the concentration of inflammatory factors. Double luciferase reporter gene detection and rip detection confirmed mir-654-5p and Interaction between Circ_0003972.

**References:**


**Table 1.** Table showing a summary of the results. Ct: confidence interval

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ACAAs</th>
<th>AUC (CI 95%; p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td>0.586 (0.504-0.668; 0.044)</td>
</tr>
<tr>
<td>CTZ</td>
<td></td>
<td>0.594 (0.512-0.676; 0.028)</td>
</tr>
<tr>
<td>ABA</td>
<td></td>
<td>0.680 (0.638-0.720; 0.015)</td>
</tr>
<tr>
<td>TCZ</td>
<td></td>
<td>0.675 (0.649-0.700; 0.017)</td>
</tr>
</tbody>
</table>

**Disclosure of Interests:** None declared.

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**POS0438**

**IDENTIFICATION OF ANTI-CYTOKINE AUTOANTIBODIES WITH POTENTIAL TO PREDICT FLARE IN RHEUMATOID ARTHRITIS PATIENTS UNDERGOING BIOLOGICAL THERAPIES: A DISCOVERY STUDY**

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**Background:** The presence of anti-cytokine autoantibodies (ACAAs) seems to be a physiological mechanism to control the immune response and regulate cytokine activity. Biological therapies also regulate cytokine activities and have greatly improved the quality of life of RA patients. However, alteration of the cytokine network by the use of these treatments may lead to a disbalance in the regulatory system of ACAAs. We hypothesize the ACAAs network may influence the course of immune response in RA patients and may be useful to predict the therapy efficacy.

**Objectives:** We aimed to explore the potential of circulating ACAAs to predict flare in a cohort of RA patients treated with biological therapy.

**Methods:** We employed sera at baseline from 194 RA patients of the clinical trial OPTIBIO (A Coruña), whose primary endpoint is to evaluate the usefulness of standardized protocol strategies of dose reduction in patients with RA in remission treated with biologics. These patients were treated with TNF inhibitors (Etanercept, N=47; Infliximab, N=12; Adalimumab, N=35; Certolizumab (CTZ), N=17; Golimumab, N=5), Tocilizumab (TCZ, N=68) and Abatacept (ABA, N=18). Patients were in clinical remission (DAS 28 <2.6 or SDAI <5 or ACR/EULAR 2011 criteria) at least from 6 months. Patients were followed during a minimum period of one year and maximum period of 3 years. Flare was considered when remission criteria were not fulfilled. The bead-based antibody array MILLPLEX MAP Human Cytokine Autoantibody Magnetic Bead Panel was used for the simultaneous detection and quantification in sera of anti-BAFF; anti-IFN-γ, anti-IFN-α, anti-IFN-β, anti-IFN-ε, anti-IL-1α, anti-IL-1β, anti-IL-6, anti-IL-8, anti-IL-10, anti-IL-12 (p40), anti-IL-15, anti-IL-17A, anti-IL-17F, anti-IL-18, anti-IL-22, and anti-TNFα. Non-parametrical tests, ROC curves and logistic regressions were performed for the statistical data analysis using SPSS. P-value < 0.05 was considered statistically significant.

**Results:** The levels of anti-17A and anti-IL-16 were increased in the sera from patients who suffered a flare during the follow-up period (N=76), compared to those who remained in remission (N=118), showing an area under the curve (AUC) of 0.586 and 0.594, respectively. Segregating by treatment, the levels of anti-17A were specifically increased in those relapsing patients under CTZ (N=6), ABA (N=12) and TCZ (N=20) treatment. The AUC of anti-17A within these three therapies was 0.867, 0.903 and 0.682, respectively.

**Conclusion:** Logistic regression analysis also associated the levels of anti-17A with the risk of suffering a flare in TCZ-treated patients (OR=1.11; p=0.015, for 100 MFI increase). In addition, the TCZ-treated patients who suffered a flare also showed higher levels of anti-IL-17F, anti-IL-1α, and anti-IL-18 compared to those that remained in remission, showing AUCs of 0.689, 0.657 and 0.698, respectively. Anti-IL-18 was also associated with the risk of flare in these patients (OR=1.65; p=0.028, for 100 MFI increase). The presence of these ACAAs was also higher in the TCZ-treated patients who suffered a flare compared to those in remission. These two subpopulations are characterized by a higher proliferation and inflammation and apoptosis.

