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Altered serum profile of the interleukin-22 system in inflammatory bowel disease

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A B S T R A C T

Background and Aim: Interleukin-22 (IL-22), plays a vital role in the mucosal repair of inflammatory bowel disease (IBD). Serum levels of IL-22 and IL-22 binding protein (IL-22BP), a soluble inhibitory IL-22 receptor, were measured in patients with IBD to investigate the profile of IL-22 in the systemic circulation.

Methods: Blood samples from 92 healthy subjects, 98 patients with ulcerative colitis (UC), and 105 patients with Crohn's disease (CD) were analyzed for serum levels of IL-22, IL-22BP, human β-defensin 2 (hBD-2), and serum inflammatory parameters. Disease activity was assessed by the partial Mayo score and Harvey-Bradshaw index for UC and CD, respectively.

Results: Serum IL-22 level was lower in UC ($P < 0.001$) and CD ($P < 0.001$) vs control and its decrease was more pronounced in CD than in UC ($P = 0.019$). Serum IL-22BP level was lower in UC ($P < 0.001$) and CD ($P < 0.001$) vs control and correlated with inflammatory parameters (albumin and C-reactive protein (CRP) in UC; hemoglobin, albumin, and CRP in CD). Serum IL-22/IL-22BP ratios were higher in UC ($P = 0.009$) vs control and correlated with inflammatory parameters (albumin and CRP). Serum hBD-2 level was higher only in CD ($P = 0.015$) but did not correlate with serum IL-22 levels, IL-22BP levels, IL-22/IL-22BP ratios, or inflammatory parameters.

Conclusions: Dysregulation of the IL-22 system in the blood may play a role in the pathogenesis of IBD. Further studies are needed to understand the pathogenic and clinical significance of the blood IL-22 system in IBD.

1. Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) [1] and Crohn's disease (CD) [2], is a chronic relapsing disorder affecting the gastrointestinal tract. Its pathogenesis is complicated, but current research suggests that intestinal injury may surpass the capacity for repair in genetically susceptible individuals [3]. In IBD, the dysregulated immune response is associated with the production of a range of immune mediators, in particular, cytokines [4].

Interleukin-22 (IL-22) is mainly produced by T cells and group 3 innate lymphoid cells at barrier surfaces and is crucial to maintaining epithelial integrity. IL-22 functions in tissue repair by induction of epithelial cell proliferation and in the promotion of antimicrobial activity by induction of antimicrobial peptides, such as human β-defensin 2 (hBD-2) [5,6]. IL-22 has been shown to contribute to epithelial remodeling of the IBD mucosa [7]. Therefore, IL-22 is considered a promising therapeutic agent in IBD [7]. In fact, a natural inducer of IL-22, Indigo naturalis, is currently being evaluated in a clinical trial of patients with UC with successful results [8].

IL-22 binds to not only a heterodimeric receptor composed of IL-10R2 and IL-22R1 but also a soluble receptor known as IL-22 binding protein (IL-22BP, also called IL-22Ra2 or CRF2-10) [9–11]. IL-22 has 20- to 1,000-fold higher affinity to IL-22BP compared to the membrane-bound IL-22R1 [12,13]. As IL-22 activity is tightly regulated by IL-22BP, the expression profile of IL-22BP is essential to evaluating the role of IL-22 in IBD. Previous studies have described the serum profile of IL-22BP in multiple sclerosis [14], psoriasis [15], and acute-on-chronic liver failure [16] to be significantly elevated. However, the expression level of IL-22BP in patients with IBD still remains unknown.

In the present study, to better understand the function of IL-22 in

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Table 1
Baseline characteristics of the study population.

	Ulcerative colitis	Crohn's disease	Healthy subjects
No. of patients	98	105	92
Sex (male/female)	54/44	60/45	52/40
Age (years)	41.6	33.7	39.6
(median, IQR)	(33.0–59.7)	(24.3–52.1)	(30.4–49.6)
Disease distribution	Proctitis/Left-sided colitis/Pancolitis	Ileitis/Colitis/Ileocolitis	–
	15/37/46	25/30/50	
Disease duration (years)	4.37	9.15	–
(median, IQR)	(1.0–9.0)	(2.8–15.9)	
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)	–
None (%)	9 (9.1%)	9 (8.5%)	

IBD, we assessed the characteristics of serum IL-22 and IL-22BP. We also evaluated the serum IL-22/IL-22BP ratio as a marker of the biological activity of IL-22 [15,16] and measured serum hBD-2 as an IL-22-induced biological product [17]. These measurements may offer clues for determining the pathogenic role of IL-22, thus aiding in the development of therapeutic options and identification of new biomarkers.

