Interestingly, around 20% of patients experience chronic postoperative pain and the reason for this is not fully understood. Pain in OA is multifactorial and low-grade chronic inflammation has been indicated as a potential cause. Pre-clinical evidence suggests that pro-inflammatory mediators, such as interleukin 6, can sensitize the peripheral and central nerves and these molecules might be associated to clinical pain. Clinical evidence has demonstrated differences in pro-inflammatory mediators when comparing patients with OA and healthy individuals. A recent study has linked these profiles to chronic postoperative pain after TKR but an in-depth analysis, as seen for neuropathic and widespread pain, is needed to advance the field. Currently, no specific biomarkers have been identified, despite initiatives on focused molecular inflammatory mediators. In an attempt to advance the field, a comprehensive analysis of an extensive network of cytokines, chemokines, and growth factors could identify the role of low-grade inflammation in patients with OA and potentially stratify patients more prone to experiencing pain.

**Objectives:** This study aimed 1) to evaluate preoperative serum levels of 92 inflammatory biomarkers in KOA patients compared to healthy controls, 2) to investigate preoperative differences of inflammatory biomarkers within different subgroups of patients with KOA and link these subgroups to clinical pain before and after TKR surgery.

**Methods:** Blood samples from preoperative patients with KOA scheduled for TKR (n=200) and healthy participants (n=39) were collected. After centrifugation of the serum was frozen at -80°C until analysis. Serum samples were analyzed for inflammatory markers using the OLINK inflammation panel, which included 92 protein markers. Clinical pain was assessed using a Visual Analog Scale (VAS). Moreover, patients completed the Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire before and 12 months after TKR. Multivariate data analysis was performed to identify differences between patients and controls. Hierarchical cluster analysis (HCA) and Orthogonal Partial least squares discriminant analysis (OPLS-DA) was used for comparing groups (patients vs controls) and to identify subgroups within patients. T-tests were used to evaluate difference within the KOA cohort in terms of VAS and KOOS scores before and 12 months after TKR.

**Results:** Multivariate analysis showed that 12 proteins were differentially expressed between patients and controls (P<0.05). Hierarchical cluster and OPLS-DA analysis identified two patient subgroups (pat-1, n = 46; pat-2, n= 72) and 23 proteins were dysregulated comparing these two groups (p<0.01). Post-operative VAS and KOOS assessments were significantly different between the two subgroups (p<0.05).

**Conclusion:** The present study suggest a low-grade inflammation in patients with KOA when compared to healthy pain free subjects. Additionally, this study suggests that a high inflammatory subgroup for patients with KOA exist and this group is likely to have more clinical and worst function 12-months after TKR.

**Acknowledgements:** Supported by the Danish National Research Foundation (DNRF121).

**Disclosure of Interests:** None Declared.

**DOI:** 10.1136/annrheumdis-2023-eular.4017

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**AB0040 DIFFERENTIALLY EXPRESSED GENES IN PATIENTS WITH OSTEOARTHRITIS**

**Keywords:** Biomarkers, -omics, Osteoarthritis

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**Background:** Osteoarthritis (OA) is a chronic degenerative disease that affects the joints, causing symptoms of arthralgias, swelling, loss of flexibility, etc. It is the most common rheumatic disease with an estimate of 27 million patients in the US [2]. Its pathophysiology is not yet well understood, as it is thought to be a result of multiple factors that induce metalloproteases, synovial angiogenesis and inflammatory cytokines that leads to inflammation and cartilage destruction [3]. To better understand the molecular mechanisms underlying this disease, we performed a meta-analysis with integrative bioinformatics.

**Objectives:** We aimed to obtain and assess overlapped differentially expressed genes (DEGs) in datasets of patients with OA through functional enrichment and protein-protein interactions (PPI).

**Methods:** We designed a search strategy in the Gene Expression Omnibus platform to identify datasets of gene expression profiling by array of patients with osteoarthritis. The inclusion criteria were: 1) Presence of healthy controls in the datasets, and 2) Analysis of data with GEO2R. The exclusion criteria were: 1) Incomplete information in the datasets, 2) Failure to identify case and controls, and 3) Gene expression by RNAseq, DEGs were selected when p < 0.05 and Logfold change > 2. We assessed the genes with DAVID database, and PPI

with String and Cytoscape; and performed a prediction analysis of possible therapeutic targets.

