopsies of post-bulbar duodenum should be performed.<sup>3</sup> Duodenal bulb is the first contact point of gluten and in 9–13% of patients may be the only location of villous atrophy.<sup>3,14,15</sup> However, inflammatory changes of peptic injury and distortion of villi in areas overlying Brunner glands or lymphoid follicles may cause certain difficulties in interpretation of duodenal bulb specimens.<sup>12,15</sup>

### Lymphocytic duodenosis

Histological changes associated with CD can be classified according to Marsh/Oberhuber or Corazza classification (Table 1).<sup>3</sup> Marsh I lesion, also known as lymphocytic duodenosis (LD), is characterized by normal villous architecture and > 25 IELs per 100 enterocytes.<sup>16</sup> This is a common condition, with a prevalence of 5.4% in the general population.<sup>17</sup> Revealing LD in duodenal biopsy with positive serology may represent CD, but further investigation is required to support the diagnosis.<sup>3</sup>

Different studies evaluated CD prevalence in 9% up to 40% of patients with LD.<sup>18</sup> Common reasons for this pathology are also *Helicobacter pylori* and other gastrointes-

tinal infections, small intestinal bacterial overgrowth, use of nonsteroidal anti-inflammatory drugs or proton pump inhibitors, hypogammaglobulinemia, autoimmune or chronic inflammatory disorders. In most cases no cause for LD is found and these changes usually disappear on repeated biopsy.<sup>6,16,18,19</sup> There is a high prevalence of patients fulfilling irritable bowel syndrome (IBS) criteria in this group.<sup>16</sup> In fact, the association between IBS and LD has been reported in several studies, where immunocytochemical staining for T cells (CD3) was performed.<sup>16,19-21</sup> However, in the study in which standard haematoxylin and eosin (H&E) staining was used none of the investigated samples exceeded 25 IELs per 100 enterocytes.<sup>22</sup>

### Borderline cases

As mentioned above, serology correlates with degree of mucosal injury; therefore, negative serology does not exclude CD in patients with LD.<sup>16,18</sup> In equivocal cases, the patient’s history should be revisited. If there is no apparent cause of LD and CD is suspected, HLA typing and repeated biopsy after gluten challenge should be performed.<sup>16</sup> Recent study shows that intake of ≥ 3 g of gluten a day (amount equal to 1.5 slices of bread) will induce histopathological findings consistent with celiac disease in approximately 90% of patients after 14 day trial.<sup>23</sup> Extending the challenge for another 6 weeks in patients who tolerate the challenge well may further improve diagnostic sensitivity. If repeated biopsy and serology after gluten challenge are negative, CD is unlikely. Final follow-up serologic test is performed after 6-12 months of gluten containing diet.<sup>23</sup> Another approach, where initial response to a GFD is assessed seems less accurate, as 38% of patients with LD who improve on a GFD are HLA DQ2/DQ8 negative.<sup>16</sup>

### Non-celiac gluten sensitivity

In the case of negative testing for CD and wheat allergy (WA), non-celiac gluten sensitivity (NCGS) should be considered. This condition may also reveal mildly inflamed mucosa, although IELs count is not as high as in patients with CD. Typically, symptoms disappear after gluten elimination. An open gluten challenge (monitored reintroduction of gluten containing food) or preferably double blinded gluten challenge is performed after at least 3 weeks on a GFD. Relapse of symptoms, with onset hours to days after gluten exposure, confirms the diagnosis.<sup>24,25</sup>

Table 1. Summary of histological classifications frequently used for celiac disease<sup>3</sup>

Marsh modified (Oberhuber)	Histologic Criterion			Corazza
	increased intraepithelial lymphocytes*	crypt hyperplasia	villous atrophy	
Type 0	no	no	no	none
Type 1	yes	no	no	grade A
Type 2	yes	yes	no	
Type 3a	yes	yes	yes (partial)	grade B1
Type 3b	yes	yes	yes (subtotal)	
Type 3c	yes	yes	yes (total)	

\* > 40 intraepithelial lymphocytes per 100 enterocytes for Marsh modified (Oberhuber); > 25 intraepithelial lymphocytes per 100 enterocytes for Corazza.

