

Therefore, we developed a new algorithm for automated and standardised analysis of multiplex FCM data.

**Materials and Methods** Automation included asinh-transformation of data, cell grouping, population detection and population feature extraction. For grouping of cells, an unbiased unsupervised model based t-mixture approach with Expectation Maximisation (EM)-iteration was applied. Populations were detected and identified by meta-clustering of several experiments according to position and extension of cell-clusters in multi-dimensional space and by including a General Procrustes Analysis (GPA) step. For validation, peripheral leukocytes from healthy donors and patients with rheumatoid arthritis (RA) were prepared by hypoosmotic erythrocyte lysis and stained with different sets of lineage-specific antibodies, including CD3, CD4, CD8, CD56, CD19, CD14 and CD15. In parallel, different leukocyte samples were depleted of one of these populations by magnetic beads. Qualitative and quantitative characteristics of major populations were compared with conventional manual analysis.

**Results** Whole blood leukocytes stained simultaneously with up to 7 markers were correctly distinguished in all major populations including granulocytes (CD15+), T-cells and their subpopulations (CD3+, CD4+, CD8+), monocytes (CD14+), B-cells (CD19+), and NK-cells (CD56+). The result was comparable to the “gold standard” of manual evaluation by an expert. The new technology is able to detect subclusters and to characterise so far neglected smaller populations based on the new parameters generated. Automated clustering did not require fluorescence compensation of data. Cell-grouping is applicable even for large FCM datasets of at least 10 parameters and more than 1 million events. Comparing the cell-clusters between RA and healthy controls, differences were detectable in several cell (sub-)populations, stable enough to perform correct classification into controls and disease.

**Conclusions** Our approach reveals first promising results for the analysis of large datasets as generated by multiplex FCM analysis in an automated and time-saving way. Defined clustering algorithms avoid operator-induced bias. In addition, our unsupervised procedure is able to detect unexpected sub-clusters and to characterise so far neglected smaller populations, which may help not only to distinguish normal from disease but also to develop markers for disease activity and therapeutic stratification.

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#### A10.5 COMPARATIVE EFFECTIVENESS OF BIOLOGICAL THERAPIES IN RHEUMATOID ARTHRITIS IS INFLUENCED BY RESPONSE MEASURES AND DISEASE ACTIVITY STATE

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**Background and Objectives** Several biological therapies have become available in the last years in the management of rheumatoid arthritis (RA). Two of the most common drug classes include anti-tumour necrosis factor (TNF) and anti-interleukin-6 (IL-6) agents, which target central cytokines in the disease pathway. We have previously shown that the proportion of patients achieving remission

was higher in the tocilizumab group, an anti-IL-6 agent, compared to anti-TNF therapies, but the magnitude of the effect was associated with the disease activity measure used, namely DAS28, CDAI or SDAI. The aim of this study is to assess whether this difference remains significant in other RA disease activity states.

**Materials and Methods** We included biologic-naïve RA patients registered in the Rheumatic Diseases Portuguese Register, Reuma.pt, who have started therapy with anti-TNF (adalimumab, infliximab, golimumab) and anti-IL-6 (tocilizumab) monoclonal antibodies after 1<sup>st</sup> January 2008. Our primary outcome was the proportion of patients in each disease activity state (remission, low, moderate, high) at 6 months, applying DAS28, CDAI and SDAI. Univariate and multivariate logistic regressions were performed to compare the groups.

**Results** 220 RA patients were enrolled, 180 treated with anti-TNF monoclonal antibodies and 40 treated with tocilizumab. Both groups had similar baseline characteristics but tocilizumab-treated patients had significantly higher SJC, DAS28, SDAI and CDAI as well as shorter disease duration. At 6 months, a significantly higher proportion of patients in the tocilizumab group had reached the DAS28 (n = 21, OR 0.16, p < 0.0001, 95%CI 0.06–0.38) and SDAI (n = 9, OR 0.29, p = 0.03, 95%CI 0.09–0.91) remission thresholds, but no significant difference was seen for CDAI (n = 8, OR 0.41, p = 0.12), in the adjusted logistic multivariate model. Moreover, the proportion of patients with moderate (n = 85, OR 3.49, p = 0.006, 95%CI 1.44–8.43) and high disease activity (n = 30, OR 6.13, p = 0.028, 95%CI 1.32–30.89) was higher in the anti-TNF group only according to DAS28. No differences were seen in the low disease activity class.

