NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

SCHOOL OF HEALTH SCIENCES

DEPARTMENT OF MEDICINE

SECTION OF BASIC SCIENCES
DEPARTMENT OF PHYSIOLOGY

Director: Academic - Professor George Kollias

DOCTORAL THESIS

Prevalence and clinical implications of Scleroderma-specific autoantibodies in Sjögren's syndrome

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Doctoral Thesis of candidate Nikolaos Marketos in the Medical School of the National and Kapodistrian University of Athens, Greece, on the subject:

"Prevalence and clinical implications of Scleroderma-specific autoantibodies in Sjögren's syndrome"

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Three-member Advisory Committee:

1. Supervisor: Associate Professor Clio P. Mavragani

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The thesis approval from the Medical School of the University of Athens does not constitute acceptance of the opinions of the author (Organization University of Athens, Article 202 of Law 5343)

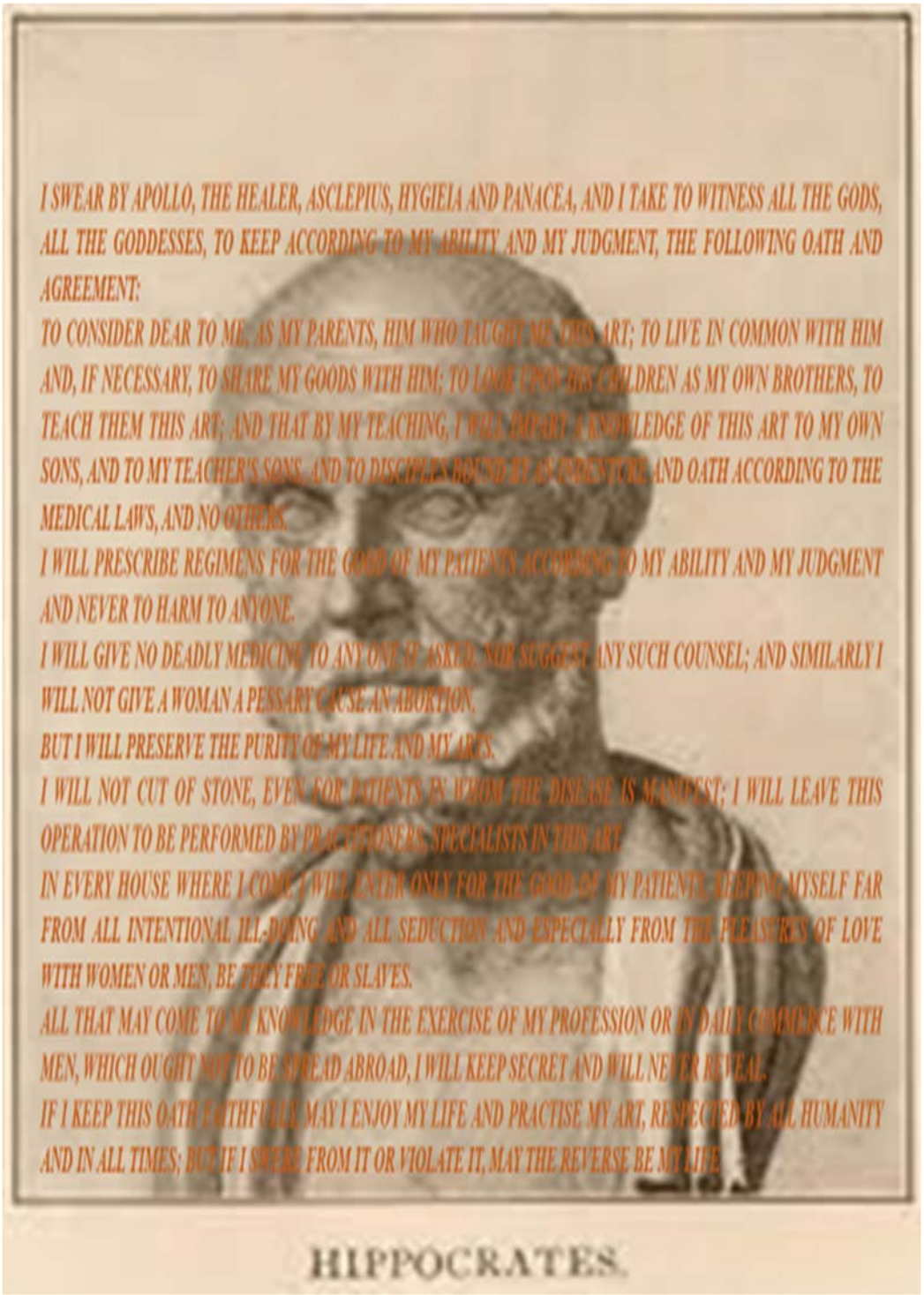
Αφιερώνεται με αγάπη και εκτίμηση:

Στους γονείς μου, που με στήριξαν στις επιλογές μου

Στη σύζυγό μου Νεκταρία, για τις πολύτιμες συμβουλές της στην εκπόνηση της Διατριβής και τη συμπαράστασή της

Στο Μάκη και τη Χριστινέλλα για την υπομονή και την αγάπη τους

OATH OF HIPPOCRATES



ΙΠΠΟΚΡΑΤΕΙΟΣ ΟΡΚΟΣ

ΟΜΝΥΜΙ ΑΠΟΛΛΩΝΑ ΙΗΤΡΟΝ, ΚΑΙ ΑΣΚΛΗΠΙΟΝ, ΚΑΙ ΥΓΕΙΑΝ, ΚΑΙ ΠΑΝΑΚΕΙΑΝ, ΚΑΙ ΘΕΟΥΣ ΠΑΝΤΑΣ ΤΕ ΚΑΙ ΠΑΣΑΣ, ΙΣΤΟΡΑΣ ΠΟΙΕΥΜΕΝΟΣ, ΕΠΙΤΕΛΕΑ ΠΟΙΗΣΕΙΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ ΟΡΚΟΝ ΤΟΝΔΕ ΚΑΙ ΞΥΓΓΡΑΦΗΝ ΤΗΝΔΕ. ΗΓΗΣΑΣΘΑΙ ΜΕΝ ΤΟΝ ΔΙΔΑΞΑΝΤΑ ΜΕ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ ΙΣΑ ΓΕΝΕΤΗΣΙΝ ΕΜΟΙΣΙ, ΚΑΙ ΒΙΟΥ ΚΟΙΝΩΣΑΣΘΑΙ, ΚΑΙ ΧΡΕΩΝ ΧΡΗΙΖΟΝΤΙ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΑΣΘΑΙ, ΚΑΙ ΓΕΝΟΣ ΤΟ Ἐξ ΩΥΤΕΟΥ ΑΔΕΛΦΟΙΣ ΙΣΟΝ ΕΠΙΚΡΙΝΕΕΙΝ ΑΡΡΕΣΙ, ΚΑΙ ΔΙΔΑΞΕΙΝ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ, ΗΝ ΧΡΗΙΖΩΣΙ ΜΑΝΘΑΝΕΙΝ, ΑΝΕΥ ΜΙΣΘΟΥ ΚΑΙ ΞΥΓΓΡΑΦΗΣ, ΠΑΡΑΓΓΕΛΙΗΣ ΤΕ ΚΑΙ ΑΚΡΟΗΣΙΟΣ ΚΑΙ ΤΗΣ ΛΟΙΠΗΣ ΑΠΑΣΗΣ ΜΑΘΗΣΙΟΣ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΑΣΘΑΙ ΥΙΟΙΣΙ ΤΕ ΕΜΟΙΣΙ, ΚΑΙ ΤΟΙΣΙ ΤΟΥ ΕΜΕ ΔΙΔΑΞΑΝΤΟΣ, ΚΑΙ ΜΑΘΗΤΑΙΣΙ ΣΥΓΓΕΓΡΑΜΜΕΝΟΙΣΙ ΤΕ ΚΑΙ ΩΡΚΙΣΜΕΝΟΙΣ ΝΟΜΩ ΙΗΤΡΙΚΩ, ΑΛΛΩ ΔΕ ΟΥΔΕΝΙ. ΔΙΑΙΤΗΜΑΣΙ ΤΕ ΧΡΗΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ ΚΑΜΝΟΝΤΩΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ, ΕΠΙ ΔΗΛΗΣΕΙ ΔΕ ΚΑΙ ΑΔΙΚΙΗ ΕΙΡΞΕΙΝ. ΟΥ ΔΩΣΩ ΔΕ ΟΥΔΕ ΦΑΡΜΑΚΟΝ ΟΥΔΕΝΙ ΑΙΤΗΘΕΙΣ ΘΑΝΑΣΙΜΟΝ, ΟΥΔΕ ΥΦΗΓΗΣΟΜΑΙ ΞΥΜΒΟΥΛΙΗΝ ΤΟΙΗΝΔΕ. ΟΜΟΙΩΣ ΔΕ ΟΥΔΕ ΓΥΝΑΙΚΙ ΠΕΣΣΟΝ ΦΘΟΡΙΟΝ ΔΩΣΩ. ΑΓΝΩΣ ΔΕ ΚΑΙ ΟΣΙΩΣ ΔΙΑΤΗΡΗΣΩ ΒΙΟΝ ΤΟΝ ΕΜΟΝ ΚΑΙ ΤΕΧΝΗΝ ΤΗΝ ΕΜΗΝ. ΟΥ ΤΕΜΕΩ ΔΕ ΟΥΔΕ ΜΗΝ ΛΙΘΙΩΝΤΑΣ, ΕΚΧΩΡΗΣΩ ΔΕ ΕΡΓΑΤΗΣΙΝ ΑΝΔΡΑΣΙ ΠΡΗΞΙΟΣ ΤΗΣΔΕ. ΕΣ ΟΙΚΙΑΣ ΔΕ ΟΚΟΣΑΣ ΑΝ ΕΣΙΩ, ΕΣΕΛΕΥΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ ΚΑΜΝΟΝΤΩΝ, ΕΚΤΟΣ ΩΝ ΠΑΣΗΣ ΑΔΙΚΙΗΣ ΕΚΟΥΣΙΗΣ ΚΑΙ ΦΘΟΡΙΗΣ, ΤΗΣ ΤΕ ΑΛΛΗΣ ΚΑΙ ΑΦΡΟΔΙΣΙΩΝ ΕΡΓΩΝ ΕΠΙ ΤΕ ΓΥΝΑΙΚΕΙΩΝ ΣΩΜΑΤΩΝ ΚΑΙ ΑΝΔΡΩΩΝ, ΕΛΕΥΘΕΡΩΝ ΤΕ ΚΑΙ ΔΟΥΛΩΝ. Α Δ' ΑΝ ΕΝ ΘΕΡΑΠΕΙΗ Η ΙΔΩ, Η ΑΚΟΥΣΩ, Η ΚΑΙ ΑΝΕΥ ΘΕΡΑΠΗΙΗΣ ΚΑΤΑ ΒΙΟΝ ΑΝΘΡΩΠΩΝ, Α ΜΗ ΧΡΗ ΠΟΤΕ ΕΚΛΑΛΕΕΣΘΑΙ ΕΞΩ, ΣΙΓΗΣΟΜΑΙ, ΑΡΡΗΤΑ ΗΓΕΥΜΕΝΟΣ ΕΙΝΑΙ ΤΑ ΤΟΙΑΥΤΑ. ΟΡΚΟΝ ΜΕΝ ΟΥΝ ΜΟΙ ΤΟΝΔΕ ΕΠΙΤΕΛΕΑ ΠΟΙΕΟΝΤΙ, ΚΑΙ ΜΗ ΞΥΓΧΕΟΝΤΙ, ΕΙΗ ΕΠΑΥΡΑΣΘΑΙ ΚΑΙ ΒΙΟΥ ΚΑΙ ΤΕΧΝΗΣ ΔΟΞΑΖΟΜΕΝΩ ΠΑΡΑ ΠΑΣΙΝ ΑΝΘΡΩΠΟΙΣ Ἐς ΤΟΝ ΑΙΕΙ ΧΡΟΝΟΝ. ΠΑΡΑΒΑΙΝΟΝΤΙ ΔΕ ΚΑΙ ΕΠΙΟΡΚΟΥΝΤΙ, ΤΑΝΑΝΤΙΑ ΤΟΥΤΕΩΝ.

ΙΠΠΟΚΡΑΤΕΙΟΣ ΟΡΚΟΣ

(απόδοση στη Νεοελληνική)

ΟΡΚΙΖΟΜΑΙ ΣΤΟΝ ΑΠΟΛΛΩΝΑ ΤΟΝ ΙΑΤΡΟ ΚΑΙ ΣΤΟΝ ΑΣΚΛΗΠΙΟ ΚΑΙ ΣΤΗΝ ΥΓΕΙΑ ΚΑΙ ΣΤΗΝ ΠΑΝΑΚΕΙΑ ΚΑΙ Σ΄ ΟΛΟΥΣ ΤΟΥΣ ΘΕΟΥΣ ΕΠΙΚΑΛΟΥΜΕΝΟΣ ΤΗ ΜΑΡΤΥΡΙΑ ΤΟΥΣ, ΝΑ ΤΗΡΗΣΩ ΠΙΣΤΑ ΚΑΤΆ ΤΗ ΔΥΝΑΜΗ ΚΑΙ ΤΗΝ ΚΡΙΣΗ ΜΟΥ ΑΥΤΟ ΤΟΝ ΟΡΚΟ ΚΑΙ ΤΟ ΣΥΜΒΟΛΑΙΟ ΜΟΥ ΑΥΤΟ. ΝΑ ΘΕΩΡΩ ΑΥΤΟΝ ΠΟΥ ΜΟΥ ΔΙΔΑΞΕ ΑΥΤΗ ΤΗΝ ΤΕΧΝΗ ΙΣΟ ΜΕ ΤΟΥΣ ΓΟΝΕΙΣ ΜΟΥ ΚΑΙ ΝΑ ΜΟΙΡΑΣΤΩ ΜΑΖΙ ΤΟΥ ΤΑ ΥΠΑΡΧΟΝΤΑ ΜΟΥ ΚΑΙ ΤΑ ΧΡΗΜΑΤΑ ΜΟΥ ΑΝ ΕΧΕΙ ΑΝΑΓΚΗ ΦΡΟΝΤΙΔΑΣ. ΝΑ ΘΕΩΡΩ ΤΟΥΣ ΑΠΟΓΟΝΟΥΣ ΤΟΥ ΙΣΟΥΣ ΜΕ Τ΄ ΑΔΕΛΦΙΑ ΜΟΥ ΚΑΙ ΝΑ ΤΟΥΣ ΔΙΔΑΞΩ ΤΗΝ ΤΕΧΝΗ ΑΥΤΗ ΑΝ ΘΕΛΟΥΝ ΝΑ ΤΗ ΜΑΘΟΥΝ, ΧΩΡΙΣ ΑΜΟΙΒΗ ΚΑΙ ΣΥΜΒΟΛΑΙΟ ΚΑΙ ΝΑ ΜΕΤΑΔΩΣΩ ΜΕ ΠΑΡΑΓΓΕΛΙΕΣ, ΟΔΗΓΙΕΣ ΚΑΙ ΣΥΜΒΟΥΛΕΣ ΟΛΗ ΤΗΝ ΥΠΟΛΟΙΠΗ ΓΝΩΣΗ ΜΟΥ ΚΑΙ ΣΤΑ ΠΑΙΔΙΑ ΜΟΥ ΚΑΙ ΣΤΑ ΠΑΙΔΙΑ ΕΚΕΙΝΟΥ ΜΕ ΔΙΔΑΞΕ ΚΑΙ ΣΤΟΥΣ ΑΛΛΟΥΣ ΜΑΘΗΤΕΣ ΠΟΥ ΕΧΟΥΝ ΚΑΝΕΙ ΓΡΑΠΤΗ ΣΥΜΦΩΝΙΑ ΜΑΖΙ ΜΟΥ ΚΑΙ Σ΄ ΑΥΤΟΥΣ ΠΟΥ ΕΧΟΥΝ ΟΡΚΙΣΘΕΙ ΣΤΟΝ ΙΑΤΡΙΚΟ ΝΟΜΟ ΚΑΙ ΣΕ ΚΑΝΕΝΑΝ ΑΛΛΟ ΚΑΙ ΝΑ ΘΕΡΑΠΕΥΩ ΤΟΥΣ ΠΑΣΧΟΝΤΕΣ ΚΑΤΑ ΤΗ ΔΥΝΑΜΗ ΜΟΥ ΚΑΙ ΤΗΝ ΚΡΙΣΗ ΜΟΥ ΧΩΡΙΣ ΠΟΤΕ, ΕΚΟΥΣΙΩΣ, ΝΑ ΤΟΥΣ ΒΛΑΨΩ Ή ΝΑ ΤΟΥΣ ΑΔΙΚΗΣΩ. ΚΑΙ ΝΑ ΜΗ ΔΩΣΩ ΠΟΤΕ ΣΕ ΚΑΝΕΝΑ, ΕΣΤΩ ΚΙ ΑΝ ΜΟΥ ΤΟ ΖΗΤΗΣΕΙ, ΘΑΝΑΤΗΦΟΡΟ ΦΑΡΜΑΚΟ, ΟΥΤΕ ΝΑ ΔΩΣΩ ΠΟΤΕ ΤΕΤΟΙΑ ΣΥΜΒΟΥΛΗ. ΟΜΟΙΩΣ ΝΑ ΜΗ ΔΩΣΩ ΠΟΤΕ ΣΕ ΓΥΝΑΙΚΑ ΦΑΡΜΑΚΟ ΓΙΑ Ν΄ ΑΠΟΒΑΛΕΙ. ΝΑ ΔΙΑΤΗΡΗΣΩ ΔΕ ΤΗ ΖΩΗ ΜΟΥ ΚΑΙ ΤΗΝ ΤΕΧΝΗ ΜΟΥ ΚΑΘΑΡΗ ΚΑΙ ΑΓΝΗ. ΚΑΙ ΝΑ ΜΗ ΧΕΙΡΟΥΡΓΗΣΩ ΠΑΣΧΟΝΤΕΣ ΑΠΟ ΛΙΘΟΥΣ ΑΛΛΑ Ν΄ ΑΦΗΣΩ ΤΗΝ ΠΡΑΞΗ ΑΥΤΗ ΓΙΑ ΤΟΥΣ ΕΙΔΙΚΟΥΣ. ΚΑΙ Σ΄ ΟΠΟΙΑ ΣΠΙΤΙΑ ΚΙ ΑΝ ΜΠΩ, ΝΑ ΜΠΩ ΓΙΑ ΤΗΝ ΩΦΕΛΕΙΑ ΤΩΝ ΠΑΣΧΟΝΤΩΝ ΑΠΟΦΕΥΓΟΝΤΑΣ ΚΑΘΕ ΕΚΟΥΣΙΑ ΑΔΙΚΙΑ ΚΑΙ ΒΛΑΒΗ ΚΑΙ ΚΑΘΕ ΓΕΝΕΤΗΣΙΑ ΠΡΑΞΗ ΚΑΙ ΜΕ ΓΥΝΑΪΚΕΣ ΚΑΙ ΜΕ ΑΝΔΡΕΣ, ΕΛΕΥΘΕΡΟΥΣ ΚΑΙ ΔΟΥΛΟΥΣ. ΚΑΙ Ο,ΤΙ ΔΩ Ή ΑΚΟΥΣΩ ΚΑΤΑ ΤΗΝ ΑΣΚΗΣΗ ΤΟΥ ΕΠΑΓΓΕΛΜΑΤΟΣ ΜΟΥ, Ή ΚΙ ΕΚΤΟΣ, ΓΙΑ ΤΗ ΖΩΗ ΤΩΝ ΑΝΘΡΩΠΩΝ, ΠΟΥ ΔΕΝ ΠΡΕΠΕΙ ΠΟΤΕ ΝΑ ΚΟΙΝΟΠΟΙΗΘΕΙ, ΝΑ ΣΙΩΠΗΣΩ ΚΑΙ ΝΑ ΤΟ ΤΗΡΗΣΩ ΜΥΣΤΙΚΟ. ΑΝ ΤΟΝ ΟΡΚΟ ΜΟΥ ΑΥΤΟ ΤΗΡΗΣΩ ΠΙΣΤΑ ΚΑΙ ΔΕΝ ΤΟΝ ΑΘΕΤΗΣΩ, ΕΙΘΕ Ν΄ ΑΠΟΛΑΥΣΩ ΓΙΑ ΠΑΝΤΑ ΤΗΝ ΕΚΤΙΜΗΣΗ ΟΛΩΝ ΤΩΝ ΑΝΘΡΩΠΩΝ ΓΙΑ ΤΗ ΖΩΗ ΜΟΥ ΚΑΙ ΓΙΑ ΤΗΝ ΤΕΧΝΗ ΜΟΥ, ΑΝ ΟΜΩΣ ΠΑΡΑΒΩ ΚΑΙ ΑΘΕΤΗΣΩ ΤΟΝ ΟΡΚΟ ΜΟΥ ΝΑ ΥΠΟΣΤΩ ΤΑ ΑΝΤΙΘΕΤΑ ΑΠΟ ΑΥΤΑ.

Curriculum Vitae

Name: Nikolaos Marketos

Date of birth: 14/10/1976

Nationality: Greek

Professional Address: 17-19 Feidippidou street, 11526, Athens, Greece

Email address: nickmarketos@yahoo.com

Phone number: (+30) 6974371409

WORK EXPERIENCE

16/12/2016 – Current – **Rheumatologist consultant**

Henry Dunant Medical Centre and Iatriko Medical Centre

Main activities and responsibilities:

Consultant rheumatologist at the outpatient clinic, inpatient wards

01/10/2016 – Current - **Rheumatology specialist**

Main activities and responsibilities:

Clinical and research activities

01/01/2013 – 01/09/2016 - **Rheumatology intern and specialist, Sweden**

Region i Östergötland, Reumatologiska kliniken, Universitetssjukhuset i Linköping

Main activities and responsibilities:

- Consultant rheumatologist at the outpatient clinic, inpatient wards, emergency department, intensive care unit
- Active participation in educational departmental meetings
- Active participation in local, national, and international meetings (ACR 2012, SCR 2012 and EULAR 2016)

15/06/2011 – 14/12/2012 - **Internal Medicine and Rheumatology intern**

Landstinghet i Kalmar län, Medicinska kliniken, Oskarshamnssjukhus

Address: Kalmar, Sweden

Main activities and responsibilities:

- Trainee rheumatologist at the outpatient clinic, inpatient wards, emergency department, intensive care unit
- Active participation in educational departmental meetings

1/2/2008 – 1/6/2011 – **Medical assistant**

Sismanoglion Hospital, Rheumatology Department

Primary subject / occupational skills covered:

- Trainee in Rheumatology at the outpatient clinic, inpatient wards
- Active participation in educational departmental meetings

02/05/2006 – 01/02/2008 - **Internship in Internal Medicine**

Konstantopoulio Hospital "Agia Olga", Internal Medicine department

Primary subject / occupational skills covered:

- Trainee internal medicine at the outpatient clinic, inpatient wards, emergency department, intensive care unit
- Active participation in educational departmental meetings

01/02/2005 – 01/11/2005 - **Medical doctor / Military service**

Greek military

Address: Arta, Athens, Chios, Thiva; Greece

Main activities and responsibilities:

- Responsible for the well-being of all military personnel in each camp
- Training and lectures on thermonuclear, biological, and chemical warfare to soldiers
- Examining patients at the camp and the emergency department in each case

01/08/2004 – 30/08/2004 - **Medical doctor / Volunteer at the Olympic Games**

Athens 2004

Address: Athens, Greece

Main activities and responsibilities:

- Examining spectators at the games

26/09/2003 – 30/09/2004 - **Medical doctor / Rural service**

Greek Ministry of Health Care

Address: Rahoula, Karditsa, Greece

Main activities and responsibilities:

- Treating patients at the village, the outpatients, and the emergency department of the municipal hospital
- Active participation in educational departmental meetings

EDUCATION

01/09/2009 – 18/07/2012 - **Master in Metabolic Bone Diseases**

National and Kapodistrial University of Athens, Medical School

- Excellence (8,85/10) in writing and presenting the subject: **The significance of comorbidities of autoimmune diseases and fracture risk**

01/10/1996 – 01/02/2003 - **Medical degree**

Grade: Very well

National and Kapodistrial University of Athens, Medical School

LANGUAGE SKILLS

Mother tongue(s): Greek

Other language (s): English (fluently), Swedish (fluently), German (adequately)

PUBLICATIONS

[2017] **Biomarkers of diabetic nephropathy: A 2017 update**

https://doi.org/10.1080/10408363.2017.1377682

Reference: Nektaria Papadopoulou-Marketou, Christina Kanaka-Gantenbein, **Nikolaos Marketos**, George P. Chrousos

[2018] **Diabetic nephropathy in type 1 diabetes**

http://doi.org/10.23736/S0026-4806.17.05496

Reference: Nektaria Papadopoulou-Marketou, Stavroula A . Paschou, **Nikolaos Marketos**, Sofia Adamidi

[2018] **Canakinumab for refractory RA: a case report**

https://doi.org/10.31138/mjr.29.3.170

Reference: Nikolaos Marketos, Ilias Bournazos, Dimitrios Ioakimidis

[2019]

Type I interferon signature in Sjögren's syndrome: pathophysiological and clinical implications

https://pubmed-ncbi-nlm-nih-gov.e.bibl.liu.se/31376268/

Reference: Nikolaos Marketos, Ilir Cinoku, Anna Rapti, Clio P. Mavragani

[2019]

Molecular and clinical spectrum of four pedigrees of TRAPS in Greece: results from a national referral center

https://www.doi.org/10.1093/rheumatology/kez424

Reference: Adrianos Nezos, Ourania D Argyropoulou, Eleni Klinaki, **Nikolaos Marketos**, Panagiota Karagianni, Elias Eliopoulos, Panayiotis Vlachoyiannopoulos, Despoina N Maritsi, Athanasios G Tzioufas

[2019]

Biologics in Sjögren's syndrome

https://doi.org/10.1016/j.phrs.2019.104389

Reference: Charalampos Skarlis, Nikolaos Marketos, Clio P. Mavragani

[2020]

Chapter SS in Book Absolute Rheumatology Review

https://link.springer.com/chapter/10.1007/978-3-030-23022-7_10

Reference: Anna Rapti, **Nikolaos Marketos**, and Clio P. Mavragani

[2020]

Anakinra for Adult-Onset Still's Disease Despite Old Age

https://www.doi.org/10.1097/RHU.0000000000001534

Reference: Nikolaos Marketos, Amalia Patelli, Nektaria Papadopoulou-Marketou, and Dimitrios Ioakeimidis

[2020]

The Role of Novel Autoantibodies in the Diagnostic Approach and Prognosis of Patients with Raynaud's Phenomenon

https://doi.org/10.31138/mjr.31.4

Reference: Vasiliki Koulouri, Adrianos Nezos, **Nikolaos Marketos**, Evangelia Argyriou, Kyriaki Boki

[2021]

+3179G/A Insulin-Like Growth Factor-1 Receptor Polymorphism: A Novel Susceptibility Contributor in Anti-Ro/SSA Positive Patients with Sjögren's Syndrome: Potential Clinical and Pathogenetic Implications

https://www.mdpi.com/2077-0383/10/17/3960

Reference: Charalampos Skarlis, **Nikolaos Marketos**, Adrianos Nezos, Asimina Papanikolaou, Michael Voulgarelis, Michael Koutsilieris, Haralampos M. Moutsopoulos, and Clio P. Mavragani

[2022]

Scleroderma-specific autoantibodies: Should they be included in the diagnostic work-up for Sjögren's syndrome?

https://doi.org/10.1016/j.semarthrit.2022.152026

Reference: Nikolaos Marketos , Vasiliki Koulouri , Evangelia P. Piperi , Maria E. Georgaki , Nikolaos G. Nikitakis , Clio P. Mavragani

CONFERENCES

- Poster presentation granted with bursary: EULAR 6/2016.
- Poster presentation Swedish Rheumatology Society annual symposium: 9/2016
- Poster presentation Greek Rheumatology Society annual symposium: 12/2016
- 1st prize KEPETZI in the Greek Rheumatology Society annual symposium 2020 for the article: "Prevalence and clinical implications of Scleroderma specific autoantibodies in seronegative patients with sicca complaints."
  "Scleroderma specific autoantibodies in sicca.
- Oral Presentation in Spring Rheumatology days in 2017, 2022
- Poster in the Greek Rhumatology Society annual symposium 2020
- Poster in Hellenic Society of Physiology, 2022
- Oral presentation in Autoimmunity Congress, 2022

PROJECTS

- Participation in the Tozultra study as co-investigator 2015-2016.
- Primer Investigator SERENA study: 8/2017, ongoing
- Primer Investigator SENSE study [23/05/2019 – 30/05/2019]
- Primer Investigator Zefxis study [2021 – ongoing]

Certifications

GCP 2015

COURSES

- EULAR ONLINE COURSE ON THE RHEUMATIC DISEASES: OCT 2014 - OCT 2017
- MITOS course on musculoskeletal ultrasound: 12/2016

PREFACE

Funny as it may seem, one does not know if it is destiny, fate, or pure luck that leads one's life. And, of course, no one would be more accurate on the subject than our great poet Konstantinos Kavafis with his most acknowledged creation of Ithaca, signaling that it is the journey, not the destination, that is important. And that one should hope the journey to be a long one.

Sjögren's syndrome (SS) always seemed to me a somewhat overestimated autoimmune entity, perhaps as I was influenced by other physicians who believe that more debilitating and life-threatening rheumatological diseases such as vasculitis and systemic lupus erythematosus (SLE) ought to keep our scientific interest and efforts to alleviate symptoms. However, not even in 2013, when I studied during my rheumatology specialty in Sweden, the land from which Sjögren spawned, and even more in Linköping, close to Jonköping, Sjögren's hometown, under the supervision of my mentor and Sweden's "Doctor House" Per Eriksson, an expert on SS and vasculitis, did I realize the bond I would have to this autoimmune disease. It was first when my steps brought me to Clio P. Mavragani's doorstep in early 2018 that I realized the considerable effort invested globally in expanding our knowledge on SS, recognized nowadays as the platform on which pathogenetic mechanisms of autoimmunity can be studied. After that, with the help of the Professor and the other members of the Molecular and Applied Physiology Unit, in the Department of Physiology of the Medical School of Athens, Greece, my interest in SS began, leading to the present study of Scleroderma (SSc)-specific autoantibodies in SS, revealing such exciting results, fueling our interest for further research.

I would like to take this opportunity and thank Professor Michalis Koutsilieris of the Department of Experimental Physiology, Medical School of Athens, Greece, who accepted me at his laboratory and supported my research. Additionally, I would like to thank Associate Professor Antonis Chatzigeorgiou for his support and comments on the subject. Associate Professor Panagoula Aggelogianni, although recently added to my team of supervisors, never refused to help complete this doctoral thesis.

Additionally, I would like to thank Charalampos Skarlis and Vassiliki Koulouri from the Department of Experimental Physiology, Molecular and Applied Physiology Unit, for their support and help with this project, as well as their valued friendship.

Last but not least, I would like to thank Clio P. Mavragani for her inspiration, wholehearted support, guidance, brilliant thoughts, suggestions, and friendship. Dr. Mavragani has the gift of simplifying the most complex pathogenetic mechanisms and lighting the sparkle of research interest in all members of our laboratory team. With her help, anything can be accomplished.

Finally, I would like to express my deep appreciation to my wife, Nektaria Papadopoulou, an endocrinologist researcher herself, who has encouraged and supported me in pursuing my dream of this doctoral thesis from the very beginning.

SUMMARY

Sjögren's syndrome (SS) is a chronic, autoimmune disease with an indolent course characterized by ocular and oral dryness, as a result of lymphocytic infiltration of salivary and lachrymal glands. Although it is generally considered a benign syndrome affecting mainly middle-aged women, many patients suffer from fatigue, pain, and extra glandular symptoms, often worsening quality of life. Though exact pathogenesis is still unclear, over the last decades, it was consistently shown that type I interferon (IFN) pathway activation is a crucial event with type I IFN inducible genes being upregulated in both peripheral blood and salivary gland tissues derived from these patients contributing to B cell activation and autoantibody production against Ro/SSA and La/SSB antigens.

While anti-Ro/SSA and/or anti-La antibodies have been a major component in previously adopted classification criteria for SS, in a significant subset of SS patients these are absent. Moreover, patients with systemic sclerosis (SSc) classicaly characterized by Raynaud's phenomenon and hardening of the skin have been also shown to display sicca symptomatology, with a subset of SS pateints having in their serum antibodies against centromere antigens. Over the last years, an array of novel specific SSc antibodies was identified in association with distinct clinical and prognostic phenotypes among patients with SSc. In view of this data, we aimed to elucidate the prevalence and possible clinical implications of these SSc-specific autoantibodies in consecutive patients presenting with sicca complaints. Towards this end, we evaluated the presence of serum specific autoantibodies in consecutive patients with sicca complaints referred to the Molecular and Applied Physiology Unit, Department of Physiology, Medical School of Athens, National and Kapodistrian University of Athens, Greece. We found that SSc-specific autoantibodies were often present in sicca individuals [41.7% (90/216) patients evaluated (19% at strong, 22.7% at medium titers)]. Of note, SSc-specific autoantibodies at strong titers were significantly more often detected in patients with minor salivary gland (MSG) biopsies compliant with current SS histopathological criteria (30% vs. 12.5%, p=0.009), an association that remained significant after adjustment for antibodies against Ro/SSA and La/SSB autoantigens [OR 95% (CI): 4.1 (1.5-10.6)]. Theoretically, if SSc-specific autoantibodies at strong titers held the same burden in SS classification as anti-Ro/SSA antibodies, approximately one-third of seronegative (anti-Ro/SSA negative) sicca patients with negative or unknown MSG biopsy would be characterized as

suffering from SS. Furthermore, MSG biopsies from sicca patients with strong positive SSc-specific autoantibodies weew characterized by remarkable periductal fibrosis independently of the presence of lymphocytic infiltration. This finding fuels an alternative hypothesis of the mechanism of MSG dysfunction in SS, remaining unknown at present if we are upon a distinct clinical entity or a subgroup of SS. A prospective matched and blinded study quantitating the amount of fibrosis in MSG biopsies from sicca patients with distinct serological reactivities would help elucidate this hypothesis.

Our main findings are summarized in the following previously presented abstracts in the published articles:

Abstract of the article "Type I interferon signature in Sjögren's Syndrome: Pathophysiological and clinical implications."

https://pubmed-ncbi-nlm-nih-gov.e.bibl.liu.se/31376268/

Reference: Nikolaos Marketos, Ilir Cinoku, Anna Rapti, Clio P. Mavragani

Type I interferons (IFN) have long been recognized as mediators of innate immune defense mechanisms against viral threats. Robust evidence over the last 15 years revealed their significant role in the pathogenesis of systemic autoimmune diseases, including systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS). Despite the progress, methods of detection, initial triggers, biological functions, and clinical associations in the setting of autoimmunity remain to be fully clarified. As therapeutic options for SS are currently limited, neutralizing specific targets of the type I IFN pathway seems a promising alternative. In this review, we summarize the current evidence regarding the role of type I IFN in SS.

Abstract of the article: "Scleroderma-specific autoantibodies: Should they be included in the diagnostic work-up for Sjögren's syndrome?"

https://doi.org/10.1016/j.semarthrit.2022.152026

Reference: Nikolaos Marketos , Vasiliki Koulouri , Evangelia P. Piperi , Maria E. Georgaki , Nikolaos G. Nikitakis , Clio P. Mavragani

Objectives: Sicca complaints are a frequent reason for rheumatologic consultation. Testing for specific antibodies against Ro/SSA and La/SSB antigens and minor salivary gland (MSG) biopsy are among the main tools implemented in the diagnostic work-up. Anticentromere antibodies and sicca manifestations are frequently detected in Sjögren's syndrome (SS) and systemic sclerosis (SSc), respectively. Herein, we aimed to determine the frequency and clinical associations of a wide spectrum of scleroderma (SSc)-specific autoantibodies in consecutive patients referred for evaluation of possible SS.

Methods: Demographic, clinicopathological, and laboratory data were recorded in 216 consecutive patients with sicca complaints. All study participants were tested for SSc-specific autoantibodies (against CENP, PM/Scl, Scl-70, Ku, NOR90, RP11, RP155, fibrillarin, PDGFR, and Th/To) using a commercially available immunoblot kit. According to band intensity, the identified autoantibodies were further classified in those with strong and medium titers.

Results: SSc-specific autoantibodies were detected in 41.7% (90/216) patients evaluated (19% at strong, 22.7% at medium titers) without significant differences between anti-Ro/SSA positive and negative groups. At strong titers was significantly higher in patients with MSG biopsies fulfilling SS histopathological criteria (30% vs. 12.5%, p=0.009). This association remained significant after adjustment for antibodies against Ro/SSA and La/SSB autoantigens [OR 95% (CI): 4.1 (1.5-10.6)].

Conclusion: SSc-specific autoantibodies are frequently detected among patients presenting with sicca complaints and at strong but not medium titers are independently associated with MSG biopsy positivity. Taken together, these data imply a useful role of SSc antibody testing in the diagnostic work-up and possibly in the classification criteria for SS.

