Fecal Calprotectin Level as Diagnostic Marker for Intestinal Inflammation in Inflammatory Bowel Disease Patients

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ABSTRACT

Background: *Inflammatory bowel disease (IBD) diagnosis was still based on invasive examination, such as endoscopy and histopathology. Fecal calprotectin was a non-invasive intestinal inflammation marker, but several study give a different result in its diagnostic value and correlation to inflammatory bowel disease. This research was aimed to prove that fecal calprotectin examination has a high diagnostic value in diagnosing inflammatory bowel disease, and also correlate to its clinical stages.*

Method: *This is a cross sectional study to do a diagnostic test in several hospital in Jakarta, from September 2014 to February 2015. Receiver operating characteristic (ROC) curve was made to get fecal calprotectin diagnostic level and Krusskal Wallis test was performed to identify fecal calprotectin difference among each inflammatory bowel disease clinical stages.*

Results: *A total of 71 patients with inflammatory bowel disease was invoved in this research, based on colonoscopic examination result. Among them, 57 patients was confirmed to have intestinal inflammation based on histopathology result. Fecal calprotectin level was found to be higher in patients with inflammatory bowel disease than patients without intestinal inflammation (553.8 µg/g vs. 76.95 µg/g, $p < 0.001$). A cut off point of 179.3 µg/g was gathered, with 96% sensitivity (95% CI: 0.88-0.99), 93% specificity (95% CI: 0.69-0.99), and 99.5% area under curve (AUC) 99.5% (95% CI: 0.98-1.00). A significant difference was found between fecal calprotectin in each inflammatory bowel disease clinical stages ($p < 0.001$).*

Conclusion: *Fecal calprotectin has a high diagnostic value for inflammatory bowel disease (IBD) and strongly correlate to its disease clinical stages.*

Keywords: *fecal calprotectin, histopathology, inflammatory bowel disease, cut off points*

ABSTRAK

Latar belakang: *Diagnosis penyakit radang usus masih didasarkan pada pemeriksaan invasif seperti endoskopi dan histopatologi. Fecal calprotectin merupakan petanda inflamasi intestinal non-invasif yang dapat digunakan untuk membedakan penyakit radang usus dengan penyakit intestinal non-inflamasi, namun studi-studi*

yang ada masih memberikan perbedaan nilai diagnostik dan hubungannya dengan derajat penyakit radang usus. Penelitian ini bertujuan untuk membuktikan bahwa pemeriksaan fecal calprotectin memiliki nilai diagnostik yang tinggi untuk mendiagnosis penyakit radang usus serta berhubungan dengan derajat klinis penyakit radang usus.

Metode: *Penelitian ini adalah studi potong lintang untuk melakukan uji diagnostik yang dilakukan di beberapa rumah sakit di Jakarta mulai bulan September 2014 hingga Februari 2015. Kurva receiver operating characteristic (ROC) dibuat untuk mendapatkan nilai diagnostik fecal calprotectin dan uji Krusskal Wallis untuk menilai perbedaan kadar fecal calprotectin menurut derajat penyakit radang usus.*

Hasil: *Terdapat 71 pasien penyakit radang usus berdasarkan pemeriksaan kolonoskopi diikutkan dalam penelitian. Dari pasien tersebut didapatkan sebanyak 57 pasien ditetapkan secara pasti menderita penyakit radang usus berdasarkan pemeriksaan histopatologi. Kadar fecal calprotectin lebih tinggi bermakna pada pasien penyakit radang usus dibanding yang bukan penyakit radang usus (553,8 µg/g vs. 76,95 µg/g, p < 0,001). Didapatkan nilai titik potong 179,3 µg/g dengan sensitivitas 96% (95% CI: 0,88-0,99), spesifisitas 93% (95% CI: 0,69-0,99) dan area under curve (AUC) 99,5% (95% CI: 0,98-1,00). Didapatkan perbedaan bermakna kadar fecal calprotectin pada masing-masing derajat penyakit radang usus (p < 0,001)*

Simpulan: *Pemeriksaan fecal calprotectin memiliki nilai diagnostik yang tinggi untuk mendiagnosis penyakit radang usus serta berhubungan dengan derajat penyakit radang usus.*

