Eosinophils in pathohistological reports of pediatric gastrointestinal biopsies at the University hospital of Split : a three year retrospective study

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EOSINOPHILS IN PATHOHISTOLOGICAL REPORTS OF PEDIATRIC GASTROINTESTINAL BIOPSIES AT THE UNIVERSITY HOSPITAL OF SPLIT: A THREE YEAR RETROSPECTIVE STUDY

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LIST OF ABBREVIATIONS

Anti-tTG – anti-tissue transglutaminase

5-ASA – 5-Aminosalicylates

ASCAs – anti-*Saccharomyces cerevisiae* antibodies

CD - Chrohn's disease

CRP – C-reactive protein

ECP – Eosinophilic cationic protein

EDN – Eosinophil derived neurotoxin

EGD – Esophagogastroduodenoscopy

EPO – Eosinophil peroxidase

ESR – Erythrocyte sedimentation rate

FS – Flexible sigmoidoscopy

GER – Gastroesophageal reflux

GERD – Gastroesophageal reflux disease

IBD – Inflammatory bowel disease

IL-5 – Interleukin 5

JAK – Janus kinase

LES – Lower esophageal spincter

MBP-1 – Major basic protein 1

p-ANCA – Perinuclear antineutrophil cytoplasmic antibodies

PEG – Polyethylene glycol

PPIs – Proton pump inhibitors

PSC – Primary sclerosing cholangitis

UC – Ulcerative colitis

1. INTRODUCTION

1.1 Eosinophils

1.1.1 Development of eosinophils

The two major types of leukocytes are agranulocytes and granulocytes; the granulocytes are further subdivided into neutrophils, basophils and eosinophils. They develop from hematopoietic stem cells (1). Multiple transcription factors and cytokines contribute to the differentiation from the myeloid progenitor cells through several intermediate stages to the fully matured eosinophil (2, 3). It has been shown that the major transcription factors to influence this development are C/EPB members (CCAAT/ enhancer-binding protein family), GATA-1 (belonging to zinc finger family) and PU.1 (ETs family member) (1-3). Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Interleukin-3 (IL-3) and Interleukin-5 (IL-5) are the most important cytokines which are necessary for the eosinophilic differentiation, IL-5 being the most specific one for eosinophils (1-4). This process takes part in the bone marrow, once the eosinophils leave the bone marrow, they do not further differentiate (1, 4).

The diameter of an eosinophil is in the range of 12-17 μm (2). They have a bilobular nucleus and within the cytoplasm they contain specific acidophilic granules, which when stained are responsible for their characteristic red-pink appearance (Figure 1) (2, 3). In the bloodstream eosinophils comprise about 1-3% of the present leukocytes (2). Their circulatory half-life is approximately eight to twelve hours, but after migrating into the tissues they can survive there for about eight to twelve days (2). The tissues where eosinophils are most abundant are the thymus, the lower gastrointestinal tract, the upper respiratory tract, the uterus, the spleen, the lymph nodes and the adipose tissue (2, 4, 5).

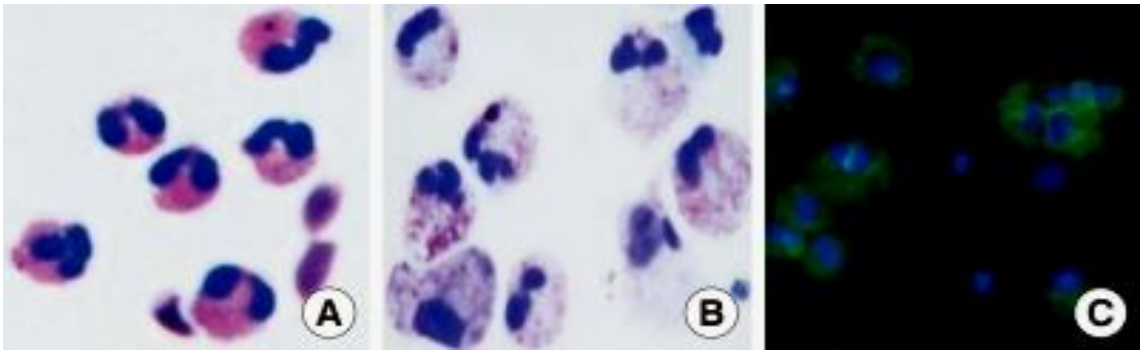

Figure 1. A: Eosinophils from peripheral blood, B and C: Eosinophils from cord blood (2).

1.1.2 Function of eosinophils

Eosinophils are part of the innate and adaptive immune system (4). They play a grand role in defending the body against parasitic infections and intracellular pathogens as well as in the mediation of hypersensitivity reactions and allergies (1, 2, 4). In response to different stimuli eosinophils migrate and adhere to the tissues where they are required (1, 4). There are numerous types of receptors for cytokines, complement proteins, immunoglobulins and toll-like receptor ligands on the surface of eosinophils through which they can be activated (1). This activation triggers degranulation of the large granules stored in the cytoplasm (1). The granules are composed of a crystalloid core made of the major basic protein (MBP-1) and a matrix containing eosinophilic cationic protein (ECP), eosinophil derived neurotoxin (EDN) and eosinophil peroxidase (EPO). MBP-1 activates basophils, neutrophils, epithelial cells, mast cells and their degranulation and also increases smooth muscle reactivity. Additionally due to its ability to increase the membrane permeability it has a cytotoxic effect on pathogens but also on host cells. EDN promotes the proliferation of T- and B-cells. It has neurotoxic properties and plays an antiviral role since it is a potent RNAse. ECP is also an RNAse, but it is weaker than EDN. It has as well neurotoxic abilities and acts cytotoxic towards pathogens and host cells. EPO exerts its effects by the generation of reactive oxygen species which are toxic to extracellular pathogens. It further possesses proinflammatory as well as anti-inflammatory capacities and it is able to activate epithelial cells (1-4). Besides degranulation eosinophils like neutrophils are capable of phagocytosis and antigen-presentation to T-cells (1, 4). Eosinophils do not only react to cytokines, they also produce cytokines and growth factors especially in an inflammatory environment (1, 3).

1.1.3 Abnormal values of eosinophils

The normal peripheral blood value for eosinophils is $<0.5x10^9$/L (6). A patient is considered to have eosinopenia if there are $<0.05x10^9$/L eosinophils in the circulating blood, which can be induced by certain drugs including beta-blockers and corticosteroids (4, 6). Other reasons known to cause decreased values of eosinophils are the exposure to stress and in some cases bacterial and viral infections (4, 6, 7). The opposite is present in the state of eosinophilia. This condition is categorized into mild eosinophilia: $0.5\text{-}1.5x10^9$/L, moderate eosinophilia, also called hypereosinophilia: 1.5-5x10^9/L and severe eosinophilia: $>5x10^9$/L (3, 4, 8). There can either be an increase of circulating eosinophils, an increase of tissue eosinophils or both simultaneously (3).

Eosinophilia is divided into primary and secondary types, the latter being the more common one (4, 7). A number of conditions are summarized under the term hypereosinophilic syndromes which belong to the primary type. They are characterized by constantly elevated numbers of eosinophils ranging between moderate to severe eosinophilia and are connected to tissue and organ damage (3). Secondary eosinophilia is used as an umbrella term for the diseases in which the eosinophil count is elevated due to an underlying cause (4, 7). Allergies, especially involving the respiratory tract and the skin, are often associated with mild to moderate eosinophilia (1, 2, 8). Asthma and allergic rhinitis usually cause mild eosinophilia, while chronic sinusitis can provoke moderate to severe elevation of the eosinophilic levels (4, 7). It has been shown that eosinophils take part in eliciting the symptoms and luminal obstruction in asthma. Drug allergies are also capable of triggering eosinophilia (4, 7). Sulfonamides and penicillins are the drugs which are responsible for most cases of drug-related eosinophilia. Usually the affected patients have no significant clinical consequences and do not require therapy (7). It is well known that protozoal and helminthic infections raise the levels of eosinophils above normal, although helminthic infections account for an exceedingly higher number of cases with eosinophilia associated to parasitic infections in comparison to protozoal infections (1, 4, 7). Severe rheumatoid arthritis and systemic lupus erythematosus are examples from a group of rheumatologic and autoimmune diseases which are connected to an elevated number of eosinophils in the peripheral blood and tissues (7). In autoimmune diseases the eosinophils are recruited to the inflammatory environment by other inflammatory cells, cytokines and chemoattractants where they contribute to the tissue damage and destruction by degranulation, activation of tissue remodeling resulting in fibrosis as well as T- and B-cell activation (3). Opposing to the organ destruction eosinophils are also

known to participate in tissue repair and angiogenesis by releasing Transforming Growth Factor α (TGF-α), Transforming Growth Factor β (TGF- β), Platelet Derived Growth Factor (PDGF) and Vascular Endothelial Growth Factor (VEGF) (4). Eosinophilia can be malignancy related as in acute and chronic eosinophilic leukemia (7). The causes of eosinophilia in myeloid and lymphoid malignancies differ from each other. In lymphoid malignancies the defective lymphoblast secretes more IL-5 than the normal lymphocyte which in turn promotes the production of eosinophils, while in myeloid malignancies a genetic lesion leads to increased differentiation in the direction of the eosinophil lineage (4).

Due to the cytotoxic properties of the content of the eosinophilic granules, longstanding eosinophilia can result in tissue damage. Consequences of permanent eosinophilia can include encephalopathy, respiratory symptoms, eosinophilic enteritis, eosinophilic cholangitis, endomyocardial damage and myocardial necrosis. After the detection of eosinophilia the clinical history of the patient is important to differentiate all the possibilities and detect the underlying cause. Parts of the diagnostic workup when investigating eosinophilia, are laboratory and allergy testing, taking biopsies if indicated and conducting imaging procedures (7).

