Evaluation of Serum Calprotectin Levels in Patients with Inflammatory Bowel Disease

ATSUSHI MORI*, KEIICHI MITSUYAMA*,**, RYOSUKE SAKEMI†, SHINICHIRO YOSHIOKA*, SHUHEI FUKUNAGA*, KOTARO KUWAKI*, RYOSUKE YAMAUCHI*, TOSHIHIRO ARAKI*, TETSUHIRO YOSHIMURA*, HIROSHI YAMASAKI*, KOZO TSURUTA*, TAKU MORITA*, SAYO YAMASAKI*, OSAMU TSURUTA* AND TAKUJI TORIMURA*

* Division of Gastroenterology, Department of Medicine,
** Inflammatory Bowel Disease Center, Kurume University School of Medicine, Kurume 830-0011,
† Department of Gastroenterology, Tobata Kyoritsu Hospital, Kitakyushu 804-0093, Japan

Summary: *Background*: Fecal calprotectin has been proposed as a useful biomarker of disease activity in inflammatory bowel disease (IBD). However, the role of calprotectin in systemic circulation is not well established. Thus, this study aimed to quantify serum calprotectin levels to identify a potential inflammatory marker for IBD.
Methods: Ninety-eight patients with ulcerative colitis (UC) and 105 patients with Crohn's disease (CD) were prospectively enrolled and clinically scored. Ninety-two healthy, age-matched subjects served as controls. Blood samples from UC and CD patients and controls were analyzed for serum calprotectin levels and routine laboratory parameters. Disease activity was assessed by partial Mayo score and Harvey-Bradshaw index for UC and CD, respectively.
Results: Serum calprotectin levels were higher in CD and UC patients than in controls and were higher during active disease than during inactive disease in CD but not in UC. In UC, serum calprotectin levels were correlated with C-reactive protein (CRP) but not with other laboratory parameters or disease activity. In CD, serum calprotectin levels were positively correlated with disease activity, serum CRP, and platelet count. In UC and CD, serum calprotectin and CRP levels increased during the acute phase and decreased towards remission.
Conclusions: Serum calprotectin is an inflammatory marker in IBD but might be more effective in evaluating patients with CD than those with UC. Further studies are needed to confirm these findings and to better determine the specific uses of serum calprotectin in routine practice.

Keywords calprotectin, Crohn's disease, enzyme-linked immunosorbent assay, inflammatory bowel disease, serum, ulcerative colitis

INTRODUCTION

Inflammatory bowel diseases (IBD), consisting of ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory disorders involving the gastro-intestinal tract, in which the migration and activation of inflammatory cells and the production of inflammatory mediators play important roles [1].

There is a need for a reliable marker capable of mirroring intestinal inflammation, that can be used as

Corresponding Author: Keiichi Mitsuyama, M.D., Ph.D., Inflammatory Bowel Disease Center, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. Tel: +81-942-31-7561, Fax: +81-942-34-2623, E-mail: ibd@med.kurume-u.ac.jp

Abbreviations: 5-ASA, 5-aminosalicylic acid; ANCA, anti-neutrophil cytoplasmic antibody; AZA, azathioprine; CD, Crohn's disease; CRP, C-reactive protein; HBI, Harvey-Bradshaw index; IBD, inflammatory bowel disease; LPS, lipopolysaccharides; PSL, prednisolone; TNF-α, tumor necrosis factor-alpha; UC, ulcerative colitis.

a substitute for endoscopy. Blood biomarkers provide a non-invasive estimation of the inflammatory activity in IBD [2]. However, relatively few blood markers have been extensively validated in clinical settings.

