Profiling the autoantibody repertoire in ANCA associated vasculitis with multiplex antigen arrays to predict relapse

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BACKGROUND

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic autoimmune disease characterized by the inflammation and destruction of small blood vessel causing organ damage and failure. The two major antigens targeted by ANCAs are proteinase 3 (PR3) and myeloperoxidase (MPO). A major unmet need in the disease management is a tool to predict which patients will suffer from relapses, and to select patients in whom immunosuppression may be stopped early. The aim of our study was therefore to profile the autoantibody repertoire in AAV patients at remission to identify novel autoantibodies capable to distinguish patients at high risk of relapses from those who remain in remission (Long-Term-Remission-Off-Therapy; LTROT).

RESULTS

- The overall reactivity showed similar number of reactive autoantibodies in AAV patients at remission classified as LTROT and relapsing (Fig. 1). In both groups, anti-MPO positive patients show a median number of reactive autoantibodies higher than anti-PR3 positive patients (non-significant; Fig. 2).

- AAV patients at remission classified as relapsers showed a higher prevalence of antibodies towards ATF3 (Activating Transcription Factor 3), MTTL6 (METhylTransferase-Like protein 6), KCNK4 (Potassium Channel Subfamily K Member), and bMERM-1 (bMERB Domain Containing 1) (Fig. 3).

- To our knowledge, none of the selected antigens has been previously reported in the context of AAV. However, ATF3 is a stress-induced transcription factor reported to be upregulated in presence of kidney injury, while KCNK4 is part of the potassium channel family which is reported to have a role in modulating B cells in Granulomatosis with Polyangiitis.

- Most of the samples show reactivity to more than one of the identified targets (Fig. 4)

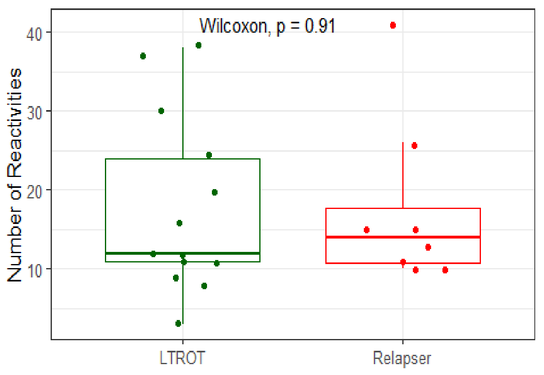


Fig 1. Total number of reactive autoantibodies per sample in LTROT and relapsing patients.

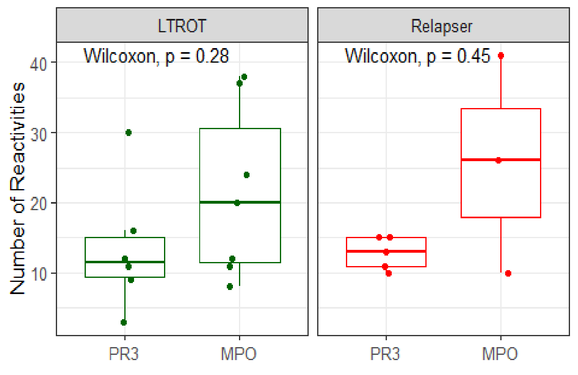


Fig 2. Total number of reactive autoantibodies per sample in LTROT and relapsing patients classified in anti-MPO+ and anti-PR3+.

