a cross-sectional analysis in TIP-RA of DNA methylation signatures in peripheral blood cells in ERA, at-risk anti-CCP+ individuals and demographically matched CCP- controls. **Methods:** Genomic DNA was isolated from two independent cohorts of CCP- (cohorts 1 and 2, respectively: B cell: $n = 17/34$; memory T cell: $n = 21/34$; and naïve T cell: $n = 21/33$), CCP3+ (B cell: $n = 18/37$; memory T cell: $n = 20/36$; and naïve T cell: $n = 20/35$), and CCP3+ ERA (B cell: $n = 4/18$; memory T cell: $n = 5/18$; and naïve T cell: $n = 5/18$) after separating PBMCs using antibodies and magnetic beads. Methylation was measured by Illumina Infinium MethylationEPIC chip. Differentially methylated loci (DMLs) were identified using Welch's t-test and mapped to gene promoter regions to define DM genes (DMGs). Principal component analysis (PCA) was used to represent relationship among groups. Pathway analysis was applied by Reactome. **Results:** For the initial cohort, 1494, 1097 and 1330 DMLs were identified among CCP+, CCP- and ERA in B cells, memory T cells and naïve T cells, respectively. For the confirmatory cohort, 523, 793 and 548 DMLs were found in corresponding cell populations. The DML overlap between the 2 cohorts was highly significant ($p = 2.48E-77$). The DMLs were combined for both groups and corresponded to 411, 412, and 351 DMGs in B cells, memory T cells and naïve T cells. Of these, we found 246, 198 and 195 DMGs between CCP3+ and ERA in each peripheral blood cell population, respectively. PCA showed separation of CCP+, CCP- and ERA in each of the three blood cell types by DMLs (Fig. 1). DMGs were mapped to biological pathways to identify DM pathways. Although most were not significant, there were several highly significant differences comparing CCP+, ERA and CCP- in memory T cells involving pathways, including "Interferon gamma signaling" (FDR 7.48E-14), "PD-1 signaling" (FDR 8.71E-10), "Translocation of ZAP-70 to Immunological synapse" (FDR 4.75E-10), and "Phosphorylation of CD3 and TCR zeta chains" (FDR 8.71E-10).

Figure 1. PCA shows the separation of CCP+, CCP- and ERA patients in memory T cells in confirmatory cohort.

Conclusion: We identified reproducible methylation signatures of CCP-, CCP+, and ERA in peripheral blood B cells, memory T cells and naïve T cells in initial and confirmatory cohorts. The methylome of ERA also demonstrated a distinctive pattern from CCP+, indicating that progression to RA is accompanied by epigenetic remodeling, especially in T cell signaling and interferon responses. These signatures identify critical pathways in CCP positivity and classifiable RA and could provide the basis of novel interventions to prevent disease.

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OP0338

MASS SPECTROMETRY IDENTIFIES NOVEL BIOMARKERS IN GIANT CELL ARTERITIS, USEFUL IN PATIENTS ON INTERLEUKIN-6 RECEPTOR BLOCKADE

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Background: Acute phase reactants (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP]) have limited utility in GCA, even in patients treated with prednisone alone. Furthermore, the lack of reliable biomarkers in patients receiving interleukin (IL)-6 blockade therapy is a major unmet need.

Objectives: To identify biomarkers of disease activity in GCA patients treated with prednisone monotherapy and with prednisone in combination with tocilizumab (TCZ).

Methods: We mapped the serum proteome of GCA patients with active and inactive disease in an unbiased manner using high-throughput multiplexed mass spectrometry. Proteomic analyses were performed in 5 µl serum samples with 11-plexed tandem mass tag (TMT) technology using an Orbitrap Lumos mass spectrometer. A SEQUEST-based database search engine was employed for peptide identification. Quantification was based on TMT reporter ion intensities. All patients were sampled during their participation in the GACTA trial,¹ in which they received TCZ plus 26 weeks of prednisone (TCZ group) or placebo plus 26 or 52 weeks of prednisone (PRED group). Active disease was defined as the presence of cranial or PMR symptoms requiring treatment intensification regardless of ESR and CRP levels. Samples were selected if patients were in clear states of active or inactive disease at GACTA systematic sample collection timepoints (baseline and weeks 4, 12, 24, 48). An exhaustive leave-2-out strategy was used to identify classification markers. All possible pairs of samples were isolated as test samples and the remaining training samples were used to identify the protein markers. Proteins with an absolute log2 fold concentration difference ≥ 0.5 between active and inactive samples and a P-value < 0.1 were retained and sorted based on the metric $-\log_{10}(P\text{-value}) \times \text{absolute}(\log_2 \text{ fold change})$. Top markers within each training set were selected to generate normalized ranks {0,1} across all samples. A mean rank was calculated for every sample. The set of normalized ranks for the test samples across all sets of top markers were bootstrapped for each test sample 100 times with replacement. The bootstrapped rankings were evaluated by determining areas under the curves (AUC) of receiver operator characteristic (ROC) curves.

