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FRI0366 **SUBSTANTIAL CHANGES IN BONE FORMATION REGULATORS BIOMARKERS IN EARLY AXIAL SPONDYLOARTHRITIS – ROLE OF TNF INHIBITORS**

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Background: The hallmark of advanced axial spondyloarthritis (SpA) is spine ankylosis, due to an excess of bone formation. Our hypothesis was that pathways of bone formation regulators changed over time and that their changes were related to inflammation or use of “anti-inflammatory” drugs (like non-steroidal anti-inflammatory drugs (NSAIDs) and/or Tumor Necrosis Factor inhibitors (TNFi)).

Objectives: This prospective study aimed to describe the 5-year serum changes of bone formation regulators markers (sclerostin, bone morphogenetic protein 7 (BMP-7) and Dickkopf-1 (DKK-1)) in early axial SpA and to assess determinants of their changes.

Methods: The DESIR cohort is a prospective, multicentre French study of 708 patients (34 ± 9 years, 58% HLA B27 positive, BASDAI 45 ± 20) with early (> 3 months and < 3 years) inflammatory back pain suggestive of axial SpA. Sclerostin, BMP-7 and DKK-1 serum levels were assessed at baseline, two and five years. Change in bone formation regulators over time was analysed using mixed linear models, first in complete-cases and then in available-cases analysis. Determinants of serum biomarkers change were analysed using mixed linear models and we searched for an interaction between time and determinants of serum biomarkers.

Results: Serum levels of each biomarkers at baseline, two and five years are reported in Table 1. Serum BMP-7 significantly increased over time, with a mean of 0.17 pg/mL per month (Figure 1A). Median relative change of BMP-7 at five years was 53.0% [IQR -31.6%, 286.8%]. Serum BMP-7 levels was undetectable in 337 patients (59.6%) at baseline, in 111 patients (48.0%) at two years and in 59 patients (20.2%) at five years. At baseline, serum sclerostin was significantly correlated with age (r = 0.28, p < 0.001), weight (kg) (r = 0.10, p = 0.007), CRP level (mg/mL) (rS = -0.14, p < 0.001), mSASSS (r = 0.08, p = 0.03), number of syndesmophytes (r = 0.15, p < 0.001), bone mineral density in hip and lumbar spine (r = 0.19, p < 0.001 and r = 0.18, p = 0.001 respectively). Serum sclerostin significantly increased over time, with a mean of 0.001 ng/mL per month (Figure 1B). Median relative change of sclerostin at five years was 14.8% [-7.9%, 41.4%]. Serum DKK-1 did not significantly vary over time. In multivariate analysis, serum BMP-7 increased with moderate or high disease activity (ASDAS-CRP) (0.19 pg/mL per month, p = 0.01), in men (5.3 pg/mL, p = 0.004) and decreased with the duration of TNFi use (-0.04 per month of TNFi use, p < 0.001). Unlike in serum BMP-7, there was not a significant interaction between time and determinants of serum sclerostin. In multivariate analysis, serum sclerostin increased with time (0.001 ng/mL per month, p < 0.001), age (0.007 ng/mL per year, p < 0.001) and in men (0.05 ng/mL, p = 0.001) but decreased with the use of TNFi (-0.037 ng/mL, p < 0.001), in patients with a Z score ≤ -2 at at least one site (-0.036 ng/mL, p = 0.005) and with serum BMP-7 levels (-0.0006 ng/mL per unit of serum BMP-7, p = 0.03).

Conclusion: Serum BMP-7 levels significantly increased over time with a rapid and substantial change. Serum sclerostin levels significantly increased over time but to a lesser degree than serum BMP-7. Serum BMP-7 changes were related to the use of TNFi.

Table 1. Serum levels of bone biomarkers over time

	SOST (ng/mL)	DKK-1 (pg/mL)	BMP-7 (pg/mL)
Baseline	0.45 ± 0.18	5413.0 ± 2370.0	11.0 ± 27.5 2.1 [0 – 15.7]
Two years	0.46 ± 0.15	5621.3 ± 2571.0	20.3 ± 19.2 16.2 [5.2 – 27.7]
Five years	0.51 ± 0.19	4998.2 ± 2889.3	20.9 ± 17.6 19.5 [7.2 – 30.6]

Results are expressed by mean ± standard deviation and median [IQR]

Figure 1:

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FRI0367 **INFLAMMATORY PROCESSES IN EXPERIMENTAL SPONDYLOARTHRITIS ARE ACCOMPANIED BY FORMATION OF ECTOPIC LYMPHOID STRUCTURES IN THE BONE MARROW AND ALTERATIONS IN THE B CELL LINEAGE**

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Background: Tumour necrosis factor (TNF) is important in immune-mediated inflammatory diseases such as spondyloarthritis (SpA). SpA patients have extensive angiogenesis in inflammatory and bone forming regions. Transmembrane (tm)TNF-transgenic (tg) mice (1) that overexpress tmTNF develop SpA symptoms, including inflammation, bone destruction and bone formation. Interestingly, these mice also develop lymphoid aggregates in the bone marrow (BM) of the axial and peripheral skeleton.

