

Sclerostin and Antisclerostin Antibody Serum Levels Predict the Presence of Axial Spondyloarthritis in Patients with Inflammatory Bowel Disease

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ABSTRACT. Objective. The early diagnosis of inflammatory bowel disease (IBD)-associated spondyloarthritis (SpA/IBD) in patients affected by IBD represents a major topic in clinical practice; in particular, to date there are no available serum biomarkers revealing the presence of joint inflammation in these patients. Sclerostin (SOST), an antagonist of the Wnt/ β -catenin pathway, and antisclerostin-immunoglobulin G (anti-SOST-IgG) have been recently studied in patients with ankylosing spondylitis (AS) as a putative marker of disease activity.

Methods. SOST and anti-SOST-IgG serum levels were assayed in 125 patients with IBD, 85 with axial or peripheral SpA, and in control groups (patients with AS and rheumatoid arthritis, and healthy individuals). The diagnostic performance in discriminating the presence of SpA/IBD was assessed for both candidate biomarkers.

Results. Patients affected by SpA/IBD with axial involvement displayed significantly lower levels of SOST and higher levels of anti-SOST-IgG compared to patients with only peripheral arthritis, IBD, and controls. Moreover, SOST and anti-SOST-IgG serum levels were inversely correlated and were associated with the duration of articular symptoms. Both biomarkers showed good accuracy in predicting the presence of axial SpA in patients with IBD.

Conclusion. We demonstrated that in patients with IBD, SOST and anti-SOST-IgG might represent novel biomarkers to assess the presence of axial joint involvement. Moreover, the development of anti-SOST-IgG and the subsequent decrease of SOST serum levels could play a role in the pathogenesis of SpA/IBD. (First Release February 1 2018; J Rheumatol 2018;45:630–7; doi:10.3899/jrheum.170833)

Key Indexing Terms:

INFLAMMATORY BOWEL DISEASE
SCLEROSTIN

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Arthritis is the most frequent extraintestinal manifestation in inflammatory bowel diseases (IBD) and may develop before,

simultaneously with, or after the diagnosis of overt IBD^{1,2}. IBD-associated spondyloarthritis (SpA/IBD) is included in

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the group of SpA, together with ankylosing spondylitis (AS), reactive arthritis, undifferentiated arthritis, and psoriatic arthritis³.

SpA/IBD represents an important issue in clinical practice, particularly with regard to pathogenesis, early diagnosis, and therapeutic management⁴.

It has been emphasized in previous studies^{4,5} that the coexistence of joint and gut inflammation calls for multidisciplinary management, including the development of clinical and laboratory tools that may contribute to the early diagnosis and the followup of the patients.

Regarding SpA/IBD, the soluble biomarkers that are currently used to identify and measure inflammation by monitoring articular disease activity lack diagnostic accuracy in the presence of an overt or hidden active IBD.

Sclerostin (SOST) is a novel molecule that seems to have promising features in clinical practice, as well as in the pathogenesis of SpA. It binds to the lipoprotein receptor-related protein and antagonizes the canonical Wnt signaling^{6,7} that normally activates the tumor necrosis factor (TNF)- α , constituting a positive-feedback loop^{8,9}. Through this mechanism, SOST downregulates osteoprotegerin (OPG) in a dose-dependent manner, causing an increase in the receptor activator of nuclear factor- κ B ligand/OPG mRNA ratio, having a catabolic action through the promotion of osteoclast formation and activity from the osteocytes¹⁰.

It was previously observed that patients with AS have lower SOST serum levels than do healthy controls, and SOST concentration is significantly correlated to radiographic progression¹¹. Moreover, the presence of circulating immunoglobulin G (IgG) antibodies that bind SOST (anti-SOST-IgG) have been referred to as the cause of lower SOST serum levels, playing a putative pathogenic role in the development of articular disease¹².

Considering the lack of useful biomarkers that could be used to diagnose early SpA/IBD, we investigated whether SOST serum levels and/or the presence of anti-SOST-IgG could have a role in the diagnosis, and maybe pathogenesis, of SpA in patients affected by IBD.

MATERIALS AND METHODS

Patients. The presence of SpA/IBD was assessed in a prospective cohort of 302 consecutive patients with IBD screened for the presence of articular symptoms in the outpatient gastroenterology clinic¹³.

Upon rheumatologic evaluation, SpA/IBD was diagnosed in 85 patients, according to the Assessment of SpondyloArthritis International Society (ASAS) criteria^{3,14,15}; 51 (60%) were affected by Crohn disease (CD), and 34 (40%) by ulcerative colitis (UC). Supplementary Figure 1 (available from the authors upon request) details the working procedure used for the classification of patients. Of the 85 patients, 40 (47%) were classified as having peripheral SpA/IBD (per-SpA/IBD), while 45 patients (53%) were classified as having axial SpA/IBD (axSpA/IBD), according to the imaging arm of the ASAS criteria^{3,14,15}. In all the patients affected by SpA/IBD, the presence of the HLA-B27 was assessed by our institution's laboratory (Hystotype SSP-B27 kit, BAG Health Care GmbH-Zentrale).

