OA-FLS and MSC on proteome level revealed 598 differentially expressed proteins. We observed no differences in the expression of classical fibroblast markers such as vimentin, tenascin C and decorin as confirmed on RNA level. Remarkably, fibronectin, which is mainly produced by fibroblasts, is significantly lower expressed at both protein and RNA levels in OA-FLS together with collagen type 1 and CD106. Conversely, CD9, CD54 and fibroblast-specific protein-1 were expressed significantly higher in FLS at both levels, while hyaluronan synthase 1-3 remained unchanged. Of note, in terms of mitochondrial function, human OA-FLS show a significantly lower basal respiration and ATP production than MSC, but a comparable spare respiratory capacity and cellular mitochondrial dehydrogenase activity (NADH amount) per cell. Additionally, we identified the pyruvate dehydrogenase kinase (PDK) 3 to be highly expressed in OA-FLS, while the expression of mitochondrial ATP synthase subunits, electron transport chain complexes and glycolytic enzymes was comparable with MSC. Finally, inhibition of PDK by using DCA resulted in a significant increase in oxygen consumption rate and ATP production in OA-FLS. Thus, our data newly suggest, that PDKs may play a crucial role in the pathogenesis of OA and possibly RA.

Conclusion: Our data provide evidence that, although the classical fibroblast markers do not discriminate between MSC and FLS, the latter demonstrate a significantly higher expression of PDKs, known to inhibit the pyruvate entry into the TCA cycle which finally limits the mitochondrial ATP production. Therefore, shifting the metabolism of FLS from glycolysis to mitochondrial respiration via inhibition of PDKs might be a novel approach in OA for the development of disease-modifying osteoarthritis drugs in order to unleash the metabolic flow.

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AB0041

TO ESTIMATE THE PREVALENCE OF ACETABULAR OSSIFICATIONS IN THE ADULT POPULATION WITH ASYMPTOMATIC, MORPHOLOGICALLY NORMAL HIPS AT CT AND TO DETERMINE WHETHER THE PRESENCE OF LABRAL OSSIFICATIONS IS ASSOCIATED WITH PATIENT-RELATED OR HIP-RELATED PARAMETERS

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Background: In clinical practice, ossifications adjoining the acetabular rim are frequently seen in healthy-appearing hip joints, at all ages. The origin of these acetabular rim ossifications (ARO) is unclear. While ARO have been described by some authors as variants that may mimic marginal acetabular osteophytes, others have hypothesized that ARO may be the cause or the consequence of femoroacetabular impingement. Overall, the literature on ARO is limited, in particular in the healthy population, and their characteristics and origin not fully understood.

Objectives: To estimate the prevalence of acetabular rim ossifications in the adult population with asymptomatic, morphologically normal hips at CT and to determine whether the presence of these ossifications is associated with patient-, or hip-related parameters.

Methods: We prospectively included all patients undergoing thoracoabdominal CT over a 3-month period. After exclusion of patients with a clinical history of hip pathology and/or with signs of osteoarthritis on CT, we included a total of 150 hips from 75 patients. We analyzed the presence and the size of ossifications around the acetabular rim. The relationships between the size of acetabular rim ossifications and patient-related (sex, age, BMI), or hip-related parameters (joint space width, and cam- and pincer-type femoroacetabular impingement morphology) were tested using multiple regression analysis.

Results: The prevalence of acetabular rim ossifications in this population of asymptomatic, non-osteoarthritic hips was 96% (95%CI=[80.1;100.0]). The presence of ossifications and their size were correlated between right and left hips (Spearman coefficient=0.64 (95%CI=[0.46;0.79]), p<0.05). The size of acetabular rim ossifications was significantly associated with age (p<0.0001) but not with BMI (p=0.35), gender (p=0.05), joint space width (p≥0.53 for all locations) or any of the qualitative or quantitative parameters associated with femoroacetabular morphology (p≥0.34).

