Abstract A10.24 Figure 1

Objective To visually assess the relationship between the reason for stopping the first biologic and the period of time when the biologic was started.

Methods All patients with rheumatoid arthritis undergoing treatment with a biologic are registered in the clinical database of a single centre with seven treating rheumatologists. The biologic used, as well as the reason to stop it is recorded, as well as all dates regarding the start and the last dose. We performed a multiple correspondence analysis with the Burt method of adjusted inertias and standard normalisation with the variables: first biologic, time period (1998–2001, 2002–2005, 2006–2009), and reason to stop (adverse event, inefficacy, others). Correspondence analysis provides a means of displaying or summarising a set of data with categorical variables in two-dimensional graphical form.

Results The total inertia of the exploratory analysis was 0.10. The first two dimensions added to 57% of the inertia, so that all dimensions were accounted for. The major drives of the relationship between the three variables were “infliximab” and the time period “1998–2001” (% inertia 0.146 and 0.177, respectively). The distance between other categories of the three variables was not relevant enough to make any suggestions of an association. The figure shows the correspondence map of the relationship.

Conclusions If to any biologic at all, time has affected the decision to start infliximab, with more treatments started in the earlier times than in the later ones. The reason to stop any first biologic does not clearly relate to the time neither to the biologic itself in this single centre dataset.

A10.25 SYNOVIAL TISSUE TRANSCRIPTOME AND SYNOVIAL FLUID PROTEOME HAVE STRONGER DISCRIMINATORY POWER THAN SERUM IN DISSECTING INFLAMMATION IN RA

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Background and Objectives Rheumatoid arthritis (RA) is a chronic disease associated with polyarticular inflammation, cartilage and/or bone destruction. Current diagnosis of RA is based on clinical criteria and measurement of non-specific markers of inflammation in the blood. A main challenge in diagnosis of RA is to establish objective criteria able to provide a more detailed insight into

joint pathobiology. In search for convenient biomarkers, gene-expression profiles from synovial tissues of RA and osteoarthritis (OA) patients were generated. These profiles were used for selection of candidate biomarkers that were measured at the protein level both in synovial fluid and matched serum samples from RA and OA patients.

Materials and Methods Gene-expression profiles from synovial tissues of RA and OA patients were generated by Affymetrix HG-U133A arrays. The BioRetis database was used for array analyses and selection of RA candidate genes. ELISA and multiplex immunoassays were used for validation of the candidate markers at the protein level.

Results Transcriptome analyses of synovial tissues from RA and OA patients revealed more than 1000 differentially expressed genes. Genes involved in chemotaxis, inflammation, cell adhesion and activation were strongly up-regulated in patients with RA. In total, 20 genes out of 1000 were used for validation at the protein level, including 1) cytokines: IL8, MIF, MIG, CXCL13, CCL18; 2) the shedded form of membrane molecules: sCD14, sCD163, sCD44, uPAR, sICAM1, sVCAM1, P-Selectin, E-Selectin; and 3) markers related to bone destruction and bone formation: NTxI and PINP. These molecules were measured both in SF and matched serum samples from RA and OA patients. Protein levels of all these markers were elevated in SF of RA patients, showing an obvious inflammatory profile that clearly discriminated RA from OA. Serum levels of these markers were elevated in RA patients, compared to OA and/or healthy donors (ND) as well. Among all serum markers CXCL13, sCD14, CCL18, PINP and NTxI disclosed the highest level of specificity and sensitivity. Nevertheless, the potential of serum markers in general to discriminate RA from OA and/or ND was far weaker compared to those in SF.

Conclusions Disease-specific profiles were evident in the joint where both synovial tissue transcripts and related SF proteins revealed a clear differences between RA and OA. Despite blood is a favourable diagnostic material, the results from this study indicated that the capacity of blood to dilute and neutralise inflammation is robust enough to limit discrimination between RA and OA.

A10.26 SYNOVIAL Wnt AND WISP1 EXPRESSION INDUCES EXPRESSION OF CARTILAGE-DEGRADING METALLOPROTEINASES IN THE SYNOVIUM

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Background Many osteoarthritis (OA) patients show synovial involvement. We found strong upregulation of Wnts 2b and 16 and the downstream protein WISP1 in knee joints in experimental OA. The role of the synovium in the induction of OA pathology under the influence of Wnt signalling is unclear. Here we investigated the potential of Wnt signalling to increase the expression of cartilage-degrading enzymes in the synovium.