## Villous atrophy

Definite diagnosis of CD in adults is made in the presence of villous atrophy (VA) in duodenal biopsy.<sup>2</sup> VA is defined as flattening of surface secondary to the shortening and blunting of the intestinal villi and is associated with an increase in crypt cell mitoses and crypt elongation.<sup>12</sup> To correctly assess VA in duodenal biopsy, good quality samples, properly oriented by qualified technician are essential.<sup>2,12</sup> Tangential sectioning and insufficient size of samples may lead to misinterpretation and overdiagnosis of VA.<sup>26</sup> Although CD is the most common cause of villous atrophy, there are many other conditions with similar histopathology.<sup>27</sup> Therefore, in the absence of positive serology, this condition, defined as seronegative villous atrophy (SVA), should be further investigated. Performing HLA DQ2/DQ8 typing in the first step can exclude CD in case of negative result. Careful review of the patient's history may deliver clinical clues to narrow down the possible causes. Additional testing for parasites, bacterial or viral infections, anti-enterocyte antibodies or serum immunoglobulin level is suggested. Finally, biopsy specimens should be reviewed by experienced gastrointestinal pathologists in search for specific histological features (Table 2).<sup>10,28</sup> In previously conducted research, definitive etiology for VA was found in 85% of patients, of which most

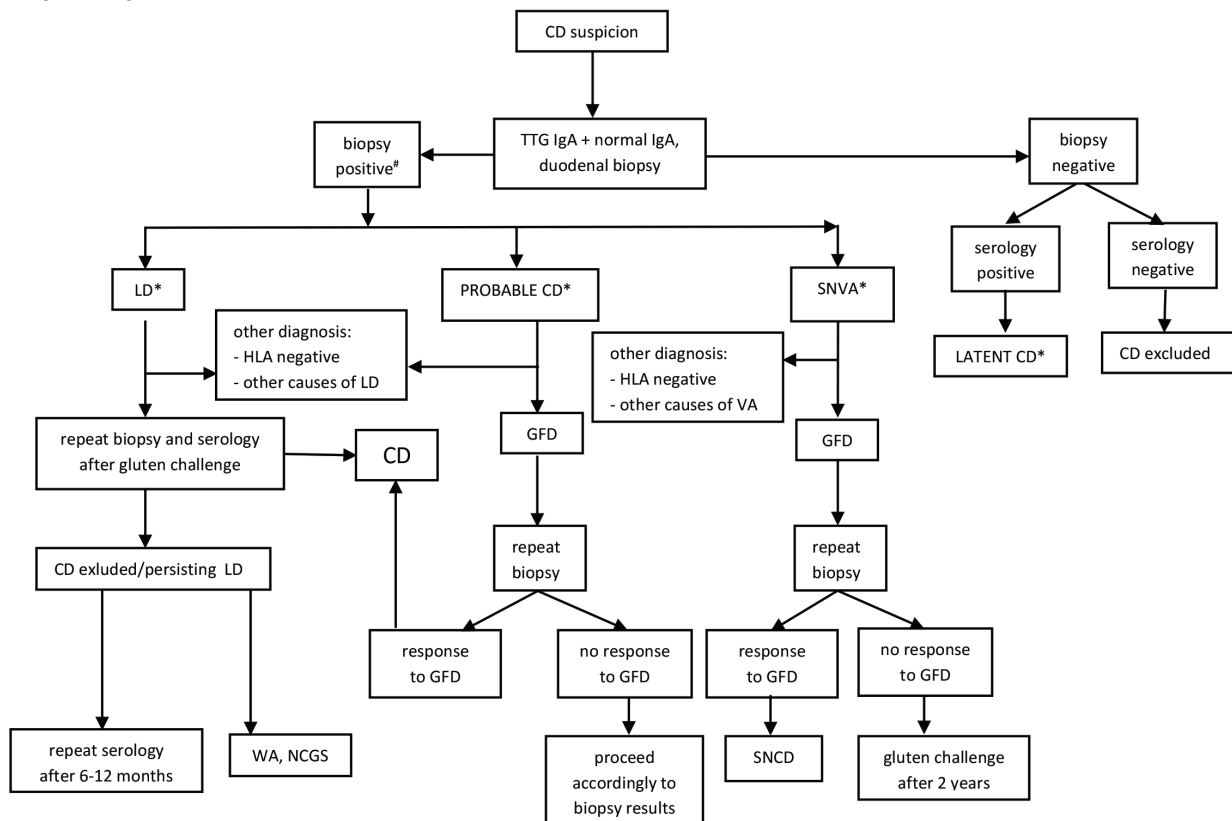
common were seronegative celiac disease, medication related villous atrophy, common variable immunodeficiency (CVID), autoimmune enteropathy, giardia infection and lymphoma.<sup>10</sup> Immune mediated enteropathy, differently called unclassified sprue, was a diagnosis of exclusion.<sup>10</sup>