Objectives: Characterization of lymphoid aggregates in tmTNF tg mouse BM in the context of angiogenesis and bone formation.

Methods: Ankles, femora, tibiae, vertebrae and spleens from tmTNF tg mice and wild-type (WT) littermates (6 weeks, 12 weeks, and 8 months old; n=5 per age per group) were dissected and analyzed by confocal microscopy. In addition, 12 week old mice (n=5 per group) were analyzed by flow cytometry. To study the importance of TNF-R signaling in these processes, tmTNF tg mice lacking TNF-RI (tmTNF tgTNF-RI^{-/-}) or TNF-RII (tmTNF tgTNF-RII^{-/-}) (n=4 per group) were investigated.

Results: Immunofluorescent (IF) evaluation demonstrated that BM of tmTNF tg mice contained extensive lymphoid aggregates, both in the vertebrae and the ankles, but not in the femurs or spleen. IF microscopy demonstrated that the aggregates in the BM contain characteristics of ectopic lymphoid structures (ELS) and consisted of B220⁺ B cells and FDC-M1⁺ follicular dendritic cells that are in close proximity of MECA79⁺ high endothelial venules (HEVs). Flow cytometric analysis revealed that most B220⁺ B cells are IgD⁺ naive B cells and that tmTNF tg vertebrae

contain significantly more IgD⁺CD95⁺ germinal center B cells ($P < 0.01$) with a higher expression of the costimulatory molecules CD80 and CD86 compared to WT. The vertebrae also contained more CXCR5⁺PD-1⁺FoxP3⁺CTLA4⁺ T regulatory cells and a trend towards an increase in CXCR5⁺PD-1⁺FoxP3⁺CTLA4⁺ T follicular helper cells in tmTNF tg mice. Meanwhile, B cell development in the BM of tmTNF tg hind limbs was not altered. Furthermore, BM, spleen and vertebrae from tmTNF tg mice contained significantly more IgA⁺ plasma cells compared to WT littermates. Of note, tmTNF tgxTNF-RI^{-/-} mice did not display lymphoid aggregates or HEVs in the BM, while tmTNF tgxTNF-RII^{-/-} mice did, although to a lesser extent than tmTNF tg mice.

Conclusion: tmTNF overexpression in mice results in extensive lymphoid aggregates in the BM that are often organized in ELS, which may be mediated via TNF-RI signaling. These ELS might result in an increase of IgA⁺ plasma cells in tmTNF tg mice. Ongoing studies will look further into this and may indicate whether these findings contribute to the pathology observed in these mice.

REFERENCE:

[1] Alexopoulou L, et al. Eur J Immunol 1997; 27(10):2588-92

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FRI0368 VASCULAR CALCIFICATION BY INFLAMMATION COULD BE AN IMPORTANT CAUSE OF CARDIOVASCULAR DISEASE IN PATIENTS WITH ANKYLOSING SPONDYLITIS

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Background: Patients with ankylosing spondylitis (AS) have a higher risk of cardiovascular disease. Atherosclerosis is a main pathological process of cardiovascular disease and calcified plaque is a characteristic feature of atherosclerosis. Inflammation plays a potential role in vascular calcification. There is no previous study revealing the mechanism of vascular calcification in AS.

Objectives: We investigated the relationship between vascular calcification and inflammation in patients with AS.

Methods: Sixteen male patients aged over 20 years with AS were enrolled. They fulfilled the modified New York criteria and each of their ankylosing spondylitis disease activity score was more than 2.1. Sex and age matched nine healthy controls were also recruited. Mouse MOVAS (American Type Culture Collection, ATCC[®], CRL-2797TM) vascular smooth muscle cells were stabilized in maintenance medium for 24 hours. Then the amount of serum equivalent to 10% of maintenance medium from subjects was added and cells were stimulated for another 72 hours. We exchanged this medium with calcification medium every third day and cells were cultured for another 11 days. After 2 weeks, cells were stained with Alizarin Red S and the optical density (OD) was measured while being compensated by cell viability. For Western blotting, cells were stabilized for 24 hours and stimulated for another 72 hours through the same procedure as that of Alizarin Red S staining. Then we measured the level of protein expression in PPAR-gamma, beta-catenin, TNF- α , interleukin (IL)-23, and MMP7.

