Development of a qualitative ELISA for the detection of anti-BICD2 autoantibodies in 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.1,2 We recently conducted autoantibody profiling studies in SSc using the Protagen SeroTag® process and found novel SSc-associated autoantibodies with the potential to improve the diagnosis of SSc and other systemic autoimmune diseases (SARD). Beyond the classical markers such as anti-centromere and anti-Scl-70 antibodies, which are generally exclusive of the other, novel autoantibodies were identified. Here, we report the development of the qualitative ELISA for the detection of human BICD2 autoantibodies. BICD2 (Protein bicaudal D homolog 2) is an evolutionarily conserved motor adaptor protein that is involved in dynein-mediated transport by linking the dynein motor complex to various cargoes.

Methods

We developed an ELISA using a highly purified recombinant fragment of human BICD2, the Protagen® MULTILISA® BICD2-ELISA. The assay principle is described in Fig. 1. Two lots of kit material were independently manufactured using two different lots of purified BICD2 protein. Quality control was performed with a quality control panel of 8 samples.

The MULTILISA® BICD2-ELISA was calibrated using receiver operating characteristics (ROC) analysis to 95% specificity.

Diagnostic performance

A mixed cohort consisting of SSc, SARD and healthy donor samples (n=394) were tested with the Protagen® MULTILISA® BICD2-ELISA. Data were analyzed using ROC analysis (Fig. 2).

Linearity analysis

Four positive samples were stepwise diluted with a negative sample and measured using the Protagen® MULTILISA® BICD2-ELISA. The calculated index (ratio from OD sample and OD substrate) was plotted over sample amount (Fig. 3). The samples show linear dilution behavior in a range between I=0.2 and I=4. This covers an OD range between 0.150 OD and 2.600 OD.

Added value

173 SSc-samples were analyzed with one lot of the Protagen® MULTILISA® BICD2 ELISA. SSc patients tested positive for anti-BICD2, anti-Scl-70 and anti-CENPB autoantibodies are shown in a Venn-Diagram (Fig. 3).

The prevalence of a-Scl70, a-CENPB and a-BICD2 autoantibodies were comparable in this cohort. The anti-BICD2 assay identifies 7 additional patients, who were tested negative for anti-Scl70 and anti-CENPB.

Verification data

Two lots of material were used to verify the assay and to determine the lot-to-lot consistency, intra- and inter-assay-variability, robustness against interference substances and the accelerated stability.

Lot-to-lot consistency

The preliminary results show high correlation of all results to ELISA (data not shown).

Intra-/inter-assay variance

Robustness against interference substances

Accelerated stability at 37°C

Assay portability studies

Test portability was verified using bead based assay, and Western blot / Line immunobossay studies. The preliminary results show high correlation of all results to ELISA (data not shown).

Conclusion

We developed a qualitative ELISA for detecting anti-BICD2 autoantibodies, which occur in 30% of SSc patients. The assay was validated by analyzing SSc samples and SARD control samples. The availability of a validated, qualitative BICD2-ELISA may improve the specific detection of SSc and reduce the diagnostic gap. Verified RuO-Kits of the Protagen® MULTILISA® BICD2-ELISA will be available in November 2015.

References