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ORIGINAL ARTICLE

Soluble ST2: A new and promising activity marker in ulcerative colitis

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Abstract

AIM: To correlate circulating soluble ST2 (sST2) levels with the severity of ulcerative colitis (UC) and serum levels of pro-inflammatory cytokines, and to demonstrate the predictive power of sST2 levels for differentiation between active and inactive UC.

METHODS: We recruited 153 patients: 82 with UC, 26 with Crohn's disease (CD) and 43 disease controls [non-inflammatory bowel disease (IBD)]. Subjects were excluded if they had diagnosis of asthma, autoimmune diseases or hypertension. The serum levels of sST2 and pro-inflammatory cytokines [pg/mL; median (25th-75th)] as well as clinical features, endoscopic and histological features, were subjected to analyses. The sST2 performance for discrimination between active and inactive UC, non-IBD and healthy controls (HC) was determined with regard to sensitivity and specificity, and Spearman's rank correlation coefficient (r). To validate the method, the area under the curve (AUC) of receiver-operator characteristic (ROC) was determined (AUC, 95% CI) and the total ST2 content of the colonic mucosa in UC patients was correlated with circulating levels of sST2.

RESULTS: The serum sST2 value was significantly higher in patients with active [235.80 (90.65-367.90) pg/mL] rather than inactive UC [33.19 (20.04-65.32) pg/mL], based on clinical, endoscopic and histopathological characteristics, as well as compared with non-IBD and HC ($P < 0.001$). The median level of sST2 in CD patients was 54.17 (35.02-122.0) pg/mL, significantly higher than that of the HC group only ($P < 0.01$). The cutoff was set at 74.87 pg/mL to compare active with inactive UC in a multicenter cohort of patients. Values of sensitivity, specificity, and ability to correctly classify UC, according to activity, were 83.33%, 83.33% and 83.33%, respectively. The AUC of the ROC curve to assess the ability of this molecule to discriminate between active *vs* inactive UC was 0.92 (0.86-0.97, $P < 0.0001$). The serum levels of sST2 in patients with UC significantly correlated with endoscopic and histo-

pathological scores (r = 0.76 and r = 0.67, P < 0.0001, respectively), and with the pro-inflammatory cytokine, tumor necrosis factor-α (r = 0.69 and r = 0.61, respectively, P < 0.0001). Interestingly, we found a direct correlation between total intestinal ST2 content and serum levels of sST2, adjusted to endoscopic activity score in patients with mild (r = 0.44, P = 0.004), moderate (r = 0.59, P = 0.002) and severe disease (r = 0.82, P = 0.002). Only patients with inactive UC showed no significant correlation (r = 0.45, P = 0.267).

CONCLUSION: sST2 levels correlated with disease severity and inflammatory cytokines, are able to differentiate active from inactive UC and might have a role as a biomarker.

Key words: Inflammatory bowel disease; Ulcerative colitis; Soluble ST2; Biomarkers

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INTRODUCTION

Inflammatory bowel diseases (IBDs) belong to the group of chronic diseases that cause intestinal inflammation. Ulcerative colitis (UC) and Crohn's disease (CD) are the two most important diseases in this group. Their characteristics are mainly episodes of active inflammation or remission. In order to provide a differential diagnosis of these diseases, it is necessary to know the clinical, endoscopic, histological, radiologic and serologic characteristics, as well as their course throughout time[1].

Currently, classifications of IBD are based on epidemiologic (age, gender, race), clinical (activity rate, localization and phenotype) and genetic [single nucleotide polymorphism (SNP)][2,3] parameters, and the presence of biological markers[4,5]. However, due to the high percentage of non-classifiable IBD (10%-15%) and the difficulty of a differential diagnosis, it has become necessary to search for new markers for these diseases.

One ideal characteristic of an IBD biomarker is the specificity; however, it also has to be easy to detect, tests must be minimally invasive, low-cost, quick to perform and replicable across laboratories[6,7]. In addition, the biomarker should be able to identify individuals at risk of developing the disease, detect the activity, monitor the effect of the treatment and, finally, have a prognostic value for the reactivation of the disease[7,8]. Current biomarkers for IBD include serological levels of specific antibodies (ASCA, ANCA, anti-OmpC, anti-Cbir, anti-glycans)[9-12], serum (CRP and cytokines)[13-15] and fecal proteins (calprotectin and lactoferrin)[5,7,16-20]. Nevertheless, the majority of these markers show a low sensitivity and/or specificity, and they cannot reflect the real intestinal damage.

In this context, sST2 protein has recently been identified as a new and reliable biomarker of heart failure[21-26]. High serum levels of sST2 have been described in patients with chronic inflammatory diseases, such as autoimmune diseases[27,28] and asthma[29].

ST2 belongs to the interleukin (IL)-1R super-family, is coded in human chromosome 2 and is expressed as two splice variants: one membrane bound, ST2L, which is a receptor of IL-33; and a soluble protein, sST2[30-32].

