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Role of Calprotectin, IL-6, and CRP in Distinguishing Between Inflammatory Bowel Disease and Diarrhea Predominant Irritable Bowel Syndrome

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ABSTRACT

Background: The early establishment of prophylaxis and immediate administration of anticoagulant therapy upon the diagnosis of venous thromboembolism should be the treatment objectives in these patients. **Objective:** The study aimed to determine the optimal cut-off point of Calprotectin, IL-6 (interleukin-6), CRP (C reactive protein) to differentiate UC, IBS-D. **Methods:** A cross-sectional descriptive study of 335 individuals ≥15 years old was performed, including 31 healthy controls, 215 with IBS-D, 71 diagnosed with UC, and 18 diagnosed with CD. Receiver Operating Characteristics (ROC), sensitivity, specificity, and area under curve (AUC) were computed. **Results:** The results showed that the median value of calprotectin (IQR) in healthy participants was 20.0 (6.0 - 34.0) µg/g; 17,7 (8,7-38,9) µg/g in IBS-D group; 1710.0 (588 - 4260,0) µg/g in UC group; and 560.5 (177.8 - 1210.0) µg/g in CD group. Calprotectin concentration in IBD group including UC and CD was higher than IBS-D with p<0.05. The median value of CRP (range IQR) was 1,3 (0,9 – 2,3) mg/L in IBS-D group; 7.0 (2.4 -16.6) mg/L in UC group; and 10.1 (2.2 - 42.5) mg/L in CD group. CRP concentration in IBD group including UC and CD was higher than IBS-D with p<0.05. The median value of IL-6 (range IQR) was 2.3 (1.6 – 5.7) pg/mL in IBS-D group; 16.8 (9.4 - 47.0) pg/mL in UC group; and 9.4 (7.9 - 11.0) pg/mL in CD group. Calprotectin concentration in IBD group including UC and CD was higher than IBS-D with p<0.05. The optimal cut-off point of calprotectin that differentiated IBS-D from IBD was 110.5 µg/g, with sensitivity and specificity of 93.3% and 91.4%, respectively; of IL-6 was 7.2 pg/mL with sensitivity and specificity of 92.0% and 78.0%, respectively; of CRP of 2.4 mg/L had specific sensitivities of 83.3% and 86.0%, respectively. **Conclusion:** The Calprotectin immunoassay has the best value in discriminating between IBD and IBS-D.

Keywords: Calprotectin, IL-6; CRP, Ulcerative colitis bleeding, Diarrhea predominant irritable bowel syndrome syndrome, Crohn's disease, Inflammatory bowel disease.

1. BACKGROUND

IBS and IBD are both common diseases and affect the gastrointestinal tract, psychological well-being, and quality of life. The prevalence of IBS is 5%-20% of the population among countries across the world (1). In the United States, patients were evenly distributed between IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), and IBS with a mixed bowel pattern (IBS-M), while in Europe, studies have found IBS-C (45.9%) or IBS-D (50%) to be the majority (1). The disease is not life-threatening but prolonged which causes the patient to worry, stress, lose sleep, and fear of experiencing other serious diseases of the intestines. The disease has been known for a long time, but the pathogenesis of the disease is still unclear. There is no radical treatment method, and the treatment is still difficult. In fact, colonic mucosal biopsies of several patients with IBS revealed dense mast cell infiltration, which releases many mediators such as serine proteases. This can be the cause of the nervous excitement and symptoms of IBS. Furthermore, food components are thought to be allergens that cross the epithelial barrier, leading to mastocyte infiltration and activation. Mast cells are activated like an allergic mechanism; however, the allergen detection and desensitization are not very sensitive; thus, it is still

a difficult problem to determine the cause and treat it completely. There are very limited data in the English literature regarding the prevalence and characteristics of IBS in the Vietnamese population (2)

IBD refers to chronic or relapsing inflammatory bowel diseases and includes primarily ulcerative colitis (UC) and Crohn's disease (CD). UC and CD are the major forms of inflammatory bowel disease of unknown etiology. Both diseases develop a complex pathology that mainly affects the gastrointestinal tract and causes different clinical symptoms. IBD has emerged as a global disease with an estimated worldwide prevalence of over 6,5 million people in 2027 (3). In Vietnam, IBD was a rare disease in the 70-80s, but recently, the disease has been on the rise. Vietnam is a rapidly developing country in Southeast Asia. the Vietnamese, like many of their Asian counterparts, are increasingly adopting Westernized diets, lifestyles, and behaviors that can increase the risk of IBD and UC is more common than Crohn's disease. However, there is still no accurate data on the incidence of UC in Vietnam because there is no statistical study on UC. Patients with IBD often have a reduced quality of life because the disease usually develops at a young age with symptoms such as abdominal pain, diarrhea, and bloody stools and progresses cyclically with cycles of relapse and remission. In addition, it is possible to develop extra-gastrointestinal complications in systemic organs such as joints, skin, and eyes. The incidence of colorectal cancer is significantly increased in UC patients with extensive lesions in long duration. UC is not considered a disease that significantly affects the survival prognosis of patients, although UC patients have a slightly shorter survival prognosis than the general population.