Calprotectin is a calcium-binding protein of the S100 family which is mainly found within neutrophils and monocytes/macrophages [3]. In the human body, this protein exists in blood, urine, feces, saliva, and cerebrospinal fluid and plays a role in various biological activities, such as inflammation and immunoregulation [4]. In inflammation, calprotectin functions as an alarm by indicating inflammatory activities mainly induced by neutrophils. Calprotectin plays its role by binding to its receptors on the cell surface, triggering signal transductions and inducing leukocyte migration and cytokine production in inflammatory regions [5,6]. Interestingly, a recent report using calprotectin-knockout mice showed that calprotectin promotes inflammatory leukocyte-renal cell interaction, which is critical for the development of nephritis [7]. These findings lead us to speculate that calprotectin may be involved in the pathophysiology of IBD.

An increased serum level of calprotectin is found in patients with many inflammatory diseases [8-14]. At present, fecal calprotectin is recognized as a useful marker for evaluating disease activity of IBD [15,16]. However, the role of calprotectin in the systemic circulation is not well established [17]. Thus, this study aimed to compare the association between serum calprotectin, traditional markers of inflammation, and clinical disease activity in patients with IBD.

PATIENTS AND METHODS

Ethical Consideration

The study protocol was reviewed and approved by the Ethics Committee of Kurume University School of Medicine (No. 13098). Informed consent was obtained from each subject or their parents before enrollment in this study.

Patients

Between July 2016 and April 2018, serum samples were collected from 98 patients with UC and 105 with CD. The diagnoses were based on characteristic clinical, endoscopic, radiological, and histological features, as shown in Table 1. Among the patients with UC, there were 54 men and 44 women with a median age of 41.6 years, and median disease duration of 4.37 years. In terms of disease range, 15 patients had proctitis, 37 had left-sided colitis, and 46 had pancolitis. Among the patients with CD, there were 60 men and 45 women, with a median age of 33.7 years and median disease duration of 9.15 years. The disease affected the ileum alone in 25 patients, the colon alone in 30 patients, and both the ileum and the colon in 50 patients. These patients had received adequate medical therapy. Ninety-two healthy, age-matched subjects served as normal controls.

TABLE 1.
Demographic and disease data on the patients in this study

	Ulcerative colitis	Crohn's disease	Healthy subjects
No. of Patients	98	105	92
Sex (male/female)	54/44	60/45	52/40
Age, years (median, IQR)	41.6 (33.0-59.7)	33.7 (24.4-51.7)	39.6 (30.4-49.6)
Disease extent	Proctitis/Left-sided colitis/ Pancolitis 15/37/46	Ileitis/Colitis/Ileocolitis 25/30/50	–
Disease duration, years (median, IQR)	4.37 (1.0-9.0)	9.15 (3.0-15.9)	–
Treatments			
5-aminosalicylic acid (%)	Oral 72 (73.4), Topical 26 (27.0)	Oral 85 (80.9)	–
Prednisolone (%)	Oral 17 (17.3), Topical 8 (8.1)	Oral 14 (13.3)	–
Immunomodulator (%)	22 (22.4)	39 (37.1)	–
Leukocytapheresis (%)	6 (6.1)	6 (6.1)	–
Anti-tumor necrosis factor (%)	7 (7.1)	65 (61.9)	–
Indigo naturalis (%)	19 (19.3)	0 (0)	–

Evaluation of Disease Activity

For the evaluation of disease activity, clinical activity in patients with UC was graded using the partial Mayo score (inactive disease was defined as a score ≤ 2 with no individual sub-score > 1 point) [18]. Patients with CD were graded according to the Harvey-Bradshaw index (HBI), with inactive disease being defined as a score < 5 points [19].

Measurement of Serum Calprotectin

The serum calprotectin levels were quantified using an ELISA (sCAL™ ELISA; Bühlmann Laboratories AG, Schönenbuch, Switzerland) [20], and all procedures were performed at room temperature. In brief, blood samples (0.5 mL) were collected into plain tubes and centrifuged for 15 min at 1800 × g. The resulting serum samples were frozen at –20°C for subsequent measurement. Then, 100 μL of each diluted serum sample (1:100 with incubation buffer) was incubated for 30 min onto a microtiter plate coated with a monoclonal capture antibody highly specific to calprotectin. The plate was then washed with washing solution three times, and 100 μL of monoclonal anti-calprotectin antibody conjugated with horseradish peroxidase was added to each well and incubated for 30 min. The washing procedure was performed a further five times, before adding 100 μL of enzyme substrate solution to each well. The plate was incubated for 15 min, and 100 μL of stop solution was added to all wells. The optical density was read at 450 nm. The serum calprotectin concentration was calculated from standards of known concentration and expressed as μg/mL.