Sacroiliitis was present in all the patients with axSpA/IBD. Among them, 7 patients (16%) fulfilled the modified New York criteria and all were

HLA-B27-positive, whereas 38 (84%) were affected by nonradiographic (nr-) axSpA/IBD and HLA-B27 was negative^{3,14,15,16}.

In the SpA/IBD (considering together peripheral and axial SpA) and IBD cohorts, intestinal disease was active in 59.3% and 60% of patients with CD, respectively, and 50% and 40% of patients with UC, respectively (Table 1 gives percentages of active disease in per-SpA/IBD, axSpA/IBD, and IBD patients).

For control groups, serum samples were used from 40 IBD patients without articular symptoms, 20 from patients with AS, 20 from patients with rheumatoid arthritis (RA), and 20 from healthy controls (HC). Both patients and controls were not taking TNF inhibitors upon entry in the study and/or for ≥ 3 months before the collection of biological samples. Corticosteroids (< 16 mg/day), methotrexate (< 20 mg/wk), mesalazine, sulfasalazine, and azathioprine were allowed. The use of cyclooxygenase-2 inhibitors, which could induce IBD flare, was not allowed, except occasionally for the relief of pain. Table 1 shows the patient characteristics.

Serum collection. Serum samples were collected on first evaluation for both patients and controls while they were taking only the allowed drugs mentioned. The sera of all patients and controls were immediately centrifuged at 1200 rpm for 10 min at 4°C, and aliquots were stored at -80°C until analysis.

Determination of SOST by ELISA. SOST serum levels were measured in all patients using a commercial ELISA kit according to the manufacturer's instructions (ICL Lab Inc.). All measurements were performed in duplicate for each sample and mean values were used for all analyses.

Immunoprecipitation of serum SOST circulating unbound or bound with IgG. Sequential immunoprecipitation experiments (IP) were carried out to isolate serum SOST in 2 forms: bounded with serum IgG (b-SOST) or circulating unbound, or free (f-SOST).

In the first IP round, total serum IgG were immunoprecipitated from 20 μl of serum diluted in 1000 μl of IP buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1% Nonidet P-40, 0.1 mg/ml PMSF, and 1 mM sodium orthovanadate) and incubated with 40 μl of protein A/G plus agarose (Santa Cruz Biotechnology) overnight at 4°C. Thereafter, samples were centrifuged and the pellets, containing b-SOST, were immediately resuspended with 20 μl of LDS sample buffer and stocked at -80°C .

The supernatants were kept and used in the second round of IP to isolate the f-SOST. Rabbit polyclonal anti-SOST antibody (Santa Cruz Biotechnology) was added to the supernatants and incubated with 40 μl of protein A/G plus agarose (Santa Cruz Biotechnology) overnight at 4°C. After 3 washings and centrifugations, these protein A/G beads complexes (containing f-SOST) were eluted with 20 μl of LDS sample buffer and analyzed with b-SOST by Western blotting analysis as described below.

Western blot analysis. The immunocomplexes obtained as described above were denatured, centrifuged, and loaded onto 4–12% NuPAGE gels (Invitrogen, Thermo Fisher Scientific) and transferred to nitrocellulose membranes. Membranes were incubated with 5% nonfat milk powder in phosphate buffered saline with 0.05% Tween 20 (PBST) for 1 h at room temperature, and then overnight at 4°C with primary antibody, a rabbit polyclonal anti-SOST, 1:500 (Santa Cruz Biotechnology) in 5% nonfat milk powder PBS. The day after, they were washed with PBST and incubated with anti-rabbit secondary antibodies conjugated with horseradish peroxidase (HRP), diluted to 1:1000. Membranes were washed 3 times in PBST, and the detection of protein bands was performed with the ECL-Western blotting detection kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The intensity of the bands (densitometric analyses) was quantified using Quantity One (Bio-Rad Laboratories).

The relative amounts of SOST unbound and/or bounded with IgG were normalized against human IgG brought down in the immunoprecipitation and hybridization procedures.

Determination of anti-SOST-IgG with peptide binding/ELISA. The determination of serum anti-SOST-IgG was carried out with a peptide-binding/ELISA as previously described¹². Briefly, 55 $\mu\text{g}/\text{ml}$ of recombinant peptide SOST-146 (sequence TRF HNQ SEL KDFG; Pepsan), generated

Table 1. Demographic and clinical characteristics of the patient cohorts. Values are n (%) or mean ± SD unless otherwise specified.