1.2 Most common pediatric gastrointestinal diseases

1.2.1 Gastroesophageal reflux disease (GERD)

Gastroesophageal reflux (GER), also called regurgitation, is the retrograde passage of gastric contents from the stomach into the esophagus. It is a physiological mechanism which can occur up to multiple times a day in adults and children (9, 10). The regurgitations are caused by a lower esophageal sphincter (LES) relaxation during which the intragastric pressure is transiently higher than the LES pressure (11). If it turns into a pathological condition associated with symptoms and complications it is called gastroesophageal reflux disease (GERD) (10). Independent of high-, middle- and low-income countries the prevalence of GER in infants is high. In 50% of 0-3 month old infants GER occurs at least once a day. This number increases further to 67% for infants between 4 and 6 months. After that age the prevalence drops to 21% in 7-9 months old infants and even more to 5% once 10-12 months of age are reached (9). If GER newly occurs after the age of 18 months or continues from early infancy past that age it is considered pathological. However only 5-9% of the infants having regular regurgitations actually develop GERD. During childhood the prevalence of GERD increases from 1.8% in 3-9 year old children to 3.5% in the 10-17 year olds until it reaches 20-40% in adults (9). The symptoms GERD can elicit are divided into esophageal and extraesophageal (10, 12). If an infant presents with growth failure and signs for esophagitis

like problems with feeding and sleeping, irritability, long periods of crying and anaemia, the suspicion for GERD should be raised. The most common extraesophageal symptoms for infants are choking, wheezing and coughing episodes (9, 10). The presentation of GERD in older children and adolescents is similar to adults. The leading esophageal symptoms are heartburn, epigastric and/or chest pain, especially during the night, and regurgitations that leave a sour taste (9, 10). Extraesophageal signs include wheezing, hoarseness, a sore throat, recurring pneumonia, laryngitis and dental erosions. Alarm symptoms that require prompt investigation are the presence of bile or blood in the regurgitations, forceful vomiting, hepatosplenomegaly, seizures and choking (9, 10, 12). Some children are more prone to develop severe GERD due to predisposing conditions. Examples for these conditions are anatomical malformations like esophageal atresia with or without a tracheoesophageal fistula and neurodevelopmental disorders such as cerebral palsy and congenital myopathies (9, 13).

In the diagnostic process it is important to differentiate between GER and GERD, which particularly in infants can be difficult (9, 10). No specific test has found to be the golden standard for diagnosing GERD, it is rather a combination of properly assessing the medical history and carrying out a physical examination and after that deciding if additional diagnostic measures are needed (10). Multiple GER questionnaires according to different age groups have been developed and can be useful for symptom evaluation and distinguishing GER from GERD (9). 24h- pH-metry and multiple intraluminal impedance (MII) pH-impedance monitoring is usually used in patients who express only extraesophageal symptoms of GERD to document their reflux patterns (12). If esophagitis is suspected or the patient presents with unclassical symptoms, an endoscopy with biopsy is indicated. Dysphagia in children might point to anatomical malformations, so in these cases a barium contrast series is recommended (9). To reduce the quantity of regurgitations in infants there are a few modifications that parents can try, before pharmaceutical and/or surgical therapy come into question (10, 13). They have to receive feeding advices related to the infant's position during feeding, the possibility of thickening the feeds with rice or corn and giving smaller feeding volumes while increasing the number of feedings (14). All of these measures can lead to decreased GER. In some infants a cow's milk protein allergy can trigger GER, so a 2-4 week empirical change to a dairy free diet or a hypoallergenic formula can be tried to in- or exclude this diagnosis (11, 15). If the infant does not respond to the prior described changes, a 4-6 weeks course of acid suppression is recommended, provided that the infant is at least one year old, since for younger children proton pump inhibitors (PPIs) are not approved (Figure 2) (9).

In older children and adolescents lifestyle modifications are the first line of treatment. Weight reduction if necessary is an important factor and also the avoidance of caffeine, chocolate, alcohol and tobacco consumption (9, 10, 13). Sleeping in the left lateral decubitus position can help to decrease the nocturnal symptoms (9). Additionally a 12 week period of acid suppression is indicated, usually with PPIs which have proven more potent than histamine 2 receptor antagonists (9). Antacids can be used for symptomatic relief, but are not recommended for prolonged therapy in children. If lifestyle modifications and medical therapy fail or there are life-threatening complications of GERD, surgery is considered (12, 13). The method of choice is the Nissen fundoplication procedure during which a part of the gastric fundus is wrapped around the distal part of the esophagus to increase the pressure of the LES and thus avoid regurgitations (12). However, children who are neurologically impaired need the surgical option the most, but at the same time they are the subset of patients in which the surgery most often fails (9).

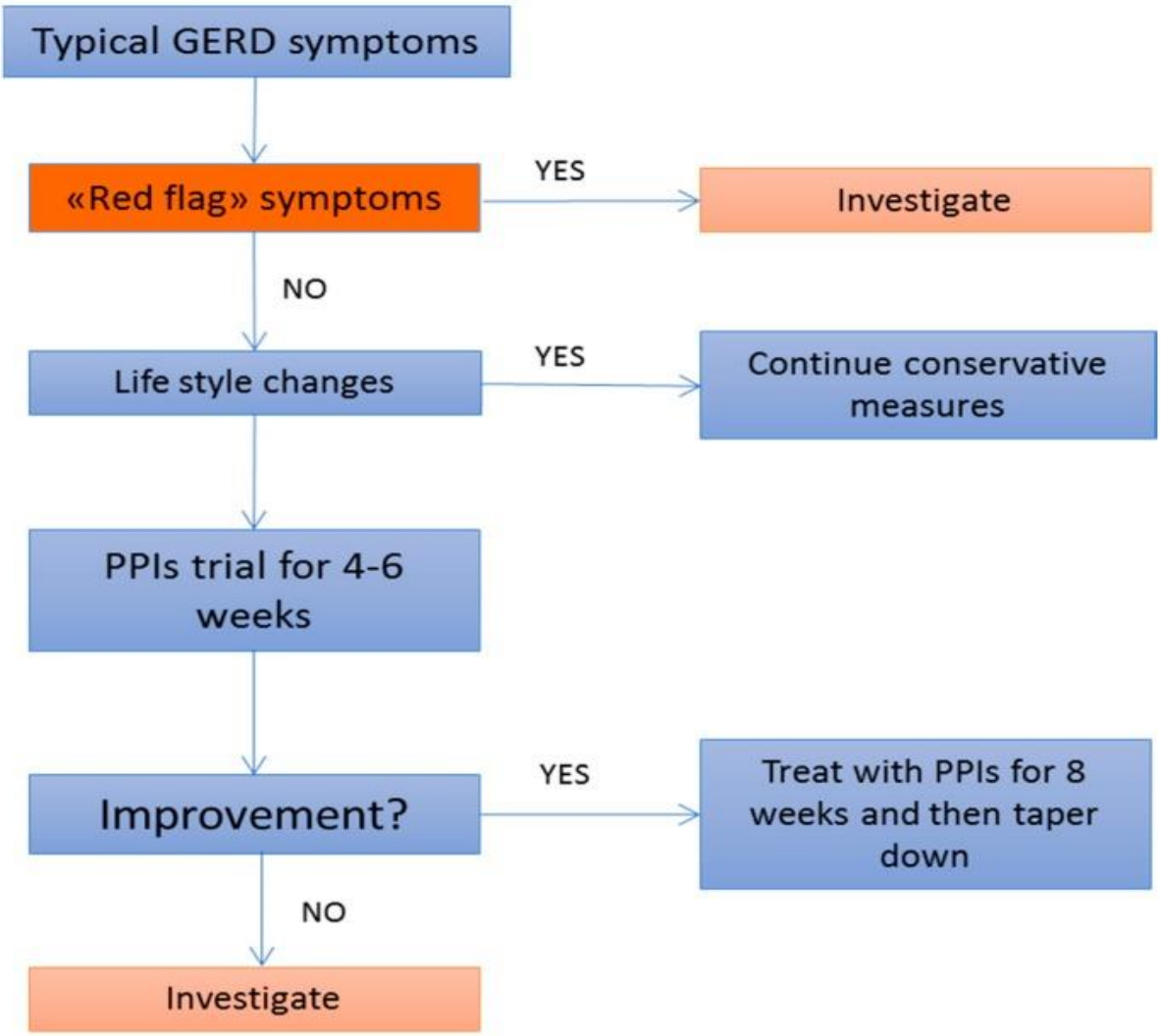

Figure 2: Clinical management of GERD in children older than 1 year (10).

PPIs: Proton pump inhibitiors

1.2.2 Eosinophilic esophagitis

Eosinophilic esophagitis, in comparison to many other diseases which have been known for a long time, is a quite recently discovered disease. Since then the interest in that field and the studies done to acquire more information about the condition is constantly increasing, but still the diagnosis in many patients is estimated to be delayed about 4-6 years (16, 17). The prevalence of the disease ranges between 10-57 in 100.000 and many studies have shown a 3:1 male predominance (16, 18, 19). Eosinophilic esophagitis is characterized by inflammation of the esophageal mucosa which is infiltrated especially by eosinophils (19). The etiology is not entirely clear but eosinophilic esophagitis is thought to be an immunologic disease which is triggered in genetically susceptible individuals by antigen sensitization, mostly from food allergens (19). The activation of the immune system leads to the production of inflammatory cytokines and the eosinophils infiltrating the mucosa degranulate and release their content which as described above contains toxic and destructive products. The consequence of this process is tissue injury and fibrosis of the esophagus over time when chronic disease develops (16). Other conditions that can lead to an increase of eosinophils in the esophageal mucosa are GERD, hypereosinophilic syndromes, Crohn´s disease, infections and connective tissue disorders (16, 19).

The leading symptoms for eosinophilic esophagitis in children as well as adults are dysphagia and esophageal food impactions (18, 19). In younger children eosinophilic esophagitis should also be suspected if they present with nausea, vomiting, refusal to eat and chest and/or abdominal pain (16, 20). Additional common symptoms in adults include atypical chest pain and heartburn which is commonly enhanced by the ingestion of alcohol and often resistant to empirical PPI therapy (17, 19). In a large proportion of the patients regardless of the age group a typical sequence of atopic manifestations can be traced back in their medical history consisting of atopic dermatitis, food allergies, asthma and allergic rhinitis, which if occurring one after another are called “atopic march” (16, 21). The diagnosis of eosinophilic esophagitis consists of a combination of the clinical picture, laboratory results, the findings during endoscopy and the correspondent biopsies and contrast radiography (19). The laboratory results of about 50% of patients show peripheral blood eosinophilia, however the specificity of that finding is compromised if other atopic diseases are involved. During endoscopy typically edema with a loss of vascular markings, esophageal rings, punctuate exudates and longitudinal furrows in the esophageal mucosa are encountered (16, 20). The histological findings demonstrate eosinophilic infiltration of the esophageal squamous

epithelium, defined by >15 eosinophils per high-power field (Figure 3) (16, 19). The ongoing inflammation eventually leads to basal cell hyperplasia and fibrosis of the lamina propria which also becomes evident in the histologic examination (19). Eosinophilic esophagitis is associated with multiple complications including narrowing of the esophagus, food impaction, esophageal strictures and perforation (20). To determine the involvement of GERD in the disease after diagnosis, the first therapeutic approach is a trial of PPIs (16, 19). In 30-50% of cases of eosophageal eosinophilia the disorder is PPI responsive and the eosinophilic infiltration of the esophageal mucosa decreases or disappears completely. If the symptoms persist regardless to PPI administration, empirical elimination diets are tried with the goal to find the potentially triggering allergen in the food, usually starting with the most common food allergies like milk, nuts, wheat, soy, seafood and eggs (19, 22). It works by the systemic elimination of either a single component, e.g. peanuts, of the diet or a small group of similar food components, e.g. all nuts, and then slowly reintroducing them into the diet to determine whether the temporary elimination has positively affected the symptoms (22). The elimination diets are often preceded by allergologic testing, aiming to already exclude certain allergens (16, 19). An alternative to the empirical elimination diets are swallowed topical glucocorticoids, while systemic glucocorticoids are reserved for severe cases that are resistant to the other aforementioned therapies and for patients with complications of the disease (19). In patients with progressive fibrostenosis with marked dysphagia, relief can be provided by esophageal dilation, initial concerns about the risk of perforation have been disproven in more recent studies (19, 22).

