Review

Towards the identification of novel autoantibodies in Sjögren's syndrome

T. Witte[1], F. Engelke[1], J. Ritter[2], T. Dörner[2], S. De Vita[3], A.V. Goules[4], A.G. Tzioufas[4]

[1]*Klinik für Rheumatologie und Immunologie, Medical School Hannover, Germany;*
[2]*Department of Medicine and Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, and German Rheumatism Research Centre (DRFZ), Berlin, Germany;*
[3]*Clinica di Reumatologia, DPMSC, Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine, Italy;*
[4]*Department of Pathophysiology, School of Medicine, National University of Athens, Greece.*

Torsten Witte, MD
Fiona Engelke
Jacob Ritter, cand med.
Thomas Dörner, MD
Salvatore De Vita, MD
Andreas V. Goules, MD
Athanasios G. Tzioufas, MD

Please address correspondence to:
Torsten Witte,
Klinik für Rheumatologie und Immunologie,
Medical School Hannover,
Carl-Neuberg-Str. 1,
30625 Hannover, Germany.
E-mail: witte.torsten@mh-hannover.de

Received on May 29, 2022; accepted in revised form on July 11, 2022.

Clin Exp Rheumatol 2022; 40: 2395-2397.

Key words: Sjögren'syndrome, HarmonicSS, cluster, autoantibodies

Competing interests: T. Witte has received honoraria from AbbVie, Aesku, Chugai, Galapagos, GSK, Janssen, Lilly, Medac, Novartis, Pfizer, Roche, UCB and Astra Zeneca. T. Dörner has received study support and honoraria for scientific advice from Eli Lilly, Janssen, Novartis, Roche, UCB, Sanofi and BMS. The other authors have declared no competing interests.

ABSTRACT

Primary Sjögren's syndrome may be difficult to diagnose when antibodies against Ro/SSA are lacking, and can be grouped in at least four clusters indicating different pathophysiological pathways. Novel biomarkers, in particular autoantibodies, would be helpful in diagnosing Sjögren's syndrome and in further identification and characterisation of the clusters.

In this review, we describe new technologies that may be utilised in the rapid identification of novel autoantibodies, and an example of how well characterised patients, here from the HarmonicSS cohort, are a prerequisite in the discovery of clinically meaningful biomarkers. This translational approach hold promise to optimise the diagnosis and treatment of individual pSS patient subsets.

Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disorder characterised by a typical histology including lymphocytic infiltration of the salivary and lachrymal glands and, as a consequence of that, oral and ocular dryness. The prevalence of pSS, which is affecting mostly middle-aged women, has been estimated to be in the range between more than 1% (1) and 0.1% (2), respectively. However, it may be even more common, since many of the patients do not complain of dry eyes and mouth symptoms and then are not likely to be diagnosed (3).

In addition to that, there are further challenges in the management of pSS. Diagnosing pSS can be difficult in patients without the most important laboratory marker antibodies against Ro/SSA, which is found in approximately 40-68% of the patients (4).

PSS is not a uniform disease, but subsets of the disease have been described. The most important differentiation is disease limited to glandular and extraglandular disease manifestations. Furthermore, four clusters were characterised based on transcriptomic, genomic, epigenetic, cytokine expression and flow cytometry data, combined with clinical parameters (5). The latter discovery suggests, that the pathomechanisms leading to pSS may differ. As a consequence, individualised therapeutic strategies taking the potential distinct pathomechanisms into account may hold promise to improve diagnostics and outcome.

Therefore, novel biomarkers are needed that help to identify pSS subsets among Ro/SSA negative patients, which were found among subclusters of pSS and/or predicted the response to immunomodulatory therapies. Autoantibodies appear to be attractive for that purpose since they are easy to measure and rheumatologists are familiar with their interpretation.

In order to identify and characterise such novel autoantibodies, two major advances have recently been made:

1. A huge very well characterised pSS cohort with data from 7551 patients has been established in the "HARMONIsation and integrative analysis of regional, national and international Cohorts on primary Sjögren's syndrome" (HarmonicSS) consortium (6, 7).
2. Technologies for the fast screening of huge numbers of potential autoantibodies have been developed.

HarmonicSS

In this project funded by the European Union 21 clinical partners from the largest Sjögren's centres in Europe as

well as 13 technical partners, joined and established a database including 7551 patients. This database is unique, since the data of the patients was harmonised to ensure maximal quality. Currently, information on clinical manifestations, treatment, laboratory parameters as well as demographic parameters are available. The members of the consortium can be contacted and asked to provide biomaterials of the patients in order to participate, including the search for novel biomarkers (7).

