Serum ST2 in inflammatory bowel disease: a potential biomarker for disease activity

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ABSTRACT

ST2, a specific ligand of interleukin 33, was described as a biomarker protein of inflammatory processes and overexpression of ST2 in ulcerative colitis (UC) was shown previously. We aimed to investigate the potential relationship of serum ST2 levels with the clinical, endoscopic and histopathological activity scores in UC and Crohn's disease (CD). Serum ST2 levels were determined in 143 patients with inflammatory bowel disease (IBD) (83 UC and 60 CD), in 50 healthy controls (HC), and in 32 patients with irritable bowel syndrome (IBS). Serum ST2 levels were elevated in IBD (56.8 (41.9–87.2) pg/mL) compared to HC and IBS (30.7 (20.2–54.3), p<0.001 and 39.9 (25.9–68.7) pg/mL, p=0.002, respectively). No significant difference was found between UC (54.2 (41.3–93.0) pg/mL) and CD (63.8 (42.7–88.4) pg/mL) and between IBS and HC. Serum ST2 levels were significantly increased in active UC compared to inactive UC (72.5 (44.1–99.5) vs 40.0 (34.7–51.6) pg/mL, p<0.001) and in active CD in comparison with inactive CD (63.8 (42.7–88.4) vs 48.4 (29.6–56.9) pg/mL, p=0.036). Patients with CD showing fistulizing behavior had significantly higher ST2 levels compared to patients with inflammatory and stricturing CD (p<0.001). Clinical activity scores of patients with UC and CD were correlated with serum ST2 levels (r=0.692, p<0.001 and r=0.242, p=0.043, respectively). Serum ST2 levels showed stepwise increases with the increasing histopathological scores of patients with UC and CD (p<0.001 for both). The present study highlights significant associations between ST2 and IBD presence and activity and demonstrates elevated serum ST2 levels in patients with active CD as a novel finding.

Significance of this study

What is already known about this subject?

- Non-invasive assessment of disease severity is considered as the ideal mainstay of routine disease monitoring of inflammatory bowel disease (IBD).
- ST2 protein, a receptor of interleukin 33, has recently been identified as a new and reliable biomarker of various inflammatory diseases.
- Increased ST2 expression was found in inflamed mucosa of patients with IBD.
- Significant elevation of serum ST2 levels was reported in active ulcerative colitis (UC) compared to inactive UC.

What are the new findings?

- By enrolling a considerable number of patients with Crohn's disease (CD) for the first time in the literature, significantly higher serum ST2 levels in active CD compared to inactive CD were demonstrated as a novel finding.
- Furthermore, serum ST2 levels were found significantly elevated in fistulizing CD compared to stricturing and inflammatory CD.
- The significant relation of serum ST2 levels to the widely used clinical and histopathological activity scores in both of the major subtypes of IBD (CD and UC) was shown.

How might these results change the focus of research or clinical practice?

- This study highlights a significant association between ST2 and IBD presence and activity.
- These results indicate that ST2 may be a key component of inflammation in IBD.
- By conducting further longitudinal studies, the ability of ST2 to diagnose IBD and to grade its activity can be better understood and ST2 may find a place in clinical practice as an objective measure of disease activity and can diminish the need for colonoscopy.

INTRODUCTION

Inflammatory bowel diseases (IBDs), ulcerative colitis (UC), and Crohn's disease (CD) are life-long relapsing–remitting gastrointestinal inflammatory disorders that are thought to result from an exaggerated mucosal immune response to intestinal pathogens in a genetically susceptible host.[1] IBD is an important public health problem that affects millions of people worldwide and leads to substantial morbidities and high health costs.[2 3] Moreover, the intestinal

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inflammation associated with IBD is an established risk factor for colonic malignancy.[4]

Currently, the diagnosis of IBD is based on radiological, endoscopic, and histological examination. After diagnosing the disease, colonoscopy is further needed to monitor disease activity and assess responses to standard therapies. However, besides being associated with significant procedural risks, colonoscopy is an invasive and expensive procedure. Therefore, non-invasive assessment is considered as the ideal mainstay of routine disease monitoring and many efforts have been made to identify laboratory biomarkers that can be used as an objective measure of disease activity and can help to diminish the need for colonoscopy.[5 6]

Although the exact etiology of the chronic inflammation in IBD is unknown, it is widely accepted that an imbalance of proinflammatory and anti-inflammatory mediators is the key factor in pathogenesis.[7] In support of this hypothesis, selective blockade of proinflammatory cytokines is currently one of the most effective strategies to downregulate mucosal inflammation in IBD; the best example is the use of antitumor necrosis factor (TNF) therapies to successfully treat both CD and UC.[8] Another evidence that supports the theory that chronic IBD is the result of dysfunctional immunoregulation manifested by the inappropriate production of mucosal cytokines is the imbalance between the production of interleukin 1 (IL-1) and its natural antagonist, the IL-1 receptor antagonist.[9]

In this setting, the formerly orphaned IL-1 receptor-related protein, ST2, was discovered to be the specific ligand of IL-33, which is a novel IL-1 family member. ST2 exists in two different splice variants leading to the synthesis of ST2L, a transmembrane receptor that confers IL-33's biological effects, and sST2, a soluble molecule that most likely serves as a decoy receptor for IL-33 and can be measured in serum.[10] Serum ST2 was recently described as a biomarker protein of inflammatory processes, such as heart failure,[11] systemic lupus erythematosus,[12] ankylosing spondylitis,[13] idiopathic pulmonary fibrosis,[14] bronchial asthma,[15] septic shock, and trauma.[16] High ST2 serum levels detected in chronic inflammatory processes suggest that sST2 might be involved in controlling the progression of the disease. Lately, the role of IL-33/ST2 axis in the pathogenesis of IBD was investigated by several studies, and increased ST2 expression in inflamed mucosa[17 18] and serum[19] of patients with IBD and the significant correlation of serum ST2 levels with total ST2 levels of colonic mucosa[19] were reported.