Results: The PRED group included 21 patients (active, $n = 16$; inactive, $n = 5$) and the TCZ group included 21 patients (active, $n = 14$; inactive, $n = 7$). Using high-throughput sample preparation methods without applying any depletion of known highly abundant serum proteins, we quantified 760 proteins across all samples and 344 proteins in at least half the samples. Compared to inactive PRED-treated patients, active PRED-treated patients showed significant overexpression of several acute phase reactants including serum amyloid A1 and 2 (SAA1, SAA2) and complement factor H (CFH) (Fig. 1a). The magnitude of concentration change and the level of statistical significance observed for SAA1, SAA2 and CFH in PRED-treated patients were higher than those of CRP (Fig. 1a). Compared to inactive TCZ-treated patients, active TCZ-treated patients demonstrated significant overexpression of multiple biomarkers including haptoglobin, haptoglobin precursor, SAA2 and complement factor 4A, and underexpression of peptidase inhibitor 16 (Fig. 1b), a protein involved in vascular and regulatory T cell biology. Sets of 10 biomarkers resulted in a classification of active versus inactive disease with ROC AUCs of 0.89 (95% CI 0.79-0.96) in the PRED group (Fig. 2a) and 0.97 (95% CI 0.95-0.97) in the TCZ group (Fig. 2b).

Conclusion: We identified several differentially expressed serum proteins in GCA patients with active and inactive disease receiving prednisone monotherapy or TCZ-based treatment regimens. In both treatment groups, a signature of biomarkers classified disease activity status with high accuracy. Haptoglobin, a readily available laboratory test, may be useful in monitoring disease activity in GCA patients receiving IL-6 blockade therapy.

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Figure 1. Differentially expressed serum proteins in giant cell arteritis patients with active and inactive disease

(a) Top 10 markers in patients treated with prednisone monotherapy (PRED group); (b) top 10 markers in patients treated with tocilizumab and prednisone (TCZ group)

Figure 2. Accuracy of top biomarkers for discrimination between active and inactive giant cell arteritis

(a) Top 10 markers in patients treated with prednisone monotherapy (PRED group); (b) top 10 markers in patients treated with tocilizumab and prednisone (TCZ group)

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LB0002

A FIRST IN CLASS THERAPEUTIC NANOPARTICLE FOR SPECIFIC TARGETING OF ANTI-CITRULLINATED PROTEIN ANTIBODY AMELIORATES SERUM TRANSFER AND COLLAGEN INDUCED ARTHRITIS

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Background: Rheumatoid arthritis (RA) is an immune mediated inflammatory disease with autoimmune features, including antibodies to citrullinated proteins and peptides (ACPAs). Several in vitro studies have suggested a pathogenic role of ACPAs in RA. However, in vivo proof of this concept has been hampered by the lack of therapeutic strategies to reduce or deplete ACPA in serum and synovial fluid. Previously, we constructed a chitosan-hyaluronic acid nanoparticle formulation with the ability to use neutrophil recruitment as a delivery mechanism to inflamed joints. Specifically, nanoparticles got phagocytosed and then released to synovial fluid upon death of the short-lived neutrophils.

Objectives: We hypothesized that reducing ACPA levels would have a therapeutic effect by blocking cytokine production. In this study, we prepared and tested a series of therapeutic nanoparticles for specific targeting of ACPA in synovial fluid.

Methods: Nanoparticles were prepared by the microdroplet method and then decorated with synthetic cyclic citrullinated peptide aptamer PEP2, PEG/hexanoic acid and fluorophore (Cy5.5). Nanoparticles were characterized by dynamic light scattering (DLS), scanning electron microscopy (SEM) and high-performance liquid chromatography (HPLC). Nanoparticles were then used in a series of in vitro assays, including cell uptake with flow cytometry (FACS) detection, and in vivo studies including disease activity scores, cytokine measurements and near-infrared imaging.

