

# Primary Intestinal Malignant Lymphomas Associated With Celiakia – A Pathologist’s Review

Lukáš Plank\*

Department of Pathology, Comenius University in Bratislava Jessenius Medical Faculty and University Hospital in Martin and Martin’s Biopsy Center, Ltd., Slovakia

\*Corresponding author: plank@jmed.uniba.sk

Received February 25, 2015; Revised March 05, 2015; Accepted March 16, 2015

**Abstract** Celiakia represents a prototype of chronic antigenic stimulation and autoimmune dysregulation leading to the accumulation of reactive cytotoxic intraepithelial lymphocytes (IEL) and small intestinal mucosal injury. These lymphocytes possess a potential of transformation, gaining an aberrant phenotype and genotype during their clonal expansion. The development of type I enteropathy –associated T-cell lymphoma (EATL) in patients with celiac disease might represent a gradual multistep transformation process starting from the increasing accumulation of IEL, and developing through the refractory celiac disease I and refractory celiac disease II, the last representing probably a lymphoma in situ. These transformational stages of the progressing celiac disease are showing increasing risk of type I EATL development, and decreasing survival of the patients. The whole process of progressing EATL lymphomagenesis resembles the hyperplasia-adenoma-dysplasia-adenocarcinoma sequence of colorectal carcinomas. Similar to the already more understood multistep developmental process from CD to type I EATL, an overt type II EATL lymphoma might represent a final step in a process of progression from IEL, representing the lymphoma precursors. However, the patients with the type II lymphoma show signs of celiakia neither clinically nor morphologically and the pathogenetical link to CD is missing. The lymphomagenesis of EATL type II seems to utilize activation of other pathways than that of type I. The recent data support the attempts to set apart the type II from the EATL category and to rename it.

**Keywords:** Celiakia, refractory celiac disease, primary intestinal lymphoma, enteropathy-associated T-cell lymphoma type I, enteropathy-associated T-cell lymphoma type II, monomorphic CD56+ intestinal lymphoma, lymphoma in situ

**Cite This Article:** Lukáš Plank, “Primary Intestinal Malignant Lymphomas Associated With Celiakia – A Pathologist’s Review.” *International Journal of Celiac Disease*, vol. 3, no. 2 (2015): 59-68. doi: 10.12691/ijcd-3-2-1.

## 1. Introduction

While Hodgkin lymphomas, with a very few exceptions, do not arise at the extranodal sites, the primary intestinal malignant lymphomas are represented by a variety of B-cell and T-cell non-Hodgkin lymphomas. The adjective “primary intestinal” means, that:

a).lymphoma arises in the intestine and per definitiam is present in the intestinal localization without evidence of its dissemination into the liver, spleen, peripheral lymph nodes, and bone marrow; however, at the time of diagnosis up to 10% of the cases may show bone marrow involvement and the dissemination may appear later in the disease’s course,

b).a secondary intestinal tract involvement by either primary nodal or extranodal lymphoma of other than intestinal origin during its dissemination was excluded [1,2].

The category of primary intestinal lymphomas is most often represented by tumors arising in the small intestine and less common in the colorectum. This category

includes a few nosologic entities, such as enteropathy-associated T-cell lymphoma (further EATL), and immunoproliferative small intestinal disease (IPSID), that, essentially, arise only in the gastrointestinal tract [3]. More common are those primary intestinal lymphomas, which might arise also as either primary nodal, or primary extranodal lymphomas in other mucosal sites.

In majority of lymphomas, a clonal lymphomatous proliferation is either genetically or immunologically driven. Its immunological lymphoproliferative “drivers” might include infectious agents and/or specific non-infectious antigens, activated due to autoimmune inflammatory syndromes. Based on the contribution of Peter Isaacson and his school [1], the primary gastrointestinal lymphomas have led the way in understanding the steps in pathogenesis of lymphoma from accumulation of reactive mucosa-associated lymphoid tissue (MALT) through its potential clonal transformation to an overt lymphoma. Bacteria, or at least immune responses to bacterial antigens, have been implicated in the pathogenesis of MALT lymphoma. These include *H. pylori* in gastric MALT lymphoma and *Campylobacter jejuni* in intestinal MALT lymphoma

associated with alpha heavy chain disease [1]. In a similar way, the celiakia might represent a prototype of chronic antigenic stimulation and immune/autoimmune dysregulation leading to mucosal injury and accumulation of cytotoxic T-cells, with a potential to clonal expansion and development of specific primary intestinal lymphoma [4]. The association between malabsorption and intestinal lymphoma is known for almost 80 years [5]. Only approx. 50 years later it was proved, which of them is the cause and the consequence, respectively. The designation “EATL” introduced by O’Farely et al [6] replaced the extensively previously used and already obsolete term “malignant histiocytosis” [1,7].

The celiakia (celiac disease, gluten-sensitive enteropathy synonymously) causes serious health problems, including an increased risk of EATL development (see further). The increased risk was previously also reported for other malignancies, e.g. oropharyngeal, oesophageal and small intestine carcinoma, melanoma, etc. [2,8]. In contrast, some recent data suggest no increase in the incidence of solid tumors in CD, only increase in lymphomas [9].

However, “EATL” does not represent a nosologic unit, as according to the WHO lymphoma classification [10] two EATL forms should be distinguished, which are defined as follows:

- “EATL” as a tumor of cytotoxic intraepithelial lymphocytes (IEL), showing varying degrees of transformation but usually presenting as tumor of large lymphoid cells, often with inflammatory background and enteropathic changes in the adjacent small intestinal mucosa. In this WHO classification, this entity is referred to simply as “EATL”, however in majority of reports as type I EATL, or “classical EATL”. Although it is not absolute, the type I EATL shows a strong association with CD; the pathogenetical link to CD has been proved in a great majority of cases (see further); therefore it might be considered to represent a CD-related and associated tumor,

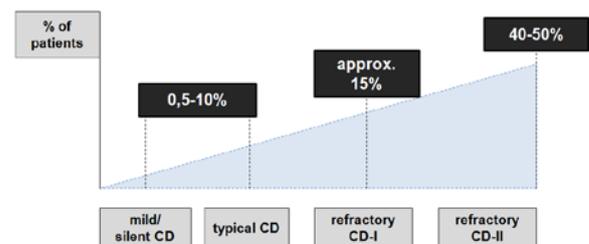
- “monomorphic variant”, or type II EATL acc. to WHO lymphoma classification [10]. This lymphoma represents also an intestinal tumor of intraepithelial cytotoxic T-lymphocytes, however, composed of small to medium-sized monomorphic lymphoid cells. In patients with type II EATL, the evidence of CD, including presence of enteropathic changes in adjacent small intestinal mucosa is usually missing; that means that a pathogenetical link to CD is usually absent. That is the reason why the International Lymphoma Study Group proposed to set apart type II EATL from the category EATL and to rename it [3,11]; this might become true in the expected new edition of WHO lymphoma classification. In the in 2010 published WHO classification of the digestive tract tumors [12], the tumor has already been referred to as simply “*monomorphic CD56+ intestinal T-cell lymphoma*”, based on the characteristic morphologic and phenotypic patterns of this tumor. In addition, in some recent reports from Asia, the expression “*epitheliotropic intestinal T-cell lymphoma*” as synonymic term to type II EATL has been used [13]. Accepting the still valid terminology of lymphoma classification according to the WHO, the designation type II EATL is used also in this review.