In light of the data presented above, we hypothesized that serum ST2 levels may be associated with the severity of disease in IBD. Therefore, we aimed to investigate the potential relationship of serum ST2 levels with the widely used clinical, endoscopic, and histopathological activity scores in UC and CD to see if there can be any clinical role of serum ST2 in determining disease activity in IBD.

MATERIALS AND METHODS

Patients and controls

Between January 2014 and September 2015, 143 patients with IBD (83 with UC and 60 with CD), 50 healthy controls (HC), and 32 patients with irritable bowel syndrome (IBS) were enrolled in the study. Patients with IBD had a definitive diagnosis of UC or CD confirmed by clinical, endoscopic, radiological, and histological workup, based on standard criteria.[20 21] All patients with IBS fulfilled the ROME III criteria.[22] Patients with malignancy, hepatic insufficiency, renal dysfunction, indeterminate colitis, non-classifiable inflammatory disease, cardiovascular disease, history of autoimmune diseases, and celiac disease were excluded. The control group had no illness, no inflammatory disease, no history of smoking, no usage of alcohol, drug, or herbal substances, and no history of previous intestinal or autoimmune diseases. All patients and controls were of Turkish descent.

Endoscopic, clinical, and histopathological assessments

In the case of UC, endoscopic activity was determined by the Rachmilewitz endoscopic activity index based on granulation, vascular pattern, mucosal vulnerability, and mucosal damage. The score range from 0 to 12, with a higher score indicating more severe disease and endoscopic remission, was defined as ≤4 points by this index.[23] Endoscopic activities of CD cases were determined by the Simple Endoscopic Score for Crohn's Disease (SES-CD). [24] To calculate SES-CD, the intestine was divided into five segments (the ileum, right colon, transverse colon, left colon, and rectum), and the degree of disease activity in each segment was evaluated by four parameters: the presence and size of ulcers (score 0–3), the extent of the ulcerated surface (score 0–3), the extent of the affected surface (score 0–3), and the presence and type of narrowing (score 0–3). The SES-CD score was calculated as the sum of the disease activity scores from all five segments, ranging from 0 to 60, with a higher score indicating more severe disease. Endoscopic remission was defined as ≤2 points by this index.

Clinical activity in patients with UC was determined by the clinical colitis activity index (CCAI).[23] A CCAI score exceeding 4, on a 0–29 scale, was compatible with active disease while different degrees of clinical activity were defined as follows: inactive (remission) 0–4, mild activity 5–10, moderate activity 11–17, and high activity >18 points.

CD activity index (CDAI),[25] a scoring system used to quantify the symptoms of patients with CD, was used to assess clinical activity in patients with CD. A CDAI score <150 points was compatible with inactive disease (remission), while different degrees of clinical activity were defined as follows: mild activity 151–220, moderate activity 221–450, and high activity >450 points.

The location of biopsies was determined by endoscopists and at least 4 biopsies were obtained from each of the following segments: terminal ileum, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum. If the colonic segments were endoscopically normal, the biopsies were taken randomly and if the segments were diseased, the biopsies were taken from the lesions. All biopsies were fixed in 10% neutral formalin, processed, and sections stained with H&E. An experienced gastrointestinal pathologist blinded to clinical information evaluated the biopsies and used histopathological score for the evaluation of intestinal inflammation in both diseases. Each biopsy was graded on a scale of 0–3 (grade 0=inactive, grade 1=cryptitis-crypt abscesses in <50% of mucosa, grade 2=cryptitis-crypt abscesses in >50% of

mucosa, and grade 3=ulceration). On the basis of evaluation of all of the individual biopsies according to the following system, each subject had an overall histopathological classification as inactive (grade 0), mild (grade 1), moderate (grade 2), or severe (grade 3). Subjects were considered inactive if all biopsies were grade 0. Subjects were considered mild if there were one or more segments that had biopsies of maximum grade 1. Patients were considered moderate if >1 segment had grade 2 inflammation and severe if one or more segments had grade 3 inflammation.[5]

Ethical considerations

The study protocol was approved by our local Ethics Committee, and all subjects gave their written informed consent to participate in the study.

Laboratory studies

All subjects underwent physical examination, anthropometric measurements, and biochemical screening. The weight and height of the participants were measured with a calibrated scale after patients had removed their shoes and any heavy clothing. Body mass index (BMI) was computed as body weight/(height)^2. Standard laboratory parameters of all patients including white cell count (WCC), platelet count, high-sensitive C reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), serum ferritin, and serum albumin were routinely determined at the central laboratory of our center.

For ST2 analyses, all blood samples were collected from an antecubital vein between 8:00 and 9:00 after overnight fasting just before the colonoscopy procedure. Blood was drained to a serum separator tube and allowed to clot for 30 min. After sufficient clotting, samples were centrifuged for 15 min at 1000*g*. Then the serum was removed immediately and stored frozen at −80°C until analyzed.