**Disclosure of Interests:** None declared.

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**POS0439**

**STROMAL B-CELL CROSS-TALK PROMOTES THE ESTABLISHMENT OF SYNOVIAL B CELL NICHE THROUGH THE SELECTION, ACTIVATION OF NATURALLY OCCURRING EBV+ B CELLS**

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**Background:** Rheumatoid Arthritis (RA) is characterized by the formation of ectopic lymphoid structures (ELS) in the synovial tissue, which can promote B cells activation and local production of autoantibodies. B cells exert an essential role in RA immunopathogenesis, as demonstrated by therapeutic effects of Rituximab (1). We previously showed that ELS in the RA joints frequently accumulate Epstein Barr virus (EBV)-infected B cells displaying evidence of both latent (LMP2A) and early lytic viral reactivation in locally differentiated plasma cells (PCs)(2). RA synovial fibroblasts (SFs) can sustain B cells activation, proliferation and maturation into high affinity antibodies producing cells, mimicking B cells physiological differentiation in germinal centres (3). Whether RASFs can also promote preferential selection of naturally-occurring EBV+ B cells is currently unknown.

**Objectives:** Here, we aim to a) demonstrate SFs role in EBV+ B cells selection b) phenotypically characterize B cells after co-culture with SFs c) dissect the molecular mechanisms behind the B cells SFs crosstalk.

**Methods:** Long-term in vitro B cells SFs co-cultures have been established, followed by phenotypical characterization of B cells in flowcytometry. Supernatant were then screened by ELISA at different timepoints, to measure IgG, IgM and IgA production. EBV infection status on B cells were analysed by qRT-PCR after EBV markers in CD58+/CD23high cells. The high proliferation rate of these B cells allowed – on a specific experiment - the establishment of a cell line, named

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Comparison of Cluster 2 (C2) with the signature of 16 chemokines was significantly enriched (CCL-3, -4, -10, -20, -23; CXCL1; CXCL1, -10, -11, -5, -6; MCP-1, -3, -4). Clinically, 25% of the non-responders’ patients was included in C2, while 75% was included in C1, suggesting that a prominent circulating chemokinesis profile prior therapy is associated with a poor clinical outcome. These data were similarly observed in patients before receiving DMARDs, where a signature of upregulated chemokines and pro-inflammatory mediators characterised a cluster with a high % of non-responders patients.

Conclusion: A pro-inflammatory signature, where chemokines are predominantly enriched, associated with the serum of RA patients before therapy, is associated with a poor clinical outcome. This newly identified signature, which deserves a more in-depth analysis, might be clinically useful guiding precision medicine and novel therapeutic approaches.

Acknowledgements: Supported by ISCIII (PI12/00599 y RICOR-R212/0002/0033) co-financed by FEDER, Fundación Andalucía de Reumatología (FAR) and Consejería de Conocimiento, Investigación y Universidad de la Junta de Andalucía (P20_01367). Disclosure of Interests: None declared. DOI: 10.1136/annrheumdis-2022-eular.4466

POS0440  IN-DEPTH ANALYSIS OF AXL AND MERTK EXPRESSION PATTERNS AND REGULATION BY BIOLOGIC TREATMENTS IN RHEUMATOID ARTHRITIS

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Background: Tyrosine kinases receptors MerTK and Axl have been implicated in the pathogenesis of several autoimmune diseases. Despite sharing significant structural homology and having common ligands, Axl and MerTK have distinct features and biological functions [1]. A growing body of evidence suggests that both Axl and MerTK play a crucial role in Rheumatoid Arthritis (RA) pathogenesis and progression and may be exploited as novel therapeutic targets [2]. However, numerous unanswered questions remain to be addressed.

OBJECTIVES: i. To define common and distinct gene-partners of Axl/MerTK and quantify their expression in RA synovial tissue.
ii. To assess the co-expression of Axl/MerTK by synovial cells.
iii. To outline the longitudinal variation in Axl/MerTK expression upon treatment intervention.

Methods: Synovial tissue samples were collected by US-guided biopsy from: i. Patients with early (<12 months) RA DMARDs/steroid-naive [n=87]; and ii. RA patients who failed the first-line biologic with TNF-inhibitors (TNFi) before and 16 weeks after receiving either Rituximab (RTX) or Tocilizumab (TOC) [n=164] [3]. Gene expression was obtained by bulk RNAseq performed on an Illumina HiSeq2500 platform. Axl/MerTK-modules were defined using STRING networks and the module expression determined by the mean z-score of regularized log transformed expression for all genes in the set. Axl-MerTK, CD55, CD90, CD68 protein expression was analysed by immunofluorescence staining.