IBS-D and IBD can be differentially diagnosed based on symptoms, although many cases may be similar. Colonoscopy is still the gold standard for the differentiation, assessment of inflammation, progression as well as monitoring of treatment of disease treatment. However, endoscopy is an invasive, expensive, and painful procedure. In particular, endoscopy performed in children can lead to complications, be time-consuming and be quite complicated. Performing noninvasive tests such as calprotectin, C-reactive protein (CRP), and Interleukin 6 (IL-6) could help in the differential diagnosis of IBD, IBS without limiting endoscopic procedures.

Calprotectin is considered a suitable marker to differentiate these two diseases. Calprotectin is a protein complex made of calcium and zinc bound to proteins, capable of inhibiting the growth of bacteria. Calprotectin accounts for more than 60% of the total protein in the cytoplasm of neutrophils. Following phagocytosis, neutrophils and macrophages rupture and release calprotectin that invades the intestinal mucosa as part of the inflammatory response. Fecal calprotectin is a biomarker specific for intestinal inflammation, a non-invasive and effective parameter for diagnosing IBD, with high sensitivity and specificity (4). Calprotectin is also a useful marker for monitoring treatment and early detection of disease recurrence of these types of inflammation. Multiple studies have shown that increased fecal Calprotectin correlated with all major endoscopic endpoints - SES-CD (5), UCEIS (6) và Mayo Endoscopic Subscore (MES) (7). Some manufacturers recommend a fecal Calprotectin threshold of 50 μg/g for normal adults. However, some difficulties have arisen. Firstly, different methods cannot give similar quantitative results given the differences in clinical practices, population characteristics and eating habits. On the other hand, in the clinical context, using a reference value for the general population alone is unlikely to differentiate IBD from IBS; thus, a reference value for determining IBD and IBS needs to be found.

2. OBJECTIVE

This study evaluated the diagnostic value of Calprotectin, CRP and IL-6 in differentiating IBS-D and IBD.

3. MATERIAL AND METHODS

Study settings

A cross-sectional study was conducted at three tertiary hospitals in Hanoi, Vietnam (Hanoi Medical University Hospital, Thanh Nhan Hospital, and 108 Hospital) from June 2021 to December 2022. Selection criteria included a) the patient was confirmed as IBS-D based on the ROME IV diagnostic criteria; b) Patients with UC and CD were confirmed based on clinical symptoms, colonoscopy and histopathology; c) The patients with IBS-D, UC, CD all had colonoscopy. Suspicious cases were performed with histopathological examination to confirm the diagnosis. Exclusion criteria included pregnant women associated with bacterial enteritis, a history of colorectal surgery, use of certain drugs such as NSAIDs, and prednisolone. A convenient sampling method was utilized. IBS-D and IBD patients had done medical records and control tests on the first day of admission, stool samples were taken before colonoscopy. The healthy group was selected from people with physical examinations who did not have digestive diseases or other chronic iseases.

The analysis was performed on 335 people ≥ 15 years of age, of which 31 people were healthy, 215 patients with IBS-D, 71 diagnosed with UC, 18 diagnosed with CD.

Sample collection methods

The LIAISON Calprotectin assay was utilized, using chemiluminescent immunoassay (CLIA) technology with two monoclonal antibodies to capture and detect calprotectin. This method was intended for in vitro diagnostic use to quantify calprotectin in human feces. First, calprotectin was extracted from a mixture of the stool sample with an extraction buffer, using either the weighing procedure or the LIAISON Calprotectin Stool Extraction Device. Next, extracted samples/calibrators/controls were incubated with test buffers and magnetic beads coated with a monoclonal antibody that specifically recognizes the calprotectin complex. After annealing, all unbound materials were removed by a wash cycle. Next, the conjugate (monoclonal antibody recognizing calprotectin bound to an isoluminol derivative)