Serum ST2 concentrations were measured using the Human ST2 ELISA kit (Catalog number KE1248, IMMUNOWAY, Newark, Delaware, USA) according to the manufacturer's instructions. The assay is based on the method of quantitative sandwich enzyme immunoassay, and the intra-assay and inter-assay coefficients of variation were 4–6% and 8–10%, respectively.

Statistical analysis

All analyses were performed using SPSS V.21.0 for Windows (IBM Corp, Armonk, New York, USA). Descriptive statistics were used for the overall sample and patient groups. Variables were investigated using visual (histograms, probability plots) and analytical methods (Shapiro-Wilk test) to determine whether or not they are normally distributed. The variables were expressed as means±SDs for the normally distributed data, and as medians and first and third quartiles in the brackets for the non-normally distributed variables. Since serum ST2 was not normally distributed, it was handled by the Mann-Whitney U test and Kruskal-Wallis test when a statistically significant difference between the subgroups was noted. For post hoc comparisons, p values were calculated by Bonferroni correction acccording to the number of variables included. The Student t test was used to evaluate differences between the two study groups in normally distributed continuous variables like age, BMI, WCC, albumin, ferritin, and platelets. Correlations among the study variables were analyzed by the Pearson and Spearman tests depending on normality of variables. The capacity of serum ST2 levels in differentiating IBD from IBS, active IBD from inactive IBD, active UC from inactive UC, and active CD from inactive CD was analyzed using receiver operating characteristic curve (ROC) analysis and areas under the curves (AUCs) were computed. The optimum cut-off values were determined as the points on ROC curves that are closest to the upper left corner. Sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated for these cut-off values. A p value of <0.05 was considered to show a statistically significant result.

RESULTS

A total of 225 participants were enrolled in the study. Of these, 143 were patients with IBD (83 (58%) UC, 60 (42%) CD), 32 had IBS, and 50 were HC. The demographic and baseline characteristics of UC, CD, IBS, and HC groups are shown in table 1.

Serum ST2 in IBD and control groups

Serum ST2 levels were elevated in patients with IBD (56.8 (41.9–87.2) pg/mL) compared to patients with HC and IBS (30.7 (20.2–54.3), p<0.001 and 39.9 (25.9–68.7) pg/mL, p=0.002, respectively). When compared separately, serum ST2 levels of patients with UC (54.2 (41.3–93.0) pg/mL) and CD (63.8 (42.7–88.4) pg/mL) were significantly higher than concentrations found in patients with HC and IBS (UC: p<0.001, p=0.004; CD: p<0.001, p=0.003, respectively). There were no significant differences between patients with UC and CD (p=0.838) and between patients with HC and IBS (p=0.08) in terms of serum ST2 levels. Serum ST2 levels of UC, CD, IBS and HC groups are shown in figure 1.

Serum ST2 levels with regard to endoscopic activity

According to the Rachmilewitz endoscopic activity index, 58 (69.9%) of the UC cases were active and 25 (30.1%) of them were classified as inactive. With respect to SES-CD, 52 (86.7%) of the CD cases were active and 8 (13.3%) of them were inactive. In total, 110 (76.9%) of IBD cases were endoscopically active and the rest of them (n=33, 23.1%) were inactive. Significantly higher levels of serum ST2 levels were observed in patients with active IBD compared to patients with inactive IBD (66.3 (44.1–95.7) vs 42.3 (34.1–56.5) pg/mL, p<0.001). Similarly, serum ST2 was significantly higher in active UC compared to inactive UC (72.5 (44.1–99.5) vs 40.0 (34.7–51.6) pg/mL, p<0.001) and in active CD compared to inactive CD (63.8 (42.7–88.4) vs 48.4 (29.6–56.9) pg/mL, p=0.036). Serum ST2 levels of active UC and active CD cases were statistically significantly higher than those for the IBS (p<0.001 and p=0.002, respectively) and HC (p<0.001 for both) groups. Patients with inactive UC and inactive CD did not show any statistically significant difference compared to the IBS (p=0.974 and 0.761, respectively) and HC groups (p=0.096 and 0.207, respectively). Serum ST2 levels of active UC, inactive UC, active CD, inactive CD, IBS, and HC groups are shown in figure 2.

Table 1 The main clinical characteristics and laboratory parameters of patients and controls

	IBD	UC	CD	IBS	HC
Patients	143	83	60	32	50
Gender F/M	84/59	48/35	36/24	21/11	25/25
Age	40.2±11.7	39.9±11.5	40.6±12.4	41.3±12.8	40.3±11.8
BMI, kg/m^2	24.9±4	25.4±4.2	24.2±3.9	25.2±4.3	23.2±3.8
Disease duration	4 (1.0–10.0)	4.0 (2.0–10.0)	4 (0.25–11.5)	–	–
Serum ST2, pg/mL	56.8 (41.9–87.2)	54.2 (41.3–93.0)	63.8 (42.7–88.4)	39.9 (25.9–68.7)	30.7 (20.2–54.3)
WCC, /mm^3	7660±2493	7821±2682	7274±2285	6754±1873	6797±1539
hs-CRP, mg/L	6.21 (3.5–18.0)	5.0 (3.5–12.0)	8.8 (3.3–30.1)	1.3 (1.08–3.3)	4.9 (3.1–7.1)
ESR	15.0 (9.0–30.0)	12.0 (8.0–24.0)	21.0 (11.0–43.3)	4.5 (2.3–11.0)	8.5 (2.8–13.0)
Ferritin, ng/mL	34.7 (13.2–65.0)	25.0 (8.75–55.0)	42.6 (22.4–82.6)	15.8 (5.2–30.2)	21.2 (12.1–52.3)
Albumin, g/dL	4.5 (4.0–4.7)	4.5 (4.1–4.7)	4.4 (4.0–4.7)	4.6 (4.2–4.9)	4.6 (4.3–4.8)
Platelet, ×10^3/mm^3	274 (231–359)	285 (232–362)	265 (227–348)	243 (213–299)	250 (215–305)

