Assessment of semaphorin 3A and its role in bone remodelling in a group of ankylosing spondylitis patients

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ABSTRACT

Objective. *Several molecules are involved in the pathogenesis of new bone formation in ankylosing spondylitis (AS). The aim of the present study was to evaluate serum levels of semaphorin 3A in AS and to investigate any correlations with radiographic damage, disease activity, function and treatment.*

Methods. *AS patients who fulfilled the modified New York criteria were enrolled for this study. Healthy subjects were also enrolled as control group. BASDAI, ASDAS-CRP, BASMI, BASFI, patients and physician VAS, C-reactive protein and erythrocyte sedimentation rate were evaluated at baseline visit. Radiographs of the spine and pelvis performed within six months from the enrolment in the study were collected in all patients. Spinal damage was assessed using the mSASSS. Serum concentrations of semaphorin3A were assessed at baseline and after four months of therapy in patients who started an anti-TNF.*

Results. *Twenty healthy subjects and forty AS patients were enrolled in the study. Of these patients, 15 started anti-TNF therapy the day of baseline visit. Semaphorin3A serum concentrations [median (25th–75th)] were similar in AS patients [0.26 (0.20–0.31) ng/ml] and controls [0.28 (0.26–0.3) ng/ml; p=ns). No significant correlation was found between semaphorin 3A serum levels and radiographic damage index. Semaphorin 3A serum levels positively correlated with ESR values (rho=0.37, p=0.049) and with disease activity assessed by the physician VAS (rho=0.47, p<0.01). No differences were found in the semaphorin3A serum levels after 4 months, compared to baseline values.*

Conclusion. *The results of the present study could contribute to the intriguing topic of bone remodelling in AS.*

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterised by inflammation and new bone formation at axial and peripheral entheseal sites. In the disease course, the development of syndesmophytes, enthesophytes and spinal fusion is associated with chronic pain, functional impairement and disability. Despite the recent progress in AS therapy with biologic drugs, the pathogenesis of bone remodelling and the treatment of radiographic progression seem to be unclear (1).

Several mechanisms involving osteocytes, chondrocytes, immune cells, inflammatory cytokines and cellular pathways seem to be responsible for new bone formation in AS (1, 2). Semaphorins represent a group of proteins implicated in cell-to-cell communications divided in eight main classes (3). Semaphorins, in particular semaphorin 3A (Sema3A), are involved in nervous system development and cancer progression, by affecting chemotaxis, viability, tumorigenesis, metastasis, and angiogenesis (3). Moreover, semaphorins are critical for various phases of the immune response: it has been shown that Sema3A receptor complex signaling may modulate regulatory T cell functions. In addition, it was recently show that Sema3A is involved in the entry of dendritic cells into the lymphatic system (4, 5) and several studies indicated that a reduction of Sema3A expression is involved in the exacerbation of autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus (6, 7). Furthermore, semaphorins, in particular Sema3A, are also involved in bone and cartilage formation. Knockout Sema3A mouse model showed severe osteopenia, abnormal bone and cartilage development, and spinal fusion with partial duplication of the ribs, suggesting the role of this molecule in bone homeostasis (8).

Sema3A expression is localised mainly at osteoblastic cell line level, while the expression of receptor complex has been identified both on osteoblastic cells and on osteoclast precursors. The link between Sema3A and Neuropilin-1 receptors seems to result in abrogation of differentiation and migration of osteoclasts precursors. On the other hand, Sema3A seems to stimulate osteoblast differentiation through the activation of the canonical Wnt/β-catenin pathway and thus, promoting new bone formation (9). The role of Sema3A in the pathogenesis of bone formation and its relationship with the inflammatory process in AS is still unknow. The hy-

pothesis of the present study was that, in AS, Sema3A could play a role in bone remodelling, acting in the stimulation of the osteoblast responsible for new bone formation.

Therefore, moving from these evidences, the primary objective of this study was to evaluate serum levels of Sema3A in a group of patients affected by AS, and to investigate any correlations with radiographic damage, disease activity, and function. The secondary objective was to evaluate any modification of Sema3A serum levels after treatment with TNF antagonists.