Characteristics	IBS-D	IBD	Control	p
n	215	86 (UC:71; CD:18)	31	
Gender (n%)				
Male	103 (47.9)	35 (39.3)	15 (48.4)	0,3
Female	112 (52.1)	54 (60.7)	16 (51.6)	
Age, mean (SD)				
	48.7 (13.2)	44.2 (14.5)	46.1 (11,9)	0,02

Table 1. Characteristics of patients

was added to the reaction and incubated. Non-binding components were removed by a second wash cycle. After that, the substrate was added, and the chemiluminescence reaction began. The photomultiplier measured the light signal as a relative light unit (RLU) and was proportional to the calprotectin concentration present in the calibrator/control/patient sample. Repeatability and reproducibility were validated in accordance with CLSI Guideline EP15A3, with results meeting manufacturer claims. The limitation of the technique was that patients with loose stools needed to accurately weigh stools; leading to collecting samples many times. In addition, stool samples were stable at -200C for 1 month. If left for longer, the calprotectin concentration would be different from that in the original sample, especially since this only occurred in groups with high calprotectin concentration. The Liaison DiaSorin XL analyzer performs a quantitative Calprotectin test.

IL-6 performed by ACCESS Immunoassay reagent on UniCel DxI 800 Systems. The Access IL-6 assay is a paramagnetic particle, chemiluminescent immunoassay and is intended for use on Access immunoassay analyz-

Characteristics	IBS-D (1)	IBD		p-value
		UC (2)	CD (3)	
Common symptoms (n%)	225	71	18	
Fever	0 (0%)	7 (9.9%)	2 (11.1%)	
Stomach-ache	177 (82.3%)	65 (91.5%)	15 (83.3%)	*0.06
Bloody stools	13 (6.0%)	50 (70.4%)	9 (50.0%)	*<0.001
Blood test (median (IQR)				
WBC count (G/l)	6.0 (5.0 –7.0)	8.2 (6.0 – 10.8)	6.5 (5.0 10.7)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.14
Neutrophil (%)	57.8 (51.7 – 62.8)	67.0 (58.0 – 75.0)	66.2 (55.5 – 82.2)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.7
Lymphocytes (%)	30.3 (26.0 – 35.5)	25.0 (17.6 – 31.1)	21.0 (12.2 – 30.0)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.3
Erythrocyte sedimentation rate (mm)	8.0 (5.5 – 16.0)	23.0 (12.0 – 31.1)	13.5 (10 – 41)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.2
CRP (mg/L)	1.3 (0.9 – 2.3)	7.0 (2.4 -16.6)	10.1 (2.2 - 42.5)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.2
IL-6 (pg/mL)	2.3 (1.6 – 5.7)	16.8 (9.4 – 47.0)	9.4 (7.9 – 11.0)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.1
Calprotectin (µg/g)	17.7 (8.8 – 38.9)	1710.0 (588.0 - 4260.0)	560.0 (177.8 - 1210.0)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.005
Protein (g/L)	75 (71.5 – 77.1)	66.7 (60.0 –75.2)	68.5 (62.7 -73.1)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.8
Albumin (g/L)	43.3 (41.2 – 45.8)	37.4 (33.3 - 41.6)	37.0 (32.5 -39.3)	
Sodium (mmol/L)	139 (137.0 – 140.0)	138.0 (136.0 -139.0)	137 (135 – 139.2)	**(1), (2), (3) = 0.001 ***(2), (3) = 0.9
Hemoglobin (g/L)	145.0 (138.0 – 155.0)	129.0 (110 -145)	119.0 (98.7 – 144.3)	**(1), (2), (3) = 0.00 ***(2), (3) = 0.3
pH	6.0 (5.0 – 6.5)	5.9 (5.0 – 7.0)	5.5 (5.0 – 6.3)	**(1), (2), (3) = 0.92 ***(2), (3) = 0.8
Intestinal microbiota: n (%)				
Scattered	1 (0.5%)	10 (14.1%)	1 (5.6%)	0.04
Mainly Gram-negative bacteria	156 (72.6%)	30 (42.3%)	6 (33.3%)	
Mainly Gram-positive bacteria	25 (11.6%)	24 (33.8%)	4 (22.2%)	
Bala4	33 (15.3%)	7 (9.9%)	7 (38.0%)	

Table 2. Symptoms of patients. *One-way ANOVA; ** Kruskal – Wallis test; * Mann-Whitney test, Abbreviation: IBS-D: Irritable bowel syndrome-diarhea; IBD: Inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease, Table 3 shows that Calprotectin had the highest AUC value for differentiating IBS from IBD at 0.978. IL-6 and CRP were also tests that could determine IBS from UC with p<0.05, AUC of 0.87 and 0.88, respectively.**

