# EVALUATION OF T-LYMPHOCYTE SUBTYPES IN THE DIAGNOSIS OF CELIAC DISEASE

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*Summary*. The objectives of this study were tot verify comparatively clinical age reported antecedents and the morphological aspect of the intestinal mucosa in patients sensitive to gluten, hospitalised at IOMC ("Alfred Russescu" Hospital) in the last 7 years. Celiac disease is an inflammation most frequently affecting the proximal small intestine, depending on the presence of gluten in the diet, whose pathogenesis seems to be immunological in nature. 107 cases were divided in three groups following clinical manifestations types at hospitalisation time: typical digestive, untypical digestive and extra digestive manifestations. Intestinal biopsies, made with Crosby probe, in children aged between 1.3 and 8 years (one single case was diagnosed as late as at the age of 15), regardless of gender. Then we analysed morphologically (HE usual and PAS histochemical staining) and immunohistochemically (lymphocytes B, T with possible subtypes). The lesions were counted at the first biopsy according to the Marsh score. The immunohistochemical tests have indicated the prevalence of T lymphocytes (UCHL1, CD3, CD4, CD8, gamma-delta) both in the luminal epithelium with various degrees of aggression in *lamina propria* and also spread in stroma. B-lymphocytes (L26) are distributed prevalently nodular in stroma. In conclusion, it is CD4 T cells that are present in particular in the control of the gluten immune response in patients with Marsh I and Marsh III lesions.

*Key words*: celiac disease, Marsh score, T-lymphocytes, B-lymphocytes.

## INTRODUCTION

The malabsorbtion accompanies or follows a big number of bowel diseases. It secondary generates malnutrition, the fearsome cause of infantile morbidity and mortality. The malnutrition could consequently follow:

I. An unballanced diet.

II. The maldigestion (primary gastric, intestinal, exocrine pancreatic, lever and cholecystic lesions, cystic fibrosis of the pancreas).

III. Malabsorbtion or intracellular metabolic disorders (Figure 1), that affects patients with,

a) Post partum evidentiable abnormalities, (usually congenital ones), concerning the enterocyte (enzymatic systems disorders interfering with the final

stage of digestion, with the absorption and intracellular metabolism), as well as the intestinal associated lymfoid tissue (hypersensibility for the dietary components).

b) Aggressions of the enterocyts of the intestinal villosity, or those of the crypts, that appear modified or metaplasied.

c) Local organic diseases or postsurgical associated lesions that are followed by a reduction of the absorption surface.

d) Insufficient determined complex origin lesions.

This classification is based on anamneses (weight loss, diarrhoea, oedema) laboratory examinations (anaemia, steatorhhea, hypoalbuminemia) radiological examinations and other techniques (including intestinal biopsy).

The intestinal biopsy is electively indicated when the clinical suspicion, places the patient in the third group, subgroup c).

The duodenal biopsy analysis is an accurate and certain diagnostic method. In our country, though, the accessible instruments for drawing mucosal fragments act in a "bild" way under the radiological screen, so errors are likely to appear especially when lesions are divided in small plots; a single negative biopsy does not exclude the diagnosis.

The duodenal biopsy evaluates:

*The normal aspect* for this diagnosis the presence of a suite of minimum 4-digitiform intestinal villosity (tubular, without diameter altering) with the villosity height versus crypts length (Lieberkuhn glands) ratio 3/1 (or 4/1 in older children) is required. Normality is also characterised by the 8/1 ratio e/c enterocytes/ caliciform cells; rare intraepithelyal lymphocytes, rare plasmocytes in *lamina propria*, stroma`s particularities (absence of oedema, inflamatory infiltrate) or fibrose.

The criteria established by Morson and Dawson in 1990 and Marsh in 1999 show that the intestinal mucosa reacts at the varied causes of aggression (see classification) by constitutive modifications that form a tripod:

– transformation of the architecture and quality of the enterocytes;

– progressive thickening of the Lieberkuhn crypts (varies with the aetiology);

– quantitative presence of lymfoplasmocytes in chorion.