Values are presented using means±SDs for normally distributed data and medians and first and third quartiles in the brackets for the non-normally distributed variables. BMI, body mass index; CD, Crohn's disease; ESR, erythrocyte sedimentation rate; F, female; HC, healthy controls; hs-CRP, high-sensitive C reactive protein; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; M, male; UC, ulcerative colitis; WCC, white cell count.

The AUC for serum ST2 levels to differentiate patients with IBD from patients with IBS was found to be 0.679 with a p value of 0.002 (figure 3A). At the cut-off value of 46.5 pg/mL with a sensitivity of 62.9%, specificity of 68.7%, PPV of 66.8%, and NPV of 64.9%. When the capacity of serum ST2 levels to differentiate active IBD from inactive IBD was evaluated, AUC was 0.758 with p<0.001 (figure 3B). At the cut-off level of 60.2 pg/mL, sensitivity was 55.9%, specificity was 90.8%, PPV was 85.9%, and NPV was 67.3%.

While we aimed to investigate differentiation between active UC and inactive UC, the AUC for ST2 was 0.808 with a p<0.001 (figure 3C). The optimum ST2 cut-off value was 47.1 pg/mL with sensitivity, specificity, PPV, and NPV values of 72.4%, 72.0%, 72.1%, and 72.3%, respectively. When serum ST2 levels were evaluated to differentiate active CD from inactive CD, AUC was 0.732 with a p=0.036 (figure 3D). At the cut-off level of 57.6 pg/mL, sensitivity was 55.8%, specificity was 100%, PPV was 100%, and NPV was 69.3%.

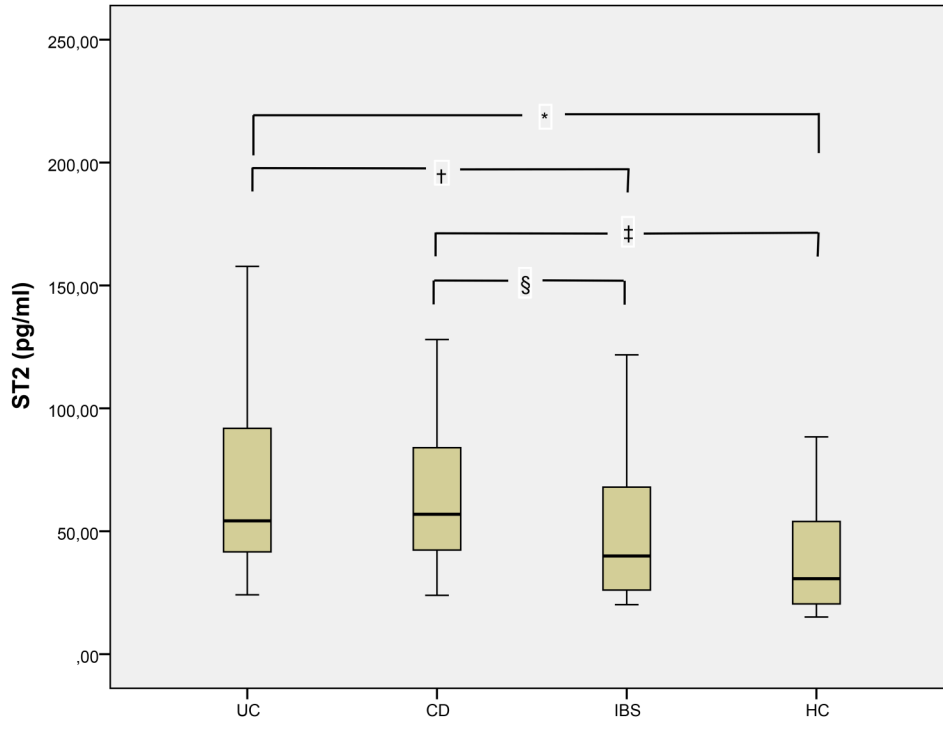

Figure 1 Median serum ST2 levels of UC, CD, IBS, and HC groups. The graph shows the IQR (box), median (thick line), and range (thin lines) of serum ST2 levels. The length of the box represents the IQR within which 50% of the values were located. *UC vs HC: p<0.001, †UC vs IBS: p=0.004, ‡CD vs HC: p<0.001, §CD vs IBS: p=0.003. CD, Crohn's disease; HC, healthy controls; IBS, irritable bowel syndrome; UC, ulcerative colitis.