## Uncertain cases management

As mentioned above, in patients remaining on a gluten containing diet, positive serology and obvious celiac histopathology is sufficient to confidently state diagnosis of CD and introduce a GFD. In equivocal cases, further investigations should be performed to exclude other potential causes for present pathologies (Table 3). If CD remains possible, these patients should embark on a GFD.<sup>15</sup> Histological changes in response to a GFD in patients with VA strongly support diagnosis.<sup>3</sup> In contrast to previous recommendations, follow-up biopsy should not be performed before 6 months on a GFD as in this period a complete recovery of duodenal mucosa is infrequently achieved.<sup>29</sup> In questionable cases, gluten challenge after at least 2 years on a GFD can be performed to support the diagnosis (Fig. 1).<sup>9</sup>

Symptomatic improvement on a GFD or exacerbation after gluten challenge has very low positive predictive value and should not be used as diagnostic criteria.<sup>3,9</sup>

Fig. 1. Diagnostic algorithm for celiac disease



CD – celiac disease; GFD – gluten free diet; LD- lymphocytic duodenosis; SNVA – seronegative CD; # - normal architecture and increased intraepithelial lymphocytes (IELs) ( $\geq 25/100$  enterocytes) or villous atrophy +/- increased IELs; \* definitions and management in Table 3.

**Table 2.** Histological mimics of CD in seronegative patients with normal architecture and increased intraepithelial lymphocytes (IELs) ( $\geq 25/100$  enterocytes) or villous atrophy +/- increased IELs

Suspected etiology	Villous morphology	Other histological findings	Clues
<b>Drugs</b>			
Medication related	increased IELs (PPIs) +/- VA (e.g. azathioprin, colchicine, ipilimumab, mycophenolate, NSAIDs, olmesartan <sup>32</sup> , telmisartan <sup>33</sup> , methotrexate <sup>10</sup> )	prominent neutrophilic inflammation (NSAIDs) <sup>34</sup> , +/- increased subepithelial collagen (olmesartan) <sup>32</sup>	improvement after drug discontinuation
<b>Infections</b>			
<i>Helicobacter pylori</i> (HP) gastritis	increased IELs in an architecturally normal duodenal mucosa <sup>32</sup>	–	improvement after HP eradication <sup>35</sup>
<i>Giardia lamblia</i> infection	usually normal mucosa, variable VA in a minority of cases <sup>32</sup> . IELs not markedly increased <sup>36</sup>	trophozoites on the surface of villi, reactive lymphoid follicles <sup>36</sup>	positive stool for parasites and ova exam (2-3 samples) and/or antigen detection <sup>37</sup>
Post viral enteropathy	diffuse, moderate to marked VA with increased IELs <sup>32</sup>	increased crypt mitoses <sup>12</sup>	acute, self-limiting illness <sup>28</sup> , anti-gliadin antibodies may be slightly increased <sup>12</sup>
Small intestinal bacterial overgrowth	normal to moderate blunting of villi, may have an increased number of IELs and/or neutrophils <sup>38</sup>	normal to increased number of plasma cells in lamina propria <sup>38</sup>	positive hydrogen breath test, positive duodenal aspirate
AIDS enteropathy	normal villous architecture to partial VA <sup>12</sup>	depletion in mucosal CD4 T lymphocytes, increase in CD8 lymphocyte count, increased crypt depth with normal mitoses per crypt <sup>12</sup>	opportunistic infections, such as microsporidiosis, cyclosporidiosis, leishmaniasis, isosporiasis, cryptosporidiosis, mycobacterial or CMV infections may be present <sup>12</sup>
Whipple's disease	shortened, blunted villi, increased IELs	infiltration of foamy macrophages into lamina propria that contain intracellular PAS positive granules, bacterial rods within macrophages, plasma cells or in extracellular space <sup>34</sup>	other clinical findings include arthropathies, lymphadenopathy, fever, and hyperpigmentation of sun-exposed skin, cardiovascular and neurologic pathologies <sup>39</sup>
Tropical sprue	mild to moderate blunting of villi, increased IELs <sup>38</sup> total VA is rare <sup>32</sup>	changes are equally prominent in the jejunum and ileum in addition to the duodenum <sup>32</sup> , increased numbers of plasma cells and eosinophils in lamina propria <sup>38</sup>	travel to endemic areas (Central and South America and South and Southeast Asia), B <sub>12</sub> and folate deficiencies, megaloblastic anemia, atrophic glossitis, megaloblastic cytological changes, response to antibiotics <sup>38</sup>
<b>Autoimmune disease</b>			
Autoimmune enteropathy	usually flat villi, may have slightly increased number of IELs, usually has cryptitis (neutrophils) <sup>38</sup>	GVHD-like apoptosis with lymphocytes infiltrating crypt bases, lack of goblet cells, endocrine cells and Paneth cells if anti-goblet cell antibodies present <sup>38</sup>	history of autoimmune conditions <sup>28</sup> , lack of any triggering food protein <sup>12</sup> , presence of anti-enterocyte antibodies, anti-goblet cell antibodies, anti-parietal cell antibodies <sup>38</sup> , anti-gliadin antibodies may be present <sup>12</sup> , associated with immunodeficiency states and thymomas <sup>38</sup>
Extraintestinal autoimmune disorders	increased number of IELs and variable degree of VA <sup>32</sup>	–	Hashimoto thyroiditis, Graves' disease, rheumatoid arthritis, lupus erythematosus, multiple sclerosis, psoriasis, ankylosing spondylitis or progressive systemic sclerosis, type 1 diabetes

