Evaluation of Serum Calprotectin Levels in Patients with Inflammatory Bowel Disease

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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...
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>
<thead>
<tr>
<th>No. of Patients</th>
<th>Ulcerative colitis</th>
<th>Crohn’s disease</th>
<th>Healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>98</td>
<td>105</td>
<td>92</td>
</tr>
<tr>
<td>Age, years (median, IQR)</td>
<td>41.6 (33.0-59.7)</td>
<td>33.7 (24.4-51.7)</td>
<td>39.6 (30.4-49.6)</td>
</tr>
<tr>
<td>Disease extent</td>
<td>Proctitis/Left-sided colitis/ Pancolitis 15/37/46</td>
<td>Ileitis/Colitis/Ileocolitis 25/30/50</td>
<td>–</td>
</tr>
<tr>
<td>Disease duration, years (median, IQR)</td>
<td>4.37 (1.0-9.0)</td>
<td>9.15 (3.0-15.9)</td>
<td>–</td>
</tr>
<tr>
<td>Treatments</td>
<td>5-aminosalicylic acid (%)</td>
<td>Oral 72 (73.4), Topical 26 (27.0)</td>
<td>Oral 85 (80.9)</td>
</tr>
<tr>
<td>Prednisolone (%)</td>
<td>Oral 17 (17.3), Topical 8 (8.1)</td>
<td>Oral 14 (13.3)</td>
<td>–</td>
</tr>
<tr>
<td>Immunomodulator (%)</td>
<td>22 (22.4)</td>
<td>39 (37.1)</td>
<td>–</td>
</tr>
<tr>
<td>Leukocytapheresis (%)</td>
<td>6 (6.1)</td>
<td>6 (6.1)</td>
<td>–</td>
</tr>
<tr>
<td>Anti-tumor necrosis factor (%)</td>
<td>7 (7.1)</td>
<td>65 (61.9)</td>
<td>–</td>
</tr>
<tr>
<td>Indigo naturalis (%)</td>
<td>19 (19.3)</td>
<td>0 (0)</td>
<td>–</td>
</tr>
</tbody>
</table>
Evaluation of Disease Activity
For the evaluation of disease activity, clinical ac-
tivity 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]. Pa-
tients 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 us-
ing an ELISA (sCAL™ ELISA; Bühlmann Laborato-
ries AG, Schönenbuch, Switzerland) [20], and all pro-
cedures 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 re-
sulting serum samples were frozen at −20°C for sub-
sequent measurement. Then, 100 µL of each diluted
serum sample (1:100 with incubation buffer) was in-
cubated for 30 min onto a microtiter plate coated with
a monoclonal capture antibody highly specific to cal-
protectin. 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 fur-
ther five times, before adding 100 µL of enzyme sub-
strate 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 pa-
tient and used to measure various laboratory para-
eters. The platelet count, serum levels of hemoglobin,
albumin, and C-reactive protein (CRP) were deter-
mined 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). Dif-
fences were considered significant if p < 0.05.

RESULTS
Serum Calprotectin Levels
Figure 1 shows serum levels of calprotectin in pa-
tients 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 pa-
tients 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 be-	ween patients with active and inactive disease ac-
cording to the disease extent (Figure 2). In patients
with UC, serum calprotectin levels in those with ac-
tive disease and inactive disease were comparable re-
gardless 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
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.**

<table>
<thead>
<tr>
<th></th>
<th>Ulcerative colitis</th>
<th>Crohn’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemoglobin</strong></td>
<td>$r = 0.10$</td>
<td>$r = -0.06$</td>
</tr>
<tr>
<td><strong>Platelet count</strong></td>
<td>$r = 0.05$</td>
<td>$r = 0.41$</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>$r = -0.11$</td>
<td>$r = -0.33$</td>
</tr>
<tr>
<td><strong>C-reactive protein</strong></td>
<td>$r = 0.320$</td>
<td>$r = 0.505$</td>
</tr>
</tbody>
</table>

$p$-value $< 0.05$ indicates statistical significance

**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-neutrophil 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.

CONFLICT OF INTEREST: No

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