Recently, in our laboratory, we have described for the first time increased levels of sST2 in serum and total ST2 in the colonic mucosa in IBD patients, and also its distribution in epithelial and infiltrating cells from colonic mucosa. In addition, we showed that serum sST2 levels significantly correlate with total ST2 levels in colonic mucosa[33]. Supporting our results, other groups also have shown evidence that the ST2/IL-33 system could be participating in the development of IBD[34-36].

To date, there are no studies that correlate levels of sST2 with severity of the UC.

The aims of the present study were to determine in another cohort of UC patients whether serum sST2 and intestinal total ST2 levels correlate with the severity of the disease, based on endoscopic and histological activity rates, and with serum levels of pro-inflammatory cytokines.

MATERIALS AND METHODS

Participants were recruited from the Gastroenterology Departments at "*Clínica Las Condes*", "*Hospital Clínico de la Universidad de Chile*" and "*Hospital Clínico de la Pontificia Universidad Católica de Chile*", respectively. Patients were diagnosed based on standard clinical, endoscopic and histological criteria. The study was approved by the Ethics Committee/Ethics Review Board of each participating center, and all patients signed an informed consent prior to their participation in this study.

During the study process, between January 2008 and December 2009, 153 patients were subjected to colonoscopy. Procedures were carried out by gastroenterologists with more than 5 years of experience in colonoscopy (co-authors RQ, MA-L), and findings were classified according to the clinical criteria of the Montreal Classification. Inclusion criteria for the study were: IBD diagnosed patients, > 18 years, blood specimens collected just before colonoscopy, biopsies taken and informed consent.

Exclusion criteria were: non-classifiable inflammatory disease, indeterminate colitis, infectious ileocolitis, asthma, history of autoimmune diseases, celiac disease and hypertension.

Patients were grouped based on endoscopic and histological criteria: Group UC (n = 84) and CD (n = 26), and non-IBD controls (irritable bowel syndrome, colorectal cancer, family history of colorectal cancer, diverticular disease and chronic diarrhea; n = 43). In addition, a group of healthy subjects (n = 40, between 18 and 45 years old) were included to determine reference levels of sST2.

A 5 mL blood specimen was obtained from each patient, and 3 to 4 biopsies were immediately frozen in liquid nitrogen and stored at -80℃ until analysis. From the healthy subjects, only a blood sample was obtained for analysis.

In the case of UC, endoscopic activity was determined in the most swollen area using the endoscopic Mayo Score[37]. In the case of CD, clinical activity was determined according to the Harvey-Bradshaw Index (HBI)[38], and for endoscopic activity, we used the Simple Endoscopic Score for Crohn's Disease (SES-CD)[39]. Histopathological score was used for the evaluation of intestinal inflammation in both diseases. Each biopsy was graded on a scale of 0-3 (0 = normal; 1 = mild; 2 = moderate; 3 = severe and included those patients with active ulceration) according to Gomes *et al*[40].

Quantification of serum sST2 and total intestinal ST2 levels

Levels of sST2 and total intestinal ST2, in serum and protein extract of colonic mucosa, respectively, were determined using a commercial enzyme-linked inmunosorbent assay (ELISA) kit for human ST2 (DuoSet, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Serum samples obtained from 5 mL of blood were subjected to a treatment with protein A/G PLUS-Agarose (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Protein extracts were obtained from each sample by homogenization using a lysis buffer supplemented with a protease inhibitor cocktail (Complete Mini, Roche Diagnostics, Basel, Switzerland) and subsequent disruption by sonication. Levels of total intestinal ST2 were adjusted to the total protein concentration determined by Bradford protein assay. All samples were analyzed in duplicate and each determination was expressed in pg/mL. The detection limit of the technique, provided by the kit's manufacturer, is 20 pg/mL.

Measurement of serum inflammatory cytokines

Serum levels of IL-33 (Apotech, Geneva, Switzerland), IL-6 (Human IL-6 ELISA Ready-Set-Go, eBioscience, San Diego, CA, USA), and tumor necrosis factor (TNF)-α (Human TNF-α ELISA Ready-Set-Go, eBioscience) were measured using an ELISA kit, according to the manufacturer's instructions. The detection limits provided by the kit manufacturers are 5 pg/mL, 2 pg/mL and 4 pg/mL for IL-33, IL-6 and TNF-α, respectively. Samples were prepared as previously described for ST2 determination.

Statistical analysis

Data were analyzed using the statistical software GraphPad Prism4 (La Jolla, CA) and are presented as average ± SD for variables with a normal distribution, and as a median (25th-75th) in the case of non-parametric distributions. Differences and significances among analyzed groups were established by multiple comparisons using the non-parametric Kruskal-Wallis tests. Further comparisons of individual groups *vs* control were performed by using Bonferroni-Dunn statistics and a 5% significance level. Sensitivity and specificity for levels of sST2, and their respective confidence intervals of 95%, were calculated according to endoscopic activity and compared to a non-inflammatory condition. The best cut-off value for sST2 that discriminated between inactive UC and active UC patients and the different levels of endoscopic activity was determined by area under the curve (AUC). Uni and bivariate analyses were carried out to determine the risk factors associated with each one of the following demographic and clinical parameters: gender, age, extent of the disease and medication at endoscopy. The associations between serum levels of sST2 and total intestinal ST2, and serum cytokines, were analyzed using Spearman's rank correlation coefficient (r). For each statistical test that was used, values of $P \leqslant 0.05$ were considered significant.

