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# Relationship between Interleukin-6 Expression with the Progressive and Severity of Ulcerative

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## Abstract

**Background.** Based on international statistics, the incidence of Inflammatory Bowel Disease (IBD) is about 2.2-14.3 cases per 100.000 people per year for Ulcerative Colitis (UC). In Indonesia there is no epidemiological study of IBD. To assess the degree of severity associated with the ulcerative colitis disease can be used a variety of tools, including the Truelove Witts score which has sensitivity and specificity that high enough for UC in the phase of active disease. Interleukin-6 (IL-6) is a pro-inflammatory cytokine that plays a very important role in self-defense mechanism and acts as an acute phase protein. Serum levels of IL-6 and increased expression in the colonic mucosa of active IBD patients indicate the level of IBD disease activity.

**Aims.** The aim of the study was to determine the relationship between Interleukin-6 expression (IL-6) and the severity of UC disease using Truelove Witts classification.

**Methods.** This study used a cross sectional method that analyzed the relationship between IL-6 expression and UC weighing based on Truelove Witts classification using colon tissue biopsy results from UC patients who met inclusion and exclusion criteria.

**Results.** There were 26 subjects studied and performed an endoscopic analysis and anatomical pathology. Mean age of research subjects were 52.73 + 11.11 years with men's favored subjects (n = 15). Subjects were severe UC 16 (61.5%), moderate 7 (26.9%) and mild 3 (11.5%). More male subjects in mild UC, 12 vs. 3 and more female subjects in moderate-severe UC, 4 vs. 7 (p 0.032). Significant differences in IL-6 expression in defecation were > 4 times/day, IL-6 300 (285-400) versus defecation < 4 times/day, IL-6 295 (212.1-340) p 0.039. Significant difference in IL-6 expression between mild UC 295 (212.1-340) with moderate- severe UC 301.66 (235.57-400) with p value 0.032.

**Conclusion.** IL-6 expression in moderate-severe UC subjects were higher than mild UC subjects based on Truelove Witts criteria.

**Keywords:** *Inflammatory Bowel Disease, Ulcerative Colitis, Truelove Witts classification, Interleukin-6.*

## Abstrak

**Latar belakang.** Berdasarkan statistik internasional, insiden Inflammatory Bowel Disease (IBD) sekitar 2,2-14,3 kasus per 100.000 orang per tahun untuk untuk kolitis ulseratif (KU). Di Indonesia belum ada studi epidemiologi tentang IBD. Untuk menilai derajat keparahan yang berhubungan dengan fase penyakit kolitis ulseratif dapat digunakan berbagai perasat, diantaranya adalah skor Truelove Witts yang memiliki sensitivitas dan spesifisitas yang cukup tinggi untuk KU pada fase penyakit aktif. Interleukin-6 (IL-6) adalah sitokin pro-inflamasi yang berperan sangat penting pada mekanisme pertahanan diri dan berfungsi sebagai protein fase akut. Kadar serum IL-6 dan peningkatan ekspresinya pada mukosa kolon pasien IBD aktif menunjukkan tingkat aktivitas penyakit IBD.

**Tujuan Penelitian.** Untuk mengetahui hubungan antara ekspresi IL-6 dengan keparahan penyakit KU menggunakan klasifikasi Truelove Witts.

**Metode.** Penelitian ini menggunakan metode cross sectional yang menganalisa hubungan antara ekspresi IL-6 dengan beratnya KU berdasarkan klasifikasi Truelove Witts menggunakan hasil pemeriksaan biopsi jaringan kolon dari pasien KU yang memenuhi kriteria inklusi dan eksklusi.

**Hasil Penelitian.** Sebanyak 26 subyek penelitian diteliti dan dilakukan analisa secara endoskopi serta patologi anatomi. Rerata usia subyek penelitian  $52,73 \pm 11,11$  tahun dengan subyek terbanyak pria ( $n=15$ ). Subyek dengan severitas KU 16 (61,5%), sedang 7 (26,9%) dan berat 3 (11,5%). Lebih banyak subyek pria pada KU derajat ringan 12 vs. 3 dan lebih banyak subyek wanita pada KU derajat sedang-berat 4 vs. 7 ( $p 0,032$ ). Perbedaan bermakna pada kriteria BAB  $> 4$  kali/hari, kadar IL-6 300 (285-400) berbanding dengan BAB  $< 4$  kali/hari, IL-6 295 (212,1-340)  $p 0,039$ . Perbedaan bermakna ekspresi IL-6 antara KU derajat ringan 295 (212,1-340) dengan KU derajat sedang-berat 301,66 (235,57-400) dengan nilai  $p 0,032$ .