*The minor villar aggression (Marsh I-st grade* is morphologically defined by the presence of aggressionated enterocytes. They raise their mitotic activity (ratio e/c = 31/1); parcels of young enterocytes appear on the villosity; intraepithelial lymfocites are present (20–30/100 enterocytes); the stroma bears little modifications (oedema) (Figures 5, 6, 7, 8, and 9).

*The medium villar aggression (Marsh II-nd grade)*. Intestinal villosity are shortened and thickened, the enterocytes of the cryptes are hyperplasiated, determining the thickening of the basal layer; the intraepithelial lymphocyte are present. The stroma may contain an increased plasmocyte number. This aspect is typical, representing the usual pattern of the reaction of the aggressed duodenal mucosa (Figure 10).

*Severe villar aggression (Marsh III-rd grade)*. Its architecture is defined by parcelar atrophies; the intestinal villosity is uneven atrophied with hyperplastic crypts; the enterocytes suffer the same altering as those that appear in the gluten induced entropathy (especially the celiac disease).

This aspect alternates with normal areas, in post viral enteritis, milk or soya protein induced enteritis, herpetiform dermatitis, or post medication enteritis. Main drugs generating this aspect are cloramfenicol, aureomicin, penicilin compounds, sulfamides).

Other features seen in this grade of aggression are the entrerocytes with congenital atrophy of the microvillosity; or the microvillosity inclusion disease; the increasement of the number of the intraepithelial lymphocytes (as in the milk cow proteins or soya protein sensibility).

*Lamina propria* contains miscellaneous lymphonuclears, PME and some rare PMN (cow milk and soya proteins induced entheropathy, the post-viral entheropathy; the allergic enteropathy shows predominance of PME that are also found intraepithelialy).

The absence of the plasmocytes characterises hypogammaglobulinemia and the selective deficiency of Ig A. Other feature is the presence of very much dilated limfatic vessels (intestinal limfangiectasy).

Marsh IIIa score (Figure 11) – partially atrophied intestinal vilosities: it is shortened, flattened, with deeper crypts and intense regeneration of the epithelium cells and frequent interepithelial lymphocytes (>30).

Marsh IIIb score (Figures 12, 13, and 14) - destructive lesions (subtotal intestinal vilozities, clearly atrophied villi, still recognoscible, widened hyperplasic crypts containing immature epithelial cells with a higher growth rate and influx of inflammatory cells).

Marsh IIIc score (Figures 15, 16, and 17) – hyperplasic lesions (total VA completely absent villi, with severe atrophy, atrophied, hyperplastic glands and infiltrating lesions).

Celiac disease (CD) is an intestinal disorder with multifactorial aetiology. HLA and non-HLA genes together with gluten and possibly additional environmental factors are involved in disease development.

Evidence suggests that CD4 (+) T cells are central in controlling an immune response to gluten that causes the immunopathology, but the actual mechanisms responsible for the tissue damage are as yet only partly characterized.

## MATERIAL AND METHODS

This study is focused on the morphological aspect of the intestinal mucosa in patients sensitive to gluten, hospitalised at IOMC (“Alfred Russescu” Hospital) in the last 5 years. The retrospective survey involved 97 children (43 boys and

54 girls) aged between 1.3 and 8 years, only one of them being 15 years old (Figure 2).

The clinical symptoms in the early stage fell under 3 categories (Figure 3):

– Group A (32.3%): typical digestive manifestations (steatorrhea, lax abdomen, malnutrition, growth failure).

– Group B (64.6%): atypical digestive manifestations (vomiting, abdominal pain, constipation).

– Group C (3.07%): extradigestive manifestations (delayed psychomotor growth, iron deficiency anaemia, growth failure).

One can note that Group A prevails in the under 3 years age category, and Groups B and C are initial manifestations more frequently found in older children (Figures 4 and 5).

## DIAGNOSIS METHODS

Small intestinal biopsy is considered the main approach for diagnosis of classical celiac disease. In addition, IgA antibodies against gliadin and endomysium are valuable tools for the detection of patients with celiac disease and for therapy control (maize or rice flour diet). Intestinal biopsies, made with Crosby probe were analysed morphologically (HE usual and PAS histochemical staining) and immunohistochemically (lymphocytes B, T with possible subtypes).