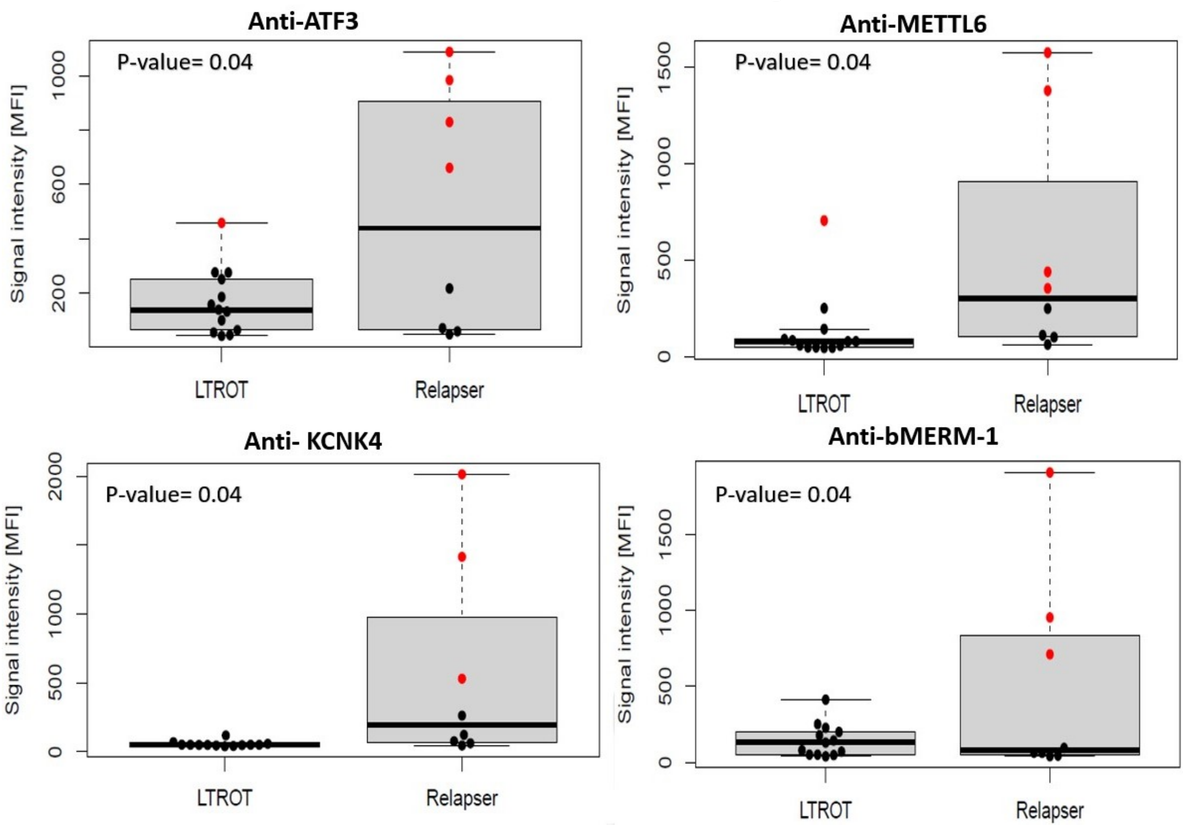


Fig 3. Intensity signals of the four autoantibody targets showing higher prevalence of reactivity in relapsing patients compared to LTROT at remission. In red are indicated the samples passing the cutoff for reactivity. P-values refer to Fisher´s exact test. MFI = Median Fluorescence Intensity.

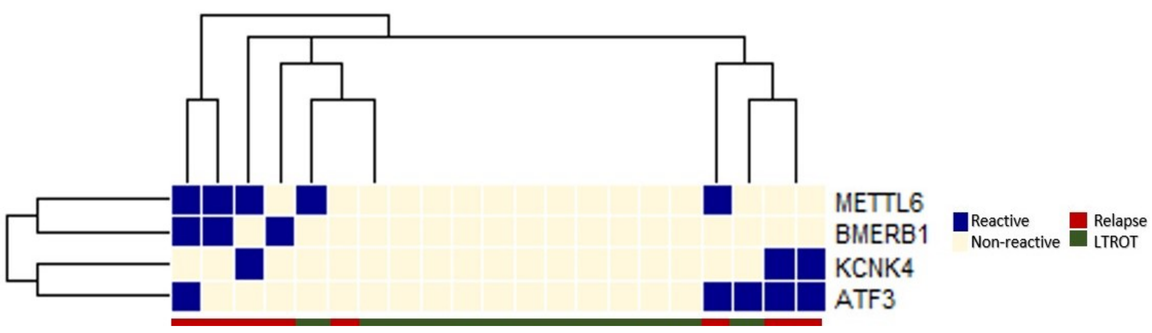
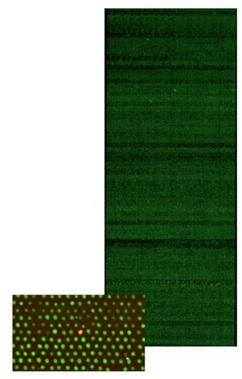


Fig 4. Heatmap representing the distribution of reactivities across the sample cohort.

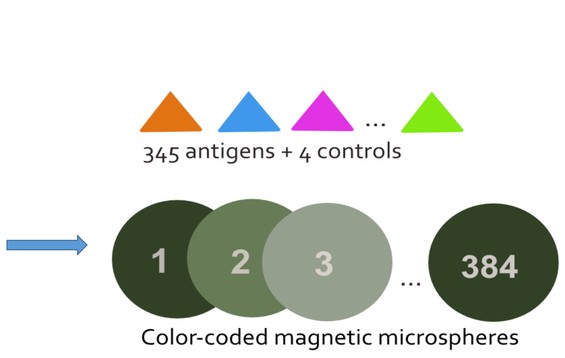
STUDY COHORT AND METHOD

Cohort	LTROT*	Relapse*
Total number	13	8
Age median [range]	53 [30-69]	53 [25-69]
Sex F/M	6/7	5/3
Autoantibodies MPO/PR3	7/6	3/5

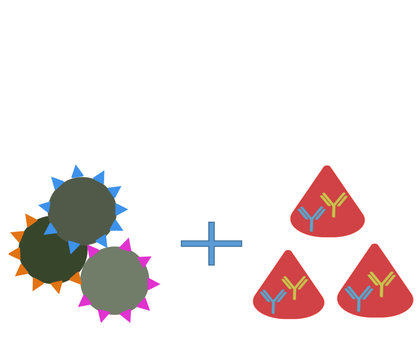
* AAV patients in remission



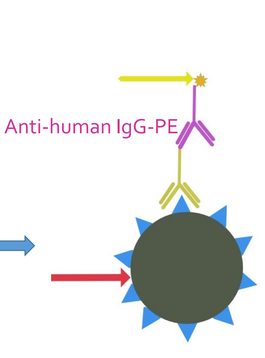
1. Proteome-wide screening of LTROT and Relapse sample pools on planar array (42000 antigen 19000 unique proteins)



2. Selection of interesting antigens and generation of a bead-based antigen array for verification



3.Incubation of plasma samples (RKD Biobank) with bead-based array



4. Detection and read-out by FlexMap3D system (Luminex)

CONCLUSION

We have identified four putative candidate autoantibodies that could be helpful in classifying AAV patients at high risk of long-term relapse. To our knowledge, these four protein targets were not previously reported in the context of AAV. The results will be validated in larger sample cohorts.

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SCAN ME

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