Background: Genome damage has been related to the induction of autoimmune processes, chronic inflammation, and apoptosis. Recent studies suggest that some rheumatological disease are associated with overall genomic instability in the T cell compartment [1,2]. However, no data regarding leucocytes abnormalities in synovial fluid (SF) and their relationship with inflammation are available.
Objectives: The aim of this study was to investigate cellular phenotypes in SF collected from patients with different inflammatory arthropathies, including rheumatoid arthritis (RA), psoriatic arthritis (PsA), crystal-induced arthritis (CIA) and non-inflammatory arthropathies such as osteoarthritis (OA).
Methods: SFs were collected by arthrocentesis from swollen knees of untreated adult patients with RA (n = 6), PsA (n = 12), CIA (n = 10), and OA (n = 7). Total white blood cell (WBC) count was performed using a Bürker counting chamber. Differential leucocyte count was performed by microscopic examination of 300 cells on May Grunwald Giemsa-stained preparations (MGG). Crystal search was performed using compensated polarized light microscopy. MGG staining was used for studying cellular morphology and to perform a cytogenic evaluation of leucocytes. All slides were examined for micronuclei (MN) and nuclear abnormality (NA) included binucleated cells, and karyolitic, karyorrhectic, and pyknotic cells. The following cytokines, chemokines and growth factors were measured in SFs using commercially available ELISA kits: interleukin (IL)-1β, IL-6, IL-8, IL-10, TGFβ, and TNFα. The expression levels of factors involved in apoptosis such as BCL-2, BAK, BID, BAD and BAX were measured by real-time quantitative PCR (qPCR). Caspase-3 activity was determined using commercially available colorimetric assay kit.
Results: We found high percentage of MN in SF from CIA and RA compared to the OA group ($p<0.05$) and a high frequency of pyknotic cell in RA ($p<0.05$) and CIA patients ($p<0.01$). The percentage of karyorrhectic and karyolitic cells were higher in RA patients with respect to OA and PsA patients ($p<0.05$). A correlation between pyknosis and immature polymorphonuclear cells with local inflammatory indices was observed. A strong positive correlation between local inflammatory cellular indices including WBC and PMN and the cytokines IL-1β, IL-6, IL-8, IL-10 was found. The study of apoptosis process revealed an increased BAX expression in CIA ($p<0.05$) and RA ($p<0.08$) compared to OA and PsA, while Bcl-2 was higher in CIA ($p<0.05$). Caspase-3 activity was increased in SF from RA patients compared to OA and PsA ($p<0.05$) and correlates with inflammatory and anti-inflammatory cytokines (IL-1β, IL-8, IL-10).
Conclusion: Our results showed that inflammatory SF are associated with genomic instability and abnormal cell subsets. A deeper knowledge of different abnormal cell subsets and their function could lead to a better understanding of the mechanisms underlying in the resolution of inflammation in these diseases.
REFERENCES:
[1] Shao, L. DNA Damage Response Signals Transduce Stress From Rheumatoid Arthritis Risk Factors Into T Cell Dysfunction. Front Immunol 2018, 9, 3055.
[2] Galita, G., Brzezińska, O., Gulbas, I., Sarnik, J., Poplawska, M., Makowska, J., Poplawski, T. Increased Sensitivity of PBMCs Isolated from Patients with Rheumatoid Arthritis to DNA Damaging Agents Is Connected with Inefficient DNA Repair. Journal of clinical medicine 2020, 9(4), 988.
Acknowledgements: NIL.
Disclosure of Interests: None Declared.
DOI: 10.1136/annrheumdis-2023-eular.5655

AB0029 DISBALANCE OF SYNOVIAL FLUID CYTOKINES IN CHRONIC KNEE SYNOVITIS

Keywords: Synovium, Cytokines and chemokines

M. Karimov[1], T. Nuriakhmetova[2], A. Nuriakhmetov[3], I. Akhtiamov[4]. *[1]Tashkent Medical Academy,Traumatology, Orthopedic and Military Surgery,Tashkent, Uzbekistan*; *[2]Kazan State Medical University, Hospital therapy, Kazan, Russian Federation*; *[3]Republican Clinical Hospital, Orthopedics No. 2, Kazan, Russian Federation*; *[4]Kazan State Medical University,Traumatology, Orthopedic and Military Surgery, Kazan, Russian Federation*