## RESULTS

The lesions counted according to the Marsh score were at the first biopsy:

a) *37 Marsh I infiltrating lesions* (normal mucosa architecture, villi epithelium infiltrated with lymphocytes; lymphocytosis score: 30 lymphocytes/ 100 enterocytes).

b) *15 Marsh II infiltrating lesions* (almost normal villi, enlarged crypts with immature epithelium and infiltrated with lymphocytes).

c) *12 Marsh IIIa destructive lesions (partial villi atrophy (VA)* = shortened, flattened villi, slight infiltration with interepithelial lymphocytes and hyperplasic crypts).

d) *18 Marsh IIIb destructive lesions (subtotal VA* = clearly atrophied villi, still recognisable, widened hyperplasic crypts containing immature epithelial cells with a higher growth rate and influx of inflammatory cells).

e) *25 Marsh IIIc hyperplasic lesions (total VA = completely* absent villi, with severe atrophy, atrophied, hyperplastic glands and infiltrating lesions).

The immunohistochemical tests have indicated the prevalence of T lymphocytes (CD3, CD4, CD8, gamma-delta) both in the luminal epithelium with various degrees of aggression and in *lamina propria*.

**DISCUSSIONS**

*The celiac disease* is a gluten-induced sensibility, the patients do not tolerate the gilaidne or they are hypersensible at it (a glycoprotein found in grain, rye, barley). The actual mechanisms responsible for the tissue damage are as yet only partly characterized. There are evident proofs concerning the direct immunological cytotoxicity against the epithelial cells of the small intestine. The celiac disease defines a long-standing gluten-induced intolerance, but the complaints may appear several times at a very young age.

The suspicion of celiac disease is raised by the anamneses. Next stage for the positive diagnose is the macroscopical examination of the feces (bulky, foamy, white-yellowish, floating on the water surface.). Next come selective techniques proofing the malabsorbtion by the D-xylose test. The faeces lipid balance (demonstrates steatorheea), and other investigations for excluding other malabsorbtion causes.

The positive diagnosis of the celiac disease is a exclusion, the drawing of the intestinal biopsy is performed after the exclusion of other diagnostics, as for example the cystic fibrosis of the pancreas (Figure 18).

The gastroenterologysts consider that 3 intestinal biopsies are necessary:

– first biopsy in drawn with a diagnostic purpose (the diet contains gluten-visible aspect).

– second biopsy is drawn after a strict, 6–12 months gluten free diet, and the microscopical analysis shows minimum aggression or normal aspects, except the lymphocyte intraepithelial number which is still high.

– third biopsy is drawn 1–2 months after the previous one, in order to show the histological relapse at the gluten load. Frequently the symptoms relapse immediately after the gluten ingestion. Unfortunately, endomysial antibodies, which are present in 90% of patients, are not traced out in our hospital.

Because of the increasement of the risk of gastrointestinal lymphoma, malignant histiocytosis, digestive cancer, the association of some cutanate lesions at the affected adults, the diagnosis must be certain and the patient must be pursued for the lifetime. The carcinogenesis is a progressive process: it begins by the presence in *lamina propria* of a mix cellular infiltrate, with high number of lymphocytes, atipical and activated lymphocytes.

*Viruses* are the most frequent aetiology of gastroenteritis in children. Particular problems are raised by children that show an apparent healing after un acute gastroenteritis episode, followed by an clinical and morphological syndrome (see morphological aspect described in Figure 4). For some of those children, the milk protein intolerance may be shaded by an acute gastroenteritis and misinterpreted.

*Milk and soya proteins induced enteropathy* is diagnosed based on four complex gastrointestinal symptoms: malabsorbtion with occult blood loss in

gastrointestinal tract, feriprive anaemia, protein loss enteropathy, alergic proctitis. This protein loss enteropathy is symptomatically since the first 6 months of life (vomiting, diarrhoea, growth failure, hypoproteinemia, oedema, steatorheea). The intestinal byopsy evidentiates parcelar atrophy of the villosities (see aspect Figures 4 and 5) with the presence of PME intraepithelial and in *lamina propria* (specific diagnostic help but un aspect not always present). It differs from the celiac disease by the presence of uneven lesions of the duodenal mucosa, and the presence of PME.

