THE SYNOVIAL TISSUE TRANSCRIPTOME REVEALS COMBINATIONS OF PROTEIN BIOMARKERS FOR UNAMBIGUOUS IDENTIFICATION OF RA PATIENTS FROM SYNOVIAL FLUID AND FOR QUANTIFICATION OF DISEASE ACTIVITY IN SERUM

Biljana Smiljanovic¹, Bruno Stuhlmüller¹, Marc Bonin², Silvial Pade³, Marina Backhaus¹, Gerd R. Burmester¹, Andreas Radbruch², Andreas Grützka², Thomas Häupl¹
¹Department of Rheumatology and Clinical Immunology, Charité, Berlin, Germany; ²Deutsches Rheuma – Forschungszentrum Berlin, Germany

Background:
❖ A main challenge in disease-management of rheumatoid arthritis (RA) is to establish objective criteria relevant for diagnosis and therapeutic stratification of patients. The commonly used disease activity score 28 (DAS28), autoantibodies (RF, ACPA), or the joint ultrasound score US7 only insufficiently characterize the diversity of chronic inflammation in RA.
❖ To address these challenges we analysed synovial tissue transcriptomes, synovial fluid (SF) and serum proteome from long-lasting RA patients, and serum proteome from early RA patients.

Results:
1. From RA synovial tissue transcriptome to validation at the protein level in synovial fluid (SF) and serum from RA patients

(1) SYNOVIAL TISSUE PROFILE

- Synovial tissue (ST) biopsies of patients with RA (n=10) and OA (osteoarthritis; n=10)
- RA gene expression profile was determined by 1200 differentially expressed genes
- ST profile in RA indicates infiltration of cell types: MuMTF, T-, B-, NK-cells

(2) SELECTED MARKERS

- 23 up-regulated genes that allowed separation between RA and OA patients were selected: cytokines, surface molecules, enzymes: CCL2, MIF, CXCL9, CXCL10, CXCL13, CCL18, CD14, CD163, CD44, ICAM1, SELP, ICAM1, CXCL13, CCL18, S100P and LBP

(3) SELECTED MARKERS WERE MEASURED AT THE PROTEIN LEVEL

- 23 selected markers were measured in SF and matched serum from patients with long-lasting RA, OA and ND patients
- In SF: 20 markers, identified to be significant by ANOVA
- In serum: 15 markers were identified to be significant by ANOVA

(4) PREDICTION ANALYSES OF MICROARRAY (PAM) DIFFERENTIATED RA FROM OA AND IDENTIFIED COMBINATION OF TOP MARKERS IN SF AND SERUM

RA DISEASE PATTERN IN SF
- PAM for 20 markers measured in SF
- Reduced RA disease pattern in SF

RA DISEASE PATTERN IN SERUM
- PAM for 16 markers measured in Serum
- Reduced RA disease pattern in serum

- 5-Markers score in sum of log-transformed and normalised concentrations in SF of: sCD14, CXCL13, CCL18, S100P and LBP

CORRELATION OF CLINICAL DATA WITH 5-MARKERS SCORE IN SF

- Correlation of clinical data with 5-markers score in SF

- Correlation of clinical data with 5-markers score in serum

2. Combination of top candidate markers in serum from patients with early RA showed "weak" power to discriminate RA from ND

- 10 patients with early RA
- Combination of 7 markers measured in serum from patients with early RA before and during treatment
- Correlation of 5-Markers score with clinical data in serum from patients with early RA

Take home message:
❖ Disease management of RA is currently mainly based on counting the number of tender (TJC) and swollen (SJC) joints:
  DAS 28 = 0.56 x TJC + 0.28 x SJC + 0.7 x Ln (ESR) + 0.014 x VAS
❖ Aim of this study was to identify combination of biomarkers as objective criteria relevant for diagnosis and activity measure of long-lasting and early RA
❖ In long-lasting RA different body compartments disclosed the systemic nature of disease both at transcriptional and protein level
❖ The tested synovial fluid proteome in long-lasting RA greatly resembles the transcriptome data for the secreted proteins
❖ Serum from long-lasting RA also reflected the disease-specific characteristics of this multi-parameter testing when compared to OA and ND
❖ In serum from early RA patients the multi-parameter testing faces limitations.
❖ More sensitive approaches that might increase sensitivity and specificity are needed such as following the alterations of specific cell population from peripheral blood in a more comprehensive way

Materials and Methods:
❖ Gene-expression profiles from synovial tissues biopsies were generated by Affymetrix HG-U133A arrays. The BioRetis database was used for array analyses.
❖ ELISA and multiplex immunoassays were used for validation of the markers at the protein level.
❖ Samples from two groups of RA patients were analysed: (1) synovial tissue, SF and serum from long-lasting RA and OA (as a control); (2) serum from early established RA patients before and after treatment with corticosteroids and methotrexate (MTX). Serum from healthy donors (ND, n=14) were used as controls, both for patients with long-lasting and early established RA.