ΠΕΡΙΛΗΨΗ

Το σύνδρομο Sjögren's (σS) είναι μια χρόνια αυτοάνοση ασθένεια με βραδεία εξέλιξη που χαρακτηρίζεται από συμπτώματα ξηροφθαλμίας και ξηροστομίας ως αποτέλεσμα λεμφοκυτταρικής διήθησης των σιελογόνων και δακρυικών αδένων. Παρότι κατά βάσιν θεωρείται ένα καλόηθες σύνδρομο, το οποίο προσβάλλει κυρίως γυναίκες μέσης ηλικίας, πολλοί ασθενείς υποφέρουν από κόπωση, πόνο, και εξωαδενικά συμπτώματα, τα οποία συχνά οδηγούν σε επιδείνωση της ποιότητας ζωής τους. Αν και η ακριβής παθογέννεση του συνδρόμου είναι ακόμη αδιευκρίνιστη, κατά τις τελευταίες δεκαετίες έχει επανειλημμένα δειχθεί ότι η ενεργοποίηση του μονοπατιού της ιντερφερόνης τύπου Ι αποτελεί καίριο γεγονός καθώς τα επαγόμενα από ιντερφερόνη τύπου Ι γονίδια υπερεκφράζονται τόσο σε περιφερικό αίμα όσο και ιστούς σιελογόνων αδένων των ασθενών αυτών, οδηγώντας σε ενεργοποίηση Β λεμφοκυττάρων και παραγωγή αυτοαντισωμάτων έναντι Ro/SSA και La/SSB αντιγόνων.

Παρότι τα anti-Ro/SSA και/ή anti-La/SSB αντισώματα αποτελούν σημαντικό στοιχείο των παλαιότερων κριτηρίων ταξινόμησης για σS, αυτά είναι απόντα σε ένα σημαντικό μέρος των ασθενών με σS. Επιπλέον, οι ασθενείς με συστηματικό σκληρόδερμα (ΣΣκ) -κλασσικά χαρακτηριζόμενο από φαινόμενο Raynaud και σκλήρυνση του δέρματος- έχει δειχθεί ότι εμφανίζουν συμπτώματα ξηρότητας, ενώ ένα μέρος των ασθενών με σS έχουν στον ορό τους αντισώματα έναντι κεντρομεριδίου. Κατά τα τελευταία χρόνια μια σειρά από νέα ειδικά αντισώματα ΣΣκ έχουν αναγνωριστεί σε σχέση με διακριτούς κλινικούς και προγνωστικούς φαινότυπους μεταξύ των ασθενών με ΣΣκ.

Ενόψει αυτών των δεδομένων, αποφασίσαμε να μελετήσουμε τον επιπολασμό και πιθανές κλινικές επιπτώσεις αυτών των ειδικών αυτοαντισωμάτων ΣΣκ σε διαδοχικούς ασθενείς που παραπονούνται για συμπτώματα ξηρότητας. Για το σκοπό αυτό, μελετήθηκαν διαδοχικοί ασθενείς με συμπτώματα ξηρότητας που είχαν παραπεμφθεί στη Μονάδα Μοριακής και Εφαρμοσμένης Φυσιολογίας, στο Εργαστήριο Φυσιολογίας, της Ιατρικής Σχολής, του Εθνικού και Καποδιστριακού Πανεπιστημίου Αθηνών. Φάνηκε ότι τα ειδικά αυτοαντισώματα ΣΣκ ήταν συχνά παρόντα σε άτομα με συμπτώματα ξηρότητας [41.7% (90/216) ατόμων που εκτιμήθηκαν (19% ισχυρώς θετικά, 22.7% μετρίως θετικά)]. Να σημειωθεί ότι ειδικά αυτοαντισώματα ΣΣκ σε ισχυρούς τίτλους ανιχνεύονταν στατιστικά σημαντικά πιο συχνά σε ασθενείς με βιοψίες ελασσόνων σιελογόνων αδένων (ΕΣΑ) που πληρούσαν τα ισχύοντα παθολογοανατομικά

κριτήρια για σS (30% έναντι 12.5%, p=0.009), μια συσχέτιση που παρέμενε στατιστικά σημαντική ακόμα και μετά τροποποίηση για αντισώματα έναντι Ro/SSA και La/SSB αυτοαντιγόνα [OR 95% (CI): 4.1 (1.5-10.6)]. Θεωρητικά, αν τα ειδικά αυτοαντισώματα ΣΣκ σε ισχυρούς τίτλους είχαν την ίδια βαρύτητα στην κατάταξη ασθενών με σS, περίπου ένα τρίτο των οροαρνητικών (anti-Ro/SSA αρνητικών) ατόμων με αρνητική ή μη διαθέσιμη βιοψία ΕΣΑ θα κατατάσσονταν ως σS. Επιπλέον, παρατηρήσαμε ότι βιοψίες ΕΣΑ ασθενών με συμπτώματα ξηρότητας που είχαν ισχυρά θετικά αυτοαντισώματα ΣΣκ παρουσίαζαν στοιχεία περιπορικής ίνωσης εκτός από λεμφοκυτταρική διήθηση όπου αυτή υπήρχε. Το εξαιρετικό αυτό εύρημα δίνει βάση σε εναλλακτική υπόθεση του μηχανισμού δυσλειτουργίας των ΕΣΑ στο σS, άγνωστο προς το παρόν αν αφορά μια καινούργια νοσολογική οντότητα ή μια υποκατηγορία των ασθενών με σS. Διενεργώντας μια προοπτική, σταθμισμένη και τυφλή μελέτη θα μπορούσε να διερευνηθεί περαιτέρω αυτή η υπόθεση.

Τα βασικά ευρήματά μας συνοψίζονται στις ακόλουθες περιλήψεις ήδη δημοσιευμένων άρθρων:

Περίληψη του άρθρου " Η υπογραφή ιντερφερόνης τύπου Ι στο σύνδρομο Sjögren's: παθοφυσιολογικές και κλινικές επιπλοκές"

https://pubmed-ncbi-nlm-nih-gov.e.bibl.liu.se/31376268/

Συγγραφείς: Νικόλαος Μαρκέτος, Ηλίας Cinoku, Άννα Ράπτη, Κλειώ Π. Μαυραγάνη

Οι ιντερφερόνες τύπου Ι έχουν από καιρού αναγνωριστεί ως μεσολαβητές των αμυντικών μηχανισμών φυσικής ανοσίας έναντι απειλών από ιούς. Ακράδαντα στοιχεία κατά τα τελευταία 15 έτη αποκαλύπτουν το σηματικό τους ρόλο στην παθογένεση συστηματικών αυτοανόσων νοσημάτων, συμπεριλαμβανομένων του συστηματικού ερυθηματώδους λύκου (ΣΕΛ) και του συνδρόμου Sjögren's (σS). Παρόλη την πρόοδο, μέθοδοι για την ανίχνευση, αρχικοί εκκινητές, βιολογική λειτουργία, και κλινικές συσχετίσεις τους στα πλαίσια της αυτοανοσίας δεν έχουν εξακριβωθεί πλήρως. Καθώς οι θεραπευτικές επιλογές για το σS παραμένουν περιορισμένες, η αδρανοποίηση συγκεκριμένων στόχων της ιντερφερόνης τύπου Ι φαίνονται ως μια υποσχόμενη επιλογή. Σε αυτή την ανασκόπηση συνοψίζουμε τα μέχρι στιγμής δεδομένα σχετικά με τον ρόλο της ιντερφερόνης τύπου Ι στο σS.

Περίληψη του άρθρου: "Αυτοαντισώματα ειδικά του σκληροδέρματος: θα έπρεπε να συμπεριλαμβάνονται στο διαγνωστικό αλγόριθμο του συνδρόμου Sjögren's?"

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Συγγραφείς: Νικόλαος Μαρκέτος, Βασιλική Κουλούρη, Ευαγγελία Π. Πιπέρη, Μαρία Ε. Γεωργάκη, Νικόλαος Γ. Νικητάκης, Κλειώ Π. Μαυραγάνη

Σκοπός: Τα συμπτώματα ξηρότητας αποτελούν συχνό αίτιο παραπομπής σε ρευματολόγο. Ο έλεγχος για τα ειδικά αντισώματα έναντι Ro/SSA και La/SSB αντιγόνων καθώς και βιοψία ελασσόνων σιελογόνων αδένων (ΕΣΑ) αποτελούν κύρια εργαλεία που χρησιμοποιούνται στη διαγνωστική προσέγγιση. Αντικεντρομεριδιακά αντισώματα και εκδηλώσεις ξηρότητας ανιχνεύονται συχνά σε σύνδρομο Sjögren's (σS) και συστηματικό σκληρόδερμα (ΣΣκ), αντίστοιχα. Επί του παρόντος, στοχεύσαμε στη διευκρίνιση της συχνότητας και των κλινικών συσχετίσεων ενός ευρεός φάσματος ειδικών αυτοαντισωμάτων ΣΣκ σε διαδοχικούς ασθενείς που παραπέμφθηκαν προς διερεύνηση πιθανού σS.

Μέθοδοι: Καταγράφηκαν δημογραφικά, κλινικοπαθολογικά, και εργαστηριακά δεδομένα 216 διαδοχικών ασθενών με συμπτώματα ξηρότητας. Όλοι οι συμμετέχοντες ελέγχθηκαν για ειδικά αυτοαντισώματα ΣΣκ (έναντι CENP, PM/Scl, Scl-70, Ku, NOR90, RP11, RP155, fibrillarin, PDGFR, and Th/To) χρησιμοποιώντας ένα εμπορικά διαθέσιμο κιτ ανοσοαποτύπωσης. Σύμφωνα με την ένταση του σήματος, τα αναγνωρισμένα αυτοαντισώματα κατετάγησαν περαιτέρω σε εκείνα με ισχυρώς θετικούς και μετρίως θετικούς τίτλους.

Αποτελέσματα: Ειδικά αυτοαντισώματα ΣΣκ ανιχνεύθηκαν σε 41.7% (90/216) ασθενείς που αξιολογήθηκαν (19% σε ισχυρά θετικούς, 22.7% σε μετρίως ισχυρά θετικούς τίτλους) χωρίς σημαντικές διαφορές μεταξύ anti-Ro/SSA θετικών και αρνητικών ομάδων. Ισχυρά θετικοί τίτλοι ήταν σημαντικά συχνότεροι σε ασθενείς με βιοψίες ΕΣΑ που πληρούσαν τα ιστοπαθολογικά κριτήρια για σS (30% έναντι 12.5%, p=0.009). Αυτή η συσχέτιση παρέμεινε σημαντική μετά από τροποποίηση για αντισώματα έναντι Ro/SSA και La/SSB αυτοαντιγόνα [OR 95% (CI): 4.1 (1.5-10.6)].

Συμπεράσματα: Τα ειδικά αυτοαντισώματα ΣΣκ ανιχνεύονται συχνά σε ασθενείς που προσέρχονται με συμπτώματα ξηρότητας, και σε ισχυρά θετικούς αλλά όχι μετρίως ισχυρά

θετικούς τίτλους εμφανίζουν συσχέτιση με τη θετικότητα βιοψίας ΕΣΑ. Συνολικά, τα δεδομένα αυτά υπονοούν ένα χρήσιμο ρόλο του ελέγχου για αντισώματα ΣΣκ στη διαγνωστική προσέγγιση και πιθανότατα στα διαγνωστικά κριτήρια για σS.

TABLE OF CONTENTS

Prevalence and clinical implications of Scleroderma-specific autoantibodies in Sjögren's syndrome

1. GENERAL PART

1.1 INTRODUCTION

It has now been almost a century since the first extensive description by Henrik Sjögren, a Swedish ophthalmologist, of an autoimmune disease that affects lacrimal and salivary glands, which subsequently was named after him (Sjögren's syndrome, SS)[1]. This autoimmune entity, the second more frequent autoimmune disease after rheumatoid arthritis (RA), affects mainly middle-aged women[2] at a 9-fold rate in comparison to men, is of unknown etiology, and has diverse phenotypical expression. The reported incidence rate varied between 0.05 and 0.09%, while prevalence rates were estimated between 0.44% and 0.77%[3]. An earlier, more comprehensive study revealed a prevalence between 0.1% and 4.8%, depending on the population studied and the classification criteria used[2]. Greek studies, however, had shown an estimated incidence of 5.3 cases per year per 10^5 inhabitants (95% confidence interval [CI] 4.5–6.1), as well as a prevalence of 92.8 cases per 10^5 inhabitants (95% CI 83.7–101.9)[4]. These data were collected in a north-western part of Greece and have not yet been confirmed from a national registry. Additionally, they were based on the use of 2002 ACR-EULAR classification criteria for SS[5], which have since 2016 been exchanged with the more recent 2016 ACR-EULAR classification criteria for SS[6]. Nevertheless, until this day, no epidemiological data on SS according to the latest criteria have been reported worldwide.

1.2 ETIOLOGY OF SS

Exploration of disease mechanisms is essential for disclosing its etiology, identifying biomarkers for diagnosis, assessing disease process, monitoring response but also identifying targets for treatment, and finally defining critical items in classification criteria[7]. The etiology and pathophysiology of SS remain elusive despite the intense

research and technological advance of recent years. The fundamental pillars recognized in the pathogenesis of SS so far are stress, environmental, genetic, and epigenetic factors, as well as menopause as a mechanism of estrogen deficiency. Environmental factors, in addition to predisposing genetic susceptibility in the presence of stressful events as well as anti-Ro/SSA and anti-LA/SSB autoantibodies, and allegedly estrogen deficiency, have been thought to interact in yet unknown ways in the pathogenetic mechanisms of SS[8].

1.2.1 The environment in SS

Environmental factors seem to contribute to the development of SS in susceptible individuals. Skin or urogenital infections in the presence of antibodies against Ro/SSA and La/SSB epitopes, as well as respiratory infection regardless of these antibodies, were shown to contribute to SS development via molecular mimicry, epitope spreading, bystander activation, and B cells that become immortal[9]. As far as viruses or bacteria are concerned to be a trigger of the disease, although not completely understood, infection with a virus is still thought to increase the likelihood of the disease, especially regarding Coxsackie, Epstein-Barr virus (EBV), and Hepatitis C virus (HCV)[10,2]. Similarities between viral proteins and Ro/SSA and La/SSB antigens have spawned the theories for molecular mimicry as an initiating mechanism[11]. Association of active EBV infection to ectopic lymphoid structures in salivary glands of SS patients seems to confer in the growth and differentiation of autoreactive B-cells locally[12]. Augmented innate and adaptive immune response after vaccination, especially B cell hyperactivity after H1N1 vaccination[13], may pose another clue for the role of viral loading as an epigenetic factor resulting in SS[14].

Of particular interest, endogenous virus-like genomic repeat elements, such as the long interspersed nuclear element-1 (LINE-1), have gained the scientific community's attention in SS as factors engaged in disease pathogenesis[15,16]. Overexpression of LINE-1 in minor salivary gland (MSG) tissue from SS patients was associated with increased expression of type I interferon (IFN) and L1 DNA demethylation. Experimental transfection of plasmacytoid dendritic cells (pDCs) or monocytes with L1 DNA or RNA resulted in induction of type I IFN, which was successfully abrogated when Toll-like

receptor (TLR)-7/TLR-8 in the first case and IKKe/TANK-binding kinase 1 in the latter were inhibited. Increased methylation of promoters for genes involved in the secretory machinery in MSG tissue of SS patients has also been shown, resulting in lower mRNA expression of these genes, thus recognizing another mechanism in disease pathogenesis[17]. The endpoint of this procedure is the increased production of type I IFN and, thereafter, B cell activation, the renowned hallmark of SS[18,19,20].

1.2.2 Genes as contributing factors in SS

Genetic factors have been implicated in SS's pathogenesis and pathophysiology, given SS familial aggregation[21,22,23,24,25]. In addition, murine models have helped elucidate aberration in salivary gland homeostasis and integrity before disease development[26]. Genomic studies have identified HLA, IRF5, STAT4, BLK, IL12A, CXCR5, and TNIP1 as genome-wide association regions in Europeans[27], with the addition of other loci in Asians[28,29]. Of interest, only 12 loci have to date an established GWAS association with SS, the strongest being HLA, IRF5, and STAT4[7], while in Europeans, a large GWAS study recently identified ten novel associations (CD247, NAB1, PTTG1-MIR146A, PRDM1-ATG5, TNFAIP3, XKR6, CRHR1, CHMP6, TYK2, SYNGR1)[7].

The pathways through which genes are implicated in SS pathogenesis include i) IFN signaling, ii) B cell function and antibody production, and iii) NFκB signaling through apoptotic and inflammatory genes[30]. Regarding the burden of genes in not only disease initiation but even lymphoma predilection in SS, it has been shown that deranged immune response in individuals of younger age (<40 years old) may explain their increased risk for the disease, as functional Leucocyte immunoglobulin-like receptor 3 (LILRA3) gene variant was statistically more often detected in this subgroup[31].

1.2.3 Epigenetics of SS

In epigenetic mechanisms, on the other hand, it has been shown that Ro/SSA reactive T cells may be activated from oral[32] and gut microbiota[33], incriminating typical human microorganisms as the trigger factor for SS. Furthermore, altered expression of methylating enzymes, [especially DNA methyltransferase 3 (DNMT3A) and lymphoid-

specific helicase (LSH)][34], modifications of histones, and identification of non-coding RNAs modulating gene expression in SS[35], were shown to lead to immune dysregulation and eventual distinct clinical phenotypes.

1.2.4 Autoantibodies and SS

Autoantibodies against Ro/SSA and La/SSB antigens are essential for SS diagnosis and are a fundamental component of the latest 2016 ACR/EULAR classification criteria for the disease[6]. In a Swedish retrospective study, these autoantibodies, besides antinuclear antibodies (ANA) and rheumatoid factor (Rf), were detected in a median of 4 years before symptom onset in 2/3 of patients who later developed SS[7]. Furthermore, the presence of at least one of each ANA, RF, anti-Ro/SSA, and anti-La/SSB in declining prevalence was detected in 4/5 of these patients up to 20 years before the diagnosis of SS. It was associated with early-onset, more severe disease course, with anti-Ro/SSA exhibiting the highest predictive value[36].

Another mechanism of disease occurrence has been hypothesized through autoantibodies to muscarinic receptor 3 (M3R)[37]. Using a rabbit model, experimentally raised antibodies to the second extracellular loop of the MR3 receptor seemed to bear the same properties as autoantibodies to these receptors in SS patients. These inhibit cholinergic transmission at the postsynaptic level, thus mediating parasympathetic dysfunction and sicca symptomatology[38].

1.2.5 Stress in SS

Finally, another disease mechanism has been elucidated through stressful events in the life of individuals with inadequate coping mechanisms, leading to defective anti-inflammatory mechanisms and eventually an exaggerated immune response. Especially when the hypothalamic-pituitary-adrenal (HPA) axis is chronically suppressed, as shown by lower levels of adrenocorticotropic hormone (ACTH) and cortisol in SS patients[39], the effect of this dysregulation becomes more apparent.

1.3 PATHOPHYSIOLOGY – MECHANISMS OF DISEASE

Murine models have provided insights into possible disease initiation and progression mechanisms. In addition, it has been shown that estrogens impede the expression of intercellular adhesion molecule-1 (ICAM-1) by IFN γ by binding to their respectable receptors (ERα, ER$\beta 1$, ER$\beta 2$), leading to the protection of salivary and lacrimal glands against autoimmune damage[40]. Reinforcing these findings, ovariectomy increases apoptotic epithelial cells in salivary glands of previously healthy mice[41]. These and many more experiments add value to the role of estrogens in protection against sicca symptoms and at least partially explain the higher prevalence of SS in postmenopausal women.

Apart from the recognized role of B and T lymphocytes, when referring to the pathogenesis of SS, a special tribute is mandatory to epithelial cells, the main target of lymphocytes infiltrating exocrine glands and parenchymal organs in SS; hence the term autoimmune epithelitis often used to describe SS[42,43]. Apoptotic molecules have been observed in ductal, acinar, and mononuclear infiltrating cells of MSG biopsies derived from SS patients. Additionally, it was shown that epithelial cells in these patients undergo apoptosis. At the same time, epithelial cell repair and proliferation markers were also detected, indicating a continuing circle of destruction and regeneration with the epithelial cell in its centre[44]. Increased apoptosis of these cells releases antigens (Ro/SSA, La/SSB) and produces their respectable autoantibodies. The consequence of the immunocomplexes created is the type I IFN production by pDCs in genetically susceptible individuals[45], resulting in amplification of epithelial activation, B cell-activating factor (BAFF) overexpression, and production of autoantibodies. These epithelial cells have a role in producing cytokines and antigen-presenting cells (APCs), in turn further activating B and T lymphocytes[46,47,48,49,50]. At its utmost, activating these lymphocytes leads to aggregates with proliferating cells along with follicular dendritic cells and activated endothelial cells forming ectopic lymphoid structures, defined as germinal centers (GCs) [51] producing anti-Ro/SSA and anti-La/SSB antibodies. The formation of GCs was later proposed as a highly-predictive marker of non-Hodgkin lymphoma (NHL) in SS patients[52,53]. Other predictors of lymphoma development included male sex, splenomegaly, sensorimotor neuropathy, and cryoglobulinemia. An earlier study had proposed a predictive risk score for NHL development in SS patients, without obtaining MSG biopsy and thus clinically

more helpful, consisting of 3 clinical (salivary gland enlargement, lymphadenopathy, Raynaud's phenomenon) and four serological (anti-Ro/SSA or/and anti-La/SSB autoantibodies, RF, monoclonal gammopathy and C4 hypocomplementemia[54]. In this study, the probability of NHL development in those with 3 to 6 risk factors was calculated to be 39.9%(OR (95%CI): 16.6 [6.5–42.5], P<0.05), while in the presence of all seven risk factors the corresponding probability reached 100% (OR [95%CI]: 210.0 [10.0–4412.9], P<0.0001).

1.4 CLINICAL PHENOTYPE OF SS

Clinically SS is characterized by both organ-specific and systemic manifestations[55]. The acclaimed hallmark of the disease is impairment in salivary and lacrimal gland function along with inflammation mainly led by lymphocytes in various organs, especially the lung, kidney, and liver, resulting in obstructive bronchiolitis, interstitial nephritis, and autoimmune cholangitis, respectively. The most frequent lung involvement encountered in SS is mild small airway obstruction, usually lymphocytic bronchiolitis, characterized by dry cough in 40% according to studies and eventual xerotrachea[56]. Other rare entities include diffuse lymphoid hyperplasia (follicular bronchiolitis and lymphoid interstitial pneumonitis), lymphomatoid granulomatosis, and lymphoma. Of note, seldom is the impairment of lung function clinically significant.

As far as the kidney is concerned, lymphocytic infiltrates around tubules are shown to occupy the interstitium, thus leading to type I renal tubular acidosis (RTA I). This condition is often only discovered in SS patients at late stages and may lead to kidney insufficiency[57]. Finally, infiltration of lymphocytes around cholangial ducts leads to inflammation and rarely lymphoproliferation. The condition resembles the lymphocytic infiltration of the organs mentioned above and requires prompt recognition and treatment[58].

These manifestations are attributed to lymphocytic infiltration of the epithelia of the involved organs, apart from exocrine glands. On the other hand, extra-epithelial manifestations such as palpable purpura, glomerulonephritis, and peripheral neuropathy are thought to occur due to B cell hyperactivity and subsequent immune complex

deposition at various sites. The latter, often more severe systemic manifestations, are linked to increased morbidity and elevated lymphoma risk.

Through the years, it has been argued that SS is either primary or secondary to other autoimmune diseases such as RA, SLE, DM, or SSc, with conflicting reports[59,60,61]. However, it has now been acknowledged that SS is a primary pathological entity but may accompany other autoimmune diseases, as mentioned above.

1.5 CHRONOGRAPHY OF SS

Since the first description of the clinicopathological characteristics attributed to the autoimmune entity designated SS[1], several attempts have been made to agree on classification criteria. However, apart from early attempts to describe SS classification criteria to study the disease universally[62], it was first in 1968 when Chisholm and Masson came forward with the description of the technique used for evaluation of minor salivary gland (MSG) biopsy[63], which remains essentially one of the main components for the diagnosis of SS until this day. Several classification criteria for SS were published in the following years, with more exceptional the ones of Greek[64], Danish[65], American[66], and Japanese researchers[67].

For a patient to be classified as having SS according to the Greek criteria, 2/3 of the following criteria should be fulfilled: xerophthalmia defined by subjective complaints, Schirmer's test less than or equal to 5 mm/5 min, or positive Rose-Bengal staining on slit-lamp examination, xerostomia defined by subjective complaints and parotid flow rate less than or equal to 1 cc/5 mm/gland and, presence or history of parotid gland enlargement. In addition, all patients' labial minor salivary glands histology showed round cell infiltrates greater than or equal to 2+ (Tarpley's classification). Therefore, it seems that these criteria did not demand the presence of either positive antibodies against Ro/SSA or La/SSB or a positive MSG biopsy[64].

According to the American classification criteria for SS, objective ocular dryness (evident by using Rose Bengal or fluorescein dye staining), objective oral dryness, MSG biopsy with a focal score of ≥ 2 (focal infiltrates/4mm^2), along with antibody positivity for rheumatoid factor (RF) or antinuclear antibodies (ANA) should be obtained. If 3 of these

criteria were present, SS diagnosis would be possible, while it would be definite if all four were present[66]. Unfortunately, few details are currently available for either the Danish or the Japanese criteria.

A more unified set of criteria, endorsed by EULAR (European League Against Rheumatism) and ACR (American College of Rheumatology), emerged in 2002. According to these criteria, six different domains were assessed: i) ocular symptoms, ii) oral symptoms, iii) ocular signs (Schirmer's test or Rose-Bengal, iv) MSG biopsy positivity, v) salivary gland involvement, indicated by diminished unstimulated whole salivary flow (UWSF) (<1.5 ml/15 min), or parotid sialography, or salivary scintigraphy, and finally vi) antibodies to Ro/SSA or La/SSB autoantigens. Additionally, a list of exclusion criteria should also be fulfilled: Past head and neck radiation treatment, HCV infection, acquired immunodeficiency syndrome (AIDS), pre-existing lymphoma, sarcoidosis, graft versus host disease, use of anticholinergic drugs (since a time shorter than 4-fold the half-life of the drug). The presence of 4/6 of these features, including either MSG biopsy or anti-Ro/SSA or anti-La/SSB autoantibodies positivity, supports the classification of a patient as SS in the absence of the exclusion criteria[5].

Since 2016 a new set of classification criteria for SS has emerged. These are based on the weighted sum of 5 items: anti-Ro/SSA antibody positivity and focal lymphocytic sialadenitis with a focus score of 1 foci/4 mm^2, each scoring 3 points; an abnormal ocular staining score of 5 (or van Bijsterveld score of 4), a Schirmer's test result of <5 mm/5 minutes, and an unstimulated salivary flow rate of <0.1 ml/minute, each scoring 1 point. Individuals with signs or symptoms suggestive of SS with a total score of 4 points for the above items meet the criteria for primary SS. Sensitivity and specificity against clinician-expert–derived case/non-case status in the final validation cohort were high, i.e., 96% (95% confidence interval [95% CI] 92–98%) and 95% (95% CI 92–97%), respectively[6].

The evolution of these classification criteria through time reveals the effort to include the patients suffering from the disease using the best and economically available diagnostic tools at each timepoint. A particular advantage is the use of unified criteria, acknowledged by EULAR and ACR since 2016. Up to date, no large epidemiologic study on SS according to these criteria has been published. Nevertheless, prevalence and incidence may differ between timepoints, as different criteria have been used. Recently, an effort to

include ultrasound of the parotid and minor salivary glands (salivary gland ultrasound or SGUS) has been observed[68,69]. However, the usefulness and prognostic value of MSG biopsy is still unquestionable[70]. Of course, this leaves the field open for using other, non-incisional diagnostic methods in exchange for MSG biopsy, as many patients are reluctant to undergo this procedure.

1.6 DIFFERENTIAL DIAGNOSIS

Medical conditions and medications that induce xerostomia, keratoconjunctivitis sicca, and parotid gland enlargement are part of the differential diagnosis for SS. Medications have strong adverse effects on salivary and lacrimal gland function. They may induce reduced secretion, including atropine, atropine antiparkinsonian drugs, anticholinergic, and anti-histaminic drugs. Furthermore, apart from anxiety and depression, antidepressants such as amitriptyline and monoamine oxidase inhibitors, as well as neuroleptics and analgesics in the form of morphine, codeine, and tramadol, may have the same result. Additionally, various drugs such as botulinum toxin type A, class IA anti-arrhythmic (disopyramide), and isotretinoin may cause adverse effects of xerostomia and xerophthalmia. This result may also be induced by psychotropic drugs and the use of substances from tobacco to ecstasy, cannabis, and cocaine. Several other medicines may cause similar symptoms at a moderate level. These include heart medication (β and α blockers, calcium channel blockers, diuretics), benzodiazepines, serotonin reuptake inhibitors, H_1-antihistaminic drugs, and some anti-retroviral drugs. Despite the profound effect of these drugs on lachrymal and salivary secretion, their use should not exclude further evaluation of a patient presenting with sicca symptoms.

Sarcoidosis should be considered when evaluating a patient presenting with sicca symptoms. Finding non-caseating granulomas in MSG biopsy and negative anti-Ro/SSA and anti-La/SSB serology help exclude this condition. HIV and HCV must always be considered among infections that may present sicca symptoms. In the first case, positive testing for virus antibodies, male predominance, absence of antibodies against Ro/SSA and La/SSB, and discovery of CD8 T lymphocytes in MSG biopsies help differentiate these patients. In the case of HCV, one notes a higher mean age of patients, seldom parotid

gland enlargement, and more often abnormal liver function tests (LFTs). A variety of other conditions considered SS mimics include anxiety-depressive syndromes, head, and neck radiotherapy, hyperlipoproteinemia (types II, IV, and V), diabetes mellitus, IgG4-related sialadenitis, chronic graft-versus-host disease, lymphoma, and amyloidosis. The physician exploring the possible causes of sicca symptoms in his patient should not forget more prominent ones such as aging, especially in women post-menopausal and estrogen-deficient, as well as the prolonged use of contact lenses as far as dry eye disease is concerned[71]. Inflammation (chronic blepharitis or conjunctivitis, pemphigoid, or Stevens-Johnson syndrome) ought also to be considered[72], along with various other conditions (corneal anesthesia, blink abnormality, hypovitaminosis A, eyelid scarring, or trauma), especially in isolated ocular gland dysfunction.

Of note, autoimmune thyroid disease has also been attributed to the cause of sicca symptoms in some patients. For example, previous studies have shown that 35% of patients with autoimmune thyroid disease (ATD) test positive for anti-nuclear antibodies (ANA) in contrast to 9% of healthy controls. Additionally, SS was diagnosed in 10% of ANA+ ATD patients[73]. According to these findings, 26% of Hashimoto patients had sicca symptoms in another cohort[74], even when the thyroid function was normal.

Despite the extensive evaluation and the strong suspicion of the presence of underlying SS, on several occasions, often the diagnosis of SS cannot be definitely drawn, and sicca complaints cannot always be fully explained in the context of a well-defined clinical entity. These patients not fulfilling classification criteria for SS[6] were previously designated as suffering from dry eyes and mouth syndrome (DEMS)[75] or SAPS (sicca, asthenia, and polyalgia syndrome)[76]. This group of patients was found to have non-specific chronic musculoskeletal pain, Raynaud's phenomenon (RP), and subjective sicca symptoms at a similar rate to their sex- and age-matched SS counterparts. Interestingly, 59% of these patients tested positive for antibodies against thyroid peroxidase. A mild interstitial infiltrate, and the presence of perivascular infiltrates in MSGBs were the main histopathological findings[77]. Therefore, fibromyalgia-like syndromes along with SAPS and DEMS should be considered in evaluating patients with sicca complaints.

1.7 BASIC TREATMENT PRINCIPLES

The treatment of SS patients expands from alleviating ocular and salivary discomfort to managing dangerous complications, mainly lymphoma[78]. Because of various factors (lack of randomized controlled trials, the inefficacy of current medication used in other autoimmune systemic diseases, and diverse local traditions in disease management), the scientific community has not yet agreed on treatment guidelines. Nevertheless, recently EULAR recommendations for treating SS patients with topical and systemic therapies were released due to a multidisciplinary task force[79]. The classic triplet of sicca symptoms, fatigue, and pain is at the core of therapy, followed by systemic disease. The basic principles suggest that treatment should be administered by health professionals with expertise in SS.

Additionally, symptomatic relief by using local remedies should be the first-line therapy for ocular and oral dryness, as no treatment regimen has shown efficacy in curing ocular or lacrimal dryness. Furthermore, systemic therapy such as glucocorticoids, antimalarials, and other disease-modifying antirheumatic drugs (DMARDs), intravenous immunoglobulin, and biologics should be withheld for active systemic disease, as assessed by using the EULAR Sjögren's syndrome disease activity index (ESSDAI)[80]. It is recommended that systemic treatment is applied in two stages, the first as induction of remission by using intensive immunosuppression and the second with milder immunosuppression for maintenance of remission. Due to a lack of data, the duration of these stages must be decided individually for every patient. It should also be emphasized that due to a lack of data from large RCTs, some recommendations on treatment are based on extrapolation from earlier trials that implemented the 2002 ACR/EULAR SS classification ctriteria[5], as they are considered by many almost equivalent to the more recent 2016 criteria[6] in terms of SS group description.

1.7.1 Treatment of xerostomia

Concerning lacrimal dysfunction, a careful and objective assessment of salivary flow must be recorded before any treatment. In case of mild impairment, gustatory stimulants such as sugar-free acid candies, lozenges, xylitol, and mechanical stimuli in the form of sugar-free chewing gum are recommended since these patients have a present although

decreased salivary function. However, these benefits are based on subjective relief of symptoms as no randomized clinical trials (RCTs) exist. When the secretory function is moderately impaired or non-pharmacological therapy is not eligible, the use of pharmacological agents is suggested. Mainly the muscarinic agonists pilocarpine and cevimeline[81] (the latter not yet available in Europe) are considered, again based on limited data on subjective improvement. A gradual, step-up increase of treatment to a maximum of 20mg/d in separate doses is preferred to avoid excess sweating, the main side effect of these muscarinic agents, apart from seldom nausea, diarrhea, and palpitations.

Furthermore, the use of choleretic (anetholtrithione) or mucolytic drugs such as bromhexine or N-acetylcysteine has exerted some benefit without side effects in intolerant or non-responding patients, although in early trials[82]. Finally, salivary substitution is offered in patients with no residual lacrimal function, or without satisfactory effect of pharmacological or non-pharmacological stimulation, with regimens that have neutral PH and contain fluoride, among other electrolytes, to achieve the formulation of normal saliva. As stated above, these oral sprays, gels, and rinses have shown subjective improvement in limited trials, without side effects[83].

1.7.2 Treatment of xerophthalmia

Ocular discomfort in the setting of SS can be a debilitating symptom. Earlier studies have shown the efficacy and safety of ocular regimens[84]. It is recommended that patients suffering from ocular dryness at any degree use artificial tears containing methylcellulose or hyaluronate at a frequency adjusted to symptom alleviation and that preservative-free regimens are considered for those who need frequent use of ocular lubricants[85]. When the problem is not adequately addressed and the patient suffers from severe/refractory ocular dryness, treatment from an ophthalmologist with expertise in dry eye disease should be suggested. An initial course of short-term (approximately 2-4 weeks maximum) of topical non-steroidal anti-inflammatory drugs (NSAIDs), alternatively low dose corticosteroids may be administered, with caution on side effects with prolonged use in the form of corneal-scleral melts, perforation, ulceration, and severe keratopathy from the former, as well as infections, glaucoma, and cataract from the latter, respectively.

Further down the line, topical administration of Cyclosporin A (CyA) was approved by FDA in 2002[86], although a systematic review of the literature has shown inconsistent efficacy of this regimen[87]. Finally, serum tear-drop regimens bear many risks and difficulties. Therefore, they should only be used in patients refractory to CyA drops, while tacrolimus drops showing initially favorable results need to be evaluated in larger trials[87].

1.7.3 Treatment of systemic manifestations

1.7.3.1 Role of conventional disease-modifying antirheumatic drugs (cs DMARDS) in SS

Moving on to more systemic complaints, fatigue and pain are frequently reported in the context of SS. Regarding fatigue, aerobic exercise is recommended[88], while the treating physician must assess concurrent disease states such as fibromyalgia. Short-duration treatments with NSAIDs for full 7-10 days may be administered in case of musculoskeletal pain. Hydroxychloroquine can be effective as a longer-duration regimen concerning its efficacy on arthralgias and arthritis in SLE[89]. However, the use of biologic agents to address these symptoms is not currently warranted. Furthermore, aerobic exercise and physical activity are recommended for non-inflammatory pain[90]. Neuropathic pain may benefit from regimens such as gabapentin, pregabalin, and amitryptiline, while opioids are ineffective and thus contraindicated[91].