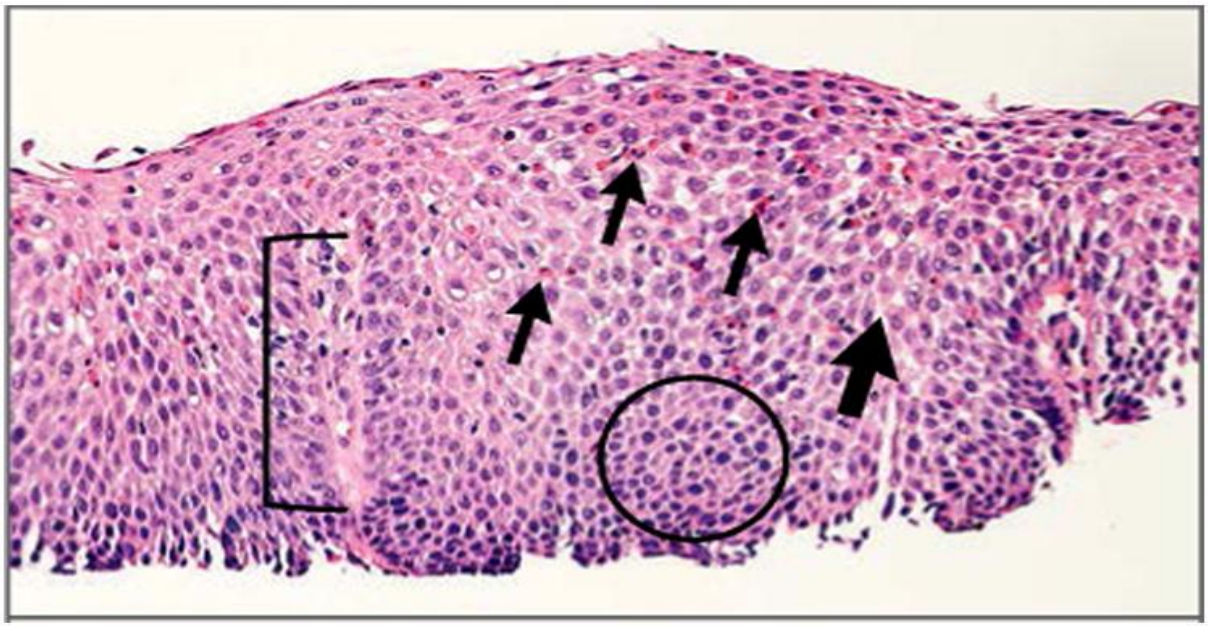

Figure 3: Mucosal eosinophilic infiltration in eosinophilic esophagitis (19).

1.2.3 Celiac disease

Celiac disease is an autoimmune disorder in which the digestion of dietary gluten triggers an immune response that leads to injury of the small intestine (23). It is also known as nontropical sprue, celiac sprue and gluten-sensitive enteropathy (24). The prevalence is reported to be 0.5-1% of the general population and the female to male ratio ranges between 2:1 and 3:1 (23, 24). There are genetic as well as immunologic and environmental factors that contribute to the development of the disease (23). People carrying the HLA class 2 haplotype DQ2 and DQ8 are generally predisposed for celiac disease (23, 24). Ingestion of gliadin, a component of gluten which is found in rye, barley and wheat, is the leading environmental factor (23). The amount of gluten which is ingested at the introduction of gluten into the diet is also important, the risk being higher the more gluten is ingested (24). Gastrointestinal infections, early introduction to antibiotics and alterations of the intestinal microbiota have also been identified as risk factors (24). The immunologic part of the disease is comprised of several autoantibodies which are detectable in the patient's serum: IgA antigliadin, antiendomysial and anti-tissue transglutaminase (anti-tTG) antibodies (23, 25). According to the different clinical pictures celiac disease has been divided into three types (23, 26). The classical type, also called intestinal type, has the highest prevalence in children younger than three years and is in general more common in children than in adults (23, 27). The leading symptom of the classical type is diarrhea, others include abdominal pain and distention, loss of appetite and failure to thrive due to the malabsorption of nutrients from the injured bowel

which can lead to anemia and decreased bone density (23, 24). The presentation in older children and adults is similar, however in that part of the population the nonclassical type is more common. The nonclassical form lacks the diarrhea and instead presents with a number of extraintestinal symptoms: In children the combination of iron deficiency anemia with a short stature is often found, while in adults there also is iron deficiency anemia but frequently together with dermatitis herpetiformis cutaneous lesions (23, 27, 28). Other extraintestinal manifestations are comprised of fatigue, decreased bone density, aphtous ulcers and headaches (23, 24). The third type is called subclinical type and usually gets detected due to screening of asymptomatic patients (23). A few other conditions are associated with a higher occurrence rate of celiac disease such as Turner's and Down's syndrome, selective IgA deficiency, diabetes mellitus and other autoimmune diseases in general (24, 28).

The golden standard, for the diagnosis of celiac disease, besides the clinical examination and evaluation, is a mucosal intestinal biopsy and the serologic status which tests for anti-tTG, deaminated gliadin peptide antibodies and anti-endomysium antibodies (23, 26). First the serologic status is taken in clinically suspicious individuals and based on the results the decision is made whether taking biopsies is necessary. When transglutamase IgA antibodies and endomysium IgA antibodies are detected together, they almost reach sensitivity and specificity of 100% for celiac disease (23). Anti-gliadin antibodies are only recommended to be taken in younger children, since their sensitivity and specificity is low in older children and adults (27). If there is a known or suspected IgA deficiency, IgG antibodies should be measured (25). Duodenal biopsies should be taken while the patient still includes gluten in his diet to get an accurate histological picture (24, 26). The biopsy usually reveals villous atrophy of the mucosa of the small intestine which is characteristic for the disease, increases crypt cell proliferation which leads to crypt hyperplasia and an increased number of intraepithelial lymphocytes as well as plasma cells (Figure 4) (25-27, 29). The severity of the mucosal changes increases from proximal to distal and is assessed with the Marsh-Oberhuber classification (27).

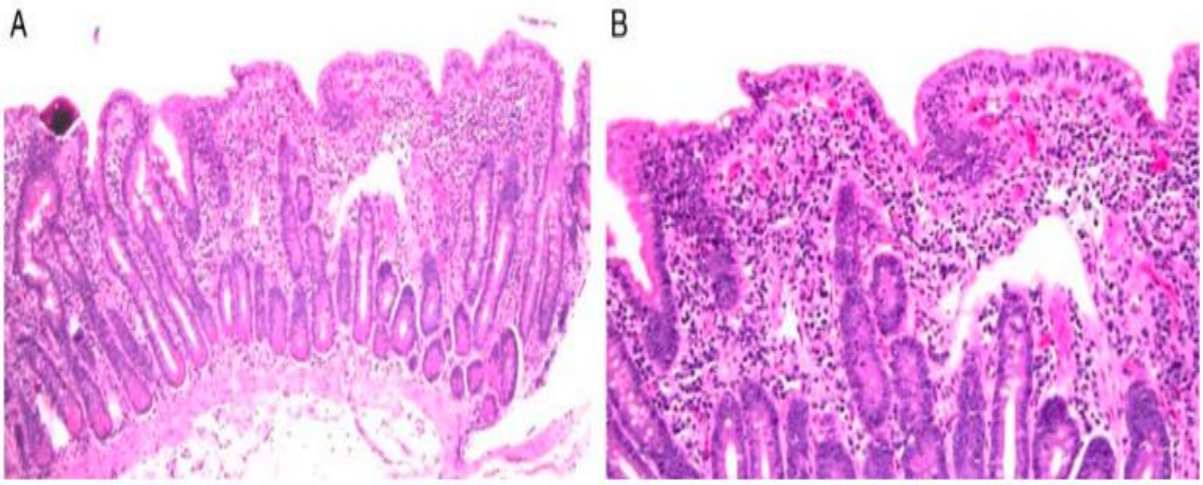

Figure 4. Characteristic pathohistology for celiac disease: A: Villous atrophy and crypt hyperplasia, B: Intraepithelial lymphocytes (29)

HLA typing is most useful for excluding the diagnosis of celiac disease; if the patient is negative for HLA DQ2 and DQ8 he will not develop the disease (25, 27). If the patient sticks to a gluten free diet, which is the therapeutic hallmark for celiac disease, the pathologic changes of the bowel wall are reversible, the villi grow back and both the intestinal and extraintestinal symptoms decrease or resolve entirely (24). Also the amount of autoantibodies decreases (23, 24, 27). The regression of symptoms, autoantibodies and histologic changes finalizes the diagnosis since the histologic picture can overlap with other conditions like eosinophilic enteritis, acid hypersecretion, Chrohn's disease, milk-protein intolerance in children and bacterial overgrowth (23, 25). Regular check-ups on the clinical presentation and the serologic status are recommended for monitoring the course of celiac disease, during which many patients experience relapses and spontaneous remissions (23, 24). Complications that can occur involve the failure to respond to gluten restriction, which is called "refractory celiac disease" or ulcerations of the injured intestinal tissue (23). The most hazardous complication of celiac disease is the development of neoplasms. The incidence for intestinal but also for extraintestinal cancer is increased the population of patients with celiac disease (23, 24).

