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Alimentary Tract

Tissue and peripheral eosinophilia as predictors for disease outcome in children with ulcerative colitis

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ABSTRACT

Background: Eosinophils are implicated in the pathogenesis of ulcerative colitis.
Aims: To evaluate the magnitude of mucosal and blood eosinophils in newly diagnosed pediatric ulcerative colitis patients and its significance in predicting disease outcomes.
Methods: We retrospectively evaluated colorectal biopsies of 96 pediatric patients with ulcerative colitis and 50 age- and sex-matched controls. Samples were taken from diseased areas of the colon and examined by a gastrointestinal pathologist. The most inflamed site was used for assessment of mucosal eosinophils.
Results: Samples from 96 diagnostic and 70 follow-up colonoscopies were evaluated. Median age was 13.3 years (IQR 10.1–15.3). Median duration of follow-up was 12.8 years (IQR 7.2–17.1). Median number of tissue eosinophils at diagnosis was 45 (IQR 22–73) compared to 10 eosinophils (IQR 8–25) during histologic remission ($p < 0.0001$). Peripheral absolute eosinophil counts correlated with tissue inflammation and eosinophilia ($p = 0.001$). Mucosal eosinophilic infiltration ($p = 0.02$) and peripheral eosinophilia ($p = 0.04$) was associated with clinical severity at diagnosis. Multivariate analysis showed that severe eosinophilic infiltration is associated with corticosteroid therapy following diagnosis ($p = 0.04$) but not with long-term risk for step-up therapy or colectomy.
Conclusion: Tissue and peripheral eosinophilia correlate with ulcerative colitis severity at diagnosis and with short-term corticosteroid requirement but not with long-term outcomes.

1. Introduction

Ulcerative colitis (UC) is characterized by a chronic relapsing and remitting inflammatory process involving the colonic mucosa, which results in chronic mucosal inflammation, architectural distortion, and an inflammatory cell infiltrate including neutrophils, lymphocytes, plasma cells, macrophages, eosinophils and mast cells [1]. Compared to adult disease, pediatric UC is characterized by a more severe phenotype, reflected by more extensive disease and a higher rate of acute severe exacerbations [2].

A considerable body of evidence supports a possible deleterious role of eosinophils in inflammatory bowel disease (IBD) [3] and in UC in particular [4]. Animal studies have suggested a role for eosinophil cationic proteins in the pathogenesis of IBD [5–7]. Mucosal release of eosinophil degranulation products such as cationic protein (ECP), eosinophil peroxidase (EPO), and eosinophil protein x (EPX) was found to be significantly increased in patients with active colitis and proctitis compared with inactive UC and controls [8–10] and these were correlated with the endoscopic score [10,11]. Similar findings were demonstrated in a cohort of pediatric UC patients [12]. Patients with active as well as inactive UC were shown to have higher numbers of active mucosal eosinophils compared to controls. Interestingly, during inactive UC, the number of activated eosinophils was even larger [13]. Mucosal eosinophils have also diagnostic value as their number were shown to be higher in affected colonic segments of UC patients compared to patients with Crohn's colitis [14].

Few studies have demonstrated an association between mucosal eosinophilic infiltration and poorer clinical outcome in UC patients. Severe eosinophilic infiltration in colonic biopsies was a significant predictor of poor response to medical therapy [15] while increased number of eosinophils and neutrophils in the lam-

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ina propria were observed to be associated with significantly higher relapse rate [16]. Interestingly, peripheral eosinophilia is common in UC patients and correlates with high eosinophilic count in colonic biopsy samples [17]. Lastly, peripheral eosinophilia was found to be associated with severe colitis, risk for colectomy and primary sclerosing cholangitis [18].

The predictive role of mucosal eosinophils in the long-term outcome of pediatric UC is not well characterized and the above data were not studied comprehensively in the pediatric population. Thus, we aimed to assess the presence of eosinophils in the colonic mucosa and in blood samples of pediatric patients with newly diagnosed UC, and to investigate its association with disease phenotype at diagnosis and with long-term outcomes including response to medical therapy and the risk for colectomy.