Characteristics	axSpA/IBD, n = 45	per-SpA/IBD, n = 40	IBD, n = 40	p
Sex, male	25 (54.8)	9 (21.7)	22 (55)	0.023[#]
Age, yrs, median (IQR)	43 (37.5–45)	51 (37–61.5)	44.5 (36.3–59.5)	0.474
Smoker ^o	15 (33)	8 (20)	10 (25)	0.094 [#]
IBD, CD:UC	28 (61):17 (39)	23 (57.5):17 (42.5)	25 (62.5):15 (37.5)	0.928
Active IBD, CD:UC [§]	30 (68):15 (33)	18 (46):22 (64)	24 (60):16 (40)	0.476 (CD), 0.268 (UC)
Duration of IBD, yrs, median (IQR)	12 (5–14.5)	5 (0.5–16)	1 (0.8–6.3) ^{*∞}	< 0.001*
Undergoing corticosteroid therapy	10 (22.6)	10 (26.1)	18 (45)	0.109 [#]
Duration of articular symptoms, mos, median (IQR)	24 (12–60) ^o	10 (3–16)	NA	< 0.001*
Nonradiographic axSpA ^{+3,14}	38 (84)	NA	NA	NA
AS ^{+3,14}	7 (16)	NA	NA	NA
CDAI ¹⁷	358.89 ± 150	301.09 ± 180	334.14 ± 80	0.110
pMAYO ¹⁸	5.8 ± 1.50	6 ± 0.92	4.8 ± 2.53	0.089
ASDAS-CRP ¹⁹	2.87 ± 1.00	2.66 ± 0.63	NA	0.984
BASDAI ²⁰	5.32 ± 2.16	4.47 ± 1.81	NA	0.152
BASFI ²¹	2.54 ± 2.44	2.30 ± 1.98	NA	0.872
CRP	2.05 ± 2.65	2.09 ± 3.80	1.98 ± 1.80	0.818

All data were recorded at the time of evaluation. ^oConsidered also as previous habit. [§]IBD activity evaluated with CDAI for CD and pMayo score for UC. Statistical analysis performed with *Kruskal-Wallis test (in the case of $p < 0.05$, the result of the multiple comparisons is shown in bold face) or [#]Fisher's exact test. [∞]p value axSpA/IBD vs p value IBD = 0.015. ^op value axSpA/IBD vs p value per-SpA/IBD = 0.001. ⁺According to ASAS and modified New York criteria. ASAS: Ankylosing Spondylitis Assessment Society; IBD: inflammatory bowel disease; SpA/IBD: IBD-associated spondyloarthritis with articular axial (ax-) or peripheral (per-) involvement (radiographic or magnetic resonance imaging); IQR: interquartile range; CD: Crohn disease; UC: ulcerative colitis; axSpA: axial spondyloarthritis; AS: ankylosing spondylitis; CDAI: Crohn Disease Activity Index; pMAYO: partial Mayo score; ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score-C-reactive protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; NA: not applicable.

with an amino-terminal cysteine residue, were diluted in 100 μ l of binding buffer (BB; 0.1 M sodium phosphate, 0.15 M sodium chloride, 10 mM EDTA pH 7.2) and immobilized onto a 96-well maleimide-activated plate overnight at 4°C. Then, after washing, the plate was incubated with cysteine solution at 10 μ g/ml (200 μ l/well) for 1 h at room temperature. After 3 washings, serum was diluted to 1:10 in BB and 100 μ l/well was added for 1 h at room temperature. After 4 washings, 100 μ l/well of HRP-conjugated anti-human IgG (Bethyl Laboratories Inc.) were diluted to 1:35,000 and added for 1 h at room temperature. The reaction was developed with 100 μ l of tetramethylbenzidine (BioFX) for 15 min at room temperature, and then stopped with 100 μ l of stop solution (BioFX). The optical density was measured at 450 nm.

In each assay, the calibration curve was made by diluting a sample of patients with AS, in which anti-SOST antibodies had been previously detected by IP. All measurements were performed in duplicate for each sample, and a mean value was calculated. For normalization of results, a standard curve was included on each plate and created using serial dilutions of serum from 1 patient with AS (Supplementary Figure 2, available from the authors upon request).

Statistical analysis. Baseline characteristics of the patients were evaluated according to the presence and type of articular involvement (axSpA/IBD, per-SpA/IBD, or IBD). Quantitative variables were summarized as mean (\pm SD) and the Kruskal-Wallis test was used to evaluate differences between groups. Qualitative variables were expressed as absolute and percentage frequencies, and comparisons were performed by Fisher's exact test.

The difference of SOST and anti-SOST-IgG serum levels between experimental and control groups was analyzed using the Kruskal-Wallis test with Dunns posthoc test. The association between SOST and anti-SOST-IgG levels, duration of articular symptoms, and duration of IBD were evaluated by the Spearman correlation analysis.

A multivariate quantile regression analysis (adjusted for age, sex, tobacco use, duration of articular and gastrointestinal (GI) disease, and ongoing therapy) was used to test whether median values for SOST could be

predicted by anti-SOST-IgG levels. Results were expressed as estimated regression coefficients and 95% CI; when 95% CI did not include zero values, the regression coefficients were considered significantly different from zero.