1.2.4 Inflammatory bowel disease (IBD)

IBD is classically divided into Ulcerative Colitis (UC) and Crohn´s Disease (CD). Both of them represent immune-mediated gastrointestinal chronic relapsing and remitting inflammatory disorders (30-32). 20-30% of IBD cases are diagnosed in childhood and adolescence with 5% of all IBD patients being younger than 10 years. Very early onset IBD occurs in children under the age of 6 years and infantile IBD in children younger than 2 years (33). Infantile IBD and very early onset IBD commonly have a familial association and patients often present with a more severe and extended course of the disease (33). As many other diseases IBD consists of genetic, immunological and environmental factors (30). Smoking is an important environmental risk factor for CD while controversially for UC it seems to have a protective effect (31, 32). Further risk factors include early introduction in life to antibiotics, infectious gastroenteritis, and a diet rich in animal protein, sweets, oils, sugars, fish and shellfish, while breastfeeding and vitamin D are thought to protect against the development of IBD (31, 34, 35). In 5-10 % IBD is a familial disease and in most of these patients it represents a polygenic disorder (30, 32). The gastrointestinal system contains its own endogenous microbiotic flora, which under normal circumstances does not elicit a response from the mucosal immune system. The current hypothesis states that IBD develops due to a defective reaction of the immune system in genetic predisposed individuals to this endogenous flora, which leads to uncontrolled inflammation and tissue damage caused by the production of inflammatory cytokines from immune cells (31, 32). These cytokines are normally released in infection, but in IBD they have no proper regulation and thus trigger fibrogenesis, production of collagen and activate tissue metalloproteinases and additional inflammatory cells (31).

Although the diseases overlap in many aspects UC and CD also differ in some points from each other in their pathology, symptoms laboratory, endoscopic and radiographic features which complicates making an accurate diagnosis (30). UC is a solely mucosal, continuous disease and there are different levels of extension, however the rectum is almost always involved (30, 34). In some individuals UC extend proximally up to the rectosigmoid, beyond the sigmoid colon or it includes the whole colon (32, 34, 36). The term “backwash ileitis” is used when the inflammation extend a few centimeters into the terminal ileum. The classical symptoms of UC are bloddy diarrhea, tenesmus, rectal bleedings, passage of mucus and crampy abdominal pain (32, 34). The diarrhea often occurs after a meal or at night (30). Anorexia, nausea, fever and weight loss are systemic symptoms that can occur (34). The

laboratory analyses commonly show the elevation of C-reactive-protein (CRP), erythrocyte sedimentation rate (ESR), fecal calprotectin and lactoferrin, which are indicators for active intestinal inflammation, and decreased hemoglobin during an active phase of UC (30, 32). Also the serologic marker perinuclear antineutrophil cytoplasmic antibody (pANCA) is positive in some cases (30, 32). If the disease is severe, it also leads to hypoalbuminemia (32). Depending on the expected extend of UC either a sigmoidoscopy or a colonoscopy is performed for further diagnostic purposes and assessment of the severity of the disease and multiple biopsies are taken (34). Mild, moderate and long standing disease have different endoscopic appearances. In mild disease there is erythematous mucosa, in moderate the mucosa presents with hemorrhage, edema and ulcerations and in long-standing disease inflammatory polyps may form, while the mucosa is atrophic (32, 37). The histological examination of the taken biopsies often presents with deranged architecture of the crypts and also in some cases with basal plasma cells (Figure 5) (34, 38). The vessels of the mucosa are congested and edematous and inflammatory cells might be present (Figure 6) (32, 37, 38). One of the most dangerous complications of UC is the development of a toxic megacolon alongside with perforation and resulting peritonitis (36). Other possible complications are massive hemorrhage, stricture formation, anal fissures, perianal abscesses and hemorrhoids.

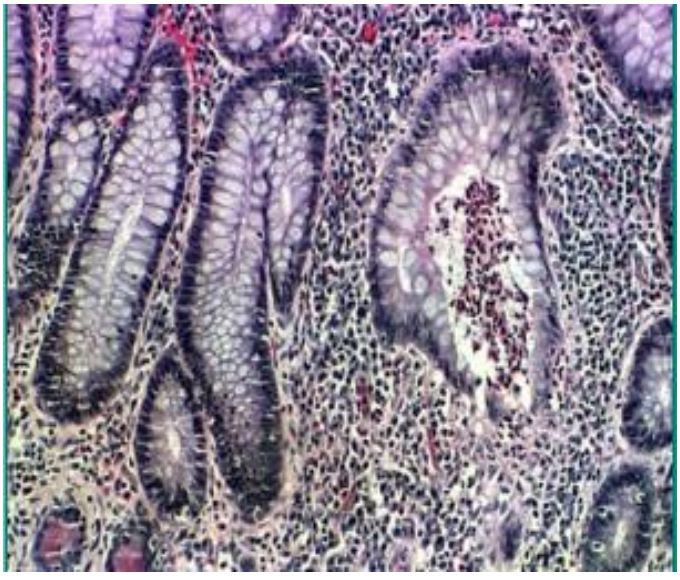

Figure 5: Characteristic features of ulcerative colitis: Distorted crypt architecture, crypt abscesses and mucosal lymphocytic infiltration (34).

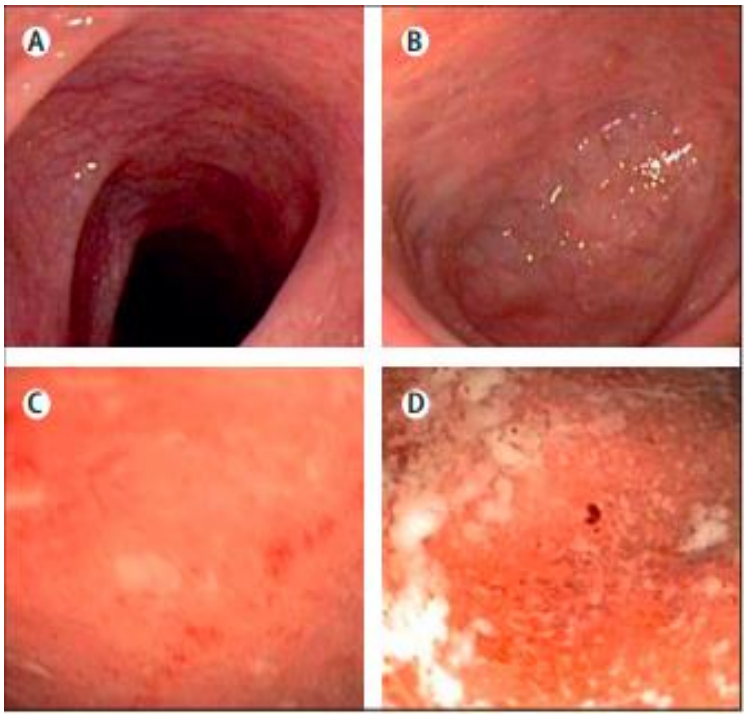

Figure 6: Endoscopic features of progressive ulcerative colitis: A: Normal mucosa, B: Eryhema and decreased vascular pattern, C: More pronounced erythema, erosions and absent vascular pattern, D: Bleeding (38).

CD in contrast to UC can involve every part of the entire gastrointestinal tract, although the rectum is spared in a lot of cases and the most frequently involved part is the terminal ileum (30, 35). CD has characteristic skip lesions, meaning between the diseased bowel parts there are unaffected areas. While, as discussed, UC only affects the mucosa, CD is a transmural disease (35, 39). CD might be confused with acute appendicitis at the beginning because it presents with diarrhea and lower right quadrant pain and sometimes a palpable inflammatory mass in that region (30). The pain is usually of colicky nature, can be relieved by emptying of the bowel and is often accompanied by fever and leukocytosis (35). The combination of diarrhea, pain and the consequent fear of eating often leads to significant weight loss (30). At the beginning of the disease the bowel wall is edematous and spastic movements can lead temporary obstruction which increases the pain, especially after eating. As the disease progresses the constant inflammation remodels the bowel wall until it gets a fibrostenotic narrow appearance with strictures that can lead to bowel obstruction instead of diarrhea (30, 39). Laboratory findings are similar to these in UC, with elevated ESR and CRP, fecal calprotectin and lactoferrin and leukocytosis, anemia and hypoalbuminemia in more progressive disease (30, 35). A different serologic marker than in UC is commonly elevated in

CD: anti-*Saccharomyces cerevisiae* antibodies (ASCAs) (30). During endoscopic interventions, stages of the disease can be differentiated by appearance like in UC. Small superficial or aphtous ulcerations are characteristic for mild disease, in moderate disease the typical "cobblestone" appearance is created by the fusion of transverse and longitudinal ulcerations and inflammatory polyps can develop (35, 39). In severe disease fistula tracts form which heal and leave fibrotic tissue and strictures. The bowel wall eventually thickens and narrows which can lead to bowel obstructions (39). The histological evaluation presents with aphtous ulcerations, crypt abscesses and characteristic noncaseating granulomas made of macrophages throughout the entire bowel wall, which are the earliest lesions to appear (Figure7) (35). Later lymphoid aggregates develop, fistulas originate from deep fissures and local abscesses form (39). The cobblestone appearance, along with the thickened bowel wall and aphtous ulcerations, can also be detected on radiographic imaging as well as strictures, fissures, decreased luminal diameter, fistulas and abscesses (39, 40). Usually either CT enterography or MR enterography are used for imaging (30, 40).

Complications arise frequently and include stricture, fistula and abscess formation, intestinal obstruction, massive hemorrhage, severe perianal disease and perforation (Figure 8) (30, 35). Some infectious as well as noninfectious conditions can mimic IBD. The infectious diseases can have a bacterial, viral, fungal or parasitic etiology. Diverticulitis and ischemic colitis belong to the noninfectious entities that are difficult to differentiate from IBD (35). Both UC and CD are associated with extraintestinal manifestations, one third of all IBD patients have at least one. Erythema nodosum occurs more often in CD, while pyoderma gangrenosum is usually represented in UC and less frequently in CD (32, 34). Due to the tendency to severe perianal disease, patients with CD commonly present with perianal skin tags and they also may suffer from oral mucosal aphtous lesions (SFL). Rheumatologic conditions like ankylosing spondylitis and peripheral arthritis develop in UC and CD (32, 34). Hepatic steatosis is a common finding in IBD patients with or without cholelithiasis. 50-70% of individuals with primary sclerosing cholangitis (PSC) are also affected by UC (30). Other extraintestinal manifestations of IBD are comprised of conjunctivitis, uveitis, ureteral obstruction, ileal bladder fistulas, decreased bone density, increased risk for arterial and venous thrombosis and cardiopulmonary disease, like endocarditis, myocarditis and interstitial lung disease (30). UC and CD are both associated with an elevated risk for the development of cancer. The risk increases further with long duration and progressive severity of the disease as well as with the development of complications like stricture formation (34,

35, 41). In UC the concomitant presence of PSC is known to be an additional risk factor for gastrointestinal malignancies.