Kata kunci: *fecal calprotectin, histopatologi, penyakit radang usus, titik potong*

INTRODUCTION

Intestinal inflammation was the main underlying cause of inflammatory bowel disease (IBD) that manifest as sign and symptom found in patients. Nowadays, endoscopic examination was still the gold standard of intestinal inflammation diagnosis.[1,2] Other non-invasive and less expensive examination that was still used to predict intestinal inflammation was erythrocyte sedimentation rate and C reactive protein.[3,4] But, this examinations were not sensitive nor specific to show an intestinal inflammation.[4]

Previous study reported a development of decal inflammation marker to diagnose IBD. Calprotectin, found by Fagerhol in 1980, was the first intestinal inflammation in fecal. Calprotectin was a 36 kD protein that bind to calcium and zinc. Most of calprotectin derived from neutrophil and monocyte with antimicrobe and antiproliferation activity. In intestinal inflammation, calprotectin concentration in fecal was 4-6 times higer than plasma, and significantly higher in IBD patients.[5,6] Is that fecal calprotectin (FC) has a high value in diagnosing IBD? Previous study have shown a different diagnostic value with different cut off point for fecal calptrotectin.[7,8,9] One of the study conducted in Indonesia reported a significant difference between fecal calprotectin in IBD and gastrointestinal functional disorder, but did not investigate its cut off points and its correlation with IBD severity.[10]

Fecal calprotectin concentration was found to have a correlation to IBD clinical severity, especially in ulcerative colitis patients.[11,12,13] Otherwise, several studies were not found any correlation between fecal calprotectin and IBD clinical severity.[14,15,16] This study was aimed to investigate fecal calprotectin level that helps to diagnose IBD, and its application to set IBD severity, and also act as treatment evaluation and prognostic tools.

METHOD

This is a cross sectional study to find a new cut off points in diagnostic test. This study was conducted in Cipto Mangunkusumo Hospital, Jakarta and other hospitals, such as Islam Hospital Jakarta, MMC Hospital Jakarta, and Pelni Hospital Jakarta from September 2014 to February 2015. Target population was suspected IBD patients with symptoms of chronic diarrhea, hematoschezia, and abdominal pain that come to gastroenterology division and Cipto Mangunkusumo Hospital and other hospitals wards. Population was patients fulfil the inclusion criteria and willing to participate on this study. Subject was patients with IBD findings during colonoscopy examination. Non-probability sampling with consecutive sampling was used. Inclusion criteria was patients with clinical symptoms of IBD (chronic diarrhea, hematoschezia, and abdominal pain), willing to participate on this study by signing informed concent form, willing to undergo colonoscopy examination, and IBD findings was found during colonoscopy. Exclusion criteria was patients using NSAID, PPI, or in IBD medication.

Patients with IBD findings during colonoscopy was underwent a biopsy in Pathology Department of

Cipto Mangunkusumo Hospital and other hospital to get the definite diagnosis, and the fecal was taken. All fecal taken from the patients was examined in Prodia Laboratory Clinic in Jakarta to get fecal calprotectin concentration level. During laboratory examination, fecal was homogenized and saved in liquid form in -20°C. Fecal calprotectin examination was using enzyme link immune sorbent assay (ELISA), PhiCal^{R} calprotectin ELISA Kit, by quantitative sandwich enzyme immunoassay technique with dual monoclonal antibody specific to bind to human calprotectin. Conoloscopy examination was performed by internist under supervision of gastroenterology-hepatologist consultant where diagnosis was made by the consultant during the colonoscopy examination.

Data was analysed using SPSS software version 21. Fecal calprotectin cut off point was known from ROC curve. Data presented in 2 x 2 table to get positive predictive value (PPV), negative predictive value (NPV), positive possibility ratio (PPR), and negative possibility ratio (NPR). Differences between mean fecal calprotectin level and IBD severity was analysed using Kruskal Wallis test, continued by post hoc analysis using Mann Whitney test, considered positive if $p < 0.05$.