Conclusion: Acetabular rim ossifications are highly prevalent in asymptomatic, non-osteoarthritic adult hips at all ages. Their size is not correlated with any patient-, or hip-related parameters except for the age. These findings suggest that ossifications at the acetabular rim, when present in isolation, should not be considered as signs of osteoarthritis or femoroacetabular impingement morphology.

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Table 1. Association between the average severity size of ossifications and patient-related, and hip-related parameters

		Regression coefficient	p-value
Patient-related parameters	BMI	0.02	0.35
	Age	0.04	<0.001
	Gender	-0.43	0.05
Joint space width	Apical	0.17	0.53
	Supero-medial	0.17	0.65
	Supero-lateral	0.06	0.82
Cam morphotype parameters	Angle alpha 45	0.00	0.81
	Offset	0.09	0.34
Pincer morphotype parameters	Acetabular version angle	-0.01	0.83
	Lateral center-edge angle	0.02	0.56
	Acetabular index	0.03	0.53
	Crossover sign	0.26	0.41
	Posterior wall sign	-0.25	0.47

Figure 1. Coronal reformats of CT examinations showing different sizes of labral ossifications in the posterosuperior quadrant (arrows). (a) No labral ossification, (b-c-d-e) Increasingly large labral ossification from left to right. Two lines are visible at the location of the ossification, formed by the contour of the acetabular rim and of the ossification (double line-sign).

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Rheumatoid arthritis - aetiology, pathogenesis and animal models

AB0042

THREE HEMATOLOGIC/IMMUNE SYSTEM-SPECIFIC EXPRESSED GENES ARE CONSIDERED AS THE POTENTIAL BIOMARKERS FOR THE DIAGNOSIS OF EARLY RHEUMATOID ARTHRITIS THROUGH BIOINFORMATICS ANALYSIS

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Background: Rheumatoid arthritis (RA) is a common chronic autoimmune connective tissue disease that mainly involves the joints. The incidence of RA is 5 to 10 per 1000 people[1]. Early diagnosis and treatment of RA can effectively prevent disease progression, joint damage, and other complications in 90% of patients[2]. At present, serum biomarkers used in the diagnosis of established RA are rheumatoid factor and anti-cyclic citrullinated peptide antibody[3]. However, early RA especially serum RF and anti-CCP antibody-negative is difficult to diagnose due to the lack of effective biomarkers. Therefore, it is vital to identify new and effective biomarkers for the early diagnosis and treatment of RA.

Objectives: This study aimed to identify new biomarkers and mechanisms for RA disease progression at the transcriptome level through a combination of microarray and bioinformatics analyses.

Methods: Microarray datasets for synovial tissue in RA or osteoarthritis (OA) were downloaded from the Gene Expression Omnibus (GEO) database, and differentially expressed genes (DEGs) were identified by R software. Tissue/organ-specific genes were recognized by BioGPS. Enrichment analyses were performed and protein-protein interaction (PPI) networks were constructed to understand the functions and enriched pathways of DEGs and to identify hub genes. Cytoscape was used to construct the co-expressed network and competitive endogenous RNA (ceRNA) networks. Biomarkers with high diagnostic value for the early diagnosis of RA were validated by GEO datasets. The ggpubr package was used to perform statistical analyses with Student's t-test.

Results: A total of 275 DEGs were identified between 16 RA samples and 10 OA samples from the datasets GSE77298 and GSE82107. Among these DEGs, 71 tissue/organ-specific expressed genes were recognized. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis indicated that DEGs are mostly enriched in immune response, immune-related biological process, immune system, and cytokine signal pathways. Fifteen hub genes and gene cluster modules were identified by Cytoscape. Eight hematologic/immune system-specific expressed hub genes were verified by GEO datasets. GZMA, PRC1, and TTK may be biomarkers for diagnosis of early RA through combined the analysis of the verification results and the receiver operating characteristic (ROC) curve. NEAT1-miR-212-3p/miR-132-3p/miR-129-5p-TTK, XIST-miR-25-3p/miR-129-5p-GZMA,

and TTK_hsa_circ_0077158- miR-212-3p/miR-132-3p/miR-129-5p-TTK might be potential RNA regulatory pathways to regulate the disease progression of early RA.