Determination of Laboratory Parameters

A blood sample was also obtained from each patient and used to measure various laboratory parameters. The platelet count, serum levels of hemoglobin, albumin, and C-reactive protein (CRP) were determined by routine laboratory analysis.

Statistical Analysis

The results were presented as the median and range. All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows software 14.0 (SPSS Inc., Chicago, IL, USA). The statistical analyses were performed using nonparametric Mann–Whitney U tests. Correlations between variables were estimated using the two-tailed Spearman's rank order correlation coefficient (r). Differences were considered significant if $p < 0.05$.

RESULTS

Serum Calprotectin Levels

Figure 1 shows serum levels of calprotectin in patients with UC, patients with CD, and normal controls. Serum calprotectin levels were higher in patients with UC (median [interquartile range], 2.565 [2.56-3.36], $p < 0.001$) and CD (2.565 [1.38-3.66], $p < 0.001$) than those in normal controls (1.04 [0.69-1.58]). CD patients with active disease had a higher level of serum calprotectin than those with inactive disease ($p < 0.001$), but this was not the case in UC patients ($p = 0.12$). In addition, no significant difference was found in serum calprotectin levels between patients with UC and CD ($p < 0.738$).

Serum calprotectin levels were also compared between patients with active and inactive disease according to the disease extent (Figure 2). In patients with UC, serum calprotectin levels in those with active disease and inactive disease were comparable regardless of disease type, including proctitis ($p = 1.00$), left-sided colitis ($p = 0.357$), and pancolitis ($p = 0.275$). In patients with CD, serum calprotectin levels were higher in those with active disease than those with inactive disease regardless of the disease type, including ileitis ($p = 0.022$), colitis ($p = 0.002$), and ileocolitis ($p = 0.035$).

Correlation with Clinical Disease Activities

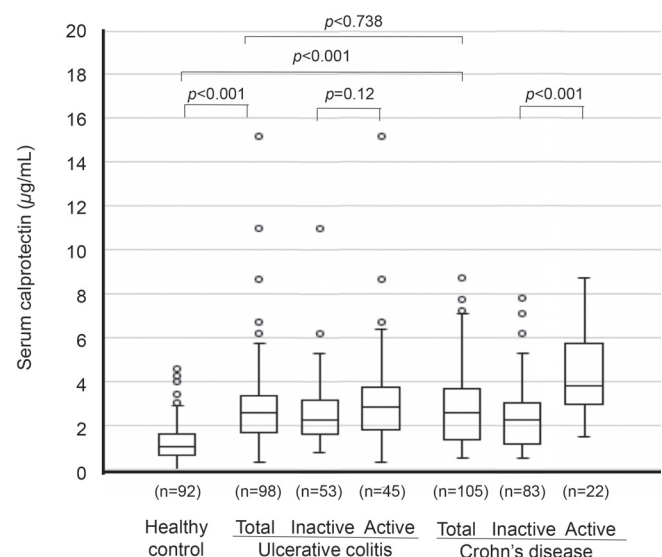We also analyzed the correlation between serum

Fig. 1. Serum levels of serum calprotectin in patients with ulcerative colitis and Crohn's disease and in normal controls. Solid line in middle of box represents median, boxes extend up to interquartile range of 25% to 75%.

calprotectin levels and clinical disease activities (Figure 3). No significant association was observed between the serum calprotectin and clinical disease activities as assessed by partial Mayo scores in patients with UC ($r = 0.143$, $p = 0.160$). In contrast, a significant correlation was observed between serum calprotectin level and clinical disease activities, as assessed by HBI in patients with CD ($r = 0.547$, $p < 0.001$).