Finally, the receiver-operating characteristic (ROC) curve method was used to assess the accuracy of SOST and anti-SOST-IgG serum levels as diagnostic biomarkers of SpA in patients with IBD. A probability of 5% was used to assess statistical significance. Data were analyzed using the R statistical package.

Ethics, consent, and permissions. Our study was carried out in compliance with the Declaration of Helsinki on ethical principles for medical research. Informed consent for biological sampling and analysis was obtained for all the participants in the study. A formal institution's research ethics board approval and registration was waived because it is not required in cases of retrospective studies on human samples.

RESULTS

SOST serum levels are significantly associated with axial joint involvement. SOST serum levels were significantly lower in the group of patients affected by SpA/IBD (185.6 \pm 76.33 pg/ml) than in patients with IBD (242.5 \pm 87.48 pg/ml, $p < 0.01$) and RA (249.6 \pm 61.33 pg/ml, $p < 0.05$), and in HC (263.6 \pm 75.5 pg/ml, $p < 0.01$).

In agreement with previous studies^{11,12}, the levels of SOST results in patients with AS were significantly lower (126.4 \pm 36.93 pg/ml) than in IBD, RA, and HC ($p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively), and were lower than in the whole SpA/IBD cohort of patients ($p < 0.05$; Figure 1).

Then we performed a further analysis wherein patients

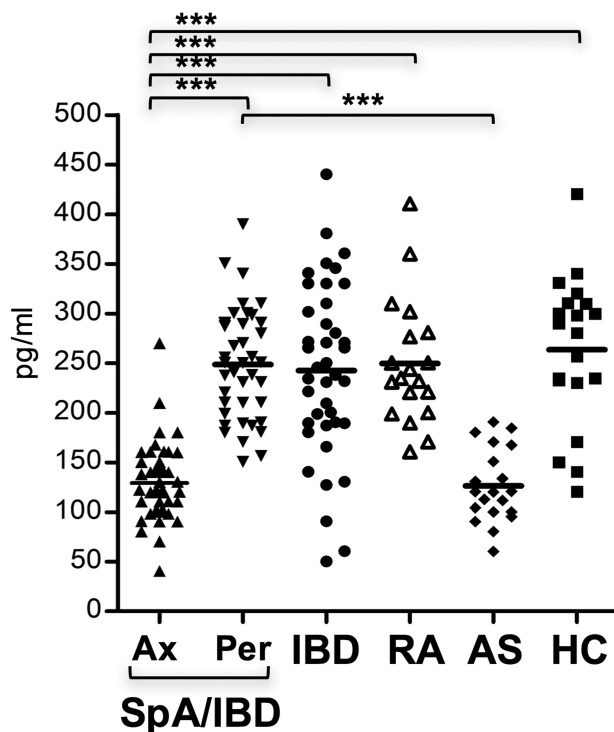


Figure 1. Analysis of SOST serum levels by ELISA in patients affected by ax- or per-SpA/IBD. *** $p < 0.001$. If not reported, p value is nonsignificant. SOST: sclerostin; IBD: inflammatory bowel disease; SpA/IBD: IBD-associated axial (ax-) or peripheral (per-) spondyloarthritis; RA: rheumatoid arthritis; AS: ankylosing spondylitis; HC: healthy controls.

with per-SpA/IBD and axSpA/IBD were considered separately. Interestingly, in patients with axSpA/IBD, SOST levels were comparable to those observed in AS (129.3 ± 38.6 vs 126.4 ± 36.93 pg/ml, respectively; $p =$ nonsignificant), but significantly lower than for patients with per-SpA/IBD (248.9 ± 55.7 pg/ml, $p < 0.001$), who displayed serum levels similar to those observed in patients with IBD and RA, and in HC ($p < 0.001$ for all comparisons; Figure 1).

Thus it is only in axSpA/IBD, not in per-SpA/IBD, that SOST levels were significantly lower than in control groups and were similar to those in patients with AS.

Presence of axSpA/IBD is associated with high levels of anti-SOST-IgG in patients with IBD. Considering the results described above and previously published data¹², we assessed the presence of anti-SOST-IgG in patients affected by SpA/IBD, IBD, and AS, and in HC.

Immunoprecipitation assays and densitometric analysis confirmed that the concentration of the SOST circulating in sera (f-SOST) of patients with axSpA/IBD was similar to that observed in AS, but consistently lower than in per-SpA/IBD and IBD ($p < 0.01$) patients, and HC ($p < 0.01$; Figure 2A).

Conversely, the concentration of SOST bound to serum IgG (b-SOST) was significantly higher in axSpA/IBD and in patients with AS than in control groups (Figure 2A),

suggesting that the decrease of f-SOST observed in axSpA/IBD is likely to be caused by specific anti-SOST-IgG, as previously demonstrated in AS¹².