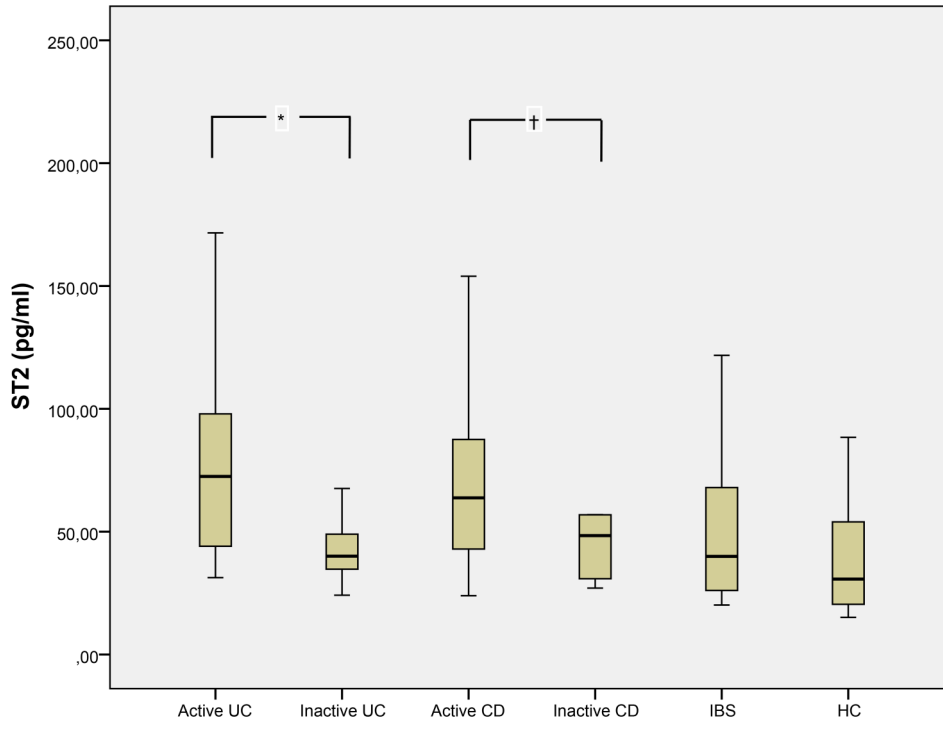

Figure 2 Median serum ST2 levels of active UC, inactive UC, active CD, inactive CD, IBS, and HC groups. The graph shows the IQR (box), median (thick line), and range (thin lines) of serum ST2 levels. The length of the box represents the IQR within which 50% of the values were located. *Active UC versus inactive UC: p<0.001, †Active CD versus inactive CD: p=0.036. CD, Crohn's disease; HC, healthy controls; IBS, irritable bowel syndrome; UC, ulcerative colitis.

Serum ST2 levels with regard to clinical and histopathological activities

Considering clinical activity, serum ST2 levels showed a good correlation with CCAI scores of patients with UC (r=0.692, p<0.001) and even though it was weaker, the correlation of serum ST2 levels with CDAI scores of patients with CD was still significant (r=0.242, p=0.043). Serum ST2 levels were further analyzed with regard to different degrees of disease activity, to investigate ability in discriminating inactive from mild, mild from moderate, and moderate from severe forms of UC and CD as defined by CCAI and CDAI, respectively. This analysis showed a significant ability of serum ST2 levels in discriminating inactive from mild and mild from moderate stages of UC, but such a finding was not seen between the CD patient subgroups defined according to CDAI. The statistical results of these findings compared to hs-CRP and ESR are shown in tables 2 and 3.

Considering pathological activity, serum ST2 levels showed a stepwise increase with the increasing histopathological scores of patients with UC (normal (score 0, n=11): 36.9 (33.1–44.1), mild (score 1, n=41): 46.8 (39.7–70.3), moderate (score 2, n=17): 75.9 (53.1–102.3), severe (score 3, n=14): 94.4 (60.9–155.6) pg/mL, p<0.001). When assessed for patients with CD, serum ST2 levels once again showed a significant increase with the increasing histopathological scores (normal (score 0, n=16): 45.7 (35.6–56.9), mild (score 1, n=27): 52.3 (39.9–67.9), moderate (score 2, n=5): 83.4 (36.3–132.3), severe (score 3, n=12): 99.1 (90.9–125.4), p<0.001).

Serum ST2 levels with regard to laboratory parameters

When the correlations of serum ST2 levels with the routinely used biochemical parameters including WCC, platelets, albumin, ferritin, hs-CRP, and ESR, and continuous variables regarding patient characteristics including patient age, BMI, and disease duration were investigated, serum ST2 levels correlated with disease duration (r=0.327, p=0.041), hs-CRP (r=0.427, p<0.001), ESR (r=0.347, p=0.001), and albumin (r=−0.343, p=0.002) in UC cases. In CD cases, hs-CRP and ESR were the only significantly correlated parameters, although the correlations were weaker (r=0.247, p=0.021 and r=0.223, p=0.047, respectively).

Serum ST2 levels of potential covariables regarding patient and disease characteristics

Potential variables including gender, smoking status, disease extent (for UC: proctitis, left sided, pancolitis), disease location (for CD: ileum, colon, ileocolonic, ileum+upper gastrointestinal tract), disease behavior (for CD: inflammatory, stricturing, fistulizing), endoscopic activity (active, in remission), medications (5-aminosalicylic acid, steroids, azathioprine, anti-TNF agents, no treatment), presence of surgical treatment, and presence of extraintestinal manifestations were further analyzed in terms of serum ST2 levels. The only statistically significant difference was seen between the disease behavior pattern of patients with CD showing significantly higher serum ST2 levels in patients with CD with a fistulizing behavior compared to patients who have inflammatory and stricturing CD (p<0.001).