Treatment of IBD is a complex combination of bowel rest, intravenous fluids and nutrition and the attempt to reach remission with additional pharmacologic therapy (42). For mild to moderate disease in UC the first line medication for induction and maintenance of remission are 5-Aminosalicylates (5-ASA) either orally or rectally in the form of enemas (34). If that is not sufficient corticosteroids can be used to induce remission, but are not recommended for maintenance therapy due to their side effects, so 5-ASA for maintaining remission is preferred (32, 36). In moderate to severe UC, 5-ASA is not sufficient for treatment and corticosteroids are the required medication for the induction of remission. For the maintenance of remission in moderate to severe disease immunosuppressants including methotrexate, azathioprine and 6-mercaptopurine are recommended (32, 42). For induction and maintenance therapy for severe UC resistant to other pharmacologic agents, the use of biologic agents, especially tumor necrosis factor inhibitors, is indicated (36, 42). Janus Kinase (JAK) inhibitors also support the maintenance therapy in moderate to severe UC by preventing the JAK enzymes from activating specific inflammatory cells (43). In cases in which pharmacologic and supportive therapy is ineffective or if the disease presents with hazardous complications like massive hemorrhage, surgery in the form of partial or total colectomy is needed (32, 44).

5-ASA can be given for induction and maintenance of remission in mild CD, but are more commonly used in UC (35). Corticosteroids are required for mild to moderate disease and to treat acute relapses, but as for UC are not recommended for maintenance therapy (42). If the disease is refractory to the aforementioned medications 6-mercaptopurine, azathioprine and methotrexate are used for maintenance therapy (42). Biologic therapy is important for severe CD where other medications have proven ineffective. With tumor necrosis factor inhibitors the goal of remission induction and maintenance is tried to be achieved (35, 42). If excessive perianal disease is present in CD, antibiotics are used for the prevention and treatment of infections (42). As for UC, surgery is indicated for severe disease resistant to pharmacologic therapeutic interventions or associated with complications. The type of surgery is dependent of the location of the disease in the gastrointestinal tract (45, 46). Supportive therapy like anti-diarrheal medications and the supplementation of vitamins and minerals is an important adjunct in the therapy of both UC and CD (42).

Figure 7: Chrohn's disease: Granuloma formation (35).

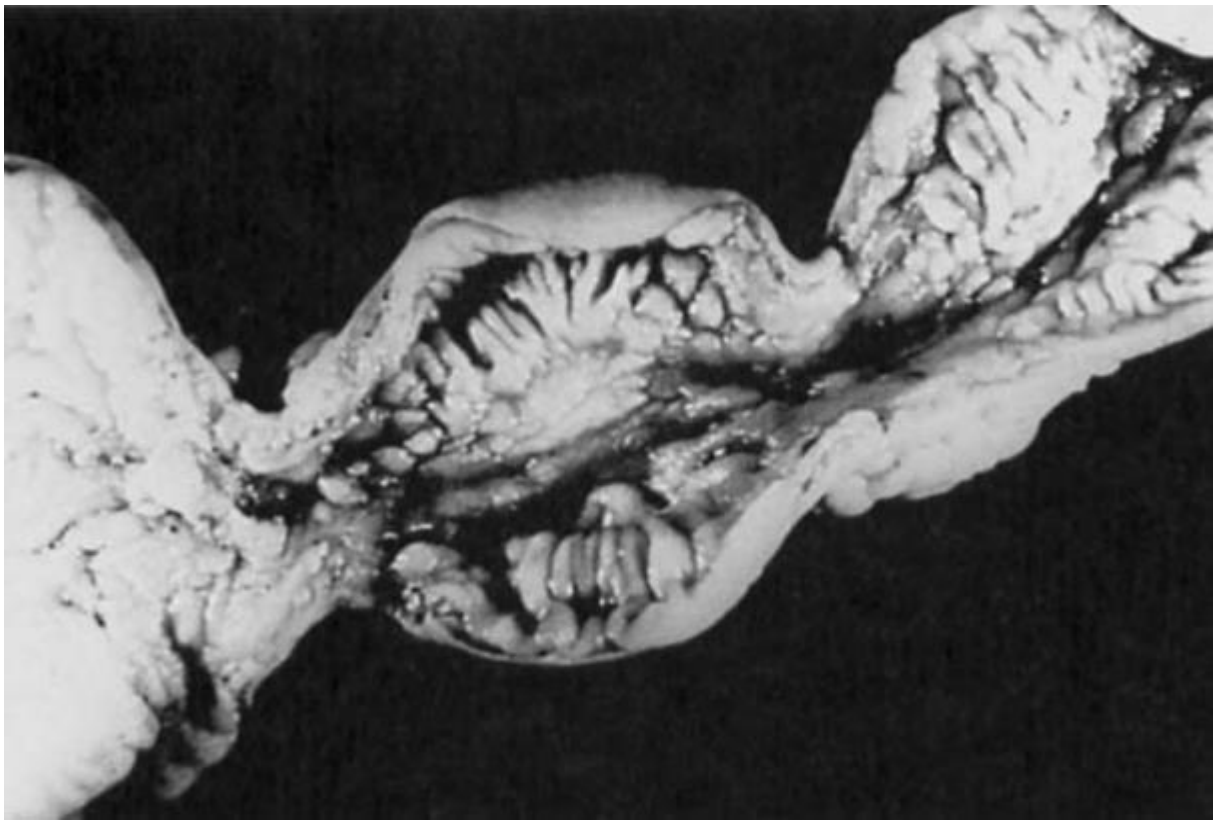

Figure 8: Small bowel stricture formation in Chrohn's disease (39).

1.3 Eosinophils in gastrointestinal diseases

The occurrence of eosinophils in the gastrointestinal tract is normal except in the esophagus (47). The highest numbers are found in the cecum and the appendix and throughout the gastrointestinal tract eosinophils reside mostly in the lamina propria (48). They participate in gut immunity and the development of inflammation although the exact "how" is still unknown (48). During the last decades the association with elevated numbers of eosinophils in gastrointestinal disorders, especially inflammatory diseases, became more evident. Although different hypotheses exist, the causality of the increased eosinophilic infiltrations and the effect on the clinical course of these diseases is still unclear (47). Eosinophilic gastrointestinal diseases are comprised of eosinophilic esophagitis, gastritis, gastroenteritis and colitis, eosinophilic esophagitis being the most common one by far. Due to the absence of eosinophils in the esophagus in healthy individuals, is it obvious that the eosinophilia found in eosinophilic esophagitis somehow belongs to the pathological changes of the disease (47, 49). It is thought that the release of the toxic granule content contributes to the mucosal inflammation and pathological features which are present in eosinophilic esophagitis (47). Not only eosinophilic infiltration of the mucosa is typical, but also the finding of peripheral eosinophilia. However peripheral eosinophilia is nonspecific for the disease since it can also result from other atopic diseases which are frequently associated with eosinophilic esophagitis (47). Eosinophilic gastritis, gastroenteritis and colitis have a much lower prevalence than eosinophilic esophagitis. They are as well characterized by dense mucosal infiltration of eosinophils and also peripheral eosinophilia can be detected in some cases (Figure 9) (47, 48). Usually the number of eosinophils increases from proximal to distal in the affected gastrointestinal tract, but it is difficult to state a proper treshold for the diseases because not enough about the normal gastrointestinal eosinophilic numbers in healthy individuals is known and still needs to be investigated (49). The current cut off values for diagnosis are defined as >25-30 eosinophils per high power field for eosinophilic gastritis and >65 eosinophils per high power field for eosinophilic colitis (47). The diagnosis of these diseases is challenging since they present with nonspecific gastrointestinal symptoms and can be confused with multiple entities (49). So mostly they are diagnosed by exclusion of the differential diagnoses.

Celiac disease and eosinophilia also have been linked (47). The infiltration is mostly present in the duodenal mucosa and commonly in the advanced stages so the eosinophilia is thought to contribute to the mucosal injury. Many patients with celiac disease also suffer from

eosinophilic esophagitis so a hypothesis that the diseases might be linked to one another was formed, but the results of later studies showed that the concomitant existence of the diseases is rather incidental (47, 50). For the role of eosinophilia in IBD patients two hypotheses have been stated. The first, more supported one, suggests that the degranulation of eosinophils in the injured tissue leads to further tissue injury while the other one hypothesizes that the eosinophils fullfill an immunologic role and help to heal or prevent inflammation. The latter one is based on the fact that the mucosal eosinophilia occurs before the onset of IBD (47, 51). Current studies evaluate the prognostic properties of the concentration of eosinophilic granule proteins which were described above, suggesting an increase in concentration might indicate a relapse and vice versa (47). Generally more investigation and research is needed about the role of eosinophilia in gastrointestinal diseases.

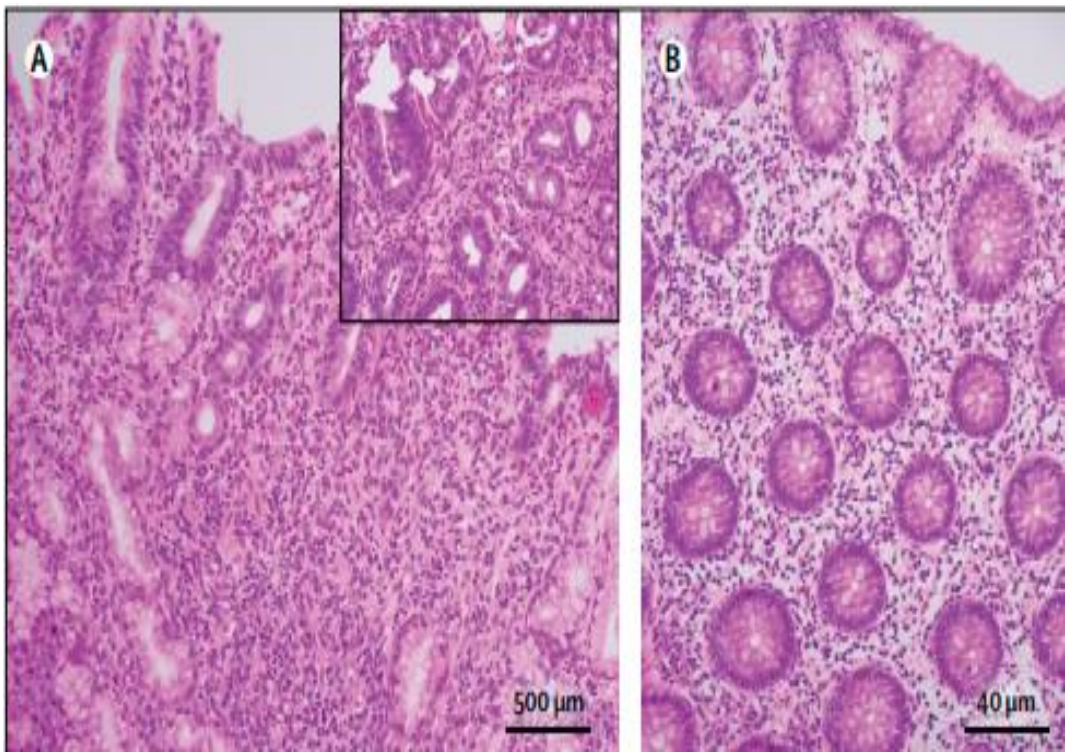

Figure 9: Eosinophilic infiltrations of the mucosa in eosinophilic gastrointestinal diseases, A: Eosinophilic gastritis, B: Eosinophilic colitis (51).