Treatment of specific disease manifestations should be chosen according to ESSDAI index severity with a goal of ≥3 points decrease. In contrast, the treating physician must also consider features of the disease not captured by ESSDAI, such as Ro/SSA-associated congenital heart block, Raynaud's phenomenon, or serositis. Glucocorticoids have no documented favorable effect on SS. Nevertheless, they are used in everyday practice in doses of 0.5mg/kg/d in severe cases and lower amounts in moderate ones; hence, rapid, as clinically feasible as possible tapering is warranted. Immunosuppressive agents acting as cortisone-sparing drugs are therefore widely used. Nevertheless, not only has monotherapy with these agents without simultaneous use of cortisone been tried, but no head-to-head study for the efficacy or safety of DMARDs is available. Besides

hydroxychloroquine (HCQ)[92] and methotrexate (MTX)[93] -two DMARDs that have been studied in the context of SS- leflunomide, azathioprine, mycophenolate mofetil and cyclophosphamide (CyC) are used upon indication despite lacking sufficient evidence in the literature regarding safety and efficacy in SS. Therefore, HCQ is used as a first-line treatment in arthritis, synovitis, and diffuse annular erythema with or without concomitant GCs, and second line for arthralgias refractory to NSAIDs. GCs are considered, as previously stated, as 1st line therapy in more severe cases such as polyarthritis, cutaneous involvement and vasculitis, interstitial lung disease, renal involvement with moderate or high ESSDAI, multineuritis, CNS vasculitis, thrombocytopenia and/or hemolytic anemia, while they are reserved as 2nd line treatment for glandular involvement. The other immunosuppressives are used as 2nd line treatment for articular, cutaneous, moderate renal involvement and multineuritis, where no evidence exists as to prefer one over the other regimen. CyC is considered as 2nd line therapy for gromerulonephritis with high ESSDAI, CNS vasculitis and/or neuromyelitis optica (NMO), as well as rescue therapy for cutaneous vasculitis, interstitial lung disease with high ESSDAI, multineuritis, ganglionopathy and hemolytic anemia. As we progress further up the treatment pyramid, intravenous immunoglobulin (IVIG) is restricted to 1st line therapy for ganglionopathy, severe (Hb<8mg/dl) hemolytic anemia and complete heart block.

1.7.3.2 The era of biologics in SS therapy

The use of B-cell targeted therapies, especially rituximab (RTX), has been more widely studied in SS in recent years and shown efficacy with a good safety profile in patients with severe, refractory systemic disease[92,94–106]. Global therapeutic response, organ-specific response, change in ESSDAI score, and prednisolone dose reduction was evaluated in these studies. At least one of these parameters was successfully addressed by RTX. It is therefore recommended as 2nd line therapy in cryovasculitis, multineuritis, glomerulonephritis, and low Hb-hemolytic anemia, as well as rescue therapy for acute glandular involvement, polyarthritis, and interstitial disease with high ESSDAI and CNS vasculitis.

In the era of precision medicine, classifying SS patients into specific subgroups in terms of molecular factors would be of great value in making the correct treatment choice for each case. For example, a recent study identified four groups of SS patients by using transcriptomic, genomic, epigenetic, cytokine expression, and flow cytometry data[107]. The first cluster exhibited IFN-related pathways, pattern recognition receptors, and IFN regulatory factor (IRF) activation. In contradiction, the second cluster was characterized by molecular features closer to healthy individuals except for IFN-related genes. Additionally, B cell activation in the form of B cell receptor signaling and B cell development was evident in the third cluster. In contrast, in the fourth cluster, the modules revealed genes associated with inflammation and neutrophils and a more severe clinical phenotype. Therefore, treatment with type I IFN response inhibitors seems rational for patients being classified in all clusters except for the second, while those belonging to the third might also benefit from B cell depletion therapy.

As of when this text is written totally 80 trials are active, 56 of which are currently recruiting and assessing various treatment approaches to SS, according to *clinicaltrials.gov*. As far as emerging therapies are concerned, several biologic drugs for SS are in different stages of development, and at this timepoint, 125 clinical trials are reported to be completed. Here we report the most significant findings of these trials.

Given the central role of BAFF in the activation and proliferation of B cells, which are crucial components in SS pathogenesis, Belimumab, a BAFF inhibitor successfully used in SLE, has gained the attention of physicians as a possibly helpful tool against SS[108,109]. Normalization of B cell frequency, phenotype, and function was also shown in another clinical trial of belimumab in SS[110]. A more aggressive approach targeting B cells with both rituximab and belimumab was investigated in a recent clinical trial *(ClinicalTrials.gov Identifier: NCT02631538)*. The study examined the safety and efficacy of co-administration compared to rituximab monotherapy, belimumab monotherapy, and placebo. All-cause mortality was higher in the combination arm of the study, but serious adverse events occurred at a similar rate in all medicine arms. In terms of efficacy, co-administration of the two drugs showed better results than monotherapy vs. placebo, as well as in comparison to each of the two drugs when administered alone, primarily against belimumab monotherapy. These results have not yet been published, but

safety issues must be carefully considered despite the encouraging efficacy of this co-administration of B cell-depleting therapies.

Abatacept, a biologic that blocks the interaction between CD80/CD86 on APCs and CD28 on T cells, thus impeding activation of the latter cell group, has known efficacy in RA[111,112]. However, a recent phase III clinical trial did not show efficacy in SS patients as neither the primary (ESSDAI and ESSPRI score reduction) nor critical secondary endpoints were achieved[113]. Nevertheless, although a single-center, randomized, double-blind, placebo-controlled phase 3 trial of the same drug failed to reach its objectives at six months[114], the extension of this study for another six months while all SS patients received abatacept showed improvement, with 50% of patients reaching low disease activity (ESDDAI<5)[115], reviving the interest of the scientific community for the drug.
A phase 2, double-blind, placebo-controlled study was performed to evaluate the impact of 8 intravenous infusions of RSLV-132, an RNAse Fc fusion protein, in 28 patients with primary Sjogren's syndrome *(NCT03247686)*. Fatigue, which also correlated with the expression of selected IFN-inducible genes, was improved in all patients who received the drug[116].

VAY736 (Ianalumab) is a monoclonal antibody directed against the BAFF receptor. Ianalumab has two modes of action: direct lysis of B cells by antibody-dependent cellular cytotoxicity and BAFF receptor blockade that interrupts BAFF-mediated signaling for B-cell maturation, proliferation, and survival. A proof of concept study showed that a single intravenous dose of Ianalumab reduced disease activity, key symptoms, and B-cell concentrations in patients with primary Sjögren's syndrome[117]. VAY736A2201 was a randomised, parallel, double-blind, placebo-controlled, phase 2b dose-finding study. Participants were randomly assigned (1:1:1:1) to receive a subcutaneous placebo or Ianalumab (5 mg, 50mg, or 300mg once monthly for six months) (NCT02962895). The ESSDAI score decreased from baseline in all Ianalumab groups, with the maximal ESSDAI score change from baseline observed in the Ianalumab 300 mg group[118]. Three other studies of the drug have been initiated but not yet recruiting.

Furthermore, a multi-center, randomized, double-blind, placebo-controlled, parallel-group study was performed to evaluate the safety, tolerability, pharmacokinetics, and

preliminary efficacy of CFZ533 (Iscalimab) -an anti-CD40 agent- in SS *(NCT02291029)*. Statistically significant results were shown only in the 10mg/kg i.v. regimen. Trials assessing the safety and efficacy of multiple doses in SS patients *(NCT03905525)* and safety and tolerability *(NCT04541589)* are currently recruiting.

Prezalumab/AMG557/MEDI5872 is a human antibody directed against ICOS/CD278 ligand (ICOSL), expressed on T cell surface[119]. In the context of SS, a phase IIa, randomized, placebo-controlled, proof of mechanism, safety, and efficacy study, was designed. ESSDAI improved in participants receiving the drug, while no deaths were reported, and only 1/31 had a severe adverse event in the form of a gastrointestinal polyp *(ClinicalTrials.gov Identifier: NCT02334306)*. However, these results have not yet been published.

Unfortunately, failures are more frequent than successful results in clinical trials evaluating new treatment options for SS.

Despite preliminary encouraging results from animal models[120], a multicentre, double-blind, randomized placebo-controlled trial of Tocilizumab, an IL-6 antibody and receptor inhibitor, also failed to demonstrate an improvement in clinical picture after six infusions in patients with SS[121]. Equally, despite preliminary encouraging results of the administration of petesicatib -an inhibitor of Cathepsin S, the cysteine protease involved in major histocompatibility complex (MHC) II processing and T cell stimulation- in biocompartments of SS patients[122], a recent multicenter, randomized, double-blind, placebo-controlled, phase IIa study of RO5459072 in 75 SS patients after 12 weeks of treatment did not demonstrate any significant changes in ESSDAI *(ClinicalTrials.gov Identifier: NCT02701985)*.

Previous studies have elucidated the role of phosphorylating enzymes or kinases in the transduction of intracellular signals in SS. More specifically, Janus, spleen tyrosine, and Bruton's kinase (JAK, TYK2, and BTK, respectively) have been more extensively studied in hematological malignancies where B cells have a central role in pathogenesis[123,124] and seemed therefore like a possible target for SS treatment. In addition, Baricitinib, an oral inhibitor of JAK1/JAK2 that is already being used for the treatment of RA[125], has shown efficacy in SS in a prospective pilot study (*ClinicalTrials.gov identifier: NCT04916756*)

and is now being investigated in a multi-center, prospective, open-label, randomized study *(NCT05016297)*.

On the other hand, a randomized, phase 2, double-blind, placebo-controlled study was undertaken to assess the safety and efficacy of filgotinib, lanraplenib, and tirabrutinib (a Janus kinase-1 inhibitor, a spleen tyrosine kinase inhibitor, and a Bruton's tyrosine kinase inhibitor, respectively) in SS patients *(NCT03100942)*. Unfortunately, none of the drugs examined met either the primary or secondary endpoints of the trial[126].

Dysregulation of the phosphoinositide 3-kinase δ (PI3Kδ) signaling pathway has been implicated in the pathogenesis of SS[127]. CDZ173 (leniolisib), an inhibitor of PI3Kδ, was studied in another trial. Regarding safety, no deaths were reported, and only 1/20 of participants who received the study drug exhibited severe adverse events. At the same time, ESSDAI, ESSPRI, fatigue, and physician and patient estimation of disease activity improved. These results have not yet been published *(ClinicalTrials.gov Identifier: NCT02775916)*. Open-Label Phase 2 Study of Parsaclisib (INCB050465) in SS is completed without any results published yet *(ClinicalTrials.gov Identifier: NCT03627065)*.

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2. SPECIAL PART

Since the first description of SS early in the 20th century, much has been revealed regarding the pathogenesis of this entity and potential treatment options. Type I IFN seems to play a significant role in both initiation and prolongation of disease. Stimulation of pDCs in susceptible individuals releases IFN stimulated genes from PBMCs, mononuclear and B cells, inducing their activation and proliferation, thus augmentation of inflammation. In the case of B cells, this is evident with autoantibody production, in this case, anti-Ro/SSA and anti-La/SSB, which contribute in disease pathogenesis. Nevertheless, assessment of other autoantibodies implicated in autoimmune diseases closely related to SS, such as SSc, has not yet been thoroughly investigated. The pathophysiological and clinical implications of type I IFN signature in SS and the prevalence and clinical implications of SSc-specific autoantibodies and their potential role in disease diagnosis are further elucidated below.

2.1 Type I interferon signature in Sjögren's Syndrome: Pathophysiological and clinical implications

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Abstract

Type I interferons (IFN) have long been recognized as mediators of innate immune defense mechanisms against viral threats. Robust evidence over the last 15 years revealed their significant role in the pathogenesis of systemic autoimmune diseases including systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS). Despite the progress, methods of detection, initial triggers, biological functions and clinical associations in the setting of autoimmunity remain to be fully clarified. As therapeutic options for SS are currently limited, neutralizing specific targets of the type I IFN pathway seems a promising option. In this review we summarize the current evidence regarding the role of type I IFN in SS.

Introduction - Overview

Sjögren's syndrome (SS) is a chronic autoimmune entity affecting typically the exocrine glands of middle-aged women. Infiltration of immune cells in the salivary and lacrimal glands leads to loss of secretory function and eventually to desiccation of oral and ocular mucosa. In a significant proportion of patients, extra glandular manifestations are present involving mainly the joints, kidneys, lung, and peripheral nervous system (1)(2). Activation of innate immune pathways has long been considered as central pathogenetic contributor, with type I interferon (IFN) attracting particular interest over the last decade (3).

Type I IFNs -members of the class II family of α-helical cytokines, along with type II IFNs and numerous other cytokines- include 13 IFNα subtypes, β, ε (expressed in placenta, supposedly having a role in reproduction), κ (in keratinocytes), ω, δ, and τ (the two latter not found in humans). They mainly signal through IFNα/β receptor (IFNAR), consisting of two subunits IFNAR1 and IFNAR2. Upon binding with IFNs, autophosphorylation of the Janus protein kinases Tyk2 and Jak1 -attached to IFNAR1 and IFNAR2 subunits respectively-, occurs. As a result, signal transducer and activator of transcription (STAT) 1 and 2 are phosphorylated leading to the formation of a STAT2/STAT1 heterodimer, which following binding of interferon-regulatory factor 9 (IRF-9), leads to the formation of a complex namely IFN-stimulated gene factor 3

(ISGF3). The latter translocates to nucleus and following binding to specific elements (interferon stimulated response elements) on the promoters of IFN stimulated genes (ISGs) leads to their transcription. The encoded ISG proteins are characterized by a wide variety of antiviral properties including prevention of the assaulting virus from entering the cells and subsequently being replicated, translated, assembled and released (4)(5).

Though the main action is antiviral/antineoplastic, several immunomodulatory functions have also been attributed to type I IFNs including induction of B cell activating factor (BAFF) (6), immunoglobulin switching (7), increased antigen presentation, T-cell mediated and natural killer cell (NK) cytotoxicity (8). As a result of these immunostimulatory properties, a tight control of type I IFN activation is mandatory in order to ensure adequate host defenses avoiding collateral tissue damage by excessive responses. Thus, checkpoints at several levels of type I IFN pathway have been detected including mainly post-transcriptional modifications of the signaling pathway (phosphorylation) and regulation of type I IFN expression by epigenetic modifications including DNA methylation, histone modification and non-coding RNA effects (9).

While virtually any cell is able to produce type I IFNs (10) following stimulation by exogenous or endogenous nucleic acids either through toll-like receptors (TLRs) or cytoplasmic sensors, plasmacytoid dendritic cells (pDCs) are considered to be the main IFNα producing cells. They are characterized by increased constitutive expression of TLR7 and TLR9 -stimulated by ssRNA/endogenous RNA and unmethylated DNA respectively- and a rapid and robust response following triggering by nucleic acids (11). Upon ligation, they signal through the myeloid differentiation primary response 88 (MyD88) activating IRF7, which has a central role in type I IFN production (12).

The main cytoplasmic receptors able to detect endogenous nucleic acids include the DNA sensors absent in melanoma 2 (AIM2) and cyclic GMP-AMP synthetase (cGAS) sensing dsDNA as well as the retinoic acid-inducible gene I (RIG-I) like receptor (RLR) family comprising of RIG-I and Melanoma differentiation associated gene 5 (MDA5) triggered by dsRNA (13). The downstream signaling pathway mediates the phosphorylation of IRF3, 7 and Nuclear Factor-kappa B (NFκB) and subsequent type I IFN and induction of proinflammatory cytokine genes (14).

Activation of type I IFN pathway in SS

The first indication of type I IFN activation in SS dates back to late seventies (15). Since then, a growing body of evidence supports a significant role for type I IFN in the pathogenesis of SS, similarly to what was observed in other systemic autoimmune diseases such as systemic lupus erythematosus (SLE) (16), dermatomyositis (17) and systemic sclerosis (18).

Using microarrays and real time PCR studies, upregulation of ISGs was initially detected in SS disease targets such as minor salivary gland (MSG) biopsies (19)(20)(21) and ocular epithelial cells (19) compared to healthy controls. After immunoblot testing using specific probes for either type I or type II IFN related proteins (IFIT-3 and GBP-2, respectively) the heterogeneity of IFN activity at the level of MSG tissues has been appreciated (22). In a follow up study, using validated probes for type I or type II IFN pathway, three IFN patterns in MSG biopsies were detected including either a predominantly type I or type II, or a mixed type I/II IFN; IFIT-3 (another type I IFN inducible gene) mainly stained salivary duct epithelial cells, while GBP-2 (a type II IFN related gene) was found in both lymphoid and duct epithelial cells surrounded by inflammatory cells by immunohistochemistry (23).

Beyond salivary gland tissues, the presence of type I IFN signature was next evaluated at systemic level. Thus, increased expression of type I IFN inducible genes or proteins has been revealed in SS derived peripheral blood mononuclear cells (PBMCs) (24)(25), whole blood (24)(26)(27), monocytes (28)(29)(30) and recently B-cells (31)(25). Based on previous work on lupus (32) and SS derived salivary gland tissues (23), Bodewes et al revealed 3 distinct IFN patterns in peripheral blood from SS patients, a type I IFN predominant, a type I and II IFN mixed pattern as well as an inactive one (33). The presence of a heightened type I IFN signature in SS ranges from 53% to 81% in several gene expression studies (34)(35)(27)(33).

Measurement of IFNs in periphery has traditionally been elusive (36). While initial reports failed to detect the presence of IFNα in serum (37), possibly due to the presence of many type I IFN subtypes currently undetectable by commercial ELISAs (38), the introduction of bioassays allowed the detection of systemic type I IFN activity in SS

serum (28) or plasma (6), which seems to account for the upregulated type I IFN signature. Whether IFNα (39)(40) or IFNβ (28) account for the increased type I IFN activity in SS peripheral blood remains to be clarified. In a recent study an advanced ELISA with single molecule array (SIMOA) digital technology was used in order to measure attomolar IFNα levels in different groups, including adult and juvenile SLE, diabetes mellitus (DM), and IFNopathies (41). This technique, though promising, has not been applied in SS yet.

Given the apparently conflicting data between studies in regard to whether type I IFN or II predominate in SS and taken into account SS phenotypic heterogeneity, the overlapping regulation of many ISGs by both type I and II IFNs and the type of biological sample implemented, we proceeded to quantitation of transcripts predominantly induced either by type I or type II IFN in both peripheral blood and MSG tissue from well characterized SS patients. Thus, while in peripheral blood a type I IFN predominant signal was observed, in salivary glands type II IFN related genes were mainly overexpressed. Moreover, increased IFNγ but low IFNα transcripts were detected in MSG tissue derived from SS patients with lymphoma compared to SS with no lymphoma and sicca controls; as a result the IFNγ/IFNα ratio has been shown to be a potential biomarker for identification of lymphoma among SS patients with high area under the curve values in the Receiver-operating characteristic analysis (ROC) (27).

Additionally, recent data support a contributing role for type III IFN -namely IFNλ- in the pathogenesis of SS. All three subtypes IFN-λ1/IL-29, IFN-λ2/IL-28A and IFN-λ3/IL-28B signal through the heterodimeric IFN-λ R1/IL-28Ra receptor predominantly expressed in pDCs. SS patients with intermediate lesions in MSGB tissue were found to have increased both IFN-λ2/IL-28A epithelial expression and IFN-λ1/IL-29 levels in the periphery in comparison to sicca controls (42). Furthermore, in another study the addition of IFN-λ1/IL-29 to IFNα led to even more enhanced stimulation of the ISGs BAFF and CXCL10 as well as the prolongation of phosphorylation of STAT in the immortalized human salivary gland ductal cell line NS-SV-DC, implying a synergistic effect of IFNα and IFNλ in the pathogenesis of SS (43), although studies in a larger scale are needed to confirm these results.

Of interest, proteins induced by type I IFNs, such as sialic acid binding Ig like lectin 1 (SIGLEC1), a cell surface protein on monocytes and macrophages detected by flow

cytometry in peripheral blood, was shown to correlate with EULAR Sjogren Syndrome Disease Activity Index (ESSDAI) score and discriminate SS patients with glandular and extraglandular manifestations (30). In a recent report, soluble SIGLEC5 –a transmembrane member of immunoglobulin superfamily expressed in neutrophils (44)- was found to be elevated in saliva but not serum in SS patients compared to controls, in association with impaired salivary secretion, increased ocular damage and higher serum IgG levels (45).

Though the source of systemic and local IFN production has not been yet fully elucidated, several lines of evidence point toward pDCs, the professional type I IFN producing cells as chief contributors of IFN signature in SS. Thus, reduced (28)(46) but activated pDCs (28) in peripheral blood of SS patients together with their identification at the level of salivary gland tissue (19)(27)(37), imply a potentially significant role in both systemic (28) and local IFN activity (19)(27)(37).

Contributors of exaggerated type I interferon production in SS

The initial trigger of type I IFN production in the setting of autoimmunity remains an area of intensive research, a growing body of data so far strongly support endogenous nucleic acids as a potential source for the intrinsic activation of type I IFN system in the absence of exogenous viruses. In SS, similarly to studies in lupus (47), immunocomplexes consisting of RNA-containing apoptotic bodies and antibodies against ribonucleoproteins derived from SS sera were shown to induce type I IFN production, following pDC stimulation (37).

Previous studies revealed that DNA derived from SLE patients was characterized by repetitive sequences (48), was enriched in CpG nucleotides and displayed high homology to retroviruses (49). Moreover, Perl et al (50) noted the presence of antibodies to endogenous retroviruses in sera of patients with several autoimmune diseases including SS. A potential source of endogenous retroviral material include Long interspersed nuclear elements (LINEs; L1), which comprise 17% of the human genome and are able to translocate within the genome, once they are fully transcribed (51). The typical structure of L1 elements includes open reading frames (ORF) that can encode an

endonuclease and a reverse transcriptase. L1 promoter methylation is one of the major mechanisms that keep it suppressed, though other controlling mechanisms have been also described (52)(53). We have previously shown that L1 transcripts were found to be overexpressed in SS patients compared to sicca controls in strong correlation with both IFNα and β at the level of MSG tissues. Failure of upregulation of L1 expression following stimulation of healthy PBMC and other cell lines with IFNα and TLR7/ TLR9 ligands excluded the possibility of Type I IFN-induced L1 overexpression. On the other hand, transfection of pDCs or $CD14^+$ monocytes with L1-carrying plasmids or L1-RNA led to type I IFN pathway activation through both TLR dependent and independent pathways, evidenced by the abrogation of type I IFN production following incubation with a TLR7/TLR8 or a TNF receptor associated factor NF-κB activator (TBK1)/IKKε inhibitor (54). Of interest, L1 expression was increased in patients with uncomplicated local SS which usually occurs in patients with advanced age (55). In line with these observations, a recent study has shown that L1 can drive type I IFN production in senescent cells (56).

Compatible with these findings, SS patients with heightened type I IFN scores in peripheral monocytes were shown to display increased transcript levels of both endosomal and cytoplasmic nucleic acid receptors such as TLR7, MDA5, RIG-I and protein kinase R (PKR) in both pDCs and monocytes, implying their contributory role in type I IFN production (57). Moreover, increased basal phosphorylation levels either in B cells (58) or T and Natural Killer (NK) cells together with enhanced B cell signaling through TLR7 and TLR9 ligation (25), have been proposed as potential drivers of augmented IFN responses in these patients.

Possible mechanisms accounting for L1 derepression remain of particular interest. A negative correlation between L1 expression and methylation levels of the L1 promoter were highly suggestive of impaired methylation mechanisms in SS patients (54). In order to further explore underlying abnormalities accounting for the decreased methylation levels, we measured gene expression of several members of the methylation machinery and found a positive correlation between DNMT1, DNMT3B and MeCP2 and L1 expression in SS salivary glands and a negative correlation with lymphoid specific helicase (LSH). These observations imply a potential compensatory role for DNMT1,

DNMT3B and MeCP2 in controlling inappropriate L1 overexpression with decreased LSH production potentially being responsible for the hypomethylation of L1 promoter (59).

Another restricting mechanism implicated in both viral infections and L1 control include members of the APOBEC family (60)(61). We have recently shown upregulation of APOBEC3A transcripts in SS MSG tissues in strong correlation with both L1 and IFNα mRNA, reflecting a potentially compensatory role against endogenous retroelements (62).

Finally, genetic influences have been shown to have an impact on type I IFN responses in the setting of autoimmunity. Thus, similarly to lupus (63), the rs10774671 variant of the 2'-5'-oligoadenylate synthetase 1 (OAS1), a viral RNA degrading enzyme, previously shown to be a risk allele for SS, has been shown to be related to a dysfunctional transcript. Thus, upon viral infection, a defective clearing mechanism of virus possibly occurs, leading to perpetuation of type I IFN responses due to the ongoing activation by viral remnants. In view of the implication of several viral triggers in SS pathogenesis (64), this mechanism might provide a functional explanation in the pathogenesis of SS. Moreover, in another study we have shown that protein tyrosine phosphatase non-receptor 22 (PTPN22) –an allele associated with other autoimmune diseases such as SLE, DM and Grave's disease- is increasingly found in SS patients in comparison to healthy individuals, especially those with low type I IFN signature (65).

Type I IFN activation in SS- Clinical associations

Ocular and oral dryness are considered to be the most characteristic clinical features of SS, but extraglandular manifestations can occur in at least one third of patients. The effort to associate specific clinical phenotypes with high or low IFN signature score follows the more well-established correlation between autoantibody/serological profiling and histopathological information from MSG biopsies, on the one hand and disease severity or even lymphomagenesis probability, on the other (66)(67).

A high type I IFN signature in CD14 monocytes was previously shown to identify SS patients with higher ESSDAI scores (34). However, total ESSDAI was not linked to high IFN activity (type I or mixed I/II) in a more recent study (33). SS individuals

characterized by high type I IFN score have also exhibited higher anti-Ro52, anti-Ro60, anti-La, RF and serum IgG, as well as lower C3 levels and lower lymphocyte and neutrophil counts (34). Given that anti-Ro, anti-La and RF positivity has been associated with earlier disease onset, heavier glandular involvement and extraglandular manifestations, it is clear that there are clinical implications behind this association (68).

Despite the extensive knowledge on IFNα administration adverse effects, with flu-like symptoms and fatigue being the main complaints of patients receiving this treatment for various conditions, fatigue in the context of SS does not seem to correlate with IFN related genes (33)(69). As for IFN-induced protein expression on glandular tissue level, results from immunoblotting and immunohistochemistry showed a correlation between high IFN type I, II or mixed type I/II scores and more severe glandular secretory insufficiency, leukopenia and high ANA, anti-Ro/SSA, IgG and IgA titers. Last but not least, MSG biopsies focus score was remarkably higher in the high IFN type II group compared to low IFN altogether or high IFN type I groups (23).

All of the above lead to the impression that while both IFN types share some common pathways in the pathogenesis of SS, such as BAFF upregulation, it is the predominance of type II over type I IFN in the glandular tissue that tips the balance and promotes MALT lymphomagenesis. High focus score and low C4, seen more frequently in type II IFN-predominant MSG tissue group, have previously been identified as risk factors for lymphomagenesis in SS (23). Furthermore, IFNγ/IFNα mRNA ratio in MSG tissue has emerged as a histopathological biomarker for the prediction of in situ lymphoma development (27).

Last but not least, the presence of autoantibodies to interferon inducible protein-16 (IFI16) has been proven in various autoimmune diseases, including SS, in which it seems to be quite common. Anti-IFI16 antibody positivity characterizes more frequently patients with abnormal Schirmer's test, elevated IgG levels and high ANA titers, as well as higher focus scores and germinal center-like structures in their MSG biopsies (70). To be noted that IFI16 is an IFNγ-inducible protein which acts as a DNA detector in case of infection and regulates IFN type I transcription, with IFI16-knockout cells producing dramatically reduced IFNα (71).

A recent study revealed higher EULAR Sjogren's Syndrome Patient Reported Index (ESSPRI) total and ESSPRI sicca and pain domain scores among patients with the highest cumulative smoking consumption. Interestingly, type I IFN signature showed an inverse correlation with these domains. However, never, former or current smokers did not show remarkable controversy regarding type I IFN positivity or ESSDAI total score (72). Finally, in a recent study of medication-free SS patients receiving vaccination against H1N1, increased levels of protective antibodies were observed mainly as a result of IFNα induced B cell hyper responsiveness (73).

Treatment

Given that type I IFN overexpression seems to play a central role in disease initiation and progression in both SLE and SS, therapeutic attempts to downregulate its effect are gaining increasing attention over the last years (74).

As endogenous RNAs have been considered as primary drivers of type I IFN in systemic autoimmunity, RNA degradation seems to be a logical target. RSLV-132, a fully human biologic Fc fusion protein comprising of an RNase fused to the Fc domain of IgG1, has been shown to inhibit type I IFN production. This agent is presently being tested in a phase II, double-blind, placebo-controlled study of 28 SS patients in light of promising results in a recently published clinical study of 32 SLE patients (75). The SS study is complete but no results have been as yet published (ClinicalTrials.gov Identifier: NCT03247686).

Of interest, administration of the antiviral agent lamivudine in an experimental aged model has been shown to downregulate type I IFN production through inhibition of L1 reverse transcriptase (56). These data might be relevant in SS patients, in which an upregulation of L1 retroelements have been observed at the level of salivary gland tissue (54).

Given that pDCs are viewed as a major cellular source of type I IFN, targeting of this cell population is also a promising anti-IFN strategy. The humanized monoclonal antibody 24F4A binds to the C-type lectin BDCA2, which is a specific receptor for pDCs, thus inhibiting IFN production. Clinical trials of BII059, another BDCA2 ligand, showed

encouraging results in SLE, but not in SS patients yet. Immunoglobulin-like transcript 7 (ILT7)/LILRA4/CD85g -a member of the immunoglobulin-like transcripts (ILTs) or leucocyte immunoglobulin-like receptors (LIR) gene family- is a molecule predominantly expressed on the surface of pDCs (76). It has been shown that activation of ILT7 leads to differentiation of pDCs from an IFN producing to an antigen presenting phenotype via downregulation of TLR7/TLR9 mediated IFN production (77)(78)(79). Stimulation of ILT7 by an antibody (MEDI7734/VIB7734) was tested in 36 patients of type I IFN-mediated autoimmune diseases including SS. Although the study was completed, no results have as yet been published (ClinicalTrials.gov Identifier: NCT02780674).

TANK is another promising target in view of the significant role it holds a in type I IFN production through interferon IRF-3 and IRF-7 ligation. Administration of BX795 -a TANK inhibitor- in PBMCs derived from SS patients resulted in downregulation of type I IFN inducible genes (80).

The idea to use monoclonal antibodies (mAbs) against IFNα in a way similar to that of TNFα inhibition in RA or even to produce anti-IFNα antibodies through vaccination has been proven in SLE with moderate results, failing to downregulate fully the IFN signatures. This might occur either because the antibodies fail to target all IFNα variants or do not affect other types of IFNs (81)(82). Targeting IFNAR has shown encouraging results in SLE patients with high IFN signature, but at the cost of upper respiratory tract infections and herpes zoster reactivation (83).

As discussed earlier, JAK kinases have been shown to mediate type I IFN effects following ligation of IFNAR. Small molecule JAK inhibitors are in clinical trials against SLE and SS (ClinicalTrials.gov Identifier: NCT03100942), with filgotinib showing encouraging results (84) while tofacitinib, already approved for RA, has shown efficacy in murine NZB/NZW F1 and MRL/lpr SLE models (85). No data in SS are currently available.

BAFF levels are heightened in SS and associate positively with both type I and II IFN signatures at both peripheral blood and salivary gland tissue (27). Belimumab, a fully humanized monoclonal antibody towards the soluble B lymphocyte stimulator (BLyS), was tested in SS in two European centers, Paris, France and Udine, Italy

(ClinicalTrials.gov Identifier: NCT01160666 and NCT01008982 respectively), with promising results. In a follow-up assessment of SS patients participating in the initial BELISS study a clinical and immunological deterioration was observed 6 and 12 months after cessation of treatment (ClinicalTrials.gov Identifier: NCT01008982). Interestingly, in a subgroup of the patients recruited for the BELISS study increased blood and salivary NK cell numbers in association with a worse response to treatment with belimumab was reported (86), while increased type I IFN scores at baseline were associated with improved outcomes, such as reduced IgG, IgM and RF serum levels (87). The authors based on these findings proposed the existence of two distinct subsets of SS: one with a predominant type I IFN-BAFF-B cell axis, representing good responders to belimumab; and one with a predominant type II IFN-NK cell axis, representing non-responders (86)(87).

Despite the major success in many rheumatic diseases, TNF inhibition failed to demonstrate efficacy in patients with SS (88)(89). Augmentation of the already upregulated type I IFN activity and the ensuing increased BAFF levels has been postulated as a potential reason for TNF failure in these patients (40).

Conclusion

With regard to SS, it seems that type I IFN dysregulation and overexpression is implicated in disease pathogenesis making its blockade an attractive therapeutic target. Although no concrete data exist as yet on medications against type I IFN currently on clinical trials, current evidence indicate that inhibition of this pathway at various stages could alter the course of the disease for SS patients. In the future, as our knowledge on its pathogenetic role in SS expands, more advanced agents will be targeting this pathway in an attempt to restore the balance of the immune system in these patients.

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2.2 Scleroderma-specific autoantibodies: Should they be included in the diagnostic work-up for Sjögren's syndrome?

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Abstract

Objectives: Sicca complaints are a frequent reason for rheumatologic consultation. Testing for specific antibodies against Ro/SSA and La/SSB antigens and minor salivary gland (MSG) biopsy are among the main tools implemented in the diagnostic work-up. Anticentromere antibodies and sicca manifestations are frequently detected in Sjögren's syndrome (SS) and systemic sclerosis (SSc), respectively. Herein, we aimed to determine the frequency and clinical associations of a wide spectrum of scleroderma (SSc)-specific autoantibodies in consecutive patients referred for evaluation of possible SS.

Methods: Demographic, clinico-pathological, and laboratory data were recorded in 216 consecutive patients with sicca complaints. All study participants were tested for SSc-specific autoantibodies (against CENP, PM/Scl, Scl-70, Ku, NOR90, RP11, RP155, fibrillarin, PDGFR, and Th/To) using a commercially available immunoblot kit. According to band intensity, the identified autoantibodies were further classified in those with strong and medium titers.

Results: SSc-specific autoantibodies were detected in 41.7% (90/216) patients evaluated (19% at strong, 22.7% at medium titers) without significant differences between anti-Ro/SSA positive and negative groups. At strong titers was significantly higher in patients with MSG biopsies fulfilling SS histopathological criteria (30% vs 12.5%, p=0.009). This association remained significant after adjustment for antibodies against Ro/SSA and La/SSB autoantigens [OR 95% (CI): 4.1 (1.5-10.6)].

Conclusion: SSc-specific autoantibodies are frequently detected among patients presenting with sicca complaints and at strong but not medium titers are independently associated with MSG biopsy positivity. Taken together, these data imply a useful role of SSc antibody testing in the diagnostic work-up and possibly in the classification criteria for SS.

Keywords: Scleroderma-specific autoantibodies, Sjögren's syndrome, anti-Ro/SSA, anti-La/SSB, Sicca

Highlights

- Systemic sclerosis (SSc) specific autoantibodies are frequently detected in unselected patients with sicca complaints evaluated for Sjögren's syndrome
- At strong titers, they are associated with histopathological features compatible with Sjögren's syndrome (SS), independently of anti-Ro/SSA and La/SSB status
- These data possibly imply SSc antibodies as an additional tool in the diagnosis and classification of Sjögren's syndrome

Introduction

Differential diagnosis in patients presenting with dry eyes and dry mouth (sicca) symptoms is broad and often challenging for the practicing physician. As Sjögren's syndrome (SS) is the leading cause of autoimmune-related dryness symptoms, patients with sicca complaints undergo meticulous examination for the detection of objective oral and ocular dryness, serological testing for autoantibodies against Ro/SSA and La/SSB antigens and minor salivary gland (MSG) biopsy. For the exclusion of important SS mimics, such as chronic viral infections, sarcoidosis and IgG4 related sialadenitis, viral testing for hepatitis C and human immunodeficiency virus, chest X-Ray and IgG4 serum levels are also considered. Use of medications such as antidepressants and antihistamine among others are common causes of dry complaints and should be always considered in the differential diagnosis [1].

Despite the extensive evaluation and the strong suspicion for the presence of underlying SS on several occasions, the diagnosis of SS cannot be drawn, and sicca complaints cannot be always fully explained in the context of a well-defined clinical entity. These patients not fulfilling classification criteria for SS[2] were

previously designated as suffering from dry eyes and mouth syndrome (DEMS)[3] or SAPS (sicca, asthenia, and polyalgia syndrome)[4]. This group of patients was found to have non-specific chronic musculoskeletal pain, Raynaud's phenomenon (RP), and subjective sicca symptoms at a similar rate to their age- and sex-matched SS counterparts, together with a higher prevalence of anti-thyroid antibodies. A mild interstitial infiltrate along with the presence of perivascular infiltrates in MSG biopsies were the main histopathological findings [5].

Other autoantibodies previously found to be detected in a subgroup of SS patients include those directed against centromere antigens (ACA), namely centromere proteins (CENP). Though they are classically detected in limited forms of systemic sclerosis (SSc), earlier reports revealed their presence in a subset of SS[6] sharing features with limited SSc (lcSSc)[7–13] in association with characteristic ultra-sonographic salivary gland findings such as hyperechoic bands [14].

Beyond anticentromere antibodies, many SSc-specific reactivities directed against nuclear and nucleolar antigens have been identified in recent years and included among others, antibodies against topoisomerase I (Scl-70), RNA-polymerase III (RP), fibrillarin, nucleolar organizing region 90 (NOR90), Th/To ribonucleoprotein (Th/To) and polymyositis/scleroderma (PM/Scl) autoantigens. These antibodies were shown to be associated with distinct clinical features in the setting of SSc, such as puffy hands, RP, esophageal dysfunction, scleroderma renal crisis, interstitial lung disease (ILD)[15]. However, no data on the associations of these antibodies with sicca features have been so far reported.

In the present study we aimed to evaluate the prevalence of SSc-specific autoantibodies in a cohort of consecutive patients presenting with sicca complaints. Moreover, the association of these antibodies with clinical, serological, and histopathological features were investigated.

