

• BRIEF REPORTS •

## Plasma interleukin-18 reflects severity of ulcerative colitis

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### Abstract

**AIM:** The aim of this study was to evaluate the association between ulcerative colitis activity and plasma or mucosal concentrations of interleukin (IL)-18.

**METHODS:** IL-18 concentrations were measured in plasma and mucosal samples from 15 patients with active ulcerative colitis (UC).

**RESULTS:** The mean plasma concentration of IL-18 measured in all patients ( $422 \pm 88$  pg/mL) doubled the mean value in healthy controls ( $206 \pm 32$  pg/mL); however, the difference was not statistically significant. Plasma IL-18 levels revealed a significant positive correlation with scored endoscopic degree of mucosal injury, disease activity index, clinical activity index and C-reactive protein concentration. The mean concentration of plasma IL-18 was significantly higher in patients with severe ulcerative colitis ( $535 \pm 115$  pg/mL) than in patients with mild ulcerative colitis ( $195 \pm 41$  pg/mL), and in healthy controls. Although the mucosal mean IL-18 concentration in severe ulcerative colitis ( $2\,523 \pm 618$  pg/mg protein) doubled values observed in mild one ( $1\,347 \pm 308$  pg/mg protein), there was no statistically significant difference.

**CONCLUSION:** Plasma IL-18 can be considered as a surrogate marker helpful in evaluation of ulcerative colitis activity.

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**Key words:** Ulcerative colitis; Interleukin-18

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### INTRODUCTION

The pathogenesis of ulcerative colitis (UC) is still unknown, but it seems to be related to the abnormal immune response and diminished ability of mucosal protection and regeneration. Signaling between epithelial cells, involving the complex

network of bioactive substances plays a crucial role in these processes<sup>[1-4]</sup>. Cytokines are involved in the modulation of the immune system related to the pathogenesis of inflammatory bowel disease<sup>[5-7]</sup>, and are rapidly synthesized and secreted by inflammatory cells upon stimulation. They induce the production of adhesion molecules, reactive oxygen metabolites, prostaglandins, leukotrienes, nitric oxide and platelet activating factors. Interleukin (IL)-18 is a potent inducer of interferon gamma production and plays an important role in inducing of both Th1 and Th2 immune responses<sup>[8,9]</sup>. According to a recent study by Ishikura *et al*<sup>[10]</sup>, its excessive production could exacerbate colitis in an experimental model. On the other hand, we know from a previous research by Siegmund *et al*<sup>[11]</sup>, that neutralization of IL-18 with specific antibodies could reduce the severity of experimental colitis. Elevated serum concentration of IL-18 has recently been demonstrated in humans with Crohn's disease (CD)<sup>[12]</sup>. The role of IL-18 in the pathogenesis of inflammatory bowel diseases (IBD) was studied mostly in experimental models. The aim of this study was to evaluate the association between ulcerative colitis activity and plasma or mucosal concentrations of interleukin-18.