Conclusion: This work identified three haematologic/immune system-specific expressed genes, namely, GZMA, PRC1, and TTK, as potential biomarkers for the early diagnosis and treatment of RA and provided insight into the mechanisms of disease development in RA at the transcriptome level. In addition, we proposed that NEAT1-miR-212-3p/miR-132-3p/miR-129-5p-TTK, XIST-miR-25-3p/miR-129-5p-GZMA, and TTK_hsa_circ_0077158-miR-212-3p/miR-132-3p/miR-129-5p-TTK are potential RNA regulatory pathways that control disease progression in early RA.

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AB0043 EFFECT OF ANTI-INFLAMMATORY MEDICATION ON INTERACTION OF SYNOVIAL FIBROBLASTS WITH MACROPHAGES IN RHEUMATOID ARTHRITIS

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Background: One of the key mechanisms in the pathogenesis of rheumatoid arthritis (RA) is the interaction of macrophages and synovial fibroblasts during joint inflammation. Increased synergistic proinflammatory activity of both cell types leads to the release of high levels of proinflammatory cytokines, especially of interleukin-6 (IL-6), and of matrix degrading enzymes. If this mechanism is uncontrolled, progressive destruction of articular cartilage and bone will take place.

In active disease, immediate anti-inflammatory treatment with glucocorticoids is usually replaced by disease-modifying anti-rheumatic drugs (DMARDs), especially by methotrexate (MTX) and biologics such as TNF- α - or IL-6-inhibitors. This led to great improvements in prognosis and outcome for RA patients. However, about 40% of patients experience no remission or suffer from side effects of medication. To optimize established substances and to develop new treatment strategies, it is necessary to understand the mechanisms underlying the limited therapeutic effects.

Objectives: Evaluation of the effect of prednisolone, MTX, adalimumab, tocilizumab on IL-6 secretion by RA synovial fibroblasts (RASf) and macrophages.

Methods: RA synovium was used for RASf isolation. Peripheral blood mononuclear cells (PBMCs) were isolated from blood of healthy donors and RA patients by using Ficoll medium followed by density gradient centrifugation. Mononuclear cells were seeded on six well plates (6x10⁶/well) and incubated for one week. Then they were stimulated with Interferon- γ (20 ng/ml) and LPS (50 ng/ml) for 48h to initiate differentiation into proinflammatory M1 macrophages. The M1 macrophages were co-cultured with RASf (100.000/well) and different treatments added (prednisolone: 10, 25, 50, 75, 100 nM, 1 μ M; adalimumab: 100, 500 μ g/ml; tocilizumab: 1, 5 μ g/ml; MTX: 0.5, 1, 5, 10, 100 nM, 1 μ M). After 24h culture supernatants were collected and IL-6- and TNF- α -ELISAs were performed.

Results: IL-6 concentrations of untreated controls were comparable, regardless whether M1 macrophages from healthy donors or RA-patients were used for co-culture. Prednisolone reduced co-culture-induced IL-6 up to 56% (p<0.001)

in co-culture of RASf and M1 macrophages of healthy donors and up to 60% (p<0.001) in co-culture of RASf and RA M1 macrophages. Adalimumab reduced IL-6 up to 28% (p<0.05) in M1 of healthy donors and up to 45% (p<0.01) in RA M1 macrophage co-cultures. A minor reduction by 10-20% of IL-6 was observed with tocilizumab and no significant effect could be achieved after treatment with MTX.

Conclusion: Prednisolone and adalimumab clearly decrease but do not eliminate proinflammatory synergistic activity of RASf and M1 macrophages. These results confirm the clinical observation, that there is a large number of RA-patients that independent of anti-inflammatory treatment still suffer from low-level joint inflammation.

The synergistic proinflammatory activity of M1 macrophages and RASf seems to be a complex and multifactorial mechanism that is difficult to eliminate by a single treatment substance. Since it is one of the key mechanisms in RA pathogenesis, there is a critical need to investigate how therapy effects could be optimized. This study confirmed RASfs as one of the leading effector cells of increased synergistic proinflammatory activity, thus underlining their promising role as a treatment target in rheumatoid arthritis.