Test	Healthy people	IBS-D	IBD		p-value
			UC	CD	
n	31	215	71	18	IBS-D vs IBD: p <0.001 IBS-D vs UC : p < 0.001 IBS-D vs healthy people: p = 0.6 UC and CD : p = 0.005
Min/Max	5.0/141.0	2.2/645.2	11.6/29800	5.6/5800	
Median (IQR)	20 (6.0 – 34.0)	17.7 (8.8 – 38.9)	1710.0 (588.0 - 4260.0)	560.0 (177.8 - 1210.0)	

Table 3: Concentrations of calprotectin (µg/g) in healthy and IBS-D, UC and CD patients

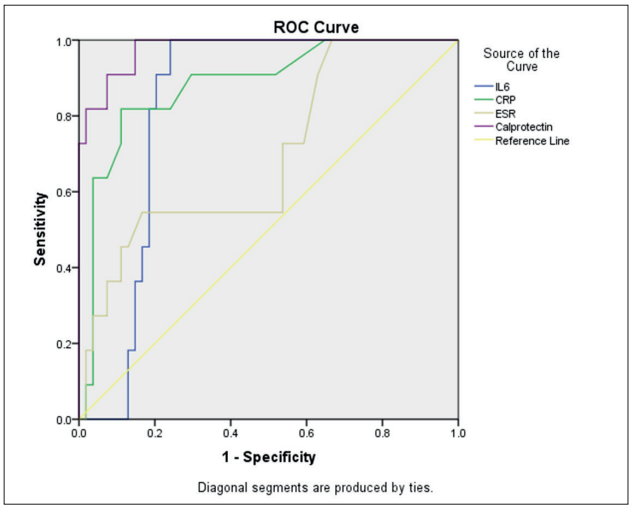

Figure 1. Diagnostic values of Calprotectin (µg/g); IL-6 (pg/mL); CRP (mg/L) and Erythrocyte sedimentation rate in differentiating IBS-D and IBD

ers (8). CRP performed on AU 680 system by Beckman Couter CRP Latex reagent (9)

Statistical analysis

Data were analyzed using SPSS 20.0 software. $\chi 2$ test, One-way ANOVA, Kruskal-Wallis and Mann-Whitney test were used to compare characteristics among IBS-D, UC and CD patients. The sensitivity and specificity of different biomarkers were computed for differentiating IBS-D and IBD. The receiver operating characteristic (ROC) curve and Area under curve (AUC) was used to determine diagnostic values of Calprotectin, CRP and IL-6 in differentiating IBS-D and IBD. Multivariate logistic regression was performed. A p-value of less than 0.05 was used for detecting statistical significance.

Ethical approval

The Hanoi People's Committee approved the study under Decision No. 3723/QD-UBND dated January 27, 2021, and approved by the Ethics Committee of Thanh Nhan Hospital for the study according to Decision No. 31/BVTN-HDĐĐ.

4. RESULTS

Table 1 shows no difference was found regarding gender and age among IBS-D, UC and CD patients (p>0.05). There was no difference in symptoms of abdominal pain in patients with IBS, UC, and CD. Results of erythrocyte sedimentation rate, blood leukocyte counts, IL-6, and CRP levels were higher in the UC, and CD groups compared with IBS. There was no difference in these inflammatory indices in the UC group compared with the CD group. The results of lymphocytes in the IBS group were higher than in the UC and CD groups (p<0.05). The determination of intestinal microbiota was mainly Gram-negative (p<0.001).

Table 2 shows that the concentration of calprotectin in human feces was higher in the UC group than in the IBS-D group (p<0.05). Calprotectin concentrations were not different in the UC and CD groups, IBS and healthy groups

Table 3 shows that Calprotectin had the highest AUC value for differentiating IBS from IBD at 0.978. IL-6 and CRP were also tests that could determine IBS from UC with p<0.05, AUC of 0.87 and 0.88, respectively.

Table 4 presents the results of multivariable logistic regression analysis, showing that calprotectin concentration was a predictor of IBD with p<0.05, OR=1.007, 95%CI=1.002-1.013.