Results: We screened a series of citrullinated peptide epitopes and identified a fibrinogen-derived 21-amino-acid-long citrullinated peptide showing high selectivity toward autoantibodies in RA samples. We incorporated this aptamer in the chitosan-hyaluronic acid nanoparticle formulation previously described. Average nanoparticle size was 230nm $\pm$ 10nm by DLS and SEM; z potential was -0.0012. Purity by HPLC was over 95%. Attachment efficiency of the aptamer was 92% by HPLC. FACS study showed selective uptake of Cy5.5 labelled aptamer-nanoparticle conjugates by neutrophils in the concentration range 0.5-4nM. Similar to previous studies,¹ there was no apparent immunogenicity for this nanoparticle formulation measured by cytokine secretion from human peripheral blood leukocytes. In vivo, over 50% reduction of disease activity was achieved in three weeks treatment using as little as 1 nM drug candidate (dosed every 48 hours) in the collagen-induced (CIA) mouse model of RA (N=30; p<0.001 for treated vs placebo). Same was observed in the serum transfer model (N=10). The aptamer-nanoparticle conjugate significantly reduced IL-6 and TNF α levels in the mouse sera (p<0.01). The effects were not inferior to tocilizumab treated controls (N=30). To confirm mode of action, we applied Cy5.5-labelled aptamer-nanoparticles in the collagen-induced mouse model (N=10) and analyzed the resulting uptake by near-infrared imaging. We confirmed over 6-fold higher signal accumulation in inflamed vs healthy joints (p<0.01), which strongly supports the fact that the aptamer is highly specific to the inflammatory process.

Conclusion: Overall, we have designed a first-in-class therapeutic nanoparticle drug for specific targeting of anti-citrullinated protein antibodies. The marked effect of this nanoparticle observed in vivo holds promise for targeting ACPAs as a therapeutic option in RA.

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Science communication skills for young rheumatologists

OP0339

ALTMETRIC - DOES SOCIAL MEDIA IMPACT CITATION RATE IN RHEUMATOLOGY JOURNALS?

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Background: The coloured altmetrics donut has become a standard feature of online publications. The colours depict different online sources by which an article was mentioned, while the number in the donut, the Altmetric Score (AS), reflects the summarised attention an article has received. The Dimensions database joins citations from any kind of scientific or mainstream publication. Studies analysing the link between the AS and the citation rate of an article suggest that this connection is strongly dependent on the field of research, the type of article and the type of analysis used.

Objectives: To analyse the connection between AS and citation rate in articles published in rheumatology journals.

Methods: We retrieved data on article usage, AS and citations of articles published in ARD and RMD Open between January 2015 and November 2019. For time-dependent analyses on the influence of AS on citations, articles published in 2019 were excluded. Forward-stepwise regression models were used to explore factors influencing total citation rates. We performed subanalyses, dividing articles in categories of correspondence, original research and editorials/viewpoints. We dichotomised articles by reaching the top 25% in terms of citation count within the first, second, third and fourth year after publication according to their category. We explored the risk of reaching these top 25% in dependency of AS using logistic regression (log transformed AS) and receiver operating curve analyses (ROC, reported cut-offs were identified coinciding with 80% specificity).

Results: We used 1597 articles published in ARD and 409 articles of RMD Open with complete data on AS and article usage within the mentioned timeframe. AS are higher in more recently published articles (p=0.04, β : 1.3 per year), but the number of Dimensions citations is lower in more recently published articles (β : -8.5 per year, p<0.001). Twitter shows by far the highest activity among the AS subcategories (highly correlating with AS r=0.8, p<0.001). The total number of twitter mentions increased by 2.8/year from 2015 to 2019, indicating that more recently published articles were more often picked up on twitter. Changes in R² in the regression model indicated that besides time since publication and AS, also the type of article influences citation count. For original research and editorials, AS may significantly add to the variability of the citation count, which was not the case for correspondences. The influence of AS on citation count of editorials added 16% to the 12% variability explained by publication time. Both factors showed similar β -coefficients (months: β : 0.76; β : AS: 0.83). This effect was smaller in original articles (month: β =0.74; AS: β =0.11, Total R²: 23.7%). AS significantly coincides with reaching the top 25% of citation counts according to time since publication. For the first year those articles with AS >15 showed a positive Likelihood Ratio (+LR; 95%CI) of 1.6 (1.4-1.9) to reach the top 25%, the second year AS>15: +LR: 1.9 (1.6-2.2), the third year AS>13: +LR 2.3 (1.9-2.7) and in the fourth year AS>12 +LR: 2.1 (1.7-2.7). This effect was again different between publication categories, with no effect of AS in correspondence articles. Figure 1 highlights that AS influences citations of editorials to a larger extent than of original articles, except within the first year of publication.

Figure 1: Odds Ratio of reaching the top 25% of cited papers in the respective year since publication by Altmetric score, separately shown for original and editorial/viewpoint articles.

Conclusion: We could show, that Altmetric scores are higher in more recent publications and seem to have an influence on the number of citations, particularly early citations in the first two years after publication. The early implementation of published articles in social media could help to increase overall citation.

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