Absolute Eosinophil Counts and Colonic Mucosal Eosinophils Based on Inflammatory Bowel Disease Severity

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ABSTRACT

Background. The inflammatory process in Inflammatory Bowel Disease (IBD) involves various types of inflammatory cells, including eosinophils. Eosinophils are not only associated with the severity of IBD but are also associated with clinical improvement. In contrast to the confirmed role of neutrophils, the role of eosinophils either in the blood or in the colonic mucosa of IBD patients have not been fully understood and confirmed.

Objectives. To determine the difference in absolute eosinophil counts in the blood based on the severity of IBD and to determine the differences in the density of colonic mucosal eosinophil based on the severity of IBD.

Methods. This is a cross-sectional study with consecutive sampling in the period August 2020 - July 2021. The research subjects were IBD inpatient or outpatient at Dr. Sardjito Hospital who met the inclusion and exclusion criteria. Researcher assessed data on absolute eosinophil counts in peripheral blood and eosinophil density in the colonic mucosa. The number of biopsies and the location of the biopsy were determined based on a colonoscopy performed by a Gastroentero-Hepatology Consultant. The calculation of eosinophils in the colonic mucosal tissue was carried out by an Anatomical Pathologist Specialist. IBD severity was assessed using the Truelove Witts score for Ulcerative Colitis (UC) and Crohn's Disease Activity Index (CDAI) for Crohn's Disease (CD) patients. Analysis using a computer program with a p value <0.05 was considered statistically significant.

Results. There were 50 research subjects with a median age of 55 years (24-77 years). There was not a big difference between the number of male and female patients, namely 26 people (52%) vs 24 people (48%). The number of UC patients were dominant, namely 48 people (96%). The severity of IBD were divided into three groups with the number of patients with mild IBD were 22 (44%), moderate were 11 (20%), and severe IBD were 18 (36%). The number of patients with eosinophilia were 7 patients (14%) and colonic mucosal eosinophilia were 5 patients (10%). Kruskal Wallis test found that the mean absolute eosinophil blood in mild IBD was 21.45 cells/μL, moderate IBD was 25.50 cells/μL, and severe IBD was 30.44 cells/μL (p = 0.152). The mean of colonic mucosal eosinophils in mild IBD was 19.48 cells/hpf, in moderate IBD was 27.00 cells/hpf, in severe IBD was 32.03 cells/hpf (p = 0.023) and there was a statistically significant moderate correlation (r = 0.392; p = 0.005).

Conclusion. There is no significant difference in absolute eosinophil counts in the blood based on the severity of IBD and there is a significant difference in the density of colonic mucosal eosinophil based on the severity of IBD.

Keywords. Inflammatory Bowel Disease (IBD), absolute eosinophil counts, colonic mucosal eosinophils

ABSTRAK

Latar Belakang. *Proses inflamasi pada* Inflammatory Bowel Disease *(IBD) melibatkan berbagai jenis sel inflamasi, termasuk eosinofil. Eosinofil tidak hanya berhubungan dengan keparahan IBD tetapi juga berhubungan dengan perbaikan klinis. Berlawanan dengan peran netrofil, peran eosinofil baik di darah ataupun di mukosa kolon pada penderita IBD belum sepenuhnya dipahami dan terkonfirmasi.*

Tujuan Penelitian: *Mengetahui adanya perbedaan kadar eosinofil absolut di darah berdasarkan keparahan IBD dan mengetahui adanya perbedaan kepadatan eosinofil di muksa kolon berdasarkan keparahan IBD.*