2. Methods

2.1. Ethical considerations

The study protocol was reviewed and approved by the Ethics Committee of Kurume University School of Medicine. Informed consent was obtained from each subject or their parents before enrollment in this study.

2.2. Patients

Between July 2016 and April 2018, serum samples were collected from 98 patients with UC and 105 with CD (Table 1). The diagnoses were based on characteristic clinical, endoscopic, radiological, and histological features. 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.

2.3. 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 $\leq$ 2 with no individual sub-score > 1 point) [18]. Patients with CD were graded according to the Harvey-Bradshaw index, with the inactive disease being defined as a score $<$ 5 points [19].

2.4. 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.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Serum concentrations of IL-22 and IL-22BP were measured using ELISA kits from eBioscience (BMS2047TEN, San Diego, CA, detection range 31.3–2,000 pg/mL, sensitivity 5.0 pg/mL) and MyBioSource Inc. (MBS915792, San Diego, CA, detection range 31.25–2,000 pg/mL, sensitivity 7.81 pg/mL), respectively. Serum hBD-2 was determined by hBD ELISA kits (EK-072–37, Phoenix Pharmaceuticals, Inc. Burlingame, CA).

2.6. Statistical analysis

Statistical analysis was performed with GraphPad Prism Software (GraphPad Software, San Diego, CA) or SPSS Statistics 23.0 software (IBM, New York). All values are expressed as medians (interquartile range [IQR]). Differences between groups were compared using the Mann-Whitney *U* test. Correlations were calculated using the Spearman rank correlation coefficient. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Serum IL-22 levels

Serum levels of IL-22 in 92 healthy subjects, 98 patients with UC, and 105 patients with CD are presented in Fig. 1. The median (IQR) level of serum IL-22 (pg/mL) was 14.6 (5.0–45.5) in normal controls, 6.3 (5.0–32.4) in UC, and 5.0 (5.0–17.3) in CD. Serum IL-22 levels were significantly lower in UC ($P < 0.001$) and CD ($P < 0.001$) compared with healthy controls. Interestingly, the decrease of serum IL-22 was more pronounced in CD than UC ($P = 0.019$). No significant difference was found in serum IL-22 levels between active and inactive diseases in UC ($P = 0.64$) and CD ($P = 0.42$).

3.2. Serum IL-22BP levels

We measured serum IL-22BP levels in patients with IBD for the first time. As shown in Fig. 2, the median (IQR) level of serum IL-22BP (pg/mL) was 9156.4 (5851.0–14723.0) in normal controls, 5287.1 (1848.5–9872.1) in UC, and 4410.8 (2303.8–7951.3) in CD. Serum IL-22BP levels were lower in UC ($P < 0.001$) and CD ($P < 0.001$) compared with those in healthy controls. No significant difference was observed in serum IL-22BP levels between UC and CD ($P = 0.332$) and between active and inactive diseases of UC ($P = 0.98$) and CD ($P = 0.88$).

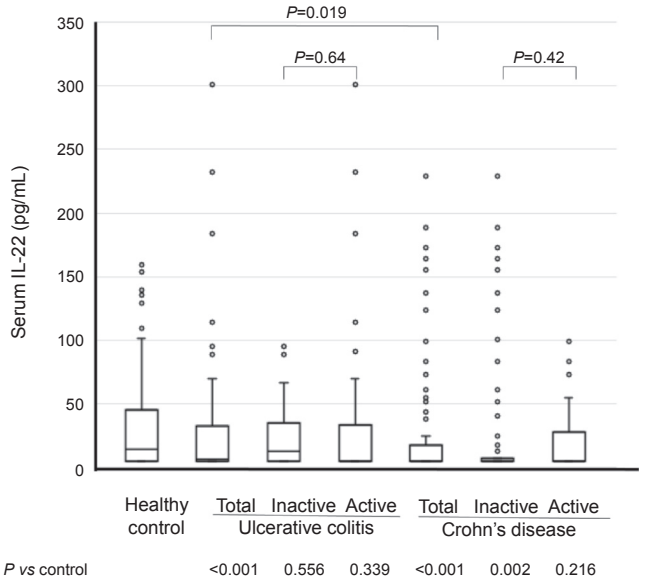

Fig. 1. Serum interleukin (IL)-22 levels in patients with ulcerative colitis (UC) or Crohn's disease (CD), and in healthy controls. Boxes represent the interquartile range (IQR) between the first and third quartiles and the line inside the bar represents the median. Whiskers indicate the lowest and highest values within 1.5 X IQR from the first and third quartiles. Circles represent outliers beyond whiskers.