RESULTS

Main characteristics of IBD patients

During the study period, a total of 153 patients were recruited. Of these, 84 (54.9%) corresponded to UC, 26 (16.9%) to CD and the other 43 (28.1%) to non-IBD controls. Table 1 summarizes the main characteristics of IBD patients following the Montreal classification and also indicates gender and age distribution among groups, as well as medication at endoscopy. At the time of the procedure, 40 UC (47.6%) and 12 CD patients (46.1%) were active according to endoscopic Mayo and SES-CD criteria, respectively.

Determination of reference levels and cut-off value for sST2 in patients with IBD

The reference level of sST2 in serum, determined in the healthy subject group (HC), was 32.40 (19.00-49.00) pg/mL; in the case of the non-IBD, CD and UC groups, levels were 46.33 (26.00-74.66) pg/mL, 54.17 (35.02-122.0) pg/mL and 67.59 (30.78-199.1) pg/mL, respectively, with significant differences ($P < 0.001$) between UC *vs* HC and CD *vs* HC (Figure 1A). Due to the low number of patients with each of the CD phenotypes, such as inflammatory, penetrating and stenosing, we decided to focus on the analysis of sST2 levels restricted to the group of UC patients. Analysis of the levels of sST2 in the serum of the UC group, according to the Mayo Score of endoscopic activity (active ⩾ 2) resulted in concentrations of

Table 1 Clinical characteristics of the patient groups *n* (%)

	IBD						Controls	
	UC				CD		Non-IBD	HC
No. of patients	84				26		43	40
Female (%)	48 (57.1)				13 (50)		23 (53.4)	18 (45)
Age (mean ± SD, yr)	38 ± 12.6				42.6 ± 15.5		49.8 ± 18.3	30.8 ± 5.4
Location of disease								
Ulcerative proctitis, E1	16 (19)							
Left-sided colitis, E2	16 (19)							
Extensive colitis, E3	52 (62)							
Small bowel, L1					4 (15.5)			
Colon, L2					15 (57.6)			
Ileocolonic, L3					7 (26.9)			
Disease behavior								
Inflammatory					25 (96.1)			
Stenosing					1 (3.9)			
Penetrating					0			
Index disease	0	1	2	3	≤ 8	≥ 9		
Endoscopic Mayo score	8 (9.5)	40 (47.6)	25 (29.7)	11(13.2)				
Histopathological score	8 (9.5)	35 (41.6)	32 (38.1)	9 (10.7)				
Harvey-Bradshaw Index					22 (84.6)	4 (15.4)		
SES-CD					24 (92.3)	2 (7.7)		
Medication at endoscopy								
No medication	14 (16.6)				5 (19.2)			
Topical 5-ASA	16 (19)				2 (7.7)			
Systemic 5-ASA	24 (28.5)				7 (26.9)			
Systemic steroids	10 (11.9)				3 (11.6)			
5-ASA + steroids	13 (15.5)				0			
5-ASA + azathioprine	7 (8.5)				4 (15.4)			
Azathioprine	0				5 (19.2)			

Data regarding location of disease, disease behavior, index disease and medication at endoscopy are represented as number of patients (%). UC: Ulcerative colitis; CD: Crohn's disease; HC: Healthy subjects; SES-CD: Simplified endoscopic activity score for Crohn's disease; 5-ASA: 5-aminosalicylic acid derivatives; IBD: Inflammatory bowel diseases.

Table 2 Sensitivity, specificity and cut-off values for sST2 in ulcerative colitis patients compared to other groups

	Inactive UC				Active UC			
	Cut-off value (pg/mL)	Sensitivity (%)	Specificity (%)	AUC (95% CI)	Cut-off value (pg/mL)	Sensitivity (%)	Specificity (%)	AUC (95% CI)
HC	33.08	54.29	52.08	0.58 (0.46 -0.71)	64.25	94.29	91.67	0.98 (0.95-1.00)[b]
Non IBD	42.67	60.98	64.58	0.58 (0.45-0.70)	76.29	80.49	83.33	0.90 (0.84-0.97)[b]
iUC	-	-	-	-	74.87	83.33	83.33	0.92 (0.86-0.97)[b]

[b]$P < 0.0001$. iUC: Inactive ulcerative colitis; HC: Healthy subjects; IBD: Inflammatory bowel disease; AUC: Area under the curve.

33.19 (20.04-65.32) pg/mL for inactive UC and 235.8 (90.65-367.9) pg/mL for active UC, with significant differences between active UC *vs* HC, non-IBD and inactive UC ($P < 0.0001$) (Figure 1B).