Therefore, specific ELISA immunoassays were carried out to confirm this finding and assess serum levels of putative anti-SOST-IgG in the study groups. Anti-SOST-IgG were found in the sera of all the groups.

The serum levels of anti-SOST-IgG were significantly higher in the whole SpA/IBD cohort (32.96 ± 16.73 IU/ml) than in HC, but similar to patients with IBD (27.27 ± 11.77 IU/ml, $p =$ nonsignificant), and significantly lower than for patients with AS (63.56 ± 18.47 IU/ml, $p < 0.001$).

However, the separated analysis of the axSpA/IBD from the per-SpA/IBD group demonstrated that anti-SOST-IgG serum levels were significantly higher in axSpA/IBD than in IBD (43.29 ± 13.74 vs 27.27 ± 11.77 IU/ml, $p < 0.01$) and more importantly, than in patients with per-SpA/IBD (21.33 ± 11.33 IU/ml, $p < 0.001$), in whom serum levels were similar to those found in HC (Figure 2B).

Similar to what was observed in the SOST analysis, serum levels of anti-SOST-IgG were similar between patients with axSpA/IBD and AS (43.29 ± 13.74 vs 63.56 ± 18.47 IU/ml, $p =$ nonsignificant; Figure 2B).

Inverse correlation of serum levels of SOST and anti-SOST antibodies and association with duration of articular symptoms in patients with SpA/IBD. The relationship between SOST and anti-SOST-IgG serum levels was evaluated using Spearman correlation analysis, which demonstrated a significant inverse association between SOST and anti-SOST-IgG levels in patients with IBD globally considered, with or without articular inflammation ($r = -0.47$, 95% CI -0.65 to -0.27 ; Table 2A). No significant association was found between IBD duration and SOST or anti-SOST-IgG levels (Table 2A).

In the group of patients with SpA/IBD, the correlation between SOST and anti-SOST-IgG serum levels was stronger than in the IBD group ($r = -0.62$, $p < 0.001$; Table 2B and Figure 3).

The duration of articular symptoms was negatively associated with SOST ($r = -0.42$, 95% CI -0.63 to -0.16) and directly correlated with anti-SOST-IgG ($r = 0.48$, 95% CI 0.25 – 0.68 ; Table 2B). SOST and anti-SOST-IgG serum levels presented a modest but not significant inverse correlation in axSpA/IBD ($r = -0.34$, $p = 0.02$; Table 2B and Figure 3).

No significant correlations were found between SOST and anti-SOST-IgG serum concentrations with GI and articular disease activity (data not shown).

Anti-SOST antibodies serum levels predict SOST serum levels in multivariate analysis. In the multivariate quantile regression model, adjusted for age, sex, tobacco use, duration of articular and GI disease, and ongoing therapy (corticosteroids and disease-modifying antirheumatic drugs), anti-SOST significantly predicted SOST serum levels (coefficient = -1.85 , 95% CI -4.27 to -1.05), confirming that higher