**Kesimpulan.** Ekspresi IL-6 pada subyek KU derajat sedang-berat lebih tinggi dibandingkan dengan KU derajat ringan berdasarkan kriteria Truelove Witts.

**Kata kunci:** Inflammatory Bowel Disease, kolitis ulseratif, klasifikasi Truelove Witts, Interleukin-6.

## Introduction

Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease that involves the gastrointestinal tract, is remission and relapse with the exact cause so far not known clearly. Broadly speaking IBD consists of ulcerative colitis (UC), Crohn's disease (PC) and when it is difficult to distinguish both, it is included in the category of indeterminate colitis.<sup>1</sup>

Based on international statistics, IBD incidence is around 2.2-14.3 cases per 100.000 people per year for UC and 3.1 to 14.6 cases per 100.000 people per year for Crohn disease.<sup>2</sup> The incidence of ulcerative colitis in the United States is about 15 per 100.000 people respectively and remains constant. This prevalence estimated at 200 per 100.000 population.<sup>3</sup> There are no epidemiological study of IBD1 for UC incidence at Sardjito General Hospital in 2014 from medical record obtained 35 inpatients and 14 outpatients (Sardjito General Hospital medical records data, 2014).

Ulcerative colitis just like Crohn's disease has phases in the course of the illness that is the active phase or remission. In the active

phase, there is a decrease in oral nutrition intake due to abdominal pain and anorexia. Inflammatory bowel mucosa may accompanied by diarrhea causing loss of protein, blood, minerals, electrolytes and various elements and nutrient mal-absorption. As a result, in this active phase patients are at risk for malnutrition.<sup>4</sup> Assessment of this degree of severity especially in the active phase is important, as it will determine treatment considerations, counseling and prognosis in IBD patients.<sup>5</sup>

Practices for assessing the degree of severity associated with the ulcerative colitis phase can be used in various traps, including Truelove Witts score, Mayo score and the classification of Montreal.<sup>6,7</sup>

The Truelove Witts classification is one of the criteria that assess the degree of UC clinic based on frequency of diarrhea, presence/absence of fever, degree of severity of anemia and rate of sedimentation or C-reactive protein (CRP).

The Truelove Witts classification compared to the Mayo and Montreal criteria

has considerable sensitivity and specificity for in the active disease phase<sup>7</sup>. UC patients have an activated immune response, both the congenital immune system (macrophages, neutrophils) and the acquired immune (T cells and B cells), which lose tolerance to commensal bacteria in the intestinal mucosa. In the immune system, T cells play an important role in regulating the proliferation and differentiation of B cells into antibody-producing cells.

Further research by purification and cloning showed that BSF-2 consisted of 184 amino acids weighing 26 kDa, which based on agreement finally named interleukin 6<sup>8</sup>. Interleukin 6 is a more prominent mediator than other cytokines for the systemic response of a localized inflammation. Interleukin 6 is a pro-inflammatory cytokine that plays a very important role in self-defense mechanism and acts as an acute phase protein that induces the differentiation of B cells and T cells so that it can be an indicator for the assessment of the diseases of UC<sup>9</sup> disease. Levels of serum interleukin 6 and increased expression in colonic mucosa of active IBD patients indicate the level of IBD<sup>10</sup> disease activity.

The purpose of this study was to determine the relationship between IL-6 expression and the severity of UC disease using Truelove Witts classification.

## Methods

The subjects of this study were patients aged over 18 years who declared ulcerative colitis based on endoscopy results between December 2016 and July 2017 at Dr. Sardjito Hospital, PKU Muhammadiyah Bantul Hospital and willing to follow the research by signed informed consent. Exclusion criteria of

this study were patients who did not experience the disease of colorectal malignancy.

The gastro-entero hepatology polyclinic patients and Dr. Sardjito General Hospital ward that fulfill the inclusion and exclusion criteria got an explanation of the research. Patient data record included complaint manifestation of ulcerative colitis (frequency of diarrhea with or without blood), vital signs (blood pressure, pulse, and respiratory rate and body temperature), age, sex, weight, height, BMI. Patients also have taken blood samples to obtain Hb and LED values. Data obtained from the history, physical examination and laboratory examination were recorded in the study form. Degree classification of Truelove Witts, interleukin 6 expressions and statistical analysis were assessed.