Correlation with Laboratory Parameters

Table 2 summarizes the correlation coefficients and significance values between the serum calprotectin and the indicated laboratory parameters. In patients with CD, serum calprotectin significantly correlated with platelet count ($r = 0.41$, $p < 0.01$), serum albumin ($r = -0.33$, $p < 0.01$), and serum CRP ($r = 0.505$, $p = 0.001$). However, in patients with UC, calprotectin correlated with serum CRP only ($r = 0.320$, $p = 0.001$).

Serial Measurements of Serum Calprotectin

Figure 4 shows the time course of serum calprotectin followed longitudinally in two patients with UC and two patients with CD. Eventually, the disease activity in all patients was controlled by treatment. In these cases, serum levels of calprotectin and CRP increased during the acute phase and decreased gradually as patients went into remission, regardless of the treatment modality.

DISCUSSION

There are several reports concerning non-invasive biomarkers for IBD. Of these, fecal calprotectin has been suggested as a useful marker of inflammatory activity in IBD. Little is known, however, about the role of serum calprotectin in IBD. In this study, we examined the serum calprotectin level from patients with IBD and assessed the relationship with clinical and laboratory parameters, with the aim of evaluating the efficacy of serum calprotectin as a potential inflammatory biomarker for IBD.
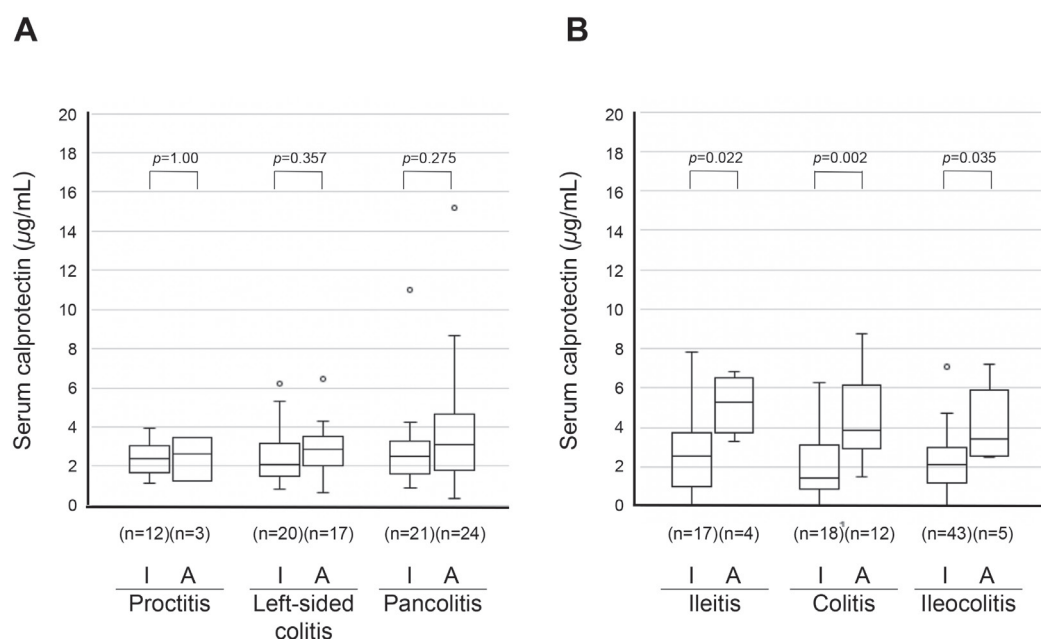
TABLE 2.
Correlation coefficients (r) and significant differences (p) between serum calprotectin levels and laboratory measurements of hemoglobin, platelet count, albumin and C-reactive protein in patients with ulcerative colitis and with Crohn's disease. A p-value < 0.05 indicates statistical significance