However, it must be mentioned, that although the risk among coeliakia patients is greatest for primary intestinal lymphomas of T-cell lineage of EATL type, some large

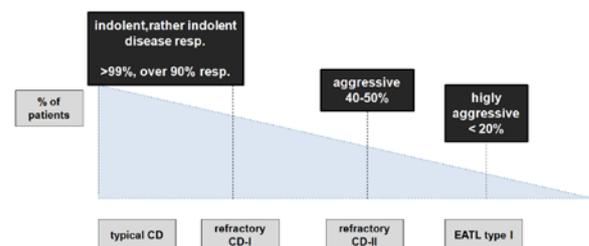
population-based studies noticed also an increased risk for B-cell lymphomas inside and outside the intestinal tract as well [9,14].

## 2. Enteropathy of Celiakia and/or of other Origin and Its Relation to EATL Precursor Lesions and an Overt Lymphoma

Although in spite of very extensive reasearch many facets of celiakia disease remain unclear, the close association and pathogenetical relation of type I EATL to the celiac disease are world-wide definitively accepted. The consensus is based on accumulation of epidemiologic and genetic data, clinical experiences and knowledge of the disease pathophysiology, on histopathological findings in the biopsies of patients with CD and EATL, as well as phenotypical and genotypical analysis of the lymphoid population infiltrating intestinal mucosa [10]. The CD8-positive intraepithelial lymphocytes in the intestinal mucosa of CD patients seem to undergo conversion to CD8-negative and CD30-positive lymphoid population of type I EATL, and so to represent a cellular origin of type I EATL. The development of type I EATL might represent a gradual multistep transformation process starting from CD and developing through the refractory CD-I and refractory CD-II, the last representing probably a lymphoma in situ [10,15]. These transformational stages of the progressing celiac disease are showing increasing risk of type I EATL development, and decreasing survival of the patients (Figure 1 and Figure 2). The whole process is in a way similar to the “hyperplasia-adenoma-dysplasia-adenocarcinoma” sequence of epithelial neoplasms, however, it is still not fully understood, whether all of these stages are necessary intermediate in the multistep process [2,12].



**Figure 1.** Percentual risk of type I EATL development in 5 years (details see in the text)



**Figure 2.** Percentual rate of type I EATL patients' survival in 5 years (details see in the text)

The development of celiac disease is supported by environmental influences, as well as genetical and familiar dispositions. Celiac disease is associated with

permanent intolerance to gluten (and its two fraction - prolamin and glutenin), which is found in some cereals [16]. The disease arises in genetically predisposed people, after exposure of the small intestine to a group of proteins (called gliadin, secalin, hordein and secalin resp.) representing pathogenetically most important prolamin fractions of gluten. These proteins arise in the small intestine by digestion of wheat (containing gliadin), rye (secalin), barley (hordein) and oats (avenin) [9,16]. The gluten derivatives trigger a maladaptive autoinflammatory immune response, consisting of cellular and humoral antibody-mediated immune reactions and resulting in the intestinal mucosal injury and leading to malabsorption of nutrients. However, recently a wider spectrum of gluten-related disorders seems to be discussed, which might include not only CD as an autoimmune-based disease, but also “gluten-sensitivity” as a separate and immune mediated condition, together with wheat allergy [14].

The cellular response in CD is represented by activation of specific CD4+ T-lymphocyte intraepithelial population (IEL), targeting multiple endogenous autoantigens, including enterocytes, and breaching the mucosal epithelium [17]. The exogenous gluten products are probably not directly toxic to mucosa in CD patients and the IEL actively contribute to mucosal damage leading to villous atrophy, crypt hyperplasia and increasing accumulation of T-lymphocytes, both in the epithelium and in lamina propria. These CD-related IEL are present also in mucosa adjacent to lymphoma of type I EATL patients. The humoral immune mechanisms also play a central role in celiakia, as it has been documented by a number of antibodies detectable in CD patients, such as antigliadin, antireticulin, antiendomysial antibodies and TTG antibodies [18]. These antibodies contribute to our understanding of CD pathogenesis and to the diagnostic procedures.

The familial nature of the CD has been documented by an increased incidence of CD in first-degree relatives of symptomatic patients [9]. The genetic predisposition of CD is reflected by the findings of “genetic contributors” to the disease - most cases of CD are associated with human leucocyte antigen (HLA) haplotype DQ2 (DQA1\*0501/DQB1\*0201), whereas HLA-DQ8 (DQA1\*0301/DQB1\*0302) is present in just a minority of patients. In addition to associations with HLA, other non-HLA regions of the genome, such as 5q31–33, as well as other non-HLA genes (*CCR3*, interleukin focus *IL2/IL21*) seem to confer some risk for coeliac disease [9,17]. The vast majority (more than 90%) of type I EATL patients have identical HLA haplotypes, as seen in patients with celiakia, the frequency in both the groups is almost identical. This association has not been identified in patients with EATL type II, as these patients show the 30-40% frequency of HLA-DQ2/- DQ8 corresponding to that found among the “healthy” Caucasian population [3,4,10,17].

The percentage of celiakia patients who might develop in the course of disease a lymphoma, differs from 0.5% in some series, to 5-10% in others (see also Figure Nr. 1). Generally, the risk of type I EATL following clinically milder (or silent) forms could be lower than in typical CD.

It has been accepted, that the only effective treatment for CD is lifelong adherence of patient to a strict gluten-free diet and this diet seems to diminish also the risk of development of many of cancers known to be associated

with CD. However, the data on the protecting role of a gluten-free diet from EATL development are not consistent. A strict adherence to gluten-free diet, at least that beginning already in childhood probably confers protection from development of lymphoma [9]. In contrast, a poor compliance with a gluten-free diet is associated with an increased risk. However at least minimal risk of secondary EATL development persists despite a serious adherence to gluten-free diet of adult CD patients [8,14]. All these discussions support the opinion that the role of immune modulation by lymphoma in patients with long-standing celiac disease may be even more complex [19]. In this association it might be mentioned, that primary intestinal T-cell lymphoma with similar features to those of EATL has been described also in patient with the recently described autoimmune enteropathy, arising in young children and/or in adults [18].