Metode Penelitian. *Penelitian ini merupakan studi yang dilaksanakan menggunakan metode potong lintang dengan* consecutive sampling *dalam kurun waktu Agustus 2020 - Juli 2021. Subjek penelitian adalah penderita IBD yang menjalani rawat inap atau rawat jalan di RSUP Dr. Sardjito yang memenuhi kriteria inklusi dan kriteria eksklusi. Peneliti melakukn penilaian data kadar eosinofil absolut di darah perifer dan kepadatan eosinofil pada mukosa kolon. Jumlah biopsi dan lokasi pengambilan biopsi ditentukn berdasarkan kolonoskopi yang dilakukn oleh Konsultan Gastroentero-Hepatologi. Perhitungan eosinofil dalam jaringan mukosa kolon dilakukan oleh Spesialis Patologi Anatomi. Penilaian keparahan penyakit IBD menggunakan skor Truelove Witts untuk pasien Kolitis Ulseratif (KU) dan* Crohn's Disese Activity Index *(CDAI) untuk pasien Penyakit Crohn (PC). Analisis menggunakan program komputer dengan nilai p <0,05 dianggap bermakna secara statistik.*

Hasil Penelitian. *Terdapat 50 subjek penelitian dengan median usia 55 tahun (24-77 tahun). Jumlah pasien laki-laki vs perempuan tidak jauh berbeda yakni 26 orang (52 %) vs 24 orang (48 %). Jumlah pasien KU dominan, yakni 48 orang (96%). Keparahan IBD dibagi menjadi tiga kelompok dengan jumlah pasien IBD ringan 22 orang (44%), sedang 11 orang (20%), dan berat 18 orang (36%). Jumlah pasien yang mengalami eosinofilia sebanyak 7 pasien (14%) dan eosinofilia mukosa kolon sebanyak 5 pasien (10%). Analisis menggunakan uji Kruskal Wallis didapatkan* mean rank *eosinofil absolut darah pada IBD ringan sebesar 21,45 sel/μL, pada IBD sedang sebesar 25,50 sel/μL, dan pada IBD berat sebesar 30,44 sel/μL (p = 0,152).* Mean rank *eosinofil mukosa kolon pada IBD ringan sebesar 19,48 sel/hpf, pada IBD sedang sebesar 27,00 sel/hpf, dan pada IBD berat sebesar 32,03 sel/hpf (p = 0,023). Terdapat korelasi sedang yang bermakna secara statistik (r = 0,392; p = 0,005).*

Kesimpulan. *Tidak terdapat perbedaan bermakna kadar eosinofil absolut di darah berdasarkan keparahan IBD dan terdapat perbedaan bermakna kepadatan eosinofil di mukosa kolon berdasarkan keparahan IBD.*

Kata kunci. Inflammatory Bowel Disease *(IBD), eosinofil absolut darah, eosinofil mukosa kolon*

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic idiopathic disease, which is thought to be mediated by immune processes in the body against the digestive tract. The two major types of this disease are ulcerative colitis (UC) and Crohn's disease (CD).[1] The inflammatory process in IBD involves various types of inflammatory cells. The role of neutrophils in IBD has been confirmed, namely their role in the active state.[2] In contrast to the confirmed role of neutrophils, the role of eosinophils is still not fully proven.

Eosinophils are multifunctional leucocytes involved in the pathogenesis of various inflammatory processes.[3] Eosinophils in the gastrointestinal tract participate in physiological mechanisms, namely: (1) maintenance of the barrier function of the gastrointestinal mucosa; (2) providing immune response to pathogens in the intestinal lumen; (3) interaction with the enteric nervous system; (4) linking natural and adaptive immune responses. In the gastrointestinal tract there is a role for eotaxin-1 which modulates the accumulation of eosinophils in the gastrointestinal tract.

Serum eotaxin-1 was reported to be elevated in active IBD.[3,4] Active eosinophils will last longer, eosinophils will degranulate resulting in the release of cytotoxic granules and proinflammatory cytokines which will cause damage to enterocytes and colonocytes.[5] However, eosinophils can also have a repair effect.[6] The role of eosinophils in the tissue repair process is associated with the activation function of fibroblasts, where fibroblasts are the main target cells for tissue healing and remodeling.[7.8]

This study aims to determine the difference in absolute eosinophil counts in the blood based on the severity of IBD and to determine the differences in the density of eosinophils in the colonic mucosa based on the severity of IBD.