Patients and Methods

Patients

Two hundred and seventy-nine consecutive patients with sicca complaints were referred for evaluation of possible SS between 01.01.2017 to 30.08.2021 at the Molecular Physiology-Clinical Applications Unit, Department of Physiology, School of Medicine, National and Kapodistrian University of Athens. Sixty-three patients were lost to follow up and were excluded from the study. Therefore, the final study group

consisted of 216 patients, all of whom gave informed consent according to the declaration of Helsinki before they participated in the study.

Evaluation of sicca symptoms

Demographic data, clinical manifestations, medication history, as well as hematological, biochemical, and immunological parameters were recorded. In more detail, subjective and objective oral and ocular dryness measures (documented by unstimulated salivary flow rates and Schirmer's test/Lisammine Green staining, respectively) were obtained. Moreover, testing for chronic viral infections (hepatitis C, human immunodeficiency virus), as well as for several autoantibodies [(Rheumatoid factor, Antinuclear antibodies (ANA), anti-Ro/SSA and La/SSB antibodies] and complement levels was offered. Antibodies against Ro/SSA were determined by chemiluminescent immunoassays as previously described[16]. MSG biopsy was also performed, following patient consent. The latter was considered positive when focus score (FS) was higher than 1 (≥50 lymphocytic infiltrates/4mm^2) of salivary gland tissue examined [17].

SSc-specific autoantibodies

Sera from all 216 patients were evaluated for the presence of SSc-specific autoantibodies by using a commercially available kit [EUROLINE Systemic Sclerosis (Nucleoli) profile (IgG) (EUROIMMUNE Medizinische Labordiagnostika AG)] against the following antigens: Scl-70, CENP A, CENP B, RP11 & RP155, Fibrillarin, NOR90, Th/To, PM/Scl against 75kd & 100kd proteins, Ku, and Platelet-Derived Growth Factor Receptor (PDGFR). According to signal intensity, the SSc-specific autoantibodies were divided into those with strong titer (≥26) and those with medium titer (11-25). A band intensity below 11 was considered to denote negative autoantibody titers.

Statistics

Comparison of categorical variables was performed using a chi-square test. Numerical variables were compared with a t-test or the Mann-Whitney test when data did not follow a normal distribution. Backwards stepwise logistic regression analysis was implemented to explore the independent association

between SSc-specific autoantibodies and MSG biopsy positivity following adjustment for antibodies to Ro/SSA and La/SSB antigens. The SPSS v.26 statistical program was used for the analysis.

Results

Prevalence of primary SS in the sicca cohort

Supp Table 1 summarizes the clinical, laboratory and histopathological characteristics of all study participants. As shown in Fig.1, among the 216 patients of the final study sample, anti-Ro/SSA antibodies were detected in sera of 85 (39.4%) patients (seropositive), while the rest 131 (60.7%) were seronegative. MSG biopsies were available in 72 out of 85 (84.7%) in the anti-Ro/SSA positive group and 70/131 (53.4%) in the anti-Ro/SSA negative group. Classification criteria for primary SS were fulfilled in 88 out of 216 participants (40.7%) [82.4% (70/85) in the seropositive and 13.7% (18 out of 131) in the seronegative group] according to the 2016 ACR/EULAR classification criteria for primary SS [2]. In the anti-Ro/SSA- group without available MSG biopsies (n=61), the diagnosis of primary SS cannot be excluded.

Prevalence of SSc-specific autoantibodies in sicca patients

Among 85 anti-Ro/SSA (+) patients by chemiluminescent immunoassays, 73 (85.9%) tested positive for anti-Ro52 according to the EUROLINE immunoassay implemented. The remaining anti-Ro/SSA positive sera were most likely reactive against Ro-60 autoantigen. Given the previously reported occurrence of sicca complaints in the context of SSc[8] we next sought to determine the prevalence of a wide spectrum of SSc-specific antibodies in our cohort. As shown in Fig.2A, the overall frequency of SSc-specific antibodies was 41.7% (19% at strong titers and 22.7% at medium titers). Following stratification according to the anti-Ro/SSA status, the corresponding frequencies for the seropositive vs. seronegative group were 44.7% vs. 39.7% for all SSc-specific antibodies, 18.8% vs. 19.1% for the SSc-specific antibodies at strong titers and 25.9% vs. 20.6% for those at medium titers (non-significant differences between anti-Ro/SSA+ and anti-Ro/SSA- subsets were detected). The type and frequencies of SSc specific antibodies at strong and

medium titers and according to anti-Ro/SSA status are displayed in Suppl. Tables 2-4. Of note, while anticentromere staining pattern on the immunofluorescent ANA testing was detected in 11 out of 216 patients (5.1%), reactivities against CENPA and CENPB were present in 21 out of 216 sera tested by Euroline immunoblot (9.7%).

Association of SSc-specific antibodies with MSG biopsy positivity

We next sought to explore potential associations between MSG biopsy positivity and SSc-specific antibodies (strong and medium titers). As shown in Fig.2B and Table 1, significantly increased rates of SSc-specific antibodies at strong titers were detected among patients with positive MSG biopsies compared to those without (30% vs 12.5%, p-value: 0.009), [OR 95% CI: 3.0 (1.3-7.1)]. Such differences were not detected for medium SSc-specific titers. Other features associated with MSG biopsy positivity included anti-Ro/SSA and anti-La/SSB positivity and abnormal Rose Bengal staining (Table 1). Multivariate analysis revealed an independent association of SSc-specific antibodies at strong titers with MSG biopsy positivity, following adjustment for anti-Ro/SSA and anti-La/SSB autoantibodies [OR 95% (CI): 4.1 (1.5-10.6)]. The prevalence of SSc-specific antibodies at strong and medium titers in patients according to anti-Ro/SSA and MSG biopsy positivity is displayed in Suppl Fig 1. Theoretically, if detection of SSc-specific antibodies in the sera of anti-Ro/SSA (-) individuals with negative or unavailable MSG biopsies had the same weight as that of anti-Ro/SSA, then additional 30.3% (33/109) of patients would fulfill 2016 SS classification criteria [13.8% (15/109) for strong titers and 16.5% (18/109) for medium titers, respectively). Overall, 38.9% (51/131) of anti-Ro/SSA negative individuals presenting with sicca complaints would fulfill SS classification criteria instead of 13.7% (18/131), as displayed in Fig.1.

The type and frequency of different SSc-specific antibodies at strong titers according to MSG biopsy positivity are displayed in Fig.3 and Suppl. Table 5. While the diversity of SSc-specific antibodies was greater in the MSG (+) group compared to MSG (-), no significant differences in frequency terms were detected.

In Figs. 4 & 5, representative MSG biopsies from patients with various SSc antibodies are displayed. While histopathological criteria for SS are not fulfilled for all patients, the presence of fibrosis alone or along with lymphocytic infiltrates was detected (photos taken using Olympus Slideview VS200).

Associations of SSc-specific antibodies with distinct features

We next wished to explore whether SSc-specific autoantibodies are associated with distinct clinical and histopathological features. As shown in Supp Tables 6-8, arthralgias and myalgias occurred more frequently in patients with medium titers versus those with negative serum SSc antibody titers, while minor salivary gland histopathological focus scores ≥ 1 were more frequently detected among patients with strong SSc titers (63.3 vs 40, p=0.029).

Upon clinical indication, further investigation was performed in the SSc-specific antibody-positive patient group. High resolution thoracic computed tomography (HRCT) was performed in eighteen cases of those having SSc-specific antibodies at strong titers, of which 11 (61%) had abnormal findings including micronodules, peribronchial thickening and ground glass pattern. Additionally, 16.7% (2 out of 12) patients had abnormal pulmonary function tests (PFT) consistent with restrictive disease, while pulmonary hypertension (defined as PAH>30 mm Hg measured by heart ultrasound (HUS), reflecting PAH>20mm Hg measured by right heart catheterization)[18] was detected in 3 out of 13 (23.1%). The corresponding figures for patients with SSc specific antibodies at medium titers were, abnormal findings in HRCT for 9/15 (60%), abnormal pulmonary function tests (PFTs) for 3/9 (33.3%), and pulmonary hypertension for 5/11 (45.5%) performed tests, respectively. Telangiectasias were noted in 2/41 (4.9%) of patients with SSc antibodies at strong titers and 2/49 (4.1%) of those with medium ones, respectively. Table 2 displays the final diagnosis of the study participants following extensive work-up in the anti-Ro/SSA positive and anti-Ro/SSA negative groups. Moreover, the prevalence of SSc positivity in each diagnostic group is displayed. While SS is the prevalent diagnosis among anti-Ro/SSA positive individuals (82.4%), UCTD was the commonest diagnosis among anti-Ro/SSA negative individuals (34.4%). Of interest, the prevalence of SSc specific autoantibodies in anti-Ro/SSA negative individuals with SS and UCTD was 66.7 and 71.1, respectively.

Discussion

In the present study, we report that SSc-specific antibodies were frequently detected in a cohort of consecutive patients evaluated for sicca complaints in an outpatient rheumatology setting. Notably, SSc specific at strong -but not medium- titers were found to be independently associated with positive MSG biopsies, even after adjustment for the presence of serum antibodies towards Ro/SSA and La/SSB antigens, implying a potentially contributory role of these antibodies in SS classification. Reactivities against

centromere proteins accounted for almost half of all SSc specific antibodies detected at strong titers, with the remaining being directed against other nucleolar (NOR90, fibrillarin, PM/Scl, Th/To) or nuclear antigens (Scl70/Topoisomerase I, RNA polymerase III, Ku). Periductal fibrosis was a prominent histopathological feature in minor salivary glands of these patients either alone or in association with focal or sparse lymphocytic infiltrates. Some of these patients presented with occult scleroderma features, and follow-up may reveal those who will eventually develop systemic sclerosis. Theoretically, if SSc-specific antibodies had the same weight as anti-Ro/SSA antibodies in the classification criteria for SS, almost one third of anti-Ro/SSA negative patients with either negative or unavailable MSG biopsies would be classified as SS. Whether this patient subset represents a distinct clinical entity or a subtype of SS remains to be further explored.

While anticentromere antibodies have been extensively investigated in the context of SS [6,9,10–13,20–22], and even been proposed as a serologic marker for SS diagnosis equivalent to anti-Ro/SSA and anti-La/SSB positivity [24], this is the first attempt to evaluate the role of a wide spectrum of SSc-specific autoantibodies and their contribution to salivary gland inflammation and generation of sicca symptomatology.

Anticentromere antibodies have been previously suggested as the serological link between distinct autoimmune entities, as for PACK syndrome [PBC (primary biliary cirrhosis, ACA, CREST (Calcinosis, RP, Esophageal dysfunction, Sclerodactyly, Telangiectasias) and KCS (Keratoconjuctivitis sicca)][25]. SS patients with anticentromere antibodies have been shown to share overlapping features with SSc such as RP [7,12,21,23,26,27], dysphagia [7,12,27], sclerodactyly [12,27], telangiectasias [12,27], in addition to sicca phenotype [13,26,27]. Compared to their SS counterparts, they have seldom hypergammaglobulinemia [21], display reduced frequency of antibodies against Ro/SSA and/or La/SSB [12, 20, 25, 26] but share similar histopathological findings [12, 25, 26] in MSG tissues. While a more severe clinical phenotype [21] conferring higher lymphoma risk [23] was previously reported, this was not confirmed in other studies [28,29]. Interestingly, as much as 25% of SS patients with anticentromere antibodies may eventually be classified as limited cutaneous SSc on follow-up [10,30,31], although this was not universally detected [26].

Sicca features have been consistently reported in the setting of both limited (lc) and diffuse (dc) cutaneous SSc. In patients with lcSSc, the frequency of oral and ocular dryness is variable, ranging from 23-75% [26,32–34] and 21-100% [26,32–34], respectively; interestingly, 49% of these patients have been shown to display lymphocytic sialadenitis in MSG biopsy [34]. In dcSSc, sicca features were reported in 39-49% of patients[32,33,35,36,37], along with evidence of fibrotic changes in MSG biopsies [37]. Furthermore, recurrent SGE was evident in 11.4% of patients and MSG biopsy revealed distinct groups of significant fibrosis in

either periductal, perilobular or intralobular regions; fibrosis was absent in the patients with mixed connective tissue disease, RP or healthy controls [37]. Another study of 44 dcSSc patients showed that SGE was present in 44.4% and one fifth had MSG findings compatible with SS, while 38.6% had mild fibrotic changes [38]. Earlier reports have estimated an SS prevalence ranging from 0- 88% in the context of SSc [35,37,39–41].

Features of objective mucosal dryness have been also reported in 57% of patients with idiopathic pulmonary fibrosis (IPF)[42]. Sicca symptomatology seems to increase disease burden in SSc patients as it shows strong association to the SF-36 quality of life score [43]. Along the same lines, sicca complaints have been suggested [44] to be included in the Scleroderma Clinical Trials Consortium-Damage Index (SCTC-DI)[45].

While sicca features in patients with positive MSG biopsies are attributed to the local inflammatory process and ensuing impaired glandular function, the underlying mechanisms accounting for the generation of sicca complaints in the absence of classical histopathological SS findings are not fully elucidated. Neurosecretory dysfunction as the result of an inflammation-related dampened neurotransmitter release, or autoantibodies against muscarinic 3 receptor[32,46], as well as fibrosis and vascular inflammation has been proposed as having a contributory role for dry features in the context of SS and SSc [47].

Given the association of abnormal findings in salivary gland ultrasound (SGUS) with positive MSG biopsy in SS patients [48], abnormal parotid gland SGUS in patients with antibodies against Ro/SSA and La/SSB antigens has been shown to have high predictive value for SS [49]. Notably, abnormal findings in SGUS were observed in one third of SSc patients, the majority of which were positive for anticentromere antibodies[43], further supporting an SS/SSc overlap phenotype [50]. Furthermore, abnormal ultrasonographic features of salivary glands were present in 51.6% of SS and 62.7% of SSc patients, respectively[51]. Nevertheless, the two modalities (US and MSG biopsy) are considered complementary in pursuit of SS diagnosis and cannot replace one another[52]. Therefore, the presence of SSc-specific autoantibodies at strong titers in sicca patients is the sole non-invasive modality so far shown to be independently associated to MSG biopsy positivity irrespective of the presence of anti-Ro/SSA and anti-La/SSB antibodies.

The major limitation of the current study is the lack of available minor salivary gland biopsies in all study participants, mainly due to reluctancy of some patients to undergo labial gland biopsies. Moreover, one could argue that MSG biopsies were more often performed in the anti-Ro/SSA positive rather than the anti-Ro/SSA negative group. However, given the increased risk for lymphoma development among anti-

Ro/SSA positive individuals, anti-Ro/SSA patients with sicca complaints are routinely offered an MSG biopsy, despite they fulfill ACR 2016 criteria for SS. Additionally, while the presence of fibrosis in MSG tissue has been earlier shown in patients with SSc[37], and even detected in one third of patients in another prospective study of SSc patients who underwent MSG biopsy[53], a prospective matched and blinded study quantitating the amount of fibrosis in MSG biopsies from patients with distinct serological reactivities should be performed.

In conclusion, these data suggest that SSc-specific antibodies are frequently detected in patients presenting with sicca complaints and at strong titers they are independently associated with MSG positivity. Although our observations must be confirmed in larger cohorts, we propose that testing for SSc-specific autoantibodies could be a useful tool in the diagnostic work-up and potentially in future classification criteria for SS.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethics

Our study complies with the Declaration of Helsinki. The locally appointed ethics committee has approved the research protocol. Written informed consent has been obtained from the subjects.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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Table 1. Association of objective measures of ocular/oral dryness and antibodies against Ro/SSA and La/SSB antigens with MSG biopsy positivity. MSG: minor salivary gland biopsy; SSc Abs: Scleroderma Specific autoantibodies

%	MSG (+) (n=70)	MSG (-) (n=72)	p-value	95% Confidence Interval (Odds ratio)
Abnormal ocular staining score	43.8	21.6	0.049	2.8 (1.0-8.1)
Abnormal Schirmer's test	50	58.8	0.38	0.7 (0.3-1.6)
Abnormal unstimulated whole salivary flow (<0.5ml/5min)	50	25	0.30	3.0 (0.4-24.9)
Anti-Ro/SSA	68.6	33.3	<0.001	4.4 (2.2 – 8.8)
Anti-La/SSB	37.3	5.9	<0.001	9.5 (3.1-29.3)
SSc-specific Abs (strong titers)	30	12.5	0.01	3.0 (1.3-7.1)
SSc-specific Abs (medium titers)	28.6	26.4	0.77	1.1 (0.5-2.3)

Table 2. Final diagnoses in the anti-Ro/SSA+ and anti-Ro/SSA- groups and rates of SSc+ sera in each diagnosis following extensive diagnostic work up.

Final diagnosis n (%)	Sicca cohort (n=216)	SSc+ positive sera (n=90)
Anti-Ro/SSA (+) group (n=85)		
SS	70 (82.4)	31/70 (44.3)
UCTD	5 (5.8)	3/5 (60)
RA	2 (2.4)	1/2 (50)
SLE	5 (5.8)	1/5 (20)
DM	1 (1.2)	1/1 (100)
PBC	1 (1.2)	0/1 (0)
SSc	1 (1.2)	1/1 (100)
Anti-Ro/SSA (-) group (n=131)		
UCTD	45 (34.4)	32/45 (71.1)
Hashimoto	29 (22.1)	2/29 (6.9)
SS	18 (13.7)	12/18 (66.7)
SLE	13 (10)	2/13 (15.4)
RA	11 (8.4)	2/11 (18.2)
Dermatomyositis	3 (2.2)	1/3 (33.3)
Fibromyalgia	3 (2.2)	1/3 (33.3)
Non-specific sialadenitis	3 (2.2)	0/3 (0)
Sclerosing sialadenitis	1 (0.8)	0/1 (0)
Chronic blepharitis	1 (0.8)	0/1 (0)
MGUS	1 (0.8)	0/1 (0)
PBC	1 (0.8)	0/1 (0)
PsA	1 (0.8)	0/1 (0)
RP	1 (0.8)	0/1 (0)

Sjögren's syndrome, UCTD: undifferentiated connective tissue disease, SLE: systematic lupus erythematosus, MGUS: monoclonal gammopathy of unknown significance, PBC: primary biliary cirrhosis, PsA: psoriatic arthritis, RA: rheumatoid arthritis, RP: Raynaud's phenomenon.

Figure 1. Flow-chart of our consecutive sicca cohort and primary SS classification according to EULAR/ACR 2016 classification criteria (MSGB: minor salivary gland biopsy, SS: Sjögren's syndrome)

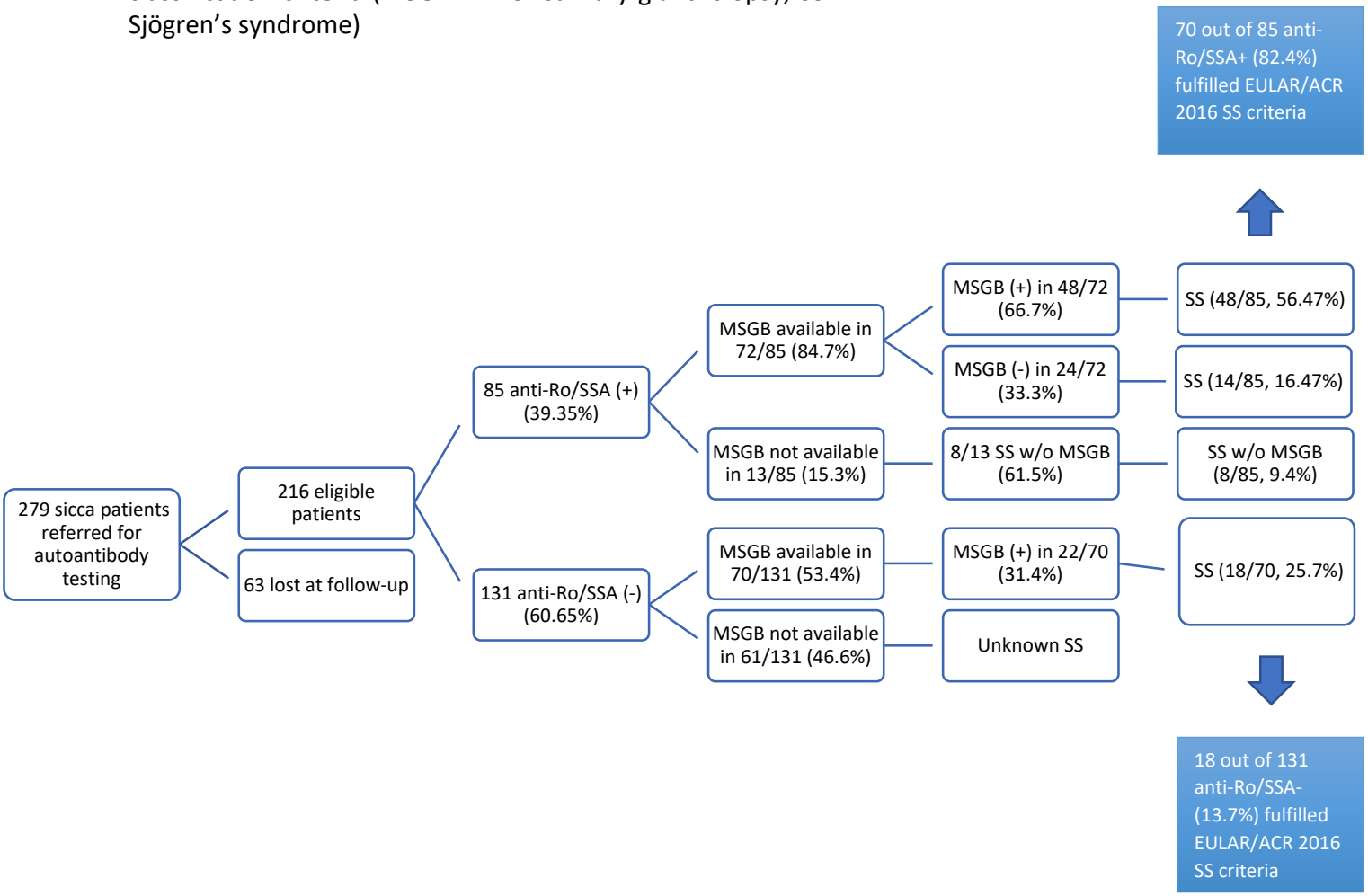


Figure 2. High prevalence of SSc-specific antibodies at strong and medium titers in the sicca cohort (panel A) and association with MSG tissue positivity (defined by a lymphocytic focus score≥1) (panel B).

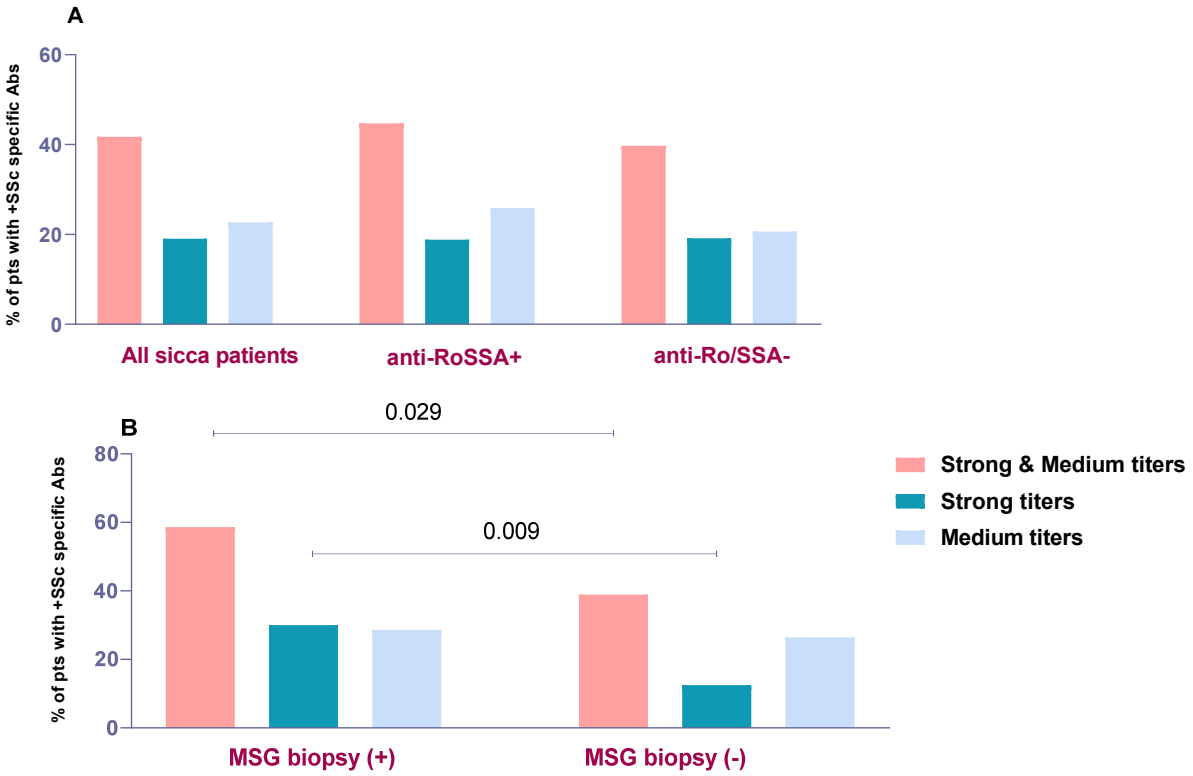


Figure 3. Type and frequency of different SSc-specific antibodies at strong titers according to MSG biopsy positivity (SSc: systemic sclerosis; MSG: minor salivary gland; CENPA, CENPB: centromere protein A, B Scl-70: Scleroderma-70 or Topoisomerase I; RP: RNA-polymerase III; NOR 90: Nucleolar Organizing Region 90; PM/Scl: Polymyositis/Scleroderma).

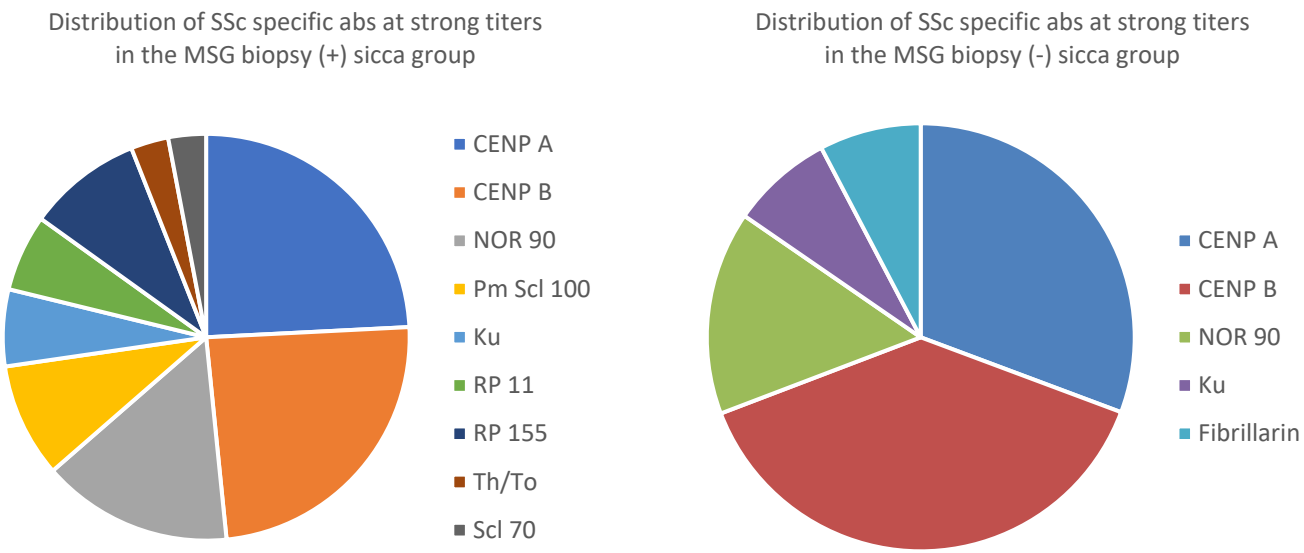


Figure 4. Representative MSG tissue biopsies from sicca patients with positive SSc specific antibodies at strong titers (hematoxylin/eosin staining). A. anti-Ku+, anti-Ro/SSA- FS: 0.65; B. anti-CENPB+, anti-Ro/SSA-, FS:0; C. anti-fibrillarin+, anti-Ro/SSA-, FS: 0; D. anti-Scl70+, anti-Ro/SSA-, FS: 0.63; E. anti-NOR90+, anti-Ro/SSA- , FS: 1.01, F. anti-CENPA+, anti-CENPB+, anti-Ro/SSA-, FS: 1.35.

(MSG: minor salivary gland; NOR90: Nucleolar Organizing Region 90; SSc: systemic sclerosis; SS: Sjögren's syndrome; CENPA, CENPB: centromere protein A, B; FS: focus score)

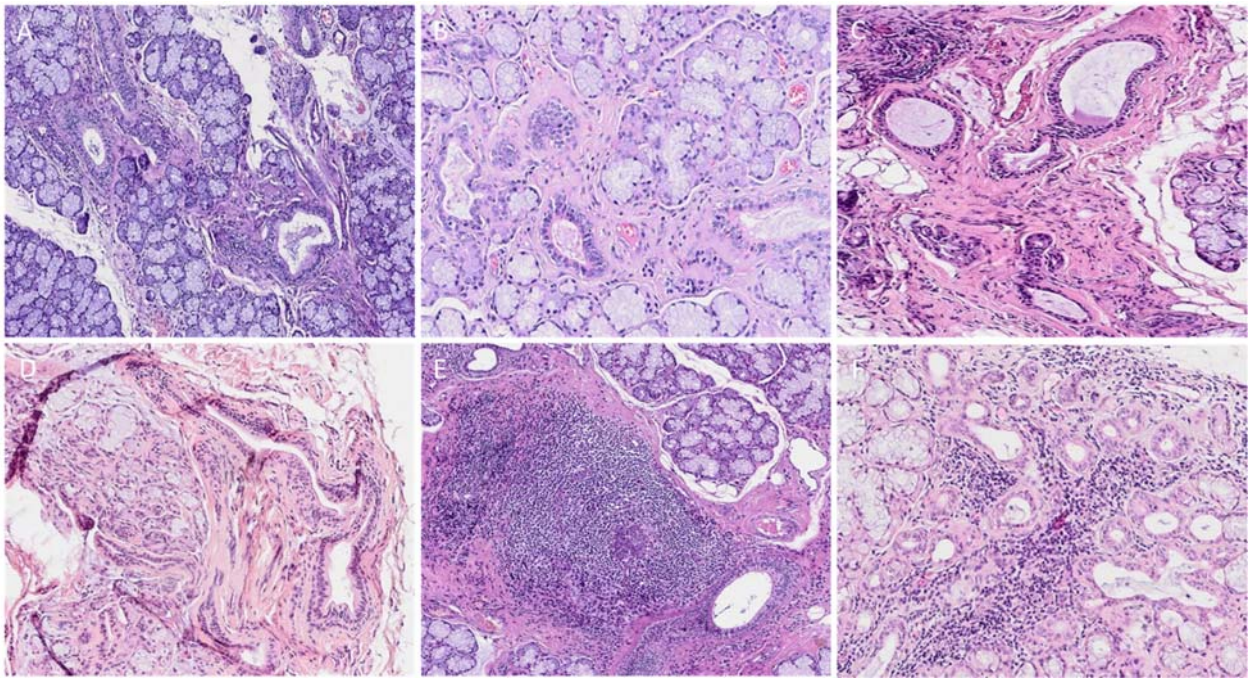
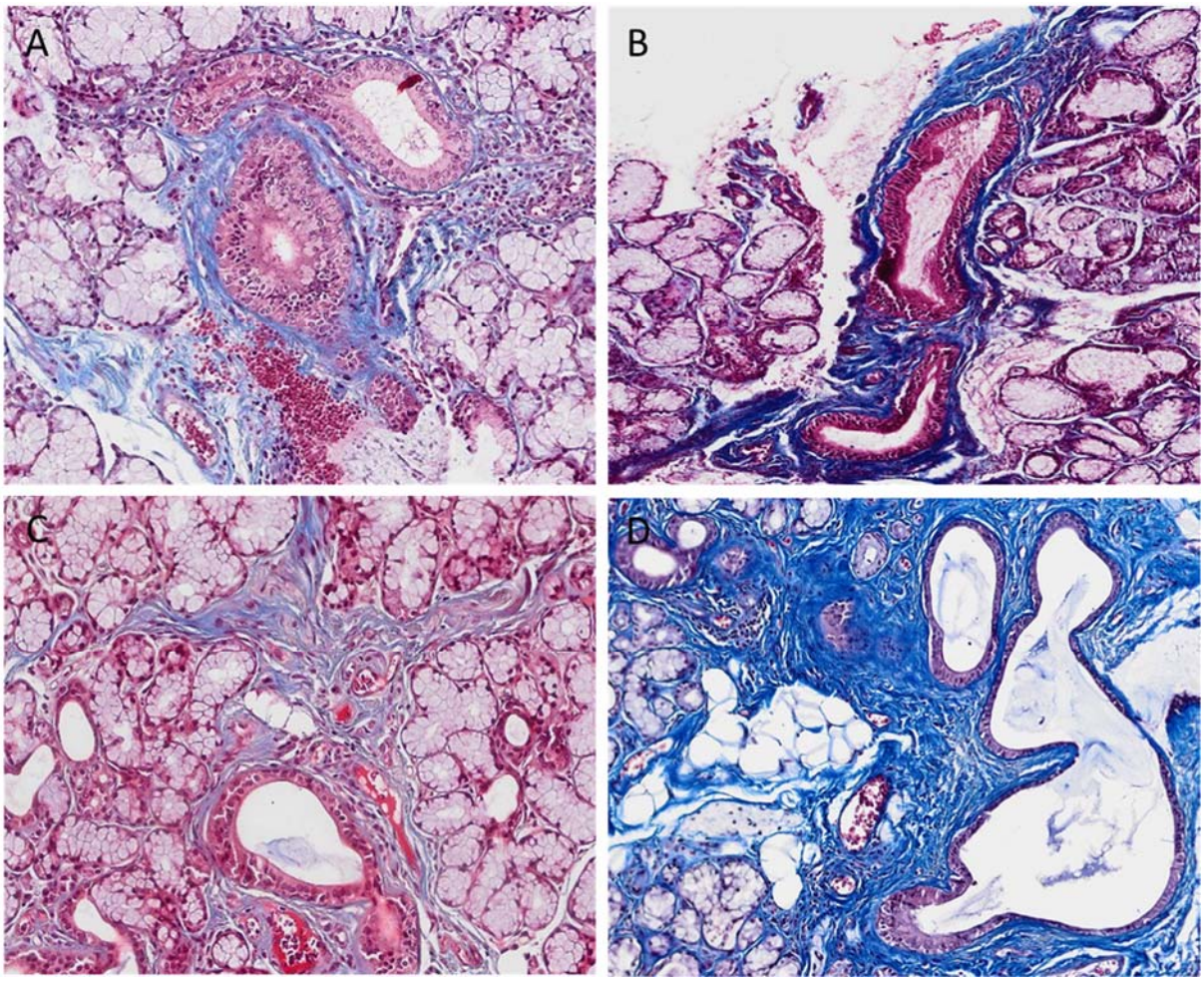
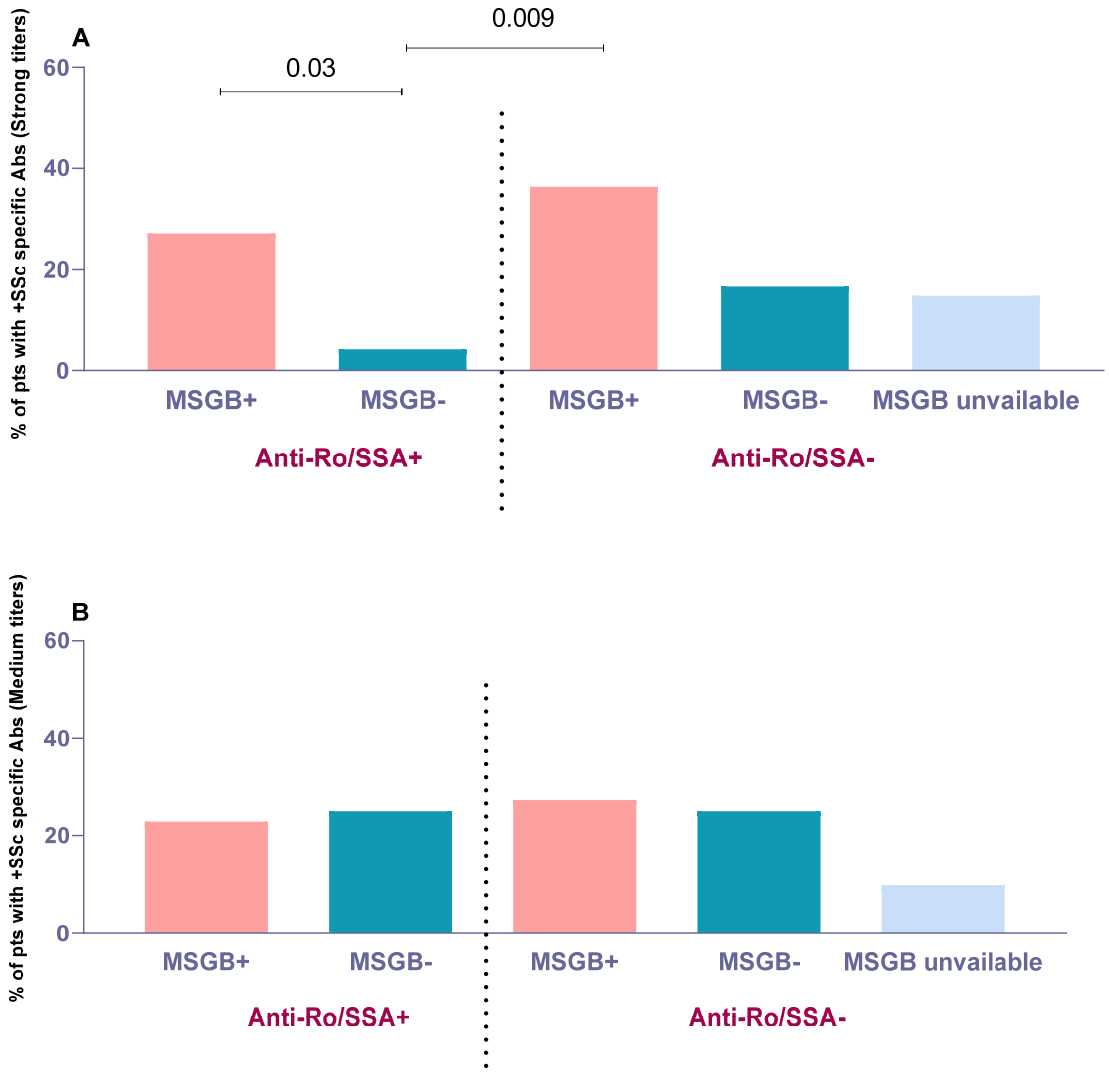


Figure 5. Representative MSG tissue biopsies depicting the presence of fibrosis using Masson staining from the same sicca patients (A-D), displayed in Figure 4.