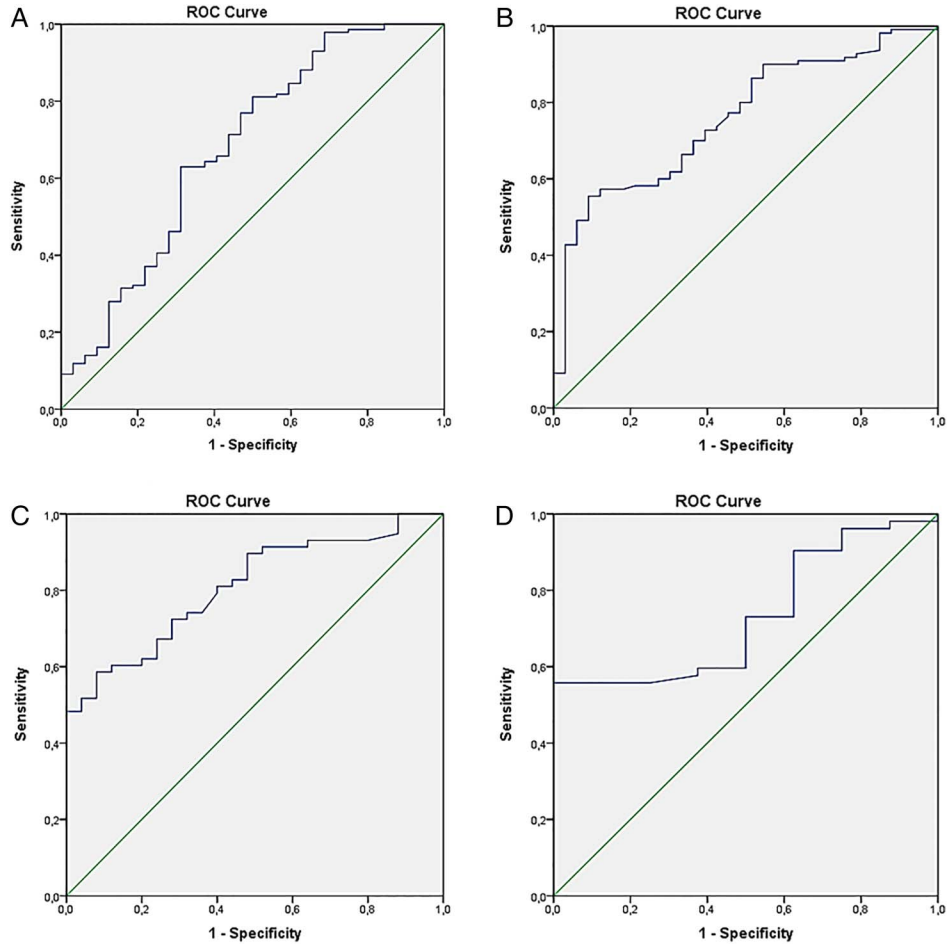

Figure 3 ROC curves exhibiting ability of serum ST2 to differentiate (A) IBD from IBS, (B) active IBD from inactive IBD, (C) active UC from inactive UC, (D) active CD from inactive CD groups (areas under the curves=0.679, 0.758, 0.808, and 0.732, respectively). CD, Crohn's disease; HC, healthy controls; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; ROC, receiver operating characteristic; UC, ulcerative colitis.

Serum ST2 levels of each category and related p values are shown in table 4.

DISCUSSION

The search for biomarkers that characterize specific aspects of IBD has received substantial interest in the past years and is moving forward rapidly as the demand for biomarkers in the routine clinical care of patients with IBD increases. Even though the exact cause of IBD is still unknown, an abnormal immune response causing a chronic inflammation is presumed as the final impact and it is thought to be due to an imbalance of pro-inflammatory and anti-inflammatory mediators. Since IBD has an immunological background and researches in the field of immunology showed great progression in past decades, immunological markers are increasingly being proposed to improve management of patients with IBD.

Table 2 Correlation of the CCAI with serum ST2, hs-CRP, and ESR

CCAI	Inactive (0–4)	p Value	Mild (5–10)	p Value	Moderate (11–17)	p Value	Severe ≥18
N/%	43/51.8		31/37.3		7/8.4		2/2.4
ST2, pg/mL	42.3 (34.7–56.2)		70.7 (48.3–95.7)		157.8 (127.5–184.4)		153.6 (114–193.1)
p Value		<0.001*		<0.001*		1.000	
hs-CRP, mg/L	3.9 (3.0–10.0)		6.21 (3.5–12.1)		11.2 (3.5–58.5)		29.3 (16.6–42.0)
p Value		0.053		0.483		0.667	
ESR	10.0 (6.0–18.0)		17.0 (9.0–24.0)		18.0 (8.0–66.0)		42.5 (34.0–51.0)
p Value		0.049*		0.658		0.500	

Values are presented using medians and first and third quartiles in the brackets.
*Statistically significant.
CCAI, clinical colitis activity index; hs-CRP, high-sensitive C reactive protein; ESR, erythrocyte sedimentation rate.

Table 3 Correlation of the CDAI with serum ST2, hs-CRP, and ESR

CDAI	Inactive (0–150)	p Value	Mild (151–220)	p Value	Moderate (221–450)	p Value	Severe >450
N/%	49/81.7		8/13.3		3/5		0/0
ST2, pg/mL	56.8 (40.3–81.6)		71.5(44.8–140.1)		82.0 (63.7–104.9)		–
p Value		0.161		0.683		–	
hs-CRP, mg/L	7.6 (3.1–26.2)		21.3 (2.4–68.7)		43.3 (39.8–57.6)		–
p Value		0.535		0.497		–	
ESR	19.0 (10.5–37.5)		34.0 (16.5–48.5)		7.0 (7.0–37.5)		–
p Value		0.324		0.630		–	

Values are presented using medians and first and third quartiles in the brackets.
CDAI, Crohn's disease activity index; ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitive C reactive protein.