METHODS

This study is a cross sectional study with consecutive sampling in the period August 2020 to July 2021. The research subjects were IBD patients undergoing inpatient or outpatient treatment at Dr. Sardjito Hospital who met the inclusion criteria (adult aged > 18 years; diagnosed with IBD based on clinical criteria, colonoscopy and histological findings; have complete medical record clinical data based on the case report form; underwent a colonoscopy at RSUP Dr. Sardjito; sign the informed consent) and exclusion criteria (having malignancies such as: colorectal, gynecological; comorbid such as: tuberculosis, HIV, autoimmune diseases; infections of the gastrointestinal tract such as: worms, fungi; radiation history). This study assessed data on absolute eosinophil counts in peripheral blood and eosinophil density in the colonic mucosa. The number of biopsies and the location of the biopsy were determined based on a colonoscopy performed by a Gastroentero-Hepatology Consultant. The calculation of eosinophils in the colonic mucosal tissue is carried out by an Anatomical Pathologist Specialist. IBD severity was assessed using the Truelove Witts score for UC patients and the Crohn's Disease Activity Index (CDAI) for CD patients. Bivariate analysis for numerical data uses the T-test for normally distributed data and the Mann Whitney test for non-normally distributed data. Multivariate analysis for normally distributed data uses ANOVA and Kruskal Wallis for non-normally distributed data. Data were processed using a computer program with a p value <0.05 considered statistically significant.

RESULT

Table 1 shows that there were 50 study subjects with a median age of 55 years (24-77 years). The number of male vs female patients were not much different, namely 26 people (52%) vs 24 people (48%). UC patients were dominant, namely 48 people (96%). The severity of IBD were divided into three groups with the number of patients with mild

IBD 22 (44%), moderate 11 people (20%), and severe 18 people (36%). The number of patients with eosinophilia were 7 patients (14%). The median eosinophilia value was 130 cells/μL (0-1470 cells/μL). The number of patients with colonic mucosal eosinophilia were 5 patients (10%). The median colonic mucosal eosinophil value was 8.5 cells/hpf (0-172 cells/hpf).

Table 1. Basic Characteristics of Research Subjects

Subject Characteristics	N(%)	Median (min-max)
Age (years)		55 (24-77)
Gender		
- Man	26 (52%)	
- Woman	24 (48%)	
IBD Type		
- Ulcerative Colitis	48 (96%)	
- Crohn's disease	2 (4%)	
IBD Severity		
- Mild	22 (44%)	
- Moderate	10 (20%)	
- Severe	18 (36%)	
Blood absolute eosinophils		130 (0-1470)
Eosinophilia (absolute eosinophilia of blood > 350 cells/μL)	7 (14%)	
Colonic mucosal eosinophils		8.5 (0-172)
Colonic mucosal eosinophilia (eosinophils > 25 cells/hpf)	5 (10%)	
Allergy History	11 (22%)	

Differences in absolute blood eosinophil counts and eosinophil density in the colonic mucosa based on the severity of IBD are shown in Tables 2, 3, 4, 5, 6, and 7. Kruskal Wallis test found the mean absolute blood eosinophil count for mild IBD was 21.45 cells/μL, moderate IBD was 25.50 cells/μL, and severe IBD was 30.44 cells/μL ($p = 0.152$). The mean of colonic mucosal eosinophils of the mild IBD was 19.48 cells/hpf, the moderate IBD was 27.00 cells/hpf, and the severe IBD was 32.03 cells/hpf ($p = 0.023$).

Table 2. Analysis of Differences in Blood Absolute Eosinophil Counts and Eosinophil Density in Colonic Mucosa based on Severity of IBD

	IBD Severity (*Mean Rank*)			p
	Mild (22)	Moderate (10)	Severe (18)	
Blood Absolute Eosinophils	21.45	25,50	30.44	0.152
Colonic Mucosal Eosinophils	19.48	27,00	32.03	0.023

Kruskall's Wallis test

In this study, bivariate analysis of severity was performed using the Mann Whitney test. The results of the analysis of differences in absolute blood eosinophil counts and eosinophil density in the colonic mucosa based on the severity of IBD which

were divided into two groups are shown in Table 3, 4, and 5.