	AUC	95% CI	P	Cut-off Calprotectin	Sensitivity (%)	Specificity (%)
Calprotectin (µg/g)	0.990	0.97-1.0	<0.001	110.5	93.3	91.4
IL-6 (pg/mL)	0.86	0.77-0.95	<0.001	7.2	92.0	78.0
CRP (mg/L)	0.89	0.8-0.97	<0.001	2.4	83.3	86.0
erythrocyte sedimentation rate (mm)	0.70	0.59-0.81	0.001	16.4	64.2	78.0

Table 4: Diagnostic values of IBD and IBS-D of calprotectin, IL6, CRP, and erythrocyte sedimentation rate. Abbrev: AUC: Area under the curve

Characteristics	OR	95%CI	p
White blood count (G/l)	1.5	0.64-1.57	1.00
Calprotectin (µg/g)	1.007	1.002 – 1.013	0.01
CRP (mg/L)	1.04	1.01 – 1.07	0.4
Erythrocyte sedimentation rate (ESR) (mm/h)	0.995	0.86 – 1.14	0.8
IL-6 (pg/mL)	1.028	0.98 – 1.073	0.5

Table 5: Analysis of the value of calprotectin, CRP, IL-6 in predicting Inflammatory bowel disease

Table 5 shows that Calprotectin and white blood cell count varied according to the severity of UC (Based on the Mayo scale). There was no difference in Calprotectin and other inflammatory indices according to lesion site.

5. DISCUSSION

Epidemiologically, IBS occurs at all ages, most commonly in women, IBD occurs at ages 15-30 regardless of gender (10).. In this study, the average age of IBS-D patients was higher than IBD (Table 1). According to research by Vicente-Steijn, R et al., median age of IBS patients: 36 (17), UC: 47 (30), CD: 37 (25). In general, research around the world suggests that the majority of patients with inflammatory bowel disease are first diagnosed between the ages of 30 and 50 (11). The age of diagnosis is within the working age, so it affects the quality of life. quality of life and social labor productivity. The average age at diagnosis is higher in Asian countries than in Western countries (12). We found that the proportion of women with the disease was higher than that of men in both IBD and IBS groups. According to research by Vicente-Steijn R, the incidence in women is higher than in men in both IBS, UC and CD groups (13). According to Jha, A. K. et al., the age of IBD onset is 35 years (range 14–60), with a male-to-female ratio of 2: The rate of men and women suffering from the disease varies between regions, possibly due to eating habits, stress, anxiety, and changes in microbiota in different geographical areas. Most studies show that there are no gender differences in patients with IBD and IBS and that the disease can occur in both men and women (14).

IBS-D and UC share many similarities in symptoms, such as abdominal pain, bloating, and diarrhea, resulting in difficulty in diagnosis. After assessing the clinical symptoms, we found that abdominal pain was present in most of the patients, and there was no difference in the IBS-D, UC, and CD groups. Although there was a difference in the rate of fever in the UC group compared with the IBS-D group, the proportion of patients with fever was low; therefore, fever had little value in distinguishing these two groups of diseases. Fatigue, weight loss, and bloody stools were more common in the IBD group than in the IBS-D group. In the opinion of clinicians, alarm signs such as fever, weight loss, anorexia, bloody stools, vomiting, and anemia are characteristic features of IBD (10). Pain symptoms in IBS are often due to stretching of the intestinal wall and can go away after defecation, while pain symptoms in IBD are often related to intestinal nerves, the pain is more persistent and does not go away after defecation.

In cases of UC, electrolyte disturbances, especially hyponatremia, were observed due to fecal sodium loss. The study results showed that protein, sodium, and Hb levels decreased in the UC and CD groups compared with the IBS group. We found a reduction in hemoglobin in the CD group compared with UC, although the rate of hematuria was lower in the CD group than in the UC group. Anemia is closely related to quality of life and is an important issue in treating chronic patients. There are many causes of anemia in UC patients. Chronic blood loss from the gastrointestinal tract leads to anemia and iron deficiency. Some inflammatory cytokines that can inhibit erythropoietin synthesis also lead to anemia. Folic acid deficiency can be caused by diet or as a side effect of sulfasalazine and methotrexate. In Crohn's disease, anemia is also caused by decreased absorption of vitamin B12 and folic acid in the terminal ileum and duodenum and decreased iron absorption in the duodenum (15). In severe cases, massive gastrointestinal blood loss can lead to severe anemia.

The pathological causes of IBD and IBS-D are both unclear. The complex pathogenesis of IBS-D includes many factors, such as changes in intestinal motility, visceral sensation, alterations in the gut-brain axis, microbiota, bile acid metabolism, and intestinal permeability. In addition, immune activation may be associated with mild inflammation. Colonic mucosal biopsies of approximately two-thirds of patients with IBS-D showed dense mast cell infiltration, which released multiple mediators, such as serine proteases, which might be the cause of euphoria and IBS-D symptoms. Furthermore, food components and antigens are thought to cross the leaky epithelial barrier, leading to mast cell infiltration and activation and IBS-D symptoms. In UC patients, increased immune system activity, altered enteric nervous system, increased ganglion cells, and inflammatory cell infiltration were observed.