	Ulcerative colitis		Crohn's disease	
	r	p	r	p
Hemoglobin	0.10	0.31	-0.06	0.54
Platelet count	0.05	0.58	0.41	<0.01
Albumin	-0.11	0.26	-0.33	<0.01
C-reactive protein	0.320	0.001	0.505	0.001

Fig. 2. Comparison of serum calprotectin levels between active and inactive disease according to the disease type in patients with ulcerative colitis (A) and Crohn's disease (B). Solid line in middle of box represents median, boxes extend up to interquartile range of 25% to 75%. A, active; I, inactive; n.s, not significant.
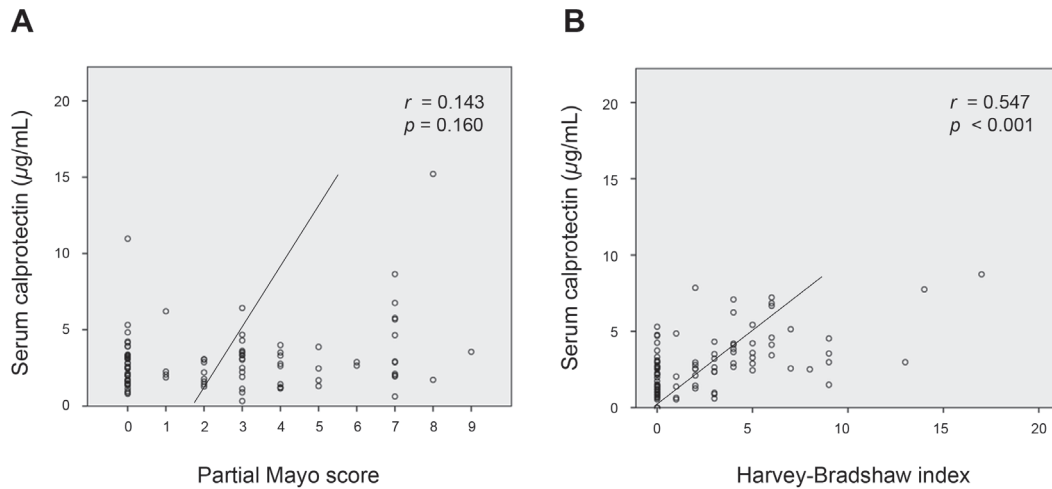
Fig. 3. Serum calprotectin levels in patients with ulcerative colitis and Crohn's disease according to the clinical disease activity as assessed using the Mayo score (A) and the Harvey-Bradshaw index (B), respectively.
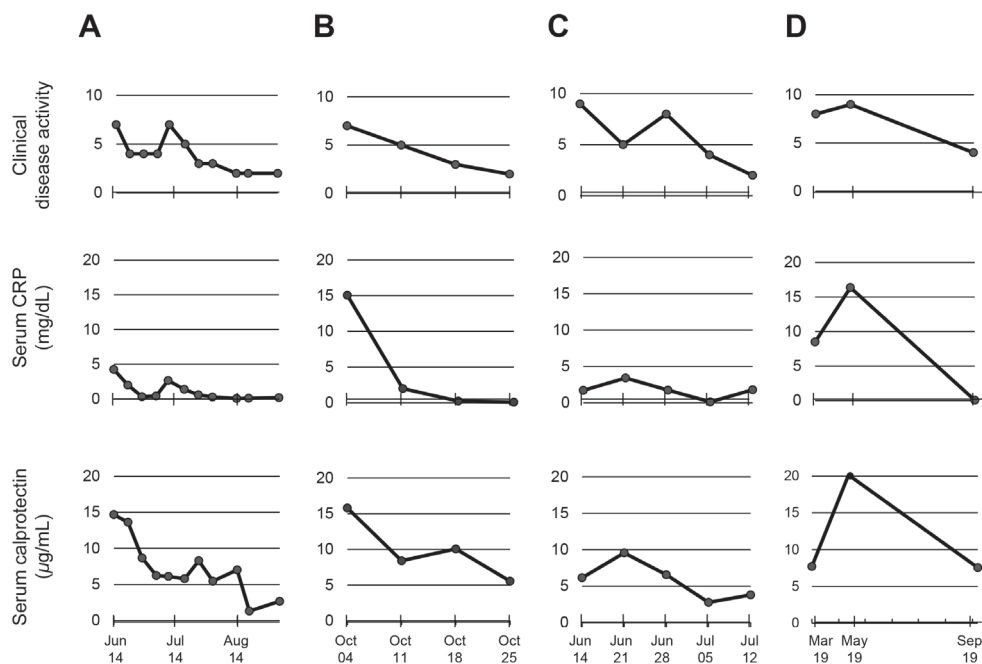