Suppl. Figure 1. Prevalence of SSc-specific antibodies at strong (Panel A) and medium (Panel B) titers. As displayed, SSc-specific antibodies at strong titers were significantly higher among anti-Ro/SSA(+) /MSGB(+) and anti-Ro/SSA(-) /MSGB(+) patients (13/48, 27.08% and 8/22, 36.4%, respectively), compared to the anti-Ro/SSA(+)/MSGB(-) patients [1/24, 4.2% (p=0.026 and p=0.0086, respectively)] but not anti-Ro/SSA(-)/MSGB(-) group [8/48, 16.7%, (p=0.26)] (Panel A). No significant associations were observed for SS-specific antibodies at medium titers between these groups (Panel B).



Supplementary Tables

Supplementary Table 1. Demographic, clinical and laboratory characteristics of the study participants.

Patient characteristics	**(n=216)**
Demographics	
Age at study entry (years) (mean±SD)	57.0 ±13.1
Female sex (%)	94.4
Subjective & Objective measures of oral/ocular dryness	
Dry mouth subjective (%)	85.2
Dry eyes subjective (%)	85.6
Salivary gland enlargement (%)	15.6
Abnormal Schirmer's test (%)	51.4
Ocular Stain positive (%)	26.7
Abnormal unstimulated whole salivary flow (<0.5ml/5min) (%)	79.9
Focus score≥ 1 (%)	45.8
Systemic features	
Arthralgias-Myalgias (%)	68.5
Arthritis (%)	15.9
Raynaud's Phenomenon (%)	36.5
Palpable Purpura (%)	3.4
Laboratory parameters	
Anti-Ro/SSA (%)	39.4
Anti-La/SSB (%)	15.1
RF positivity (>20 IU/ml) (%)	22.5
C3 mg/dl (mean ± SD)	109 ±21
C4 mg/dl (mean ± SD)	23.9 ±8.6
WBC baseline (mean ± SD)	6173±1795
Neutrophil number (mean ± SD)	3675 ±2780
Lymphocyte number (mean ± SD)	2002 ±703
ESR (mm/h) (mean ± SD)	22.5 (±17.7)
IgG (mg/dL) (mean ± SD)	1295 ±434
IgM (mg/dL) (mean ± SD)	152 ±145
IgA (mg/dL) (mean ± SD)	236 ±127
LDH U/L) (mean ± SD)	217 ±73

ANA: anti-nuclear antibodies, ESR: erythrocyte sedimentation rate, HGB: hemoglobin, RF: rheumatoid factor, IgG, IgA, IgM: immunoglobulins G, A, M

Supplementary table 2. Overall distribution of SSc-specific autoantibodies at strong and medium titers in the sicca cohort (CENPA, CENPB: centromere protein A, B Scl-70: Scleroderma-70 or Topoisomerase I; RP: RNA-polymerase III; NOR 90: Nucleolar Organizing Region 90; PM/Scl: Polymyositis/Scleroderma; PDGFR: Platelet-Derived Growth Factor Receptor).

Autoantigenic targets of SSc-specific antibodies	Strong titers in the whole sicca group (n=62)	Medium titers in the whole sicca group (n= 61)
CENPA (%)	22.6	6.6
CENPB (%)	24.2	1.6
NOR 90 (%)	14.5	6.6
PM-Scl 100 (%)	9.7	11.5
PM-Scl 75 (%)	1.6	6.6
Ku (%)	8.1	9.8
RP 155 (%)	8.1	11.5
RP 11 (%)	4.8	3.3
Th/To (%)	3.2	32.8
Fibrillarin (%)	1.6	8.2
Scl 70 (%)	1.6	1.6
PDGFR (%)	0	0

Supplementary table 3. Distribution of SSc-specific autoantibodies at strong titers according to anti-Ro/SSA status in the whole sicca cohort (Scl-70: Scleroderma-70 or Topoisomerase I; RP: RNA-polymerase III; NOR 90: Nucleolar Organizing Region 90; PM/Scl: Polymyositis/Scleroderma; PDGFR: Platelet-Derived Growth Factor Receptor).

Autoantigenic targets of SSc-specific antibodies (strong titers)	**anti-Ro/SSA (+) (n=26)**	**anti-Ro/SSA (-) (n=36)**	**p-value**
CENPA (%)	26.9	19.4	0.55
CENP B (%)	26.9	22.2	0.77
NOR 90 (%)	7.7	19.4	0.28
PM-Scl 100 (%)	7.7	11.1	1
PM-Scl 75 (%)	0	2.8	1
Ku (%)	7.7	8.3	1
RP 155 (%)	7.7	8.3	1
RP 11 (%)	3.8	5.5	1
Th/To (%)	7.7	0	0.17
Fibrillarin (%)	0	2.8	1
Scl 70 (%)	3.8	0	0.42
PDGFR (%)	0	0	1

Supplementary table 4. Distribution of SSc-specific autoantibodies at medium titers according to anti-Ro/SSA status in the whole sicca cohort (SSc: Systemic Sclerosis, Scl-70: Scleroderma-70 or Topoisomerase I; RP: RNA-polymerase III; NOR 90: Nucleolar Organizing Region 90; PM/Scl: Polymyositis/Scleroderma; PDGFR: Platelet-Derived Growth Factor Receptor).

Autoantigenic targets of SSc-specific antibodies (medium titers)	**anti-Ro/SSA (+) (n=30)**	**in anti-Ro/SSA (-) (n=31)**	**p-value**
CENPA (%)	6.7	6.5	1
CENP B (%)	3.3	0	0.49
NOR 90 (%)	0	12.9	0.11
PM-Scl 100 (%)	10	12.9	1
PM-Scl 75 (%)	3.3	9.7	0.61
Ku (%)	13.3	6.5	0.43
RP 155 (%)	6.7	16.1	0.43
RP 11 (%)	3.3	3.2	1
Th/To (%)	43.3	22.6	0.11
Fibrillarin (%)	6.7	9.7	1
Scl 70 (%)	3.3	0	0.49
PDGFR (%)	0	0	1

Supplementary table 5. Distribution of SSc-specific Abs according to MSG positivity (SSc: Systemic Sclerosis, MSG: minor salivary gland, Scl-70: Scleroderma-70 or Topoisomerase I; RP: RNA-polymerase III; NOR 90: Nucleolar Organizing Region 90; PM/Scl: Polymyositis/Scleroderma; PDGFR: Platelet-Derived Growth Factor Receptor).

	MSG (+)	MSG (-)	p-value	MSG (+)	MSG (-)	p-value
SSc-specific Abs	**Strong titers**			**Medium titers**		
CENP A (%)	24.2	30.8	0.72	4.5	11.1	0.62
CENP B (%)	24.2	38.5	0.47	4.5	0	0.45
NOR 90 (%)	15.1	15.4	1.0	4.5	3.7	1
Ku (%)	6.1	7.7	1	13.6	7.4	0.65
Pm Scl 100 (%)	9.1	0	0.55	9.1	7.4	1
Pm Scl 75 (%)	0	0	1	0	3.7	1
RP 11 (%)	6.1	0	1	4.5	3.7	1
RP 155 (%)	9.1	0	0.55	4.5	18.5	0.20
Th/To (%)	3	0	1	40.9	37	1
Scl 70 (%)	3	0	1	0	0	1
Fibrillarin (%)	0	7.7	0.28	13.6	7.4	0.65
PDGFR (%)	0	0	1	0	0	1

Supplementary Table 6. Demographic, clinical, laboratory and histopathological characteristics associated with the presence or absence of SSc specific autoantibodies in the whole cohort.

	SSc Abs + (n=90)	SSc Abs- (n=126)	p-value
Demographic characteristics			
Female sex (%)	97.6	91.7	0.2
Age at study entry (years) (mean ± SD)	57.7 ± 14.2	56.2 ± 12.4	0.43
Clinical features			
Ocular dryness (subjective) (%)	89.9	82.9	0.3
Oral dryness (subjective) (%)	84	85.9	0.7
SGE (%)	17.9	14.2	0.6
Abnormal Schirmer's test (%)	52.7	50.6	0.8
Ocular Stain positive (%)	25	27.6	0.8
Arthralgias-Myalgias (%)	77.8	62.9	0.023
Arthritis (%)	16.3	15.7	0.9
Raynaud's phenomenon (%)	40.2	34.1	0.37
Palpable purpura (%)	3.8	3.2	0.8
Laboratory features			
WBC number/mm^3 (absolute number) (mean ± SD)	6100 ± 1801	6217 ± 1796	0.65
Neutrophil number/mm^3 (absolute number) (mean ± SD)	3896 ± 4167	3542 ± 1405	0.38
Lymphocyte number/mm^3 (absolute number) (mean ± SD)	1994 ± 720	2007 ± 695	0.89
Monocyte number/mm^3 (absolute number) (mean ± SD)	431 ± 144	433 ± 204	0.92
Hgb (g/dL) (absolute number) (mean ± SD)	13.3 ± 3.7	13 ± 1.3	0.29
ESR (mm/h) (mean ± SD)	23.6 ± 19.3	21.8 ± 16.7	0.5
IgG (mg/dL) (mean ± SD)	1334 ± 362	1274 ± 469	0.47
IgM (mg/dL) (mean ± SD)	176 ± 117	138 ± 158	0.18
IgA (mg/dL) (mean ± SD)	243 ± 136	232 ± 123	0.7
LDH (U/L) (mean ± SD)	221 ± 76	214 ± 72	0.7
C3 (mg/dl) (mean ± SD)	110 ± 20	109 ± 22	0.63
C4 (mg/dl) (mean ± SD)	24 ± 9	24 ± 8	0.78
Anti-Ro/SSA antibodies (%)	41	38.3	0.7
Anti-La/SSB antibodies (%)	16.5	14.3	0.67
Histopathological features			
Focus score (number of foci/$4mm^2$) (mean ± SD)	1.19 ± 1.8	0.84 ± 1.14	0.17
Tarpley score (mean±SD)	1.1 ± 1.1	0.83 ± 1.1	0.18
Focus score≥ 1 (%)	54	39.5	0.084

C3: complement C3, C4: complement C4, ESR: erythrocyte sedimentation rate, HGB: hemoglobin, IgA: immunoglobulin A, IgG: immunoglobulin G, IgM: immunoglobulin M, LDH: lactate dehydrogenase, SGE: Salivary gland enlargement, WBC: white blood cells

Supplementary Table 7. Demographic, clinical, laboratory and histopathological characteristics associated with the presence or absence of SSc specific autoantibodies at strong titers in the whole cohort.

	SSc Abs at strong titers (n=41)	**Absence of SSc Abs (n=126)**	**p-value**
Demographic characteristics			
Female sex (%)	100	91.7	0.16
Age at study entry (years) (mean ± SD)	59.41 ± 14.3	56.21 ± 12.5	0.17
Clinical features			
Ocular dryness (subjective) (%)	89.5	82.8	0.57
Oral dryness (subjective) (%)	84.6	85.8	0.85
SGE (%)	12.9	14.3	0.85
Abnormal Schirmer's test (%)	51.7	50	0.87
Ocular Stain positive (%)	31.3	28.1	0.8
Arthralgias-Myalgias (%)	74.4	62.6	0.18
Arthritis (%)	15.4	15.9	0.94
Raynaud's phenomenon (%)	47.5	34.4	0.13
Palpable purpura (%)	0	3.2	0.26
Laboratory features			
WBC number/mm^3 (absolute number) (mean ± SD)	6175 ± 1921	6234 ± 1793	0.86
Neutrophil number/mm^3 (absolute number) (mean ± SD)	3531 ± 1556	3552 ± 1407	0.94
Lymphocyte number/mm^3 (absolute number) (mean ± SD)	1952 ± 637	2010 ± 697	0.64
HGB (g/dL) (absolute number) (mean ± SD)	12.6 ± 0.98	12.9 ± 1.3	0.28
ESR (mm/h) (mean ± SD)	26.7 ± 20.5	21.8 ± 16.8	0.15
IgG (mg/dL) (mean ± SD)	1275 ± 341	1279 ± 470	0.97
IgM (mg/dL) (mean ± SD)	182 ± 123	139 ± 159	0.3
IgA (mg/dL) (mean ± SD)	290 ± 162	234 ± 123	0.1
LDH (U/L) (mean ± SD)	234 ± 71	215 ± 72	0.24
C3 (mg/dl) (mean ± SD)	109 ± 16	109 ± 22	0.98
C4 (mg/dl) (mean ± SD)	22 ± 8	24 ± 8	0.32
Anti-Ro/SSA antibodies (%)	39	37.9	0.9
Anti-La/SSB antibodies (%)	15.4	14.4	0.88
Histopathological features			
Focus score (number of foci/$4mm^2$) (mean ± SD)	1.48 ± 2.3	0.85 ± 1.14	0.06
Tarpley score (mean±SD)	1.27 ± 1.22	0.84 ± 1.1	0.11
Focus score≥ 1 (%)	63.3	40	0.029

C3: complement C3, C4: complement C4, ESR: erythrocyte sedimentation rate, HGB: hemoglobin, IgA: immunoglobulin A, IgG: immunoglobulin G, IgM: immunoglobulin M, LDH: lactate dehydrogenase, SGE: Salivary gland enlargement, WBC: white blood cells

Supplementary Table 8. Demographic, clinical, laboratory and histopathological characteristics associated with the presence or absence of SSc specific autoantibodies at medium titers in the whole cohort.

	SSc Abs at medium titers (n=49)	Absence of SSc Abs (n=126)	p-value
Demographic characteristics			
Female sex (%)	95.3	91.7	0.68
Age at study entry (years) (mean ± SD)	55.95 ± 13.9	56.21 ± 12.5	0.9
Clinical features			
Ocular dryness (subjective) (%)	90.5	82.8	0.46
Oral dryness (subjective) (%)	83.7	85.8	0.7
SGE (%)	21.6	14.3	0.5
Abnormal Schirmer's test (%)	55.6	50	0.6
Ocular Stain positive (%)	17.6	28.1	0.39
Systemic symptoms			
Arthralgias-Myalgias (%)	81.4	62.6	0.023
Arthritis (%)	16.7	15.9	0.9
Raynaud's phenomenon (%)	32.6	34.4	0.83
Palpable purpura (%)	7	3.2	0.29
Laboratory features			
WBC number/mm^3 (absolute number) (mean ± SD)	5972 ± 1697	6234 ± 1793	0.42
Neutrophil number/mm^3 (absolute number) (mean ± SD)	4220 ± 5668	3553 ± 1407	0.23
Lymphocyte number/mm^3 (absolute number) (mean ± SD)	2026 ± 794	2010 ± 697	0.9
HGB (g/dL) (absolute number) (mean ± SD)	13.9 ± 5	12.9 ± 1.3	0.039
ESR (mm/h) (mean ± SD)	20.5 ± 17.5	21.8 ± 16.8	0.68
IgG (mg/dL) (mean ± SD)	1357 ± 384	1279 ± 470	0.5
IgM (mg/dL) (mean ± SD)	168 ± 115	139 ± 159	0.4
IgA (mg/dL) (mean ± SD)	200 ± 97	234 ± 123	0.24
LDH (U/L) (mean ± SD)	208 ± 79	215 ± 72	0.66
C3 (mg/dl) (mean ± SD)	111 ± 23	109 ± 22	0.56
C4 (mg/dl) (mean ± SD)	25 ± 9	24 ± 9	0.7
Anti-Ro/SSA antibodies (%)	44.2	37.9	0.46
Anti-La/SSB antibodies (%)	17.1	14.4	0.68
Histopathological features			
Focus score (number of foci/$4mm^2$) (mean ± SD)	0.9 ± 1.3	0.85 ± 1.14	0.83
Tarpley score (mean±SD)	0.94 ± 1.1	0.84 ± 1.1	0.68
Focus score≥ 1 (%)	44.1	41.3	0.78

C3: complement C3, C4: complement C4, ESR: erythrocyte sedimentation rate, HGB: hemoglobin, IgA: immunoglobulin A, IgG: immunoglobulin G, IgM: immunoglobulin M, LDH: lactate dehydrogenase, SGE: Salivary gland enlargement, WBC: white blood cells

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Review

Biologics in Sjögren's syndrome

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ABSTRACT

The use of biologic disease modifying antirheumatic drugs (bDMARDs) in systemic autoimmune diseases such as rheumatoid arthritis and at a lesser extent in lupus, has been well established. In Sjögren's syndrome (SS), despite the shared pathogenetic mechanisms with other autoimmune disorders, traditional immunomodulatory drugs have failed to address the main clinical features of the disease and use of biologics has been limited so far. Over the last years, our better understanding in disease pathogenesis has led to an expansion in the number of clinical trials exploring the effect and safety of biological agents with variable results. In the current review, the effect of targeting key molecular mechanisms involved in SS pathogenesis such as antigen presentation, B and T cell activation and germinal center formation is discussed.

1. Introduction

Sjögren's syndrome (SS) is an autoimmune entity of indolent course presenting with oral and ocular dryness as a result of lymphocytic infiltration of the affected organs, chiefly salivary and lacrimal glands [1]. Disease hallmarks, also shared by other systemic autoimmune diseases, include female preponderance, B cell hyperactivity expressed as hypergammaglobulinemia and the presence of several serum autoantibodies [1], as well as activation of type I and II interferon (IFN) pathways [2–4]. SS may present either alone or in association with other systemic autoimmune conditions [5]. Approximately half of SS patients present extraglandular symptoms roughly divided into nonspecific (musculoskeletal, fatigue, Raynaud's), periepithelial (lymphocytic infiltration around epithelial tissue in parenchymal organs such as lung, liver, kidney) and immune complexes mediated (purpura, glomerulonephritis and peripheral neuropathy) [1] as well as fibromyalgia and chronic pain [6]. The latter, along with salivary gland enlargement [7], cryoglobulinemia, C4 hypocomplementemia [8–10], rheumatoid factor (RF) positivity [11] and monoclonal gammopathy [12] are increasingly acknowledged as adverse predictors for lymphoma development in the context of SS, denoting a high-risk, aggressive disease phenotype. Increasing availability of biologic agents along with the better elucidation of pathogenetic pathways in recent years have fueled international efforts to conduct well-controlled studies in the setting of primary SS. In this review, we summarize the developing therapeutic strategies, as prompt identification of high-risk individuals could lead to the initiation of therapies aimed at decelerating or arresting progression to lymphoma, one of the main challenges in the current management of SS.

2. Innate immunity (Table 1)

2.1. Proinflammatory cytokines

Proinflammatory cytokines including interleukin (IL)-1, tumor necrosis factor α (TNF-α), and IL-6 have been found to be upregulated in salivary glands [13], serum [14], saliva [15,16] and tears [17] of SS patients. Non-selective blockade of proinflammatory cytokines – mainly through nuclear factor κ-B (NF-κB) inhibition – has been recently shown to be provided by iguratimod, a methane-sulfonanilide, which is a novel disease-modifying antirheumatic drug (DMARD) [18,19], successfully used in rheumatoid arthritis (RA) [20]. In SS patients, improvement of clinical parameters such as EULAR Sjogren's Syndrome Disease Activity Index (ESSDAI) as well as inhibition of B cell activating factor receptor (BAFF-R) expression and B cell antibody production has been also observed [21]. An open label, phase I & II study assessing the efficacy of iguratimod on glandular and extraglandular features of SS, at a dose of 25 mg twice daily for 24 weeks, is currently ongoing (ClinicalTrials.gov Identifier: NCT03023592). Trials based on the selective inhibition of IL-1, TNF and IL-6 pathways are presented below.

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Table 1
Innate immune pathways inhibition by bDMARDs in SS.

Pathway	Registration number – Reference	Medication	Type of study	Status	Primary outcome	Study duration	Primary outcome fulfilled
Proinflammatory cytokines							
Non-selective							
IL-1, IL-6, TNF-α	NCT03023592	Iguratimod	A single center, open label, phase I & II study	Ongoing	Glandular & extraglandular features of SS	24 weeks	Awaiting results
Selective							
IL-1 receptor	NCT00683345	Anakinra	A double-blind placebo-controlled clinical trial	Completed	Reduction in fatigue by 50%	4 weeks	Yes
TNF-α	Mariette et al. [29]	Infliximab	Randomized, multicenter, double blind, placebo-controlled study	Completed	Joint pain, fatigue, dryness	22 weeks	No
	Türkoğlu et al. [30]	Infliximab	Prospective, single center, open label study	Completed	Objective ocular tests	6 months	No
	NCT02987686	Infliximab	A phase I, prospective, single-center, open label study	Currently recruiting	Adverse events and percentage of completion	12 weeks	Awaiting results
TNF-α receptor	Zandbelt et al. [31]	Etanercept	Pilot, single center, open label study	Complete	Objective ocular tests, fatigue	12 weeks	No
	NCT00001954/Sankar et al. [32]	Etanercept	Randomized, double-blind, placebo-controlled trial	Complete	Subjective/objective oral & ocular tests	12 weeks	No
IL-6 receptor	NCT01782235	Tocilizumab	Randomized double-blind placebo-controlled, phase III trial	Ongoing	Improvement in ESSDAI score of ≥3 points	24 weeks	Awaiting results
Interferons							
RNAse	NCT03247686	RSLV-132	A phase II, double-blind, placebo-controlled study	Complete	Type I IFN inducible gene downregulation	3 months	Yes
ILT7	NCT02780674	MEDI7734/VIB7734	A multicenter, double blind, placebo controlled, phase I trial	Complete	Safety and tolerability	3 months	Awaiting results
	NCT03817424	MEDI7734/VIB7734	A phase I randomized, placebo-controlled, blinded, multiple ascending dose study	Currently recruiting	Safety and pharmacokinetics	10 months	Awaiting results
JAK, TYK inhibitors	NCT03100942	Filgotinib, GS-9876 (Lanraplanib)	Randomized, phase II, double-blind, placebo-controlled	Active but not currently recruiting	Protocol-specified response criteria	48 weeks	Awaiting results
Inflammasome							
$P2X_7R$	Khalafalla et al. [64]	A438079	Pilot study – mouse model	Complete	Improvement of salivary production	10 days	Yes

2.1.1. IL-1

Since IL-1 has been previously linked to sickness behavior [22] – frequently encountered in SS patients mainly as depression and fatigue [23–25] – IL-1 receptor antagonist anakinra was given subcutaneously for the treatment of fatigue in these patients. In the context of a double-blind, placebo-controlled, clinical trial (ClinicalTrials.gov Identifier: NCT00683345), twenty-six patients were randomized in two groups, either to receive anakinra or placebo for 4 weeks. A reduction in fatigue by 50% was evident in the active-drug group compared to placebo in a following post hock analysis, despite the initial failure to reach primary endpoint [26]. Moreover, topical administration of anakinra in a 50 μg/mL concentration for 14 days in a murine model suffering from autoimmune-mediated aqueous-deficient dry eye disease resulted in improvement of ocular surface integrity. The latter was evidenced by stabilization of Lissamine green staining [27].

2.1.2. TNF-α

Infliximab, an anti-TNF-α monoclonal antibody, has been used against several autoimmune rheumatic diseases with variable results [28]. A randomized, multicenter, double blind, placebo-controlled study of 103 SS patients over 22 weeks failed to reach both primary (joint pain, fatigue, dryness), as well as secondary endpoints (objective markers of dryness, focus score, inflammatory markers) [29]. Similarly, in a more recent, prospective, single center, open label study of intravenous use of infliximab in 22 SS patients, no significant changes regarding objective ocular tests were recorded neither in the 3rd nor in the 6th month of treatment [30]. Local use of Infliximab in the form of ocular drops (10 mg/mL × 4/d) is currently tested in a phase I, prospective, single-center, open label 12 week study for the treatment of corneal melt, an SS-related ocular complication (ClinicalTrials.gov Identifier: NCT02987686).

Etanercept, a soluble fully human TNF-α-p75- receptor fusion protein acting as TNF-α inhibitor, also failed to demonstrate significant improvement in objective ocular tests (Rose Bengal cornea staining, Schirmer's test and tear break up time), focus score and percentage of IgA-containing plasma cells in minor salivary gland biopsies (MSGBs), salivary flow tests and fatigue after a 12 week administration in SS patients [31]. These findings were confirmed in a randomized, double-blind, placebo-controlled trial [32] (ClinicalTrials.gov Identifier: NCT00001954). Incompetence to regulate TNF-α levels in SS [33], or exacerbation of IFN-α pathways [34] have been postulated as potential reasons of etanercept failure in SS. Interestingly, topical administration of etanercept in an SS patient with pyoderma gangrenosum resulted in substantial clinical and histological improvement [35].

2.1.3. IL-6

Tocilizumab, a recombinant humanized monoclonal antibody against IL-6R, has been used successfully in the treatment of other autoimmune diseases, such as RA [36], large vessel vasculitis (LVV) [37] and systemic sclerosis (SSc) [38]. Recently, tocilizumab has been proven to be efficacious in isolated case reports of SS associated neuromyelitis optica [39,40] and refractory organizing pneumonia [41]. A randomized, double-blind, placebo-controlled, phase III trial assessing the efficacy of tocilizumab in SS patients is currently ongoing. The improvement in ESSDAI score of ≥3 points (NCT01782235) represents the primary study outcome. However, no results have been published yet.

2.2. Interferons

Studies in SS patients' peripheral blood as well as MSGBs has brought to light an upregulation of genes induced by type I IFN (mainly IFNα and β), type II (IFNγ), or both—the so-called IFN signature [3,42–45]. Plasmacytoid dendritic cells (pDCs) are considered the main type I IFN producers while Th1 $CD4^+$ helper and natural killer (NK) cells are regarded as the main type II IFN producers in SS salivary gland tissues [46–48]. Endogenous nuclear elements, such as Long Interspersed Nuclear Element 1 (LINE-1; L1) genomic repeats, have been postulated to induce type I IFN production through activation of either Toll-like receptor (TLR) dependent or independent pathways in SS salivary glands [49]. In this context, RNA cleavage by RSLV-132, a novel fully human biologic Fc protein comprised of an RNase fused to the Fc domain of IgG1, eventually inhibits type I IFN production. This agent, already tested in systemic lupus erythematosus (SLE) patients with encouraging results [50], is currently under evaluation in a phase II, double-blind, placebo-controlled study of 28 SS patients. Primary endpoint was expression of type I IFN induced genes in peripheral blood mononuclear cells (PBMCs) after 3 months and secondary endpoints ESSDAI, EULAR Sjögren Syndrome Patient Reported Index (ESSPRI), Functional Assessment of Chronic Illness Therapy (FACIT) – fatigue, Profile of Fatigue (PRO-F), neuropsychological tests which were also assessed after 3 months (ClinicalTrials.gov Identifier: NCT03247686). Preliminary results have shown improvement in the mental component of PRO-F score, as well as the Digit symbol substitution test, which assesses cognitive function, while results of the analyses for primary and secondary endpoints are awaited. No serious adverse events are reported [51].

Another molecule involved in the regulation of type I IFN pathway is immunoglobulin-like transcript 7 (ILT7)/LILRA4/CD85g, a member of the immunoglobulin-like transcripts (ILTs), or leukocyte immunoglobulin-like receptors (LIR) gene family, predominantly expressed on the surface of pDCs [52]. Activation of ILT7 downregulates TLR7/9 mediated IFN production and induces differentiation of pDCs to antigen presenting cells (APCs) instead of interferon presenting cells (IPCs) [53–55]. Stimulation of ILT7 by an antibody (MEDI7734/VIB7734) was tested in 36 patients of type I IFN-mediated autoimmune diseases including SS, in a multicenter, double blind, placebo-controlled, phase I study. Patients were allocated to receive either a single, subcutaneous dose of the study drug or placebo and followed up for a 3-month period. Primary outcome included adverse events, while secondary outcomes anti-drug antibody measurement and pharmacokinetics. The study was completed but no results are as yet published (ClinicalTrials.gov Identifier: NCT02780674). Moreover, a phase I study evaluating safety and pharmacokinetics of multiple ascending doses of MEDI7734/VIB7734 is currently in recruiting phase (ClinicalTrials.gov Identifier: NCT03817424). Despite positive results in clinical trials of IFN antagonists in SLE [56] – the prototype type I IFN mediated disease [57] – these agents have not yet been tested in SS.

Upon binding of type I IFN on its receptor, autophosphorylation of receptor-associated kinases, namely janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) occurs, leading to further activation of the STAT pathway resulting in upregulation of Interferon stimulated genes (ISG) [58]. Therefore, inhibition of the JAK-STAT pathway seems a promising target in SS. In this context, lanraplanib (formerly GS-9876) – a Tyk2 inhibitor – along with the JAK1 inhibitor filgotinib are currently being tested in a randomized, phase II, double-blind, placebo-controlled study which aims to assess their safety and efficacy in 140 adult subjects with active SS (ClinicalTrials.gov Identifier: NCT03100942).

Given the crucial role of the TBK1-TNF receptor associated factor NF-κB activator (TANK) in type I IFN production through Interferon Related Factor (IRF)-3 and IRF-7 ligation, administration of BX795 – a TANK inhibitor – resulted in downregulation of type I IFN inducible genes in PBMCs derived from SS patients [59]. No clinical trials are conducted yet.

2.3. Inflammasome

The purinergic $P2X_7$ receptor ($P2X_7R$) is an ATP-gated ion channel, which has an essential role in the innate immune response through activation of the Nod-like receptor family protein 3 (NLRP3) inflammasome, leading to release of mature IL-1β and IL-18 [60,61]. Gene expression of $P2X_7R$, NLRP3, apoptosis-associated speck-like

Table 2
Adaptive immune pathways inhibition by bDMARDs in SS.

Pathway	Registration number	Medication	Type of study	Status	Primary outcome	Study duration	Primary outcome fulfilled
Antigen presentation							
Cathepsin S	NCT02701985	RO5459072 (Petesicatib)	Multi-center, randomized, double-blind, placebo-controlled, parallel-group, phase IIa	Completed	ESSDAI	12 weeks	No
Costimulation							
CD 80/86 (B7.1/B7.2)/ CD28	Verstappen et al. [87]	Abatacept	Single center, randomized, placebo controlled, open label trial	Completed	ESSDAI, B- and T-cells, T-cell related cytokines	48 weeks	Yes
	NCT02915159	Abatacept	A phase III randomized, double-blind, placebo-controlled trial	Active, not recruiting	ESSDAI	12 months	Awaiting results
	NCT02067910	Abatacept	A phase III randomized, double-blind, placebo-controlled trial (ASAP III)	Active, not recruiting	SDAI at 1 year	12 months	Awaiting results
CD28	NCT02843659	BMS-931699 (Lulizumab pegol)	A phase II, randomized, multi-center, double-blind, placebo-controlled study	Terminated	ESSDAI	12 weeks	No
ICOSL/ICOS (B7h)	NCT02334306	AMG557/MEDI5872 (Prezalumab)	Randomized, placebo controlled, phase IIa	Completed	ESSDAI	24 weeks	Awaiting results
CD40/CD40L	NCT02291029, Fischer et al. [105]	CFZ533 (Iscalimab)	A multi-center, randomized, double-blind, placebo-controlled, parallel group, phase II study	Completed	ESSDAI	12 weeks	Yes
ICAM-1/LFA-1	NCT00344448	Efalizumab	Pilot, proof of concept, randomized, double-blind, placebo-controlled study	Terminated	Objective ocular and salivary tests	12 weeks	No (↑PML risk associated with Efalizumab in other studies)
B-cell activation							
BAFF/BAFF-R axis							
BAFF	NCT01160666 and NCT01008982	Belimumab	Open label proof of concept study	Completed	Oral dryness, parotid swelling, systemic activity as well as RF titers, Igs and cryoglobulinemia	28 weeks	Yes
BAFF-R	NCT02614716	LY3090106 (Tibulizumab)	Multiple ascending dose study	Completed	Safety, tolerability, pharmacokinetics and pharmacodynamics	197 days	Awaiting results
	NCT02149420, Dörner et al. [138,139]	VAY736 (Ianalumab)	Randomized, parallel group, double-blind, placebo-controlled	Completed	Efficacy and safety: B cell depletion, ↓ BAFF levels, ↓ parotid gland stiffness	24 weeks	Yes
	NCT02962895	VAY736 (Ianalumab)	Randomized parallel assessment study	Ongoing, recruiting	Efficacy and safety	24 weeks	Awaiting results
B cell/plasma depletion							
CD22	Steinfeld et al. [162]	Epratuzumab	Open label study	Completed	Fatigue VAS, Schirmer's test and stimulated whole saliva flow in 50%	6 months	Yes
CD20	Devauchelle-Pensec et al. [145]	Rituximab	Randomized, placebo-controlled, parallel-group	Completed	2/4 items: disease activity, joint pain, fatigue and dryness	24 weeks	No
	NCT00740948	Rituximab	TEARS	Completed	As above, improved only fatigue	24 weeks	Partially
	ISRCTN65360827	Rituximab	TRACTISS	Completed	As above, improved only fatigue	48 weeks	Partially
Combination of Blys blockade and B cell depletion	NCT02631538	Belimumab & Rituximab vs Belimumab	Double-blind, randomized, placebo-controlled	Active, not recruiting	Safety & tolerability	2 years	Awaiting results
BCR signaling							
PI3Kδ inhibitor	NCT02610543	Seletalisib–UCB/UCB5857	Double-blind, phase II, multicenter, placebo-controlled	Terminated	ESSDAI – efficacy, safety and tolerability	12 weeks	No
	NCT03627065	INCB 050465	Open-label phase II study	Recruiting	% participants with ≥1-point change on SGUS score	12 weeks	Awaiting results
	NCT02775916	CDZ173, Leniolisib	Double-blind randomized, placebo-controlled, parallel group study	Completed	ESSPRI and ESSDAI	12 weeks	Awaiting results
BTK inhibitor	NCT03100942	Filgotinib, GS-9876// Tirabrutinib GS-4059	Randomized, phase II, double-blind, placebo-controlled	Active but not currently recruiting	Protocol-specified response criteria	48 weeks	Awaiting results

(*continued on next page*)

Table 2 (*continued*)

Pathway	Registration number	Medication	Type of study	Status	Primary outcome	Study duration	Primary outcome fulfilled
T cell proliferation							
T regs restoration	NCT02464319	Low-dose human recombinant IL-2	Randomized controlled study	Completed	ESSDAI	24 weeks	Awaiting results
IL-7/IL7R	NCT03239600	GSK2618960	Phase II, randomized, placebo controlled	Withdrawn	Adverse events	29 weeks	No (portfolio prioritization)
Ectopic germinal center formation							
LTbR antagonist	NCT01552681	Baminercept	Randomized, double-blind, placebo-controlled phase II clinical trial	Terminated	ESSDAI, salivary flow	24 weeks	No (liver toxicity)

protein (ASC) and caspase-1 has been shown to be increased in salivary glands of SS patients compared to non-SS and controls in association with higher focus score [62] as well as lymphoma development [63]. Intraperitoneal injection of $P2X_7R$ antagonist A438079 in $CD28^{-/-}$, $IFN^{-/-}$, NOD.H-2h4 mouse model led to improvement of both salivary gland inflammation and saliva secretion [64]. However, no data on extraglandular manifestations are currently available.

3. Adaptive immunity (Table 2)

3.1. Antigen presentation

3.1.1. Cathepsin S

Cathepsin S (CatS) is an APC lysosome protease involved in autoantigen presentation to CD4+ T cells or NK1.1+ T cells by interfering with major histocompatibility complex (MHC) II or CD1 molecules, respectively [65]. Tear CatS activity has been shown to be increased in lacrimal glands and tears of SS and NOD murine models [66,67]; similar results were shown in tears derived from SS patients [67,68], in association with increased degradation of proteoglycan 4 (PRG4) [69]. Since the latter is a molecule with lubricating properties, high CatS activity results in diminished eye lubrication [69]. In this context, treatment with the CatS inhibitor Clik60 in a murine model characterized by CD4+ T cell mediated lesions in lacrimal and salivary glands, resulted in both reduced autoantigen-specific T cell responses in vitro and Th1 cytokine expression, preventing lesions in salivary glands [70]. Recently, treatment with the RO5459072 (Petesicatib) – a CatS inhibitor – of PBMCs derived from SS patients with strong Ro/SSA and La/SSB-induced T cell responses, resulted in reduction of IL-10 and TNF-α secretion by $CD14^+$ monocytes [68]. However, in a recent multicenter, randomized, double-blind, placebo-controlled, phase IIa study of RO5459072 in 75 SS patients after 12 weeks of treatment no significant changes in ESSDAI were demonstrated (ClinicalTrials.gov Identifier: NCT02701985).