The tests related to inflammation that are prioritized include measuring the number of white blood cells, CRP concentration and erythrocyte sedimentation rate. The median values of white blood cell count, CRP concentration, and erythrocyte sedimentation rate in patients with IBD were higher than those in the IBS patient group (with $p < 0.05$), this result is also consistent with the study of Kaiser -T et al (16). According to the observations of Dhaliwal, A. and CS on 311 patients with functional gastrointestinal (GI) disease, IBD and IBS, the CRP concentration in the IBS patient group was 13 (SD: 4.6), active IBD. is 15 (±17), stable IBD is 14 (±43). The authors suggest that C-reactive protein and erythrocyte sedimentation rate are tests commonly used by clinicians to determine the probability of patients having inflammatory bowel disease with functional disorders. Using CRP and erythrocyte sedimentation rate has high sensitivity but low specificity to differentiate IBD and IBS, so it is valuable to monitor disease progression (17) The non-specific inflammatory manifestations increase the response of CRP, IL-6, and leukocytes in the blood. The study results showed that there was an increase in leukocytes, CRP, erythrocyte sedimentation rate, IL-6 in the IBD group, especially UC, than in the IBS-D group.

Calprotectin is a calcium and zinc binding protein of the S-100 family of proteins, mainly found in neutrophils, monocytes, macrophages and epithelial cells in the inflammatory response to inflammatory cells that are accumulated in the mucosa. The presence of calprotectin in the stool is a consequence of neutrophil migration into the digestive tissue due to an inflammatory process. Calprotectin is then released in the stool, which can be measured using an enzyme-linked immunoassay. Fecal calprotectin concentrations correlate well with enteritis, and fecal calprotectin is used as a biomarker in gastrointestinal disorders. Calprotectin is a sensitive marker for inflammation in the gastrointestinal tract and is useful in differentiating IBD from irritable bowel syndrome

IBS. In addition, Calprotectin is used to diagnose, monitor disease activity, guide treatment, and predict disease recurrence and postoperative recurrence in IBD. Fecal calprotectin's potential role in treating infectious gastroenteritis, acute appendicitis, peptic ulcer disease, cystic fibrosis, celiac disease, and graft-versus-host disease. In recent years, some studies have shown that calprotectin has a differential diagnosis value of up to 90% of IBD and IBS. However, sensitivity and specificity at a given cut-off point have optimal cut-off values. Furthermore, there are differences between assays; thus, further studies are needed to clarify the role and value of the calprotectin cut-off point in diagnosing IBD and IBS (18).

The study results showed no difference in Calprotectin levels in the healthy group and the IBS-D group, nor was there any difference in Calprotectin levels in the UC and CD groups. Several studies reported higher Calprotectin concentrations in the IBD group than in the healthy group and confirmed a reference value of 50 g/g as the upper limit. Another study suggested values up to 112 g/g in people over 60 years of age and up to 186 g/g in children 2 to 9 years of age, as the reference range for fecal Calprotectin in healthy individuals (19). The Calprotectin concentration in IBD patients observed to be significantly higher in IBS and had differences value.

better than CRP and ESR (20, 21). During the process of measuring calprotectin concentration, we recorded very high differences in some individuals of up to 40% - 200%, suggesting that a single determination of calprotectin may not be enough for diagnosis and follow-up treatment effectiveness. This high variability may partly be due to the variability of the extraction process because the relative amount of water in the stool can change during the day, on the other hand, because the correct location of the lesion (bloody mucus) may not be collected. Therefore, repeated determination of fecal calprotectin may be useful in monitoring and detecting patients whose values lie in the gray zone.

The overlap in clinical features between IBS-D and IBD leads to more frequent endoscopy, an invasive procedure that causes discomfort during the preparation for colonoscopy and up to several days. After the screening, especially in children, finding a cut-off value of Calprotectin that can distinguish between IBS-D and IBD is essential. Pous-Serrano et al. studied patients undergoing small bowel resection for Crohn's disease. They showed that preoperative fecal calprotectin values were significantly associated with the degree of histological inflammation (score of Chiorean) in lesions with IBD in surgical specimens. A cut-off value of 170 μg/g had a sensitivity of 81% and a specificity of 85% for the diagnosis of moderate or severe inflammation. Regression analysis showed the probability of more or less inflammation based on preoperative calprotectin values (22, 23, 24).