Fig. 4. Time courses for serum calprotectin, serum C-reactive protein, and disease activity levels in four patients with inflammatory bowel disease. Serum samples were obtained during active and inactive stages of disease in two patients with ulcerative colitis (UC) and two with Crohn's disease (CD). A: A 28-year-old man with left-sided UC was treated with 5-ASA, PSL, AZA. B: A 37-year-old woman with left-sided UC was treated with 5-ASA and PSL. C: A 26-year-old man with ileitis due to CD was treated with AZA and anti-TNF-α. D: A 21-year-old woman with ileocolitis due to CD was treated with 5-ASA, PSL and anti-TNF-α. The clinical activity was assessed by the partial Mayo score for patients with UC and the Harvey-Bradshaw index for patients with CD. (5-ASA, 5-aminosalicylic acid; TNF-α, tumor necrosis factor-alpha; PSL, prednisolone; AZA, azathioprine).

This study demonstrated that serum calprotectin levels were higher in CD and UC than in normal controls. In patients with CD, serum calprotectin levels were positively correlated with clinical disease activity and laboratory parameters. This was consistent with the findings of Meuwis *et al*. who measured serum calprotectin in 115 patients with CD and 40 healthy controls. Their study indicated that serum calprotectin has a similar profile to CRP and clinical disease activity (assessed by the Crohn disease activity index) [21]. In patients with UC, we found a significant correlation between serum calprotectin and CRP, but not with other laboratory parameters or clinical disease activity. However, a serial change in clinical disease activity, CRP, and serum calprotectin of UC and CD patients observed in this study seem to be related, indicating the suitability of serum calprotectin as a monitoring tool in some patients. Future studies are needed to determine their correlation, particularly in UC.

CRP is a serum acute phase reactant protein of hepatic origin with a half-life of approximately 20 h. Its concentration in the serum depends on the intensity of the pathological process stimulating its production [22]. A correlation between serum calprotectin and CRP in both CD and UC seen in this study, together with the relatively short half-life of calprotectin in the circulation (approximately 5 h) [23], suggests that serum calprotectin reflects the systemic inflammatory response associated with disease. However, the correlation between serum calprotectin and CRP was weak, which may reflect the different origins of these proteins.

The hypothesis of our study was based on the suggestion that calprotectin is more sensitive to intestinal inflammation than CRP. In clinical practice, some patients do not develop high CRP levels despite active disease. Ultimately, our longitudinal study showed that serum calprotectin was detectable in every patient even when CRP was undetectable (< 0.05 mg/dL). Although future studies are needed, these observations suggest that serum calprotectin could provide more relevant information on inflammatory response than CRP.

The precise origin of increased circulating calprotectin in IBD is unclear. Since calprotectin is a small molecule, it easily diffuses from inflamed tissue into the bloodstream [24], indicating that serum calprotectin is predominantly derived from the inflamed intestine. Somewhat surprisingly, recent studies have found no correlation between the serum and fecal calprotectin for UC or CD [21,25]. In patients with anti-neutro-

phil cytoplasmic antibody-associated vasculitis, increases in serum calprotectin levels were associated with higher cell-surface calprotectin expression levels on circulating neutrophils and monocytes [12]. Previous *in vitro* experiments have demonstrated that calprotectin is released from neutrophils in response to lipopolysaccharides (LPS) [26] and from monocytes/macrophages in response to LPS and inflammatory cytokines (*e.g*., tumor necrosis factor-α or interleukin-1β) [27]. Therefore, serum calprotectin would appear to be originated from leukocytes in response to various stimuli in the bloodstream as well as at the sites of gut inflammation.