The cow milk-privative or soya proteins free diet ameliorates the clinical and morphological aspect (the villosity towards normality, and a medium cellularity in *lamina propria*). Among primary immunodeficiences with morphological response on the intestinal mucosa the *selective deficiency of IgA* and *hypogammaglobulinemia* are more frequent. The selective Ig.A defficiency shows diffuse lesions; the absence of plasmocytes in *lamina propria* differentiates it from Celiac disease. The hypogammaglobulinemia shows parcelar aspect of the enteropathy, and exacerbated malabsorbtion if giardia suprainfection appears.

*The Kwasiorkor* may produce the atrophy of intestinal villosity and diffuse inflammation, but it is a rare disease in developed countries. The patients with protein-calorical malnutririon and gastrointestinal symptoms are usually suffering the effect of overlaid infections.

*Abetalypoproteinemia* is a metabolically autosomal recessive disease characterised by the incapacity of synthesis and transport of betalipoproteins and extraintestinal abnormalities (erythrocyte's haemolysis, retinitis pygmentosa and neuromuscular degeneration).

Malabsorbtion and diarrhoea are obvious in the first year of life and usually the most eloquent clinical manifestation of the disease. Microscopic examination shows that epithelial cells are pronounced vacuolary, due to the absorbed lipids, that cannot be transported outside the cell. Villosity architecture has a normal aspect.

Concluding, the duodenal biopsy is a real support for the clinician both in diagnosis as in dynamical pursue of a big number of diseases, which's simptomathology, radiological examinations, and laboratory information give us few dates for the evaluation of the lesional form.

## CONCLUSIONS

The celiac disease was mainly diagnosed in very young patients.

The disease has a wide clinical picture, characterised by atypical digestive disorders and extradigestive disorders.

There is a prevalence of certain gluten reactive subtypes of T-lymphocytes, in particular in Marsh I lesions.

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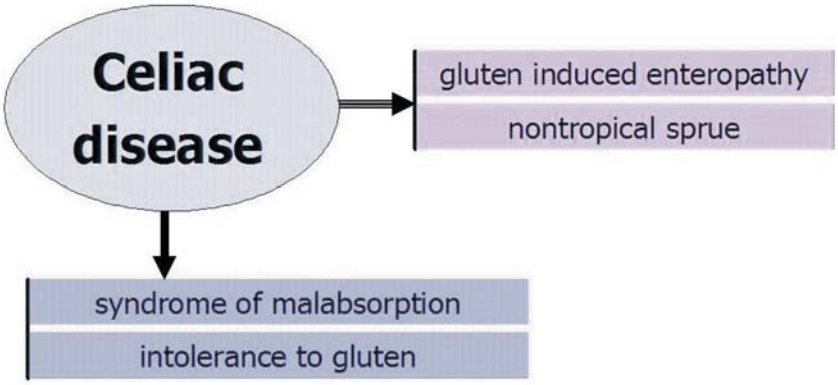