Figure 1C shows the ROC curve for sST2 levels in the active UC group in relation to the inactive UC group of patients. The optimal cut-off value estimated for sST2 that allows for the discrimination of active UC compared to inactive UC was 74.87 pg/mL. The AUC of the ROC values, cut-off, sensitivity and specificity are summarized in Table 2.

Levels of sST2, IL-33, TNF-α and IL-6 in serum and their correlation with disease activity

The range of serum sST2 concentrations for the different UC sub-groups, according to endoscopic Mayo score, is shown in Figure 2 and Table 3. Significant differences were observed among groups with moderate (Score 2) and severe activity (Score 3) comparing to inactive (Score 0) and mild activity (Score 1) sub-groups ($P < 0.001$) (Figure 2A). Regarding the histopathological compromise of the mucosa, the serum sST2 levels were significantly higher in the severe (Score 3) and moderate (Score 2) inflammation groups compared to both normal (Score 0) and mild (Score 1) sub-groups ($P < 0.001$ and $P < 0.01$, respectively) (Figure 2B). Endoscopic and histopathological scores directly correlated with serum sST2, with $r = 0.76$ and $r = 0.67$, respectively (Table 3).

Levels of serum TNF-α in the different UC sub-groups were directly proportional to endoscopic and histopathological scores. When comparing serum sST2 and cytokine levels, only TNF-α significantly correlated, both

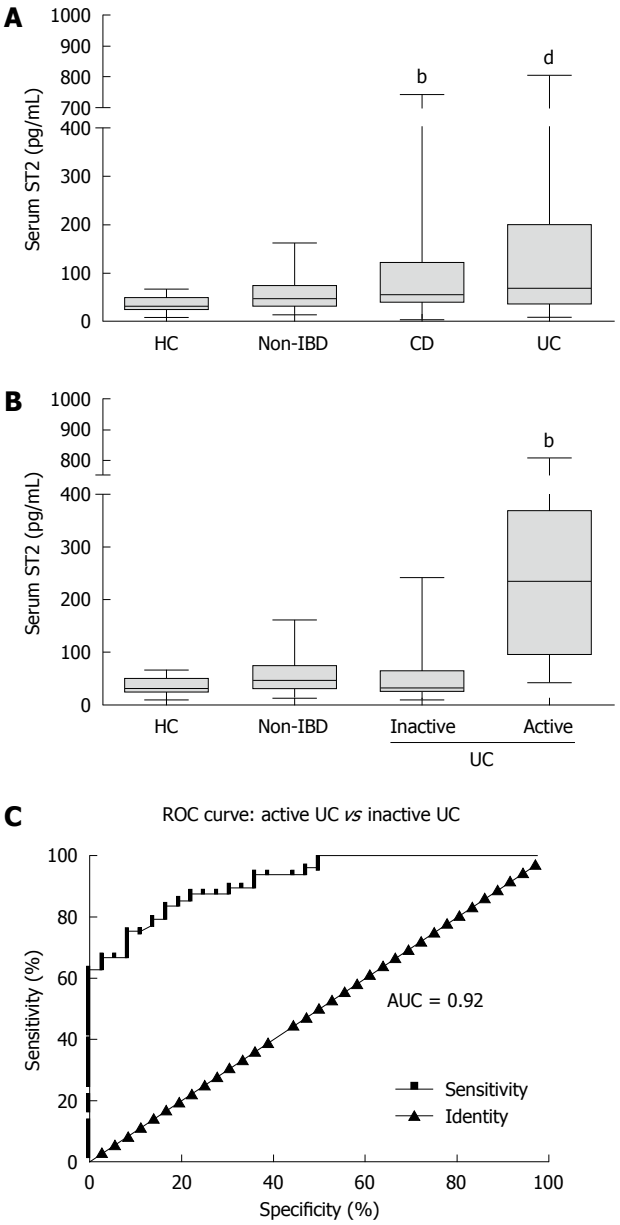

Figure 1 Distribution of serum levels of sST2 in the groups of studied patients. Box-plot showing the distribution of serum sST2 levels in healthy patients (HC) compared to non-inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC) patients (A) and comparison of HC with non-IBD and inactive and active UC patients (B). Data are represented as median and percentiles (25th-75th) and significant differences in serum sST2 levels are shown between UC and CD patients *vs* HC (A) and between active UC and other groups (B). Receiver operating characteristics (ROC) curves for sST2 cut-off point determination (C). ROC curves illustrate the specificity and sensitivity in serum sST2 level determination to differentiate active from inactive UC. A: $^{b}P < 0.01$, CD *vs* HC; $^{d}P < 0.001$, UC *vs* HC; B: $^{b}P < 0.001$, active UC *vs* HC, non-IBD and inactive UC.

with endoscopic ($r = 0.69$, $P < 0.0001$) and histopathological scores ($r = 0.61$, $P < 0.0001$) (Table 3).

