Background: The presence of autoantibodies in Rheumatoid Arthritis (RA) is a hallmark of the disease and one of main criteria for diagnosis and clinical classification. Identification of anti-citrullinated protein antibodies (ACPA) and RF-Factors in circulation of RA patients remains the most characteristic and established criteria for diagnosis. However, due to diagnostic limitation of these biomarkers, only approximately 70% of patients with RA can be identified. As a consequence, there is a lack of diagnostic biomarkers for large number of patients. Diagnosis of these patients relies mainly on assessment of their clinical symptoms such as swollen and tender joints. This problem leaves a need for diagnostic improvement and development of more sensitive biomarkers.

Objectives: In this study, we aimed to develop a sensitive biomarker assay capable to identify and quantify presence of autoantibodies against RA type II collagen in circulation of patients with RA.

Methods: The presence and levels of autoantibodies were measured in serum samples from 50 patients with moderate to severe RA who had inadequate response to Methotrexate (average age 51.2, 86% of female, 78% of white race patients). Control cohort involved serum from 42 healthy age matched patients (average age 48.6, 50% of female, 60% of white race patients). Denaturation of the type II collagen was performed by heat treatment for 15 min at 72 °C. Improvement of assay sensitivity was investigated by measurements of autoantibody levels against denatured and native type II collagen. Assay specificity was assessed by comparison of presence of autoantibodies against type II collagen versus albumin (non-sense control). The normality of data distribution was checked with Shapiro-Wilk test, significance between cohorts with Mann-Whitney nonparametric test and correlations with Spearman’s correlation coefficient (r).

Results: Serum levels of autoantibodies against denatured type II collagen were significantly higher in RA patients than in healthy controls (P < 0.0001) (Figure 1). We observed nearly 4-fold difference between both cohorts. Denaturation of type II collagen showed high improvement of assay sensitivity and increased accessibility of collagen for binding of autoantibodies. Developed assay showed specificity for detection of type II collagen autoantibodies by displaying no levels of autoimmunity against other control proteins (albumin). The levels of serum autoantibodies correlated significantly (P < 0.03) with patient disease activity (DAS28) at baseline, displaying rho = 0.3. Our assay showed good technical performance with acceptable inter- and intra- assay variations (18% and 5% respectively).

Conclusion: Present findings show that patients with RA carry upregulated levels of circulating autoantibodies directed against type II collagen. Heat-treatment of type II collagen increased exposure of immunogenic epitopes of collagen and enabled for more sensitive detection of autoantibodies directly in patient serum. Developed assay demonstrated potential for specific detection of autoantibodies and may provide additional diagnostic value in RA patients.

Disclosure of Interests: None declared.

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change from baseline to week 24, DAS28-CRP remission and ACR 20/50/70 at week 24. IPD mixed-effects meta-regressions were estimated with trial fixed effects, main effects and interaction of enrichment status and treatment type, trial-level random effects on the interaction, and baseline DAS28-CRP score for DAS28-CRP outcomes. These regressions were conducted in the full population and among ABA patients only. Sensitivity analyses defining enrichment using only criteria 3 and 4 were also conducted.

**Results:** 2,087 patients [1,328 (64%) enriched, 759 (36%) non-enriched] were included (AGREE 492 [24%], AMPLÉ 509 [24%], AVERT 339 [16%], AVERT-2 747 [36%]). Disease duration, RF+, and anti-CCP values differed as expected between the two groups, while DAS28-CRP was high regardless of enrichment status (Table 1). Among ABA-treated patients, outcomes were more favorable for enriched patients compared to non-enriched patients across all outcomes, either statistically or directionally (Figure 1, ABA treatment arm only analysis). The differences in outcomes between enriched vs. non-enriched patients were larger for ABA than for comparators across all outcomes with the exception of ACR 50, where the difference was directionally consistent (Figure 1, ABA vs. comparator analysis). The relative odds of improved efficacy of ABA vs. comparators ranged from 37% to 87% for remission and ACR responses. The results were consistent in the sensitivity analysis using only anti-CCP and RF seropositivity to define enrichment.