The results show that along with other inflammatory tests such as CRP, white blood cell count, IL-6, erythrocyte sedimentation rate, Calprotectin was still the most valuable indicator regarding sensitivity and specificity. Calprotectin cut-off value of 110.5 μg/g was found to differentiate IBD from IBS-D with sensitivity and specificity of 93.3% and 91.1%. In another study performed on 76 patients with ulcerative colitis, a sensitivity of 98% and specificity of 96% at a threshold of 188 μg/g were reported in this regard (25). The study results also showed that Calprotectin was significant in differentiating IBD and IBS-D but could not use this test to distinguish between UC and CD ($p>0.05$). Regression analysis with the participation of calprotectin and some commonly used tests to determine IBD, such as CRP, IL-6, white blood cell count, and erythrocyte sedimentation rate showed that calprotectin was an independent predictor of IBD. with IBD regardless of changes in other indicators. We have found that laboratory parameters such as CRP, IL-6, WBC, and erythrocyte sedimentation rate predicted IBD, but calprotectin was the most valuable test. In addition to the value of discriminating between IBS-D and IBD, calprotectin is also valuable in assessing the extent of the damage. The study showed that compared with other inflammatory tests, calprotectin and white blood cell count increased with lesion activity and there was no difference in lesion location. Although calprotectin effectively differentiated IBD and IBS, it could not distinguish between Crohn's disease and ulcerative colitis and could not locate lesions in the intestine (Table 6) .

6. CONCLUSION

Calprotectin test had good value in differentiating UC and IBS with clinical diarrhea. However, further research is required to confirm the diagnostic value of Calprotectin in clinical practice.

- **Author's Contribution**: B.T.T.H contributed to sample collection, biomedical data compilation and analysis, Nguyen T. Nhan manuscript writing and review; N.M.H contributed to study design, final data compilation and analysis, and manuscript writing and review; N.T.V contributed to biomedical data compilation and analysis, D.Q.M manuscript review; T.K.C contributed to final data compilation and machine learning methods; and N.T.D was the project leader. All authors read and approved the final manuscript.
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REFERENCES

1. Borghini R, Donato G, Alvaro D, Picarelli A. New insights in IBS-like disorders: Pandora's box has been opened; a review. Gastroenterol Hepatol Bed Bench. 2017 Spring; 10(2): 79-89.
2. Quach DT, Vu KT, Vu KV. Prevalence, clinical characteristics, and management of irritable bowel syndrome in Vietnam: A scoping review. JGH Open. 2021 Jul 21; 5(11): 1227-1235. doi: 10.1002/jgh3.12616.
3. GBD 2017 Inflammatory Bowel Disease Collaborators. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lan-