In many patients with EATL a deterioration of a refractory form of CD (RCD) has been observed, either with or without ulceration [12,20]. RCD is defined clinically either as a recurrence of CD symptoms after a former period of response to gluten-free diet, or deterioration verified clinically and/or on biopsies despite a strict gluten-free diet [4]. However, two variants of RCD are recognized and they show different association with EATL development. The distinction depends on the absence or presence of IEL with an aberrant phenotype; the phenotype is characterized by both pheno- and genotypic analyses. In RCD-I, the autoinflammatory immune response is already gluten independent [3]. The intestinal biopsies of the RCD-I patients show less than 20% IEL with a “normal” phenotype (identical to that of CD IEL - usually surface and cytoplasmic CD3+, CD5-, CD8+ and CD103+) and polyclonal T-cell receptor profile; this condition only rarely progress to EATL [3,7]. In contrast, in patients with RCD-II the biopsies contain increased, generally more than 20% (and not rarely more than 40%) of IEL, they express an aberrant phenotype due to downregulation of CD3 and especially of CD8 antigen similar to that of lymphocytes in mucosa adjacent to EATL: they show surface CD3- but cytoplasmic CD3+, CD8- and CD103+. They share the same monoclonal T-cell rearrangement as that of lymphocytes found in enteropathic mucosa adjacent to EATL; in addition they show partial trisomy of 1q (strongly associated with gluten resistance in CD) or 5q as they carry 1q chromosomal gains and 5q resp. in common with EATL type I [2,3,7,10,15]. The risk of EATL development is substantially increased. All these findings allow to suggest that phenotypically aberrant and clonal IEL of RCD-II constitute a neoplastic population. This may represent smoldering premalignant “praelymphomatous” (“praecancerous”) state. In agreement with a concept of other “lymphomas in situ”, e.g. follicular or mantle-cell lymphoma in situ, this state has been recently designated by various synonymous names: “*intraepithelial EATL*”, or “*cryptic/intraepithelial cryptic T-cell lymphoma*”, or “*EATL in situ*” [3,9,10]. The phenotypically aberrant lymphocytes are present throughout the whole GI tract and may cause formation of multiple mucosal ulcers, representing a morphological substrate of clinical condition called “ulcerative jejunitis”. Approx. 40-50% of such patients will develop clinically manifest type I EATL [4,12] – see also Fig. Nr.1. These pheno- and

genotypically atypical lymphocytes may occasionally involve also skin [4].

In the daily routine clinical practice, the EATL in patient with no previous history of RCD is referred as *de novo EATL*, and when there is such a history, it is designated *secondary EATL* [14]. That in practice means, that type I EATL patients are diagnosed [2,10,14]:

a).either have a history of previous CD - of childhood or adult onset CD, the history of long-standing malabsorption or celiac disease is present in approx. 50% of patients and averages 8-10 years, in some patients it is much shorter. Many of them often become resistant to gluten-free diet and develop overt lymphoma. In a proportion of these patients, there is a prodromal period of refractory CD accompanied by intestinal ulceration – s.c. ulcerative jejunitis (see further),

b).or having CD diagnosed simultaneously with detection of lymphoma during the same clinical and diagnostic episode, the diagnosis of CD is established simultaneously based on biopsy findings, celiakia serology and documentation of HLA genotypes characteristic of celiac disease. Many of these patients showing histologic evidence of enteropathy have probably subclinical CD, or are clinically asymptomatic,

c).or very rarely the enteropathy is not found, but the presence of antibodies to gliadin or endomysium or typical HLA genotypes characteristic of CD may be found.

In contrast, type II EATL usually occurs as *de novo* tumor without a history of celiac disease and the IEL in the type II represent rather a precursor lesion of lymphoma than a manifestation of CD [13,21,22].

In patients with type II EATL type II evidence of CD is missing and pathogenetical link of lymphoma to CD is usually absent. In a detailed recent study of Asian type II EATL enteropathy of other than CD origin was considered to represent a precursor lesion of type II EATL [23]. This was enteropathy of duodenitis type possibly caused by *Helicobacter pylori* infection and/or by ulcerative colitis. In this series of 26 type II EATL Japanese patients, the enteropathy was present in approximately half of the type II cases. The lymphomagenesis in these patients seems to be using activation of *c-MET*, *MAPK*, and *c-MYC* pathways in IEL. [13,23]. Similar to the already more detailed understood multistep developmental process from CD to type I EATL, an overt type II EATL lymphoma might represent a final step in a process of progression from IEL precursors.

### 3. EATL Type I and Type II - Incidence and Prevalence

Although it is an uncommon neoplasia, the EATL represents the most common T-cell primary intestinal lymphoma. The differences in the pathogenesis of type I and II EATL are reflected also by geographical different distributions of both types. Type I EATL is more prevalent among Caucasians of European descent and, in particular, among those from the United Kingdom, in individuals of Welsh and Irish descent, who share HLA haplotypes that confer an increased risk of gliadin allergy and susceptibility to gluten-sensitive enteropathy [10,14]. It represents a rare disease with an incidence of 0.10 per 100,000 inhabitants per year, accounting for approximately

80% of EATL in Europeans and its prevalence seems to be similar to that of CD [10,24]. It is rare to absent among Asians. In the regions, in which CD is frequent, it accounts for approx. 1.2% of all non-Hodgkin lymphomas and for approximately 6-9% of all peripheral T-cell and natural killer cell lymphomas [14]. In type I EATL, only a small proportion of patients have a history of childhood onset CD and most show adult onset of celiac disease [10]. The mean or median age of patients with type I EATL is therefore 50-70 years, within a wide range of 20 to 80 years, although it is unusual for this lymphoma to appear before 40 years of age. The same is true for the children and young adolescent [2,14,25]. According to some population-based studies it seems to be more prevalent in males, although uncomplicated celiac disease is more frequent in female patients [24]. In opposite, other data reported equal gender distribution of the type I EATL [4].

The prevalence of type II EATL shows a world wide geographic distribution and it is encountered as predominant or exclusive EATL typically in East-Asian countries patients (e.g. China, Taiwan, Japan, etc.). These countries are considered as non-endemic area for CD, as the celiac disease is there very rare [22,26,27,28]. In the countries with high incidence and prevalence of CD, the type II accounts for 10-20% of all EATL. Patients are middle-aged and older adults with a median age of 50-60 years and the male-to-female ratio is approximately 3.1 [14]. It seems to be plausible that factors influencing the higher incidence of T-cell and NK-cell lymphomas include racial predisposition, as also many other T-cell lymphomas are much more common in Asians than in other races.

## 4. Type I EATL

### 4.1. Site of Origin, Gross Appearance and Clinical Manifestation

In a vast majority of cases, the lymphoma is confined to abdomen at the time of presentation. Although histologic changes of CD are most prominent in the duodenum, the most common *site of origin* of EATL type I is the jejunum, followed by ileal localisation, or both in patients with multifocal manifestation. In contrast to absence of peripheral lymphadenopathy, the regional mesenteric lymph nodes may be infiltrated and therefore enlarged as well. Very rarely the lymphoma may arise in other parts of the gastrointestinal tract as duodenal, gastric and large bowel lymphoma, or even in extragastrointestinal localisations [4,7,10,25]. The extragastrointestinal manifestation has been exceptionally reported in the liver, spleen, thyroid, skin, nasal sinuses, and even brain [7,20], but one has to keep in mind a rigid adherence to a proper EATL definition and its distinction from other lymphoma entities. However, the staging procedures of patients with type I EATL sometimes reveal spread to liver, spleen and, rarely to the bone marrow [2,14].

*Macroscopic appearance* changes with the lymphoma progression. Infiltration starts in the mucosa, at the beginning it may be patchy, later on it is associated with ulcerations or strictures, especially often in the jejunal localization. The ulcers may be circumferential and be misdiagnosed as “benign ulcers” of “ulcerative jejunitis

/jejunoileitis (see further)” of patients developing type I EATL [2]. By progression, the growth is usually endophytical and takes form of plaques, or nodules with central necrotic exulceration and formation of fissures [10]. Less commonly the tumor forms large exophytically growing and into the lumen protruding polypoid masses. In the advanced cases, the infiltration is transmural and may result in perforation of the bowel.

