Utility of Autoantibodies as Biomarkers in a Well Characterised Australian Systemic Sclerosis (Scleroderma) Cohort

Background

Systemic Sclerosis is a clinically heterogeneous systemic autoimmune disease of unknown aetiology. Autoantibodies (AAs) are present in >95% of patients. Three AAs were originally considered to be highly associated with SSc; Centromere protein (CENP A or CENP B), Topoisomerase1 (Topo1) and RNA Polymerase III (RNAP3) and all were closely linked with distinct clinical manifestations. Initially it was thought that AAs were mutually exclusive and patients expressed only a single AA, however more recent technologies have demonstrated that multiple AAs can be expressed in a single patient and that other serum AAs are associated with SSc. Some of these later AAs were only available in a research setting, with their clinical associations and frequencies obscure.

Further uncertainties regarding AA's in scleroderma include the relevance of multiple AA positivity, and that of AA negative SSc. Lastly, the 2013 ACR/EULAR classification criteria for SSc showed improved diagnostic validity, but did not encompass sub-classification nor provide prognostication. Improved biomarkers for SSc subsets are sorely needed.

Aim

To determine the relationships between SSc related autoantibodies including their clinical associations in a large and well-characterized Australian patient cohort using a single diagnostic platform to detect multiple AAs.

Hypothesis

Important relationships between AAs and their clinical associations will identify and stratify AAs into clinically homogeneous subgroups.

Method

The (Euroimmun) line immunoblot assay (LIA) was used to characterise antibodies to CENP-A, CENP-B, RNAP3; epitopes 11 and 155, Topo I, NOR-90, Fibrillarin, Th/To, PM/Scl-75, PM/Scl-100, Ku, TRIM21/Ro52, and PDGFR in 505 Australian SSc sera. Supplementary LIA testing of U1RNP was also performed in selected patients.

Statistical Analyses

Patient subgroups were identified by hierarchical clustering in a principal components analysis (PCA) of quantitative autoantibody scores. Results were compared with detailed clinical data.

Results

A total of 449/505 patients were positive for at least 1 AA by LIA. Heatmap visualization of AA scores, along with PCA clustering, demonstrated strong, mutually exclusive relationships between CENP, Topo I and RNAP3. Five patient clusters were identified: CENP, RNAP3 strong, RNAP3 weak, Topo I, and 'Other'. Clinical features associated with CENP, RNAP3, and Topo I were consistent with previously published reports concerning lcSSc and dcSSc. A novel finding was the statistical separation of RNAP3 into two clusters. Patients in RNAP3 strong cluster had an increased risk of gastric antral vascular ectasia, but a lower risk of oesophageal dysmotility. Additional PCA of Cluster 4 revealed that Topo1 and CENP maintained their clinical influence even at reduced LIA staining intensity with co-expressed CENP/Topo1 patient phenotype resembling Topo1 with minimal CENP influence. A statistically significant presence of males in this and the AA negative subgroup. The AA negative subgroup phenotype was more fibrotic and less vasculopathic. Clinical associations for TRIM21 included older age at disease onset and a tendency towards ILD. SSc positive U1RNP patients and U1RNP MCTD were different, the latter having a milder phenotype.

Conclusion

Five major autoantibody clusters with specific clinical and serologic associations were identified in Australian SSc patients. Sub-classification and disease stratification using autoantibodies may have clinical utility, particularly in early disease.