New technologies for the identification of autoantibodies

The search for new autoantibodies has been greatly facilitated by technological advances over the past 20 years. Previously, the targets of new autoantibodies were often identified at length by incubating sera from patients with cell lysates and analysing the proteins bound to antibodies by Western blot procedures followed by mass spectroscopy. Today, a wealth of new technologies is available that permit rapid identification of various novel autoantibodies. Examples of such technologies include:

- Phage immunoprecipitation sequencing (PhIP seq)

In this method, all 413,000 human 36-amino acid peptides are expressed on bacteriophages (8). The bacteriophages are placed on plates on which a patient's antibodies are immobilised. Unbound bacteriophages are then washed away, but the peptides of bound bacteriophages can be analysed. Antibodies against "cytosolic 5′-nucleosidase 1A" (cN1A), among others, have been identified as markers of inclusion body myositis using this rapid screening technology (9).

- Protein arrays

In our research group we use protein arrays, *i.e.* membranes on which not peptides but almost all human proteins are immobilised. The membranes are incubated with sera from patients. Antibodies bound to the proteins are visualised by labelled antibodies against human IgG and the autoantigens can be identified by their position on the array. We have used such arrays to identify antibodies to ferritin in giant cell arteritis and to CD74 in axial spondyloarthritis, among others (10, 11). Subsequently, ELISA assays were developed in which only the relevant antigen is coupled to the plates. Using these ELISAs, we demonstrated that antibodies against CD74 are present in approximately 50% of patients with axial spondyloarthritis and are present at symptom onset. In addition, they are associated with more rapid radiographic progression of the disease (12).

However, the methodology of screening with protein arrays also has disadvantages: it is expensive, costing approximately 1,000 Euros per serum examined. In addition, "natural" antibodies are detected, because healthy individuals also have antibodies against various intracellular proteins. Therefore, the examination of a larger number of blood donors is necessary to validate whether a protein bound by antibodies of a patient is associated with a disease or is frequently recognised by antibodies of healthy individuals.

Another disadvantage of the above technologies is that the antigens are immobilised and thus lose their actual conformation. However, most autoantibodies are not directed against small linear peptide sequences, but recognise multiple peptide sequences that are adjacent to each other in the conformation of the folded protein.

In other methods suitable for screening a wide range of proteins, very large numbers of antigens are coupled to beads labelled differently depending on the antigen and then incubated with sera from patients. The proteins are thus preserved in their original conformation. Bound antibodies can be detected by a labelled secondary antibody to human immunoglobulins, and antigens bound by autoantibodies can be detected by labelling the beads to which they are bound. In large screening procedures involving 7000 different proteins, over 50 novel autoantibodies have been identified in systemic lupus erythematosus (SLE), systemic sclerosis, and Sjögren's syndrome (13). We have coupled these potential novel autoantigens that were produced in an *E.coli* expression system to Luminex beads and measured the binding of IgG antibodies to these coupled antigens on a Luminex 200, which can potentially measure up to 80 analytes simultaneously. For confirmation of the previous results (13), we analysed sera of 171 pSS patients from Hannover with a mean age of 58 years (Table I), 72 from Berlin and 36 from Udine, and compared them to sera obtained from 123 blood donors from Hannover. In the analysis, 11 novel autoantibodies were significantly associated with pSS in addition to autoantibodies against Ro60 (TROVE), Ro52 (TRIM21) and La/SSB. These 11 novel markers were found in 4% to 11% of the pSS cohort and in only 0-1.6% among blood donors. At least one of the novel autoantibodies was present in 30% of the pSS patients without antibodies against Ro60, Ro52 and La with a specificity of >95%.

Table I. Characteristics of the pSS patients from Hannover.

Feature	Proportion of patients with feature
Female	87%
Ro/SSA antibodies	66%
La/SSB antibodies	30%
Rheumatoid factor	34%
Articular involvement	39%
pulmonary involvement	19%
Renal involvement	6%
Involvement of peripheral nervous system	22%
Involvement of central nervous system	6%

The organ involvements were defined according to the descriptions in the ESSDAI (14).

Since the pSS patients have been included in the HarmonicSS database, a wealth of their clinical data including all the manifestations defined in the ESSDAI (14) was available and could be correlated with the presence of the novel autoantibodies. Two of the novel autoantibodies were associated with polyneuropathy and a further two with interstitial lung disease (ILD).