Materials and methods

AS patients fulfilled the modified New York criteria consecutely attending the Rheumatology Unit, Sapienza University of Rome, were enrolled in this study, designed as case control with a prospective phase II for 4 months follow-up after starting anti-TNF therapy. Healthy subjects, matched by age and sex, were also enrolled as control group. Exclusion criteria were the presence of history of bone fractures in the previous 24 months, history of neurological and cognitive disease and age at the time of enrolment <18 years. Subject's written consent was obtained according to the declaration of Helsinki and the study was approved by the local ethics committee.

The following data were collected: demographic data, disease duration, extra-articular manifestations and clinical pattern. The values of erythrocyte sedimentation rate (ESR, Westergren method, mm/h) and the serum concentrations of C-reactive protein (CRP, mg/l) were registered at baseline visit.

Moreover, the following indices (10-12) were evaluated:

- Bath AS Metrology Index (BASMI);
- Bath AS Disease Activity Index (BASDAI);
- Bath AS Functional Index (BASFI);
- AS Disease Activity Score (ASDAS).

Radiographs of the spine and pelvis performed within six months from the enrollment in the study were collected in all patients. Each radiograph was evaluated by expert readers (FMP, EL) using the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS). According to the New York criteria, the involvement of the sacroiliac joints was evaluated by assigning a score ranging from 1 to 4.

Table I. The main demographic clinical and x-ray characteristics of AS patients (n=40).

Male/Female	30/10
Age (median/25th-75th percentile) year	50 (40.5-56.75)
Disease duration (median/25th-75th percentile) year	12.5 (6.2-21.5)
HLA-B27 n(%)	28 (70)
CRP mg/dl (median/25th-75th percentile)	0.5 (0.2-0.9)
ESR mm/I ora (median/25th-75th percentile)	12.5 (5-20.7)
VAS global health (median/25th-75th percentile) 0-10 mm	4.75 (3-5.9)
VAS physician (median/25th-75th percentile) 0-10 mm	4 (3-5)
ASDAS-CRP (median/25th-75th percentile)	2 (1.5-3.3)
ASDAS-ESR (median/25th-75th percentile)	2.2 (1.4-3.25)
BASDAI (median/25th-75th percentile)	3.65 (2-5.2)
BASMI (median/25th-75th percentile)	2 (1-5)
BASFI (median/25th-75th percentile)	1.65 (1-3.9)
Sacroiliitis IV grade (%)	14 (35)
Sacroiliitis II-III grade (%)	26 (65)
mSASSS (median/25th-75th percentile)	10 (2.5-29.5)
Peripheral arthritis (%)	14 (35)
Presence of enthesitis (%)	12 (30)
Presence of psoriasis (%)	3 (7.5)
Presence of inflammatory bowel disease (%)	5 (12.5)
Presence of uveitis (%)	9 (22.5)
Therapy (%)	
NSAIDs	17 (42.5)
DMARDs	6 (15)
ADA	7 (17.5)
ETA	6 (15)
INF	8 (20)
GOL	1 (2.5)

CRP: C reactive protein; ESR: erytrosedimentation rate; VAS: visual analogue scale; ASDAS: ankylosing spondylitis disease activity score; BASDAI: Bath ankylosing spondylitis disease activity index; BASMI: Bath ankylosing spondylitis metrology index; BASFI: Bath ankylosing spondylitis disease functional index; mSASSS: modified Stoke ankylosing spondylitis spine score; NSAIDs: non-steroidal anti-inflammatory drugs; DMARDs: disease-modifying anti-rheumatic drugs; ADA: adalimumab; ETA: etanercept; INF: infliximab; GOL: golimumab.

Determination of Sema3A

Serum concentrations of Sema3A were assessed using commercial kit ELISA (AUROGENE srl, Rome - Product MBS705755-semaphorin 3A) according to manufacturer's instructions. The serum samples of patients were taken at the time of the visit and stored at -80°C. AS patients who started anti-TNF therapy due to high disease activity were longitudinally followed up for four months. In this subgroup of patients, Sema3A serum concentrations were evaluated at baseline (sample taken the day of starting of anti-TNF therapy) and after four months of anti-TNF therapy.

