Introduction

Autoantibodies against ACPAs (anti-citrullinated peptide/protein antibodies) show a diagnostic sensitivity of 62% and a specificity of up to 98% in established RA, whereas in early RA sensitivity of ACPA tests may be around 50%.

ACPA recognize specifically various citrullinated peptides that derive from different antigens. It has been demonstrated that autoantibodies to citrullinated peptides emerge at different stages of RA (Brink et al., 2013). Moreover, some CCP-negative RA-patients can be identified by analyzing additional citrullinated proteins or peptides (Lundberg et al., 2013). We hypothesize that autoantibodies in early RA require distinct protein sequences plus citrulline. In pre/early RA, a rather broad set of different citrullinated protein epitopes is therefore required (fine specificities). To develop a multi-epitope ACPA-test we set out to identify additional fine specificities using SeroTag screens with citrullinated proteins. Here we show validation of such novel ACPAs.

Methods

SeroTag® autoantibody profiling via the bead based Luminex FlexMAP3D® platform in over 14,000 patient samples has revealed numerous novel antigens in autoimmune indications like SSc, SLE, and RA. Whereas normal screens utilize up to 7 human proteins expressed in and affinity purified from E.coli, in RA we also use enzymatically citrullinated antigens as bait for autoantibodies. In several discovery studies 25 novel ACPAs were selected for further validation in additional early RA cohorts (Figure 2).

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. uncintrullinated and RA vs. HC. As expected, serum from patients with later stages of RA recognized a higher number of antigens (Mean ±SSD of HC), demonstrating patient specific reactivity patterns. Patients with pre-RA also recognised on average 15 novel antigens, albeit at a higher degree of diversity (fine specificities).

The graphical display 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 while reactivities against uncintrullinated antigens were not significant. In addition to known citrullinated antigens used as benchmarks (e.g. Fibronogen (FGB) or Vimentin (VIM)) the novel antigens revealed a significant difference in AAB content in sera of RA patients (Table 3, Figure 4).

Conclusions

SeroTag®-screens using citrullinated human antigens demonstrated 25 novel ACPA-candidates of which 3 were successfully validated in a large European Early RA cohort. Using those novel ACPAs 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 and by assessing both IgG and IgM. Furthermore, improved identification of ACPA in CCP-negative patients might reveal an additional diagnostic potential and might help to improve disease management for those patients.