1.4 Gastrointestinal biopsies

1.4.1 Esophagogastroduodenoscopy

One of the most common procedures performed by the gastroenterologist is the esophagogastroduodenoscopy (EGD) (52). During the procedure the patient is either conscious using topical pharyngeal anesthetics or in sedation, most commonly accomplished with propofol and lies on the left side (53, 54). This position allows the gastroenterologist to insert the gastroscope into the patients mouth and maneuver it through esophagus and stomach until the proximal duodenum (52, 54). The EGD can be used either to only visualize the mucosa of the upper gastrointestinal tract or take biopsies if there is a suspicion of a certain condition or an abnormal lesion is encountered during the EGD (54, 55). The possibility of taking biopsies and getting a histopathological examination makes the EGD a very important diagnostic tool in the field of gastroenterology (54, 56). For better visualization during the EGD the endoscopist can insufflate the parts of the upper gastrointestinal tract that are in question (52). If no complications occur, the average EGD takes 5-20 minutes, which makes it a fast intervention (52). There are numerous indications for an EGD and most of them are connected to:

1. New onset of upper GI symptoms, e.g.: weight loss, bleeding, odynophagia, persistent vomiting, dyspepsia, etc. (52);
2. Suspected neoplasia, cancer screening and cancer staging (52, 54);
3. Confirmation of radiologic abnormalities (52);
4. Follow up of chronic gastrointestinal conditions, e.g.: ulcer, inflammatory diseases, gastroesophageal reflux disease (GERD), Barrett´s esophagus, etc. (52, 56);
5. Therapeutic reasons, e.g. treatment of varices, treatment of strictures, excision of tumors, etc. (52, 56).

When upper gastrointestinal endoscopy is performed on a child, for children up to seven years a pediatric gastroscope should be used. In premature infants weighing less than two kilo it is advised to choose a nasogastroscope. The endoscopic technique and indications for an EGD in children are comparable to what was described earlier for adults (52).

1.4.2 Colonoscopy

As well as the EGD the colonoscopy is a very common procedure in the field of gastroenterology and is considered the gold standard for inspection of the entire colon (57). Before undergoing a colonoscopy the bowel of the patient has to be cleaned. This is done by giving the patient medications in liquid or tablet form that will lead to an increased frequency of bowel movements (54, 57). Commonly used bowel preparations are: polyethylene glycol (PEG), aqueous or tablet sodium phosphate and magnesium carbonate with citric acid (54, 57). During the colonoscopy the patient usually is sedated and lies in the left lateral decubitus position (54, 57, 58). Before starting the procedure the anus of the patient as well as the colonoscope are lubricated (57). After a careful initial digital rectal examination the colonoscope is inserted and passed through the anus, rectum and colon until the terminal ileum (54, 57). The technique for the colonoscopy is quite demanding since the endoscopist has to pass multiple flexures to reach the terminal ileum (57). While trying to advance through the colon abdominal palpation can help advance the colonoscope through the flexures and it is also helpful in preventing the formation of loops (57). The abilities to visualize the entire colon and take biopsies if needed make the colonoscopy an important diagnostic procedure for the lower gastrointestinal tract as the EGD is for the upper gastrointestinal tract (57). The most common indications for a colonoscopy are: constipation, lower gastrointestinal bleeding, chronic diarrhea, acute colonic pseudoobstruction (ACPO), inflammatory diseases and screening, staging and follow up of cancer (54, 57). Flexible sigmoidoscopy (FS) is a subtype of colonoscopy where only the rectum, the sigmoid colon and the descending colon is visualized (54, 58). FS has become an important diagnostic tool for colorectal cancer screening, but is also of therapeutic value if polyps need to be excised (54, 57, 58). Another indication for FS are circumstances in which a total colonoscopy constitutes a risk, e.g. in acute exacerbations of inflammatory bowel disease (IBD) or ischemic colitis (54).

In children under two years a pediatric gastroscope should be used for the colonoscopy, in children between two and ten years the instrument of choice is a pediatric colonoscope (57). To empty the bowel usually the first attempt is made with a PEG solution, if that does not work a combination of a phosphate or sodium citrate enema and a laxative is tried (57). The colonoscopy technique is as for the EGD comparable to adults. Major indications for a colonoscopy in children are: recurrent rectal bleeding, suspected IBD, gastrointestinal polyposis syndrome and colitis in immunosuppressed children (57).

1.4.3. Indications for gastrointestinal biopsies

Gastrointestinal biopsies are a very important addition to endoscopic procedures, since they allow for a histopathological evaluation of the obtained material (54, 56). The information gained from this evaluation can be crucial in leading to the correct diagnosis and the resulting treatment (56, 59, 60). Indications for taking biopsies are numerous; for upper gastrointestinal tract biopsies it is mostly due to the following conditions: GERD, Barrett´s esophagus, eosinophilic and infectious esophagitis, helicobacter pylori, gastric polyps, peptic ulcer disease, celiac disease and meta- as well as neoplasia (56, 59, 61).
The most common indications for lower gastrointestinal tract biopsies are: Microscopic colitis, diagnosis and surveillance of IBD, pouchitis, colonic polyps, acute graft versus host disease and neoplasia (55, 61).

In general if during the endoscopy anything seems abnormal it should be biopsied (54, 55). There are also situations in which the mucosa looks normal and the pathologic changes can only be found under the microscope. An example for that is microscopic colitis (55). The location and number of biopsies depends on the suspected condition (56 ,60, 61): for the diagnosis for microscopic colitis for instance it is recommended to take two or more biopsies from the ascending, the transverse, the descending and the sigmoid colon or at least three to four biopsies of the ascending as well as the descending colon, while for the diagnosis of IBD the recommendations advise to take two or more biopsies from five different locations, two of them being the ileum and the rectum (56).

To help with the decision whether a biopsy is necessary or not, there are protocols and recommendations for the indications when, where and how many biopsies should be taken. These protocols are not universal, but rather dependent locally on where the working environment is situated (56, 59, 60).

2. OBJECTIVES

2.1 Objectives

With the conduction of this study we aimed to determine the frequency of an increased number of eosinophils in gastrointestinal biopsies and to which final diagnosis these findings were most commonly connected. The endoscopic procedures with the subsequently obtained biopsies were performed in the three year time span from January 1st 2017 to December 31st 2019 at the department of Pediatrics at the University Hospital of Split and sent for pathohistological examination to the department of Pathology, Forensic Medicine and Cytology of the same hospital. Further we aimed to investigate the most common referral clinical diagnosis for the indication of endoscopy combined with biopsy, the mostly used type of endoscopy, the locations where the biopsies where taken from as well as the most common final diagnosis. As an additional outcome the average age at the time of diagnosis for female and male patients was investigated.

2.2 Hypothesis

Based on the findings of previously conducted studies we presume the most common final diagnoses with the increase of eosinophils to be celiac disease, IBD and eosinophilic esophagitis. Further we predict that the same diseases will also be the most commonly represented ones in the final diagnosis in general according to the pathohistological reports and again in the clinical referral diagnosis. We also hypothesize the most often conducted endoscopic procedure to be gastroscopy, followed by combined gastroscopy and colonoscopy and lastly colonoscopy alone. We predict that in a majority of cases the biopsies will be taken from multiple gastrointestinal locations. As well based on previous studies we hypothesize that the average age of the patients is going to be between 10 and 14 years of age.

3. SUBJECTS AND METHODS

3.1 Patients

336 patients were referred for gastrointestinal endoscopy and biopsy taking in the time span from January 1st 2017 to December 31st to the department of Pediatrics of the University Hospital of Split. The resulting pathohistological reports of these patients were reviewed. All pediatric patients referred for gastroscopy and/or colonoscopy with biopsy tissue being collected between the ages from 0 to 18 were included in the study. Excluded from the study were patients older than 18 years and endoscopy patients in which no tissue samples were obtained.

3.2 Organization of the study

We conducted a retrospective study.

3.3 Place of the study

The department of Pediatrics of the University Hospital of Split provided the medical records of the pediatric patients included in the study. The pathohistological reports with the findings of the tissue biopsies were collected from the department of Pathology, Forensic Medicine and Cytology of the University Hospital of Split.

3.4 Methods of data collection and processing

After the data from the department of Pediatrics and the department of Pathology, Forensic medicine and Cytology was collected it was further processed into tables. With Microsoft Exel and Microsoft Word Processing software the data was further analyzed.
Under this number we have the approval of the University Hospital of Split ethics committee for our study: 500-03/21-01/118

3.5 Description of the research

After collecting data about the pediatric patients age, gender, clinical diagnosis, type of the conducted endoscopy procedure, the number and location of the obtained tissue biopsies and the final diagnosis from the medical records and pathohistological reports from the department of Pediatrics and the department of Pathology, Forensic Medicine and Cytology, it was further analysed. The determination of the frequency of an increased number of eosinophils and the most common associated diseases in endoscopic procedures in combination with tissue sampling conducted between January 1st 2017 and December 31st 2019 at the department of Pediatrics at the university Hospital of Split was the primary outcome. Further outcomes included the most common clinical diagnosis for referral, the type of endoscopy procedure most often used, the locations of the. An additional secondary outcome was the average age of diagnosis in female and male patients.

3.6 Staistical analysis

The MedCalc Software (MedCalc Software, Ostend, Belgium; medcalc.org; 2019, RRID:SCR_015044) was used for the statistical analysis of the data previously collected. To interpret the statistical significance of the sampling distribution the chi-square test, as well as Mann-Whitney U test were used and $p<0.05$ was determined as the statistical significance value.

4. RESULTS

From January 1st 2017 till December 31st 2019 there were 336 pediatric pathology reports on gastrointestinal biopsies (Figure 10). 53.9% percent of the patients were female. Median age at the time of diagnosis for female patients was 13 years with range from 2 to 18years, and for male patients the median age was 12 years, with the range from 0 to18 years. There was no statistically significant difference in age between male and female patients (*P*=0.018).