Statistical analysis

Statistical evaluation was performed using the PRISM programme 5 - Graphpad. Normally distributed variables were summarised using the mean ± standard deviation (SD) and non-normally distributed variables by the median/25th-75th percentile. Categorical variables were analysed by χ-square test with Yates' correction or Fisher's exact test. The significance of the differences was determined using the Mann-Whitney test for unpaired samples and Wilcoxon's test for paired samples. Correlations among the different variables were assessed using Spearman test for non-parametric variables. Two-tailed *p*-values were reported; *p*-values less than 0.05 were considered significant.

Results

Twenty healthy subjects and forty AS patients were enrolled in the study. Of these patients, 15 started anti-TNF therapy the day of baseline visit. The main demographic, clinical, laboratory and x-ray findings of AS patients (n=40) enrolled in the present study were sum-

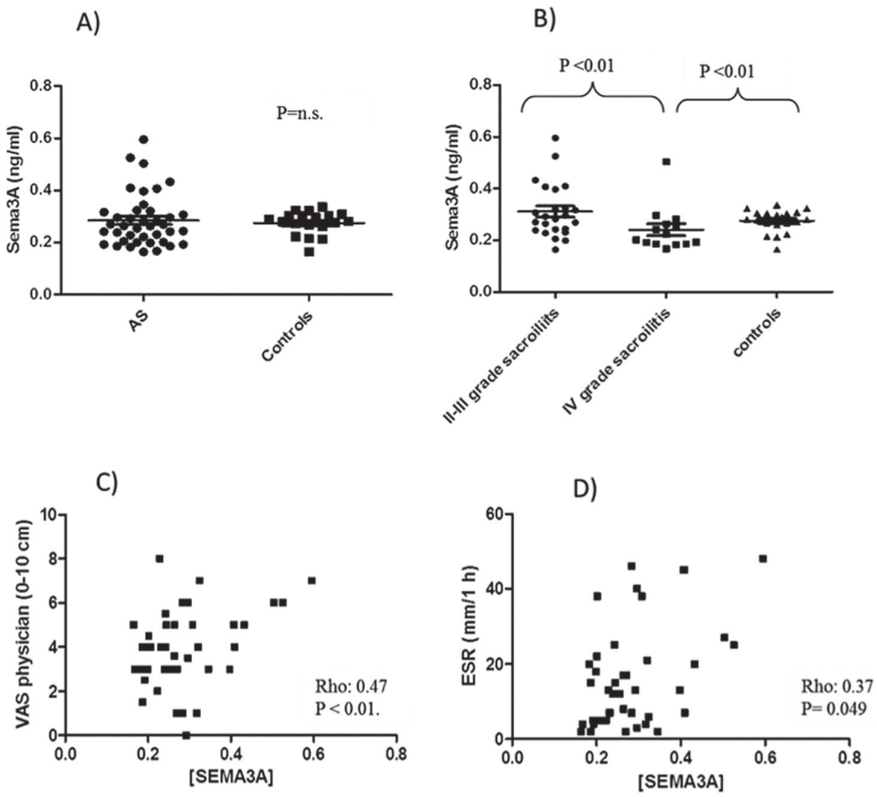

Fig. 1. **A**: Serum levels of Sema3A in AS (n=40) patients and controls (n=20); **B**: Serum levels of Sema3A in patients with II or III grade sacroiliitis compared with controls and IV grade sacroiliitis; **C**: Spearman Rho correlation between serum level of Sema3A and VAS physician; **D**: Spearman Rho correlation between serum level of Sema3A and ESR.