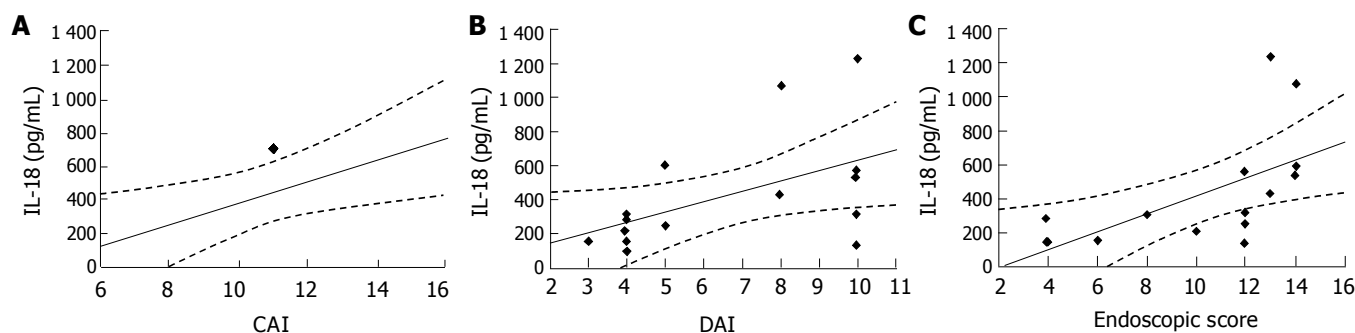
### MATERIALS AND METHODS

#### Patients

Interleukin-18 concentrations were measured in plasma and mucosal samples from 15 patients (3 females and 12 males) with active ulcerative colitis (UC), aged from 22 to 71 years (mean:  $38.9 \pm 3.3$  years). All patients had a history of diagnosed ulcerative colitis, which required typical clinical and endoscopic signs of distal part bowel involvement. Patients were treated with 5-ASA derivatives in the standard dose of 3.0 g/24 h. None of them received any steroids at the time of the study. IL-18 plasma and mucosal concentrations were compared with endoscopic pictures scored according to Meyers *et al*<sup>[13]</sup>, the disease activity index (DAI) according to Schroeder *et al*<sup>[14]</sup>, clinical activity index (CAI) according to Truelove and Witts<sup>[15]</sup>. Routine laboratory indices of the inflammatory process such as C-reactive protein (CRP), sedimentation rate (SR), white blood count (WBC) and platelet count (PLT) as well as hemoglobin, fibrinogen, total protein and albumin concentrations were also measured and compared with IL-18 values. Mucosal biopsy for histological confirmation of UC and for IL-18 measurement was taken during sigmoidoscopy performed before treatment. Patients were divided into two groups with respect to severity of the disease. They were included into the severe UC group if the values of endoscopic score, DAI and CAI exceeded the middle range of all three scales that were 8, 6, 10. Severe form of ulcerative colitis was diagnosed in 10 patients and mild one in 5. Plasma IL-18 concentrations were also compared with those in 12 healthy volunteers (5 females and 7 males with a mean age of  $40.8 \pm 2.7$  years). The study was approved by the Bioethical Committee of the Medical University of Białystok. Informed consent was obtained from each patient.

#### IL-18 measurement

Venous blood was collected on ice using vacutainer tubes with



**Figure 1** Correlations between plasma concentrations of IL-18 and values of A: CAI ( $r = 0.56$ ,  $P = 0.02$ ); B: DAI ( $r = 0.54$ ,  $P = 0.03$ ); C: endoscopic score ( $r = 0.62$ ,  $P = 0.01$ ).

K-EDTA as an anticoagulant and centrifuged at 1,000 g within 30 min after collection. Biopsy specimens were mashed with a homogenizer, and the suspension was divided and used to determine IL-18 and protein concentrations. Samples were diluted 1:5 with 0.1 mol/L phosphate buffer before assay and stored at  $-20^{\circ}\text{C}$ . Samples were incubated at room temperature and assayed in duplicate with the quantitative sandwich enzyme immunoassay (EIA) technique using microtitre wells precoated with anti-human IL-18 antibodies (MBL, Nagoya, Japan). IL-18 remained in the microtitre wells after four cycles of washing and aspiration was detected by peroxidase-conjugated anti-human IL-18 specific antibodies. The amount of peroxidase bound to each well was determined by the addition of tetramethylbenzidine substrate. The reaction was stopped by acidification with 2N sulphuric acid and the optical density was read with a microtitre plate photometer Stat Fax® 2100 (Alab/Poland) at 450 nm. The concentration of IL-18 in sample was calibrated from a dose response curve based on reference standards. Mucosal IL-18 concentration was expressed as pg per mg of protein, which was measured by the Lowry *et al* [16] method. According to a manufacturer's sensitivity of the assay, which was 12.5 pg/mL, intra-assay reproducibility C.V. range from 5.03-9.92%, inter-assay 6.25-10.1% and recovery test 80-110%.