**Conclusions** Globally, tocilizumab-treated patients had better disease activity outcomes, but the magnitude of the effect was dependent on the disease activity measure used, confirming our previous results and underlining the pronounced reduction of inflammatory markers such as ESR and CRP, translated by lower DAS28 and SDAI, respectively. Furthermore, this effect was also related to the disease activity state considered. This may be explained by the fact that these different indexes distinctly weigh the different components and/or do not classify the same patients in the same disease activity state.

#### A10.6 DISSECTING DISEASE-SPECIFIC DIFFERENCES IN RA AND OA BY TRANSCRIPTOME ANALYSES OF SYNOVIAL TISSUE, BLOOD AND BONE MARROW MONOCYTES

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**Background and Objectives** Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with joint destruction. Joint inflammation is characterised by infiltration and activation of various immune cells. To avoid difficulties in sampling synovial tissue and to avoid fluctuation in cellular composition of leukocytes, which accompanies inflammation both in synovial tissue and blood, this study was focused on transcriptome analyses of synovial tissues, blood and bone marrow monocytes. The main aim was to analyse potential of blood monocytes in dissecting inflammation in RA and in reflecting inflammation that is evident in synovial tissue. Osteoarthritis (OA), which represents a non-inflammatory disease was used as control in this study.

**Materials and Methods** Synovial tissues from 10 RA and 10 OA patients were used for gene-expression profiling by Affymetrix HG-U133A arrays. Blood and bone marrow monocytes, obtained from

8 RA and 8 OA patients undergoing hip replacement surgery, were utilised for gene-expression profiling by Affymetrix HG-U133Plus arrays. The BioRets database was used for microarray analyses and generation of RA profiles.

**Results** Transcriptome analyses of synovial tissues from RA and OA patients revealed more than 1000 differentially expressed genes. Increased expression of genes involved in chemotaxis (CCL13, CCL18, CXCL9, CXCL10, CXCL13), cell adhesion and activation (ICAM1, PECAM1, ITGAL, ITGB2, CD40, CD86) indicate to inflammation but also to infiltration of various cell types like monocytes/macrophages, NK, T- and B-cells.

By comparing transcriptome of RA and OA monocytes, both from blood and bone marrow, it was obvious that monocytes were able to disclose differences between these two diseases. The RA disease-specific gene-expression profile was evident both in blood and bone marrow and it demonstrated only a minor overlap between these two bodies compartments. Altogether, a typical RA inflammatory profile disclosed in synovial tissues was greatly silenced in blood monocytes, and almost completely absent in bone marrow derived monocytes.

**Conclusions** The RA gene-expression profile was the most specific and robust in synovial tissue, demonstrating the dominance of the inflammatory process in the joints. Nevertheless, the systemic nature of RA was also evident at the level of blood and bone marrow monocytes. Concerning that blood is a favourable and easily accessible material for diagnosis and that monocytes are able to exhibit disease-specific alterations, understanding monocyte response in different rheumatic diseases seems to be advantageous approach for biomarkers discovery. This approach should be essential for identifying the objective criteria relevant for disease and therapeutic stratification of patients with RA.

#### A10.7 DUAL EFFECTS OF SOLUBLE FASL AND MEMBRANE BOUND FASL ON FIBROBLAST-LIKE SYNOVIOCYTES CELLS (FLS) FROM RHEUMATOID ARTHRITIS (RA) PATIENTS

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**Background** Membrane-bound FasL (mFasL) is able to induce fibroblast-like synoviocytes (FLS) apoptosis. In experimental arthritis mouse models, injection of agonistic antibody (Ab) anti-Fas decreased the symptoms. However, soluble FasL (sFasL) is increased in Rheumatoid Arthritis (RA) patients and correlated with disease activity. These results indicated that mFasL could be protective whereas sFasL could be deleterious suggesting that they could have different functions.

We analysed the effect of different FasL preparations mimicking sFasL or mFasL on RAFLS proliferation and apoptosis.

**Methods** RAFLS were treated with different FasL preparations (FasL-Flag ± Ab α-Flag, FasL-Fc or sFasL) or with agonistic Ab anti-Fas. Apoptosis was then analysed by cytometry using annexinV-FITC and TOPRO-3. Proliferation was measured using tritiated thymidine. Signaling pathways was analysed by western blot and their influence was assessed using chemical inhibitors. sFasL was quantified in synovial fluids from patients using cytometric bead array.