The *clinical manifestation* is closely related to CD manifestation (already discussed in details), including weight loss and diarrhoe, as well as to the clinical stage of the tumor [25]. Many patients, some data refer that at least ½ of them, present with various complications of an “abdominal tumor” in the absence of previous CD diagnosis. The complications are often recognized during the clinical episode of an acute abdominal emergency with severe pain caused by obstructive ileus, severe intestinal bleeding and/or perforation and enterocolic fistulas [2,3,10].

## 4.2. Histology, Phenotype and Genetics

The *cytological appearance* of the *neoplastic cells* of type I EATL is not uniform and may be variable from patient to patient and also within any single case [1]. It forms a spectrum from more commonly reported pleomorphic appearance including possible formation of large and bizarre multinucleated cells up to relatively monotonous infiltrate of medium-sized to large cells (with round or irregular vesicular nuclei and prominent basophilic nucleoli), sometimes resembling immunoblasts. But the cytoplasm is pale and eosinophilic, the larger is the cell the more abundant is the cytoplasm. The tumor cells show high mitotic activity independently on their size, in cases with marked pleomorphism may show morphology close to that of anaplastic large cell lymphoma. Growth of the tumor is diffuse and within the infiltrate rests of the surface epithelium and crypts infiltrated by neoplastic cells may be visible. These are resembling “lymphoepithelial lesions” of MALT lymphoma [25], but reactive lymphoid follicles are not present. The tumor contains usually variable admixture of intermingled *inflammatory cells* of histiocytes and especially of eosinophils. The more advanced inflammatory intratumoral reaction is usually associated with more extensive necrosis [10]. In addition, some cases show granuloma formation mimicking Crohn’s disease.

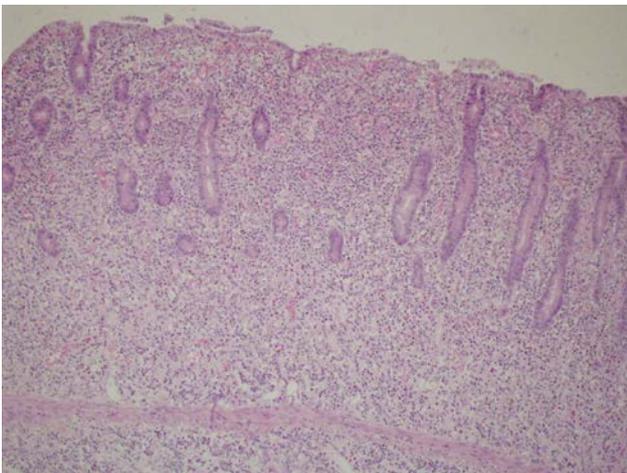


Figure 3. Type I EATL showing villous atrophy

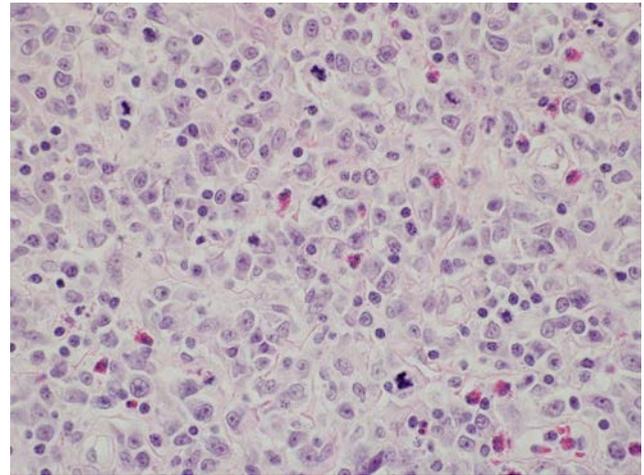


Figure 4. Type I EATL: pleomorphic neoplastic cells showing high mitotic activity and admixture of eosinophils

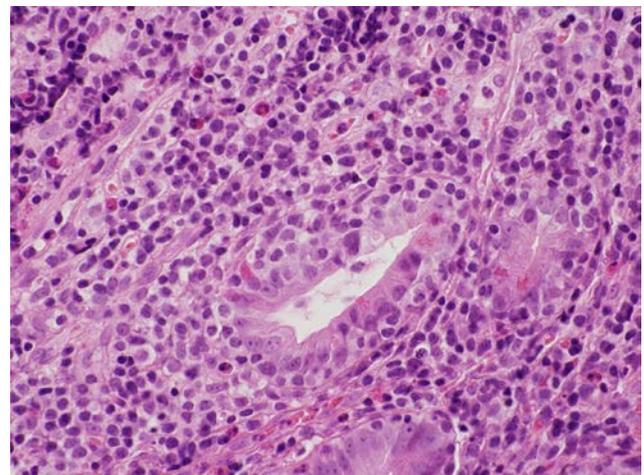


Figure 5. Type I EATL neoplastic cells infiltrating residual mucosal epithelial structures

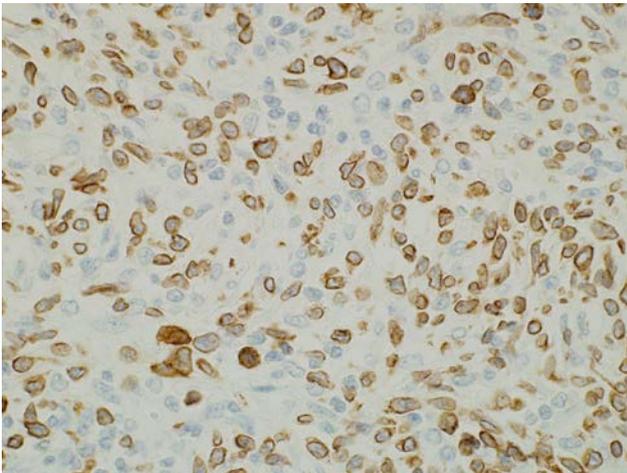
The *intestinal mucosa* away from and/or adjacent to tumor shows various CD enteropathic changes: villous atrophy, hyperplasia of crypts, increased lymphocytes and plasma cells in the lamina propria and intraepithelial lymphocytosis, both of cryptal and surface epithelium.

The IEL are small and their morphology is different from that of neoplastic cells, although phenotypically (see further) they are closely related and the destruction of the epithelial structures might be related to their cytotoxic nature [14]. In the cases with striking increase of intraepithelial lymphocytes, these obscure the mucosal epithelial cells and spill over into the lamina propria. The degree of all these enteropathic changes is individually variable, they are maximal (as usual in CD) in jejunal tumors. Because CD changes usually improve distally, the findings in mucosa adjacent to lymphoma arising in the more distal ileal may be minimal [1].

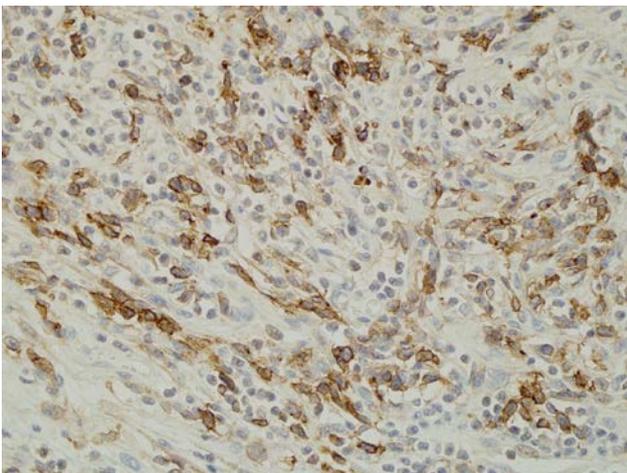
When the enlarged *lymph nodes* are examined, they show mostly histologically identifiable partial either intrasinusoidal and/or subcapsular and paracortical infiltration by neoplastic cells, sometimes associated with areas of confluent coagulative necrosis. In contrast, the nodal involvement may be histologically inapparent but recognizable by immunohistochemistry. However, the enlarged lymph nodes without neoplastic infiltration may show non-specific reactive lymphoproliferative changes,

including cystic changes containing clear serous fluid (s.c. cavitation - [14]).