Table 3, 4 and 5 show that all analyzes of differences in absolute blood eosinophil counts based on the severity of IBD did not show any significant differences ($p > 0.05$). In the analysis of differences in the density of colonic mucosal eosinophils involving moderate IBD severity (Table 3 and 4), the results were not significant ($p > 0.05$) but in Table 5 the results showed a significant difference ($p = 0.009$).

Table 3. Analysis of Differences in Blood Absolute Eosinophil Counts and Eosinophil Density in Colonic Mucosa based on Mild and Moderate IBD Severity

	IBD Severity (*Mean Rank*)		
	Mild (22)	Moderate (10)	p
Blood Absolute Eosinophils	15.57	18.55	0.404
Colonic Mucosal Eosinophils	14.84	20,15	0.136

Mann Whitney test

Table 4. Analysis of Differences in Blood Absolute Eosinophil Counts and Eosinophil Density in Colonic Mucosa based on Moderate and Severe IBD Severity

	IBD Severity (*Mean Rank*)		
	Moderate (10)	Severe (18)	p
Blood Absolute Eosinophils	12.45	15.64	0.325
Colonic Mucosal Eosinophils	12.35	16.69	0.302

Mann Whitney test

Table 5. Analysis of Differences in Blood Absolute Eosinophil Counts and Eosinophil Density in Colonic Mucosa based on Severity of Mild and Severe IBD

	IBD Severity (*Mean Rank*)		
	Mild (22)	Severe (18)	p
Blood Absolute Eosinophils	17.39	24.31	0.062
Colonic Mucosal Eosinophils	16.14	25.83	0.009

Mann Whitney test

Based on Table 3 and 4, the difference in absolute blood eosinophil counts and eosinophil density in the colonic mucosa involving moderate IBD severity had a *p* value > 0.05. We performed the analysis by combining moderate severity in one of the other two severity. In Table 6 the severity of IBD is divided into mild-moderate and severe with the results of the mean absolute blood eosinophil rank on mild-moderate IBD severity of 22.72, on severe IBD severity of 30.44 ($p = 0.072$). The mean rank of colonic mucosal eosinophils in mild-moderate IBD severity was 21.83, at severe IBD severity 32.03 ($p = 0.017$).

In Table 7 the severity of IBD is divided into mild and moderate-severe with the results of the mean absolute blood eosinophil rank

on the severity of mild IBD of 21.45, on the severity of moderate-severe IBD of 28.68 (p = 0.100). The mean rank of colonic mucosal eosinophils at the severity of mild IBD was 19.48, at the severity of moderate-severe IBD was 30.23 (p = 0.009).

This study showed a correlation between colonic mucosal eosinophil density and the severity of IBD. In Table 8, the Spearman's correlation test was obtained *Rho* with a correlation coefficient (*r*) of 0.391. This correlation was a moderate correlation which is statistically significant (p = 0.005).

Table 6. Analysis of Differences in Blood Absolute Eosinophil Counts and Eosinophil Density in Colonic Mucosa based on Severity of IBD Mild - Moderate, and Severe

	IBD Severity (*Mean Rank*)		p
	Mild – Moderate (32)	Severe (18)	
Blood Absolute Eosinophils	22.72	30.44	0.072
Colonic Mucosal Eosinophils	21.83	32.03	0.017

Mann Whitney test

Table 7. Analysis of Differences in Blood Absolute Eosinophil Counts and Eosinophil Density in the Colonic Mucosa based on the Severity of Mild and Moderate – Severe IBD

	IBD Severity (*Mean Rank*)		p
	Mild (22)	Moderate – Severe (28)	
Blood Absolute Eosinophils	21.45	28.68	0.082
Colonic Mucosal Eosinophils	19.48	30.23	0.009

Mann Whitney test

Table 8. Correlation of Eosinophils with Severity of IBD

	IBD Severity	
	r	p
Blood Absolute Eosinophils	0.277	0.052
Colonic Mucosal Eosinophils	0.391	0.005