3.2. Costimulation

Co-stimulation is a finely-tuned process which has a central role in B and T-cell activation and proliferation [71,72], crucial events in SS pathogenesis. In this context, three families of costimulatory molecules have been mainly shown to be involved in the interaction between APC, T and B cells: (i) Immunoglobulin (Ig) family, including: (a) CD80/86 (B7.1/B7.2) expressed on APCs, CD28 and cytotoxic T-lymphocyte-associated antigen 4 (CTLA4 or CD152) on T cells and (b) inducible costimulator of T cells ligand (ICOSL, B7h.2) on APCs and the ICOS on T cells, (ii) TNF/TNF-R family, including CD40 on APCs and B cells as well as CD40 ligand (CD40L) on T cells, and (iii) cell adhesion molecules, such as intercellular adhesion molecule (ICAM) on APCs and leukocyte function associated antigen-1 (LFA-1) on T cells, respectively. For full T cell activation at least 2 major interactions of costimulation are required: (i) of T cell receptor (TCR) expressed on the surface of T cells with MHC-II on APCs and (ii) of CD28 on T cells with CD80/CD86 on APCs. Upon T cell activation, the inhibitory molecule CTLA4 is upregulated on the T cell surface, outcompeting CD28, thus preventing its ligation with CD80/86 on APCs, resulting in suppression of T cell responses [73]. Once activated, interaction between CD40L on T cells and CD40 on B cells can lead to stimulation of B cells as well [74–78]. Administration of a blocking signal or inhibiting a stimulatory signal may regulate an otherwise overwhelming response [79].

3.2.1. Ig family

3.2.1.1. CD 80/86 – CD28. CD80/CD86 and ICOSL on APCs bind on CD28 and ICOS, respectively, both expressed on the surface of T cells, promoting survival, proliferation and cytokine secretion [80]. ICOS expression is induced following T cell binding to ICOSL [81].

CD80/86 are expressed on the surface of salivary gland epithelial

cells from SS patients [82], implying an antigen presenting role for these cells. Interestingly, treatment of the NFS/sld mutant murine SS model with an anti-CD86 antibody resulted in amelioration of autoimmune lesions in salivary and lacrimal glands [83].

Abatacept, is a humanized CTLA4-IgG1 fusion protein, which binds both CD80 and CD86 receptors on the APCs blocking their specific interaction with the CD28 T-cell receptor [84], leading to inhibition of T-cell activation and subsequent T-cell dependent B-cell activation. In a proof of concept study (Abatacept Sjögren Active Patients Phase Study: ASAP study) abatacept was administered in 15 SS patients for a total of 6 months resulting in decrease of EULAR Sjögren Syndrome Disease Activity Index (ESSDAI), ESSPRI, RF and IgG levels, which returned after the end of the study [85]. In a similar study, lymphocytic foci number, local FoxP3$^+$ T cells and local lymphocytic infiltrates decreased significantly, whereas salivary production increased. In addition, total lymphocytes, naïve B cells and CD4$^+$ T cells were heightened in periphery along with a reduction of gamma globulins [86]. Furthermore, intravenous administration of abatacept in 15 SS patients and equal number of age- and sex-matched healthy individuals showed that circulating T follicular helper (Tfh) and T regulatory (Treg) populations, ICOS expression, serum IL-21 and chemokine (C-X-C motif) ligand 13 (CXCL13), circulating plasmablasts and anti-Ro/SSA and anti-LA/SSB levels were significantly reduced, while the opposite was observed regarding circulating CD4$^+$ T cells. Decrease in ICOS was observed even in parotid glands. Cessation of treatment resulted in gradual return of all parameters to baseline values [87].

Studies with abatacept have also been performed in SS patients with RA. Administration of abatacept in 9 RA patients with SS features resulted in improvement of salivary secretion as well as signal intensity ratio (SIR) of parotid gland parenchyma [88], previously shown to correlate with sialography, histopathology and salivary secretion [89]. The results showed significant improvement of salivary secretion. Efficacy of abatacept was also evident in a similar population in a one year, open-label, prospective, observational multicenter study (ROSE study). Improvement in a simplified disease activity index (SDAI) as well as salivary flow both at 6 months [90] and on completion of the study at 1 year [91] was reported.

Moreover, a phase III randomized, double-blind, placebo-controlled trial of the efficacy and safety of subcutaneous abatacept given for 6 months followed by open-label administration of equal length in 253 SS patients was initiated in 2016. The study is active but not recruiting, estimated to be completed by 2023 (ClinicalTrials.gov Identifier: NCT02915159). A similar phase III study was initiated in 2014 (ASAP III Study). Study population will be divided into the usual weekly administration during a year and an initial placebo arm for 6 months followed by abatacept for another 6 months (ClinicalTrials.gov Identifier: NCT02067910). The study is active but not recruiting and no preliminary data have as yet been published [92].

In a study of 30 RA and 26 SS patients whose peripheral blood lymphocytes were evaluated, Bruton's tyrosine kinase (BTK) protein was increased in B cells from a major fraction of patients with primary SS and correlated with serum RF levels and parotid gland T cell infiltration. Interestingly, use of abatacept restored BTK protein expression in B cells to normal levels [93].

An issue that should be addressed is the fact that CD80/86-specific blocking strategies also inhibit CTLA-4 signal, which is crucial for Treg function [94]. Selective blockade of CD28 without affecting CTLA-4 is suggested as an effective strategy for modulating immune responses by preventing the maturation of pathogenic effectors while preserving the suppressive function of Tregs in a primate animal model [95]. However, a phase II clinical trial in 45 SS patients which attempted to evaluate efficacy and safety of BMS-931699 (lulizumab pegol) – a novel selective CD28 antagonist – has been terminated due to inability to meet protocol objectives (ClinicalTrials.gov Identifier: NCT02843659).

3.2.1.2. ICOS/ICOSL. Recent studies reveal that ICOS expression is elevated on PBMCs [96] derived from SS patients, while its expression on circulating Tfh cells declines after abatacept treatment and correlates with improvement on ESSDAI score [87]. Prezalumab/AMG557/MEDI5872, a fully human antibody directed against ICOS/CD278 ligand (ICOSL), expressed on T cell surface [97], has shown efficacy in a small clinical trial in patients with SLE suffering from arthritis [98]. In the context of SS, a phase IIa, randomized, placebo controlled, proof of mechanism safety and efficacy study, was designed. ESSDAI as primary outcome along with change in plasma cell (PC) and Tfh levels in both peripheral blood and MSG tissue, focus score, ESSPRI, safety and tolerability were assessed. This study is now completed, awaiting results (ClinicalTrials.gov Identifier: NCT02334306).

3.2.2. Costimulatory molecules of the TNF/TNFR family (CD40/CD40L)

CD40 is a transmembrane glycoprotein constitutively expressed on several immune (mainly APCs and B cells) and non-immune cells, such as epithelial, endothelial and keratinocytes [99]. Through its interaction with CD40L found on T cells it regulates B cell development, antibody production [100] and germinal center formation [101]. Previous studies revealed that soluble CD40L serum levels and mRNA expression in CD4$^+$T cells are increased in SS patients compared to healthy control donors [102,103], suggesting that CD40/CD40L pathway could represent a possible therapeutic target.

Iscalimab/CFZ533 is a novel blocking monoclonal antibody against CD40. In a phase II multi-center, randomized, double-blind, placebo-controlled, parallel group study, the safety, tolerability, pharmacokinetics and preliminary efficacy of CFZ533 in SS patients was tested. Study participants were randomized in 4 groups to receive either placebo or subcutaneous/intravenous dose of CFZ533, respectively. The latter group showed significant improvement regarding ESSDAI, ESSPRI, patient visual analogue scale (VAS), disease activity VAS, Short Form Survey (SF-36) and Multidimensional Fatigue Inventory (MFI) as well as reduction of CXCL13 after 12 weeks. The drug was safe and well tolerated. (ClinicalTrials gov: NCT02291029) [104]. The subgroup analysis of patients receiving induction treatment either subcutaneously or intravenously confirmed these results [105].

Furthermore, blockade of the CD40/CD40L interaction can be achieved through CD40L inhibition. To the same end, administration of MR1 – a novel anti-CD40L monoclonal antibody – in the NOD/ShiLtJ SS murine model, resulted in downregulation of CD40 and CD80 expression on B cells, abrogating their differentiation into antibody secreting phenotype, subsequently resulting in reduced anti-Ro secretion through dampened immune responses. Moreover prevention of germinal center formation through downregulation of activation-induced cytidine deaminase (AICDA) mRNA expression was detected [106]. These findings imply that CD40L could represent an alternative target of costimulation inhibition in the context of SS.

3.2.3. Cell adhesion molecules (LFA-1/ICAM-1)

LFA-1 – a β2 integrin on T cell surface – binds predominantly to intracellular adhesion molecule-1 (ICAM-1) on APCs enhancing their interaction and promoting activation and migration of T cells to inflammatory sites [107]. LFA-1 and ICAM-1 expression was found to be increased on activated lymphocytes and endothelial cells in salivary and lacrimal glands in a SS murine model (MRL/lpr) [108,109]. High mRNA and protein expression in the epithelial cells present in the conjunctival and accessory lacrimal tissues in dry eye patients are also observed [108,110]. Interestingly, blockade of the LFA-1/ICAM-1 interaction after retrograde installation of a recombinant virus encoding ICAM-1/Fc resulted in reduction of glandular inflammation in SS animal model (NOD) [111]. Moreover, treatment of the keratoconjunctivitis sicca (KCS) murine model with Lifitegrast – an LFA-1 inhibitor – led to reduction of IFN-γ and CXCL9 transcripts in animal conjunctiva [112].

Efalizumab is another recombinant humanized monoclonal antibody which inhibits the LFA-1/ICAM-1 interaction through binding to

human CD11a, the alpha-subunit of LFA-1. Efalizumab, which has already been approved by the United States food and drug administration (U.S. FDA) for treatment of mild-to-moderate psoriasis [113], was currently tested in a pilot, proof of concept, randomized, double-blind, placebo-controlled study in SS patients. Unfortunately, increased documented risk of progressive multifocal leukoencephalopathy (PML) associated with efalizumab in other studies led to early termination of the SS study (ClinicalTrials.gov Identifier: NCT00344448).

3.3. B cell activation

Hyperactivity of B cells is a cardinal feature of SS pathogenesis. About 35–40% of patients suffering from SS have hypergammaglobulinemia and circulating autoantibodies (RF, anti-Ro/SSA and anti-La/SSB) [1,114]. Given their central role in disease pathogenesis, targeting B cells provides a reasonable therapeutic approach and new drugs are acting through B cell depletion or inhibition of B cell differentiation [115].

3.3.1. BAFF

B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are cytokines, members of the TNF family with crucial role in B cell development, survival, maturation and differentiation. BAFF is present in two forms, membrane-bound and soluble, which is the biologically active form [116,117], in contrast to APRIL which exists only in soluble form [118]. The two molecules are acting through three receptor types: TACI (transmembrane activator and calcium modulator), BCMA (B cell maturation antigen) and BAFF-R (BAFF receptor). TACI and BCMA bind APRIL, whereas BAFF-R selectively binds BAFF [119–122]. BAFF and APRIL are secreted from monocytes/macrophages, DCs, pDCs, follicular DCs, neutrophils, epithelial cells (ECs) and activated T lymphocytes [123]. The contribution of BAFF in SS pathogenesis has been established in both BAFF-transgenic mice model [120] and in vivo [124]. Increased serum and salivary gland BAFF levels are associated with autoantibodies against Ro/SSA and La/SSB as well as RF [124,125]. Given that BAFF levels, as well as genetic variants of both BAFF and BAFF-R, have been linked to SS and SS-related lymphoma, B cells represent a promising therapeutic target for SS patients either complicated or not by mucosa-associated lymphoid tissue (MALT) lymphoma [3,126–129].

Belimumab is a fully humanized monoclonal antibody toward the soluble B lymphocyte stimulator (BLyS) already licensed for patients with SLE. The first open label proof of concept study to evaluate the efficacy and safety of belimumab in SS was conducted simultaneously in two European centers, Paris, France and Udine, Italy (ClinicalTrials.gov Identifier: NCT01160666 and NCT01008982, respectively) [130,131]. Improvements in oral dryness, parotid swelling, systemic activity as well as RF titers, Igs and cryoglobulinemia were observed, with salivary flow, Schirmer's test, and focus score of MSGBs being unchanged [132]. Notably, belimumab treatment led to restoration of both transitional and naïve B cells to similar levels to those observed in healthy controls and normalized BAFF-R expression levels. As a result, a decrease in serum IgM and IgG titers as well as antinuclear antibodies (ANA) and RF titers was observed. In contrast, an increase in C4 complement levels was seen [131]. In the follow-up study (ClinicalTrials.gov Identifier: NCT01008982), which included SS patients enrolled in BELLIS study, after six and twelve months cessation of belimumab, a deterioration of clinical parameters was observed including increased RF, IgM, and BAFF titers as well as circulating B cells at baseline [129]. Analysis of a subgroup (ClinicalTrials.gov Identifier: NCT01160660) of the BELISS study revealed that high blood and salivary NK cell numbers are associated with a worse response to belimumab [133], and high type I IFN scores at baseline are associated with improved outcomes, such as reduced IgG, IgM and RF serum levels [134]. According to the authors, these findings possibly imply the existence of two distinct subsets of SS: one with a predominant type I IFN-BAFF-B cell axis, representing good responders to belimumab; and one with a predominant type II IFN-NK cell axis, representing non-responders [133,134].

BAFF levels are associated with B cell clonal expansion in salivary glands of SS patients [127] and its overexpression is considered as a possible reason of failure of the B-cell depleting treatment with Rituximab in SS patients complicated with MALT lymphoma [127,135]. The simultaneous anti-BAFF and anti-CD20 treatment showed effectiveness on reducing marginal zones of B cells in the murine model for CD20 expression [136], suggesting that targeting BAFF could facilitate the action and increase the efficacy of anti-CD20 therapy in SS patients with MALT lymphoma [137]. In this context, a double-blind, randomized, placebo-controlled trial (ClinicalTrials.gov Identifier: NCT02631538) in 70 active SS patients was planned aiming at evaluating the safety and tolerability profile of belimumab/rituximab co-administration in comparison to belimumab monotherapy. This study is active but not recruiting.

In the context of targeting BAFF/BAFF-R axes, two novel biologic agents are currently tested in clinical trials. Tibulizumab (LY3090106) – a humanized monoclonal antibody targeting BAFF and IL-17a – is currently evaluated regarding safety, tolerability, pharmacokinetics and pharmacodynamics in 32 SS patients (ClinicalTrials.gov Identifier: NCT02614716), with no data available yet. Moreover, ianalumab/VAY736 is another novel, human IgG1 monoclonal antibody targeting BAFF-R, leading to both B cell depletion – possibly through antibody dependent cytotoxicity – and blockade of BAFF/BAFF-R signaling. Efficacy and safety of VAY736 has been tested in a recent, randomized, parallel group, double-blind, placebo-controlled, 24 weeks trial, in 27 SS patients. BAFF serum levels reduction and parotid gland stiffness improvement detected by ultrasound (US), has been achieved in all treated patients (ClinicalTrials.gov Identifier: NCT02149420) [138]. Moreover, improvement without reaching statistical significance regarding ESSDAI, ESSPRI, multidimensional fatigue inventory (MFI), short Form-36 (SF-36), global assessments by physician (PhGA) and patient (PaGA), as well as B cell depletion were observed. Interestingly, a particular strong response was observed in general fatigue and physical fatigue – components of MFI – especially in the 10 mg/kg single infusion group, with positive results sustained until study completion. Infusion site reactions were reported by approximately 3/4 of patients [139]. These results will be evaluated in an ongoing randomized parallel assessment study aiming at evaluating efficacy and safety of multiple doses of VAY736 in a larger cohort of SS patients with moderate to severe disease activity (ClinicalTrials.gov Identifier: NCT02962895). In order to predict responders and non-responders receiving VAY736 treatment, the Immunosignature technology has been implemented as a potential tool on drug response prediction [140].

3.3.2. CD20

CD20 is expressed on most B cell precursors and mediates their activation, proliferation and differentiation [141]. Rituximab (RTX) is a chimeric antibody directed against CD20 antigen leading to B cell depletion. As an additional mechanism for its action, recent data suggest that RTX decreases B cell-derived proinflammatory cytokines, B cell antigen-presenting activity and $FcRL4^{+}$ B cells [142]. It also hampers the production of IL-17-producing T cells [143]. B cell depletion strategy initially provided beneficial results as reported from several open-label studies, showing significant improvement in fatigue, mucosal dryness [144–150] and systemic manifestations [151]. These results were however not confirmed in subsequent randomized clinical trials which failed to reach the primary endpoints (improvement in two of four items: disease activity, joint pain, fatigue and dryness at 24 weeks) [145]. Recently, two large placebo-controlled trials TEARS (NCT00740948) [145,152] and TRACTISS (ISRCTN65360827) [153,154] have been conducted. Both studies confirmed the efficacy of RTX to improve fatigue and salivary flow but did not achieve the remaining of primary and secondary outcomes tested such as joint pain, ocular and overall dryness

[152,154]. The apparent discrepancy between different reports may be due to different size cohorts, baseline systemic disease activity and time points of measurements implemented. There is no current active clinical trial regarding RTX use in SS.

3.3.3. CD22

CD22 protein is a B cell surface molecule [155], acting both as a homing receptor and down-modulator of B cell receptor (BCR) signaling [156,157]. CD22 plays a key role in the entry of B cells into the lacrimal and salivary glands of SS patients [155,158]. Epratuzumab is a humanized monoclonal antibody against the CD22 receptors on B-cells, leading to peripheral depletion [159]. Although several clinical trials have tested the efficacy and safety of epratuzumab in SLE patients [160,161], only one small open label study has been performed in SS to date. In this study, 16 SS patients received 4 infusions of epratuzumab once every 2 weeks with a 6-month follow-up period. Epratuzumab showed efficacy in fatigue, Schirmer's test and stimulated whole salivary flow, approximately in half of the patients [162]. These promising results might be related to the drug's dual functional role as a homing receptor for recirculating B cells and a down-modulating co-receptor for BCR [162]. Thus, epratuzumab seems to hold promise in active SS, suggesting that further studies may be conducted. No randomized clinical trials are underway in order to confirm these findings.

3.3.4. BCR signaling

Growing evidence points to a critical role of a network of kinases such as spleen tyrosine kinase, phosphatidylinositol 3-kinase delta isoform (PI3Kδ) and Bruton's tyrosine kinase (BTK) in BCR signal transduction. Since signaling of PI3Kδ has been implicated in proliferation, migration, and function of B cells, targeting of these kinases was attempted in patients with B-cell lymphoma [163,164]. Due to the previously shown central role of B cells in SS pathogenesis, targeting of the PI3K signal transduction pathway seems relevant. Indeed, a decrease in lymphocytic infiltration and subsequent disruption of lymphoid aggregates was observed in a murine model treated with UCB5857, a small-molecule inhibitor of PI3Kδ [165]. Moreover, reduced transcript and protein levels of chemokines CXCL13 and CXCL12 along with downregulation of lymphoid cytokines (LTα, LTβ), BAFF and AICDA was observed in salivary gland tissue of the UCB5857 treated mice, implying a promising role of this agent in SS treatment [166]. However, a phase II, multicenter, double-blind, placebo-controlled, 12 week proof-of-concept study to assess the efficacy, safety and tolerability of Seletalisib–UCB/UCB5857 in SS patients was stopped prematurely due to enrolment challenges (ClinicalTrials.gov Identifier: NCT02610543). Another selective PI3Kδ inhibitor (INCB 050465) administered orally once daily is currently in an open-label phase II study in recruiting phase, with primary outcome being the proportion of participants with ≥1-point change on the salivary gland ultrasound (SGUS) score for parotid and submandibular glands at Week 4 and 12. Secondary outcome measures include salivary CXCL13 levels change from baseline at the same time frame (ClinicalTrials.gov Identifier: NCT03627065). In the same context, a study of an orally administered selective PI3Kδ inhibitor (CDZ173, leniolisib) for 12 weeks was tested in terms of safety, tolerability, pharmacokinetics and preliminary therapeutic efficacy. Primary and secondary outcome measures included ESSPRI and ESSDAI, physician- and patient global assessment of disease activity (VAS) change from baseline, respectively. The study was completed by March 2018, awaiting results (ClinicalTrials.gov Identifier: NCT02775916).

Given that BTK is a crucial kinase activating NF-κB pathway in diffuse large cell lymphomas (DLCL), tirabrutinib, a selective, irreversible BTK inhibitor is currently considered [167]. Safety and efficacy of the BTK inhibitor Tirabrutinib (formerly GS-4059) is currently evaluated in a randomized, phase II, double-blind, placebo-controlled study involving 150 adult subjects with active SS (ClinicalTrials.gov Identifier: NCT03100942).

4. T cell proliferation

Infiltrating T cells are critical in SS pathogenesis contributing to exocrine gland tissue damage and systemic manifestations through secretion of pro-inflammatory cytokines such as IFN-γ, IL-7, IL-17 [168,169] and B cell recruitment [170], suggesting its potential role as a prominent therapeutic target.

4.1. GSK2618960/IL-7

IL-7/IL7-R axes plays a key role in T cell ontogeny [171], homeostasis [172], survival (anti-apoptotic activity), differentiation and proliferation of mature naive and memory T cells [173]. In addition, IL-7 regulates survival and function of innate lymphoid cells including NK cells [174]. Increased IL-7R serum levels [175] and higher expression of both IL-7 and IL-7R [175–177] in labial salivary glands of SS patients compared to controls have been associated with increased inflammation [176] and higher risk for lymphoma development [175]. Interestingly, blockade of IL-7R significantly ameliorated hyposalivation and reduced IFN-γ-producing CD4+ and CD8+ T cells, B cells, and CXCL9-13 in the MSGBs of a SS mouse model [178]. Given these findings targeting IL7/IL7R pathway could represent a promising therapy.

GSK2618960 is a humanized Fc-disabled IgG1 monoclonal antibody (mAb) directed against the alpha component (IL7Rα; CD127) of the heterodimeric IL-7R. Although the safety and pharmacokinetic properties of GSK2618960 have been already tested in healthy subjects demonstrating a tolerant profile, a clinical trial regarding its use in SS treatment is withdrawn due to portfolio prioritization (ClinicalTrials.gov Identifier: NCT03239600).

4.2. Tregs restoration

IL-2 has been shown to be reduced compared to IL-6 and IL-17A cytokines, previously shown elevated in the plasma of SS patients. Th17 cells have been previously shown to be disproportionately increased in comparison to Tregs, contributing in SS pathogenesis [179]. Given the role of IL-2 in supporting the function of Treg in vivo, supplementation of this cytokine might have a beneficial role in the restoration of this imbalance [180]. In a previous study of 31 SS patients, the expression of phosphorylated STAT-5 (p-STAT5) was reduced, while p-STAT3 was increased in both SS MSGBs and PBMC – derived $CD4^+$ T cells in comparison to sicca and healthy controls, respectively. Induction of STAT5 by in vitro IL-2 treatment of PBMCs derived from SS patients led to a decrease of Th17 differentiation, implying a possible therapeutic target for SS [181]. In line with these results, administration of low-dose recombinant (rh) IL-2 treatment was shown to restore Th17/Treg imbalance cells in SS along with ESSDAI improved [182].

The safety and efficacy of a low-dose human rh IL-2 was explored in 60 SS patients during a randomized controlled study. The regimen was administered as a subcutaneous injection. Primary outcome employed ESSDAI in 24 weeks, while secondary targets included CD4+ T cells, Th17 cells and Tfh cells both before and during treatment. The trial is reported completed by March 2018 although no results have been made public as yet (ClinicalTrials.gov Identifier: NCT02464319).

5. Ectopic germinal center formation

5.1. Lymphotoxin beta receptor

Ectopic germinal centers are structures of B cells encircled by T cells inside secondary lymphoid tissue. In SS gradual formation of germinal centers is initiated by inflammatory infiltrates of T cells, B cells and plasma cells around ductal tissue [183] and have been found to associate with higher RF, and increased anti-Ro/SSA and anti-La/SSB titers [184,185]. The formation of ectopic germinal centers in the setting of SS has been shown to be highly dependent on lymphotoxin b receptor

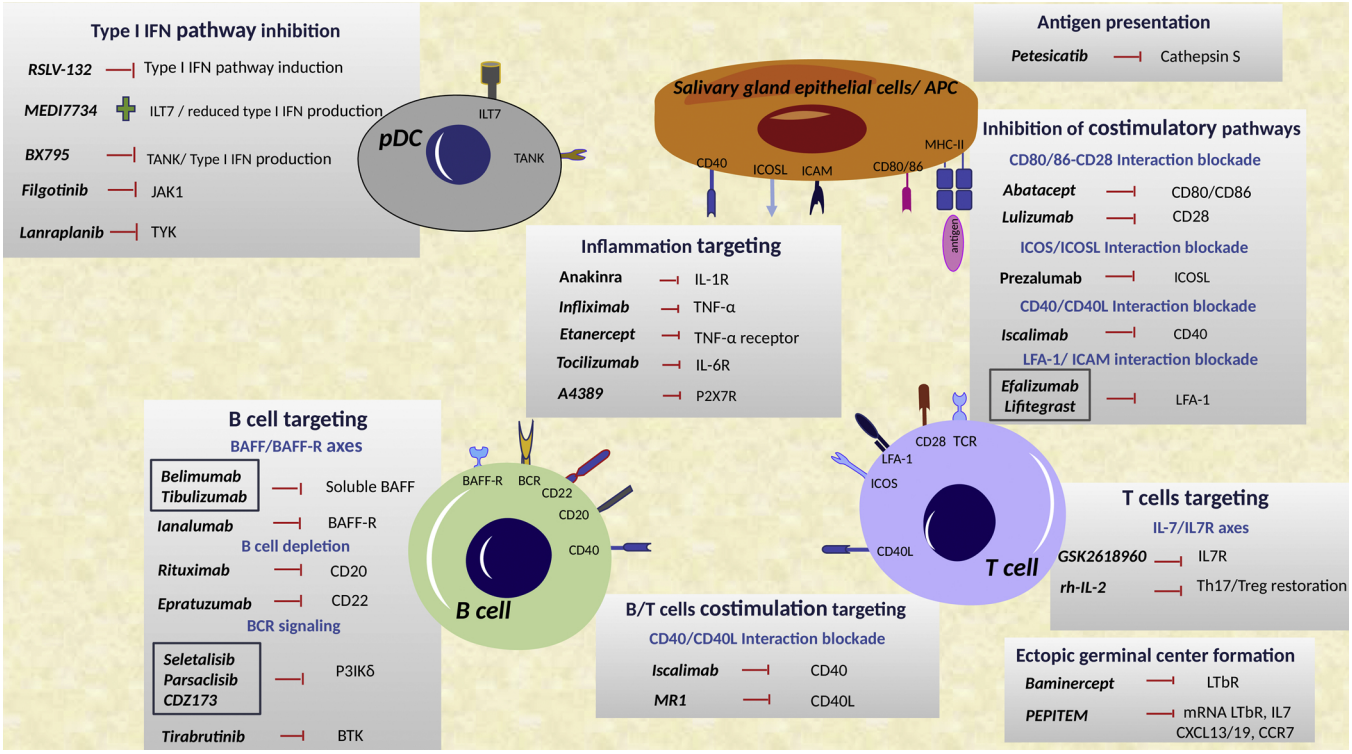

Fig. 1. Overview of the major SS pathogenetic pathways targeted by bDMARDs.
+: stimulation; ↓: reduction; ⊥: inhibition.
B cell activating factor receptor: BAFF-R; B cell receptor: BCR; T cell receptor: TCR; tumor necrosis factor alpha: TNF-α; purinergic receptor P2X7R: P2X7R; inducible costimulator of T cells: ICOS; inducible costimulatory of T cells ligand: ICOSL; Bruton's tyrosine kinase: BTK; phosphatidylinositol 3-kinase delta isoform: PI3Kδ; Lymphotoxin receptor beta: LTbR; intracellular adhesion molecule-1: ICAM-1; Lymphocyte function-associated antigen 1: LFA-1; interferon: IFN; chemokine (C-X-C motif) ligand 13/19: CXCL13/19; C-C chemokine receptor type 7: CCR7; TNF receptor associated factor NF-kB: TANK; janus kinase: JAK; tyrosine kinase: TYK; interleukin 1 receptor: IL-1R; interleukin 6 receptor: IL-6R; interleukin 7 receptor: IL-7R; major histocompatibility complex class II: MHC-II; regulatory T lymphocytes: Tregs; T helper 17 cells: Th17; recombinant human interleukin 2: rh-IL-2.

(LTbR)/(TNFRSF3) signaling [186–188]. In a NOD murine model of SS, an anti-LTbR antibody was injected intraperitoneally, resulting in reduction of B cell accumulation, elimination of high endothelial venules (HEV) and reduction of the entry rate of lymphocytes into lacrimal glands [189]. Moreover, reduction of CXCL13 was observed, while both tear-fluid secretion and ocular surface integrity score improved significantly.

Given these encouraging results, efficacy and safety of baminercept, an LTb-R fusion protein was tested on 52 SS patients in a 24 week, randomized, double-blind, placebo-controlled, phase II, clinical trial [190]. Unfortunately, unstimulated whole salivary flow rate and ESSDAI did not improve significantly. Regarding side effects, breast cancer, hepatocellular injury, abnormal liver function tests and pleurisy as well as anemia, upper respiratory tract infection and injection site reactions were reported more often in the study drug subgroup in comparison to placebo (ClinicalTrials.gov Identifier: NCT01552681).

Recent studies suggest that the B cell-derived peptide (PEPITEM) regulates T cell trafficking through secretion of sphingosine-1 phosphate [191] in RA and diabetes mellitus (DM). Intra-ductal administration of PEPITEM in a murine C57BL/6 SS model resulted in decrease of both lymphotoxin beta mRNA transcripts as well as IL-7, lymphoid chemokines (CCL19 and CXCL13) and T-cell chemokine receptor CCR7, molecules known to regulate formation of ectopic germinal centers in SS [192]. An overview of the molecular pathways along with related targeted therapies is illustrated in Fig. 1.

6. Conclusion

Despite our better understanding in SS pathogenesis, treatment of SS is still elusive. In light of the efficacy and safety of bDMARDs in autoimmune diseases of similar etiopathogenetic mechanisms, previously studied agents such as rituximab, belimumab and abatacept are currently tested in SS patients with encouraging results. Furthermore, the study of inhibition of pathogenetic pathways via other molecules is currently underway. With multiple new drugs emerging from the pipeline, the need to differentiate phenotypic subgroups on the basis of distinct pathogenetic pathways in SS seems to be crucial. Carefully tailored treatment modalities for the individual SS patient can ensure maximum efficacy with minimal adverse events.

List of compounds

1. RSLV-132
2. Filgotinib (GS-9876)
3. Belimumab
4. VAY736 (Ianalumab)
5. Rituximab
6. Seletalisib
7. CFZ533 (Iscalimab)
8. Petesicatib
9. Bamniercept
10. PEPITEM

Conflict of interest statement

All authors declare no conflict of interest.

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Type I interferon signature in Sjögren's syndrome: pathophysiological and clinical implications

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ABSTRACT

Type I interferons (IFN) have long been recognised as mediators of innate immune defense mechanisms against viral threats. Robust evidence over the last 15 years revealed their significant role in the pathogenesis of systemic autoimmune diseases, including systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS). Despite the progress, methods of detection, initial triggers, biological functions and clinical associations in the setting of autoimmunity remain to be fully clarified. As therapeutic options for SS are currently limited, neutralising specific targets of the type I IFN pathway seems a promising option. In this review we summarise the current evidence regarding the role of type I IFN in SS.

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune entity affecting typically the exocrine glands of middle-aged women. Infiltration of immune cells in the salivary and lacrimal glands leads to loss of secretory function and eventually to desiccation of oral and ocular mucosa. In a significant proportion of patients, extraglandular manifestations are present involving mainly the joints, kidneys, lung, and peripheral nervous system (1, 2). Activation of innate immune pathways has long been considered as central pathogenetic contributor, with type I interferon (IFN) attracting particular interest over the last decade (3).

Type I IFNs, members of the class II family of α-helical cytokines, along with type II IFNs and numerous other cytokines, include 13 IFNα subtypes, β, ε (expressed in placenta, supposedly having a role in reproduction), κ (in keratinocytes), ω, δ, and τ (the two latter not found in humans). They mainly signal through IFNα/β receptor (IFNAR), consisting of two subunits IFNAR1 and IFNAR2. Upon binding with IFNs, autophosphorylation of the Janus protein kinases Tyk2 and Jak1 -attached to IFNAR1 and IFNAR2 subunits respectively-, occurs. As a result, signal transducer and activator of transcription (STAT) 1 and 2 are phosphorylated leading to the formation of a STAT2/STAT1 heterodimer, which following binding of interferon-regulatory factor 9 (IRF-9), leads to the formation of a complex namely IFN-stimulated gene factor 3 (ISGF3). The latter translocates to nucleus and following binding to specific elements (interferon stimulated response elements) on the promoters of IFN stimulated genes (ISGs) leads to their transcription. The encoded ISG proteins are characterised by a wide variety of antiviral properties including prevention of the assaulting virus from entering the cells and subsequently being replicated, translated, assembled and released (4, 5).

Though the main action is antiviral/antineoplastic, several immunomodulatory functions have also been attributed to type I IFNs including induction of B cell activating factor (BAFF) (6), immunoglobulin switching (7), increased antigen presentation, T-cell mediated and natural killer cell (NK) cytotoxicity (8). As a result of these immunostimulatory properties, a tight control of type I IFN activation is mandatory in order to ensure adequate host defenses avoiding collateral tissue damage by excessive responses. Thus, checkpoints at several levels of type I IFN pathway have been detected including mainly post-transcriptional modifications of the signalling pathway (phosphorylation) and regulation of type I IFN expression by epigenetic modifications including DNA methylation, histone modification and non-coding RNA effects (9).

While virtually any cell is able to produce type I IFNs (10) following stimu-

lation by exogenous or endogenous nucleic acids either through toll-like receptors (TLRs) or cytoplasmic sensors, plasmacytoid dendritic cells (pDCs) are considered to be the main IFNα producing cells. They are characterised by increased constitutive expression of TLR7 and TLR9 -stimulated by ssRNA/endogenous RNA and unmethylated DNA respectively- and a rapid and robust response following triggering by nucleic acids (11). Upon ligation, they signal through the myeloid differentiation primary response 88 (MyD88) activating IRF7, which has a central role in type I IFN production (12).

The main cytoplasmic receptors able to detect endogenous nucleic acids include the DNA sensors absent in melanoma 2 (AIM2) and cyclic GMP-AMP synthetase (cGAS) sensing dsDNA as well as the retinoic acid-inducible gene I (RIG-I) like receptor (RLR) family comprising RIG-I and Melanoma differentiation associated gene 5 (MDA5) triggered by dsRNA (13). The downstream signalling pathway mediates the phosphorylation of IRF3, 7 and Nuclear Factor-kappa B (NFκB) and subsequent type I IFN and induction of proinflammatory cytokine genes (14).

Activation of type I IFN pathway in SS

The first indication of type I IFN activation in SS dates back to late seventies (15). Since then, a growing body of evidence supports a significant role for type I IFN in the pathogenesis of SS, similarly to what was observed in other systemic autoimmune diseases such as systemic lupus erythematosus (SLE) (16), dermatomyositis (17) and systemic sclerosis (18).

Using microarrays and real time PCR studies, upregulation of ISGs was initially detected in SS disease targets such as minor salivary gland (MSG) biopsies (19-21) and ocular epithelial cells (19) compared to healthy controls. After immunoblot testing using specific probes for either type I or type II IFN related proteins (IFIT-3 and GBP-2, respectively) the heterogeneity of IFN activity at the level of MSG tissues has been appreciated (22). In a follow-up study, using validated probes for type I or type II IFN pathway, three IFN patterns in MSG biopsies were detected including either a predominantly type I or type II, or a mixed type I/II IFN; IFIT-3 (another type I IFN inducible gene) mainly stained salivary duct epithelial cells, while GBP-2 (a type II IFN related gene) was found in both lymphoid and duct epithelial cells surrounded by inflammatory cells by immunohistochemistry (23).

Beyond salivary gland tissues, the presence of type I IFN signature was next evaluated at systemic level. Thus, increased expression of type I IFN inducible genes or proteins has been revealed in SS derived peripheral blood mononuclear cells (PBMCs) (24, 25), whole blood (24, 26, 27), monocytes (28-30) and recently B-cells (31, 25). Based on previous work on lupus (32) and SS derived salivary gland tissues (23), Bodewes *et al*. revealed 3 distinct IFN patterns in peripheral blood from SS patients, a type I IFN predominant, a type I and II IFN mixed pattern as well as an inactive one (33). The presence of a heightened type I IFN signature in SS ranges from 53% to 81% in several gene expression studies (27, 33-35).