Background: One of the most important issues of orthopedics remain long-standing synovitis despite the treatment after traumas and arthroscopy even after removal of mechanical irritating factor (meniscal resection, chondroplasty). In the long term prolonged treatment of arthritis may lead to irreversible joint damage progression. Current generally accepted strategy is to start the treatment as early as possible which affects the following disease course. Due to this, the study of pathogenetic features of posttraumatic synovitis is of interest as based on them, the choice of the most specific and effective treatment may be made.
Objectives: To study the features of cytokine level changes in synovial fluid in knee synovitis.
Methods: The prospective study of patients with long-standing knee synovitis included knee arthroscopy and outcome registration, measurement of cytokines in aspirated prior to surgery synovial fluid using flow fluorometry.
Results: The study included 41 patients (31.7% males and 68.3% females) with an average age 51.3±12.5 years. Trauma was present in 48.8% of patients. The duration of synovitis below 1 years was observed in 68.3% of patients, 56.1% of them had it for less than 6 months. Osteoarthritis stage 1 was observed in 24.4% of patients, stage 2 – in 22%, stage 3 – in 39%, stage 4 – in 14.6% of patients. The average VAS score on inclusion prior to arthroscopy was 3.56±0.72. Improvement immediately after the surgery was observed in 28 patients (68.3%). Other patients (31.7%) had long-standing synovitis (for over 2 weeks after the intervention), and 7 of them (17%) – for over 2 months. In the synovial fluid the level of IL-10 and TNFα decreased with increasing age (rs=-0,637, p=0,014 и rs=-0,481, p=0,044, respectively). The level of IL-10 positively correlated with synovitis duration (rs=0,830, p=0,011). Besides, in patients with synovitis duration more than a year, the level of IL-4 (r=0,578, p=0,024) and IL-10 (r=0,769, p=0,022) was higher. IL-4 was also higher with higher intensity of pain according to VAS score (rs=0,799, p=0,041). Also, there was a correlation of IL-6 level and presence of trauma in past medical history (p=0.05).
Conclusion: Proinflammatory TNFα and anti-inflammatory IL-10 positively correlated with the age. If synovitis was present for over a year, patients had higher levels of IL-4 and IL-10, which could be explained by proliferation processes in osteoarthritis. High pain intensity was associated with higher levels of IL-4. Trauma was associated with increased levels of IL-6 in synovial fluid, which may probably be the trigger factor of inflammation.
REFERENCES: NIL.
Acknowledgements: NIL.
Disclosure of Interests: None Declared.
DOI: 10.1136/annrheumdis-2023-eular.6348

Osteo arthritis, aetiology, pathology and animal models

AB0030 OSTEOMODULIN DOWN-REGULATION IS ASSOCIATED WITH OSTEOARTHRITIS DEVELOPMENT

Keywords: Animal models, Osteoarthritis, Cartilage

J. Zappia[1], Q. Tong[2], R. Van der Cruyssen[3,4], F. M. F. Cornelis[5], C. Lambert[1], T. Pinto Coelho[6], J. Grisart[7], E. Kague[2], R. Lories[5,8], M. Muller[9], D. Elewaut[4,10], C. Hammond[2], C. Sanchez[1], Y. Henrotin[1,7,11]. *[1]Université de Liège, Center for Interdisciplinary Research on Medicines, Liège, Belgium*; *[2]University of Bristol, School of Physiology, Pharmacology, and Neuroscience, Bristol, United Kingdom*; *[3]University of Ghent, Department of Rheumatology, Ghent, Belgium*; *[4]University of Ghent, Department of Biomedical Molecular Biology, Ghent, Belgium*; *[5]KU Leuven, Department of Development and Regeneration, Leuven, Belgium*; *[6]Université de Liège, Cardiovascular Sciences, Liège, Belgium*; *[7]Artialis, SA, Liège, Belgium*; *[8]University Hospital Leuven, Division of Rheumatology, Leuven, Belgium*; *[9]Université de Liège, Laboratoire d'Organogenèse et Régénération, Liège, Belgium*; *[10]Ghent University Hospital, Department of Rheumatology, Ghent, Belgium*; *[11]Princess Paola Hospital, Physical Therapy and Rehabilitation, Marche-en-Famenne, Belgium*