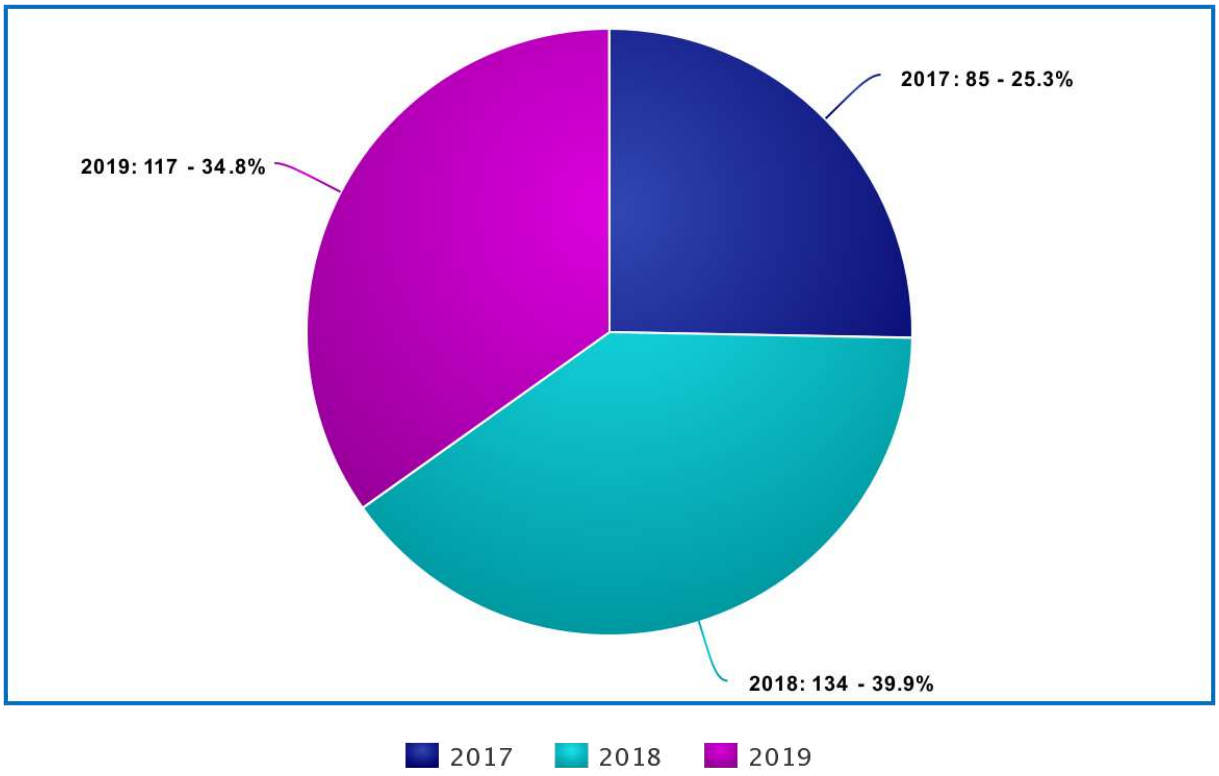

Figure 10. Number of pathology reports on gastrointestinal biopsies over the 3-year period.

The most common endoscopy procedure was gastroscopy, followed by colonoscopy and combined gastroscopy and colonoscopy (Figure 11).

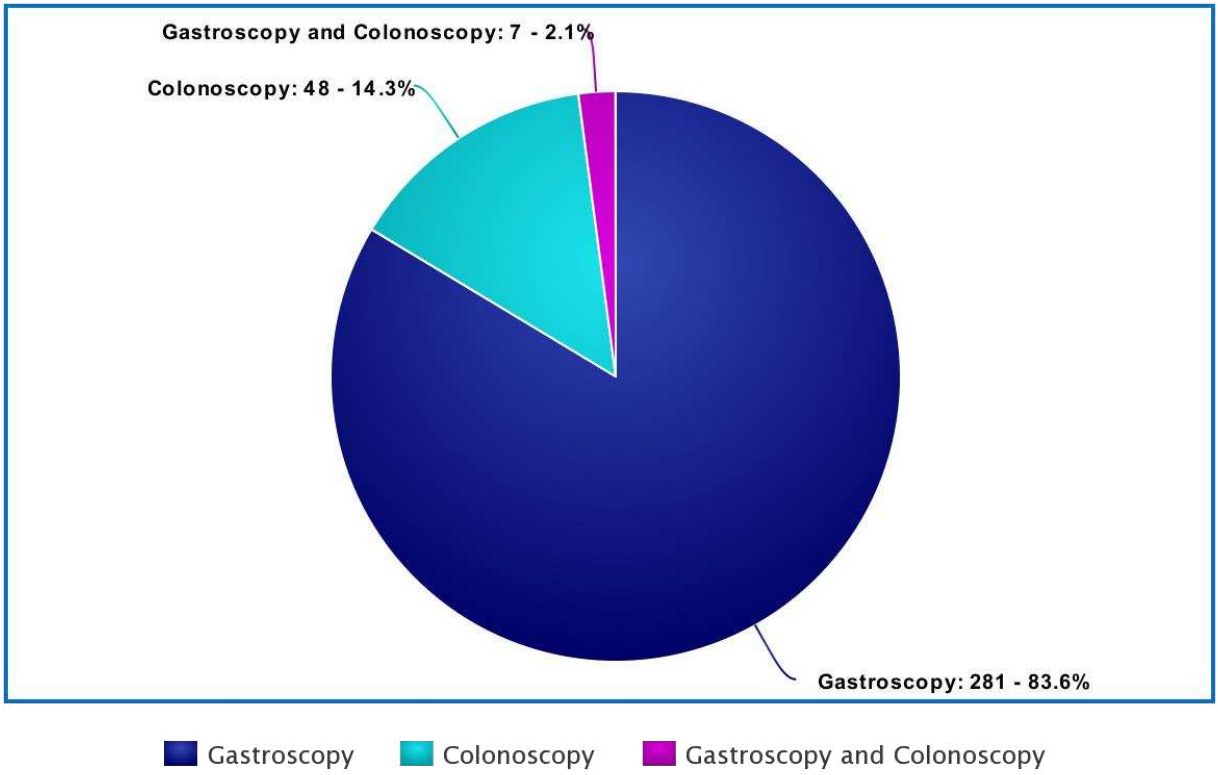

Figure 11. Distribution of the endoscopy procedures performed.

The referal clinical diagnosis were divided into 12 categories as presented at Table 1. The most common one was malabsorbtion noted in 66 cases and the least common was irritable bowel syndrome noted in 8 cases, which was statistically significant (P<0.0001).

Table 1. Referral clinical diagnosis

Clinical diagnosis	**N (%)**	**P**
Malabsortion	66 (19.6%)	
Abdominal colic	59 (17.6%)	
Gastroesphageal reflux	35 (10.4%)	
Gastropathy including gastritis	22 (6.5%)	
Inflammatory bowel disease	18 (5.4%)	
Crohn diesease	18 (5.4%)	<0.0001*
Ulcerative colitis	15 (4.5%)	
Irritable bowel syndrome	8 (2.4%)	
Others	59 (17.6 %)	
Non-GI diagnosis	9 (2.7%)	
No referal diagnosis made	27 (8%)	

* Chi-square test

In 36.9% of the cases multiple samples (from more then one gastrointestinal site) were taken during the endoscopy, and the least common biopsied site was colon (0.9%) (Table 2), which was statistically significant (P<0.0001).

Table 2. Distribution of biopsy according to biopsy site

Biopsy site	**N (%)**	**P**
Stomach	32 (9.5%)	
Duodenum	71 (21.1%)	
Ileum	93 (27.7 %)	<0.0001*
Colon	3 (0.9%)	
Inadequate sample	13 (3.9%)	
Multiple GI locations	124 (36.9%)	

*Chi-square test

Increased number of eosinophils was found in 103 cases (45.6%-47%). 53% of those cases were male patients. Accordng to biopsy site increased eosinophil number was most commonly noted in multiple biopsies, while eosinophilic increase was absent in stomach biopsies, which was statisically significant (P<0.0001) (Figure 12).

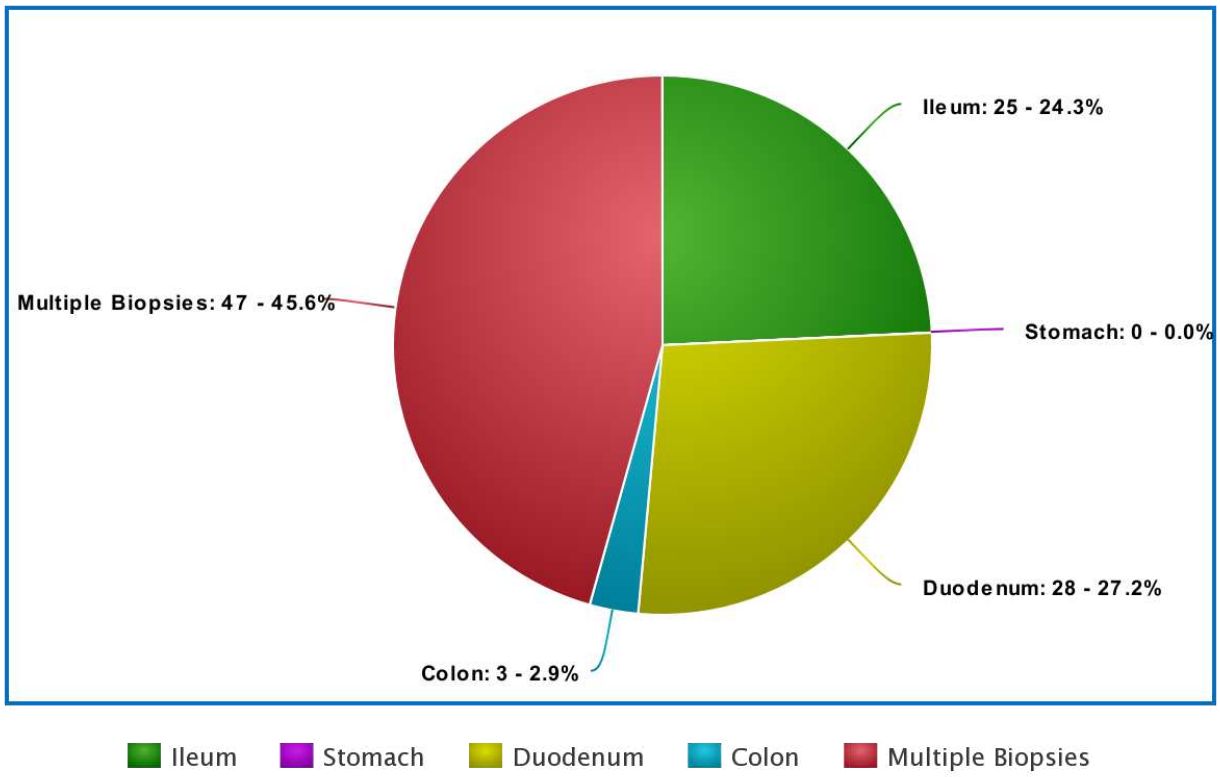

Figure 12. Increased number of eosinophils according to biopsy site.