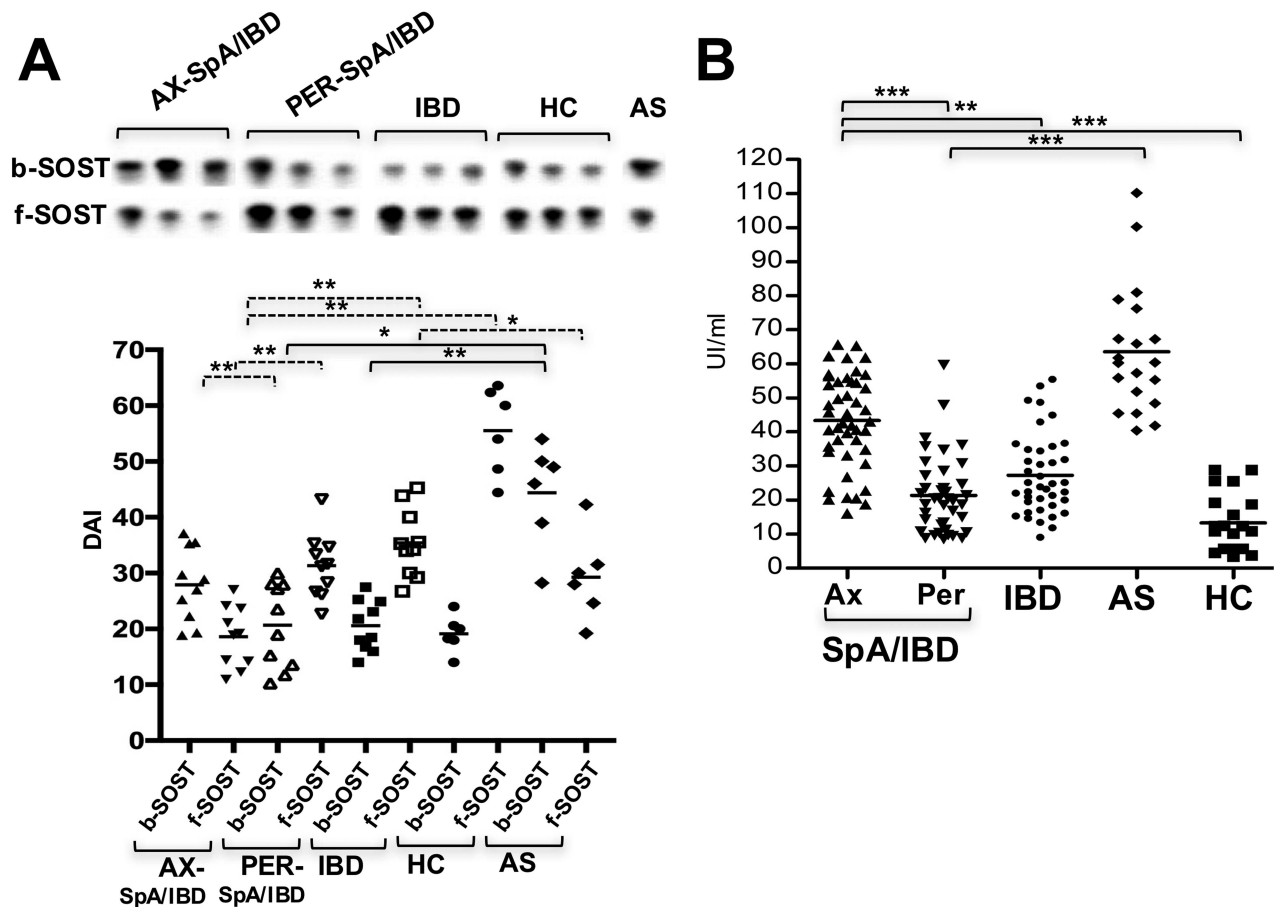


Figure 2. Immunoprecipitation and densitometric analysis of SOST serum levels circulating unbound or bound to serum immunoglobulins by ax- or per-SpA/IBD. (A) Upper panel shows Western blot analysis of f-SOST or b-SOST immunoprecipitated from sera of patients affected by ax- or per-SpA/IBD, IBD, AS, and HC. The blots show the most representative samples of 3 different patients from each group, except for the positive control patient affected by AS. Lower panel shows densitometric analysis carried out using Western blots of ≥ 6 patients from each group. (B) Anti-SOST-IgG serum levels in patients affected by ax- or per-SpA/IBD. SOST: sclerostin; anti-SOST-IgG: anti-sclerostin-immunoglobulin G; IBD: inflammatory bowel disease; SpA/IBD: IBD-associated axial (ax-) or peripheral (per-) spondyloarthritis; f-SOST: SOST circulating free; b-SOST: SOST bound to serum IgG; DAI: densitometric arbitrary units; AS: ankylosing spondylitis; HC: healthy controls. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. If not reported, p value is nonsignificant.

levels of anti-SOST are significantly associated with lower SOST serum levels (Table 3).

Diagnostic accuracy of SOST and anti-SOST antibodies serum levels for the diagnosis of axSpA in patients with IBD. ROC analysis was conducted to assess the ability of serum SOST and anti-SOST-IgG concentrations to determine the presence of axSpA in patients affected by IBD. In both cases, the area under the curve results were statistically significant (SOST 0.88, $p < 0.0001$; anti-SOST-IgG 0.84, $p < 0.0001$; Supplementary Figure 3, available from the authors upon request), indicating that both determinations may represent a novel useful biomarker for the assessment of axial involvement.

A cutoff value of SOST serum levels < 169.3 pg/ml (sensitivity 91.11%, specificity 84.62%, likelihood ratio 5.92) or a cutoff value of anti-SOST-IgG serum levels higher than 32.25 IU/ml (sensitivity 80%, specificity 79.49%, likelihood ratio

3.9) provided the best accuracy to discriminate the presence of axSpA (Supplementary Tables 1 and 2, available from the authors upon request).

DISCUSSION

SpA is considered a heterogeneous group of disabling rheumatic diseases, which includes AS, reactive arthritis, psoriatic arthritis, and SpA/IBD³.

The pathogenesis of SpA/IBD is yet to be explained and it is not known which soluble molecules are implicated in the onset of articular inflammation in patients with IBD. This aspect is closely related with the lack of soluble biomarkers that could be used in clinical practice for the early diagnosis of SpA/IBD²².

In this scenario, SOST, an antagonist of Wnt/ β -catenin pathway, has been evaluated in diseases characterized by bone reabsorption such as RA²³, monoclonal gammo-

pathies²⁴, and osteoporosis²⁵; or conversely, in diseases characterized by bone neoossification, as in AS¹⁰.

SOST has been extensively studied in AS and correlated with radiographic progression, bone damage, and clinical activity^{11,26,27}. In one study, it was found that in patients with AS, the presence of circulating anti-SOST-IgG could be the cause of lower SOST serum levels (i.e., it plays a putative pathogenic role in the development of the articular disease)¹².