Results: The level of OD of MOVAS cells treated with serum from AS patients (19.503 ± 6.422 , mean $\pm$ SD) was significantly higher than that from controls (12.724 ± 5.746) ($P=0.027$, Mann-Whitney test). The level of MMP7 expression of MOVAS cells treated with serum from AS patients (1.921 ± 0.702) was significantly higher than that from controls (0.779 ± 0.191) ($P=0.000$, Mann-Whitney test). The level of beta-catenin expression of MOVAS cells treated with serum from AS patients (1.292 ± 0.356) was significantly higher than that from controls (0.887 ± 0.310) ($P=0.025$). There was negative correlation between PPAR-gamma and TNF- α ($\rho=-0.762$, $p=0.028$, Spearman rank correlation coefficient), and between PPAR-gamma and IL-23 ($\rho=-0.601$, $p=0.039$); positive correlation between MMP7 and TNF- α ($\rho=0.833$, $p=0.010$), and between MMP7 and IL-23 ($\rho=0.797$, $p=0.002$).

Conclusion:

1. Serum from AS patients showed increased calcification of vascular smooth muscle cells than serum from controls.
2. The level of vascular calcification marker from vascular smooth muscle cells treated with serum from AS patients was significantly higher than that from controls.
3. There were negative relationship between inflammatory markers and inhibitory marker of vascular calcification but positive relationship between inflammatory markers and stimulatory marker of vascular calcification.

These findings suggest that vascular calcification by inflammation could be an important cause of cardiovascular disease in AS patients.

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FRI0369 TOWARDS MORE PRECISE ESTIMATES OF THE FAMILIAL AGGREGATION AND HERITABILITY OF ANKYLOSING SPONDYLITIS – A SWEDISH NESTED CASE-CONTROL STUDY

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Background: Ankylosing spondylitis (AS) is known to have strong familial aggregation, but studies quantifying the familial risk are few and have reached varying results. Familial risks reported from population-based studies range from 17 to 94 in first-degree relatives of AS cases.^{1,2} The heritability (i.e., the proportion of variance in AS liability explained by genetic variation) has been reported to >90%, based on very small studies of twins.³

Objectives: To assess the familial aggregation and heritability of AS among all Swedish AS patients, and to investigate if risks vary by type of first-degree relative or sex.

Methods: We identified all patients with an ICD-10 code of AS in the Swedish National Patient Register (NPR) between 1 January 2001 and 31 December 2016. To increase validity, index patients were required to have two or more visits listing an AS diagnosis, and at least one of the visits being to a specialist in rheumatology or internal medicine. Each index patient was matched on sex and birth year to 50 general population controls from the Total Population Register. First-degree relatives of index patients and controls were identified through the Multi-generation register. Their disease status was ascertained from the NPR in a similar manner as for index patients. Familial risks of AS among first-degree relatives were calculated as odds ratios (ORs) with conditional logistic regression. Based on the familial risks, the corresponding heritability was calculated for a range of plausible prevalence estimates.

Results: We identified 13 795 patients that met the inclusion criteria. Of their siblings, parents, and children, 3.6, 2.3, and 2.1% respectively also had AS. The overall familial OR when having one affected relative was 19.4 (95% CI 18.1-20.8). The estimates were similar for relatives of different kinds (see table), but having more than one affected relative resulted in a higher risk (OR 68.0 (95% CI 51.3-90.1)). Heritability, estimated from sibling risk, was 77%. There were no clear-cut differences between the sexes, but women tended to have an elevated familial risk if the affected relative was female, and slightly lower heritability compared to males. These estimates can be considered as an upper limit of heritability, as shared environmental factors have not been taken into account in calculations.

Conclusion: Familial risks of AS were similar between different types of first-degree relatives, and no significant gender difference was seen on the heritability scale. Although high compared to most other diseases, the familial risks and heritability were lower than previous reports from other countries, though in line with earlier Swedish findings using in part the same data source. The heritability proposed here might still be an overestimate of the true influence of genetics on disease risk. Thus, efforts should also be directed at identifying other risk factors for AS that are not of genetic origin.

REFERENCES:

- [1] Sundquist K, et al. Rheumatology 2008;47(8):1199-202.
- [2] Geirsson AJ, Kristjansson K, Gudbjornsson B. Ann Rheum Dis 2010;69(7):1346-8.
- [3] Brown MA, et al. Arthritis Rheum 1997;40(10):1823-8.