## Statistical Analysis

Independent variables were the degree of severity of ulcerative colitis used the Truelove Witts classification and dependent variables were IL-6 tissue expression based on immunohistochemistry. Univariate analysis of basic characteristic data was processed and exposed by descriptive method in mean  $\pm$  standard deviation (SD), median or number (%) based on existed variables and categorical in terms of number and percentage. To determine the relationship between IL-6 expression and degree of ulcerative colitis by Truelove Witts classification was excluded by Kruskal Wallis test, which was confirmed by Mann Whitney test. The data was processed using the IBM Statistical Product and Service Solutions (SPSS) version 22-computer program. The p value  $<0.05$  was considered statistically significant.

## Results

In this study, 27 research subjects checked into polyclinic in Dr. Sardjito General Hospital and PKU Muhammadiyah Bantul Hospital. After done the colonoscopy, a macroscopic ulcerative colitis image was confirmed by biopsy examination. The 27 subjects were studied by data analysis and passed the assessment the severity of ulcerative colitis based on Truelove Witts criteria (mild, moderate and severe).

The mean age of the study subjects was  $52.73 \pm 11.11$  years with 15 subjects were men (57.7%). Most subjects did not smoke 21 subjects (80.8%), average body mass index of  $22.18 \pm 4.97$  kg / m<sup>2</sup>. Mean of hemoglobin level  $12.77 \pm 1.71$  g / dl; leukocyte  $8.22 \pm 2.86$  10<sup>3</sup>/mm<sup>3</sup> and ESR 21.26 (4-63) mm/h. UC mild degree was obtained in 16 subjects (61.5%), moderate degree in 7 subjects (26.9%) and degree of weight in 3 subjects (11.5%). The median value of interleukin 6 in this study was 297.55 with the lowest score 212.1 and the highest value 400. The severity level of UC in male subjects was the highest with mild degree 13 subjects (81.25%) and moderate-severe 3 subjects (18.75%). The women subjects weighing 7 subjects (63.67%) and mild degree lightness 4 subjects (36.33%) with p value 0.032. While age, BMI, smoking status and Salofak therapy did not significantly different.

## Discussion

The significant differences were seen in the defecation criteria > 4 times/day where the IL-6 300 (285-400) levels were compared with defecation <4 times/day, IL-6 295 (212.1-340) p 0.039. Other criteria components such as pulse frequency, body temperature, Hb, ESR and CRP levels found no significant differences.

Based on table 4, we found that IL-6 levels were based on Truelove Witts criterion were 295 (212.1-340) for mild severity and 301.66 (235.57-400) for moderate-severe with a p value of 0.032, meaning that there was a significant difference between the severity mild to moderate-severe ulcerative colitis.

In this study obtained the subject of 15 male and 11 female. Epidemiologically, the incidence of ulcerative colitis in males and females found no significant difference with 5.7 point. The mean age of the entire study subjects was  $52.73 \pm 11.11$  years. According to research in the United Kingdom, UC 5 disease can affect any age but tend to be higher in the third or fourth decade.<sup>5</sup> In Indonesia, this epidemiological study has not yet been explored. Based on body mass index, the subject of study has not included the category of malnutrition. Underweight and nutrient status were commonly found in patients with Crohn disease and its lower in ulcerative colitis. One study suggested that body mass index was higher in ulcerative colitis (25.2 kg/m<sup>2</sup>) than Chron disease (22.8 kg/m<sup>2</sup>) 11.

