Validation of 3 Novel Biomarker Candidates for Systemic Sclerosis

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Introduction
Systemic sclerosis (SSc) is a systemic autoimmune disease that manifests as progressive fibrosis of the skin and internal organs. SSc is associated with the presence of several autoantibodies (aab) to intracellular targets, with the three most important SSc-specific being anti-centromere, anti-ScI70 and anti-RNA polymerase III antibodies, which occur in over 50% of SSc patients. Autoantibody specificities are strongly associated with pattern of organ involvement and disease outcome, making autoantibodies an essential tool in the clinical management of SSc. This highlights the need for additional specific and sensitive diagnostic and prognostic biomarkers in SSc. We have recently conducted high-content autoantibody profiling studies of SSc, systemic autoimmune diseases (AID), and healthy controls and found novel SSc associated autoantibodies.

Methods
Three novel identified biomarker candidates, namely Lysine (K)-specific demethylase 6B (KDM6B), Protein Phosphatase Inhibitor 2 (PPPIR2) and Bicaudal Drosophila Homolog 2 (BICD2) were developed into ELISA Kits using highly purified recombinant antigens and evaluated. Autoantibody specificities were analyzed using an independent and well characterized cohort containing sera of patients suffering from SSc (n=288), IIM (n=19), SJL (n=11), RA (n=19), SLE (n=40) and healthy blood donors (n=187). Assay threshold levels were calculated using a receiver operating characteristic analysis and set for specificities of around 95%.

Results
Using the respective cut-off for the ELISA tests we were able to find autoantibody reactivity against BICD2 in 32.6% of SSc patients and 4% of control samples. Anti-BICD2 and anti-PPPIR2 reactivities co-migrated with anti-centromere aab, but were also found in anti-centromere and anti-ScI-70 negative samples. Interestingly, both anti-BICD2 and anti-PPPIR2 aab were found in SSc samples tested negative for anti-RNA Polymerase III auto-reactivity (data not shown).

Optimized solid and liquid phase of the Multilisa PPP1R2 showed high diagnostic performance. In a reduced study cohort (n=196) Multilisa PPP1R2 reached a sensitivity of 20.6% and 94.4%, respectively.

When analyzed for Skin thickening measured by modified Rodnan Skin score (MRSS), anti-BICD2 reactivity was found elevated in patients with MRSS stages of 0 and 1 respectively, anti-BICD2 positive SSc patients seem to show a more moderate skin involvement.

Conclusion
In this study we were able to validate the diagnostic value and high specificity of the three newly discovered autoantigens using ELISA technology. Anti-BICD2 aab showed correlations with a more moderate disease course of SSc. Search for clinical associations of the other newly discovered SSc-associated autoantibodies is ongoing.