The “typical” EATL type I *morphology* may be *altered* and cause diagnostic problems due to following reasons. In some cases the admixed inflammatory cells may be so pronounced as to obscure the neoplastic population, which is difficult to recognize without immunohistochemical analyses. In addition, the morphology in cases with repeated CD exacerbations with ulcerations (s.c. benign ulcers of ulcerative jejunitis) followed by remissions with healing CD before the clinical manifestation of lymphoma may be altered due to scarring and distortion of mucosal architecture [1,29]. One has also to be aware, that a precise IHC analysis of CD30 expression might contribute to recognize histologically “invisible” neoplastic cells, not only in apparently uninvolved lymph nodes but within “benign” ulcers as well [1].



**Figure 6.** Type I EATL neoplastic cells showing surface and cytoplasmic CD3 positivity



**Figure 7.** Numerous neoplastic cell of type I EATL express CD30 positivity

*Phenotype of the neoplastic cells* (determined by flow cytometry or immunohistochemistry) is slightly variable: they express leucocyte common antigen (CD45), T-cell antigens CD2, CD7 and CD3 (surface and/or cytoplasmic CD3 positivity), but lack CD5 and usually also CD4 and CD8 antigens (although sometimes slight to moderate CD8 positivity may be demonstrated). The neoplastic cells express cytotoxic phenotype - positivity of cytotoxic granule-associated proteins (TIA-1, granzyme B, perforin,

etc.), and are usually TCR- $\alpha\beta$  positive [3,10]. Various proportion of neoplastic cells, especially those of larger size express positivity of CD30, the positivity in a majority of the cases is detected in more than 50% of the tumor cells, what is of interest due to the development of new therapeutic strategies [30].

The very high proliferation activity of the tumor cells can be proved by using Ki-67 proliferation marker. If the unfixed tumor tissue is available for IHC analysis, positivity of CD103 (integrin, seu intraepithelial homing integrin or HML-1 - human mucosal lymphocyte antigen 1) may be detected [1,4]. Very recently also relatively low percentual positivity (less than 40%) of tumor cells was described for IHC expression of MATK antigen (“megakaryocyte-associated tyrosine kinase”); the antigen is expressed in much higher percentage in the cells of EATL type II [3,31]. The small *intraepithelial lymphocytes* in areas of enteropathic CD-related changes show an abnormal phenotype identical to that of lymphoma cells, they are usually CD3+, CD5-, CD8-, CD4- [10].

**Genetics:** The vast majority of patients with type I EATL, more than 90%, have the human leucocyte antigen (HLA) haplotype HLA DQA1\*0501/DQB1\*0201 (as in CD patients - [4,17]). Similar to insufficient knowledge of molecular pathogenesis of majority of peripheral T-cell lymphomas, also the tumor cells of type I EATL, do not show any specific chromosomal translocations, which could serve as a diagnostic marker of the disease. But studies of clonality have proved a clonal rearrangement of TCR- $\beta$  and TCR- $\gamma$  genes and comparative genomic hybridization studies have shown recurrent characteristic gains and losses, providing useful ancillary data for its diagnosis. They include for type I EATL characteristically frequent gains in 1q (1q21-23 and 1q32.2-q41 resp.) and in 5q34-q3, both are rare in type II). Common complex segmental amplifications of 9q31 or alternatively deletions in 16q12.1 may be identified, but these are prevalent in both EATL types. In addition, less frequent (appearing in approx. up to ¼ of cases) are e.g. chromosomal gains of 7q, losses at chromosomes 8p, 13q21, and 9p21 [3,10,21].

## 5. Type II EATL

### 5.1. Site of Origin, Gross Appearance and Clinical Manifestation

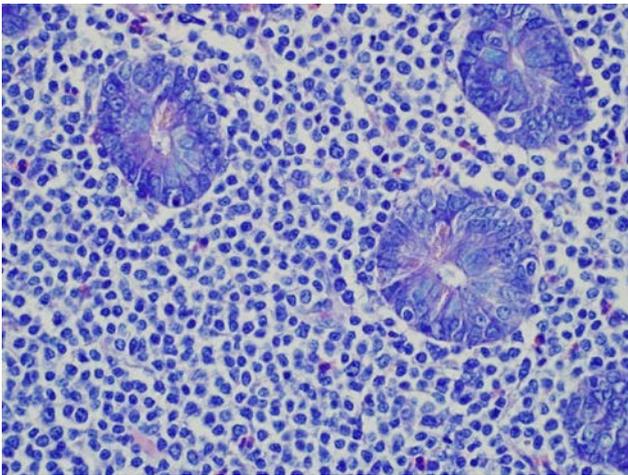
The most common *site of origin* of type II EATL is the small intestine, especially the jejunal and ileal parts, although isolated involvement of other gastrointestinal areas can be seen. The tumor is often multifocal and the regional mesenteric lymph nodes are frequently infiltrated and enlarged. By progression, a frequent secondary extragastrointestinal pulmonary involvement has been observed, at least in the cohort of Japanese patients [26]. Type II EATL may also arise uncommonly in other parts of the gastrointestinal tract as duodenal, gastric, or large intestinal lymphoma [4,23].

*Macroscopic appearance* of type II EATL is most often represented by single or multiple tumor masses, showing superficial ulceration, or transmural infiltration eventually resulting in perforation of the bowel.

The *clinical manifestation* of type II EATL is similar to that of type I and almost all patients complain of pain. The patients present usually with diarrhoea, hypoproteinemia, weight loss, and abdominal discomfort, or with clinical episodes of acute abdominal events due to intestinal obstruction and/or perforation, eventually associated with severe haemorrhage [23,25].

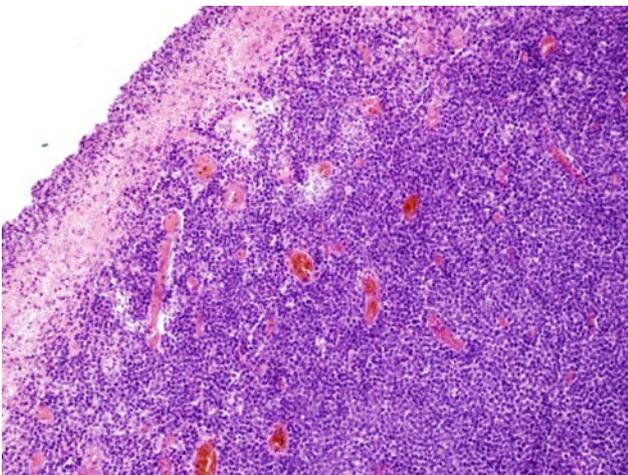
## 5.2. Histology, Phenotype and Genetics

In contrast to the type I, the *cytological appearance* of the *tumor cells* is almost always uniform, the small to medium-sized cells do not show tendency to pleomorphism. The neoplastic cells contain relatively monotonous round and darkly basophilic nuclei and narrow rim of pale cytoplasm. The tumors, according to the Asian observations (from China, Taiwan, and Japan - [22,23,26,27,28]) could be divided into three zones: a) central tumor zone composed of the monomorphic tumor cells, b) peripheral zone showing spread of tumor cells into the adjacent mucosa with marked epitheliotropism, but the residua of epithelial mucosal structure are still recognizable, and similar to type I, c) distant mucosa with cytologically normal IEL.