### Table 1. Baseline characteristics

| All patients | Non-enriched patients | Enriched patients | ABA Comparator | ABA Comparator
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>2,087</td>
<td>1,328</td>
<td>759</td>
<td>742</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.3 ± 12.9</td>
<td>49.4 ± 12.6</td>
<td>50.6 ± 12.9</td>
<td>48.6 ± 12.5</td>
</tr>
<tr>
<td>Female</td>
<td>79.3%</td>
<td>81.3%</td>
<td>83.4%</td>
<td>83.7%</td>
</tr>
<tr>
<td>Disease characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>8.5 ± 12.5</td>
<td>19.9 ± 15.8</td>
<td>13.5 ± 15.1</td>
<td>13.5 ± 15.1</td>
</tr>
<tr>
<td>RF Positive</td>
<td>91.0%</td>
<td>74.2%</td>
<td>76.1%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-CCP Positive</td>
<td>81.8%</td>
<td>52.2%</td>
<td>47.3%</td>
<td>100%</td>
</tr>
<tr>
<td>DAS28-CRP Score</td>
<td>5.71 ± 1.13</td>
<td>5.72 ± 1.19</td>
<td>5.66 ± 1.20</td>
<td>5.68 ± 1.09</td>
</tr>
</tbody>
</table>

Comparators included MTX and MTX+ADA.

**Figure 1. Analysis results.**

**Conclusion:** This post-hoc study corroborates previous evidence of improved outcomes among ABA-treated, seropositive early RA patients by applying eAM enrichment. In the sensitivity analysis using only anti-CCP and RF seropositivity to define enrichment, the outcomes among ABA-treated, seropositive early RA patients, suggesting a potential for patient-tailored treatment approaches.

**References:**


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**POS0475 INTEGRATIVE CLINICAL, MOLECULAR AND COMPUTATIONAL ANALYSES ALLOW THE IDENTIFICATION OF DISTINCTIVE PHENOTYPES OF RHEUMATOID ARTHRITIS PATIENTS RELATED TO THE CLINICAL INVOLVEMENT AND THE RESPONSE TO TNF INHIBITORS**

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**Background:** TNF inhibitors (TNFi) represent an extraordinary advance in the management of Rheumatoid Arthritis (RA). Despite their benefits, there is a percentage of patients (20–40%) that do not achieve clinical improvement. Therefore, is necessary to search for new and easily accessible biomarkers predictive of therapeutic response that might guide precision medicine.

**Objectives:** 1. To explore changes in the molecular profile of RA patients following TNFi therapy in serum samples. 2. To search for new and reliable biomarkers predictive of TNFi response, based on clinical and molecular profiles of RA patients, by using machine learning algorithms.

**Methods:** In an prospective multicenter study, 79 RA patients undergoing TNFi and 29 healthy donors (HD) were enrolled. Twenty-two RA patients were further included as a validation cohort. Serum samples were obtained before and after 6 months of treatment, and therapeutic efficacy was evaluated. Patients' response was determined following EULAR response criteria. Serum inflammatory profile was analyzed by a multiplex immunoassay, along with oxidative and NETotic profiles, evaluated by commercial kits. A circulating miRNA array was also performed by next-generation sequencing. Clustering analysis was carried out to identify groups of patients with distinct molecular signatures. Then, clinical and molecular changes induced by TNFi were delineated after 6 months of therapy. Finally, integrative clinical and molecular signatures as predictors of response were assessed at baseline by supervised machine learning methods, using regularized logistic regressions.

**Results:** Inflammatory, oxidative stress and NETosis-derived biomolecules were found altered in RA patients versus HD, closely interconnected and associated with several deregulated miRNAs. This altered network allowed the unsupervised division of three clusters of RA patients with distinctive clinical phenotypes, further linked to TNFi effectiveness. Cluster 1 included RA patients with low levels of pro-inflammatory cytokines, associated with a medium-low disease activity score and good clinical response. Clusters 2-3 comprised patients with high levels of pro-inflammatory cytokines, associated with a high disease activity and a non-response rate of 30%.

After 6 months of therapy the molecular profile found altered in RA patients was reversed in responder patients, who achieved a molecular phenotype similar to HDs. However, non-responder patients' molecular profile remained significantly deregulated, including alterations in inflammatory mediators (IL-6, L-8, TNFα, VEGF, IL-1RA, IL-5, IL-15, GMCSF, GCSF, FGF), oxidative stress markers (LPO) and NETosis-derived products (Elastase), along with specific miRNAs (miR-199a-5p). These molecular changes further correlated with changes in disease activity score. Machine-learning algorithms identified clinical (Creatinine, IgM, Vitamin D, Swollen Joints, C4, Disease Duration and Tryglicerides) and molecular (Nucleosomes, IL-10, miR-10a5p, IL-13, IL-12p70, IL-15 and Frontera, Cadiz, Spain