Background: Osteoarthritis (OA) is associated with metabolic and structural changes in all joint tissues. Subchondral bone sclerosis, cartilage degradation, and synovial inflammation are the main hallmarks of OA [1–3]. OMD is a keratan sulfate proteoglycan, a member of the small leucine-rich proteoglycan (SLRP) family. OMD was first identified in bone where it is involved in the mineralization process [4,5]. Our previous work demonstrated that in the secretome of osteoblasts, OMD was one of the most differentiating factors between osteoblasts originating from the sclerotic and non-sclerotic zone of OA subchondral bone [3]. OMD levels were lower in the supernatant of osteoblasts coming from the sclerotic area.
Objectives: The present work examined if OMD is involved in bone and cartilage changes occurring during skeletal development and in OA.
Methods: We used *Omd* knock-out (KO) or overexpressing male mice and mutant zebrafish to study *in vivo* the impact of OMD on skeletal development and aging. We investigated the influence of OMD on the severity of cartilage and bone damage induced by destabilization of the medial meniscus in these mice. We also analyzed the animals' gait using the CatWalk XT system. The effect of OMD on gene expression by human trabecular osteoblasts in monolayer culture was analyzed by RNA sequencing method. Finally, OMD binding to RANKL was assessed using a solid phase binding assay.
Results: In wild-type mice, we identified OMD mainly in bone and calcified cartilage. Tibial growth plate significantly decreased in all genotypes with age but to a lesser extent in the KO mice than in other genotypes. In KO mice, the calcified cartilage layer was thinner in the medial tibial plateau and thicker in the tibial lateral plateau than in the wild-type, while total cartilage thickness was not different between genotypes. We also demonstrated that *Omd* deficiency led to thicker and less porous bone and subchondral bone sclerosis. *Omd* knock-out mice spontaneously developed more severe OA cartilage lesions in the medial

tibial plateau than the wild-type during aging. In contrast, OMD production did not influence cartilage and bone changes induced by median meniscus destabilization. The gait pattern of mice was abnormal in KO compared to the wild-type genotype with a shorter swing and a smaller paw contact intensity. *omd*$^{-/-}$ zebrafish developed more severe cartilage lesions than wild-type. In osteoblasts culture, OMD down-regulated some genes involved in the extracellular matrix organization and up-regulated other genes responsible for the collagen network degradation. Finally, OMD bound to RANKL and inhibited osteoclastogenesis.

Conclusion: Alterations of the OMD expression modify bone and cartilage metabolism and structure. OMD helps to preserve bone and cartilage integrity and a local decrease in its production leads to the development of OA mainly by increasing subchondral bone sclerosis and thinning the calcified cartilage.

REFERENCES:

[1] Maruotti N, Corrado A, Cantatore FP. Osteoblast role in osteoarthritis pathogenesis. J Cell Physiol. 2017;**232**. doi:10.1002/jcp.25969

[2] Stewart HL, Kawcak CE. The importance of subchondral bone in the pathophysiology of osteoarthritis. Front Vet Sci. 2018;**5**. doi:10.3389/fvets.2018.00178

[3] Sanchez C, Mazzucchelli G, Lambert C, *et al.* Comparison of secretome from osteoblasts derived from sclerotic versus non-sclerotic subchondral bone in OA: A pilot study. Published Online First: 2018. doi:10.1371/journal.pone.0194591

[4] Wendel M, Sommarin Y, Heinegård D. Characterization of osteoadherin - A novel, cell binding keratan sulfate proteoglycan from bone. Acta Orthop. 1995. doi:10.3109/17453679509157655

[5] Sommarin Y, Wendel M, Shen Z, *et al.* Osteoadherin, a cell-binding keratan sulfate proteoglycan in bone, belongs to the family of leucine-rich repeat proteins of the extracellular matrix. *Journal of Biological Chemistry* Published Online First: 1998. doi:10.1074/jbc.273.27.16723

Acknowledgements: NIL.