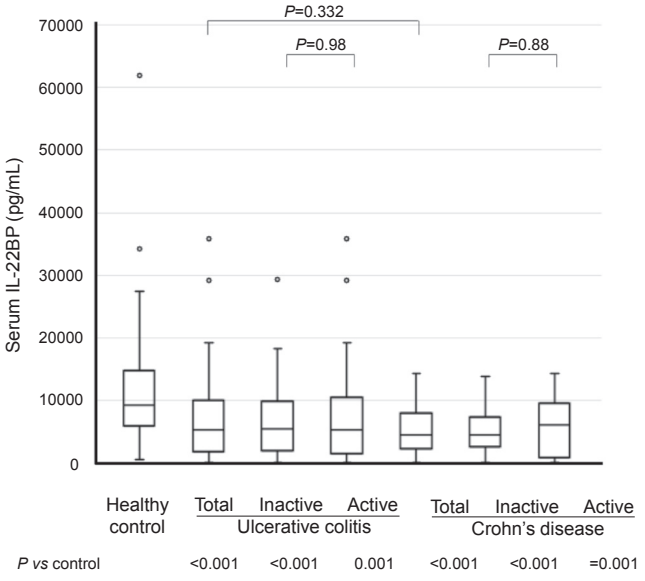

Fig. 2. Serum IL-22 binding protein (IL-22BP) levels in patients with UC or CD and in healthy controls. Boxes represent IQR between the first and third quartiles and the line inside the bar represents the median. Whiskers indicate the lowest and highest values within 1.5 X IQR from the first and third quartiles. Circles represent outliers beyond whiskers.

3.3. Serum IL22/IL22BP ratios

We examined serum IL-22/IL-22BP ratio as a predictable marker of IL-22 activity. The serum IL22/IL22BP ratio in patients with IBD is shown in Fig. 3. The median (IQR) serum IL22/IL22BP ratio was 0.00180 (0.00056–0.00544) in normal controls, 0.00354 (0.00071–0.01037) in UC, and 0.00140 (0.00079–0.00778) in CD. The serum IL22/IL22BP ratio was higher in UC (P = 0.009) compared with that in healthy controls, but this was not the case in CD (P = 0.213). No significant association was found in the serum IL22/IL22BP ratio between active and inactive diseases in UC (P = 0.954) and CD (P = 0.836).

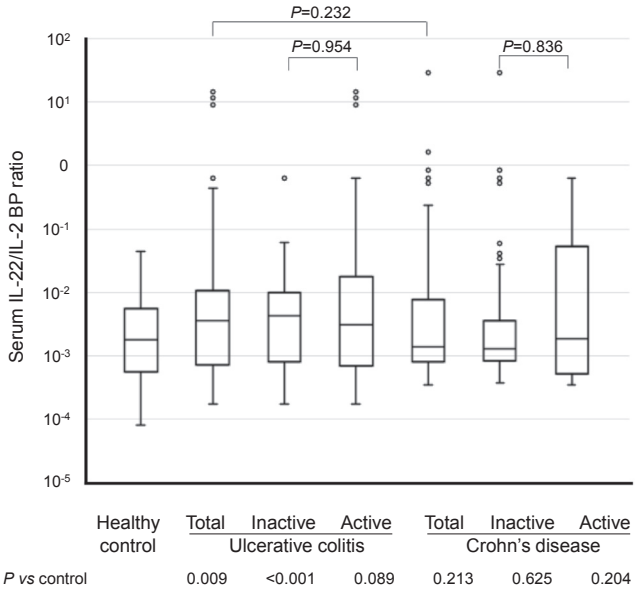

Fig. 3. Serum interleukin (IL)-22/IL-22 binding protein (IL-22BP) ratios in patients with UC or CD, and in healthy controls. Boxes represent IQR between the first and third quartiles and the line inside the bar represents the median. Whiskers indicate the lowest and highest values within 1.5 X IQR from the first and third quartiles. Circles represent outliers beyond whiskers.