In this context, sST2 protein, the receptor of IL-33, has recently been identified as a new and reliable biomarker of various inflammatory and autoimmune diseases. Moreover, increased ST2 expressions in the mucosa[17 18] and serum[19] of patients with IBD were reported. Therefore, we investigated the relationship of serum ST2 levels with disease

Table 4 Median serum ST2 values and regarding p values in UC and CD with potential covariables

	Ulcerative colitis			Crohn's disease		
	n (%)	ST2 (pg/mL)	p Value	n (%)	ST2 (pg/mL)	p Value
Total	83 (100)	54.2 (41.3–93.0)		60 (100)	56.9 (42.3–84.3)	
Gender						
Female	48 (57.8)	54.3 (40.8–94.8)	0.702	36 (60)	56.9 (40.5–83.1)	0.530
Male	35 (42.2)	54.2 (41.3–85.7)		24 (40)	59.1 (42.5–94.2)	
Disease extent						
Proctitis	11 (13.3)	53.8 (31.3–65.4)	0.223			
Left sided	41 (49.4)	48.3 (39.7–89.0)				
Pancolitis	31 (37.3)	60.2 (44.1–95.4)				
Disease location						
Ileum				24 (40)	48.4 (40.4–63.1)	0.015
Colon				12 (20)	66.0 (46.1–93.5)	
Ileocolonic				20 (33.3)	77.6 (51.2–105.8)	
Ileum+upper GI				4 (6.7)	82.3 (45.7–118.1)	
Disease behavior						
Inflammatory				30 (50)	56.8 (40.5–71.5)	0.015*
Stricturing				19 (31.7)	52.3 (42.5–82.0)	
Fistulizing				11 (18.3)	98.7 (68.7–117.7)	
Endoscopic activity						
Active		72.5 (44.1–99.5)	<0.001*		63.8 (42.7–88.4)	0.036*
Inactive		40.0 (34.7–51.6)			48.4 (29.6–56.9)	
Medication						
5-ASA	29 (34.9)	49.0 (39.1–90.6)	0.425	20 (33.3)	54.6 (35.9–74.8)	0.500
Steroids	32 (38.6)	61.9 (42.7–112.8)		2 (3.3)	47.2 (39.9–54.6)	
Azathioprine	9 (10.8)	46.8 (39.3–76.2)		16 (26.7)	57.6 (40.1–83.1)	
Anti-TNF-α	2 (2.4)	49.1 (44.1–54.2)		6 (10.0)	54.0 (45.1–83.2)	
No treatment	11 (13.3)	53.8 (33.1–70.7)		16 (26.7)	77.4 (46.7–106.0)	
Smoking						
Yes	9 (10.8)	61.9 (40.2–90.7)	0.913	11 (18.3)	76.9 (45.7–98.6)	0.468
No	74 (89.2)	54.0 (41.8–93.0)		49 (81.7)	56.9 (42.0–84.0)	
Surgical treatment						
Yes	4 (4.8)	54.8 (39.5–73.4)	0.671	11 (18.3)	52.3 (43.4–72.9)	0.640
No	79 (95.2)	54.2 (41.3–93.1)		49 (81.7)	61.1 (42.0–85.2)	
Extraintestinal manifestation						
Yes	7 (8.4)	85.7 (42.4–112.4)	0.279	18 (30)	56.9 (37.5–85.7)	0.473
No	76 (91.6)	54.0 (40.3–89.9)		42 (70)	57.6 (43.1–84.9)	

Values are presented using medians and first and third quartiles in the brackets.
*Statistically significant.
5-ASA, 5-aminosalicylic acid; anti-TNF, anti-tumor necrosis factor; CD, Crohn's diseas; GI, gastrointestinal; UC, ulcerative colitis.

activity of patients with IBD and, in addition to finding significantly higher serum ST2 levels in active UC compared to inactive UC, for the first time in the literature we showed significantly higher serum ST2 levels in patients with active CD compared to patients with inactive CD as a novel finding. Moreover, we showed correlation of serum ST2 levels with the widely used clinical activity scores and demonstrated the significant increase of serum ST2 levels with the increasing histopathological activity scores in UC and CD.

The first study investigating the IL33/ST2 receptor pathway in IBD was conducted by Seidelin *et al* in 2009.[26] They solely examined patients with UC and reported production of IL-33 by colonic epithelial cells in humans and upregulation of this production in UC. Only 1 year later, Beltrán *et al*[19] showed highly expressed ST2 and IL-33 in colonic mucosa of patients with IBD. Moreover, they found sST2 as the prevalent isoform in active IBD mucosa corresponding to the secreted one, but did not find any relationship between mucosal and serum IL-33 levels. Although they reported significantly increased serum ST2 levels in patients with active UC, owing to the very limited number of CD cases they could neither classify them as active or inactive nor evaluate such a difference in CD. Meanwhile, Pastorelli *et al*[17] reported increased circulating sST2 levels in patients with UC and CD compared to controls, but they did not further investigate the relationship serum ST2 levels with disease activity.