2. Materials and methods

2.1. Patients

We conducted a retrospective chart review of all pediatric onset UC patients, diagnosed between the ages 0 to 17 years, who were evaluated at the Schneider Children's Hospital of Israel Between 1990 and 2015. For the purpose of the study, patients with at least 3-year follow up and a complete colonoscopy at diagnosis were included. Some of the patients had more than one endoscopic evaluation during follow-up. Patients with infantile IBD (<2 years at diagnosis) or IBD-unclassified at diagnosis were excluded. Patients with allergic conditions (including bronchial asthma, allergic rhinitis, atopic dermatitis, food allergies), recent use (<30 days prior to endoscopy) of non-steroidal anti-inflammatory drugs (NSAIDs), positive stool specimens for ova and parasites were excluded as well. Diagnosis of UC was performed according to accepted criteria [19,20]. Histologic inflammatory activity and histologic remission were defined according to published consensus guidelines [21]. We also included an age- and sex- matched control group of patients who underwent colonoscopic evaluation with findings of either a single juvenile polyp but otherwise normal macroscopic findings and patients with irritable bowel syndrome (IBS). Potential control patients with known allergic conditions, as specified above, were excluded. Data were retrieved from both pediatric medical charts (Schneider Children's Medical Center) and adult medical charts for patients followed-up into adulthood (Rabin Medical Center).

2.2. Description of variables

The characteristics of UC patients were completed according to the retrospective analysis of medical charts. Age at onset; gender; ethnicity; clinical, laboratory, endoscopic and histological findings at diagnosis and timing of therapeutic regimens were thoroughly investigated by reviewing medical records. Disease activity was assessed using the Pediatric UC Activity index (PUCAI). Disease phenotype at diagnosis was categorized according to the Paris classification [22]. Main data collected during follow-up period included medical therapies, need for colectomy, number of flares and hospitalizations.

2.3. Analysis of tissue eosinophils

All patients, including controls, had a minimum of one mucosal biopsy from each colonic anatomical landmark (cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum). Paraffin embedded and hematoxylin and eosin-stained (H&E) biopsy slides from all segments well evaluated for the presence of eosinophils. Histologic specimens from patients who had more than one endoscopic evaluation during follow-up were evaluated as-well. The presence and the degree of eosinophil predominance were scored based on published data [23]: mild was defined as more than 25 and severe as more than 60 eosinophils per one high power field (hpf) covering a tissue area of 0.2 mm^2. Histological evaluation and scoring was performed by one experienced gastrointestinal pathologist who reassessed all specimens for the purpose of the study. The pathologist knew the diagnosis but was blinded to the clinical status. The quantity of eosinophils was evaluated in all biopsies by absolute counting under light microscopy [hpf, ×400]. For the purpose of the study, for the UC patients, we used the absolute count in the area of highest eosinophilic density in the most inflamed fragment. However, the analysis was also performed using the mean number of eosinophils from inflamed segments.

2.4. Analysis of peripheral eosinophils

Complete blood count results from the time of endoscopic evaluation results were available for all patients. Absolute peripheral eosinophil count was retrieved for each patient. Peripheral eosinophilia was defined by an absolute eosinophilic count greater than 0.5×10^9/L.

2.5. Data analysis

Continuous variables were evaluated for normal distribution using histogram, Q–Q plots and Kolmogorov–Smirnov test and reported as median (interquartile range, IQR) for non-normally distributed variables or mean (standard deviation, SD) for normally distributed variables. Categorical variables were reported as frequency and percentage. Continuous variables were compared using independent simple T-test or Mann–Whitney while categorical variables were compared using chi-square test or Fisher-exact test. Correlation between continuous variables was evaluated using Spearman rho correlation coefficient. Tissue and peripheral eosinophils levels in endoscopic remission versus endoscopic inflammatory activity were compared using Wilcoxon test. Univariate cox regression analysis was used to evaluate predictors for colectomy. Multivariate cox regression analysis included tissue and blood eosinophils levels, age, gender and variables with $p < 0.02$ in the univariate analysis.

Multivariate logistic regression was used for analyzing the association between tissue and blood eosinophils levels and multiple outcomes such as different types of therapies. Age, gender and eosinophils levels were forced into the regression while other potential confounders were selected using forward step-wise likelihood ratio. $p < 0.05$ was considered as statistically significant. SPSS version 23 (Armonk, NY, IBM Corp.) was used for all statistical analyses. The study protocol was approved by the Rabin Medical Center Internal Review Board which represents both the Schneider Children's Medical Center and the Rabin Medical Center.

3. Results

A total of 96 children [median age 13.3 years (IQR 10.1–15.3), 48 boys (50%)] with UC who were followed for a median duration of 12.8 years (IQR 7.2–17.1) were identified and fulfilled the inclusion criteria. Complete demographic and clinical data of UC patients at presentation is presented in Table 1.

A total of 96 diagnostic colonoscopies as well as 70 follow-up colonoscopies (21 patients underwent 3 colonoscopies while 28 patients underwent 2 colonoscopies) were performed during follow-up. Out of the follow-up endoscopies 24 demonstrated histologic remission. Median number of tissue eosinophils at diagnosis was 45 (IQR 22–73) with 25 (26%) patients showing normal tissue eosinophilia, 40 (42%) mild eosinophilia and 31 (32%) severe eosinophilia. Median eosinophil count from follow-up samples