RESULTS

During 6 months period, from September 2014 to February 2015, 71 patients with IBD suggestion was involved in this research. From all subjects, based on pathological examination, 57 (80%) subjects was diagnosed IBD (definitive), while the rest 14 patients have non-IBD diagnosis (infective colitis, reactive colitis, non-specific colitis). Forty five of IBD patients was having ulcerative colitis and 12 patients having Chron's Disease. Among all IBD diagnosed patients, disease severity was vary between mild (10 patients), moderate (18 patients), and severe (29 patients) based on Truelove and Witts classification of ulcerative colitis and The Harvey-Bradshaw classification for Chron's Disease.

In ROC curve, fecal calprotectin with 179,3 µg/g as the best cut off value with 96% sensitivity (95% CI: 0,88-0,99), 93% specificity (95% CI: 0,69-0,99), and 99,5% area under curve (AUC) (95% CI: 0,98-1,00). Cut off line identification in the curve was shown on Figure 1.

Table 1. Characteristic of patients based on IBD and non-IBD diagnosed group

Variable	IBD n = 57	Non-IBD* n = 14	p
Gender, %			
Male	32 (56.1)	8 (57.1)	
Female	25 (43.9)	6 (42.9)	
Age, median	41 (17-75)	32.5 (20-60)	
Hemoglobin, mean	11.02 (SD 1.96)	13.24 (SD 1.44)	< 0.001
Leukocyte, median	8910 (2200-39120)	7565 (4760-13310)	0.751
ESR, median	40 (10-139)	26.21 (10-55)	< 0.005
FC, median	553.8 (144-785)	76.95 (56-190)	< 0.001

*Infective colitis, reactive colitis, non-specific colitis; IBD: inflammatory bowel disease; SD: standar deviation; ESR: erythrocyte sedimentation rate; FC: fecal calprotectin

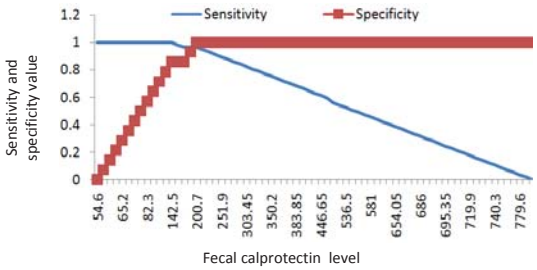

Figure 1. Curve to identify cut off line between sensitivity and specificity of fecal calprotectin level in diagnosing IBD

Based on cut off points 179.3 µg/g of fecal calprotectin level and histopathology result, a cross tabulation (2 x 2 table) was made between IBD and fecal calprotectin value to get IBD predictive value. According to cross tabulation, positive predictive value (PPV) was 98% (95% CI: 0.91-1.0), negative predictive value (NPV) was 87% (95% CI: 0.62-0.96), positive possibility ratio (PPR) was 13.51 (95% CI: 2.18-83.82), and negative possibility ratio (NPR) was 0.04 (95% CI: 0.01-0.15) (Tabel 2).

Table 2. Cross tabulation between cut off value of fecal calprotectin and IBD

Variable	IBD		Total
	Yes	No	
Fecal calprotectin			
≥ 179.3	55	1	56
< 179.3	2	13	15
Total	57	14	71

IBD: inflammatory bowel disease

Multivariate analysis using Kruskal Wallis test showed a significant difference between median fecal calprotectin and erythrocyte sedimentation rate (ESR) in each clinical stage of IBD (Table 3). This showed that the more severe IBD, the higher fecal calprotectin and ESR level found in patients. Post hoc analysis using Mann Whitney test showed a significant differences in all IBD stages: between mild and moderate IBD, mild and severe IBD, and moderate and severe IBD (Figure 2).

Table 3. Comparison between fecal calprotectin level, haemoglobin, leukocyte, and erythrocyte sedimentation rate in each IBD stages

Variable	IBD			p
	Mild	Moderate	Severe	
FC	243.10 (144-281)	399.45 (326-539)	689.7 (554-785)	< 0.001*
Hb	11.75 (SD 2.23)	10.93 (SD 1.91)	10.83 (SD 1.91)	0.440
Leukocyte	9655 (5040-39120)	7645 (2200-4270)	8730 (2300-17140)	0.171*
ESR	26.5 (15-60)	37 (10-139)	44 (10-126)	< 0.05*