**Figure 8.** Type II EATL: monomorphic small to medium-sized neoplastic cells with few intermingled dispersed eosinophils

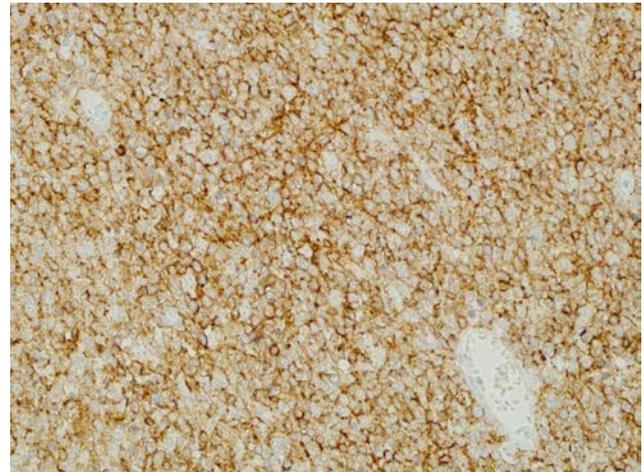
The growth is diffuse. In contrast to type I, an admixture of inflammatory cells is usually absent, the same is true for necrosis, with exception of that in the sites of ulcerations [25].



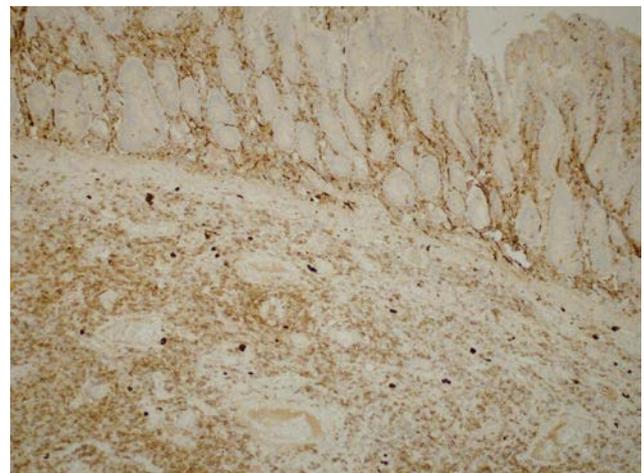
**Figure 9.** Superficial mucosal exulceration in the type II EATL

The descriptions of changes in *intestinal mucosa away from and/or adjacent* to tumor in EATL type II cases are inconsistent. Per definition, the CD enteropathic changes are not present in small intestinal mucosa at distant sites from the tumor. However, the mucosa adjacent to the tumor in its peripheral part may feature mild enteropathic changes, which have been described by various reports as “villous blunting up to atrophy and crypt hyperplasia” [10,14], or “stunted villi” [13]. This zone shows commonly a significant increase in reactive small IEL with or without villous atrophy to various extent inside and outside the IEL zone in 50 and 18% of cases respectively [23]. The heterogeneity of the reported mucosal changes require further studies respecting populational differences, as they might reflect different and today probably till insufficiently understood causes of other than CD enteropathy in the background of type II EATL.

In contrast to the EATL type I, the *neoplastic cells of type II* express distinctive *phenotype* as they express positivity of both CD8 and CD56 antigens and curiously frequent aberrant expression of CD20 in almost ¼ of cases [3,13].

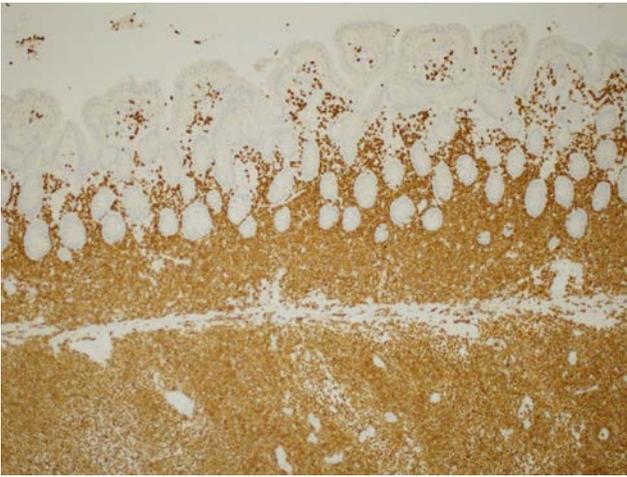


**Figure 10.** CD8 positivity of neoplastic cells of type II EATL



**Figure 11.** CD56 positivity of neoplastic cells of type II EATL

However, they show similarities to those of type I by using T-cell markers (CD2+, CD7+, but CD4- and CD5-) and by expression of cytotoxic granule-associated proteins [10].



**Figure 12.** CD2 positivity of neoplastic cells of type II EATL

The expression of CD30 is less common than in type I [3,14]. In addition, the type II tumor cells express immunohistochemically c-MET, c-MYC and MEK proteins [23], and recently, the IHC expression of MATK antigen has been documented as a novel marker of type II EATL. As already mentioned, it has been reported to be expressed in more than 80% of the type II tumor cells (and in less than 40% type I cells - [3,13,31].

The TCR immunohistochemical expression is heterogenous: while in reports from large multinational studies [32] as well as from Hong-Kong [22] most cases of type II expressed T-cell receptors  $\gamma\delta$ , and only few  $\alpha\beta$ , in a recent study of Japanese patient the TCR expression was silent or not evaluable in 40%, and in the remainder there was predominant expression of TCR- $\alpha\beta$ , over TCR- $\gamma\delta$  [13]. The phenotypic heterogeneity concerns also small *intraepithelial lymphocytes* in areas distant from the tumor, as they have been reported as either concordant or discordant with that of the type II tumor cells in the Chinese population patients in Hong Kong [22], as well as in other Asian populations [13,26,28]. In addition, they show predominantly CD8 $\alpha\alpha$  homodimer expression in  $\frac{3}{4}$  of cases [13].

**Genetics:** In the neoplastic EATL type II cells frequent characteristic gains of 8q24 chromosome allowing detection of *MYC* (8q24) gene amplification have been demonstrated, the amplification is rare in type I [25,33]. FISH analyses can be used also to prove gain of chromosomal loci 7q31 of c-MET gene, both these FISH findings are reflected by immunohistochemical expression of corresponding proteins, as summarized above [23]. In contrast, the gains of 1q and/or of 5q so common in type I, are rarely detected in type II. As already discussed above, the neoplastic cells of both types show with similar frequency the complex gains of 9q, or alternatively 16q losses. It has to be mentioned, that these findings have been previously referred to by some authors, e.g. Isacson et al. [10], as to form a common genetic link between the two types of EATL.