CD – celiac disease; VA – villous atrophy; IELs – intraepithelial lymphocytes; PPIs – proton pump inhibitors; NSAIDs – nonsteroidal anti-inflammatory drugs; ESR – erythrocyte sedimentation rate; ASCA – anti-*Saccharomyces cerevisiae* antibodies; CMSE – cow's milk protein sensitive enteropathy.

**Table 2.** Histological mimics of CD in seronegative patients with normal architecture and increased intraepithelial lymphocytes (IELs) ( $\geq 25/100$  enterocytes) or villous atrophy +/- increased IELs (cont.)

Suspected etiology	Villous morphology	Other histological findings	Clues
<b>Immune disorders</b>			
Common variable immunodeficiency	variable degree of VA +/- increased IELs <sup>40</sup>	nodular lymphoid hyperplasia, absence of plasma cells <sup>41</sup> , polymorphonuclear infiltrate, graft-versus-host disease-like lesions <sup>42</sup>	fulfilled diagnostic criteria: hypogammaglobulinaemia (IgG below 5 g/L), no other cause identified for immune defect, recurrent, severe or unusual infections, poor response to vaccination <sup>41</sup> , may coexist with <i>G. lamblia</i> infection or celiac disease
<b>Neoplasia</b>			
Enteropathy-type intestinal T cell lymphoma (EITCL) / enteropathy associated T-cell lymphoma (EATL)	VA, distorted architecture, ulcerations <sup>12</sup> clonal proliferation of phenotypically, abnormal intraepithelial lymphocytes (IELs) <sup>43</sup>	neoplastic lymphocytes, non-specific mononuclear infiltrate in lamina propria, the adjacent intact mucosa shows the histological features of CD <sup>12</sup>	signs of obstruction, perforation or haemorrhage, a palpable tumour may be found <sup>12</sup>
Immunoproliferative small intestinal disease (IPSID)	villous blunting or VA <sup>12</sup>	dense infiltration of dysmorphic plasma cells and centrocyte-like lymphocytes in the lamina propria <sup>12</sup>	thickening, erythema and nodularity of the mucosal folds may be seen in the duodenum and upper jejunum <sup>12</sup> , affects mainly older children and young adults; obstruction and abdominal masses are typical for the advanced stage presence of anomalous $\alpha$ heavy chain protein in the serum (20% to 90% of patients) common parasitic infestations <sup>44</sup>
<b>Other</b>			
Peptic duodenitis	normal IELs <sup>12</sup> , variable VA <sup>32</sup> , may be associated with VA of the mucosa of the distal duodenum <sup>32</sup>	prominent neutrophilic inflammation <sup>32</sup> , extensive gastric metaplasia and Brunner's gland hyperplasia <sup>34</sup> , oedema, acute inflammation in the lamina propria and epithelium, erosions <sup>32</sup>	peptic ulcer disease or improvement with acid suppressive therapy <sup>28</sup>
Crohn's disease	normal <sup>12</sup> / increased IELs, variable degree of architectural distortion <sup>32</sup>	focal acute inflammation (cluster of neutrophils) flanked by almost normal appearing mucosa +/- granulomas <sup>12</sup> , aphthous ulceration, fissure formation, pyloric metaplasia, fibrosis <sup>34</sup> , lymphoplasmacytosis <sup>32</sup>	elevated ESR/ASCA, multilevel involvement of the intestine <sup>28</sup>
Eosinophilic gastroenteritis	variably perturbed architecture <sup>12</sup>	eosinophilic infiltration of small bowel mucosa <sup>28</sup>	elevated peripheral eosinophil count, no evidence of parasitic, intestinal or extraintestinal disease <sup>12</sup> , multiple allergies <sup>28</sup>
Food allergies	increased IELs sometimes minimal architecture distortion, partial VA (CMSE) <sup>12</sup>	lymphonodular hyperplasia, lesions most prominent in duodenal bulb, may extend to all parts of gastrointestinal tract (CMSE) <sup>12</sup>	multiple allergies, atopy, serum IgE allergy testing <sup>28</sup>
Collagenous sprue	"flat" biopsy appearance <sup>45</sup>	subepithelial collagen deposits <sup>28</sup>	collagen deposits may also be present in the colon (i.e. collagenous colitis) or stomach (i.e. collagenous gastritis), positive endomysial antibodies <sup>45</sup>