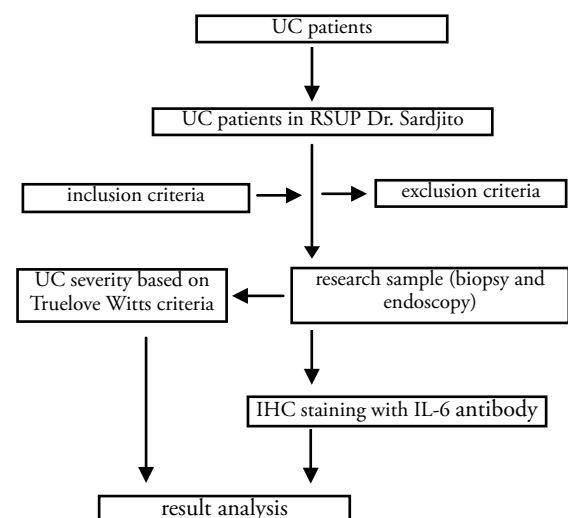


Figure 1. Research flow

**Table 1.** Baseline Characteristic

Baseline Characteristic	n (%)	Mean±SB / Media (min-max)
Age		52.73±11.11
Sex		
Men	15 (57.7)	
Women	11 (42.3)	
Smoking Status		
Non-smoker	21 (80.8)	
Active smoker	1 (3.8)	
Ex-smoker	4 (15.4)	
BMI (kg/m <sup>2</sup> )		22.18±4.97
Hemoglobin (g/dl)		12.77±1.71
Leukocyte (10 <sup>3</sup> /mm <sup>3</sup> )		8.22±2.86
ESR (mm/hour)		21.26 (4-63)
Severity		
Mild	16 (61.5)	
Moderate	7 (26.9)	
Severe	3 (11.5)	
Salofalk routine	9 (34.6)	
Interleukin-6		297.55 (212.1-400)

In this study obtained the number of subjects only as many as 27 subjects. Although theoretically, ulcerative colitis may occur at any age, but the larger number of subjects according to the sample calculations will further refine the results of the study. Another limitation of the study is 5-ASA therapy in

subjects may decrease the expression of IL-6 in the mucosa due to the anti-inflammatory potential of the drug.

## Research limitations

This study conducted to determine the criteria using only restriction medical records. The subjects of the study were 110 subjects, but only 91 subjects joined the study because of the visibility time. To ensure the routinely of PLB does the research, in addition to assist the patient's family also made regular home visits to the randomized subjects. However constraints to the distance some regular visits replaced by telephone contact.

## Conclusion

The expression of IL-6 mucosal colonic tissue were higher on moderate-severe ulcerative colitis according to Truelove Witts criteria with p 0.032. Suggestion to the further study, might be continued with a larger sample size in each degree of ulcerative colitis.

**Table 2** Baseline characteristic based on UC severity by Truelove Witts criteria UC Severity

Variable	Mild 16 (61.5%) n (%)	Moderate-severe 10 (38.5%) n (%)	p Value
Sex			
Men	12 (80)	3 (20)	0.032 <sup>a</sup>
Women	4 (36.4)	7 (63.6)	
Age (years) (mean±SB)	49.62±12.2	57.7±7.1	0.070 <sup>b</sup>
BMI (kg/m <sup>2</sup> ) (mean±SB)	22.14±4.64	22.25±5.73	0.956 <sup>b</sup>
Smoking Status			
Ex-Smoker	2 (50)	2 (50)	0.653 <sup>c</sup>
Active smoker	1 (100)	0	
Non-smoker	13 (61.9)	8 (38)	
Salofalk routine	7 (77.8)	2 (22.2)	0.210 <sup>a</sup>

<sup>a</sup>Fisher exact test; <sup>b</sup>Non-paired t test; <sup>c</sup>Chi-square test

**Table 3** Difference of IL-6 expression between Truelove Witts Components

Components	Criteria	Median	(min-max)	p-value
Defecation	>4 times/day	300	285-400	0.039*
	<4 times/day	295	212.1-340	
Pulse	>90 times/min	300	212.1-308.5	0.569*
	<90 times/min	300	235.57-400	
Temperature	>37.5	256.05	212.1-300	0.298*
	<37.5	300	235.57-400	
Hb	<11.5	280	235.57-330	0.442*
	>11.5	300	212.1-400	
ESR	>20 mm/hr	303.33	235.57-400	0.368*
	<20 mm/hr	300	260-340	
CRP	>20 mg/L	300	235.57-400	0.707*
	normal	300	212.1-400	

\*Kruskall Wallis test

**Table 4** Difference of IL-6 expression on different severity of UC according to Truelove Witts criteria

Severity (number)	Interleukin 6	P value
Mild (n=16)	295 (212.1-340)	0.032 <sup>a</sup>
Moderate-severe (n=10)	301.66 (235.77-400)	

<sup>a</sup>Mann-Whitney test

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