In our study, we have focused our attention on a different cohort of patients affected by axSpA/IBD, mostly those with nr-axSpA who are HLA-B27-negative (86%), or have per-SpA/IBD.

The rate of HLA-B27-positive patients in our cohort of axSpA/IBD has resulted in significantly lower rates of positive patients than those reported in other studies that had been carried out in radiographic or nr-axSpA^{12,28,29,30}, for which the presence of the HLA-B27 was considered an entry criterion^{28,29,30}.

In the specific case of SpA/IBD, it has been emphasized that the association between axial involvement and HLA-B27 in patients with IBD is much less conclusive than in pure AS^{31,32}. Our study reports a rate of HLA-B27-positive axSpA/IBD (16%) that is consistently in agreement with several other studies carried out on this particular set of patients (7–16.7%)^{33,34,35}.

In our cohort of mainly HLA-B27-negative patients with SpA/IBD, we have demonstrated that the serum levels of SOST are lower in patients affected by SpA/IBD than in patients with IBD who do not complain of any articular and/or periarticular inflammation, such as arthritis, enthesitis, or inflammatory back pain.

More importantly, SOST serum levels are significantly

Table 2A. Association between SOST and/or anti-SOST antibodies with the clinical characteristics of all patient cohorts affected by IBD, with or without articular inflammation.

Variables	r, n = 94	95% CI
SOST and anti-SOST	-0.47	-0.65 to -0.27
SOST and IBD duration	-0.02	-0.28 to 0.21
anti-SOST and IBD duration	-0.01	-0.25 to 0.24

Table 2B. Association between SOST and/or anti-SOST antibodies with the clinical characteristics of patient cohorts with IBD and SpA/IBD.

Variables	Ax-SpA/IBD, n = 45		Per-SpA/IBD, n = 40		SpA/IBD, n = 85		IBD, n = 40	
	r	95% CI	r	95% CI	r	95% CI	r	95% CI
SOST and anti-SOST	-0.34	-0.57 to 0.05	0.07	-0.41 to 0.50	-0.62	-0.76 to -0.40	0.09	-0.46 to 0.40
SOST and arthritis duration	0.10	-0.31 to 0.48	0.22	-0.16 to 0.59	-0.42	-0.63 to -0.16	NA	NA
anti-SOST and arthritis duration	0.31	-0.04 to 0.63	0.22	-0.18 to 0.59	0.48	0.25 to 0.68	NA	NA

Statistical analysis was performed using r Spearman correlation and 95% CI. Values in bold face are statistically significant. SOST: sclerostin serum levels; anti-SOST: anti-SOST immunoglobulin G serum levels; SpA/IBD: inflammatory bowel disease-associated spondyloarthritis, with axial (ax-) or peripheral (per-) articular involvement using radiographic or magnetic resonance imaging; IBD: inflammatory bowel disease patients without articular disease; NA: not applicable.

lower for patients with axSpA/IBD and are comparable to those observed in patients with AS. Patients with per-SpA/IBD, conversely, display SOST serum levels similar to those of patients with IBD and control groups.

Moreover, serum concentrations of anti-SOST-IgG were significantly higher in patients with axSpA/IBD involvement than for patients with per-SpA/IBD involvement and patients with IBD, and similar to those reported in patients with AS¹².

We observed a significant association between SOST and anti-SOST-IgG serum levels, suggesting that the decrease of SOST serum levels is likely driven by the presence and concentration of such anti-SOST-IgG.

Considering the duration of articular symptoms, serum levels of SOST and anti-SOST IgG are significantly correlated only in the whole group of patients with SpA/IBD but not significantly in the axSpA/IBD or per-SpA/IBD groups.

This result is important because it suggests that in all the patients affected by SpA/IBD, the increase in the level of anti-SOST antibodies during the development of the inflammation is critical. This phenomenon, causing the progressive and consistent decrease of the serum SOST, could be responsible for the development of the axial joint inflammation and not for peripheral arthritis.

Our study may have great relevance for both clinical practice and translational research. First, although there has been contrasting data so far published on the utility of SOST determination for the evaluation of disease activity in SpA^{20,35,36,37,38}, we have demonstrated that SOST and anti-SOST-IgG serum levels could represent novel biomarkers to assess the presence of axSpA in patients with IBD.

Therefore, SOST serum levels could help clinicians to select patients who will benefit from further imaging tests (i.e., magnetic resonance imaging of the sacroiliac joints) to establish an early diagnosis of SpA/IBD and improve the management of the disease. This is particularly true regarding the further choice of an appropriate treatment that, as demonstrated, could be effective on both axial and GI inflammation (i.e., TNF- α inhibitors)^{4,5,13,22}.

A further important contribution of our work includes possible new insights into the pathogenesis of arthritis in patients with IBD.