Uni and bivariate analyses of serum levels of sST2, with reference to demographic and clinical parameters such as age, gender, localization of the disease and medication at the endoscopy for each one of the analyzed groups, are shown in Table 4. In addition to the activity score, significant differences were observed for localization ($P = 0.0061$) and medication ($P = 0.0067$) of the UC group (Table 4). These results show the same trend observed in Figure 2A and B, based on endoscopic and histopathological scores, regarding gender ($P < 0.0001$), localization ($P < 0.0001$) and medication with 5-aminosalicylic acid (5-ASA) ($P = 0.0005$) (data not shown). These findings demonstrate that sST2 values exclusively depend on the severity of the disease.

Table 3 Correlation data of endoscopic and histopathological scores with serum sST2, interleukin-33, interleukin-6, tumor necrosis factor-α levels and total intestinal ST2

Disease activity index	Endoscopic score	Histopathological score
sST2 (pg/mL)		
r	0.7624	0.6762
P-value	< 0.0001	< 0.0001
IL-33 (pg/mL)		
r	0.2869	0.1687
P-value	0.0146	0.1657
IL-6 (pg/mL)		
r	0.2315	0.0888
P-value	0.0676	0.2855
TNF-α (pg/mL)		
r	0.6961	0.6112
P-value	< 0.0001	< 0.0001
Intestinal ST2 (pg/mL per mg protein)		
r	0.6267	0.6034
P-value	< 0.0001	< 0.0001

IL: Interleukin; TNF: Tumor necrosis factor; *r*: Spearman's rank correlation coefficient.

Levels of total intestinal ST2 correlate with disease activity scores and serum levels of sST2 in UC patients

In order to determine if the findings observed at the systemic level reflect the local damage, total intestinal ST2 was measured in colonic mucosa. Similarly to the serum sST2 levels, total intestinal ST2 levels in UC are closely distributed to activity endoscopic ($P < 0.0001$) (Figure 2C) and histopathological score ($P < 0.0001$) (Figure 2D). Significant differences were observed between moderate and severe activity sub-groups compared to inactive and mild (Figure 2C and D). Similarly, total intestinal ST2 levels significantly correlated with endoscopic ($r = 0.62$, $P < 0.0001$) and histopathological scores ($r = 0.60$, $P < 0.0001$) (Table 3), as seen for serum sST2 levels (Figure 2A and B).

Furthermore, serum sST2 levels and total intestinal ST2 directly correlate, according to endoscopic Mayo activity score, in the severe ($r = 0.82$, $P = 0.0027$), moderate ($r = 0.59$, $P = 0.0020$) and mild ($r = 0.44$, $P = 0.0045$) sub-groups (Figure 3).

DISCUSSION

Our group first reported that the ST2/IL-33 system, described in other inflammatory diseases, could be involved in the pathogenesis of IBD, because levels of ST2 and IL-33 in IBD patients were higher than in healthy sub-

Table 4 Baseline serum sST2 levels according to clinical characteristics of patients

	Serum sST2 (pg/mL), median (Percentiles 25th-75th)		
	UC	Non-IBD	HC
Gender			
Female	55.5 (29.3-150.1)	43.9 (22.1-73.1)	29.4 (17.0-40.3)
Male	99.7 (34.0-216.4)	59.7 (35.6-74.9)	36.2 (27.0-53.2)
Age (yr)			
18-24	190.8 (52.6-489.6)	63.2 (31.7-74.9)	29.0 (17.0-45.0)
25-34	66.2 (30.4-313.3)	46.3 (18.5-116.6)	32.4 (16.9-40.8)
35-44	125.4 (37.0-182.9)	48.1 (32.2-67.8)	44.3 (27.9-59.6)
45-54	48.2 (24.9-116.4)	43.6 (26.0-72.4)	51.0 (35.7-65.0)
⩾ 55	51.3 (19.9-90.5)	53.0 (25.9-78.8)	0
Location of disease			
Ulcerative proctitis, E1	56.5 (22.4-141.6)		
Left-sided colitis, E2	35.8 (19.4-66.2)		
Extensive colitis, E3	110.2 (34.0-345.8)[a]		
Medication at endoscopy			
No medication	36.4 (19.6-105.6)		
Topical 5-ASA	39.7 (20.4-75.7)		
Systemic 5-ASA	59.0 (23.6-141.7)		
Systemic steroids	242.4 (124.9-349.1)[a]		
5-ASA + steroids	125.4 (50.0-417.9)		
5-ASA + azathioprine	41.1 (68.9-109.0)		
Azathioprine	0		

[a]$P \leqslant 0.05$ *vs* other groups in the analysis. UC: Ulcerative colitis; HC: Healthy subjects; IBD: Inflammatory bowel disease; 5-ASA: 5-aminosalicylic acid derivatives.