3.4. Relation to disease type

We next compared the disease type of IBD with the serum IL-22 and IL-22BP levels as well as IL-22/IL-22BP ratios (Fig. 4). Serum IL-22 levels were comparable among patients with proctitis, left-sided colitis, and pancolitis in UC (Fig. 4**A**) and among patients with ileitis, colitis, and ileocolitis in CD (Fig. 4**B**). Serum IL-22BP levels were lower in patients with pancolitis compared to those with left-sided colitis (P = 0.004) and proctitis (P = 0.048) in UC (Fig. 4**C**), and they were comparable among patients with ileitis, colitis, and ileocolitis in CD (Fig. 4**D**). As for the serum IL-22/IL-22BP ratio, there was no significant difference among patients with proctitis, left-sided colitis, and pancolitis in UC (Fig. 4**E**) and among patients with ileitis, colitis, and ileocolitis in CD (Fig. 4**F**).

3.5. Relation to inflammatory parameters

We examined the association between serum IL-22, IL-22BP, and IL-22/IL-22BP ratios, and inflammatory parameters (Table 2). Serum IL-22 was not found to be associated with any inflammatory parameters in either UC or CD. In contrast, serum IL-22BP levels were associated with albumin (r = 0.394, P < 0.001) and CRP (r = -0.497, P < 0.001) in UC and with hemoglobin (r = 0.348, P < 0.001), albumin (r = 0.355, P < 0.001), and CRP (r = -0.220, P = 0.026) in CD. As for serum IL-22/IL-22BP ratios, they were associated with albumin (r = -2.52, P = 0.012) and CRP (r = 0.380, P < 0.001) in UC and with hemoglobin (r = -0.317, P = 0.001) and albumin (r = -0.262, P = 0.009) in CD. In addition, no statistical association was observed between clinical disease activity (partial Mayo score in UC and Harvey-Bradshaw index in CD) and serum IL-22, IL-22BP, or IL-22/IL-22BP ratios.

3.6. Relation to medical treatment

We also assessed the relationship between serum IL-22 levels, IL-22BP levels, and IL-22/IL-22BP ratios, and medical treatment. Although