**Results:** 6 datasets fulfilled the inclusion criteria, and 128 overlapped DEGs were identified. DAVID database yielded the top 3 Biological Processes involving those genes (Table 1). Cytoscape detected 3 clusters in which those genes interact, and iRegulon proposed CHD1 gene as a possible therapeutic target to regulate the entire cluster (Figure 1).

**Table 1. Top 3 biological processes involving overlapped DEGs among the datasets.**

<table>
<thead>
<tr>
<th>Biological Processes</th>
<th>Term</th>
<th>Gene Count</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Regulation of B-cell activation</td>
<td></td>
<td>9</td>
<td>3.7E-7</td>
</tr>
<tr>
<td>Complement activation, classical pathway</td>
<td></td>
<td>9</td>
<td>2.0E-6</td>
</tr>
<tr>
<td>Phagocytosis, recognition</td>
<td></td>
<td>8</td>
<td>5.3E-6</td>
</tr>
</tbody>
</table>

**Figure 1.** This cluster shows the genes with the strongest interactions. Cytoscape yielded other 2 clusters with a lower punctuation.

**Conclusion:** We aimed to identify and assess DEGs involved Osteoarthritis pathophysiology, as well as predict a possible therapeutic target. Our results suggest that immune processes involving B-cell activation and phagocytosis, as well as complement activation underlie OA pathophysiology. Validation of these results in patients with OA is needed since they can serve as possible diagnostic biomarkers. Further research is necessary to analyze the other clusters, and CHD1 characterization as a possible therapeutic target.

**REFERENCES:**


**Acknowledgements:** NIL.

**Disclosure of Interests:** None Declared.

**DOI:** 10.1136/annrheumdis-2023-eular.3357

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**AB0041 IDENTIFICATION AND PROPERTIES OF EXOSOMES (EX) OF SYNOVIAL FLUID (SF) WITH GONARTROSI S (GA)**

**Keywords:** Osteoarthritis, Synovium

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Background: EX - poorly studied extracellular vesicles, 30-150 nm in diameter, found in various biological fluids and have high functional activity, including immunomodulatory effects.

Objectives: The participation of EX in the development of complex connective tissue immunopathology requires further study. The aim of the work was to study EX of SF in GA.

Methods: SF of knee joints of 12 patients with GA with active synovitis and 7 conditional donors postmortem (D) were researched. EX was isolated by ultracentrifugation and double ultracentrifugation (105 g). EX was identified by transmission electron microscopy and flow cytometry. Electrometric mobility (EM) determined by the automatic microscope. Registration of the active forms of oxygen (AFO2) was carried out by EPR. The activity of Cu-Zn superoxide dismutase (Cu-Zn SOD), as well as DNA and RNA levels, was estimated by employing classical biochemical methods.

Results: The EX population undergoes significant morphofunctional changes in GA: EX become smaller, their diameter decreases by an average of 2.5 times, and the value of the negative surface charge changes to 45.5%. EM EX at GA increases by 2.4 times. Against the background of the intensification of free radical processes in EX with GA, a decrease in the activity (on average by 1.7 times) of the pathogenic protein Cu-Zn-SOD is observed. The pronounced secretion of EX in GA is accompanied by an enhanced synthesis of information molecules, especially DNA, the amount of which increases by an average of 2 orders of magnitude (Table 1). At the same time, the floating density of EX in the sucrose gradient (g/ml) is normally 1.24 ± 0.09, and at GA 2.52 ± 0.14*.

Conclusion: The molecular mechanisms of GA progression are associated with the ability of EX to influence intercellular communication, epigenetically reprogram various target cells, activate protein biosynthesis, and be carriers of a number of mediators. Violation of exosomal immune intercellular communications, apparently, underlies the development of a variety of pathologies, including systemic connective tissue diseases.

REFERENCE:

Acknowledgements: NIL.

Disclosure of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.4142

Bone diseases, aetiology, pathology and animal models.