### Statistical analysis

Values were expressed as mean $\pm$ SE. The significance of differences was calculated by non-parametric Mann-Whitney U test. For correlation analysis, the Sperman non-parametric correlation was used.  $P < 0.05$  was considered statistically significant.

### RESULTS

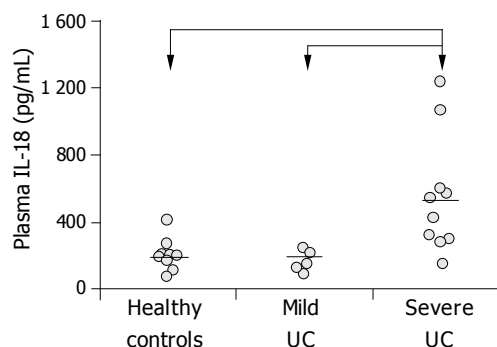
The mean plasma concentration of IL-18 measured in all patients with active UC ( $422 \pm 88$  pg/mL) doubled the mean value in healthy controls ( $206 \pm 32$  pg/mL), but the difference was not statistically significant. There was no association between plasma IL-18 and the age of patients or the disease duration. The mucosal concentration of IL-18 varied from 83 to 6 239 pg/mg protein. As demonstrated in Table 1 among laboratory markers of inflammatory activity, only the mean values of CRP and SR exceeded the upper limit of normal range. However, a significant positive correlation ( $r = 0.65$ ,  $P < 0.05$ ) was demonstrated only in respect to CRP values. Plasma IL-18 levels analyzed in all UC patients also revealed a significant positive correlation with scored endoscopic degree of mucosal injury, DAI and CAI values (Figure 1, Table 1). As shown in there was no association between mucosal concentration of IL-18 and clinical or laboratory signs of the disease activity. The mean concentration

of plasma IL-18 was significantly higher in patients with severe UC ( $535 \pm 115$  pg/mL) than in patients with mild UC ( $195 \pm 41$  pg/mL), and in healthy controls (Figure 2). Although the mucosal mean IL-18 concentration in severe UC ( $2\,523 \pm 618$  pg/mg protein) doubled the values observed in mild UC ( $1\,347 \pm 308$  pg/mg protein), there was no statistically significant difference (Figure 3).

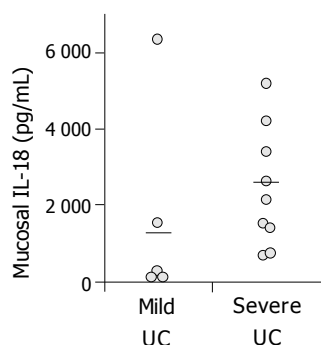
**Table 1** Laboratory and clinical markers of ulcerative colitis activity in analyzed patients

	Normal range	mean $\pm$ SE	Correlation ( $r$ )	
			plasma	mucosal
CRP [mg/dL]	0-5	18.5 $\pm$ 4.8	0.65 <sup>b</sup>	-0.13
SR [mm/h]	0-5	23.1 $\pm$ 5.0	0.36	0.20
WBC [ $\times 10^3/\mu\text{L}$ ]	4-10	6.8 $\pm$ 0.6	-0.08	0.06
hemoglobin [mg/dL]	12-18	13.2 $\pm$ 0.3	-0.36	0.07
fibrinogen [g/L]	1.8-3.5	3.5 $\pm$ 28	0.30	0.14
albumin [g/L]	3.5-5.0	2.99 $\pm$ 0.6	-0.43	0.42
protein [g/L]	6-8	5.8 $\pm$ 0.2	-0.29	0.31
PLT [ $\times 10^9/\mu\text{L}$ ]	130-400	246 $\pm$ 19.9	-0.13	0.18
CAI	0	10.7 $\pm$ 0.7	0.56 <sup>a</sup>	0.47
DAI	0	6.6 $\pm$ 0.7	0.54 <sup>a</sup>	0.29
Endoscopic score	0	3.0 $\pm$ 0.2	0.62 <sup>a</sup>	0.36

Correlations between plasma or mucosal concentrations of IL-18 and values of particular markers are demonstrated through ( $r$ ) values. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ .



**Figure 2** Individual plasma IL-18 concentrations in healthy controls and UC patients with a mild or a severe form of the disease. Statistical significance between severe UC and mild UC as well as a control is indicated by arrows ( $P < 0.05$ ). Horizontal bar denotes the mean IL-18 concentration [pg/mL] in each of studied groups.



**Figure 3** Individual mucosal IL-18 concentrations in UC patients with mild or severe form of the disease. Horizontal bar denotes mean IL-18 concentration [pg/mL] in each of studied groups (the difference is not statistically significant).

## DISCUSSION

Cytokines are the determinants of the nature of mucosal immune response. Development of IBD has been considered to be associated with the predominance of proinflammation over anti-inflammatory cytokines<sup>[2,17]</sup>. Animal models of experimental colitis exhibited clear differentiation toward Th1 or Th2 responses. CD4 T cells could produce predominant IL-2 and interferon-gamma, which are primarily involved in cellular immunity defined as Th1 response, whereas IL-4, IL-5 and IL-10 are predominantly produced by Th2 cells promoting humoral immunity<sup>[18,19]</sup>. Since it has been demonstrated by Seder *et al.*<sup>[20]</sup>, that IL-4 and IL-10 could stimulate the production of transforming growth factor (TGF)- $\beta_1$  by CD4<sup>+</sup> cells, its increased concentration may also reflect Th2-mediated T cell response in UC patients. As we demonstrated recently plasma and mucosal concentrations of TGF- $\beta_1$  were strongly associated with UC activity and successful treatment of the disease could result in a decrease of their levels<sup>[21]</sup>. In animal models, CD was predominantly related to Th1 immune response and Th2 immunity was responsible for UC development. In humans such a clear polarization of immune response in IBD pathogenesis was not observed<sup>[18,19,23]</sup>. According to Hoshino *et al.*<sup>[22]</sup> and Yoshimoto *et al.*<sup>[9]</sup>, IL-18 was involved in both Th1 and Th2 responses in UC. A recent study by Matsuzaki *et al.*<sup>[24]</sup> demonstrated in UC patients the predominance of Th1 response in severe inflammation and Th2 in mild one. Moreover, Th1 response was associated with the degree of disease activity. Since Th2 response was observed in mildly inflamed mucosae, it seemed to be involved in the early phase of UC or the initiation of inflammation.

In this study, we demonstrated the elevation of plasma IL-18 in UC patients, which doubled the normal values without statistical significance. However, there was an evident association between plasma IL-18 concentrations and the disease activity evaluated through clinical and endoscopic scoring systems. Additionally there was a significant correlation between IL-18 values and CRP - an unspecific marker of inflammation. The association with UC activity was finally proven by demonstrating a significant difference between patients with mild and severe UC. According to Furuya *et al.*<sup>[12]</sup>, IL-18 concentration was elevated in CD but not in UC patients. We divided the patients into active and inactive UC, but did not evaluate IL-18 concentrations in respect to degrees of the disease activity. Unfortunately, we were not able to carry out the study in patients with Crohn's disease, because of its extremely low incidence in this part of Europe.

There was no correlation between mucosal IL-18 concentration and clinical or endoscopic sign scores of the

disease activity as well as between mucosal and plasma values. However, mucosal IL-18 concentration was almost two times higher in our patients with severe UC than in those with mild UC. The lack of a significant difference between these groups could be related to the wide distribution of individual values (from 83 to 6 239 pg/mg protein), resulting probably from the different sites of specimens. This discrepancy between mucosal and plasma values could also be recognized as a result of a secondary stimulation of IL-18 release outside the bowel wall, that could be mediated by inflammatory and immune responses.

In conclusion, ulcerative colitis activity is strongly associated with interleukin-18 concentrations in plasma but not in colonic mucosa. Plasma IL-18 can be considered a surrogate marker helpful in the evaluation of ulcerative colitis activity.

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