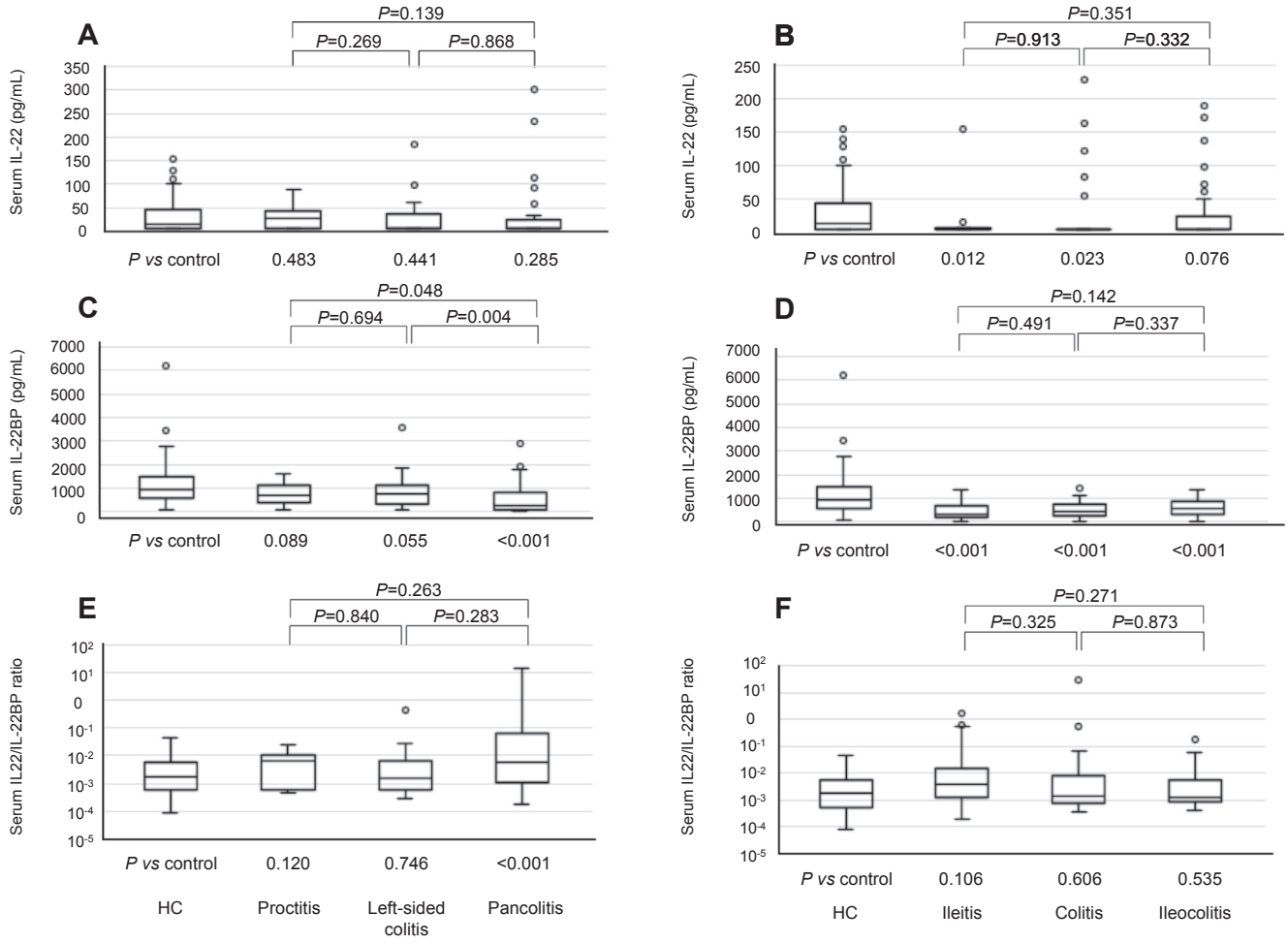

Fig. 4. Comparison of serum IL-22 levels, IL-22BP levels, and IL-22/IL-22BP ratios according to the disease extent in patients with UC **(A, C, E)** and CD **(B, D, F)**. Boxes represent IQR between the first and third quartiles and the line inside the bar represents the median. Whiskers indicate the lowest and highest values within 1.5 X IQR from the first and third quartiles. Circles represent outliers beyond whiskers.

the number of patients in each group was relatively small, no significant association was found between IL and 22, IL-22BP, or IL-22/IL-22BP ratios and cases with specific medical treatments, such as 5-aminosalicylic acid, prednisolone, immunomodulators, anti-tumor necrosis factor, and Indigo naturalis (**Supplementary** Fig. 1).

3.7. Serum hBD-2 levels

Next, we assessed serum hBD-2 levels as a marker of IL-22-induced biological product (Fig. 5). Serum hBD-2 levels were higher in CD ($P = 0.015$) than those in healthy controls, but this was not the case in UC ($P = 0.611$). The median (IQR) level of serum hBD-2 (pg/mL) was 1683.5 (344.8–4791.2) in healthy controls, 2165.2 (638.5–4021.2) in UC, and 2937.2 (1313.7–7641.0) in CD. No significant difference was found in serum hBD-2 levels between active and inactive diseases in UC ($P = 0.093$) and CD ($P = 0.711$) (Fig. 5**A**). According to the disease type, serum hBD-2 levels were higher in patients with pancolitis compared with left-sided colitis in UC (Fig. 5**B**) and they were comparable among patients with ileitis, colitis, and ileocolitis in CD (Fig. 5**C**). Serum hBD-2 levels did not correlate with serum IL-22, IL-22BP, IL-22/

Table 2
Correlation coefficients and significance of differences between serum interleukin (IL)–22 levels, IL-22 binding protein (IL-22BP) levels, and IL-22/IL-22BP ratios, and the clinical and inflammatory parameters in patients with ulcerative colitis (UC) and Crohn's disease (CD). Clinical disease activity was assessed using the partial Mayo score (p-Mayo) in UC and the Harvey-Bradshaw index (HBI) in CD. CRP, C-reactive protein; hBD-2, human β-defensin 2.