## 6. Differential Diagnosis of Type I and II EATL

The mutual distinction of both EATL types is relatively easy when the patient's clinical status is known and the

CD enteropathy is confirmed histologically, or by other laboratory tests. In addition, many morphological and phenotypical parameters of the tumor cells of both types are rather characteristic and mutually exclusive. However, in the daily practice the diagnosis of RCD and differential diagnosis of RCD types versus EATL type I respectively is challenging and requires an integrated multidisciplinary approach. Follow-up and continual monitoring of both immunophenotype and clonality of IELs is more important than one single analysis for RCD diagnosis, and could provide a useful tool for surveillance of patients at risk of EATL [34]. The demonstration of an aberrant phenotype is easier by support of flow-cytometric analyses (requiring unfixed fresh material) and/or double-stained IHC, which allow demonstration of population of CD3+/CD8- IEL above 40%. When this number is increasing during repeated examinations, or persistent at the level of more than 80% and this is supported by demonstration of monoclonality (following world-wide accepted BIOMED 2 protocols), then this represents a strong predictor of type I EATL type I [34]. For these considerations the interpretation of conventional single IHC is more challenging and may be inadequate [4].

In the absence of enteropathy, the pathologist has to consider a possibility of other T-cell lymphomas, which might manifest as primary intestinal lymphoma, especially the anaplastic large cell lymphoma and peripheral T-cell lymphoma, NOS and to follow the guidelines of the WHO lymphoma classification.

The differential diagnosis of type II EATL requires, especially in the Asian but in Caucasian population as well, a precise distinction from the EBV positive NK-cell lymphoma of the nasal type manifesting as intestinal lymphoma [26,27,28], including recently described very rare condition of "indolent NK-cell and T-cell lymphoproliferative disease of the gastrointestinal tract" [3,11]. In the case of T-cell lymphoma of the nasal type, the germline configuration of TCR and especially the positive EBER expression are mandatory for the diagnosis [7].

## 7. Prognosis of the Patients with EATL

Due to the lack of randomized clinical trials, no validated therapeutical strategies are available for the EATL patients and the treatment modalities include surgery and multiagent chemotherapy, mostly anthracyclin-based combination therapy and/or autologous stem cell transplantation. In spite of that, the median overall survival of type I patients is reported in the interval less than 10 months - less than 2 years, while 5 year survival of patients with type I EATL is less than 20% (see also Figure 1) - [3,4,25,30]. The treatment is often complicated by the poor patient's status reflecting the severity of long-standing CD and has to be discontinued [3,4]. Very rarely a long-term survival has been reported in a few cases with localized disease and in patients, that were successfully resected and/or after the bone marrow transplantation [2,3]. Therefore the recent data using the anti-CD30 targeted therapy, although based on single reports, are promising [30].

The clinical course and response to therapy of patients with type II EATL are similar to those of type I EATL,

and the prognosis is equally poor [4,14]. However, the patients with early clinical stages have significantly better prognosis than the patients with advanced disease [23].

## 8. Conclusion

Both types of EATL represent a primary intestinal lymphoma most often arising in the small intestine and rarely in other gastrointestinal localisations. Type I EATL seems to represent an unique lymphoma entity, well defined by a multiparametric approach including its clinical manifestation, morphology, phenotype and genotypical characteristics. The association of celiakia with type I EATL allows to assume a multistep process of the lymphomagenesis starting from IEL and resulting to an overt lymphoma. In contrast, the type II EATL (according to the WHO lymphoma classification) seu monomorphic CD56+ intestinal lymphoma (according to the WHO classification of the digestive tract tumors) seems to represent a different entity. It is to be expected, that in a short future it will be separated from the type I and probably also posted from the EATL category and renamed. Its lymphomagenesis apparently uses different pathways than that of type I and its relation to other than CD enteropathy remains to be clarified. The pathologists, as members of a multidisciplinary diagnostic team, are responsible for a correct biopsy diagnosis of type I and type II EATL and their precursor lesions, in spite of all the limitations inherent to biopsy sampling [3]. The better understanding of the lymphomagenesis of both the types will help to improve the treatment modalities and in the future also the poor outcome of the involved patients.

## Acknowledgement

Supported by Grant of Ministry of Health of the Slovak Rep. "2012/24-UKMA-1", and by projects of "Center of excellence CEPR II" (ITMS code 26220120036) at the Comenius University in Bratislava, Jessenius Medical Faculty in Martin, Slovakia, which are co-funded from EU sources.

## List of Abbreviations

EATL: enteropathy-associated T-cell lymphoma  
 IPSID: immunoproliferative small intestinal disease  
 MALT: mucosa-associated lymphoid tissue  
 CD: celiac disease  
 RCD: refractory celiac disease  
 WHO: World Health Organization  
 IEL: intraepithelial lymphocytes

## Statement of Competing Interest

Nothing to declare.