Methods Pathway analysis of microarray data from the synovium of a collagenase-induced OA mouse model was done using DAVID software. In vivo synovial overexpression of genes from the canonical Wnt signalling pathway was achieved by intra-articular injection of adenoviral vectors. Joint pathology was assessed by histology. Gene expression was analysed by qPCR. Human OA synovial specimens were collected from joint replacement surgery and stimulated or used for outgrowth of fibroblasts.

Results Pathway analysis showed that the Wnt signalling pathway was enriched in the synovium during experimental OA. To determine the effects of Wnt signalling on synovial tissue, we stimulated human OA synovial specimen with Wnt3a or WISP1, which resulted in increased expression of MMP3, MMP9 and MMP13,

whereas expression of TIMP1 and 3 was not altered. Next, we investigated which cell-type in the synovium may cause the increased MMP expression. Stimulation of synovial OA fibroblasts with Wnt3a increased the expression of MMP3 and MMP13, whereas WISP1 led to increased MMP3 expression. Stimulation of THP-1 cells with Wnt3a resulted in highly increased MMP3, MMP9 and MMP13 expression. WISP1 stimulation led to increased expression of MMP3. TIMP1 and 3 expression was not altered. Synovial overexpression of Wnt8a, Wnt16 and WISP1 in murine knee joints with use of adenoviral vectors led to cartilage damage in vivo. 7 days after overexpression, we found a significant induction of OA pathology at the medial margin of the medial tibial plateau, a preferential site for damage in experimental OA. Lesions were found in 92% (n = 12) of the knee joints after Wnt8a overexpression compared to 17% (N = 12) for the control virus and 80% (N = 5) for Wnt16 overexpression, but only 20% (N = 5) for the control virus. In addition, overexpression of WISP1 led to significantly increased cartilage damage after 7 days.

Conclusions Canonical Wnts produced in the synovium may play an important role in OA pathology by increasing the expression of cartilage-degrading MMPs in synovial tissue, where fibroblasts and macrophages showed comparable patterns of MMP induction. Synovium-specific overexpression of Wnt signalling members induces cartilage damage.

A10.27 SYSTEMIC SCLEROSIS – ROLE OF AGONISTIC AUTOANTIBODIES DIRECTED AGAINST THE ANGIOTENSIN RECEPTOR TYPE 1 AND THE ENDOTHELIN RECEPTOR TYPE A ON IMMUNE CELLS IN DISEASE PATHOGENESIS

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Background and Objectives Agonistic autoantibodies against the angiotensin receptor type 1 (AT1R) and the endothelin receptor

type A (ETAR) have been identified in the majority of systemic sclerosis (SSc) patients. We proved the expression of the AT1R and the ETAR on human peripheral immune cells. Here, we investigated the effects of these autoantibodies on human immune cells in vitro, the association with clinical data, and their possible role in the pathogenesis of systemic sclerosis.

Methods Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by gradient centrifugation and in vitro stimulated by affinity-purified IgG from SSc patients containing anti-AT1R and anti-ETAR antibodies as well as by affinity-purified IgG from healthy donors. After stimulation the protein expression of cytokines was measured by ELISA. T cells were isolated from PBMCs by magnetically activated cell sort (MACS) and cultivated in antiCD3/CD28 coated plates. Afterwards, migration assays were performed towards IgGs of SSc patients or healthy donors.

Results IgG of SSc patients induced a significantly increased expression and secretion of IL-8 and an abundant but due to a broad range not significant secretion of CCL18. T cell migration towards IgG of SSc patients was increased. All these effects were significantly reduced by commercial AT1R and ETAR antagonist, confirming the specificity of the biological activity of the autoantibodies. Correlation analysis with clinical data of the SSc IgG donors revealed a negative correlation of IL-8 expression with the time since disease onset, and an association of CCL18 expression with the incidence of vascular complications. The chemotactic motility of T cells correlated with the autoantibody titers of the used SSc IgG.

Conclusions SSc patient IgGs containing anti-AT1R and anti-ETAR antibodies seem to have effects on inflammation and fibrosis. IL-8 is a major inflammatory cytokine, inducing migration and inflammatory effects. CCL18 is a fibrotic cytokine, also contributing to leucocyte infiltration, and to lung fibrosis in SSc and other diseases. Thus, anti-AT1R and anti-ETAR antibodies may play an important role particularly in the early stage of SSc, contributing to leucocyte infiltration, vascular inflammation and injury, which are regarded to be the first events leading to tissue damage and fibrosis.