	IL-22				IL-22BP				IL-22/IL-22BP			
	UC		CD		UC		CD		UC		CD	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
p-Mayo	−0.04	0.66	–	–	−0.11	0.26	–	–	0.07	0.45	–	–
HBI	–	–	−0.066	0.517	–	–	0.013	0.901	–	–	0.103	0.76
Hemoglobin	−0.130	0.203	−0.159	0.109	−0.100	0.327	0.348	< 0.001	−0.04	0.694	−0.317	0.001
Albumin	0.164	0.107	−0.033	0.743	0.394	< 0.001	0.355	< 0.001	−2.52	0.012	−0.262	0.009
CRP	0.002	0.987	0.058	0.564	−0.497	< 0.001	−0.220	0.026	0.380	< 0.001	0.186	0.062
hBD-2	−0.040	0.696	0.156	0.117	−0.136	0.181	−0.096	0.339	0.124	0.224	0.184	0.065

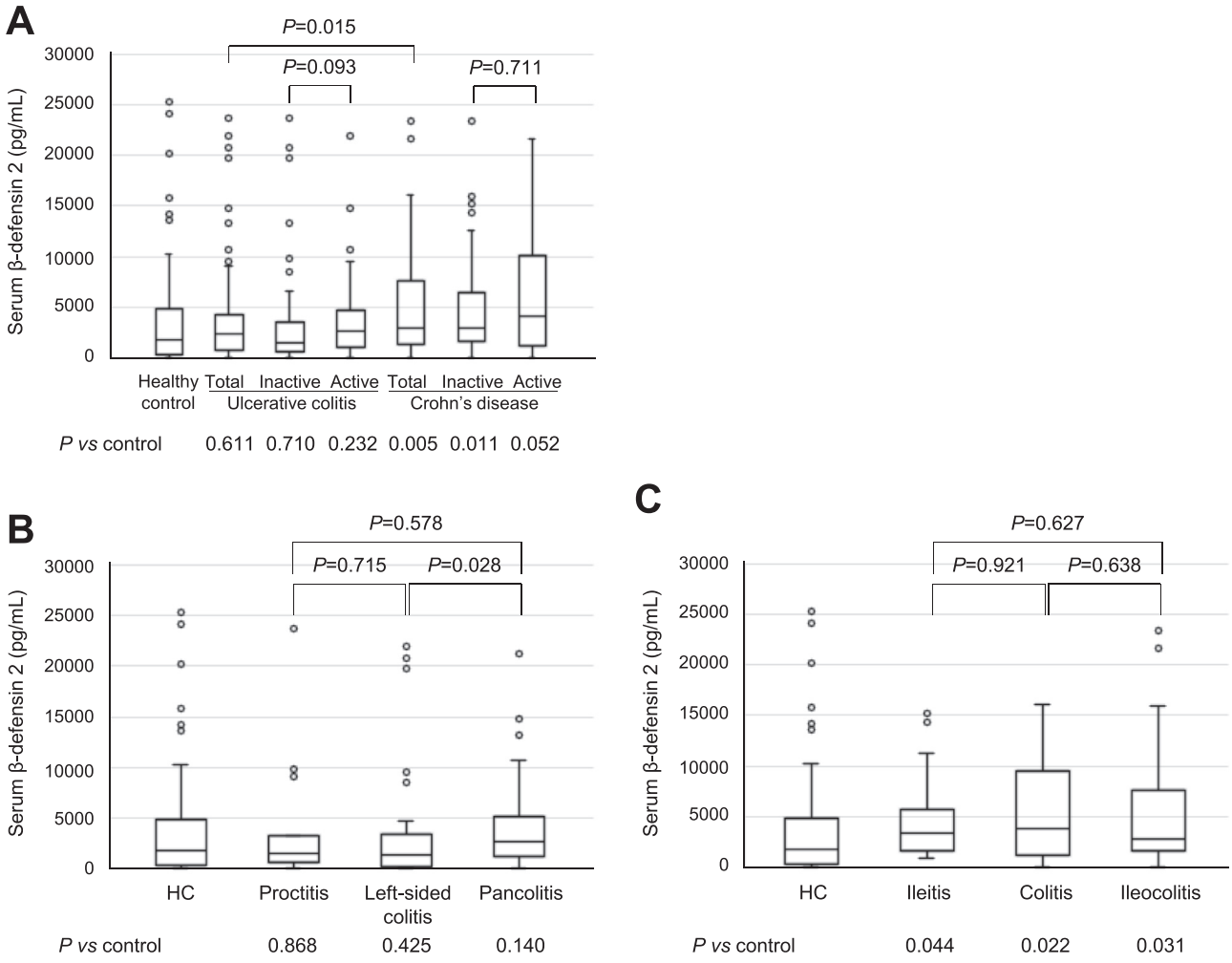

Fig. 5. (A) Serum human β-defensin-2 (hBD-2) levels in patients with UC or CD and in healthy controls. T, total (active plus inactive patients). **(B, C)** Comparison of serum hBD-2 levels according to the disease extent in patients with UC **(B)** and CD **(C)**. Boxes represent IQR between the first and third quartiles and the line inside the bar represents the median. Whiskers indicate the lowest and highest values within 1.5 X IQR from the first and third quartiles. Circles represent outliers beyond whiskers.

IL-22BP ratios, or inflammatory parameters (Table 2).

4. Discussion

IL-22 has recently been suggested to play an important role in IBD. Herein, we analyzed the interaction of IL-22 and its endogenous inhibitor IL-22BP in patients with IBD.

IL-22 is crucial to maintaining epithelial integrity with crosstalk functions between immune and epithelial cells. As for the serum profile of IL-22, previous studies showed that serum IL-22 levels are higher in rheumatoid arthritis, multiple sclerosis, Sjogren syndrome, and psoriasis; in contrast, they are lower in systemic lupus erythematosus (SLE) and sarcoidosis [20]. In IBD, our study demonstrated lower serum IL-22 levels in both UC and CD regardless of disease activity, which is very similar to the results of a study on SLE, showing no correlation between serum IL-22 levels and SLE disease activity index, as well as low serum IL-22 levels compared to normal controls [20]. This result was in contrast to a previous study showing a higher level of IL-22 in IBD, although the number of patients in each study was minimal [13,21–23]. The reason for this discrepancy is unclear but it might be partially due to the timing of blood sampling, or the difference in the