Eventually, Díaz-Jiménez *et al*[27] recently conducted a study investigating the relationship of intestinal and serum ST2 levels with the severity of IBD. Their study confirmed previous studies and showed a direct association of total intestinal ST2 levels with serum ST2 and found significantly higher serum ST2 levels in patients with UC and CD compared to controls, but again since they could enrol only a limited number of patients with CD they decided to focus on the analysis of ST2 levels restricted to the group of patients with UC. Concurrently with our findings, they found significantly higher serum ST2 levels in endoscopically active UC compared to inactive UC and reported significant correlations between serum ST2 levels and endoscopic and histopathological activities of UC patients, but missed assessment of the relationship of serum ST2 levels with clinical activity of patients.

By presenting the current study, we overcame the lacking analysis between ST2 and CD, and by enrolling a considerable number of patients with CD, for the first time in the literature, we succeeded in showing significantly higher serum ST2 levels in active CD compared to inactive CD, and in fistulizing CD compared to stricturing and inflammatory CD.

The significant increase observed in serum ST2 levels of patients with fistulizing CD is in line with the studies in the literature which pointed out the highest level of inflammation in CD cases that have fistulizing/penetrating disease behavior.[28 29] Although it must be supported by future studies on a larger scale, this finding may lead to a potential use of serum ST2 in clinical practice to select patients who do not have an evident fistulizing CD on initial presentation but may show up with overt fistulizing disease later on. Closer monitoring of these patients may yield better outcomes by early recognition of fistulas and starting advanced therapies on time.

When we evaluated the relationship of serum ST2 levels with disease location in CD, disease extent in UC, and medications used in IBD, no significant relationship was found. In contrast to our findings, increased serum ST2 levels in patients with UC with pancolitis compared to proctitis and left-sided colitis and in patients with UC receiving systemic corticoids compared to other therapies were reported in the study of Díaz-Jiménez *et al*.[27] Regarding serum ST2 concentrations, we found similar mean levels in the CD (56.9 vs 54.2 pg/mL) and control groups (30.7 vs 32.4 pg/mL) and a slightly lower level in the UC group (54.2 vs 67.5 pg/mL) compared to the study aforementioned. The optimum cut-off levels to differentiate active UC from inactive UC were not matching (47.1 vs 74.9 pg/mL), an inconsistency that can be due to measurement of serums with different ELISA kits, investigation of populations with distinct ethnicities, or usage of different endoscopic scores to categorize patients. Whatever the cause, we think that by conducting multicenter studies that use well-established scores and standard serum ST2 kits, global cut-off levels can be originated to overcome this discordance.

Even though the reason for ST2 increase during the acute phase of inflammatory diseases is not elucidated as yet, the regulatory role attributed to ST2 could be a possible answer. The presence of high serum and colonic sST2 levels is consistent with the active state of IBD and suggests that this protein, as the decoy receptor of IL-33, might be playing a negative feedback mechanism to control inflammation. Since the functions referred to the sST2 account for an immunomodulatory role in inflammatory processes, it is possible that sST2 acts as a marker of activity both in UC and in CD.

Although IL-33 has been shown to elicit a Th2 cytokine response which is dominant in UC, the ST2/IL-33 system has been described as a proinflammatory system that increases IL-5, IL-6, IL-13, and TNF-α production.[30 31] When the important roles of IL-6 in CD, IL-5, and IL-13 in UC and TNF-α in both diseases are kept in mind and recent data that suggest that the CD-Th1 and UC-Th2 paradigms are not so straight are taken into account,[32–34] it is not surprising to find ST2 acting as a biomarker in UC and also showing significant elevations correlated to the activity in CD. Coherently with the data aforementioned, for the first time we showed significantly higher serum ST2 levels in patients with active CD and a significant correlation between histopathological scores of patients with CD and serum ST2 levels.

Several caveats are inherent in this study. First, although this study includes the largest population of patients with CD to date that enables us to perform detailed analysis about serum ST2 levels of patients with CD, the relatively small sample size limits the generalisability of our conclusions. Second, since patient group consists of subjects of Turkish nationality, these results may not be extrapolated to populations from different ethnic backgrounds. Third, although a significant correlation between intestinal and serum ST2 levels was shown many times in previous studies,[17 19 27] owing to financial constraints we were unable to assess ST2 with immunohistochemical analysis in colonic biopsy materials. One can speculate that the absence of serum IL-33 assesment may be a limitation of

this study. However, conversely, since it occurs with ST2, the increase of IL-33 in mucosa was not found to be reflected in sera of patients with IBD in recent studies, suggesting that chemoattractant properties of IL-33 are restricted to inflamed tissue.[19] Therefore, we think that this finding removes the absence of IL-33 analysis as a limitation of this study.

In conclusion, this study highlights a significant association between ST2 and IBD presence and activity and demonstrates that circulating ST2 is increased with the increasing activity both in UC as well as in CD. Thus, the results of this study emphasize the involvement of ST2 in the pathophysiology of both of the major subtypes of IBD and present elevated serum ST2 levels in patients with active CD as a novel finding. Certainly, these primary results cannot yet establish ST2 assessment as a single blood test; however, they indicate that ST2 may be a key component of inflammation in IBD. Further longitudinal studies periodically updating serum ST2 levels in the follow-up of IBD cases are warranted to clarify the ability of ST2 to diagnose IBD, to grade its activity, and to predict and/or assess response to therapies.

Competing interests None declared.

Patient consent Obtained.

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