Disclosure of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.1006

AB0031 ASSOCIATION BETWEEN OMEGA-3/6 FATTY ACIDS AND OSTEOARTHRITIS: A TWO-SAMPLE MENDELIAN RANDOMIZATION STUDY

Keywords: Osteoarthritis, Validation, Biomarkers

S. Wei[1,2,3], Y. Lu[1,2,3], S. Song[1,2,3], J. Qiao[1,2,3], S. X. Zhang[1,2,3], X. Li[1,2,3]. [1]*The Second Hospital of Shanxi Medical University, Department of Rheumatology, Taiyuan, China*; [2]*Shanxi Medical University, Shanxi Provincial Key Laboratory of Rheumatism Immune Microecology, Taiyuan, China*; [3]*Shanxi Medical University, Key Laboratory of Cellular Physiology, Taiyuan, China*

Background: Osteoarthritis (OA) is the most common degenerative joint disorder worldwide. Accumulating evidence has demonstrated the associations of omega-3 and omega-6 PUFAs with the disease activity and inflammatory mediators of OA, omega-3 polyunsaturated fatty acids (PUFAs) are recognized for their anti-inflammatory properties[1], while omega-6 FAs are inflammatory mediators that are increased in joints with OA[2]. However, the evidence of causal links of omega-3 and omega-6 PUFAs on the risk for OA remains inconclusive.

Objectives: This study was conducted to evaluate the causal relationships between omega-3/6 and OA by performing the two-sample Mendelian randomization (MR) analysis.

Methods: The data set of this study was from the publicly available genome-wide association study (GWAS). The genetic instrumental variables for omega-3/6 were derived from the UK Biobank (UKB) and included 114,999 participants. Summary statistic data for OA originated from a meta-analysis of GWAS with an overall 50 508 subjects of European ancestry. In the MR Approach, the IV analysis is based on three strict assumptions, namely that IV should be strongly correlated with exposure, independent of confounding factors associated with direction and outcome, and influence outcome solely through exposure. We screened SNPs with genome-wide significance ($P < 5 \times 10^{-8}$). To ensuring that the SNPs were valid and independent, we removed the linkage disequilibrium (LD) between the SNPs at $r^2 < 0.001$, and <10,000 kb in size. Furthermore, the secondary phenotype of each SNP was retrieved to ensure that it was not associated with OA. The F statistic > 10 indicated a relatively strong estimated effect of IVs. Subsequently, two-sample Mendelian randomization analyses were conducted with inverse variance weighted (IVW), MR-Egger regression and weighted median methods. Sensitivity analyses were then conducted to assess the robustness of our results.

Results: The inverse-variance weighted (IVW) method revealed that higher omega-6 levels were correlated inversely with the risk of OA (β = -0.08, 95% CI [-0.16 to -0.02], P = 0.01), but no causal effect of omega-3 on the risk OA was observed (β = -0.04, 95% CI [-0.10 to -0.02], P = 0.15). The causal estimates from MR-Egger and weighted median methods revealed completely concurring effect directions ($\beta < 0$, $p < 0.05$). Cochran's Q of IVW analysis showed that there was heterogeneity among SNPs ($P < 0.05$), it does not affect the results of IVW, which shown the reliability of our results. MR-Egger regression analysis demonstrated that SNPs could have no-horizontal pleiotropy between omega-6 fatty acids and risk of OA ($P > 0.05$). Moreover, Results of the leave-one-out method suggested that MR results were not influenced by individual SNPs.

Conclusion: This study revealed that the high level of omega-6 predicted by genes can reduce the risk of OA. This implies that supplementing omega-6 fatty acids in our diet may be a potential nutritional modality for the prevention of OA.