Figure 1 – Celiac disease / an intestinal disorder


Figure 2 – Gender repartition of ill children

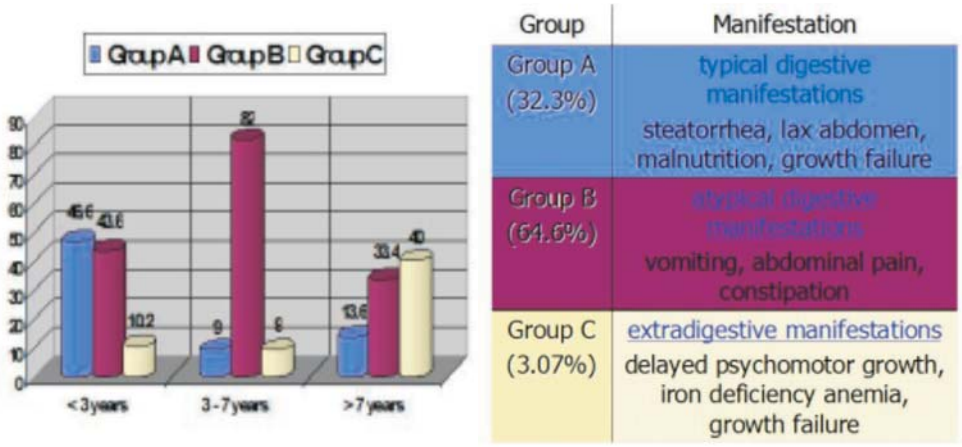



Figure 3 – Correlation age / manifestations

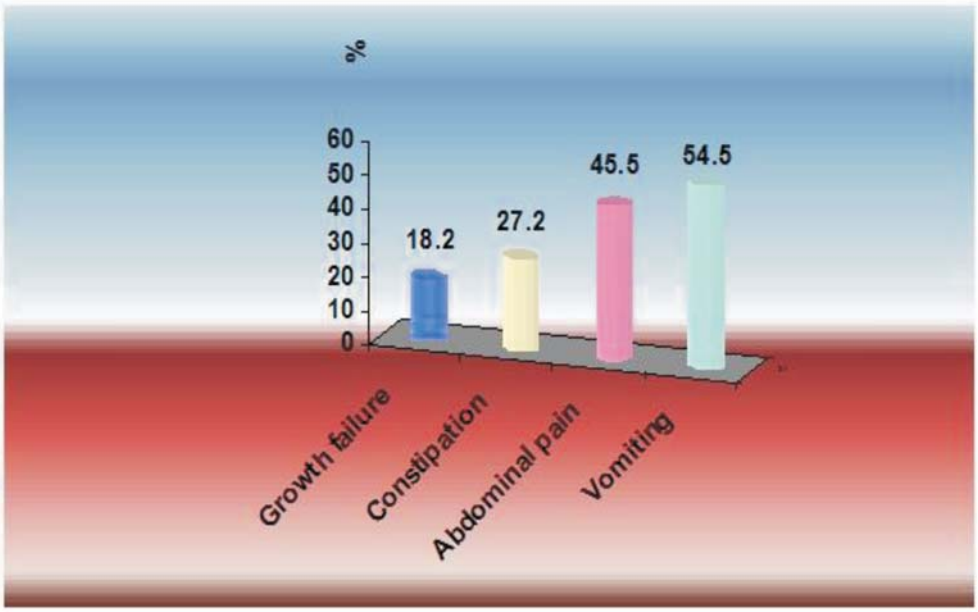



Figure 4 – Atypical manifestations

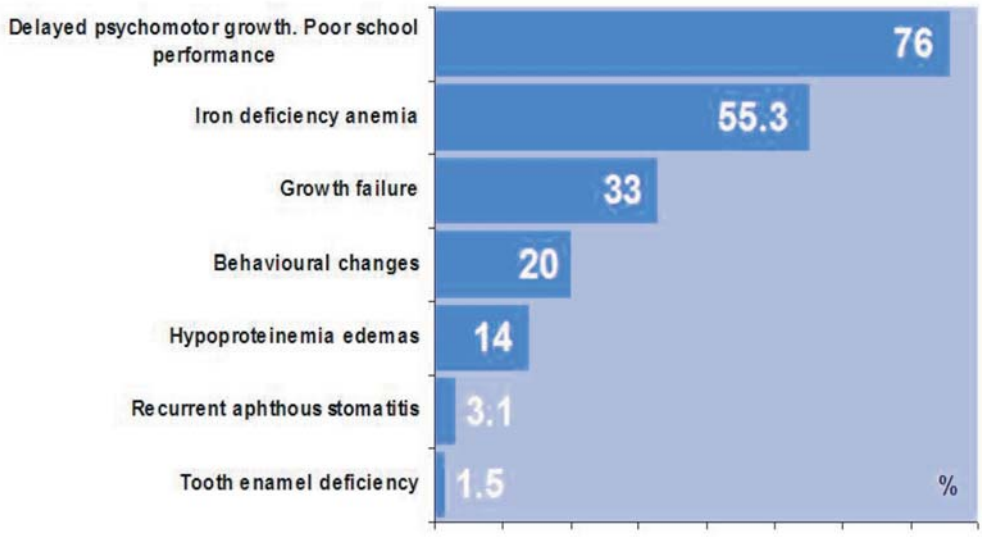



Figure 5 – Extradigestive manifestations

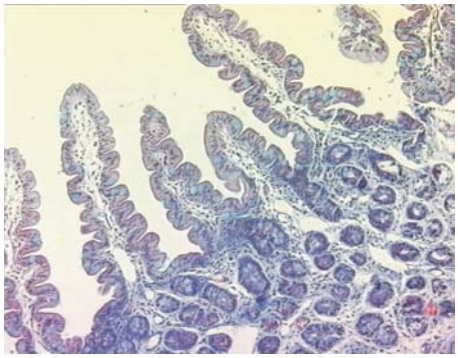


Figure 6

Figure 6 – Marsh I: unequal length of the villi, which no longer keep the 8 to 1 ratio (villus to the glandular part of the mucosa); appears the widening of the bottom part the villi and regeneration of mucosa sections which results in a lower number of caliciform cells, normal value 1 to 40 epithelial cells (Trichrome Masson, ×10)