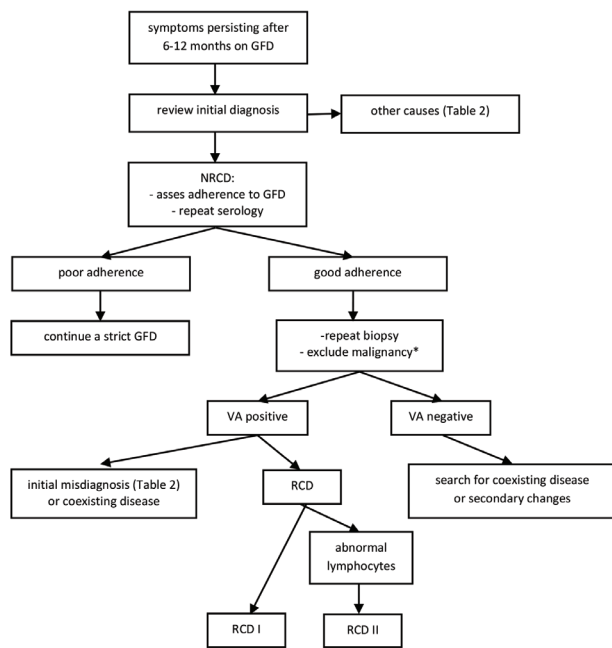
CD – celiac disease; VA – villous atrophy; IELs – intraepithelial lymphocytes; PPIs – proton pump inhibitors; NSAIDs – nonsteroidal anti-inflammatory drugs; ESR – erythrocyte sedimentation rate; ASCA – anti-*Saccharomyces cerevisiae* antibodies; CMSE – cow's milk protein sensitive enteropathy.

**Table 3.** Suggested diagnostic path in symptomatic patients diagnosed for celiac disease (CD) with equivocal results

Results	Diagnosis	Further investigations	Possible cause
sero +, VA -, IELs +	probable CD <sup>2</sup>	consider other causes of LD*, HLA typing, trial with GFD	lesions in different part of small intestine, other causes of LD*
sero -, VA +, IELs +/-	seronegative VA	consider other causes of VA*, HLA typing, asses gluten ingestion, trial with GFD	false negative serology, poor quality biopsy sample, SNCD, nonceliac villous atrophy*
sero -, VA -, IELs +	lymphocytic duodenosis (LD)	consider other causes of LD*, perform gluten challenge, HLA typing, exclude WA (specific prick test, wheat specific serum IgE), consider NCGS (gluten challenge after GFD)	NCGS, WA, other causes of LD*
sero +, VA -, IELs - <sup>#</sup>	latent/potential CD	maintain on GCD, HLA typing, repeat biopsy and serology, perform capsule endoscopy and/or enteroscopy	lesions in different part of small intestine, false positive serology

CD – celiac disease; sero – serology; VA – villous atrophy; IELs – intraepithelial lymphocytes; WA – wheat allergy; NCGS – non-celiac gluten sensitivity; SNCD – seronegative celiac disease; HLA – human leukocyte antigen; GCD – gluten containing diet; \* other causes of LD/VA in Table 2; # may present increased IELs according to some sources.<sup>10</sup>