Disclosure of Interests: None declared

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AB0044 ENDOTHELIAL CELL AND RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLAST MIGRATION AND ADHESION ARE ALTERED BY ACTIVIN/FOLLISTATIN

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Background: Activin A and follistatin belong to an anti-inflammatory auto-regulatory cycle. Patients with rheumatoid arthritis (RA) have increased activin A levels in the synovial fluid and tissue. During inflammation, activin A is released systemically, then inducing its antagonist follistatin. This negative feedback is active in different cell types but not RA synovial fibroblasts (SF). Fibroblasts interact with endothelial cells in inflamed tissues inducing angiogenesis.

Objectives: Evaluation of the role of activin A and follistatin on RASf and endothelial cell interactions.

Methods: RA synovium was used for RASf isolation, HUVEC were commercially obtained. RASf and HUVEC were stimulated in mono- and coculture. Direct: RASf together with HUVEC; indirect: inserts with HUVEC separated by a membrane from RASf. Stimuli: activin A 15ng/ml, follistatin 500ng/ml, IL-1 β 1ng/ml. Proliferation was analyzed by BrdU assay. RASf were Calcein-AM stained. Cells were transferred to 24-well plates after 18h stimulation. After adhesion for 1h, non-adherent cells were removed by shaking 3x for 5min. Afterwards, fluorescent cells were quantified. For the flow-adhesion assay, HUVEC were cultured on rattail collagen coated capillary slides. HUVEC and RASf were stimulated for 4h with TNF α , TNF α +activin A or TNF α +follistatin. After stimulation, 2x10⁶ RASf were resuspended in 20ml medium and sent through the capillaries. Two 1min videos were recorded (18.4ml/h, 30.5ml/h). Settings: TNF α -stimulated RASf on HUVEC stimulated with TNF α or activin A+TNF α or follistatin+TNF α . For migration assays, 2% FCS medium with 1x10⁵ cells were placed in inserts (8 μ m membrane) into wells with 10% FCS (control: 2%FCS vs. 2%FCS) and stimulated with, IL-1, IL-1+activinA and IL-1+follistatin. After 16h, migrated cells were quantified. For scratch-assay 4x10⁵ cells were cultured overnight, then cells were scratched and stimulated, afterwards 3 pictures per scratch were taken at start, after 10h and every 1.5h. Cells migrating into the scratch area were quantified.

Results: IL-1 induced activin A in RASf and HUVEC in all settings. IL-1-induced activin A release was reduced by follistatin in HUVEC monoculture and both cocultures compared to IL-1 alone but not in RASf monoculture. IL-1-induced IL-6 release was reduced by activin A in HUVEC and indirect coculture but not in RASf monoculture and direct coculture. Follistatin did not alter IL-6 responses. IL-1 induced VEGF in RASf but not in HUVEC and was not altered by activin A. Short-term adhesion showed no significant influence of activin A or follistatin (n=4). Flow adhesion assay showed reduced adherence/rolling of RASf on HUVEC stimulated with TNF α and activin A (n=4). Migration assays showed that IL-1 decreased migration but without significant difference between the induced effects mediated by IL-1+activinA and IL-1+follistatin (n=4). Scratch assay showed increased migration in direct coculture with greater difference between stimulated and unstimulated cells in RASf monoculture and indirect coculture (n=4). Proliferation was not altered by activin A or follistatin.

Conclusion: In direct and indirect coculture of HUVEC with RASf the effect on HUVEC was dominant leading to reduced IL-1-induced activin A release. However, the IL-1-induced IL-6 release in RASf or HUVECs was decreased by activin A in HUVEC monoculture and indirect coculture but not during cell-contact between both cell types. The direct interaction of RASf with HUVEC seems to prevent the reducing activin A effect on IL-6 release in HUVECs. Activin A seems to not to have an impact on short-term cell adhesion but first observations show, that activin A alters selectin-mediated adhesion under flow conditions. The migration assay shows that IL-1-induced effects on cell migration were enhanced by activin