Figure 7 – Marsh I Uchl l used for the identification of T-lymphocytes. T-lymphocytes are found in lamina propria, interepithelial, fragmented and in stroma (Ihc Uchl 1, ×40)

Figure 8 – Marsh I Uchl 1: the image features very rare T-lymphocytes (unexplainable) and extensive epithelial regeneration. Consequently, there was no clear-cut differentiation of the CD4 and CD8 T-lymphocytes (Ihc Uchl 1, ×40)

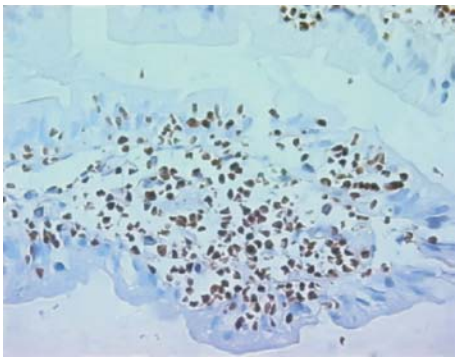


Figure 7

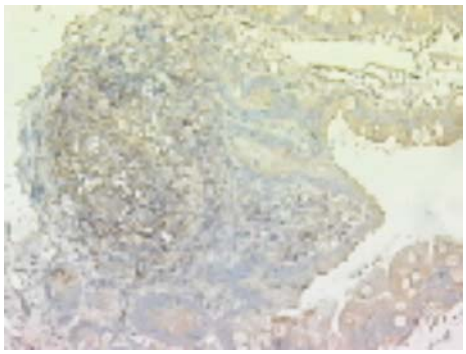


Figure 9

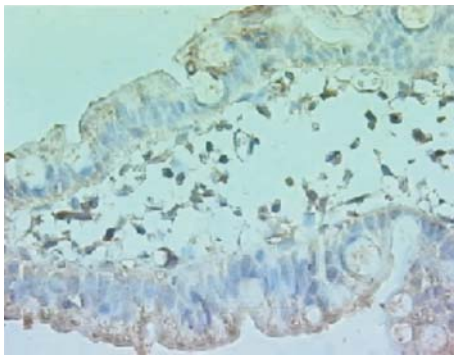


Figure 8

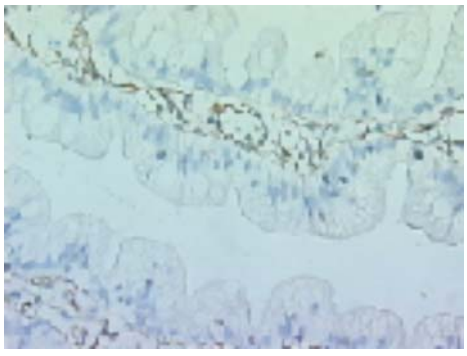


Figure 10

Figure 9 – Marsh I L26: B-lymphocytes were found in stroma; Marsh I vim: Vimentine was used to reveal the stroma. The blood vessels are clearly seen (Ihc vim, ×40). A nodular lymphatic inflammatory infiltrate (made up of B-lymphocytes) is noticeable intramucous (Ihc L 26, ×18)