**Fig. 2.** Management of non-responsive celiac disease



GFD – gluten free diet; NRCD – non-responsive celiac disease; VA – villous atrophy; RCD (I; II) – refractory celiac disease (type I; II); \*perform endoscopic or imaging procedures; such as magnetic resonance imaging (MRI); computed tomography (CT); enterography; double or single balloon enteroscopy; positive emission tomography (PET).<sup>27</sup>

## Non-responsive celiac disease

Typically, in CD patients there is a substantial clinical and serological improvement after weeks or months on a GFD.<sup>30</sup> However, as much as 4–30% of patients have persistent symptoms, signs, or laboratory abnormalities in spite of 6–12 months of treatment.<sup>2,3</sup> These patients may be affected by non-responsive celiac disease (NRCD) and should be further diagnosed to find its cause.

The first step is confirmation of CD diagnosis by reviewing patient’s initial biopsy and serology. If diagnosis is confident, then adherence to a GFD should be evaluated. Gluten ingestion is the most common cause of NRCD and is responsible for 36–51% of cases.<sup>30</sup> There is no objective laboratory method to detect gluten contamination.<sup>31</sup> Although positive CD serology often indicates gluten exposure, 19–30% of patients present positive serology despite complete gluten exclusion.<sup>30</sup> Moreover, negative serology may not reveal intermittent or low-level gluten intake.<sup>3</sup> Therefore, a detailed examination of the patient’s diet by an expert dietitian in search for potential gluten sources is necessary.<sup>2,3</sup> The next step is to repeat the small intestinal biopsy with colonic biopsies in the case of persistent diarrhea.<sup>3</sup> If there is no villous atrophy, other conditions responsible for persisting symptoms should be considered.<sup>3</sup> One scenario is that primarily asymptomatic CD coexist with other disease that have similar symptoms but no evident villous atrophy, e.g. food intolerances, small intestinal bacterial overgrowth, microscopic colitis, eosinophilic enteritis, IBS, Crohn’s disease, bile salt malabsorption, hyperthyroidism.<sup>2,3,31</sup> Another option is that secondary changes, such as lactose intolerance or pancreatic exocrine deficiency persist despite villous recovery.<sup>31</sup>

The presence of VA on repeated biopsy may result either from initial misdiagnosis (Table 2), concurrent autoimmune disease that does not respond to a GFD or refractory celiac disease (RCD).<sup>3,31</sup> Endoscopic and imaging procedures should be employed to exclude malignancy as a cause of recurrent symptoms, particularly in patients presenting significant unexplained weight loss, bowel obstruction, gastrointestinal bleeding, anorexia, pruritus, fever, nocturnal diaphoresis or abdominal pain.<sup>30</sup> Finally, RCD remains diagnosis of exclusion (Fig. 2).

## Refractory celiac disease

RCD is defined as persistent or recurrent malabsorption symptoms and signs with VA despite a strict GFD for more than 12 months.<sup>1</sup> This condition affects approximately 1–2% of CD patients and is responsible for 10–18% of NRCD cases.<sup>15,30</sup> It is typically diagnosed at the age of 50 years onwards and is exceptional in childhood.<sup>30,31</sup> The majority of patients present recurrent symptoms years after initial clinical response to a GFD (secondary RCD). Primary RCD is less common and relates to a subset of patients that initially fail to respond to a GFD.<sup>30</sup> Identifying abnormalities in IELs differentiate between 2 subcategories of RCD. Loss of CD 3 or CD 8 surface markers detected by immunohistochemistry or flow cytometry, as well as T-cell receptor chains clonal rearrangement indicated by molecular methods are characteristic for RCD type II.<sup>3,30</sup> These patients have poorer prognosis in relation to RCD type I patients, because of a much more frequent transformation into enteropathy-associated T-cell lymphoma (EATL).<sup>30</sup>

## Conclusions

In summary, although CD diagnosis is usually based on typical symptoms and consistent laboratory and histological findings, there are unclear cases in which more detailed examination is required. Each diagnostic method is susceptible to different factors influencing its outcome, which should be considered while assessing the results. Careful and accurate diagnostic approach reduces the risk of misdiagnosis. Specific histopathological image in combination with clinical clues help to differentiate CD from its mimics and allow for correct treatment. Persistence or recurrence of symptoms despite ongoing treatment requires revision of initial diagnosis, evaluation of adherence to a GFD and searching for concurrent disorders or complications.

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