Spearman's *Rho* test

DISCUSSION

A total of 50 patients who were research subjects had a median age of 55 years. The comparison of the number of male and female patients in this study was not much different, namely 26 men (52%) and 24 women (48%). UC prevalence during 2010 - 2014 at Dr. Saiful Anwar Hospital is 8.2% with 53.4% male and 46.6% female. This disease can occur at any age, but the incidence of onset based on age occurs in a bimodal pattern, with the largest peak at the age of 15-25 years and the second peak (smaller) at the age of 55-65 years.[9]

The number of UC patients in this study was 48 patients (96%) compared to CD patients, which were 2 patients (4%). The prevalence of IBD in Asia is lower than the prevalence in Europe, but the incidence of IBD in Asia is increasing rapidly. The

prevalence of UC in Taiwan in 2015 was higher than that of CD. Similar things were reported in studies in Hong Kong, South Korea, and Japan.[10]

This study found the average blood eosinophil level in IBD patients was 201.80 cells/µL. It was found that 14% of IBD patients had eosinophilia (> 350 cells/µL). The results in this study showed a smaller proportion of eosinophilia compared to previous studies. In a previous study, the proportion of IBD subjects who experienced an increase in absolute eosinophils in peripheral blood was 19.2%. In the study of the pediatric population, a larger proportion was found, i.e. 27% of patients had eosinophilia on blood examination.[12] The incidence of peripheral blood eosinophilia is more common in cases of childhood-onset IBD compared to adult-onset IBD.[13]

Several previous studies have shown a different relationship between absolute blood eosinophils and the severity of IBD. In a retrospective study of 142 subjects, there was no difference in peripheral blood eosinophil counts in UC and CD patients and there was no association with the severity of the disease based on histology. Absolute blood eosinophils were not associated with disease recurrence and hospitalization rates.[14] However, there are other studies that give different results. In a large prospective cohort study (2066 subjects), the incidence of peripheral blood eosinophilia in IBD patients

was found in only a minority of patients, but the incidence of eosinophilia was associated with inflammation and severity of IBD patients followed over a multi-year period when compared with patients without peripheral blood eosinophilia.[11] In another study with a pediatric population, peripheral blood eosinophilia was more common in UC than in CD (61.3% vs. 36.3%, $p < 0.05$). Significantly associated with disease activity of UC and CD.[15] The incidence of peripheral blood eosinophilia is more common in cases of childhood-onset IBD compared to adult-onset IBD.[13] This discrepancy in results could be due to different study designs, or to the current more effective medical therapy, thereby controlling inflammation and reducing eosinophilic signaling. The lower prevalence of peripheral blood eosinophilia in adults may be related to age-mediated differences.[11]

Peripheral blood eosinophilia in IBD is associated with younger age, comorbid asthma, rheumatic diseases, adrenal insufficiency, and primary sclerosing cholangitis.[11] Smoking can increase the incidence of UC and CD[1,15] however, smoking status alone is not associated with peripheral blood eosinophilia in IBD.[11]

This study found the average density of eosinophils in the colonic mucosal tissue in IBD patients was 13.84 eosinophils/hpf. It was found that 10% of patients had colonic mucosal eosinophilia, namely eosinophils >

25 cells/hpf were found. These results are lower when compared to the results of previous studies. The results of previous studies showed an increase in eosinophils in the tissue by 50%.[14] In another study with pediatric subjects, the results of an increase in tissue eosinophils were > 50% of the research subjects.[12]

In this study, the mean rank of colonic mucosal eosinophil density increased based on the severity of IBD. Based on this result, the researchers conducted additional analysis to determine the correlation between the two. The results of the analysis showed that there was a correlation between the density of colonic mucosal eosinophils and the severity of IBD. Spearman's correlation test *Rho* between colonic mucosal eosinophil density and IBD severity showed a correlation with the strength of the statistically significant moderate/moderate relationship (r = 0.391; *p* = 0.005).