Figure 10 – Marsh I vim: Vimentine was used to reveal the stroma. The blood vessels are clearly seen (Ihc vim, ×40)

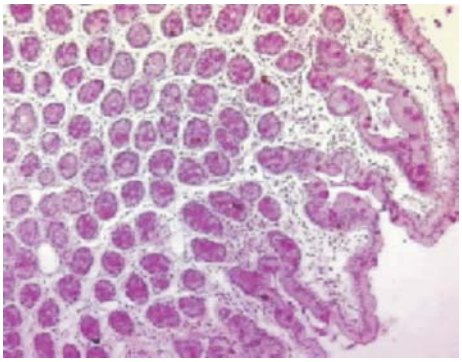


Figure 11 – Marsh II: the same elements as in Marsh I are noticeable, except that the villi are much wider, shorter, of unequal length, joining at the top. The caliciform cells (red stained with PAS) are equally rare (PAS, ×20)

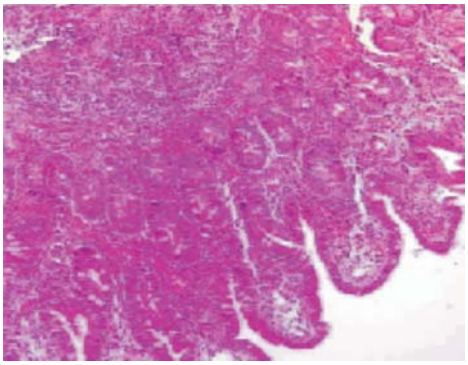
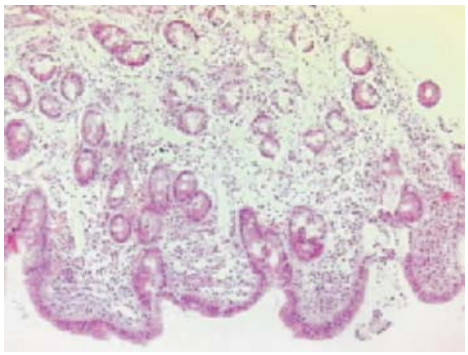


Figure 12 – Marsh IIIa: shorter and wider villi, deeper crypts. Intense regeneration of the epithelium cells and frequent interepithelial lymphocytes, >30 (HE, ×10)



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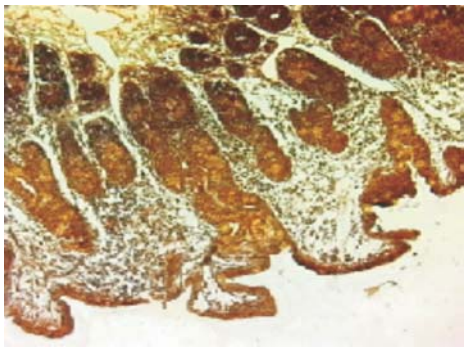

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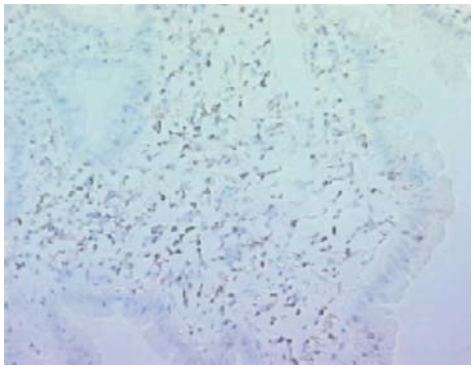

Figure 15

Figure 13 – Marsh IIIb: almost atrophied villi (forming plateaus), deepened crypts. Disruption of the glandular part because of the prevailingly B lymphocyte inflammatory infiltrate (HE, ×20)