## References

- [1] Isaacson, P.G., Norton, A.J, *Malignant lymphomas of the gastrointestinal tract*, Churchill Livingstone, Edinburg, 1994, 15-66.
- [2] Jain, D, *Lymphoproliferative disorders of gastrointestinal tract*. Walters Klewer, Philadelphia, 2014, 123-189.
- [3] Foukas, P.G., de Leval L, Recent advances in intestinal lymphomas, *Histopathology*, 66. 112-136. 2015.
- [4] Du, M.-Q., Wotherspoon, A.C, *Gastrointestinal lymphomas and lymphoid hyperplasias*, Lippincott William&Wilkins, a Wolters Kluwer business. Philadelphia, 2014, 785-813.
- [5] Fairley, N.H., Mackie, F.P.: Clinical and biochemical syndrome in lymphadenoma. *Br Med J*, 1. 375-404. 1937.
- [6] O'Farely, C., Feighery, C., O'Brian, D.S., et al, Humoral response to wheat protein in patients with coeliac disease and enteropathy associated T-cell lymphoma, *Br Med J*, 293, 908-910. 1986.
- [7] Shroff, S., Ganapathi, K.A, "Enteropathy associated T-cell lymphoma: a review of diagnostic findings and therapeutic strategies", *Diagn Histopathol*, 20 (4). 151-155. 2014.
- [8] Green, P.H.R., Fleischauer, A.T., Bhagat, G., Goyal, R., Jabri, B., Neugut, A.I, "Risk of malignancy in patients with celiac disease", *Am J Med*, 115. 191-195. 2003
- [9] Robert, M.E., Gibson, J.A, *Inflammatory disorders of the small intestine*. Elsevier, Saunders, Philadelphia, 2015, 402-435.
- [10] Isaacson, P.G., Chott, A., Ott, G., Stein, H, *Enteropathy-associated T-cell lymphoma*, IARC, Lyon, 2008, 289-291.
- [11] Swerdlow, S.H., Jaffe, E.S., Brousset, P., Chan, J.K., de Leval, L., Gaulard, Ph., Harris, N.L., Pileri, S., Weiss, L.M., on behalf of the International Lymphoma Study Group, Cytotoxic T-cell and NK-cell lymphomas: current questions and controversies, *Am J Surg Pathol*, 38. 60-71. 2014.
- [12] Müller-Hermelink, H.K., Delabie, J., Ko, Y.H., Jaffe, E.S., Nakamura, S, *T-cell lymphoma of the small intestine*, IARC, Lyon, 2010, 112-114.
- [13] Tan, S.Y., Chuang, S.S., Tang, T., Tan, L., Ko, Y.H., Chuah, K.L., Ng, Chng, W.J., Gatter, K., Loong, F., Liu, Y.H., Hosking, P., Cheah, P.L., Teh, B.T., Tay, K., Koh, M., Lim, S.T, Type II EATL (epitheliotropic intestinal T-cell lymphoma): a neoplasm of intraepithelial T-cells with predominant CD8 $\alpha$  phenotyp, *Leukemia*, 27 (8). 1688-1696. 2013.
- [14] Ferry, J.A, *Hematolymphoid tumors of the gastrointestinal tract, hepatobiliary tract and Pancreas*, Elsevier, Saunders, Philadelphia, 2015, 846-885.
- [15] Cellier, C., Delabesse, E., Helmer, C., Patey, N., Matuchansky, C., Jabri, B., Macintyre, E., Cerf-Bensussan, N., Brousse, N, "Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma". French Coeliac Disease Study Group, *Lancet*, 356 (9225). 203-208. Jul 2000.
- [16] Makovický, P., Makovický, P., Greguš, M., Klimik, M., Zimmermann, M, Histopathological Diagnosis of Celiac Disease in Adults with Functional Dyspeptic Syndrome (In Slovak). *Čes.-slov. Patol.*, 44 (1). 16-19. 2008.
- [17] Dickson B.C., Streutker, C.J., Chetty, R.: "Coeliac disease: an update for pathologists", *J Clin Pathol* 59. 1008-1016. 2006.
- [18] Greenson, J.K., Jain, D, *Small bowel mucosal disease*. Walters Klewer, Philadelphia, 2014, 929-982.
- [19] Mühr-Wilkenshoff, F., Friedrich, M., Foss, H.D., Hummel, M., Zeitz, M., Daum, S, "Immune modulation by non-hodgkin lymphoma in a patient with two primary intestinal T-cell lymphomas and long-standing celiac disease", *Digestion*, 81(4). 231-234. 2010.
- [20] Catassi, C., Bearzi, I., Holmes, G. K, "Association of celiac disease and intestinal lymphomas and other cancers", *Gastroenterology*, 128 (4 Suppl. 1). S79-86. Apr 2005.
- [21] Zettl, A., deLeeuw, R., Haralambieva, E., Mueller-Hermelink, H.-K, "Enteropathy-type T-cell lymphoma", *Am J Clin Pathol* 127. 701-706. 2007.
- [22] Chan, J. K.C., Chan, A. C.L., Cheuk, W., Wan, S.-K., Lee, W.-K., Lui, Y.-H., Chan, W.-K, "Type II Enteropathy-Associated T-Cell Lymphoma: A Distinct Aggressive Lymphoma With Frequent  $\gamma\delta$  T-cell Receptor Expression", *Am J Surg Pathol*, 35 (10). 1557-1569. Oct 2011.
- [23] Kikuma, K., Yamada, K., Nakamura, S., Ogami, A., Nimura, S., Hirahashi, M., Yonemasu, H., Urabe, S., Naito, S., Matsuki, Y., Sadahira, Y., Takeshita, M, "Detailed clinicopathological characteristics and possible lymphomagenesis of type II intestinal enteropathy-associated T-cell lymphoma in Japan", *Human Pathology*.
- [24] Wieke, H.M., Verbeek, W.H.M., Van De Water, J.M.W., Al-Toma, A., Oudejans, J.J., Chris J. J. Mulder, C.J.J., Veerle M. H, Coupé Incidence of enteropathy - associated T-cell lymphoma: A

- nation-wide study of a population-based registry in The Netherlands", *Scandinavian J Gastroenterol* 43 (11). 1322-1328. 2008.
- [25] Macon, W.R, *Peripheral T-cell lymphomas*, Elsevier Saunders, Philadelphia, 2012, 293-326.
- [26] Akiyama, T., Okino, T., Konishi, H., Wani, Y., Notohara, K., Tsukayama, C., Tsunoda, T., Tasaka, T., Masaki, Y., Sugihara, T., Sadahira, Y, "CD8+, CD56+ (natural killer-like) T-cell lymphoma involving the small intestine with no evidence of enteropathy: clinicopathology and molecular study of five Japanese patients", *Pathol Int*, 58 (10). 626-634. Oct 2008.
- [27] Chuang, S.S., Chang, S.T., Chuang, W.Y., Huang, W.T., Hsieh, P.P., Tsou, M.H., Liao, Y.L., Lin, S.H., Hsieh, Y.C., Lu, C.L., Sheu, M.J., Liu, H, "NK-cell lineage predicts poor survival in primary intestinal NK-cell and T-cell lymphomas", *Am J Surg Pathol*, 33 (8). 1230-1240. Aug 2009.
- [28] Sun, J., Lu, Z., Yang, D., Chen, J, "Primary intestinal T-cell and NK-cell lymphomas: a clinicopathological and molecular study from China focused on type II enteropathy-associated T-cell lymphoma and primary intestinal NK-cell lymphoma", *Mod Pathol*, 24 (7). 983-992. Jul 2011.
- [29] Lebwohl, B., Granath, F., Ekbohm, A., Smedby, K.E., Murray, J.A., Neugut, A.I. Green, P.H.R., Ludvigsson, J.F, "Mucosal Healing and Risk for Lymphoproliferative Malignancy in Celiac Disease: A Population-Based Cohort Study", *Ann Intern Med*. 159 (3). 169-175. 2013.
- [30] Khalaf, W.F., Meghan E. Caldwell, M.E., Reddy, N, "Brentuximab in the Treatment of CD30-Positive Enteropathy-Associated T-Cell Lymphoma", *JNCCN* 11(2). 137-140. 2013.
- [31] Tan, S.Y., Ooi, A.S., Ang, M.K., Koh, M., Wong, J.C., Dykema, K., Dykema, K., Ngeow, J., Loong, S Gatter, K., Tan, L., Lim, L.C., Furge, K., Tao, M., Lim, S.T., Loong, F., Cheah, P.L., Teh, B.T, Nuclear expression of MATK is a novel marker of type II enteropathy-associated T-cell lymphoma, *Leukemia*, 25. 555-557. 2011.
- [32] Wilson, A.L., Swerdlow, S.H., Przybylski, G.K., Surti, U., Choi, J.K., Campo, E., Trucco, M.M., Van Oss, S.B., Felgar, R.E, Intestinal gd T-cell lymphomas are most frequently of type II enteropathy-associated T-cell type, *Hum Pathol*, 44 (6). 1131-1145. 2013.
- [33] Varghese, D., Koya, H.H., Cherian, S.V., Mead K., Sharma, A., Sharma, N., Knohl, S.J., Benjamin, S, "Hemophagocytic Lymphohistiocytosis: An Uncommon Presentation of Enteropathy-Associated T-Cell Lymphoma", *J Clin Oncol* 31 (13). 226-230. May 2013.
- [34] Liu, H., Brais, R., Lavergne-Slove, A., Jeng, Q., Payne, K., Ye, H., Liu, Z., Carreras, J., Huang, Y., Bacon, C.M., Hamoudi, R.A., Save, V., Venkatraman, L., Isaacson, P.G., Woodward, J., Du, M.-Q, Continual monitoring of intraepithelial lymphocyte immunophenotype and clonality is more important than snapshot analysis in the surveillance of refractory coeliac disease. *Gut*, 59. 452-460. 2010.