Conclusion

Technological advances have simplified the discovery of novel autoantibodies. Even more important, the establishment of the HarmonicSS cohort

has created the opportunity to measure novel antibodies in very well characterised pSS patients. We can now use data and sera of additional patients in order to validate, whether or not, the novel antibodies are found across different areas of Europe at similar frequencies and if there are certain organ manifestations associated. If the current results can be confirmed, we will be able to provide new markers helpful to diagnose patients without Ro/SSA antibodies. Furthermore, if the association of novel autoantibodies and organ manifestations such as polyneuropathy and ILD can be confirmed in the overall cohort, clinicians will have a new tool to diagnose pSS manifestations or to predict the appearance of organ manifestation in the follow-up.

References

1. THOMAS E, HAY EM, HAJEER A, SILMAN AJ: Sjögren's syndrome: a community-based study of prevalence and impact. *Br J Rheumatol* 1998; 37: 1069-76. https://doi.org/10.1093/rheumatology/37.10.1069
2. TRONTZAS PI, ANDRIANAKOS AA: Sjögren's syndrome: a population-based study of prevalence in Greece. The ESORDIG study. *Ann Rheum Dis* 2005; 64: 1240-1. http://dx.doi.org/10.1136/ard.2004.031021
3. HAY EM, THOMAS E, PAL B, HAJEER A, CHAMBERS H, SILMAN AJ: Weak association between subjective symptoms or and objective testing for dry eyes and dry mouth: results from a population-based study. *Ann Rheum Dis* 1998; 57: 20-4. https://doi.org/10.1136/ard.57.1.20.
4. BALDINI C, PEPE P, QUARTUCCIO L *et al*.: Primary Sjögren's syndrome as a multi-organ disease: impact of the serological profile on the clinical presentation of the disease in a large cohort of Italian patients. *Rheumatology* 2014; 53: 839-44. https://doi.org/10.1093/rheumatology/ket427
5. SORET P, LE DANTEC C, DESVAUX E *et al*.: A new molecular classification to drive precision treatment strategies in primary Sjögren's syndrome. *Nat Commun* 2021; 12: 3523. https://doi.org/10.1038/s41467-021-23472-7
6. PEZOULAS VC, GOULES A, KALATZIS F *et al*.: Addressing the clinical unmet needs in primary Sjögren's syndrome through the sharing, harmonization and federated analysis of 21 European cohorts. *Comput Struct Biotechnol J* 2022; 20: 471-84. https://doi.org/10.1016/j.csbj.2022.01.002
7. GOULES AV, EXARCHOS TP, FOTIADIS DI, TZIOUFAS A: The clinical and technical impact of the HarmonicSS project. *Clin Exp Rheumatol* 2021; 39 (Suppl. 133): S17-9. https://doi.org/10.55563/clinexprheumatol/u7knfy
8. LARMAN HB, ZHAO Z, LASERSON U *et al*.: Autoantigen discovery with a synthetic human peptidome. *Nat Biotechnol* 2011; 29: 535-41. https://doi.org/10.1038/nbt.1856
9. SALAJEGHEH M, LAM T, GREENBERG SA: Autoantibodies against a 43 KDa muscle protein in inclusion body myositis. *PLoS One* 2011; 6: e20266. https://doi.org/10.1371/journal.pone.0020266
10. BAERLECKEN NT, LINNEMANN A, GROSS WL *et al*.: Association of ferritin autoantibodies with giant cell arteritis/polymyalgia rheumatica. *Ann Rheum Dis* 2012; 71: 943-7. http://dx.doi.org/10.1136/annrheumdis-2011-200413
11. RIECHERS E, BAERLECKEN N, BARALIAKOS X *et al*.: Sensitivity and specificity of autoantibodies against CD74 in nonradiographic axial spondyloarthritis. *Arthritis Rheumatol* 2019; 71: 729-35. https://doi.org/10.1002/art.40777
12. WITTE T, KÖHLER M, GEORGI J *et al*.: IgA antibodies against CD74 are associated with structural damage in the axial skeleton in patients with axial spondyloarthritis. *Clin Exp Rheumatol* 2020; 38: 1127-31.
13. BUDDE P, ZUCHT HD, VORDENBÄUMEN S *et al*.: Multiparametric detection of autoantibodies in systemic lupus erythematosus. *Lupus* 2016; 25: 812-22. https://doi.org/10.1177/0961203316641770
14. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjögren's syndrome. *Ann Rheum Dis* 2010; 69: 1103-9. https://doi.org/10.1136/ard.2009.110619