marised in Table I. The serum concentrations of Sema3A in AS patients and in healthy subjects were reported in Figure 1. Sema3A serum concentrations [median (25^{th}-75^{th})] were similar in AS patients [0.26 (0.20–0.31) ng/ml] and controls [0.28 (0.26–0.3) ng/ml; P=ns). Although no significant correlation was found between Sema3A serum levels and radiographic damage index evaluated by mSASSS, patients with grade IV sacroiliitis showed significantly lower levels of Sema3A, compared to patients with lower degree of sacroiliitis and healthy subjects [0.21 (0.18-0.26) ng/ml, 0.28 (0.24–0.37) ng/ml and 0.28 (0.26–0.3) ng/ml, respectively; p<0.01 for both comparisons]. Sema3A serum levels positively correlated with ESR values (rho=0.37, p=0.049) and with disease activity assessed by the physician VAS (rho=0.47, p<0.01) (Fig. 1).

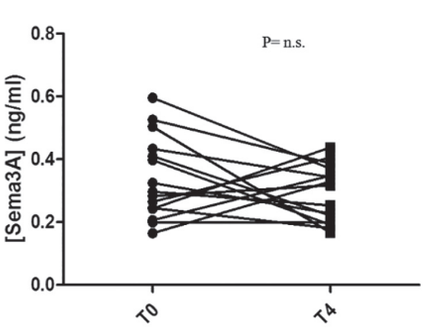

Fig. 2. Serum levels of Sema3A in patients with AS at baseline (T0) and after 4 months (T4) of therapy with anti-TNF.

In the phase II of the study, 15 AS patients were prospectively evaluated. No differences were found in the Sema3A serum levels after 4 months, compared to baseline values (Fig. 2).

Discussion

In the present study, we evaluated serum levels of Sema3A in a group of patients with AS. To our knowledge, this is one of the first contribute to this topic.

The understanding of the mechanism of bone formation in both physiological and pathological conditions should lead to the possibility to reduce disease progression in the near future and thus, studies on molecules and pathways involved in bone remodelling are need. In our study, we did not find differences in the serum levels of Sema3A among patients and controls, and, moreover, no correlations between Sema3A serum levels and the radiographic damage measured by mSASSS were found. This result could be related to the small number of patients, the type of study (cross-sectional) which could not reflect any fluctuation of serum levels, and the radiological index adopted which was not designed for measuring bone remodelling, but staging disease severity. On the other hand, a positive, significant correlation between Sema3A levels and some parameters associated with disease activity, such as ESR and physician VAS, was found. No correlations were found with ASDAS and CRP level, but these could be due to different factors (*e.g.* CRP polymorphisms) (13, 14). Moreover, the identification of significantly higher Sema3A values in patients with grade II-III sacroiliitis, compared with those showing a complete fusion of sacroiliac joints was found. The lower concentrations identified in patients with grade IV sacroiliitis could be justified by the Sema3A expression decrease in a late stage of the disease, characterised by inflammatory process resolution and new bone formation. Interestingly our study also revealed no changes in Sema3A concentrations in patients treated with anti-TNF drugs after four months of therapy. This result could suggest that Sema3A levels have not been influenced by TNF concentration. Further studies, in particular on the expression of Sema3A at histologic level or on cellular and/or animals models of spondyloarthritis are needed to establish the role of this molecule in the pathogenesis of AS. In other studies, Sema3A seems to be involved in immune responses: in rheumatoid arthritis and systemic lupus erithematosus, low Sema3A serum levels and low tissue expression of Sema3A were observed (6, 7), suggesting a suppressive role of this molecule in these diseases; furthermore, Sema3A reduces inflammation when administrated to mouse models of autoimmune arthritis (15). In AS, however, this pathogenetic mecha-

nism seems to have not an inhibitory role in the determination of systemic inflammation as shown by our results. The present study had some limitations, including the study design without the presence of a control group, the lack of histological assessment of Sema3A expression and of the assessment of damage and/or inflammation at MRI.

However, in conclusion, the results of the present study could contribute to the intriguing topic of bone remodelling in AS.

Tribute to Professor Antonio Spadaro

The present paper was also designed by Professor Antonio Spadaro who passed away on 13th May 2014. All the authors intended to dedicate this manuscript to Professor Spadaro who was a scientist, and respected and beloved physician of the Sapienza University of Rome.

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