cet Gastroenterol Hepatol. 2020 Jan;5(1): 17-30. doi: 10.1016/S2468-1253(19)30333-4.
4. Theede K, Kiszka-Kanowitz M, Nordgaard-Lassen I, Nielsen AM. [Faecal calprotectin is a useful biomarker for intestinal inflammation]. Ugeskr Laeger. 2014 Sep 8; 176(37): V04140213 (Danish).
5. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Vavricka SR, Bruegger LE, Seibold F. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol. 2010 Jan; 105(1): 162-169. doi: 10.1038/ajg.2009.545.
6. Taghvaei T, Maleki I, Nagshvar F, Fakheri H, Hosseini V, Valizadeh SM, Neishaboori H. Fecal calprotectin and ulcerative colitis endoscopic activity index as indicators of mucosal healing in ulcerative colitis. Intern Emerg Med. 2015 Apr; 10(3): 321-328. doi: 10.1007/s11739-014-1144-x.
7. Kawashima K, Ishihara S, Yuki T, Fukuba N, Oshima N, Kazumori H, Sonoyama H, Yamashita N, Tada Y, Kusunoki R, Oka A, Mishima Y, Moriyama I, Kinoshita Y. Fecal calprotectin level correlated with both endoscopic severity and disease extent in ulcerative colitis. BMC Gastroenterol. 2016 Apr 12; 16: 47. doi: 10.1186/s12876-016-0462-z.
8. https://www.fda.gov/media/142600/download.
9. https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/BAOSR6x99/01/EN_CRP%20LATEX%20REAGENT.pdf.
10. Szałwińska P, Włodarczyk J, Spinelli A, Fichna J, Włodarczyk M. IBS-Symptoms in IBD Patients-Manifestation of Concomitant or Different Entities. J Clin Med. 2020 Dec 24; 10(1): 31. doi: 10.3390/jcm10010031.
11. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011 May; 140(6) :1785-1794. doi: 10.1053/j.gastro.2011.01.055.
12. Prideaux L, Kamm MA, De Cruz PP, Chan FK, Ng SC. Inflammatory bowel disease in Asia: a systematic review. J Gastroenterol Hepatol. 2012 Aug; 27(8): 1266-1280. doi: 10.1111/j.1440-1746.2012.07150.x.
13. Vicente-Steijn R, Jansen JM, Bisheshar R, Haagen IA. Analytical and clinical performance of the fully-automated LIAISONXL calprotectin immunoassay from DiaSorin in IBD patients. Pract Lab Med. 2020 Jun 24; 21: e00175. doi: 10.1016/j.plabm.2020.e00175.
14. Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology. 2004 May; 126(6): 1504-1517. doi: 10.1053/j.gastro.2004.01.063.
15. Levine JS, Burakoff R. Extraintestinal manifestations of inflammatory bowel disease. Gastroenterol Hepatol (N Y). 2011 Apr; 7(4): 235-241.
16. Kaiser T, Langhorst J, Wittkowski H, Becker K, Friedrich AW, Rueffer A, Dobos GJ, Roth J, Foell D. Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. Gut. 2007 Dec; 56(12): 1706-1713. doi: 10.1136/gut.2006.113431.
17. Dhaliwal A, Zeino Z, Tomkins C, Cheung M, Nwokolo C, Smith S, Harmston C, Arasaradnam RP. Utility of faecal calprotectin in inflammatory bowel disease (IBD): what cut-offs should we apply? Frontline Gastroenterol. 2015 Jan; 6(1): 14-19. doi: 10.1136/flgastro-2013-100420.
18. Waugh N, Cummins E, Royle P, Kandala NB, Shyangdan D, Arasaradnam R, Clar C, Johnston R. Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation. Health Technol Assess. 2013 Nov; 17(55):xv-xix, 1-211. doi: 10.3310/hta17550.
19. Khaki-Khatibi F, Qujeq D, Kashifard M, Moein S, Maniati M, Vaghari-Tabari M. Calprotectin in inflammatory bowel disease. Clin Chim Acta. 2020 Nov;510: 556-565. doi: 10.1016/j.cca.2020.08.025.
20. Chang MH, Chou JW, Chen SM, Tsai MC, Sun YS, Lin CC, Lin CP. Faecal calprotectin as a novel biomarker for differentiating between inflammatory bowel disease and irritable bowel syndrome. Mol Med Rep. 2014 Jul;10(1):522-6. doi: 10.3892/mmr.2014.2180. Epub 2014 Apr 24. PMID: 24788223.
21. Campbell JP, Zierold C, Rode AM, Blocki FA, Vaughn BP. Clinical Performance of a Novel LIAISON Fecal Calprotectin Assay for Differentiation of Inflammatory Bowel Disease From Irritable Bowel Syndrome. J Clin Gastroenterol. 2021 Mar 1; 55(3): 239-243. doi: 10.1097/MCG.0000000000001359.
22. Pous-Serrano S, Frasson M, Cerrillo E, Beltrán B, Iborra M, Hervás D, García-Granero E, Nos P. Correlation between fecal calprotectin and inflammation in the surgical specimen of Crohn's disease. J Surg Res. 2017 Jun 1; 213: 290-297. doi: 10.1016/j.jss.2017.02.064.
23. Kennedy NA, Jones GR, Plevris N, Patenden R, Arnott ID, Lees CW. Association Between Level of Fecal Calprotectin and Progression of Crohn's Disease. Clin Gastroenterol Hepatol. 2019 Oct; 17(11): 2269-2276.e4. doi: 10.1016/j.cgh.2019.02.017.
24. Pavlidis P, Chedgy FJ, Tibble JA. Diagnostic accuracy and clinical application of faecal calprotectin in adult patients presenting with gastrointestinal symptoms in primary care. Scand J Gastroenterol. 2013 Sep; 48(9): 1048-54. doi: 10.3109/00365521.2013.816771.
25. Jha AK, Chaudhary M, Dayal VM, Kumar A, Jha SK, Jha P, Purkayastha S, Ranjan R. Optimal cut-off value of fecal calprotectin for the evaluation of ulcerative colitis: An unsolved issue? JGH Open. 2018 Aug 10; 2(5): 207-213. doi: 10.1002/jgh3.12074.