REFERENCES:

[1] P Wang, K Xiang, YY Xu, et al. Genetically Predicted Circulating Omega-3 Fatty Acids Levels Are Causally Associated With Increased Risk for Systemic Lupus Erythematosus. *Front Nutr* 2022;9:783338.

[2] AM Valdes, S Ravipati, P Pousinis, et al. Omega-6 oxylipins generated by soluble epoxide hydrolase are associated with knee osteoarthritis. *J Lipid Res* 2018;59(9):1763-70.

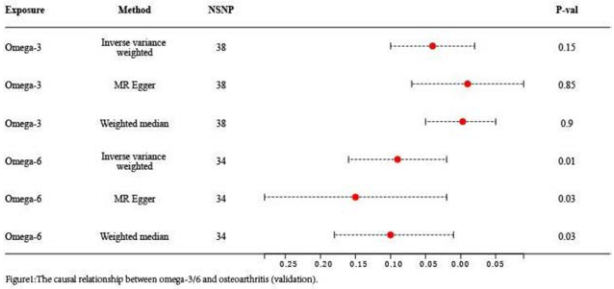

Figure 1. The causal relationship between omega-3/6 and osteoarthritis (validation).

Acknowledgements: NIL.

Disclosure of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.4230

AB0032 LOCAL EXPRESSION OF miRNAS IN OSTEOARTHRITIS PATIENTS

Keywords: Osteoarthritis, Biomarkers

R. Shumnalieva[1], D. Kachakova[2], R. Kaneva[2], Z. Kolarov[1], S. Monov[1]. [1]*Medical University-Sofia, Department of Rheumatology, Clinic of Rheumatology, Sofia, Bulgaria*; [2]*Medical University – Sofia, Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria*

Background: Microribonucleic acids (miRNAs) comprise a class of small non-coding RNAs that negatively regulate the gene expression on posttranscriptional level. Altered expression of miRNAs has been described systemically as well as locally in the inflamed joints of patients with osteoarthritis (OA) [1-3].

Objectives: The aim of our study was to evaluate the synovial fluid (SF) expression of miRNA-146a (miR-146a), miR-155, miR-193 in OA patients and to determine their role as potential diagnostic biomarkers for this disease.

Methods: 16 OA patients were included in the analysis. Expression levels of miR-146a, miR-155 and miR-193b in SF samples were determined by qPCR (SybrGreen technology) and compared to healthy controls (HCs). Relative changes of gene expression levels of the studied miRNAs were calculated by $2^{-\Delta\Delta Ct}$ method. SPSS v20 was used for statistical analysis.

Results: OA SF showed statistically significant overexpression of miR-146a (in 75.00%), of miR-155 (in 68.75%) and of miR-193b (in 75.00%) when compared to HCs and these miRNAs could be used to differentiate OA from HCs ($p=5.9x10^{-3}$, p=0.043 and $p=5.5x10^{-3}$, respectively). Receiver operating characteristic (ROC) curve analysis was constructed in order to evaluate the diagnostic accuracy of the studied miRNAs in SF to distinguish OA from HCs by using relative expression (RQ) values. Area under the curve (AUC) for miR-146a was 0.830 (95%CI=0.674÷0.985, with 75.00% sensitivity and 72.70% specificity, p=0.004), AUC for miR-155 was 0.767 (95% CI=0.582÷0.952, with 75.00% sensitivity and 54.50% specificity, p=0.020) and AUC for miR-193b was 0.801 (95% CI =0.622÷0.981, with 87.50% sensitivity and 63.60% specificity, p=0.009). There was a correlation between the SF expression levels of miR-146a and miR-193b and the radiographic stage of the disease (p=0.0071 and p=0.0105, respectively). SF levels of miR-146a and miR-155 correlated with the SF cell count ($p=5.16x10^{-3}$ and p=0.0188, respectively).

Conclusion: We found an altered SF expression of miR-146a, miR-155 and miR-193b in OA patients when compared to HCs and these miRNAs could serve as potential diagnostic biomarker for inflammatory OA. Larger sets are needed to confirm the diagnostic accuracy of the studied miRNAs in OA.