Results: Using STRING network analysis, we defined an Axl- and a MerTK-module composed of 31 predicted gene-partners of either Axl or MerTK. Thirteen genes were common to both modules and included the ligands Gas6 and ProteinS, and EGFR. Conversely, eighteen genes were uniquely present in the Axl-module (e.g., PIK3-family, IGFR1, INFR1 and STAT3) or the MerTK-module (e.g., Galectin3 and TULP). Recently discovered MerTK ligands, FCGRA1/CD64, PTPN1 and MEGF10. Axl/MerTK-modules quantified in the early-arthritis treatment-naïve RNAseq dataset showed a significant negative correlation with the synovitis score (Axl r=-0.33, p=0.0032; MerTK r=-0.33, p=0.0033). CD55, CD90, CD68-macrophages of the Lining showed notable heterogeneity between patients: they could express either Axl or MerTK alone, or co-express both. Axl was also present in most CD55+ Lining Fibroblast-Like-Cells (FLS) but not by CD90+ Subliming FLS while MerTK, as expected, was restricted to macrophages, including intra-aggregate tingible-body-macrophages. To define how Axl and MerTK vary depending on disease stage and treatment exposure, we quantified their gene expression in active RA patients inadequately responding to TNFi, prior and 16 weeks after starting second-line biologic (RTX or TOC) [3]. Different from the early-arthritis cohort, MerTK was significantly up-regulated in synovia characterised by higher degree of tissue inflammation (lympho-myeloid > diffuse-myeloid > pauci-immune, p<0.0001) and significantly positively correlated with several cytokines’ genes such as TNF, IL-6, IL27, IL-10. MerTK expression was dependent on clinical response to RTX but not TOC as assessed by EULAR response (DAS28CRP, good vs none/mod, FDRresp 0.048). Conversely, Axl expression compared with Cluster 2 (C2), where a signature of 16 chemokines was significantly enriched (CCL-3, -4, -10, -20, -23; CXCL1; CXCL1, -10, -11, -5, -6; MCP-1, -3, -4). Clinically, 25% of the non-responders’ patients was included in C2, while 75% was included in C1, suggesting that a prominent circulating chemokinesis profile prior therapy is associated with a poor clinical outcome. These data were similarly observed in patients before receiving DMARDs, where a signature of upregulated chemokines and pro-inflammatory mediators characterised a cluster with a high % of non-responders patients.

Conclusion: A pro-inflammatory signature, where chemokines are predominantly enriched, associated with the serum of RA patients before therapy, is associated with a poor clinical outcome. This newly identified signature, which deserves a more in-depth analysis, might be clinically useful guiding precision medicine and novel therapeutic approaches.

Acknowledgements: Supported by ISCIII (PI12/00599 y RICOR-R212/0002/0033) co-financed by FEDER, Fundación Andalucía de Reumatología (FAR) and Consejería de Conocimiento, Investigación y Universidad de la Junta de Andalucía (P20_01367). Disclosure of Interests: None declared. DOI: 10.1136/annrheumdis-2022-eular.4466

POS0441  THE ANALYSIS OF THE INFLAMMATORY PROTEOME IN RHEUMATOID ARTHRITIS IDENTIFIES COMMON SIGNATURES ASSOCIATED WITH THE CLINICAL RESPONSE TO DMARDS AND TNFI THERAPIES


Background: The clinical outcome of the most common therapeutic options of rheumatoid arthritis (RA) patients, such as conventional disease-modifying antirheumatic drugs (DMARDs) and TNF inhibitors (TNFi) is still unpredictable, since a high percentage of patients do not respond to the therapy. Innovative analyses combining high-throughput technologies and thorough clinical assessments are needed to gain insight about the management of this prevalent autoimmune disorder.

Objectives: To evaluate the systemic inflammatory proteome of RA patients, to identify useful biomarkers associated with distinctive clinical outcomes.

Methods: Serum samples from 140 subjects, including 40 healthy donors (HC) and 100 RA patients with high activity disease (mean DAS28=4.7), were profiled with the innovative proteomic methodology “proximity extension assay” (Olink) which analyses one panel of 92 pro-inflammatory proteins. Samples from RA active patients included 40 from newly-diagnosed RA patients before taking conventional DMARDs and 60 from biologics-naive patients (mean disease duration=10 years) before receiving TNFi drugs. Clinical outcomes were evaluated following EULAR criteria after 6 months of treatment and patients were characterized into two subgroups (C1 and C2). The molecular mechanisms behind this phenomenon are currently under investigation.

REFERENCES: