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Assessment of bone morphogenic protein 2 and interleukin-17A in patients with axial spondyloarthritis and their potential role in the new bone formation: a cross-sectional study

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ABSTRACT

Objective. *Several molecules are involved in the pathogenesis of new bone formation in axial spondyloarthritis (axSpA). The aim of this study was to evaluate the serum levels of BMP-2 and IL-17A in patients with axSpA and their possible correlations with radiographic damage, disease activity, and function.*

Methods. *AxSpA patients fulfilled the ASAS criteria and with at least New York grade 2 bilateral sacroiliitis and healthy matched controls were enrolled for this study. BASDAI, ASDAS-CRP, BASMI, BASFI and CRP were evaluated as measures of disease activity and function. Spinal damage was assessed using the mSASSS on radiographs performed within 3 months from baseline. Serum concentrations of BMP-2 and IL-17A were assessed using ELISA kit.*

Results. *Sixty patients and 30 healthy subjects satisfying the inclusion criteria were enrolled. In our axSpA group, serum BMP-2 levels [median (25^{th}–75^{th} percentile) of 589.2 (430.24–1017.1) pg/ml] did not statistically differ from controls [518.34 (450.2–1028.2) pg/ml]. However, significant correlations were found between serum BMP-2 levels and radiographic damage assessed by mSASSS, and BMP-2 levels were found to be higher in patients with grade 4 sacroiliitis when compared to patients with lower grade of sacroiliitis. Of note, serum BMP-2 levels significantly inversely correlate with IL-17A levels and CRP, and were found to be lower in patients with higher disease activity.*

Conclusion. *The results of our study may confirm a possible role of BMP-2 in the pathogenesis of new bone formation in axSpA patients. Furthermore, a link between inflammation and BMP-2 was found.*

Introduction

Axial spondyloarthritis (axSpA) are a group of chronic inflammatory diseases comprising both ankylosing spondylitis-AS (the so called radiographic axSpA) and non-radiographic axSpA, characterised by inflammation and new bone formation at axial and peripheral entheseal sites (1, 2). The development of syndesmophytes, enthesophytes and spinal fusion is associated with chronic pain, functional impairment and disability and, mainly in the AS, these manifestations impact on disease course (1). The introduction of biological therapies such as the inhibitors of tumour necrosis factor-alpha (TNF) and interleukine-17 has improved the overall outcome of patients with axSpA (3-5), letting the clinicians to act for the prevention of structural damage and to avoid loss of function and disability, especially in the early stages of the disease.

Several mechanisms involving cellular elements (immune cells, mesenchymal stem cells), inflammatory cytokines and cellular pathways, seem to be responsible for new bone formation in axSpA. However, the precise mechanism in which inflammation and new bone formation are coupling is still not fully understood (6). Furthermore, it is still almost unclear the role of biologic treatments in the inhibition of radiographic progression, even if new treatments (anti-IL-17) and new treatment strategies, through the inhibition of TNF, could lead to a better radiographic outcome (7). Bone morphogenic proteins (BMPs) are proteins belonging to the Transforming Growth Factor Beta superfamily and play a primary role in bone formation processes.

BMPs are mainly produced by mesenchymal cells and are now considered fundamental proteins for the organisation, differentiation and growth of tissues during embryogenesis (8). The potential role played by BMPs in the bone neoformation process in axSpA has recently been studied in murine DBA/1 models of spontaneous arthritis in which the pathological process is characterised by enthesitis, dactylitis, endochondral bone proliferation and ankylosis (8).

The expression of several BMPs has been demonstrated in these models: BMP-2 has been found in proliferating cells and in entheseal cells, while BMP-6 and BMP-7 in hypertrophic chondrocytes (9). Moreover, in mouse models of enthesitis and spondylitis it has been demonstrated that the overexpression of noggin, an antagonist of BMPs, is able to reduce the severity of arthritis from a clinical and histological point of view (10).

Among the different BMPs, BMP-2 seems to be the most associated to human pathological processes: in patients with SpA, BMP-2 levels correlate with disease activity and are significantly higher in patients with high mSASSS score (11, 12).

Based on these evidences, the aim of this study was to evaluate the serum levels of BMP-2 in patients with axSpA and to investigate any correlations with radiographic damage, disease activity, and function. Furthermore, the study was also aimed to investigate the possible correlation with IL-17A serum levels, a key cytokine involved in inflammatory process in axSpA.

Materials and methods

The study was designed as cross-sectional and with a group of healthy subjects as controls.

Patients affected by axSpA, referring to the Academic Rheumatology Unit-University of Molise, were consecutively enrolled during a 12 months period (1 January to 31 December 2017). Inclusion criterion was: satisfying the ASAS criteria for radiographic axial SpA (1), with at least a grade 2 bilateral sacroiliitis present at pelvis plain radiography. Healthy subjects, matched by age and sex, were enrolled as control group. Exclusion criteria were: 1. age ≤18 years, 2. the presence of history of bone fractures in the previous 24 months and 3) no treatment with bisphosphonates agents.