*Kruskal Wallis test; IBD: inflammatory bowel disease; FC: fecal calprotectin; Hb: Hemoglobin; ESR: erythrocyte sedimentation rate

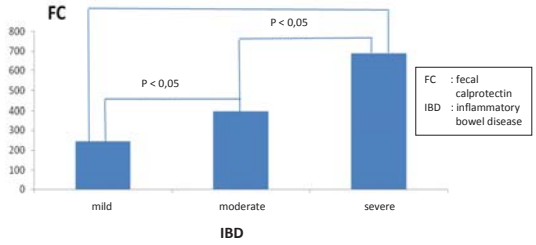

Figure 2. Difference between fecal calprotectin (FC) level in each IBD clinical stages using Mann Whitney analysis

DISCUSSION

Nowadays, endoscopy examination still become the gold standard in intestinal inflammation evaluation.[1,2] Colonoscopy diagnostic accuracy in IBD case was 89% with 4% error rate and 7% unreliable result.[1] This study showed that endoscopy still the best modalities to diagnose IBD. Median fecal calprotectin level was found to be higher in IBD patients compared to non-IBD patients which have lower intestinal inflammation (553,8 µg/g *vs.* 76,95 µg/g, respectively). All patients in this study have a fecal calprotectin level above cut off point because of the intestinal inflammation found in all patients.

Increasing fecal calprotectin level was unspecified for IBD, because this condition also found in other inflammation situation, such as infection, colorectal cancer, coeliac disease, microscopic colitis, and civerticulosis. The use of non-steroidal anti-inflammatory drugs (NSAIDs) also increase fecal calprotectin level, correlate to higher enteropathy incidence.[17] Proton pump inhibitor (PPI) was also believed to increase fecal calprotectin level.[6]

Previous study showed that fecal calprotectin was an important marker to differentiate IBD and other non-inflammatory intestinal disease.[18,19,20] Patients with non-organic disease, such as IBS, showed a lower fecal calprotectin level than organic disease, especially IBD. Several studies was using cut-off point of 50 µg/g fecal calprotectin level as positive test result to decide whether this patients have to undergo further endoscopic examination to eliminate other organic intestinal disorder.[7,21]

Fecal calprotectin level of 179,3 µg/g was choosen as the best cut-off point with 96% sensitivity (95% CI: 0.88-0.99) and 93% specificity (95% CI: 0.69-0.99) with 99.5% area under curve (AUC) (95% CI: 0.98-1.00) in diagnosing IBD. This result means that the ability of fecal calprotectin to eliminate IBD diagnosis when its level was under 179.3 µg/g was as much as sebesar 93%, and to diagnose IBD definitely when its level was above 179.3 µg/g was as much as 96%. This cut off point give a high diagnostic value of fecal calprotectin to diagnose IBD.

Although it is slightly higher (179.3 µg/g), this result was not different with other cut-off point in different study (100µg/g, 150µg/g).[8,9] A higher cut off point in this study was estimated because there was no fecal culture in all subject, except polymerase chain reaction (PCR) tuberculose (PCR-Tb) in Chron's disease suggested patients.

Immunologic mediators were played an important role in IBD etiopathogenesis. Intestinal infection and spicy hot dietary (cabage) was assumed to increase intestinal inflammation. This condition was believed to manipulate immune system to trigger chronic inflammation process resulting in IBD.[3,22] In Indonesia, with high intestinal infection prevalence, it is hard to differentiate between IBD and infection intestinal inflammation. This was caused by the high frequency of IBD and infection coincidence, even that infection was one of the cause of IBD. Infection process do not eliminate IBD diagnosis. Therefore, in this study, IBD was diagnosed based on colonoscopic findings and histopathology without infection examination. Based on those cut off points, a cross tabulation was made between IBD and fecal calprotectin level, so that positive predictive value (PPV) of 98% (95% CI: 0.91-1.0), negative predictive value (NPV) of 87% (95% CI: 0.62-0.96), positive possibility ratio (PPR) of 13,51 (95% CI: 2.18-83.82), and negative possibility ratio (NPR) of 0.04 (95% CI: 0.01-0.15).