Table 1. Some Characteristics of Synovial Fluid EX Populations in Normal (D) and in GA

<table>
<thead>
<tr>
<th>EX</th>
<th>Diameter range, nm</th>
<th>Range charge, mV</th>
<th>DNA, μg/ml</th>
<th>RNA, μg/ml</th>
<th>Cu-Zn SOD, units/mg of protein</th>
<th>AFO2, units/mg of protein</th>
<th>EM, mV/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>80 - 120 (100)</td>
<td>-15.3, -13.4 (-14.4)</td>
<td>0.014 ± 0.003</td>
<td>0.085 ± 0.017</td>
<td>17.8 ± 23.6 (20.7)</td>
<td>21.7 ± 3.7</td>
<td>0.84 ± 10.8</td>
</tr>
<tr>
<td>GA</td>
<td>30 – 50 (40)**</td>
<td>-10.2, -7.1 (-8.7)</td>
<td>1.43 ± 0.32**</td>
<td>1.31 ± 0.22**</td>
<td>9.4 ± 14.7 (12.1)**</td>
<td>48.4 ± 3.5</td>
<td>1.97 ± 10.8</td>
</tr>
</tbody>
</table>

* - p <0.05; ** - p <0.01; *** - p <0.001

Conclusion: The mechanisms of differential changes in EX are associated with the ability of EX to influence intercellular communication, epigenetically reprogram various target cells, activate protein biosynthesis, and be carriers of a number of mediators. Violation of exosomal immune intercellular communications, apparently, underlies the development of a variety of pathologies, including systemic connective tissue diseases.

Acknowledgements: We thank the French Society of Rheumatology for their funding.

Disclosure of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.286

AB0043 SERIAL ADMINISTRATION OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2 AND OSTEOPROTEGERIN-FC ENHANCES THE DIFFERENTIATION OF OSTEOSTRABLASTS

Keywords: Bone diseases, Osteoporosis

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Background: Osteoporotic bone (OP) is an intrinsic antagonist of the RANKL. A recent study reported that RANKL reverse signaling in osteoblasts may prepare osteoblasts for further maturation and that vesicular RANK stimulates osteoblast differentiation. Hence, OPG is expected to accelerate osteoblastogenesis by affecting RANKL reverse signaling.

Objectives: We compared the level of differentiation from preosteoblasts to osteoblasts by serially administrating recombinant human bone morphogenetic protein-2 (rhBMP-2), involved in bone formation and regeneration, and osteoprotegerin-immunoglobulin Fc segment complex (OPG-FC), an osteoclast inhibitor.

Methods: The MC3T3-E1 preosteoblast cell line was differentiated for one, three, and seven days with a treatment of OPG-FC in 10–200 ng/mL concentration and the cell viability was evaluated by Cell Counting Kit-8 analysis. The level of differentiation from MC3T3-E1 cells to osteoblasts was determined by alkaline phosphatase activity. The level of runt domain-containing transcription factor 2 (Runx2) and osteopontin (OPN) manifestation, involved in osteoblast differentiation, was examined by real-time polymerase chain reaction and western blotting.

Results: During MC3T3-E1 cell differentiation, the differentiation level was high with one-day treatment using 100 ng/mL OPG-FC. The treatment with 50 ng/mL rhBMP-2 for seven days, followed by one-day treatment with 100 ng/mL OPG-FC produced the highest differentiation level, which was approximately 5.3 times that of the control group (P < 0.05). The expression of Runx2 mRNA significantly increased, reaching 2.5 times the level of the control group under the condition of seven-day treatment with rhBMP-2 and one-day treatment with OPG-FC (P < 0.001). The expression of Runx2 protein significantly increased to approximately 5.7 times of the control group under the condition of seven-day treatment with rhBMP-2, followed by one-day treatment with OPG-FC (P < 0.01). The expression of OPN protein showed no change from that of the control group under various conditions of rhBMP-2 and OPG-FC combinations.

Conclusion: Differentiation ability of preosteoblasts to osteoblasts was strong with serial treatment and rhBMP-2, followed by OPG-FC. Runx2 and OPN mRNA levels and Runx2 protein levels increased. These results imply that the combination of OPG-FC and rhBMP-2 increased osteoblast differentiation efficacy.

REFERENCE:

Acknowledgements: NIL.

Disclosure of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.2280