**Results** FasL-Flag alone (mimicking sFasL) was not able to induced FLS apoptosis while proliferation was significantly activated ( $3.3 \pm 1$  fold;  $n = 5$ ;  $p < 0.05$ ). Similarly, sFasL was only able to strongly induce RAFLS proliferation ( $7 \pm 5.5$  fold,  $n = 9$   $p < 0.05$ ). Membrane bound FasL (FasL-Flag + Ab α-Flag) significantly induced RAFLS apoptosis ( $52\% \pm 18$ ;  $n = 5$ ) and a slighter but significant proliferation ( $2.2 \pm 0.3$  fold;  $n = 4$ ). Duality of mFasL was confirmed using

agonistic Ab anti-Fas (mimicking mFasL) with pro-apoptotic ( $38\% \pm 18$ ;  $n = 2$ ) and proliferative effect ( $4.4 \pm 2.0$  fold). Finally, growing concentration of FasL-Fc leads to aggregation of the protein, mimicking mFas or sFasL at high and low concentration respectively. Dose responses confirmed mFasL and sFasL effects. FasL activated Akt, JNK and ERK but also activated caspases ( $n = 5$ ). Inhibition of each pathways block FasL-induced proliferation. However, only JNK inhibition significantly increased FasL-induced apoptosis. We observed that FasL-Fc was able to induce osteoarthritis (OA) FLS apoptosis but neither FasL-Fc nor sFasL was able to significantly induced proliferation of OAFLS ( $1.4 \pm 1.3$  and  $2.6 \pm 1.1$  fold respectively,  $n = 4$ ). Synovial fluids from patients with RA ( $n = 16$ ) tends had higher sFasL concentrations compared to those with osteoarthritis ( $n = 10$ ) ( $p = 0.06$ ).

**Conclusions** mFasL induces preferentially RAFLS apoptosis, whereas sFasL only induces RAFLS proliferation. Proliferative effect of sFasL was not seen on OAFLS. According to what we have already described for TRAIL, caspases are involved in FasL-induced apoptosis and proliferation. This is the first demonstration that sFasL and mFasL have different effects on RAFLS proliferation. sFasL by enhancing RAFLS proliferation could have a deleterious role in RA. Therefore, its blockage could be a therapeutic tool to prevent RA.

#### A10.8 EVALUATION OF DISEASE ACTIVITY IN ADULT PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS

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**Background** Juvenile idiopathic arthritis (JIA) is a disease which maintains specific childish rheumatological features during whole life. There is still an open discussion which criteria of the disease activity should be used for the management of adult patients with JIA.

**Objectives** To analyse the usefulness of known disease activity and functional indices used in adult rheumatological practise for the assessment of rheumatoid arthritis (RA) and spondyloarthritis (SpA): disease activity score (DAS), disease activity score 28 (DAS28), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Spondylitis Functional Index (BASFI), Health Assessment Questionnaire – disability index (HAQ-DI), short form health survey with 36 questions (SF-36).

**Methods** 35 adult patients with JIA (polyarticular form) classified by the ILAR (International League of Associations for Rheumatology) classification criteria (Durban 1997, Edmonton 2001) were assessed clinically initially (I) and after 1 year (II) using DAS, DAS28, BASDAI, BASFI, HAQ-DI, SF-36.

**Results** 35 adult patients with JIA (polyarticular form) 19.4 (SD 1.8) years old with disease duration 6.5 (SD 4.2). 16 patients receive anti-TNF therapy and 19 patients methotrexate monotherapy. DAS 28 (I) 3.10 (IQR = 2.2) and DAS28 (II) 3.09 (IQR = 1.7),  $p = 0.3$ ; DAS (I) 2.09 (IQR = 1.6) and DAS (II) 2.03 (IQR = 1),  $p = 0.39$ ; HAQ-DI (I) 0.44 (IQR = 0.57) and HAQ-DI (II) (IQR = 0.62),  $p = 0.32$ ; BASDAI (I) 3.6 (IQR = 3.45) and BASDAI (II) 4.75 (IQR = 3.275),  $p = 0.46$ ; BASFI (I) 1.9 (3.15) and BASFI (II) 1.1 (IQR = 1.2),  $p = 0.057$ ; SF-36 physical health (I) 40.4 (IQR = 9.5) and SF-36 physical health (II) 38.7 (IQR = 11),  $p = 0.02$ ; SF-36 mental health (I) 50.1 (IQR = 27) and SF-36 mental health (II) 50.9 (IQR = 14.2),  $p = 0.6$ .

**Conclusions** Accordingly to the results, increased values of disease activity indices (usually used in adults patients with RA and SpA) show that tendons as well as peripheral joints are involved in the inflammatory process of JIA polyarticular form. Therefore the evaluation of tendons/enthese in adult patients with JIA polyarticular