**Autoantibody Profiling of Late and Early RA Patients Against Citrullinated Proteins**

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**Introduction**

The discovery of autoantibodies (AAB) against citrullinated proteins (ACPAs) and the development of serological tests using cyclic citrullinated peptide (CCP) has improved diagnosis of rheumatoid arthritis (RA). ACPAs recognize specifically various citrullinated peptides that derive from different antigens. CCP-positive RA patients show autoantibody specificities against different citrullinated antigens. It has also been demonstrated that autoantibodies to citrullinated peptides emerge at different stages of RA (Birik et al., 2013). Moreover, some CCP-negative RA-patients can be identified by analyzing additional citrullinated proteins or peptides (Lundberg et al., 2013). We follow the hypothesis that discovery of additional citrullinated antigens might improve early diagnosis of RA based on epitope-determined heterogeneity of AAB-reactivity.

**Methods**

The SeroTag® technology utilizes the bead-based luminex xMAP technology to profile the reactivity of autoantibodies in patient sera against 7000 human recombinant antigens (Figure 1). For the identification of novel RA biomarkers, a set of 417 antigens was selected from earlier studies in several autoimmune diseases like RA, SLE or SSc. The set of antigens was enzymatically citrullinated and also used in the unmodified form as control. In this study, the AAB-profiles of 76 serum samples of CCP+ and CCP- RA-patients with late-, early- or pre-RA were tested and compared to profiles of healthy volunteers and patients with early Arthritis (EA) (Table 1).

The analysis of 25 candidate antigens resulted in prominent differences in reactivity in RA patient samples and healthy controls with a higher reactivity of antibodies against citrullinated antigens (Figure 4) while reactivities against uncitrullinated antigens were not significant. In addition to known citrullinated antigens used as benchmarks (e.g. Fibrinogen (FGD) or Vimentin (VIM)) the novel antigens revealed a significant difference in AAB content in sera of RA patients (Table 3, Figure 3).

**Results**

Evaluation of the profiling data revealed significant reactivities for 25 novel antigens previously not described in RA. Activity was increased comparing both citrullinated vs. uncitrullinated and RA vs. HC. As expected, serum from patients with later stages of RA recognized a higher number of antigens (Mean = 3.3D of HC), demonstrating patient specific reactivity patterns. Patients with pre-RA also recognized on average 15 novel antigens.

**Frequency of the novel citrullinated antigen 1 was >25% in RA cohorts (69% lateRA, 36% early/RA, 27% preRA) compared to 3% in healthy controls or 2% in early arthritis patients. In contrast to that, antibodies against the respective uncitrullinated antigen could not be identified in a higher frequency in RA patient cohorts (Figure 4).**

In order to investigate the potential of novel citrullinated antigens to identify CCP-negative RA-patients the marker candidates have been analyzed in the subgroup of CCP-negative RA-patients (Table 4, Figure 5). AAB against citrullinated antigen 11 could be identified in 8-27% of CCP-negative samples of RA patients.

**Conclusion**

In this proof-of-concept SeroTag®-Screen using citrullinated human antigens 25 novel ACPA-candidates were discovered showing increased reactivity compared to non-citrullinated antigens. Using those novel ACPA an identification of early- or pre-RA patients might be possible based on increased presentation of diverse citrullinated peptide epitopes with better sensitivity and specificity. Furthermore, improved identification of ACPA in CCP-negative patients may reveal an additional diagnostic potential and might help to improve disease management for those patients.