antigen-epitope that is recognized by the anti-IL-22 antibody used in each ELISA. Our study also demonstrated that the decrease in serum IL-22 levels was more pronounced in CD than UC. This difference might be a result of variable manifestations between UC and CD: activating signals, responding cells, and related pathogenic events may be different in both diseases.

At present, the relationship between blood and gut IL-22 is still unknown. IL-22 is reported to be upregulated during inflammation in the colon [24–29] and is constitutively expressed in the small intestine [30–32]. Although it remains unclear whether blood IL-22 originated from the gut, the low blood IL-22 might be a secondary consequence of enhanced consumption of IL-22 at the disease site of IBD. Another possible explanation is the difference in the cell type producing IL-22 between the blood and gut. Cheng et al. speculated that the presence of the specific IL22-producing $CD4^+$ T cell subset in peripheral blood may be the cause of low serum IL-22 in SLE and does not correlate with disease activity [33]. A similar phenomenon may occur in IBD. IL-22 is characterized as a two-faced cytokine that has both protective and pathogenic properties [34,35]. Thus, gut and serum IL-22 might play different roles in the pathogenesis of IBD. Further studies would be necessary to better understand the functional significance of IL-22 in the blood.

We also examined serum IL-22BP, a soluble receptor antagonist of IL-22, to understand the regulation of IL-22 in the blood as is the case for the control of other cytokines, IL-1 by IL-1 receptor antagonist [36] or IL-6 by soluble gp130 [37]. In this study, we quantified serum IL-22BP in patients with IBD for the first time, demonstrated its decrease

in both UC and CD, and identified an association with serum inflammatory parameters, such as hemoglobin, albumin, or CRP. IL-22BP production is reportedly downregulated depending on the maturation of dendritic cells. In fact, IL-22BP is lower in experimental colitis in mice [38]. Therefore, the lower serum IL-22BP observed in our study may be explained partially by the maturation of dendritic cells induced by inflammatory stimuli. The quantitative assessment of serum IL-22BP, together with IL-22, may be essential to evaluate the biological activity of IL-22.