Increased number of eosinophils was most commonly noted with malabsorption as referral clinical diagnosis (22.3%) while non-GI diagnosis and gastroesophageal reflux had only 3 cases (2.9%) of increased eosinophils (P<0.0001) as shown in Table 3.

Table 3. Increased number of eosinophils according to referral clinical diagnosis

Clinical diagnosis	**N (%)**	**P**
Malabsortion	23 (22.3%)	
Abdominal colic	16 (15.5%)	
Gastroesphageal reflux	3 (2.9%)	<0.0001*
Gastropathy including gastritis	5 (4.9%)	
Inflammatory bowel disease	11 (10.7%)	
Crohn diesease	2 (1.9%)	
Ulcerative colitis	12 (11.7%)	
Irritable bowel syndrome	4 (3.9%)	
Others	20 (19.4%)	
Non-GI diagnosis	3 (2.9%)	
No referal diagnosis made	4 (3.9%)	

*Chi-square test

Final diagnosis according to pathology report were organized into 9 catergories as shown in Table 4. The most common final diagnosis was celiac disease and the least common ones were ulcerative colitis and pancolitis (P<0.0001).

Table 4. Final diagnosis according to pathology report

Pathology report	**N (%)**	**P**
Celiac disease	153 (45.5%)	
Chronic active inflammatory changes	22 (6.5%)	
Inflammatory Bowel Disease	14 (4.2%)	<0.0001*
Chronic active gastritis	11 (3.3%)	
H. pylori gastritis[a]	3 (0.9%)	
Ulcerative Colitis	2 (0.6%)	
Crohn disease	5 (1.5%)	
Pancolitis	2 (0.6%)	
Descriptive report	(36.9%)	

*Chi-square test

[a] Helicobacter pylori

Increased number of eosinophils was most commonly found when final diagnosis based on pathology report was celiac disease and none of the H.pylory gastritis had increased eosinophilic number as shown in Table 5 (P<0.0001).

Table 5. Number of cases with increased eosinophilic number according to pathology report final diagnosis

Pathology report	**N (%)**	**P**
Celiac disease	55 (53.4%)	
Chronic active inflammatory changes	11 (10.7%)	
Inflammatory Bowel Disease	10 (9.7%)	<0,0001*
Chronic active gastritis	1 (0.1%)	
H.pylori gastritis[a]	0	
Ulcerative Colitis	2 (0.2%)	
Crohn disease	5 (4.9%)	
Pancolitis	2 (0.2%)	
Descriptive report	17 (16.5%)	

*Chi-square test

[a] Helicobacter pylori

5. DISCUSSION

It has been known for a long time that increased numbers of eosinophils are associated with allergic diseases and parasitic infections (8). In the last couple of decades it has become evident that eosinophilia is also frequently present in gastrointestinal diseases including celiac disease, inflammatory bowel disease, eosinophilic esophagitis, gastritis, gastroenteritis and colitis (16, 47). The exact role of the eosinophils in the pathogenesis and course of the diseases has yet to be identified (47).

In our study we investigated the collected data including epidemiological and pathohistological characteristics of conducted gastrointestinal endoscopic procedures with subsequent obtained tissue biopsies among pediatric patients at the University Hospital of Split in a three-year time span, from January 1st 2017 until December 31st 2019.

There were 336 pathology reports on gastrointestinal biopsies, 53.9% of the patients being female. The median age of females at the time of diagnosis was 13 years, while the median age of the male patients was 12 years. The most common conducted endoscopic procedure was gastroscopy with 83.6% which was expected with the most frequent clinical diagnosis for referral to endoscopy including malabsorption, abdominal colic, gastroesophageal reflux disease and gastritis. The most often represented referral clinical diagnosis was malabsorption with 66 cases being reported. In 36.9% of all endoscopic procedures the biopsies were taken from multiple gastrointestinal locations which also correlates with the referral clinical diagnosis, since multiple diseases are represented in which it is common to take multiple biopsies (56). The least common biopsy site was the colon with only 3 cases in total. An increased number of eosinophils was present in 32.2% of the obtained biopsies, the highest frequency was observed when biopsies form multiple gastrointestinal locations were taken. We anticipated eosinophilic esophagitis to be one of the most common diseases with eosinophilic increase, however since the biopsies from the esophagus were not taken, the highest number of biopsies with eosinophilic increase was associated with celiac disease, which was also the most common final diagnosis (45.5%). The association with mucosal eosinophilia in gastrointestinal diseases has been increasingly recognized in the last decades, especially with celiac disease and inflammatory bowel disease (47). Ulcerative colitis as well as pancolitis as the final diagnosis was each only represented with 2 cases, although the diagnosis of early onset inflammatory bowel disease in childhood is often associated with more severe courses of the disease (33). Generally the number of final diagnosis of ulcerative colitis, Chrohn's disease and inflammatory bowel disease in our study was lower than expected with 21 cases in total, since 20-30% of IBD cases are already diagnosed in childhood (33). Many gastrointestinal diseases known in the adult population are

also common in pediatric patients, like IBD, celiac disease is also represented in both, the adult and the pediatric patients, different types being predominant in the different age groups (23, 24). In 17 of these 21 cases an increased number of eosinophils were found, which again correlates with our previous anticipation and the findings of other studies that inflammatory bowel disease is increasingly connected to eosinophilic findings (47). Many gastrointestinal diseases known in the adult population are also common in pediatric patients, as for example 20 to 30% of inflammatory bowel disease cases are diagnosed already in childhood (33). Celiac disease is also represented in both, the adult and the pediatric patients, different types being predominant in the different age groups (23, 24).

Our studies major limitation is the retrospective nature of the study and the small sample size of 336 pathological reports in the three year time span. The clinical diagnosis is among other factors dependent on the statements of the children and their parents about medical history and the presenting symptoms which can be inaccurate and misleading. Another limitation is that a retrospective study relies on accurate recordkeeping which is prone to bias due to possible imprecision.

6. CONCLUSION

According to the pathohistological reports the most frequent final diagnosis was celiac disease which was also most often associated with an increased number of eosinophils in the tissue samples taken during the endoscopy. More than half of the patients were female. The most often referral clinical diagnosis was malabsorption and the majority of performed procedures were gastroscopies. There were biopsies taken from specific parts of the gastrointestinal tracts, but most often the biopsies were retrieved from multiple gastrointestinal cases. There has been an increased association of eosinophilia with gastrointestinal diseases but further research has to be done to reveal the exact causality for these findings.

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8. SUMMARY

Objectives: With our study we aimed to determine the frequency of an increased number of eosinophils and the most common diseases associated with these findings in gastrointestinal biopsies. We aimed to investigate the most common referral clinical diagnosis for the indication of endoscopy combined with biopsy, the mostly used type of endoscopy, the locations where the biopsies where taken from, which final diagnosis was most commonly represented as well as the average age of the patients.

Materials and methods: We conducted a retrospective study with the collected data within the time span from January first 2017 to December 31st 2019 at the department of Pediatrics of the University Hospital of Split, including patients from 0-18 years old, who were examined by gastrointestinal endoscopy combined with biopsy taking. The collection of data included the patient data available in the archives and the pathohistological reports from the biopsies. MedCalc was used for the statistical analysis.

Results: From the 336 pathohistological reports 181 (53.9%) were from female patients with a median age at diagnosis of 13 and 155 (46.1%) from male patients with a median age at diagnosis of 12. The most common endoscopic procedure conducted was gastroscopy in 281 cases (83.6%) and in 124 cases (36.6%) biopsies were taken from multiple gastrointestinal locations which was statistically significant ($p<0.0001$). As well presenting with statistical significance ($p<0.0001$) malabsorption was the most frequent clinical referral diagnosis with 66 cases (19.6%) and after the pathohistological report the most common final diagnosis was celiac disease (45.5%). In 103 biopsies (32.2%) increased numbers of eosinophils were found and the most common final diagnosis associated with increased eosinophils was again celiac disease with 55 cases (53.4%), both of these findings being statistically significant ($p<0.0001$).

Conclusion: Celiac disease was the most common final diagnosis according to the pathohistological reports as well as the disease most often associated with an increased number of eosinophils. In order to explain the role of eosinophils in various diseases of the gastrointestinal tract further studies are necessary.

9.SAŽETAK

Ciljevi: odrediti učestalost povećanog broja eozinofila u patohistološkim nalazima biopsija gastrointestinalnog (GI) trakta, te njihovu povezanost s bolestima GI trakta. Odrediti najučestalije uputne dijagnoze koje su prethodile endoskopiji i posljedičnoj biopsiji. Odrediti najučestalja mjesta GI trakta odakle su uzete biopsije za patohistološku analizu, vrstu endoskopske pretrage, konačnu patohistološku dijagnozu, kao i prosječnu dob bolesnika.

Materijali i metode: retrospektivna studija je obuhvatila razdoblje od 1. siječnja 2017. godine do 31. prosinca 2019. godine. U studiju su uključeni pacijenti Klinike za dječje bolesti Kliničkog Bolničkog Centra Split kojima je učinjena endoskopska pretraga GI trakta u navedenom razdoblju s uzimanjem biopsije, a patohistološka analiza je učinjena na Odjelu Patologije, Kliničkog Zavoda za Patologiju, Sudsku medicine i Citologiju iste bolnice. Klinički podatci o pacijentima prikupljeni su iz arhive Klinike za dječje bolesti, a patohistološki nalazi iz baze podataka Odjela Patologije. MedCalc je korišten za statističku analizu prikupljenih podataka.

Rezultati: Analizirani su podatci 336 bolesnika, 181 (53,9%) bolesnika prosječne dobi od 13 godina te 155 (46,1%) bolesnika prosječne dobi od 12 godina. Najučestalija endoskopska pretraga je bila gastroskopija (83,6%), a u 36,6% slučajeva biopsije su uzete s više različitih lokacija GI trakta u jednom aktu, što je bilo statistički značajno ($p<0,0001$). Malapsorpcija je najučestalija uputna dijagnoza, koja je zabilježena u 19,6% slučajeva što je bilo statistički značajno ($p<0,0001$), dok je najučestalija konačna patohistološka dijagnoza bila celijakija, zabilježena u (45,5%). 32,2% svih biopsija je imalo povećan broj eozinofila, a celijakija je najučestalija patohistološka dijagnoza povezana s povećanim brojem eozinofla. Oba navedena rezultata su statistički značajna ($p<0,0001$)

Zaključak: Celijakija je najčešća patohistološka dijagnoza, ujedno i najčešća dijagnoza povezana s povećanim brojem eoznofila u biopsijama GI trakta. Daljnja istraživanja su nužna kako bi se odredila točna uloga eozinofila u različitim bolestima GI trakta.

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