Figure 14 – Marsh IIIb: staining for revealing the reticulin preceded the immunohistochemical test in order to highlight the rich reticulin network in the stroma and lamina propria (Gömöry, ×10)

Figure 15 – Marsh IIIb: you can see how rare T-lymphocytes are interepithelial, in lamina propria and stroma which it cannot be explained why (Ihc: Uchl 1, ×40)

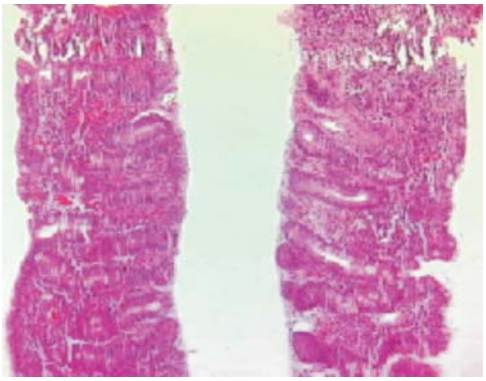

Figure 16 – Marsh IIIc: total atrophy of the villi and a bundance of inflammatory infiltrate with a nodular tendency in Mucosa and very deep crypts (HE, ×10)

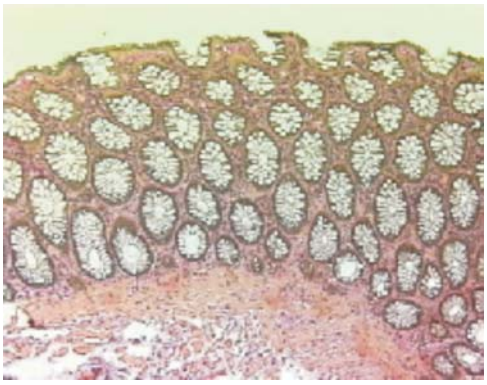

Figure 17 – Marsh IIIc: total atrophy, muscularis mucosae is noticeable and diffuse periglandular fibrosis – red (Van Giesson, ×20)

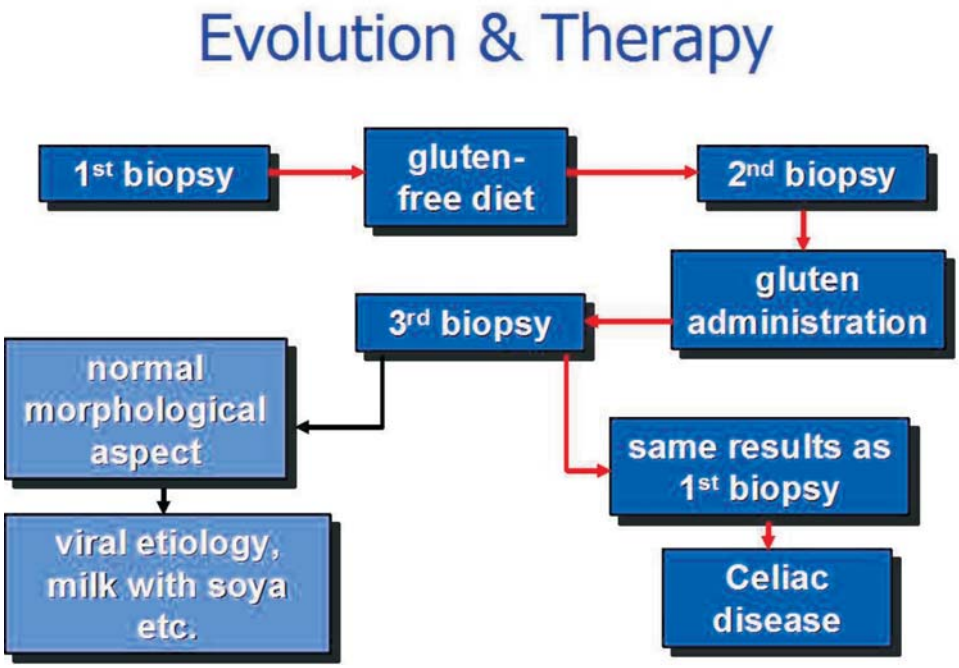


Figure 18 – The positive diagnosis of celiac disease