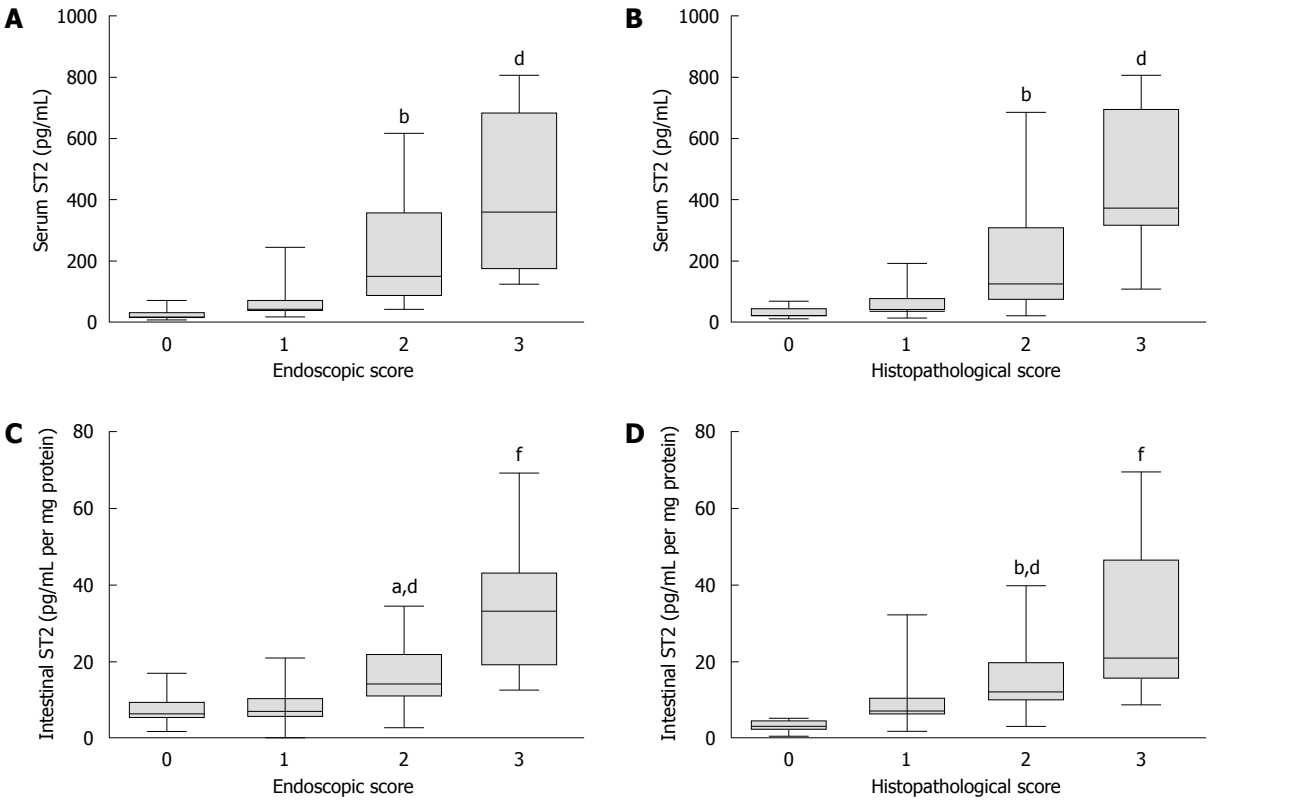

Figure 2 Analysis of serum sST2 and total intestinal ST2 levels in ulcerative colitis patients according to endoscopic and histopathological activity. Distribution of serum sST2 and total intestinal ST2 levels in ulcerative colitis (UC) patients according to the 4 rank Endoscopic Mayo Activity Score (A and C) (Activity: 0 = inactive; 1 = mild; 2 = moderate; 3 = severe) and histopathological score (B and D) (Degree of inflammation: 0 = normal; 1 = mild; 2 = moderate; 3 = severe with active ulceration). Data are represented as median and percentiles (25th-75th). Serum sST2 levels are significantly different among UC patient sub-groups of moderate and severe activity in relation to inactive and mild activity, independent of the score used. Total intestinal ST2 levels directly correlate with endoscopic activity (C) and degree of mucosal inflammation (D). A: [b]$P < 0.001$, 2 *vs* 0 and 1; [d]$P < 0.001$, 3 *vs* 0 and 1; B: [b]$P < 0.01$, 2 *vs* 0 and 1; [d]$P < 0.001$, 3 *vs* 0 and 1; C: [a]$P < 0.05$, 2 *vs* 1; [d]$P < 0.01$, 2 *vs* 0; [f]$P < 0.001$, 3 *vs* 0 and 1; D: [b]$P < 0.01$, 2 *vs* 1; [d]$P < 0.001$, 2 *vs* 0; [f]$P < 0.001$, 3 *vs* 0 and 1.