Several previous studies showed different results. In a 1987 study, a prospective study concluded that there was no correlation between tissue eosinophilia and UC severity.[17] However, in a retrospective study involving the pediatric population, it was found that tissue eosinophilia was associated with IBD severity (*p* = 0.02). The study also concluded that tissue eosinophilia and peripheral blood eosinophilia can serve as diagnostic markers for disease activity, severity and short-term outcome in the pediatric population.[12]

The difference in the results in these studies is very likely based on the existence of several roles of eosinophils in the gastrointestinal tract. The important role of eosinophils in IBD is as a pro-inflammatory and promoting agent so that it can cause effects such as diarrhea, inflammation, tissue destruction, fibrosis and stricture formation, but can also have a repair effect.[6] Eosinophils can communicate directly with T cells and mast cells in a two-way manner. Eosinophils activate T cells by acting as antigen-presenting cells and eosinophil-derived MBP can trigger mast cells. The presence of a stimulus in the form of tissue damage, viral/bacterial infection, allergens, allografts, tumors will stimulate the degranulation of eosinophils.[18] After recruitment into inflammatory tissue, eosinophils cause tissue damage by generating oxidative stress via EPO. Eosinophil peroxidase will damage the architectural organization of the extracellular matrix, promoting cell cytotoxicity.[8]

On the other hand, the role of eosinophils in the tissue repair process is associated with the activation function of fibroblasts where fibroblasts are the main target cells for tissue healing and remodeling.[7] Eosinophils can induce fibrosis directly and indirectly. Eosinophils can stimulate fibrosis directly, by releasing transforming growth factor (TGF -β), IL4, and IL13. Indirectly,

eosinophils can trigger fibrosis by stimulating epithelial cells in the tissue via MBP or EPO to express pro-fibrotic mediators.[8]

In IBD, eosinophils can usually be found among the neutrophils, suggesting a negative prognosis.[3] Eosinophils play a role in intestinal barrier dysfunction and dysregulation of the immune system in the gastrointestinal tract.[4] However, the opposite statement was concluded in other studies. In IBD with a predominance of eosinophils can reduce the risk of recurrence and treatment.[14]

In inflammatory conditions, the number of eosinophils increases. Morphological and immunohistochemical studies have revealed eosinophil activation in inflammatory diseases. However, Lampinen et al. found that eosinophil activity was higher during the inactive phase than the active/inflammatory phase. These observations suggest that eosinophils may be involved in the resolution of inflammation and repair of damaged gut tissue.[2] Another study reported that eosinophilia was more common in UC than in CD. Eosinophilia occurs more frequently in males and is of higher severity.[19]

In this study there were 22% of patients who had a history of allergies. Allergy history did not differentiate between history of allergy to drugs, food, or environmental materials. This study also looked at differences in absolute blood eosinophil counts and eosinophil density in the colonic

mucosa with a history of allergies in IBD patients. The mean absolute blood eosinophil rank in patients with a history of allergies was 25.91, in patients without a history of allergies was 25.38 ($p = 0.916$). The mean rank of colonic mucosal eosinophils in patients with a history of allergies was 27.45, in patients without a history of allergies was 24.95 ($p = 0.614$). The results of the analysis showed that there was no statistically significant difference between eosinophils in either the blood or the colonic mucosa based on a history of allergies in IBD patients.

CONCLUSION

This study concludes that there is no difference means absolute eosinophil counts in the blood based on severity IBD and there is a significant difference in the density of eosinophils in the colonic mucosa based on the severity IBD. However, this study found a disproportionate number of UC and CD patients so that the results of the study only represented UC patients. Therefore, further multicenter research is needed that considers the proportion of UC and CD patients.

REFERENCES

1. Fauci A. S., Longo D. L., Jameson L., Kasper D., Hauser S. L. 2012. Inflammatory Bowel disease. Harrison's Principles of Internal Medicine. 8th ed. The Mac-raw Hill Companies, Inc.
2. Lampinen M., Ronnblom A., Amin K., Kristjansson G., Rorsman F., *et al.* 2005. Eosinophil granulocytes are activated during the remission phase of ulcerative

colitis. *gut.* 54:1714–1720. doi:10.1136/gut.2005.066423.

3. Rothenberg M. E & Hogan SP 2006. The Eosinophil. *annu. Rev. Immunol.* 24:147–74.

4. Loktinov A. 2019. Eosinophils in the gastrointestinal tract and their role in the pathogenesis of major colorectal disorders. *World J Gastroenterol.* 25(27): 3503-3526.