In daily clinical practice, both four value above was important. Based on PPV and NPV, if patients had fecal calprotectin level higher than 179.3 µg/g the patients has a possibility to have IBD for 98%, dan if

fecal calprotectin level lower than 179.3 µg/g patients have a possibility of non-IBD for 87%. Patients with fecal calprotectin higher than 179.3 µg/g have a 13.51 times risk to have IBD than patients with low fecal calprotectin. This result showed that fecal calprotectin has a high clinical value in diagnosing IBD, so that it could be used as good early laboratory parameter in diagnosing IBD from clinical symptoms suggestive of IBD. In this study, there were also significant difference among median fecal calprotectin level in each IBD clinical stages. This means that the more severe IBD, the higher its fecal calprotectin level. Post hoc analysis showed a significant difference in fecal calprotectin level among all IBD stages, in mild-moderate, mild-severe, and moderate-severe.

IBD clinical history was characterized by active and remission phase. Remission phase was caused by therapy, but sometimes could be spontaneous. With this chronic exacerbation remision characteristic in IBD, several clinical criteria was made to identify its remission phase and to evaluate its therapy process. Truelove and Witts classification was used to identify ulcerative colitis clinical stages, divided into severe, moderate, and mild (remission) based on bloody diarrhea frequency, presence of fever, severity of anemia, and erythrocyte sedimentation rate (ESR).[2,23] The Harvey-Bradshaw score, on the other hand, was used to identify clinical stages of Chron's disease, classified as severe (markedly active), moderate (moderately active), and mild (remission) based on general condition status, abdominal pain, abdominal mass, diarrhea, and complications occured.[2,24]

This result was support several studies that reported a correlation between fecal calprotectin level with IBD clinical stages.[11,12,13] A significant difference in median fecal calprotectin level among three IBD clinical stages in this study showed that fecal calprotectin was clinically correlated to IBD stages, so that it can be used to identify IBD clinical stages. Therefore, fecal calprotectin was proposed to be a laboratory parameter used to evaluate the successful of IBD therapy and reduce the need of colonoscopy in primary settings. This study have some limitations. Patients distribution based on IBD stages was imbalance, so that it less representative. It also need a longer period of sampling to get higher sample number, so that more balance and accurate result will be gathered.

In this study, histopathology examination from biopsy specimen was done in pathology laboratory in different hospital, so that it was examined by different pathologist. This could lead to variation bias and interfere result validation. Infection examination, which is undone in this study, was estimated to have a contribution in deciding fecal calprotectin cut off point. A further study with better research methodology was needed to get more valid result, so that the result could by applicable for daily clinical practice.

CONCLUSION

Fecal calprotectin examination has a high clinical value in diagnosing inflammatory bowel disease (IBD) with 179,3 µg/g cut off points, 96% sensitivity, and 93% specificity. Median of fecal calprotectin level was significantly different among different IBD stages (mild, moderate, severe). A further prospective study using fecal calprotectin was needed to investigate its capability in IBD therapy evaluation, and also to make fecal calprotectin as prediction model of IBD diagnosis without invasive procedure. It also need a further study with better research methodology to get more valid result.

Table 4. Truelove and Witts classification [22]

	Severe	Moderate	Remission
Bloody diarrhea/day	< 4	4 or more, if	≥ 6, and
Heart rate	< 90 x/minute	≤ 90 x/minute	> 90 x/minute, or
Temperature	< 37,5 °C	≤ 37,8 °C	> 37,8 °C or
Hemoglobin	> 11,5 g/dL	≥ 10,5 g/dL	< 10,5 g/dL or
Erythrocyte sedimentation rate	< 20 mm/hour	≤ 30 mm/hour	> 30 mm/hour

Table 5. The Harvey–Bradshaw simplified Crohn's disease activity index [23]

Score	0	1	2	3	4
General status	Good	Slightly good	Poor	Very poor	Worst
Abdominal pain	No	Mild	Moderate	Severe	
Abdominal mass	No	Maybe	Yes	Confirmed with pain	
Diarrhea	1 for liquid consistency diarrhea/day				
Complication	1 for each disease : arthralgia, uveitis, erythema nodosum, pyoderma gangrenous, aphthous ulcer, anal fissure, new fistula or abscess				

Total disease activity score: ≤ 4 = mild/remission; 5-8 = moderate; ≥ 9 = severe.

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