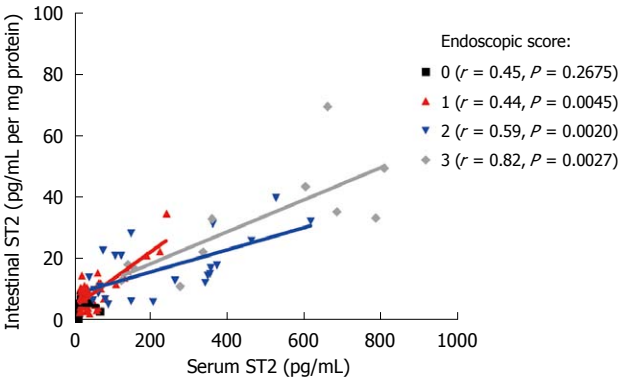

Figure 3 Graphic representation of the direct correlation between serum sST2 and total intestinal ST2 levels according to endoscopic activity scores. Activity: 0 = inactive; 1 = mild; 2 = moderate; 3 = severe. The trend lines for each analyzed group are shown. *r*: Spearman's rank correlation coefficient.

jects[33]. Recently, Pastorelli *et al*[34] also reported an increase in ST2/IL-33 system components in patients with IBD, both in the colonic mucosa as well as in serum. However, they reported that the circulating IL-33 levels in IBD patients were higher than our results and than in other diseases, even higher that those shown in sepsis[34]. Another two articles in the field[35,36] also confirmed elevated IL-33 expression in the mucosa of active IBD patients, with the limitation that these observations were conducted mainly at mRNA level, but ST2 levels were not studied.

The present study demonstrates, for the first time, a direct association between serum levels of sST2 protein and the degree of endoscopic and histopathological activity in UC.

Currently, a large number of serological, fecal or miscellaneous molecules have been proposed as indirect markers of IBD severity: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and even antineutrophil cytoplasmic antibodies (ANCA) and anti-*Saccaromyces cerevisiae* antibodies (ASCA). However, lately they have become less useful for the diagnosis and prognosis of the diseases. This is mainly due to a low sensitivity and specificity to intestinal inflammation[41] and these markers do not allow accurate differentiation between IBD and other intestinal diseases, nor do they discriminate activity status. Some molecules detected in serum are rather systemic markers that in general do not show inflammatory bowel processes[12,42]. The use of a serum inflammation marker that reflects the intestinal damage would be helpful for the management and prognosis of IBD patients. Fecal molecules, such as calprotectin and lactoferrin, represent an inflammatory neutrophilic process of the intestinal mucosa[16]. However, these are also non-specific markers of inflammation, which are increased in organic intestinal diseases such as diverticular disease[43,44] polyposis[45] and colorectal cancer[46,47].

Serum levels of sST2 allow for a highly valuable discrimination between UC patients and healthy subjects; however, this efficacy is reduced when trying to differentiate UC from organic intestinal diseases presenting any degree of inflammation. Alternatively, sST2 would allow, as happens with fecal calprotectin, the differentiation between UC and functional diseases, such as irritable bowel syndrome, chronic diarrhea and abdominal pain[5,19,48]. Many studies have shown that calprotectin significantly correlates with endoscopic and histological activity scores in CD and UC patients[49-51]. Calprotectin level decreases during clinical remission, which could be related to endoscopic mucosal healing[42,49,52], and consequently is considered a predictor of IBD reactivation. Serum sST2 levels allow for the differentiation between active and inactive UC with a high sensitivity and specificity. The cut-off value determined (74.87 pg/mL) permits the differentiation between active and inactive UC patients, as well as healthy subjects.

Similarly to fecal calprotectin, serum sST2 levels from UC patients significantly correlated with endoscopic (r = 0.76), as well as histopathological score (r = 0.67). Serum IL-33 level, another of the cytokines evaluated, did not show a direct relationship with disease activity; this might be due to the low levels detected compared to sST2, despite being the specific ligand of ST2. Serum sST2 levels in UC patients correlate with activity scores comparable with TNF-α, a commonly used serum inflammation marker. These characteristics result in the proposition of sST2 as an appropriate marker of inflammatory activity degree in UC. However, correlation of serum sST2 levels has to be achieved with other activity biomarkers previously associated with IBD, such as CRP or calprotectin.

In the case of CD patients, the analysis of serum sST2 values showed similar tendencies to those in UC, in relation to control patients (Figure 1A). The low incidence of CD in Chile[53], in addition to the exclusion criteria used in our study, account for the low number of CD patients included. Future studies will allow us to determine the association of sST2 with the inflammatory, stenosing and penetrating phenotypes of CD so as to support the concept that sST2 may also be applicable as a biomarker in CD.

Recently, ST2 has been described as a biomarker for heart failure, as serum levels correlate with hemodynamic variables, cardiac damage (BNP and pro-BNP) and inflammatory markers (CRP)[22,23,54,55]. In those studies, serum sST2 levels increase after myocardial infarction[21,56]; hence patients with a history of cardiopathies and hypertension were excluded.

In addition, some biochemical properties of sST2 support its characteristic as a reliable biomarker in UC, mainly based on its stability[57] and limited dependence on epidemiological and clinical factors, such as age, gender and diet[58].

In our study, serum sST2 levels in healthy subjects were similar to those described previously [32.4 (19-49) pg/mL *vs* 49 (4-89) pg/mL][54]. In addition, serum sST2 levels were higher in males than in females, and slightly increased between 18 and 24 years in age, as previously described[58]. However, when considering serum sST2 levels together with endoscopic activity, adjusted by gender, the distribution remained the same; therefore, we conclude that sST2

levels do not depend on these factors.