For all enrolled patients, the following data were collected: demographic data, disease duration, extra-articular manifestations (EAM) (uveitis, inflammatory bowel diseases (IBD), psoriasis) and clinical pattern (presence of peripheral arthritis, enthesitis, dactylitis). All patients underwent a clinical assessment and the following indices were evaluated: swollen/tender joint count on 66 and 68 joints respectively; Bath Ankylosing Spondylitis Metrology Index (BASMI); Bath Ankylosing Spondylitis Disease Activity Index (BASDAI); Bath Ankylosing Spondylitis Functional Index (BASFI); patient's visual analogue scale (VAS) on global disease activity spinal pain (0–100 mm).

The values of erythrocyte sedimentation rate (ESR, Westergren method, mm/h) and C-reactive protein (CRP, mg/l) were recorded. The Ankylosing Spondylitis Disease Activity Score (ASDAS) was also calculated.

Radiographs of the spine and pelvis performed within three months from the enrollment in the study were collected in all patients. Each radiograph was evaluated by two readers (FMP, EL) using the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) (13). According to the New York criteria, sacroiliac joints was evaluated by assigning a score ranging from I (doubtful) to IV (complete ankyloses).

All patients gave the informed consent and the study follows the ethical principles for medical research involving human subjects (declaration of Helsinki). The study was approved by local ethical-scientific committee of the University of Molise (n. 0001-09-2017).

Determination of BMP-2 and IL-17A serum levels

Serum samples of patients and controls were collected during the visits. Serum concentrations of BMP-2 and IL-17A was assessed using commercial kit ELISA [Human BMP-2 ELISA Kit-C8C060 (MBS175918), Human Interleukin 17A ELISA Kit-NP-002181.1 (MBS005765)]. The serum samples of patients were taken at the time of the visit and stored at -80°.

Statistical analysis

Statistical analysis was performed using the PRISM program 5 - Graphpad. Normally distributed variables were summarised using the mean ± standard deviation (SD) and non-normally distributed variables by the median / 25th-75th percentile. Percentages were used when appropriate. Mann-Whitney test was performed for unpaired categorical data and *t*-test for paired samples. The significance of the correlation was assessed by the correlation coefficient of Spearman's rank. Two-tailed *p*-values were reported. *p*-values less than 0.05 were considered significant.

Table I. Clinical characteristics of patients with axSpA (n=60).

Male/Female	40/20
Age (years - median/25th -75th percentile)	50 (40.5-56.75)
Disease duration (median/25th -75th percentile) years	12.5 (6.2-21.5)
HLA-B27 (%)	44 (73.3)
CRP mg/dl (median/25th -75th percentile)	0.5 (0.2-0.9)
ESR mm/h (median/25th -75th percentile)	12.5 (5-20.7)
PtGA (median/25th -75th percentile)	4.75 (3-5.9)
VAS Physician (median/25th -75th percentile)	4 (3-5)
ASDAS-CRP (median/25th -75th percentile)	2.2 (1.5-3.3)
BASMI (median/25th -75th percentile)	2 (1-5)
Sacroiliitis grade IV (n/%)	14 (35)
mSASSS (median/25th -75th percentile)	10 (2.5-29.5)
Patients with peripheral arthritis (n/%)	21 (35)
Treatment (%)	
NSAIDs	(42.5)
DMARDs	(15)
Anti-TNF	(57.5)

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; PtGA: patient global assessment; VAS: visual analogue scale; ASDAS: Ankylosing Spondylitis Disease Activity Score; BASMI: Bath Ankylosing Spondylitis Metrology Index; mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score; NSAIDs: non-steroidal anti-inflammatory drugs; DMARDs: disease-modifying anti-rheumatic drugs.

Results

During the one-year period, 60 axSpA patients and 30 age and sex matched controls were enrolled. The main demographic, clinical, laboratory and x-ray findings of axSpA patients enrolled in the present study were summarised in Table I. Peripheral involvement was observed in 21 patients (35%). Most of the patients showed high disease activity (median ASDAS-CRP: 2.2). The serum concentrations of BMP-2 in patients and in healthy controls were described in Figure 1. In the whole group, serum BMP-2 levels [median (25th–75th percentile)] did not differ between axSpA patients and healthy controls [589.2 (430.24–1117.1) pg/ml *vs*. 518.34 (450.2-1028.2) pg/ml; *p*=0.12] (Fig. 1).

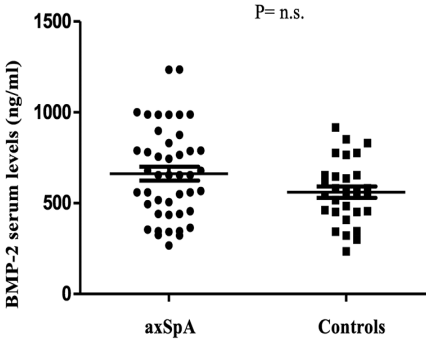

Fig. 1. BMP-2 serum levels in patients with axSpA (n=60) and in healthy controls (n=30).

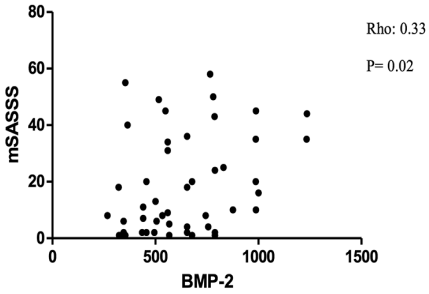

Fig. 2. Correlation between serum levels of BMP-2 and the mSASSS in patients with axSpA.

However, among axSpA patients, BMP-2 serum levels were found to be lower [470.2 (321.1–512.9) pg/ml] in patients with grade II and III sacroiliitis than patients with ankylosis of sacroiliac joints (grade IV), even if it was not statistically significant [609 (310.7–620.4) pg/ml, p=0.07). Furthermore, a significant correlation was found between serum BMP-2 levels and radiographic damage, assessed by mSASSS (Fig. 2).

IL-17A levels were found to be significantly higher in axSpA patients in respect to healthy controls and significantly correlated with CRP levels but not with mSASSS. Moreover, as expected, IL-17A levels were higher in patients with high disease activity (defined as an ASDAS-CRP ≥2.1) in respect to patients with low disease activity (ASDAS-CRP <2.1) (Fig. 3). Of note, IL-17A levels inversely correlated with BMP-2 levels, suggesting a complex link between the production of BMP-2 and inflammation (Fig. 4). We also compared the levels of BMP-2 and IL-17 between patients treated with TNF inhibitors and patients treated with NSAIDs/DMARDs. No significant differences were found.

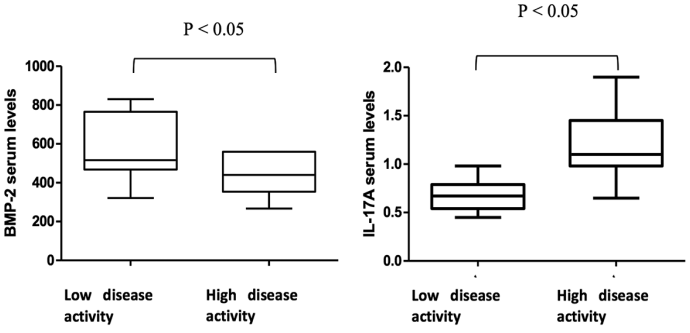

Fig. 3. Serum levels of BMP-2 and IL-17A in patients with low disease activity.

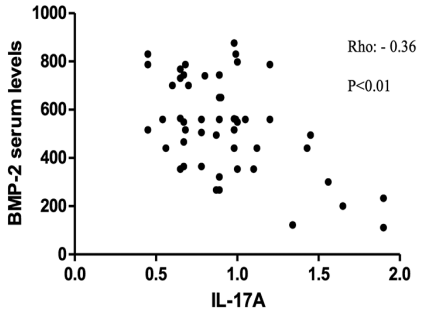

Fig. 4. Correlation between serum levels of BMP-2 and serum levels of IL-17A in patients with axSpA.

Discussion

In the present study, we evaluated the possible role of BMP-2 as a biomarker of new bone formation in radiographic axSpA patients and its relation with the pro-inflammatory cytokine IL-17A. Our results showed that BMP-2 seems to be not a potential diagnostic biomarker because the serum levels were not significantly different when compared to controls.

In previous studies BMP-2 levels were found to be higher in AS patients with spinal fusion in respect to AS patients without spinal fusion, and correlate with new bone formation assessed by mSASSS. However, the role of inflammatory cytokines inducing the expression of BMPs molecules was not completely demonstrated. Recently, it has been showed that both TNF and IL-1β could enhance BMP-2 expression in peripheral blood mononuclear cells (PBMCs) from AS patients (14). In preclinical studies on BMP-2, mRNA expression was increased with TNF alone (16-fold with TNF-α as compared with control without cytokine), with a kinetic curve demonstrating a decrease upon time (15). Fewer studies are available on the relationship between a key cytokine (IL-17A) involved in pathogenetic process of SpAs and BMPs. In the above-mentioned study, results showed that TNF increased the expression of BMP2 mRNA levels in human mesenchymal stem cells, but this effect was inhibited by IL-17A. However, the two cytokines have synergic effects on BMP-2 expression and osteogenic differentiation. Those findings demonstrated how different cytokines may interact in different manner.

Our results were in keeping with the above-mentioned evidences, showing a direct correlation between BMP-2 and radiographic damage. Furthermore, we found *in vivo*, inverse correlation between BMP-2 serum levels, CRP and IL-17A. BMP-2 serum levels were also significantly lower in patients with low disease activity assessed by ASDAS-CRP than in patients with high disease activity. Our results were in keeping with the hypothesis that inflammation and bone formation are linked but not directly coupled events, and the expression of BMP-2 may follow the resolution of inflammatory process and thus enhance new bone formation.

Conclusions

The results of our study suggest a potential role of BMP-2 in the pathogenesis of new bone formation in axSpA and put another brick in the wall of the understanding of this intriguing topic.

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