Our study has some limitations. First, this study was a single-center analysis and involved a limited number of subjects. An obvious next step is to enroll a larger number of patients and examine serial changes in serum calprotectin in relation to ongoing medical treatment. Second, this study did not include the evaluation of endoscopic disease activity. At present, mucosal healing has been proposed as the therapeutic target for IBD, since it is associated with sustained clinical remission, a reduction of hospitalization and operations, and a lower incidence of colonic carcinogenesis. Soon, we would like to evaluate whether a normalization of the serum calprotectin level reflects mucosal healing.

In conclusion, the present results suggest that serum calprotectin is an inflammatory marker in IBD, but it might be more effective in evaluating patients with CD than those with UC. Further studies are needed to confirm these findings and to better determine the potential uses of serum calprotectin in routine practice.

REFERENCES

1. Strober W, Fuss I, and Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest 2007; 117:514-521.
2. Sands BE. Biomarkers of Inflammation in Inflammatory Bowel Disease. Gastroenterology 2015; 149:1275-1285.
3. Johne B, Fagerhol MK, Lyberg T, Prydz H, Brandtzaeg P et al. Functional and clinical aspects of the myelomonocyte protein calprotectin. Mol Pathol 1997; 50:113-123.
4. Pathirana WGW, Chubb SP, Gillett MJ, and Vasikaran SD. Faecal Calprotectin. Clin Biochem Rev 2018; 39:77-90.
5. Pruenster M, Vogl T, Roth J, and Sperandio M. S100A8/A9: From basic science to clinical application. Pharmacol Ther 2016; 167:120-131.
6. Holzinger D, Tenbrock K, and Roth J. Alarmins of the S100-Family in Juvenile Autoimmune and Auto-

Inflammatory Diseases. Front Immunol 2019; 10:182.

7. Pepper RJ, Wang HH, Rajakaruna GK, Papakrivopoulou E, Vogl T et al. S100A8/A9 (Calprotectin) Is Critical for Development of Glomerulonephritis and Promotes Inflammatory Leukocyte-Renal Cell Interactions. Am J Pathol 2015; 185:1264-1274.

8. Haga HJ, Brun JG, Berntzen HB, Cervera R, Khamashta M et al. Calprotectin in Patients with Systemic Lupus Erythematosus: Relation to Clinical and Laboratory Parameters of Disease Activity. Lupus 1993; 2:47-50.

9. Soyfoo MS, Roth J, Vogl T, Pochet R, and Decaux G. Phagocyte-specific S100A8/A9 Protein Levels During Disease Exacerbations and Infections in Systemic Lupus Erythematosus. J Rheumatol 2009; 36:2190-2194.

10. Pepper RJ, Hamour S, Chavele KM, Todd SK, Rasmussen N et al. Leukocyte and serum S100A8/S100A9 expression reflects disease activity in ANCA-associated vasculitis and glomerulonephritis. Kidney Int 2013; 83:1150-1158.

11. De Rycke L, Baeten D, Foell D, Kruithof E, Veys EM et al. Differential expression and response to anti-TNFα treatment of infiltrating versus resident tissue macrophage subsets in autoimmune arthritis. J Pathol 2005; 206:17-27.

12. Frosch M, Ahlmann M, Vogl T, Wittkowski H, Wulffraat N et al. The Myeloid-Related Proteins 8 and 14 Complex, a Novel Ligand of Toll-Like Receptor 4, and Interleukin-1β Form a Positive Feedback Mechanism in Systemic-Onset Juvenile Idiopathic Arthritis. Arthritis Rheum 2009; 60:883-891.