Therapeutic strategies for IBD patients are determined according to severity and localization of the affected area. UC patients with pancolitis presented higher serum sST2 levels in relation to proctitis or left-sided colitis. UC patients receiving systemic corticoids showed an increased sST2 level when compared to other treatments. Due to the low number of patients, we were not able to determine whether corticoids affect the sST2 concentration and its correlation with activity scores. However, in UC patients, ST2 levels did not show an association with mesalazine (5-ASA) treatment and in those patients sST2 levels follow activity degree of the disease. One of the most important qualities of a biomarker is that it has to be used in clinical practice and not be affected by drug therapy[12]. One of the main limitations of calprotectin as an IBD marker is the influence of non-steroidal anti-inflammatory drugs on its level, as previously shown[59-61]. In our study, 66.3% of IBD patients were receiving 5-ASA treatment, so measurement of calprotectin in those patients may be inconclusive.

Total ST2 levels in the colonic mucosa of UC patients significantly correlated with endoscopic and histopathological activity scores. In addition to the fact that total intestinal ST2 levels are directly associated with serum sST2 levels, these findings verify it as a new and promising UC activity biomarker. The relation between serum sST2 and inflammatory bowel activity would allow, in the future, the avoidance of a colonoscopic procedure in patients that do not require it. Association studies between ST2 and other biomarkers, such as calprotectin, may confirm its use.

It is possible that sST2 not only acts as a marker of UC activity; functions attributed to sST2 account for a role as an immunomodulator in inflammatory processes. At the cellular level, sST2 has been described as an inhibitor of IL-33/ST2L signaling[62], which causes polarization of naive T cells into Th2, and further, the production of IL-5 and IL-13 that are associated with UC[63,64]. On the other hand, ST2L activation with IL-33 stimulates TNF-α, IL-6 and IL-8 secretion in mast cells[65,66] and, together with IgE, stimulates degranulation[67]. The increase of sST2 during periods of inflammation may be involved in the control of the immune response associated with IBDs such as UC.

In summary, we demonstrated that serum sST2 levels allow for the effective differentiation between the endoscopic activity degrees of UC. Determining whether serum sST2 levels could have any prognostic value for UC (and possibly for CD), whether sST2 levels could monitor the treatment impact on endoscopic mucosal healing, and whether they could predict the risk of complications in IBD course or need of surgery, are some of the questions that should be answered by further studies.

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COMMENTS

Background

Inflammatory bowel diseases (IBDs) belong to the group of chronic diseases that cause intestinal inflammation. Ulcerative colitis (UC) and Crohn's disease are the two most important diseases in this group. Their characteristics are mainly episodes of active inflammation or remission. Currently, classifications of UC are based on epidemiologic, clinical and genetic parameters, and the presence of biological markers. Therapeutic strategies for UC patients are determined according to severity and localization of the affected area.

Research frontiers

To date, there are no studies that correlate levels of soluble ST2 (sST2) with severity of UC. It is possible that sST2 not only acts as a marker of UC activity; functions attributed to sST2 account for a role as immunomodulator in inflammatory processes. At the cellular level, sST2 has been described as an inhibitor of interleukin (IL)-33/ST2L signaling. The increase of sST2 during periods of inflammation may be involved in the control of the immune response associated with IBD.

Innovations and breakthroughs

Soluble ST2 protein has been identified as a new and reliable biomarker of heart failure. High serum levels of sST2 have been described in patients with chronic inflammatory diseases, such as autoimmune diseases and asthma. Recently, in their laboratory, the authors have described for the first time increased levels of sST2 in the serum and total ST2 in the colonic mucosa of UC patients. In this study, we show that serum sST2 levels significantly correlate with total ST2 levels in the colonic mucosa. Supporting our results, other groups also have shown evidence that the ST2/IL-33 system participates in the development of IBD.

Applications

The relation between serum sST2 and inflammatory bowel activity would allow, in the future, avoidance of colonoscopy procedures in patients that do not require them. In addition, some biochemical properties of sST2, such as its stability, support its characteristic as a reliable biomarker in UC. If sST2 levels decreased during clinical remission, these could be related to endoscopic mucosal healing, and therefore be considered a predictor of UC reactivation.

Terminology

ST2 belongs to the IL-1R super-family, is coded in human chromosome 2 and is expressed as two splice variants: one membrane bound, ST2L, which is a receptor of IL-33; and a soluble protein, sST2.

Peer review

The authors examined the expression of components of the ST2/IL-33 system in serum and colonic mucosa in UC patients and correlated levels of sST2 with severity of the disease. The study revealed that sST2 levels are able to differentiate active from inactive UC and correlate with endoscopic and histopathological activity scores. In addition to the fact that total intestinal ST2 levels are directly associated with serum sST2 levels, these findings verify that circulating sST2 levels may play an important role as a new and promising biological marker in UC.

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