Measurement of IFNs in periphery has traditionally been elusive (36, 38). While initial reports failed to detect the presence of IFNα in serum (37), possibly due to the presence of many type I IFN subtypes currently undetectable by commercial ELISAs, the introduction of bioassays allowed the detection of systemic type I IFN activity in SS serum (28) or plasma (6), which seems to account for the upregulated type I IFN signature. Whether IFNα (39, 40) or IFNβ (28) account for the increased type I IFN activity in SS peripheral blood remains to be clarified. In a recent study an advanced ELISA with single molecule array (SIMOA) digital technology was used in order to measure attomolar IFNα levels in different groups, including adult and juvenile SLE, diabetes mellitus (DM), and IFNopathies (41). This technique, though promising, has not been applied in SS yet.

Given the apparently conflicting data between studies in regard to whether type I IFN or II predominate in SS and taken into account SS phenotypic heterogeneity, the overlapping regulation of many ISGs by both type I and II IFNs and the type of biological sample implemented, we proceeded to quantitation of transcripts predominantly induced either by type I or type II IFN in both peripheral blood and MSG tissue from well characterised SS patients. Thus, while in peripheral blood a type I IFN predominant signal was observed, in salivary glands type II IFN related genes were mainly overexpressed. Moreover, increased IFNγ but low IFNα transcripts were detected in MSG tissue derived from SS patients with lymphoma compared to SS with no lymphoma and sicca controls; as a result the IFNγ/IFNα ratio has been shown to be a potential biomarker for identification of lymphoma among SS patients with high area under the curve values in the Receiver-operating characteristic analysis (ROC) (27).

Additionally, recent data support a contributing role for type III IFN – namely IFNλ – in the pathogenesis of SS. All three subtypes IFNλ1/interlekin (IL)-29, IFNλ2/IL-28A and IFNλ3/IL-28B signal through the heterodimeric IFNλ R1/IL-28Ra receptor predominantly expressed in pDCs. SS patients with intermediate lesions in MSGB tissue were found to have increased both IFN-λ2/IL-28A epithelial expression and IFN-λ1/IL-29 levels in the periphery in comparison to sicca controls (42). Furthermore, in another study the addition of IFNλ1/IL-29 to IFNα led to even more enhanced stimulation of the ISGs BAFF and CXCL10 as well as the prolongation of phosphorylation of STAT in the immortalised human salivary gland ductal cell line NS-SV-DC, implying a synergistic effect of IFNα and IFNλ in the pathogenesis of SS (43), although studies in a larger scale are needed to confirm these results.

Of interest, proteins induced by type I IFNs, such as sialic acid binding Ig like lectin 1 (SIGLEC1), a cell surface protein on monocytes and macrophages detected by flow cytometry in peripheral blood, was shown to correlate with EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) score and discriminate SS patients with glandular and extraglandular manifestations (30).

In a recent report, soluble SIGLEC5, a transmembrane member of immunoglobulin superfamily expressed in neutrophils (44), was found to be elevated in saliva but not serum in SS patients compared to controls, in association with impaired salivary secretion, increased ocular damage and higher serum IgG levels (45).

Though the source of systemic and local IFN production has not been yet fully elucidated, several lines of evidence point toward pDCs, the professional type I IFN producing cells as chief contributors of IFN signature in SS. Thus, reduced (28, 46) but activated pDCs (28) in peripheral blood of SS patients together with their identification at the level of salivary gland tissue (19, 27, 37), imply a potentially significant role in both systemic (28) and local IFN activity (19, 27, 37).

Contributors of exaggerated type I interferon production in SS

The initial trigger of type I IFN production in the setting of autoimmunity remains an area of intensive research, a growing body of data so far strongly support endogenous nucleic acids as a potential source for the intrinsic activation of type I IFN system in the absence of exogenous viruses. In SS, similarly to studies in lupus (47), immunocomplexes consisting of RNA-containing apoptotic bodies and antibodies against ribonucleoproteins derived from SS sera were shown to induce type I IFN production, following pDC stimulation (37).

Previous studies revealed that DNA derived from SLE patients was characterised by repetitive sequences (48), was enriched in CpG nucleotides and displayed high homology to retroviruses (49). Moreover, Perl *et al*. (50) noted the presence of antibodies to endogenous retroviruses in sera of patients with several autoimmune diseases including SS. A potential source of endogenous retroviral material include Long interspersed nuclear elements (LINEs; L1), which comprise 17% of the human genome and are able to translocate within the genome, once they are fully transcribed (51). The typical structure of L1 elements includes open reading frames (ORF) that can encode an endonuclease and a reverse transcriptase. L1 promoter methylation is one of the major mechanisms that keep it suppressed, though other controlling mechanisms have been also described (52, 53). We have previously shown that L1 transcripts were found to be overexpressed in SS patients compared to sicca controls in strong correlation with both IFNα and β at the level of MSG tissues. Failure of upregulation of L1 expression following stimulation of healthy PBMC and other cell lines with IFNα and TLR7/TLR9 ligands excluded the possibility of Type I IFN-induced L1 overexpression. On the other hand, transfection of pDCs or CD14+ monocytes with L1-carrying plasmids or L1-RNA led to type I IFN pathway activation through both TLR dependent and independent pathways, evidenced by the abrogation of type I IFN production following incubation with a TLR7/TLR8 or a TNF receptor associated factor NF-ϰB activator (TBK1)/IKKε inhibitor (54). Of interest, L1 expression was increased in patients with uncomplicated local SS which usually occurs in patients with advanced age (55). In line with these observations, a recent study has shown that L1 can drive type I IFN production in senescent cells (56).

Compatible with these findings, SS patients with heightened type I IFN scores in peripheral monocytes were shown to display increased transcript levels of both endosomal and cytoplasmic nucleic acid receptors such as TLR7, MDA5, RIG-I and protein kinase R (PKR) in both pDCs and monocytes, implying their contributory role in type I IFN production (57). Moreover, increased basal phosphorylation levels either in B cells (58) or T and Natural Killer (NK) cells together with enhanced B cell signalling through TLR7 and TLR9 ligation (25), have been proposed as potential drivers of augmented IFN responses in these patients.

Possible mechanisms accounting for L1 derepression remain of particular interest. A negative correlation between L1 expression and methylation levels of the L1 promoter were highly suggestive of impaired methylation mechanisms in SS patients (54). In order to further explore underlying abnormalities accounting for the decreased methylation levels, we measured gene expression of several members of the methylation machinery and found a positive correlation between DNMT1, DNMT3B and MeCP2 and L1 expression in SS salivary glands and a negative correlation with lymphoid specific helicase (LSH). These observations imply a potential compensatory role for DNMT1, DNMT3B and MeCP2 in controlling inappropriate L1 overexpression with decreased LSH production potentially being responsible for the hypomethylation of L1 promoter (59).

Another restricting mechanism implicated in both viral infections and L1 control include members of the APOBEC family (60, 61). We have recently shown upregulation of APOBEC3A transcripts in SS MSG tissues in strong correlation with both L1 and IFNα mRNA, reflecting a potentially compensatory role against endogenous retroelements (62).

Finally, genetic influences have been shown to have an impact on type I IFN responses in the setting of autoimmunity. Thus, similarly to lupus (63), the rs10774671 variant of the 2'-5'-oligoadenylate synthetase 1 (OAS1), a viral RNA degrading enzyme, previously shown to be a risk allele for SS, has been shown to be related to a dysfunctional transcript. Thus, upon viral infection, a defective clearing mechanism of virus possibly occurs, leading to perpetuation of type I IFN responses due to the ongoing activation by viral remnants. In view of the implication of several viral triggers in SS pathogenesis (64), this mechanism might provide a functional explanation in the pathogenesis of SS. Moreover, in another study we have shown that protein tyrosine phosphatase non-receptor 22 (PTPN22), an allele associated with other autoimmune diseases such as SLE, DM and Grave's disease, is increasingly found in SS patients in comparison to healthy individuals, especially those with low type I IFN signature (65).

Type I IFN activation in SS clinical associations

Ocular and oral dryness are considered to be the most characteristic clini-

cal features of SS, but extraglandular manifestations can occur in at least one third of patients. The effort to associate specific clinical phenotypes with high or low IFN signature score follows the more well-established correlation between autoantibody/serological profiling and histopathological information from MSG biopsies, on the one hand and disease severity or even lymphomagenesis probability, on the other (66, 67).

A high type I IFN signature in CD14 monocytes was previously shown to identify SS patients with higher ESSDAI scores (34). However, total ESSDAI was not linked to high IFN activity (type I or mixed I/II) in a more recent study (33). SS individuals characterised by high type I IFN score have also exhibited higher anti-Ro52, anti-Ro60, anti-La, rheumatoid factor (RF) and serum IgG, as well as lower C3 levels and lower lymphocyte and neutrophil counts (34). Given that anti-Ro, anti-La and RF positivity has been associated with earlier disease onset, heavier glandular involvement and extraglandular manifestations, it is clear that there are clinical implications behind this association (68).

Despite the extensive knowledge on IFNα administration adverse effects, with flu-like symptoms and fatigue being the main complaints of patients receiving this treatment for various conditions, fatigue in the context of SS does not seem to correlate with IFN related genes (33, 69). As for IFN-induced protein expression on glandular tissue level, results from immunoblotting and immunohistochemistry showed a correlation between high IFN type I, II or mixed type I/II scores and more severe glandular secretory insufficiency, leukopenia and high ANA, anti-Ro/SSA, IgG and IgA titres. Last but not least, MSG biopsies focus score was remarkably higher in the high IFN type II group compared to low IFN altogether or high IFN type I groups (23).

All of the above lead to the impression that while both IFN types share some common pathways in the pathogenesis of SS, such as BAFF upregulation, it is the predominance of type II over type I IFN in the glandular tissue that tips the balance and promotes MALT lymphomagenesis. High focus score and low C4, seen more frequently in type II IFN-predominant MSG tissue group, have previously been identified as risk factors for lymphomagenesis in SS (23). Furthermore, IFNγ/IFNα mRNA ratio in MSG tissue has emerged as a histopathological biomarker for the prediction of in situ lymphoma development (27).

Finally, the presence of autoantibodies to interferon inducible protein-16 (IFI16) has been proven in various autoimmune diseases, including SS, in which it seems to be quite common. Anti-IFI16 antibody positivity characterises more frequently patients with abnormal Schirmer's test, elevated IgG levels and high ANA titres, as well as higher focus scores and germinal centre-like structures in their MSG biopsies (70). To be noted that IFI16 is an IFNγ-inducible protein which acts as a DNA detector in case of infection and regulates IFN type I transcription, with IFI16-knockout cells producing dramatically reduced IFNα (71).

A recent study revealed higher EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) total and ESSPRI sicca and pain domain scores among patients with the highest cumulative smoking consumption. Interestingly, type I IFN signature showed an inverse correlation with these domains. However, former or current smokers did not ever show remarkable controversy regarding type I IFN positivity or ESSDAI total score (72). Finally, in a recent study of medication-free SS patients receiving vaccination against H1N1, increased levels of protective antibodies were observed mainly as a result of IFNα induced B cell hyper responsiveness (73).

Treatment

Given that type I IFN overexpression seems to play a central role in disease initiation and progression in both SLE and SS, therapeutic attempts to downregulate its effect are gaining increasing attention over the last years (74).

As endogenous RNAs have been considered to be primary drivers of type I IFN in systemic autoimmunity, RNA degradation seems to be a logical target. RSLV-132, a fully human biologic Fc fusion protein consisting of an RNase fused to the Fc domain of IgG1, has been shown to inhibit type I IFN production. This agent is presently being tested in a phase II, double-blind, placebo-controlled study of 28 SS patients in light of promising results in a recently published clinical study of 32 SLE patients (75). The SS study is complete but no results have been as yet published (ClinicalTrials.gov Identifier: NCT03247686).

Of interest, administration of the antiviral agent lamivudine in an experimental aged model has been shown to downregulate type I IFN production through inhibition of L1 reverse transcriptase (56). These data might be relevant in SS patients, in which an upregulation of L1 retroelements have been observed at the level of salivary gland tissue (54).

Given that pDCs are viewed as a major cellular source of type I IFN, targeting of this cell population is also a promising anti-IFN strategy. The humanised monoclonal antibody 24F4A binds to the C-type lectin BDCA2, which is a specific receptor for pDCs, thus inhibiting IFN production. Clinical trials of BII059, another BDCA2 ligand, showed encouraging results in SLE, but not in SS patients yet. Immunoglobulin-like transcript 7 (ILT7)/LILRA4/CD85g -a member of the immunoglobulin-like transcripts (ILTs) or leucocyte immunoglobulin-like receptors (LIR) gene family- is a molecule predominantly expressed on the surface of pDCs (76). It has been shown that activation of ILT7 leads to differentiation of pDCs from an IFN producing to an antigen presenting phenotype via downregulation of TLR7/TLR9 mediated IFN production (77-79). Stimulation of ILT7 by an antibody (MEDI7734/VIB7734) was tested in 36 patients of type I IFN-mediated autoimmune diseases including SS. Although the study was completed, no results have as yet been published (ClinicalTrials.gov Identifier: NCT02780674).

TANK is another promising target in view of the significant role it holds in type I IFN production through IRF-3 and IRF-7 ligation. Administration of BX795 -a TANK inhibitor- in PBMCs

derived from SS patients resulted in downregulation of type I IFN inducible genes (80).

The idea to use monoclonal antibodies (mAbs) against IFNα in a way similar to that of TNFα inhibition in RA or even to produce anti-IFNα antibodies through vaccination has been proven in SLE with moderate results, failing to downregulate fully the IFN signatures. This might occur either because the antibodies fail to target all IFNα variants or do not affect other types of IFNs (81, 82). Targeting IFNAR has shown encouraging results in SLE patients with high IFN signature, but at the cost of upper respiratory tract infections and herpes zoster reactivation (83).

As discussed earlier, JAK kinases have been shown to mediate type I IFN effects following ligation of IFNAR. Small molecule JAK inhibitors are in clinical trials against SLE and SS (ClinicalTrials.gov Identifier: NCT03100942), with filgotinib showing encouraging results (84) while tofacitinib, already approved for rheumatoid arthritis (RA), has shown efficacy in murine NZB/NZW F1 and MRL/lpr SLE models (85). No data in SS are currently available.

BAFF levels are heightened in SS and associate positively with both type I and II IFN signatures at both peripheral blood and salivary gland tissue (27). Belimumab, a fully humanised monoclonal antibody towards the soluble B lymphocyte stimulator (BLyS), was tested in SS in two European centres, Paris, France and Udine, Italy (ClinicalTrials.gov Identifier: NCT01160666 and NCT01008982 respectively), with promising results. In a follow-up assessment of SS patients participating in the initial BELISS study a clinical and immunological deterioration was observed 6 and 12 months after cessation of treatment (ClinicalTrials.gov Identifier: NCT01008982). Interestingly, in a subgroup of the patients recruited for the BELISS study increased blood and salivary NK cell numbers in association with a worse response to treatment with belimumab was reported (86), while increased type I IFN scores at baseline were associated with improved outcomes, such as reduced IgG, IgM and RF serum levels (87). Based on these findings, the authors proposed the existence of two distinct subsets of SS: one with a predominant type I IFN-BAFF-B cell axis, representing good responders to belimumab; and one with a predominant type II IFN-NK cell axis, representing non-responders (86, 87).

Despite the major success in many rheumatic diseases, TNF inhibition failed to demonstrate efficacy in patients with SS (88, 89). Augmentation of the already upregulated type I IFN activity and the ensuing increased BAFF levels has been postulated as a potential reason for TNF failure in these patients (40).

Conclusion

With regard to SS, it seems that type I IFN dysregulation and overexpression is implicated in disease pathogenesis making its blockade an attractive therapeutic target. Although no concrete data exist as yet on medications against type I IFN currently on clinical trials, current evidence indicate that inhibition of this pathway at various stages could alter the course of the disease for SS patients. In the future, as our knowledge on its pathogenetic role in SS expands, more advanced agents will be targeting this pathway in an attempt to restore the balance of the immune system in these patients.

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Chapter 10
Sjögren's Syndrome

Anna Rapti, Nikolaos Marketos, and Clio P. Mavragani

Introduction

SS is a female-dominated [1], autoimmune disorder of unknown etiology and diverse phenotypical expression. Xerostomia and keratoconjunctivitis sicca (due to lymphocytic infiltration of salivary and lacrimal glands, respectively) are considered to be the clinical hallmarks, while B-cell hyperactivity is thought to be the pathophysiological cornerstone. Genetic susceptibility and environmental triggers are combined in ways yet to be defined, leading to innate and subsequently adaptive immunity over-activation [2–5]. For most patients, the disease runs an indolent course with sicca symptoms being the main complaint, along with musculoarticular pain and potentially disabling fatigue [6, 7]. However, one third of SS patients develop extraglandular manifestations, and a small percentage of them, but considerably higher compared to the one related with other systemic autoimmune disorders, proceed to develop lymphoma. Therefore, SS provides a unique study model of malignant turn in inflammatory background, caused by an autoimmune disease [8, 9]. Early prognostic tools and better understanding of the different etiopathogenetic pathways leading to divergent disease phenotypes are challenging but also the key to development of effective treatment and improvement of quality of life for these patients.

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Epidemiology – Definition

SS is encountered in approximately 0.5% of the general population, making it the second most common systemic autoimmune disease after rheumatoid arthritis. Women are affected at a 9:1 ratio in comparison to men, usually between the ages of 40 and 60 years [1, 10].

Primary and Secondary SS

SS has been traditionally classified into primary and secondary depending on whether it occurs alone or in the context of another systemic autoimmune disease (systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma, dermatomyositis) [10]. However, it has been increasingly appreciated that this classification is rather confusing since secondary forms of the disease encompass entities with distinct genetic, clinical, and serological profiles. Therefore, the replacement of the term "secondary" by the phrase "SS-associated disease" has been proposed by several investigators [11, 12].

Etiopathogenesis

SS is a disease of so far unknown etiology. Stress, as well as environmental, genetic, and epigenetic factors, seems to interact in pathogenesis (Fig. 10.1).

Fig. 10.1 A model of potential contribution of different factors in SS pathogenesis

Higher prevalence of SS around menopause fueled the hypothesis of estrogen deficiency as a possible disease mechanism. Murine experiments have shown that estrogens inhibit the IFNγ-induced expression of adhesion molecule-1 and exhibit a protective role regarding autoimmune lesions in salivary and lacrimal glands [13]. Ovariectomy led to increase of apoptotic epithelial cells in salivary glands, associated with α-fodrin cleavage, in other murine models [14]. Moreover, increased number and impact of stressful events prior to disease onset in association with inadequate coping mechanisms, coupled with a chronically suppressed hypothalamic-pituitary-adrenal axis [15], potentially leads to defective anti-inflammatory mechanisms, thus promoting an exacerbated immune response. As in many autoimmune diseases, viruses such as Coxsackie, CMV, retroviruses, HCV, and Epstein-Barr have been previously viewed as initial triggers for SS [1, 16]. Endogenous triggers, such as long interspersed nuclear element 1 (LINE-1; L1) [17, 18], have been recently shown to be over-expressed in salivary glands of SS patients possibly as a result of defective methylation [19], leading to increased production of type I interferons and B-cell activation [3–5]. The increased familial aggregation of SS and other autoimmune diseases supports the notion that genetic background is a significant contributor in disease pathogenesis and that shared susceptibility gene variants plus common environmental stimuli are the basis for a wide range of autoimmune manifestations [20–24]. Indeed, this predisposing genetic basis seems to involve genes inside and outside the MHC locus implicated in the IFN signaling pathway, others that regulate B-cell function and antibody production, as well as the apoptotic and inflammatory genes in NF-kB pathway [4, 25] (Table 10.1). Nowadays, deregulation of epigenetic mechanisms and intestinal microbial dysbiosis attract increasing attention as potential culprits in disease onset [26–28].

The observation that lymphocytes infiltrating exocrine glands and parenchymal organs surround epithelia suggests a central role of the epithelial cell in the formation and further organization of characteristic immunopathological lesions in SS [29]. Especially, salivary gland epithelial cells have been investigated and found to

Table 10.1 Association of non-MHC class genes with SS susceptibility according to distinct pathogenetic pathways

	Type I and II IFN pathways	B-cell activation	NF-κB
Genes/ chromosomes	IRF5/Chr7	BLK-FAM167A/Chr8	TNFAIP3/chr6
	IRF5/TNPO3/Chr7	CXCR5/Chr11	TNIP1/Chr5
	STAT4/Chr2	BAFF/Chr13	LTA/LTB/TNF gene clusters
	IL12A/Chr3	GTF2I/chr7	BAFF-R/Chr22
	NCR3/NKp30/Chr6	EBF1/Chr5	

IRF5 interferon regulatory factor 5; *TNPO3* transportin 3; *STAT4* signal transducer and activator of transcription 4; *IL12A* interleukin 12A; *NCR3/NKp30* natural cytotoxicity triggering receptor 3/ natural killer protein 30; *BLK-FAM167A* B-lymphocyte kinase/family with sequence similarity 167, member A; *CXCR5* chemokine (C-X-C motif) receptor 5; *BAFF* B-cell activating factor; *GTF2I* general transcription factor 2I; *EBF1* early B-cell factor 1; *TNFAIP3* tumor necrosis factor-alpha-induced protein 3; *TNIP1* TNFAIP3-interacting protein 1; *LTA/LTB/TNF* lymphotoxin gene A, lymphotoxin gene B, tumor necrosis factor; *BAFF-R* B-cell activating factor receptor

undergo increased apoptosis [30], leading to release of autoantigens (such as Ro/SSA and La/SSB) which in turn drive the production of disease-specific autoantibodies. The immunocomplexes that are generated through this process result in type I interferon (IFN) production by plasmacytoid dendritic cells (PDCs) in individuals with genetic predisposition. Subsequently, type I IFN can reinforce epithelial activation and BAFF overexpression, as well as autoantibody production. The complex role of activated epithelia as antigen-presenting cells and cells secreting chemokines and cytokines or expressing chemotactic molecules places them at the center of the immunological process. Epithelial cells ultimately contribute to further aggregation of inflammatory cells, activation of lymphocytes (both T and B), and autoantibody production, thus closing the vicious circle of autoimmunity. This series of events can possibly culminate in extensive tissue damage and even B-cell monoclonal expansion [4, 31–35]. Therefore, the term "autoimmune epithelitis," stressing the key role and active involvement of epithelial cells, has been fairly proposed to describe SS [36].

Diagnosis and Differential Diagnosis

Thorough patient history and meticulous clinical examination of all systems are of utmost importance [10]. Family history should also be recorded, as familial clustering of cases with SS and other autoimmune conditions has been recorded [20–25]. Classification criteria are commonly used in order to establish SS diagnosis, with the latest revision having taken place in 2016 by the American/European Consensus Group (Table 10.2) [37]. Differential diagnosis is summarized schematically in Table 10.3 [37–41].

Diagnostic Tests

Laboratory Tests

Routine laboratory tests (full blood count, renal and liver function tests, serum protein electrophoresis plus immunofixation in case of hypergammaglobulinemia, erythrocyte sedimentation rate, C-reactive protein, urine analysis) and immunologic markers (rheumatoid factor, anti-nuclear antibodies, complement levels, antibodies against the cytoplasmic antigens SSA/Ro and SSB/La, cryoglobulins, anti-thyroid autoantibodies) are included in the laboratory evaluation of suspected SS [42–44]. Testing for viruses (HCV, HIV, HTLV-1) and IgG4 levels is carried out for differential diagnosis purposes, and other targeted autoantibodies or supplementary laboratory tests can be requested, according to specific clinical manifestations [38–46]. A schematic presentation of associations between specific autoantibodies detected in SS patients and disease phenotypical characteristics is shown in Table 10.4 [46–63].

Table 10.2 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary SS. The classification of pSS applies to any individual who meets the inclusion criteria (a), does not have any of the conditions listed as exclusion criteria (b), and has a score of at least 4 when the weights from the five selected criteria items are summed (c). Several changes from 2002 AECG classification criteria were made. Subjective ocular or oral symptoms are now considered a prerequisite, rather than criteria contributing to the total score as they were before. Sialography and scintigraphy have been omitted, and a higher threshold for the ocular staining score has been implemented. The list of exclusion criteria was revisited, as the newly identified IgG4-related disease was added and lymphoma was removed, while more accurate techniques are required to rule out known, confounding entities (PCR confirmation of active hepatitis C). Finally, anti-SSB/La autoantibodies positivity was concluded to have no diagnostic value in the absence of anti-SSA/Ro and was therefore withdrawn. To be noted that patients on anticholinergic drugs should be objectively evaluated for their sicca symptoms after a sufficient time of these medications has elapsed

(a) Inclusion criteria
Positive response to at least 1 of the following questions:
1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than 3 times a day?
4. Have you had a daily feeling of dry mouth for more than 3 months?
5. Do you frequently drink liquids to aid in swallowing dry food?
or
Suspicion of Sjögren's syndrome from the ESSDAI questionnaire (at least 1 domain with a positive item)

(b) Exclusion criteria
1. History of head and neck radiation treatment 2. Active hepatitis C infection 3. AIDS 4. Sarcoidosis 5. Amyloidosis 6. Graft-versus-host disease 7. IgG4-related disease

(c)

Criteria items	Weighted score	SS classification
Labial salivary gland with focal lymphocytic sialadenitis and focus score ≥ 1 foci/4 mm^2	3	Score ≥ 4
Anti-Ro/SSA positivity	3	
Ocular staining score ≥ 5 (or van Bijsterveld score ≥ 4) in at least one eye	1	
Positive Schirmer's test (≤5 mm/5 min in at least one eye)	1	
Unstimulated whole saliva flow rate ≤ 0.1 ml/min	1	

Table 10.3 SS differential diagnosis. Other causes of sicca symptoms and processes infiltrating the exocrine glands need to be ruled out in order to reach the correct diagnosis. IgG4-related disease is a newly identified entity, added in the exclusion criteria list of the recently reviewed American/European Consensus SS classification criteria. It is a multi-organ, immune-mediated condition that has unified several diseases once considered to be individual. It includes Mikulicz disease (sialo/dacryo-adenitis and salivary/lacrimal gland enlargement), Küttner tumor, Riedel's thyroiditis, orbital inflammatory pseudotumor, pituitary hypophysitis, and hypertrophic pachymeningitis in the head and neck region, as well as autoimmune pancreatitis, interstitial pneumonitis, interstitial nephritis, prostatitis, retroperitoneal fibrosis, and inflammatory aortic aneurysm. Elevated serum IgG4 levels (≥135 mg/dl) and infiltration of abundant IgG4-positive plasma cells into affected organs help with differential diagnosis

Sicca symptoms (xerophthalmia and/or xerostomia)	Lymphocytic infiltration of exocrine glands	Non-lymphocytic infiltration of exocrine glands
Use of medications *Antihypertensives, antihistamines, antidepressants, isotretinoin, etc.* Previous head and neck radiation Diabetes mellitus Vitamin A deficiency Any functional or anatomical defect of the eyelid Chronic blepharitis, chronic conjunctivitis, or another chronic eye inflammation Psychogenic	Chronic viral infections *Hepatitis C, AIDS, HTLV-1* IgG4-related disease Chronic graft-versus-host disease Lymphoma	Granulomatous diseases *Sarcoidosis, tuberculosis, leprosy, syphilis* Metabolic infiltration *Hyperlipoproteinemia, diabetes mellitus, amyloidosis, hemochromatosis*

Salivary Gland Biopsy

Minor salivary gland biopsy (MSGB) (Fig. 10.2) displays a crucial role for diagnosis, prognosis, and risk stratification [64, 65]. According to American/European Consensus SS classification criteria of 2016, diagnosis cannot be established without a positive MSGB or positive anti-SSA/Ro antibodies [37]. Moreover, intense lymphocytic infiltration has been identified as an independent histopathological risk factor for NHL development [66], which is the leading cause of excess mortality in SS patients [67–69].

The presence of a lymphocytic infiltrate of ≥50 lymphocytes per 4 mm^2 of glandular parenchyma, usually located in the periductal area, is considered a positive focus score. The average number of these lymphocytic aggregates per 4 mm^2 of salivary gland tissue is the focus score (Fig. 10.3) [64]. Despite the fact that only focus score appears in the American/European consensus criteria, Tarpley score (measure of glandular architecture derangement) is also commonly used [65, 70]. Another important histopathological feature is the presence of germinal center (GC)-like structures (Fig. 10.4). The latter have been associated with higher focus score, higher frequency of extraglandular manifestations, hypergammaglobulinemia, increased RF levels, and higher prevalence of positive anti-SSA/Ro and/or anti-SSB/La autoantibodies. The presence of GC-like structures has

Table 10.4 Prevalence of specific autoantibodies in SS patients and correlation with clinical manifestations, other serological features, and disease outcomes. This table includes traditional autoantibodies for disease classification, autoantibodies identified from murine models, and autoantibodies typically associated with other autoimmune diseases. The hoped-for result of as early as possible diagnosis has recently led to the marketing of a new diagnostic kit (Sjö® test). The cumulative sensitivity of traditional (ANA, RF, anti-SSA/Ro, anti-SSB/La) and novel (anti-CA VI, anti-SP-1, anti-PSP) antibodies of Sjö® panel has been estimated to be 91.8%, whereas the sensitivities for anti-SSA/SSB alone and for the novel biomarkers alone were found to be 74.9% and 49.8%, respectively. Additionally, the cumulative specificity for the complete Sjö® panel was estimated at 79.8%. Further potential biomarkers currently under investigation are anti-kallikrein antibodies, antibodies against carbamylated proteins, and antibodies against TRIM38 proteins, among others (not shown in the table)

Type of autoantibodies	Prevalence of autoantibody positivity in pSS patients	Clinical correlation/significance
Anti-Ro/SSA Anti-La/SSB	33–74% 23–52%	Usually associated with female sex, younger age at diagnosis, more prominent lymphocytic infiltrate of the exocrine glands, and potentially a higher prevalence of extraglandular manifestations Attention needed in case of pregnancy, due to potential congenital heart block of the baby (complete heart block occurring in approximately 2% of cases)
Anti-nuclear antibodies (ANA)	59–85%	Associated with female gender, younger age at diagnosis, parotid gland enlargement, extraglandular manifestations, cytopenia, hypergammaglobulinemia, as well as increased frequency of RF, anti-Ro/SSA, anti-La/SSB, and antiphospholipid antibodies positivity
Rheumatoid factor (RF)	36–74%	Linked to earlier disease onset, female predominance, positive salivary gland biopsy, more frequent extraglandular features, and higher use of corticosteroids, among others Also, increased frequency of anti-La/SSB, anti-Ro/SSA, cryoglobulins, and ANA positivity, as well as low C3/C4 and hypergammaglobulinemia
Cryoglobulins	9–15%	One of the indisputable risk factors for non-Hodgkin's lymphoma development and SS-related death. Also, linked to earlier disease onset, higher frequency of extraglandular features (vasculitis, renal involvement, peripheral neuropathy, Raynaud's phenomenon) and cytopenia, as well as higher prevalence of parotid gland enlargement
Anti-thyroperoxidase antibodies (anti-TPO) Anti-thyroglobulin antibodies (anti-TG)	11–45% 3–100%	Autoimmune thyroid disease prevalence in SS seems to be 10–30% Furthermore, SS prevalence in already diagnosed autoimmune thyroid disease has been reported to be 3–32% (10 times higher probability of SS in autoimmune thyroid disease than in the general population) Sicca symptoms are even more frequent in the context of autoimmune thyroid disease (37% of patients develop xerostomia and 23% isolated keratoconjunctivitis sicca) Autoimmune thyroid disease-associated SS is linked to milder SS phenotype, but also to greater risk of developing further autoimmune diseases (such as autoimmune liver and inflammatory bowel diseases), requiring closer follow-up

(continued)

Table 10.4 (continued)

Type of autoantibodies	Prevalence of autoantibody positivity in pSS patients	Clinical correlation/significance
Antibodies against cyclic citrullinated peptides (anti-CCP)	3–10%	Anti-CCP-positive pSS patients do not seem to have major clinical differences from anti-CCP-negative individuals, but there is a possible association with nonerosive arthritis
Anti-mitochondrial antibodies (AMA)	1.7–27% Depending on laboratory technique for detection; indirect immunofluorescence, Western blot, or ELISA	Specific for primary biliary cirrhosis (PBC) Also, higher prevalence of Raynaud's phenomenon, peripheral neuropathy, hypergammaglobulinemia, and high ESR Valuable for separating patients with autoimmune liver involvement from those with chronic viral liver disease
Anti-smooth muscle antibodies (ASMA)	30–62%	Autoimmune hepatitis (only 1.7–4% in pSS patients)
Anti-centromere antibodies (ACA, comprising of CENP-A, CENP-B, and CENP-C)	3.7–27%	Overlapping features between SS and systemic sclerosis (SSc) Up to 40% of the ACA-positive pSS patients can progress to systemic sclerosis Associated with delayed disease onset, but increased frequency of keratoconjunctivitis sicca, Raynaud's phenomenon, peripheral neuropathy, and lymphoma Lower frequency of anti-Ro/La antibodies and higher prevalence of other coexisting autoimmune disorders, such as PBC SS patients usually recognize CENP-C alone, whereas recognition of both CENP-B and CENP-C is more frequent in SSc
Antibodies against carbonic anhydrase (anti-CA; 13 known isoenzymes)	12.5–20.8%	Anti-CA II antibodies linked to renal involvement and particularly distal renal tubular acidosis Anti-CA VI and anti-CA XIII antibodies have also been shown to correlate with urine pH and inversely with serum sodium levels (cross-reactivity between anti-CA VI and anti-CA XIII is a possible scenario, as CA VI is the only isoenzyme secreted in saliva and expressed in the parotid and submandibular glands, but not in the kidney) Anti-CA VI is considered a novel, early SS biomarker, included in commercially available Sjö® diagnostic panel. It is the most prevalent novel autoantibody of the kit among both SS and non-SS dry eye patients (52% and 43%, respectively). Anti-CA VI positivity has been linked to more severe xerophthalmia, presence of xerostomia, younger age, and negative MSGB

Type of autoantibodies	Prevalence of autoantibody positivity in pSS patients	Clinical correlation/significance
Antibodies to 21-hydroxylase (anti-21[OH])	17.50%	Anti-21[OH] positivity was not linked to overt adrenal insufficiency, but it was associated with adrenal hyporesponsiveness and evidence of more prominent B-cell activation in MSG tissue samples Decreased prevalence of subjective xerophthalmia and increased frequency of leukopenia were also noted for anti-21[OH]-positive SS individuals
Anti-muscarinic receptor antibodies	62.2–81.8%	Associated with cytopenia and higher ESSDAI scores Could partially account for the salivary gland hypofunction, the gastroesophageal symptoms, and the bladder smooth muscle hyperresponsiveness, observed in pSS patients
Antibodies against citrullinated alpha-enolase peptides (anti- CEP-1)	60% of anti-citrullinated protein antibodies (ACPA)-positive pSS patients Less than 10% of unselected pSS patients	Associated with higher urine pH levels at first evaluation (linked to distal renal tubular acidosis, nephrocalcinosis, and impaired bone health)
Antibodies against salivary protein 1 (anti-SP-1)	52% of SS patients 19% of SS patients with negative anti-Ro/anti-La	Antibodies against murine SP-1 (no known human protein analogue) seem to identify targets in human parotid glands Anti-SP-1 antibodies are considered novel, early SS biomarkers, included in commercially available Sjö® diagnostic panel Patients with lower focus scores in MSGB tend to be tested positive for anti-SP-1 more often than those with higher focus scores (who generally test positive for anti-Ro/anti-La). Especially patients expressing only anti-SP-1 antibodies (no anti-Ro/anti-La) have low or negative MSGB focus score Anti-SP-1 can also be used as a marker to separate SS-associated RA from RA not complicated with SS
Antibodies against parotid secretory protein (anti-PSP)	18%	PSP is a protein involved in the binding and clearance of infectious agents Anti-PSP is considered a novel, early SS biomarker, included in commercially available Sjö® diagnostic panel Can also be positive in non-SS dry eye disease and rarely in RA and healthy controls
Anti-α-fodrin antibodies	29% of pSS patients, but 47% of SLE patients Almost 2 times more prevalent in non-SS sicca than SS patients	Anti-α-fodrin antibodies serum concentrations have been associated with the degree of lymphocytic infiltration in salivary glands Usually found in early disease stages Most probably not useful for SS diagnostic purposes

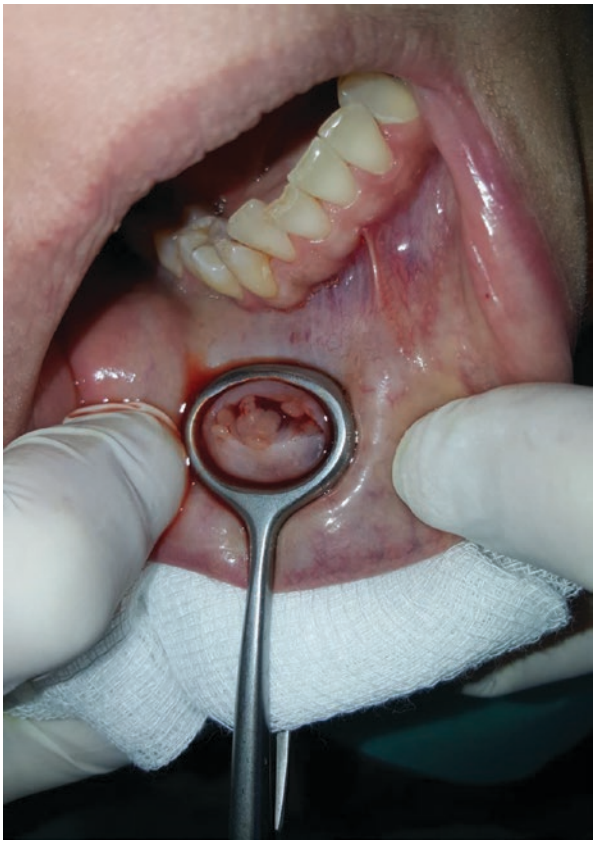

Fig. 10.2 Minor salivary gland (labial) biopsy. The procedure is simple and well-tolerated and can be done under local anesthesia on an outpatient basis. Usually 4–6 minor salivary gland lobules need to be sampled, in order for the tissue to be considered representative. Several different surgical approaches have been suggested in an effort to minimize complications. Most frequent adverse events reported in literature include temporary localized pain and bleeding, and only rarely there have been cases with persistent hypoesthesia of the lower lip (Photograph courtesy of E. Piperi, Assistant Professor in Department of Oral Pathology, School of Dentistry, UoA)

also been suggested to confer increased risk for lymphoma development. However, the contradicting results on GC-like structures significance from various studies – possibly due to poor definition on one hand and under-detection in H&E staining on the other – underline the need for uniform criteria and further research [65, 70–73].

