Introduction
Rheumatoid arthritis (RA) is an autoimmune disease typically characterized by chronic inflammation, accumulation of self-reactive B-cells and production of autoantibodies of which anti-cyclic citrullinated peptide (anti-CCP) antibodies and rheumatoid factor (RF) have diagnostic utility. However, 30% of RA patients remain sero-negative making the early diagnosis of RA more difficult.

The goal of this study is to characterize in-depth the autoantibody reactivity of RA patient samples as a source to develop and improve autoantibody-based diagnostic tests.

In order to characterize the autoantibody repertoire in patients with RA, we performed a large-scale screen against 3,068 antigens using the bead-based Luminex xMAP technology. The autoantibody signature of 75 patients with an established RA was compared against 71 healthy controls. Antibodies with high reactivity were selected and used to develop biomarker panels with improved sensitivity and specificity.

Conclusions
- Using the SeroTag® process the profiling of RA sera enabled the in-depth characterization of the autoantibody repertoire of RA patients.
- Multiple antigen/autoantibody interactions exist in serum samples of RA patients with significant differences in comparison to healthy controls. These antibodies are candidates for the development of novel diagnostic tests.
- Combining a citrullinated peptide with additional antigens improved the classification of RA-patients.
- Further studies are needed to validate the marker candidates with respect to related diseases such as osteoarthritis, psoriatic arthritis, fibromyalgia,...

References

Abbreviations
PPLS-DA: partial least squares discriminant analysis; AUC: area under the curve; S.D.: standard deviation.

Methodology
The SeroTag® technology provides a platform for the discovery and validation of novel autoantigens using an automated multiplex platform (Fig. 1). The SeroTag® technology utilizes the bead-based Luminex xMAP technology which enables to measure the reactivity of autoantibodies to thousands of different antigens in one single serum sample. A crucial component of the discovery process is the unique warehouse of currently 6,500 human proteins expressed in E.coli. The Ni-NTA purified proteins are coupled to color-coded anti-body beads enabling the multiplex analysis of up to 500 different antigens. In this study SeroTag® was utilized in a non-hypothetical driven approach to identify novel RA autoantigens.

Results
Selection of Antigen Panels
Statistical analysis was performed to distinguish RA from healthy controls including subgroup analysis with respect to CCP and RF status. The best performing single markers are visualized using Volcano plots (Fig. 3). The magnitude of the antigen reactivity in RA sera relative to the control group is shown on the x-axis and the statistical significance on the y-axis. A citrullinated peptide (citPep) of the first generation (2) as internal standard can be confirmed with the RA cohort.

Validation of Marker Candidate Antigens
For validation the putative candidate antigens were analyzed based on a second RA cohort derived from the HITHARD treatment study. The classification performance of the combination of panel <citPep compared to citPep alone was improved (Fig. 5). This indicates that new markers have been found that have the potential to identify currently seronegative RA patients.

Fig. 4: Antigens detected with elevated frequency in the RA cohort

Fig. 5: Classification performance of CCP, new panel and combination of both