5. Rothenberg ME 2004. Eosinophilic gastrointestinal disorders (EGID). *J Allergy Clin Immunol.* 113:11-28.

6. Al-Haddad S & Riddell RH 2005. The role of eosinophils in inflammatory bowel disease. *gut.* 54:1674–1675. doi: 10.1136/gut.2005.072595.

7. Xu, X., Rivkind, A., Pikarsky, A., Pappo, O., Bischoff, SC, & Levi Schaffer , F. , *et al.* 2004. Mast cells and eosinophils have a potential profibrogenic role in Crohn's disease. *Scandinavian Journal of Gastroenterology.* 39(5): 440–447. doi: 10.1080/0036552031000856.

8. Ramirez GA, Yacoub MN, Ripa M., Mannina D., Cariddi A., *et al.* 2018. Eosinophils from Physiology to Disease: A Comprehensive Review. *Hindawi BioMed Research International.* https://doi.org/10.1155/2018/909527 5.

9. Mustika S & Triana N, 2016. The Prevalence, Profile, and Risk Factor of Patients with Ulcerative Colitis at Dr. Saiful Anwar Malang General Hospital. *The Indonesian Journal of Gastroenterology, Hepatology and Digestive Endoscopy.* 17(1):16-20.

10. Hammer T & Langholz E. 2020. The epidemiology of inflammatory bowel disease: balance between East and West? A narrative review. *Dig Med Res.* 3:48

11. Click B., Anderson AM, Koutroubakis IE, Rivers CR, Babichenko D., *et al.* 2017. Peripheral Eosinophilia in Patients with Inflammatory Bowel Disease Defines an Aggressive Disease Phenotype. *Am J Gastroenterol.* doi:10.1038/ajg.2017.402

12. Morgensterm S, Brook E, Rinawi F, Shamir R, Assa A. 2016. Tissue and peripheral eosinophilia as predictors for disease outcome in children with ulcerative colitis. *Dig Liver Dis.* 49(2):170-174.

13. Prathapan KM, Rivers CR, Anderson A, Koutroumpakis F, Koutroubakis IE, *et al.* 2020. Peripheral Blood Eosinophilia and Long-term Severity in Pediatric-Onset Inflammatory Bowel Disease. *Inflamm Bowel Dis.* 26 (12): 1890-1900.

14. Alhmoud T., Gremida A., Colom Steele D., Fallahi I., Tuqan W., Nandy N., *et al.* 2020. Outcomes of inflammatory bowel disease in patients with eosinophil-predominant colonic inflammation. *BMJ Open Gastro.* 7:e000373. doi:10.1136/ bmjgast-2020-000373

15. Sadi G. , Yang Q., Dufault B., Stefanovici C., Stoffman J., *et al.* 2016. Prevalence of Peripheral Eosinophilia at Diagnosis in Children With Inflammatory Bowel Disease. *J Pediatr Gastroenterol Nutr.* 62(4):573-6.

16. Dharmika S. 2009. Inflammatory bowel disease in Sudoyo AW, Setiyohadi, B (Eds): Textbook of Internal Medicine 5 th edition. InternaPublishing. Jakarta. 591-597

17. Sarin SK., Malhotra V., Sen Gupta S., Karol A., Gaur SK, *et al.* 1987. Significance of eosinophil and mast cell counts in rectal mucosa in ulcerative colitis. A prospective controlled study. *Dig Dis Sci.* 32(4):363-7. doi:10.1007/BF01296289.

18. Hogan SP, Rosenberg HF, Moqbel R., Phipps S., Foster PS, *et al.* 2008. Eosinophils: Biological Properties and Role in Health and Disease. *Clinical and Experimental Allergy.* 38: 709–750

19. Barrie A., El-Mourabet M., Weyant K., Clarke K., Gajendran M., *et al.* 2013. Recurrent blood eosinophilia in ulcerative colitis is associated with severe disease and primary sclerosing cholangitis. *Dig Dis Sci .* 58(1): 222–228.