13. Hirono K, Foell D, Xing Y, Miyagawa-Tomita S, Ye F et al. Expression of Myeloid-Related Protein-8 and -14 in Patients With Acute Kawasaki Disease. J Am Coll Cardiol 2006; 48:1257-1264.

14. Burkhardt K, Radespiel- Tröger M, Rupprecht HD, Goppelt-Struebe M, Riess R et al. An Increase in Myeloid-Related Protein Serum Levels Precedes Acute Renal Allograft Rejection. J Am Soc Nephrol 2001; 12:1947-1957.

15. Kopylov U, Rosenfeld G, Bressler B, and Seidman E. Clinical Utility of Fecal Biomarkers for the Diagnosis and Management of Inflammatory Bowel Disease. Inflamm Bowel Dis 2014; 20:742-756.

16. Schoepfer AM, Beglinger C, Straumann A, Safroneeva E, Romero Y et al. Fecal Calprotectin More Accurately Reflects Endoscopic Activity of Ulcerative Colitis than the Lichtiger Index, C-reactive Protein, Platelets, Hemoglobin, and Blood Leukocytes. Inflamm Bowel Dis 2013; 19:332-341.

17. Azramezani Kopi T, Shahrokh S, Mirzaei S, Aghdaei HA, and Kadijani AA. The role of serum calprotectin as a novel biomarker in inflammatory bowel diseases: a review study. Gastroenterol Hepatol Bed Bench 2019; 12:183-189.

18. Schroeder KW, Tremaine WJ, and Ilstrup DM. Coated Oral 5-Aminosalicylic Acid Therapy for Mildly to Moderately Active Ulcerative Colitis. N Engl J Med 1987; 317:1625-1629.

19. Harvey RF and Bradshaw JM. A SIMPLE INDEX OF CROHN'S-DISEASE ACTIVITY. Lancet 1980; 315:514.

20. Andres Cerezo L, Mann H, Pecha O, Pleštilová L, Pavelka K et al. Decreases in serum levels of S100A8/9 (calprotectin) correlate with improvements in total swollen joint count in patients with recent-onset rheumatoid arthritis. Arthritis Res Ther 2011; 13:R122.

21. Meuwis MA, Vernier-Massouille G, Grimaud JC, Bouhnik Y, Laharie D et al. Serum calprotectin as a biomarker for Crohn's disease. J Crohns Colitis 2013; 7:e678-683.

22. Black S, Kushner I, and Samols D. C-reactive Protein. J Biol Chem 2004; 279:48487-48490.

23. Louis E, Mary JY, Vernier-Massouille G, Grimaud JC, Bouhnik Y et al. Maintenance of Remission Among Patients With Crohn's Disease on Antimetabolite Therapy After Infliximab Therapy Is Stopped. Gastroenterology 2012; 142:63-70.

24. Dale I, Fagerhol MK, and Naesgaard I. Purification and Partial Characterization of a Highly Immunogenic Human Leukocyte Protein, the L1 Antigen. Eur J Biochem 1983; 134:1-6.

25. Fukunaga S, Kuwaki K, Mitsuyama K, Takedatsu H, Yoshioka S et al. Detection of calprotectin in inflammatory bowel disease: Fecal and serum levels and immunohistochemical localization. Int J Mol Med 2018; 41:107-118.

26. Kido J, Kido R, Kataoka M, Fagerhol MK, and Nagata T. Calprotectin release from human neutrophils is induced by *Porphyromonas gingivalis* lipopolysaccharide via the CD-14–Toll-like receptor–nuclear factor κB pathway. J Periodontal Res 2003; 38:557-563.

27. Suryono, Kido J, Hayashi N, Kataoka M, and Nagata T. Effect of *Porphyromonas gingivalis* Lipopolysaccharide, Tumor Necrosis Factor-α, and Interleukin-1β on Calprotectin Release in Human Monocytes. J Periodontal 2003; 74:1719-1724.