We can speculate that the development of anti-SOST-IgG

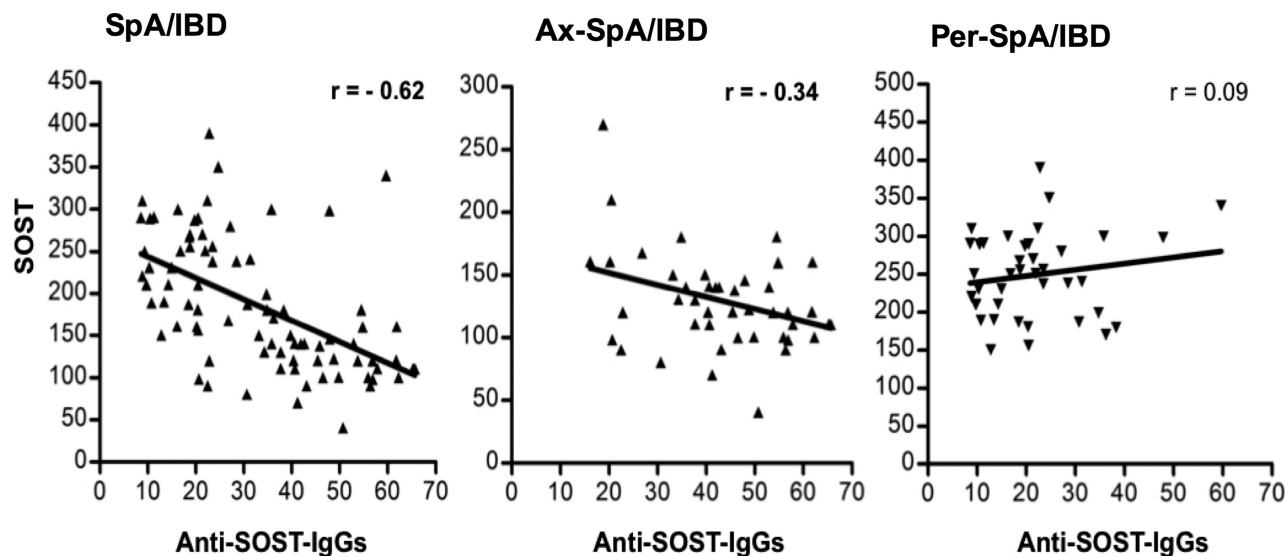


Figure 3. Correlation between SOST and anti-SOST-IgG serum levels in patients affected by SpA/IBD globally considered, and ax- or per-SpA/IBD subgroups. Statistical analysis was performed by the Spearman correlation coefficient. Bold face values are statistically significant. SOST: sclerostin; anti-SOST-IgG: anti-SOST-immunoglobulin G; SpA/IBD: inflammatory bowel disease-associated axial (ax-) or peripheral (per-) spondyloarthritis.

Table 3. Results of multivariate regression analysis for the prediction of SOST serum levels in all patient cohorts.

Independent Variables	Regression Coefficient	95% CI
Anti-SOST-IgG, IU/ml	-1.85	-4.27 to -1.05
Sex, male vs female	-13.1	-34.76 to 70.45
Age, yrs	-0.92	-2.50 to 0.36
Smoking habits, yes vs no	19.44	-49.37 to 75.68
IBD duration, yrs	1.81	-0.83 to 3.78
Articular symptom duration, mos	-0.40	-1.23 to 0.09
Corticosteroid therapy	6.70	-53.53 to 37.50
DMARD therapy	-1.33	-86.73 to 9.61

The patients analyzed were all patients affected by IBD, with or without articular inflammation. Statistical analysis was performed using quantile regression analysis. Values in bold face are statistically significant. SOST: sclerostin serum levels; anti-SOST-IgG: anti-SOST immunoglobulin G serum levels; IBD: inflammatory bowel disease; DMARD: disease-modifying antirheumatic drugs.

may be a trigger for the onset of axSpA, as previously suggested in patients with AS¹², because the presence of high levels of serum anti-SOST-IgG in patients with axSpA/IBD is inversely correlated with SOST serum levels.

In such a case, it is important to underline that this decrease of SOST in the patients' sera will account for only the phenomenon of new bone formation, which has no implications for the whole onset of acute inflammation in the spine in patients with SpA.

Moreover, we have reported a significant augmented concentration of anti-SOST-IgG and decrease of SOST, comparable with those reported in AS-HLA-B27-positive patients¹², in a consistent group of patients with nr-axSpA/IBD who were mainly HLA-B27-negative.

This interesting finding, which will have to be confirmed in a wider group of patients, suggests that the presence of HLA-B27 may not be necessary for the onset of inflammation of the spine in nr-axSpA/IBD, and its role in the development of this particular type of SpA still needs to be clarified. Further investigations on a larger cohort of patients are surely necessary to confirm the role of SOST and anti-SOST-IgG as biomarkers of SpA/IBD and to assess their role in the pathogenesis of axSpA in patients with IBD.

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