Therefore, to further examine the putative implication of IL-22 in IBD, we examined the serum IL-22/IL-22BP ratio as a conventional marker of IL-22 function [15,16]. One previous report has shown that the serum IL-22/IL-22BP ratio correlates with psoriasis disease severity [15]. Another report has revealed that low IL-22BP/IL-22 ratio was associated with acute-on-chronic liver failure and mortality of patients with cirrhosis [16]. This study showed a high IL-22/IL-22BP ratio in UC, which correlated with an increase in serum inflammatory parameters (albumin or CRP). Interestingly, our data indicate that the serum concentration of IL-22BP was approximately 100-fold higher than that of IL-22 in patients with IBD, suggesting sufficient neutralization of IL-22 by IL-22BP. Furthermore, it has been demonstrated that the binding capacity of IL-22 to IL-22BP is 20- to 1,000-fold higher than membrane-bound IL-22R1 in vitro [12,13]. Therefore, these data indicate that the IL-22-mediated immune process in the blood is under the tight control of IL-22BP.

A subgroup analysis among untreated and treated IBD patients showed that the current use of medications had no significant effects on serum IL-22 levels, IL-22BP levels, and IL-22/IL-22BP ratios. Indigo naturalis had no effect on serum IL-22 levels, although this treatment has been shown to enhance the production of IL-22 in the gut through binding with aryl hydrocarbon receptor; this discrepancy suggests different profiles of IL-22 in the blood and gut. Future studies sequentially assessing IL-22 levels, IL-22BP levels, and IL-22/IL-22BP ratios in the same patient will be required to confirm this association.

Serum IL-22 was consistently lower in IBD than normal controls irrespective of the disease phenotype (UC or CD) and disease activity (active or inactive), suggesting the fundamental dysregulation of IL-22 in patients with IBD. Although serum IL-22 was consistently lower, low IL-22BP and high IL-22/IL-22BP ratios observed in patients with IBD in correlation to the degree of some inflammatory parameters indicated the beneficial effect of the IL-22/IL-22BP system in regulation of systemic inflammatory reactions. However, other causes of sample homogeneity among patients could not be ruled out, as patients were at different stages of the disease and in several therapeutic situations.

HBDs are antimicrobial peptides important for innate immunity and contribute to the intestinal barrier. Of these, hBD-2 is expressed by epithelial cells mainly in response to IL-22 [39]. We measured the serum hBD-2 level and found that it was higher in CD but not UC, which may indicate different responses of the innate mucosal defense. At the local site of IBD, hBD-2 transcript is upregulated, suggesting that hBD-2 is induced during inflammation [40]. The lack of direct correlation between serum hBD-2 and IL-22 may indicate the involvement of different cytokines other than IL-22. In fact, serum hBD-2 levels represent the balance of hBD-2-inducing versus hBD-2-reducing factors such as IL-22/IL-1β/tumor necrosis factor-α/interferon-γ/oncostatin M versus IL-13/IL-4/thymic stromal lymphopoietin, respectively [41,42]. We should extensively examine serum hBD-2 levels and their correlations with individual cytokine levels. Moreover, hBD-2 is produced not only in the gut but also in the skin and lungs in response to various inflammatory stimuli [43], suggesting that serum hBD-2 levels may reflect systemic inflammation rather than gut inflammation alone.

Our study had some limitations. First, this study was performed as a single-center analysis. Second, functional assays that can provide further information on the possible biological activity of IL-22 were not performed. Third, this study did not include the evaluation of endoscopic disease activity. Fourth, we were not able to quantify intestinal IL-22 and IL-22BP levels. Fifth, we did not measure the serum levels of negative regulators of IL-22 other than IL-22BP, such as transforming growth factor-β [44] and IL-38 [45], and did not examine how they correlate to the serum IL-22 profile, which should be evaluated in future studies. Finally, the clinical relevance of IL-22 as a biomarker for IBD is probably rather limited in the presence of other established markers for this disease. However, the value of our study lies in exploration of the profile of this critical cytokine and its antagonist during the course of IBD.

In conclusion, these results indicate that the dysregulation of the IL-22 system in the blood may provide novel insights into the pathophysiology of IBD. It should be recognized that the pure determination of serum IL-22 has limited value without knowledge of the exact interaction between IL-22 and IL-22BP. We should examine whether the net effect of IL-22 in the blood is pro- or anti-inflammatory. Further studies are needed to explore the pathogenic and clinical significance of the blood IL-22 system in IBD.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2020.155264.

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