The advantages of MSGB include easy accessibility, avoidance of skin incisions, and local anesthesia. Parotid biopsy is reserved only to rule out lymphoma in case of persistent parotid enlargement [74, 75]. MSGB sensitivity and specificity are considered to be higher than 75% and 90%, respectively [64, 76, 77].

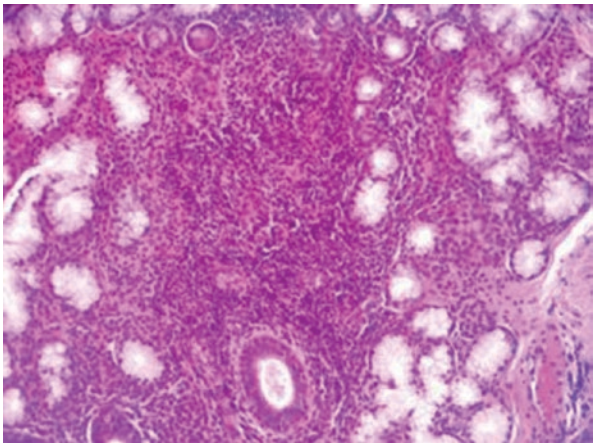

Fig. 10.3 Minor salivary gland biopsy with high focus score (Hematoxylin & Eosin staining)

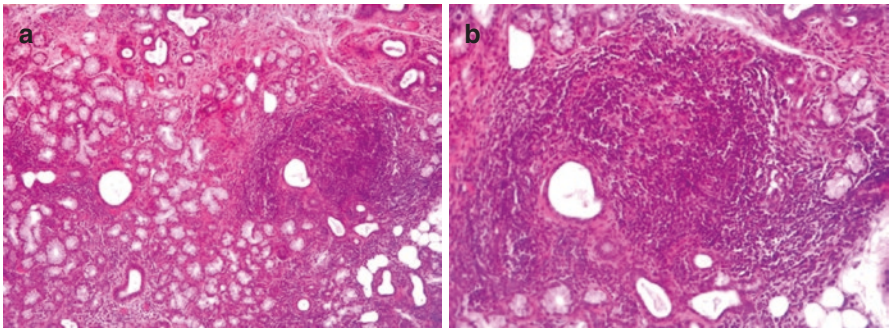

Fig. 10.4 Minor salivary gland biopsy with MALT development and germinal center-like formation in ×100 (**a**) and ×200 (**b**) magnification (Hematoxylin & Eosin staining). GC-like structures are tertiary ectopic lymphoid structures, and they are considered an advanced histopathologic lesion, previously correlated with future lymphomagenesis, extraglandular manifestations, and earlier diagnosis. However, their role in lymphomagenesis is still questioned, since more recent research has failed to demonstrate such a correlation. The prevalence of GC-like structures in SS patients is ranging from 18% to 59% according to different studies

Oral Involvement Assessment Tests

The objective evaluation of xerostomia and oral involvement in SS, except for the generally accepted MSGB, remains a challenge [75, 78]. The easiest, most common, and affordable way to assess major and minor salivary gland secretory capacity is the measurement of salivary flow or sialometry. Unstimulated whole saliva flow rate equal to or below 0.1 ml/min is considered abnormal (0.3–0.4 ml/min are expected for healthy individuals) [75].

Sialography may be used to demonstrate the morphology of the ducts, while scintigraphy can assess the salivary gland functionality. The nonspecific results of

both techniques and the involvement of radiation for the latter have led to their removal from the most recent SS classification criteria [75, 79].

Ultrasound (US), being noninvasive, inexpensive, and radiation-free, is drawing a lot of attention for the purposes of major salivary gland imaging. Multiple studies have shown good agreement and comparable results between salivary gland ultrasound and sialography and even diagnostic superiority compared to scintigraphy [80].

In addition to its value as a diagnostic tool, the prognostic value of ultrasound has also been explored. Increased parenchymal dyshomogeneity scores of major salivary glands have been found to correlate with SSA, SSB, ANA, RF, higher levels of IgG, salivary gland enlargement, cutaneous vasculitis and/or purpura, GC-like structures in salivary gland biopsy, CD4 T-cell lymphopenia, Raynaud's phenomenon, and disease activity scores. Last but not least, there is some evidence that ultrasonographic images of salivary glands change in response to treatment (rituximab versus placebo) for SS [80].

Elastography is an added feature to the classic US modality, which can increase sensitivity compared to B-mode US alone and differentiate between SS patients and sicca controls [81].

Ocular Tests

Mainly aqueous deficiency but also meibomian gland dysfunction and neuropathic pain contribute to increased tear evaporation rate, reduced tear film stability, and ocular discomfort in SS patients [82].

Keratoconjunctivitis sicca (KCS) symptoms can be quantified using patient questionnaires, like the Ocular Surface Disease Index (OSDI). The main available objective tests are Schirmer's test (Fig. 10.5), ocular surface dye staining (Fig. 10.6), and tear breakup time (TBUT) [82–84]. Among those, only the first two are included in the latest classification criteria [37].

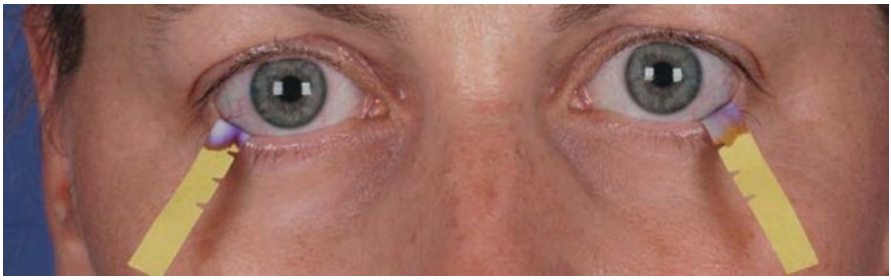

Fig. 10.5 Schirmer's test involves the measured wetting of a standardized paper strip, placed over the inferior eyelid, over a certain period of time. It is usually performed without anesthesia, and the test is considered positive when at most 5 mm of the paper are wetted in 5 min time. The cutoff point is lower (<3 mm) when the test is performed under anesthesia, as in this case we measure the basal/non-reflex tear production (Permission to re-produce kindly granted by Messmer [40])

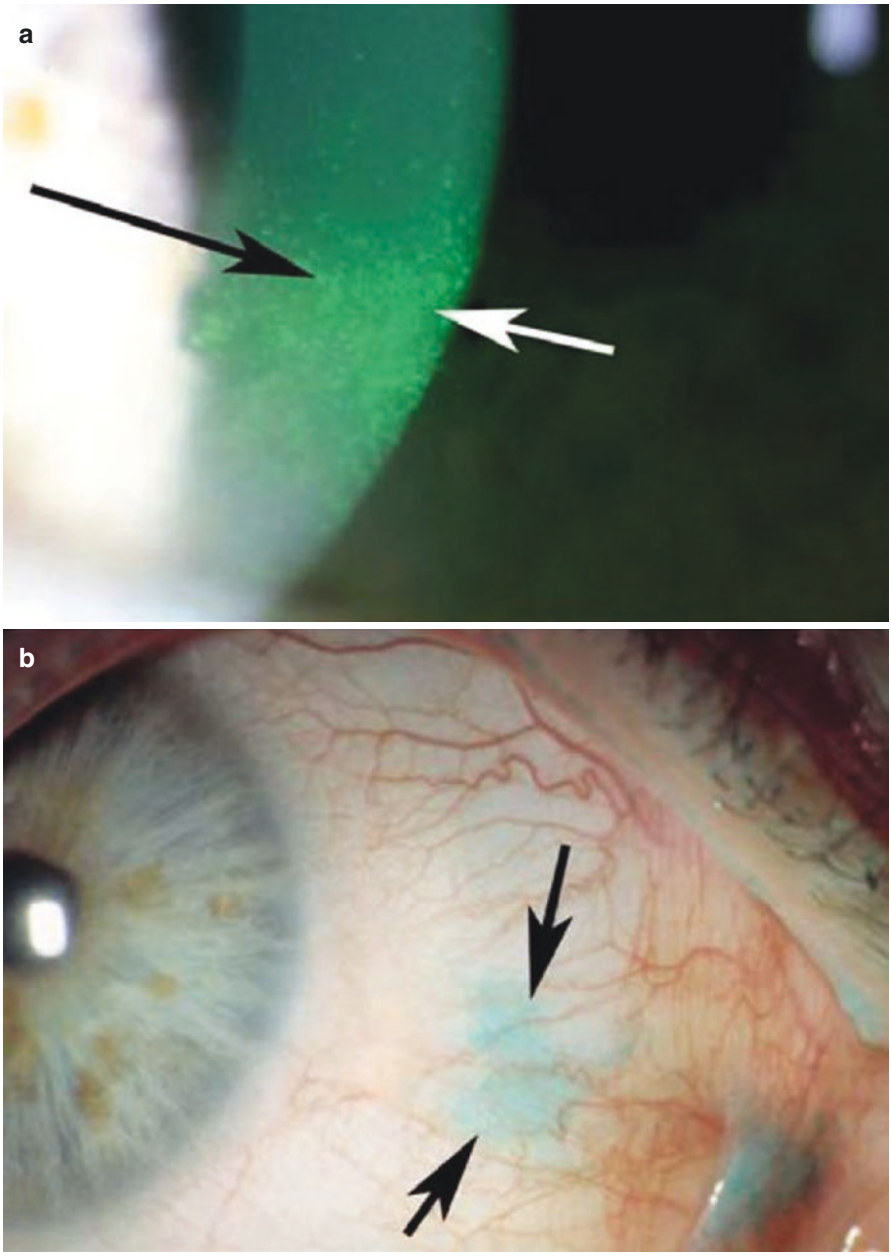

Fig. 10.6 Staining of the ocular surface in dry eye disease. Fluorescein better stains the cornea (**a**), while lissamine green (LG) is preferred for the conjunctiva (**b**). LG tends to be used nowadays instead of rose bengal (RB), due to improved toxicity and tolerance profile, given their similar staining properties. Both RB and LG bind to corneal epithelial cells that are uncoated by mucin or other proteins, and these damaged areas are easily observed under slit lamp examination. However, both dyes seem to correlate poorly to symptom severity as stated by patients in relevant questionnaires (Permission to re-produce kindly granted by Messmer [40])

Disease Activity Indexes

There are two indexes, introduced by European League Against Rheumatism (EULAR), which are used to assess SS activity from the patient's and the clinician's point of view. The first one is the EULAR Sjögren's Syndrome Patient Report Index (ESSPRI), which consists of three visual-analogue scales measuring severity of sicca symptoms, fatigue, and pain [85]. The second one is the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI), which assesses the activity in 12 different domains representing organ systems. Since its introduction, ESSDAI has become the gold standard in terms of disease activity assessment because it serves as point of reference among physicians; it is widely used in randomized controlled trials as outcome measure and has been correlated with biomarkers and lymphoma development risk [42, 43].

Clinical Manifestations and Disease Management

SS can manifest with a plethora of clinical signs and symptoms, frequently vague and definitely not pathognomonic, often resulting in diagnostic delays [86]. Oral and ocular dryness, sometimes accompanied by xeroderma, upper respiratory desiccation, and vaginal dryness with subsequent dyspareunia are among the most common complaints [10]. Extraglandular manifestations (Fig. 10.7) occur in at least one third of individuals and can schematically be divided into three groups: nonspecific, periepithelial, and immune complexes mediated [6, 87–94]. The disease often runs a benign, indolent course with the exception of severe systemic complications causing excess morbidity and most importantly lymphoma, which is one of the main causes of mortality in SS [42, 67].

With regard to management, pilocarpine is usually initiated in an effort to alleviate ocular discomfort and difficulty in everyday life caused by lack of saliva, but residual lacrimal and salivary gland function is a prerequisite in order to be effective [95, 96]. SS patients must be informed on the rare side effects of pilocarpine, such as excessive sweating, nausea, diarrhea, and palpitations; for this reason, progressive dose escalation is recommended. Natural tears and lubricants for ocular use, as well as artificial saliva and oral solutions with chlorhexidine, are frequently used by patients and recommended by ophthalmologists and dentists, respectively [97]. Interestingly, in contrast to other systemic autoimmune diseases with high inflammatory load, SS-related sicca complaints do not respond to immunosuppressive treatment [98]. Mild aerobic exercise is recommended for fatigue [99], while disease-modifying antirheumatic drugs (DMARDs) are reserved for extraglandular manifestations [100–103]. The rare cases of aggressive lymphomas are managed with cytotoxic drugs [104–106]. Tables 10.5 and 10.6 display the array of clinical manifestations and the recommended treatment options in SS patients [107–120].

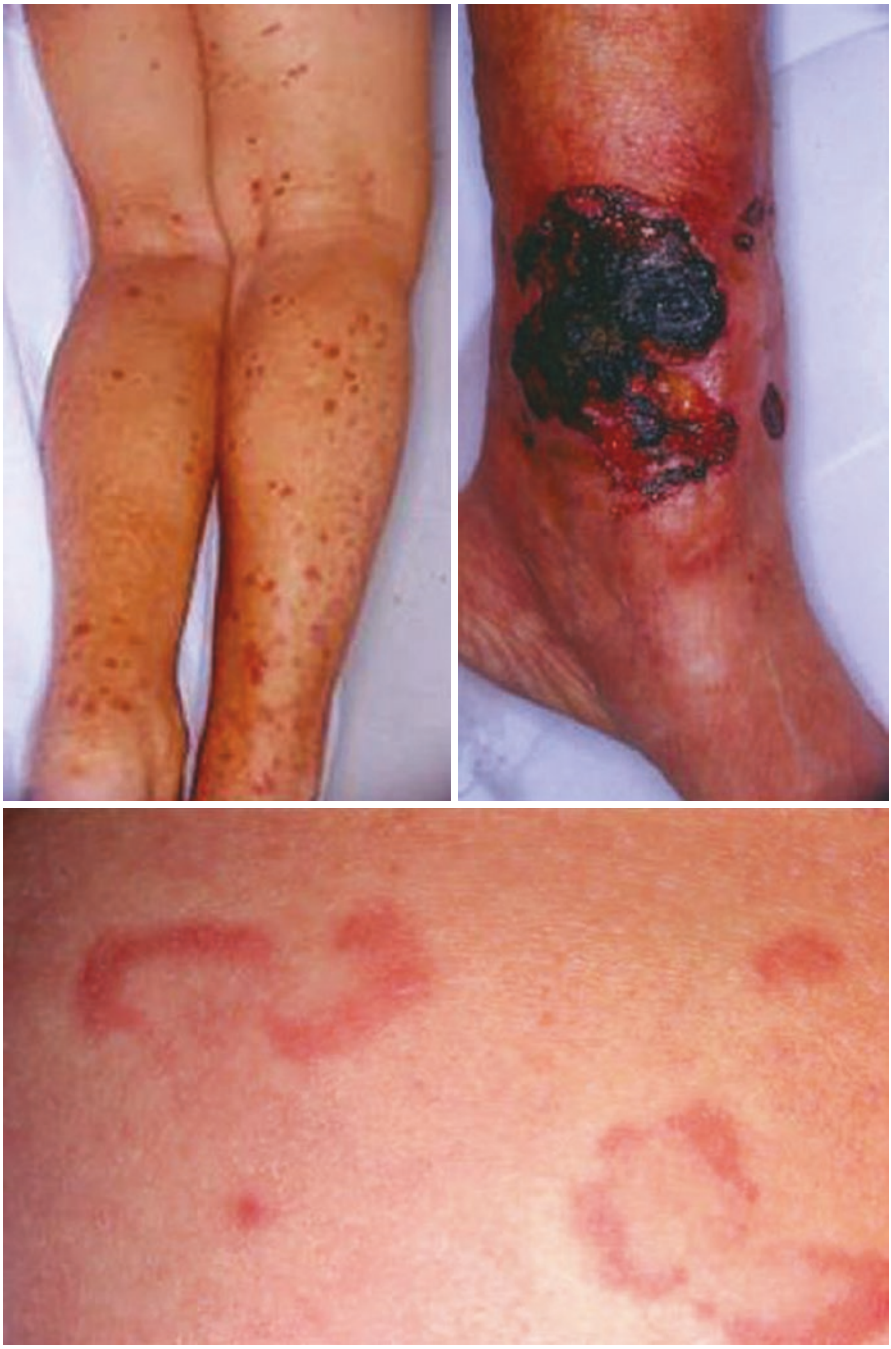

Fig. 10.7 Extraglandular features of SS. *Top left*: Palpable purpura in the lower extremities. *Top right*: Multiple necrotic cutaneous ulcers of the lower extremities in a patient with SS and cryoglobulinemia. *Bottom*: Annular urticarial lesions of the trunk (Permission to re-produce kindly granted by Hile et al. [148])

Table 10.5 Glandular manifestations and their treatment in SS patients

Organ involvement	Symptoms and signs	Therapeutic approach
Ocular	Irregularity of the corneal image, irritation, redness, photosensitivity	*No MG damage* (aqueous deficiency) Stop offending drugs, environmental changes, artificial tears, gels, ointments Ω3 suppl., CIS collyrium (0.05%) pulse steroids, punctal plugs, secretagogues, moisture chamber spectacles Topical autologous serum, contact lenses, permanent punctal occlusion Systemic anti-inflammatory medication, eyelid surgery *MG damage* (evaporative) Stop offending drugs, environment modification, lipid-rich tear substitutes, warm compress, massage CIS: 2–2.5 mg/kg/d, topical steroids, AZI, DXC, secretagogues, punctal plugs, moisture chamber spectacles Topical autologous serum, contact lenses Eyelid surgery
Oral	Dryness, caries, angular cheilitis Salivary gland enlargement	Dental fluorination, masticatory stimulation, chlorhexidine Pilocarpine hydrochloride: max 20 mg/d in divided doses Cevimeline hydrochloride: max 30 mg × 3 CS, 0.25–0.5 mg/kg/d for 10–15 d
Pancreatic	Recurrent autoimmune pancreatitis (5%)	AZA 2–3 mg/kg/d, RTX, pancreatic enzymes
Vaginal	Dyspareunia	Lubricants

MG meibomian gland, *CIS* cyclosporine, *AZI* azithromycin, *DXC* doxycycline, *CS* corticosteroids

Lymphomagenesis and Lymphoproliferation

SS is unique among autoimmune diseases as for malignant transformation risk. Those patients seem to have a 10–44-fold greater risk of developing lymphoma compared to general population, whereas systemic lupus erythematosus patients and rheumatoid arthritis patients only have a seven-fold and four-fold greater risk, respectively [67, 121]. In other words, 2.7–9.8% of SS patients are diagnosed with non-Hodgkin lymphoma (NHL) and that risk increases by 2.2% per year of age [78, 122].

Mucosa-associated lymphoid tissue (MALT) lymphomas represent 60% of cases [67]. Most common sites are the salivary glands, especially the parotid and submandibular glands, but other mucosal sites of MALT lymphoma development include the orbits, nasopharynx, stomach, thyroid, and lung [78]. Other subtypes of lymphoma found in these patients are the diffuse large B-cell lymphoma (DLBCL) and the nodal marginal zone lymphoma (NMZL), which – together with MALT – account for more than 90% of total SS-associated lymphoma cases [67].

Multiple research studies have been focusing their efforts on correlating clinical, serological, and histopathological features with risk of lymphomagenesis [66, 72, 123–126], and an attempt has also been made to formulate a predictive score for

Table 10.6 Extraglandular manifestations and their treatment in SS patients

Organ involvement	Symptoms and signs	Therapeutic approach
Nonspecific		
Musculoskeletal	Myalgias, arthralgias, Jaccoud arthropathy, rare arthritis	HCQ, MTX, or combination of both, small dose CS < 15mg qd
Raynaud's phenomenon	Cold-related color skin changes	Vasodilators, especially calcium channel blockers
Fatigue (35–50%)	Increased need for resting hours, disruption of sleep patterns	Nordic (active) walking, HCQ in some cases RTX
Periepithelial		
Bronchi (10–20%) Small airway disease (13%) ILD (17%)	Mild to moderate dyspnea and dry cough, xerotrachea	Bronchodilators AZA, RTX
Autoimmune cholangitis	LFTs abnormalities, jaundice	RTX UDCA, AZA
Renal/bladder (4–30%) Tubulo-interstitial nephritis Interstitial cystitis (0.3%)	Hypokalemic hyperchloremic distal renal tubular acidosis/ nephrocalcinosis Pollakiuria, nycturia, urinary urgency, pelvic or suprapubic pain	Urine alkalization (bicarbonate, electrolyte supplements) CS CS, CIS, surgical intervention
Immune complex-mediated disease		
Skin vasculitis Vasculitis in 5% Purpura – palpable in 5% Annular erythema	Cryoglobulinemia	CYC, AZA RTX for necrotizing vasculitis (cycles of 2gr q15 days interval/6 months)
Glomerulonephritis(rare)	Membranous or membranoproliferative	CS, RTX, CYC
Neuropathy (20%) Peripheral neuropathy CNS	Peripheral sensorimotor neuropathy or pure sensory neuropathy Motor neuropathy/ganglionopathy Mononeuritis multiplex Small fiber neuropathy MS-like	CS, 0.5–1 mg/kg, and IVIGs, RTX CS, 0.5–1 mg/kg, and CYC/ AZA, 2–3 mg/kg/d Anticholinergics, antidepressants, gabapentinoids CS, PE, RTX
Low-grade lymphoma *Disseminated lymphoma*		Wait and watch policy R-CHOP = if diffuse large B cell

HCQ hydroxychloroquine (5 mg/kg qd), *MTX* methotrexate 2.5–3 mg/15 kg qw), *CS* corticosteroids, *RTX* rituximab (cycles of 2gr q15 days interval/6 months), *AZA* azathioprine (2–3 mg/kg qd), *UDCA* ursodesoxycholic acid, *CIS* cyclosporine (2–2.5 mg/kg qd), *CYC* cyclophosphamide (750 mg–1 g/m^2), *IVIGs* intravenous immunoglobulins, *R-CHOP* rituximab-(c)yclophosphamide, (h)ydroxydaunorubicin, (o)ncovin (vincristine), (p)rednisone

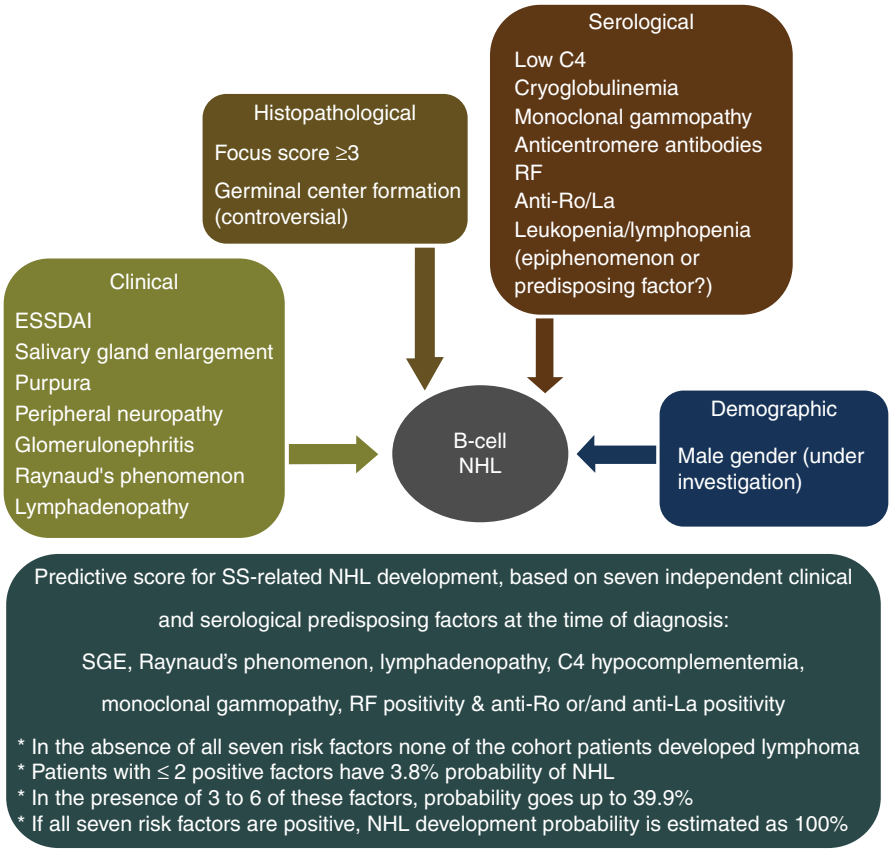

Fig. 10.8 Risk factors for lymphomagenesis in SS, as identified by various studies over the last years and a predictive score formula based on seven of them. Relevant information is gathered at the time of disease diagnosis and correlates with disease outcome, even years later, leading to the conclusion that early risk stratification is feasible for those patients. SGE salivary gland enlargement

SS-related NHL development based on data collected at the time of diagnosis [123] (Fig. 10.8). However, the molecular etiopathogenetic aspects of malignant transformation still remain largely elusive and ill-defined.

Current belief on etiopathogenesis of lymphoma focuses on four interrelated axes: chronic inflammation, B-cell activation, defective immunosurveillance, and epigenetic alterations [4, 104]. Focus score of at least 3 in MSGBs of SS patients has been identified as an independent and important predicting factor for NHL development, and the use of this threshold has a positive predictive value of 16% and a negative predictive value of 98% for that kind of malignant transformation [66]. Furthermore, the activation of P2X7 receptor-NLRP3 inflammasome complex (with subsequent increase of pro-inflammatory cytokines IL-18 and IL-1β serum levels, among others) correlates with lymphocytic infiltration severity, ESSDAI

scores, and lymphoproliferation risk. The hypothesis of inflammasome activation secondary to increased accumulation of proinflammatory nucleic acid shreds, ineffectively degraded and cleared, has recently been supported [127, 128].

Another factor promoting chronic inflammation seems to be IFNγ, as the mRNA levels in MSGBs have been associated with a higher degree of lymphocytic infiltration, shown to be a predisposing factor for lymphomagenesis [129]. Moreover, a functional variant of TNFAIP3 gene (rs2230926), encoding the A20 protein, leads to unopposed NF-κB pathway activation and is therefore involved in inflammatory process perpetuation, B-cell survival, and more aggressive disease phenotype with earlier disease onset and increased lymphoproliferation risk [130].

B-cell activating factor (BAFF), produced by various immune cells but also by the salivary epithelial cells and regulated by both type I and II IFNs, is of utmost importance for the maturation, proliferation, and survival of B cells. BAFF levels are found increased in SS patients' serum with a history of lymphoma, and high levels persist for years after treatment and remission [4, 131, 132]. Another equally important observation is the association of specific BAFF polymorphisms with SS-related lymphomagenesis and in particular the significantly different prevalence of the protective AA genotype of the rs12583006 polymorphism, as well as of the protective haplotypes TACAC and TACC and of the risk haplotype TTTC in SS patients prone to NHL development (high risk determined by the presence of adverse predictors), compared to low-risk individuals [133]. Additionally, a specific mutation (His159Tyr) of BAFF receptor (BAFF-R) is of interest, as it has been identified in more than two thirds of SS-associated MALT lymphoma. This mutation, leading to NF-kB pathway activation, was linked to earlier development of lymphoma or adverse immunological features (hypergammaglobulinemia and positive RF) [78, 134].

Finally, Fms-like tyrosine kinase 3 ligand (Flt-3 l) is a protein that acts as a cytokine and a growth factor, activating Flt-3 (or CD135) on the surface of hematopoietic progenitor cells, and therefore considered to be a mediator of B-cell survival. Higher levels of Flt-3l are strongly associated with history of lymphoma, detectable years before malignant transformation, and not affected by treatment [135].

The vicious circle of autoreactive B-cell chronic stimulation and immunocomplexes formation might finally lead to the favorable, monoclonal expansion of rheumatoid factor (RF)-reactive B cells and to the lymphomatous transformation, under defective immunosurveillance [4]. As a matter of fact, IFNα mRNA levels in MSGBs seem to strongly correlate with the expression of pro-apoptotic molecules (tumor suppressor gene p53 and auto-antigen Ro52, with the latter negatively regulating the anti-apoptotic B-cell lymphoma 2 (Bcl-2) gene) [132]. Moreover, decreased prevalence of a specific TREX1 variant (rs11797 AA genotype) in SS-related non-MALT cases was observed. This variant was shown to associate with higher mRNA IFNα levels in SS salivary gland tissues [136].

Further oncogenic mechanisms, likely to contribute to malignant turn in SS, are the over-expression of Bcl-2 due to a translocation involving chromosomes 14 and 18, leading to inhibition of apoptosis and increased B-cell survival. Apart from IFNα effect on p53 levels, specific mutations of this tumor suppressor gene were described 20 years ago in MSGBs from SS-associated NHL cases [4, 132, 137].

Epigenetic changes, involving methylating enzymes and transcription of non-coding micro-RNAs, are also implicated in lymphomagenesis [138]. MiR200b miRNAs, which regulate the expression of oncogenes and tumor suppressor genes, have been found to be downregulated in MSGBs with advanced lymphocytic infiltration and MALT lymphoma [139]. Especially miR200b-5p strongly discriminates SS patients who already have or will develop NHL from the rest of them or from sicca controls [140]. As for methylating enzymes, DNA methyltransferase (DNMT)3B and methyl-CpG-binding protein 2 (MeCP2) have been found decreased in SS-lymphoma patients [141], while methylene-tetrahydrofolate reductase (MTHFR) gene variants, leading to defective methylation and impaired stability of DNA, have lately been suggested as susceptibility factors for non-MALT lymphoma [142].

It becomes clear that the multifactorial process of lymphomagenesis is rather complicated, and the finely tuned balance between opposing forces can become deranged and lead to adverse outcomes. An example of that is the IFNγ/IFNα mRNA ratio in MSGBs, which has emerged as a histopathological biomarker for the prediction of in situ lymphoma development [132].

SS-related hematological malignancies are correlated with an eightfold higher mortality risk compared to general population [69], and one in five deaths of SS is attributable to lymphoma [68]. Follow-up every 6 months is recommended for high-risk patients. Overall, NHL 5-year survival is estimated at approximately 92%, but higher disease activity is linked to higher possibility of relapse and death [143–145]. Wait and watch strategy is suitable for MALT lymphomas localized in the salivary glands, while rituximab and chemotherapy are employed in case of disseminated or aggressive disease [106, 146, 147, 149].

Multiple-Choice Questions

1. Which of the following clinical features would set Sjögren's syndrome (SS) on top of your differential diagnosis list?

   A. A 60-year-old man with polyarthritis of the small joints of the hands, morning stiffness of >1h, and low-grade evening fever
   B. A 30-year-old woman with fatigue, hair loss, sun sensitivity of the skin, pleurisy, and oral aphthae
   C. A 50-year-old woman with ocular and oral dryness, fatigue, arthralgias of the hands and feet, and purpura
   D. A 45-year-old woman with known depression and use of relevant medication, fatigue, low-grade fever, appetite loss, and weight loss
   E. An 85-year-old woman otherwise in good health complaining for dry eyes, dry mouth, constipation, and fatigue

   Correct answer: C

Feedback:

A. Rheumatoid arthritis. Typical presentation with polyarthritis of small joints, morning stiffness.
B. Systemic lupus erythematosus presentation.
C. Sjögren's typical presentation.
D. Depression most probable; other causes must be excluded.
E. Age-related fatigue and dryness. Late onset of symptoms – autoimmune disease of lower probability.

2. Which of the following are currently believed to be implicated in disease pathogenesis?

A. UV light
B. Genes
C. Stress
D. Surgery
E. Androgens
F. All of the above
G. A + B + C + E

Correct answer: G
Feedback: All these factors except previous surgery are currently implicated.

3. Which are the main identified pathways of SS pathogenesis?

A. IFNγ
B. B-cell hyperactivity
C. NF-κB
D. All of the above
E. None of the above

Correct answer: D
Feedback: All of the above pathways are considered viable for disease pathogenesis.

4. Which of the following medications would you prescribe to a patient with ocular dryness due to pSS?
Multiple answers eligible

A. Methotrexate
B. Pilocarpine
C. Hydroxychloroquine
D. Cevimeline
E. Etanercept
F. Oral cyclosporine
G. Ocular drops of cyclosporine
H. Infliximab

Correct answer: B, D, G

Feedback:
A, C. Methotrexate and hydroxychloroquine are used for musculoskeletal manifestations, despite inadequate data.
E. TNFα inhibitors have not shown encouraging results so far.
F. Oral cyclosporine is reserved for resistant pulmonary disease, but topical treatment has been used effectively in ocular symptoms of SS.

5. Which of the following medications would worsen a patient's established SS symptoms?

   A. Artificial tears
   B. Artificial saliva
   C. Methylcellulose inserts
   D. Amitriptyline

   Correct answer: D
   Feedback: Amitriptyline is known to exacerbate oral dryness.

6. What treatment modalities would you employ to alleviate fatigue in an SS patient?
   Multiple answers eligible

   A. Corticosteroids
   B. Azathioprine
   C. Hydroxychloroquine
   D. NSAIDs
   E. Serotonin uptake inhibitors
   F. Methotrexate
   G. Pregabalin
   H. Anti-TNF agents
   I. IVIG
   J. Aerobic exercise

   Correct answer: J
   Feedback:
   A, B, C, F, H. Corticosteroids have no effect on these nonspecific symptoms nor have immunomodulatory drugs as azathioprine, hydroxychloroquine, methotrexate, or TNF inhibitors.
   J. According to guidelines only aerobic exercise is effective.

7. What treatment would you suggest to manage the same patient's oral dryness?
   Multiple answers eligible

   A. Bromhexine
   B. Pilocarpine
   C. Hydroxychloroquine
   D. Cevimeline
   E. Sugar-free fluoride-containing chewing gums
   F. Etanercept
   G. Methotrexate
   H. Infliximab

I. Regular water drinking
J. Avoidance of drying air heating systems

Correct answer: B, D, E, F, I, J
Feedback: Pilocarpine, cevimeline, chewing gums that are sugar-free, frequent water intake, and avoiding air heating may alleviate symptoms. The rest of medication on this list has no proven effect on oral dryness.

8. You prescribed pilocarpine, and 1 week later the patient is back at the office with new complaints. Which of the following could be attributable to this medication?
Multiple answers eligible

A. Constipation
B. Glaucoma
C. Acute urinary retention
D. Nausea
E. Excessive sweating
F. Diarrhea
G. Urinary infection
H. Neuropathy
I. Palpitations

Correct answer: D, E, F, I
Feedback: Constipation, glaucoma, and acute urinary retention are side effects of inhibitors of cholinergic synapses and not of agonists like pilocarpine. Urinary infection and neuropathy are not side effects of pilocarpine.

9. Which is the most likely cause of recurrent renal colic in this patient?

A. Nephrocalcinosis in the setting of distal tubular acidosis
B. Nephrocalcinosis in the setting of proximal tubular acidosis
C. Hyperparathyroidism
D. Hyperoxaluria

Correct answer: A
Feedback: Despite the fact that both hyperparathyroidism and hyperoxaluria are valid causes, nephrocalcinosis in the setting of distal and not proximal tubular acidosis seems to be the most likely cause of renal colic in the setting of SS.

10. Among the following different therapeutic strategies for SS-associated low-grade lymphoma management, which one is advised?
A. Local radiotherapy
B. Wait and watch policy
C. IV immunoglobulin
D. Combination immunochemotherapy (rituximab and CHOP)
E. Mycophenolate mofetil

Correct answer: B
Feedback: Wait and watch policy is the recommended policy. IV Ig and mycophenolate mofetil have no place on lymphoma treatment. Combination therapy

(rituximab and CHOP) is indicated in diffuse B-cell lymphomas. Current data do not support the use of radiotherapy for localized low-grade MALT lymphomas.

11. Which of the following features have been associated with increased risk for lymphoproliferation in SS?
Multiple answers eligible

A. Parotid gland enlargement
B. Positive anti-TPO/anti-TG
C. Low C4 levels
D. Distal renal tubular acidosis
E. Fibromyalgia
F. Hypergammaglobulinemia

Correct answers: A, C, F
Feedback:
B. Autoimmune thyroiditis commonly coexists with SS, and it can also be responsible for sicca symptoms without SS, but does not evoke increased risk for lymphoma.
D. Glomerulonephritis (and not distal renal tubular acidosis) confers susceptibility to lymphoma.
E. Not linked to lymphoma, but correlated with SS-associated fatigue.

12. Which of the following conditions presenting with sicca symptoms can mimic SS?
Multiple answers eligible

A. Vitamin D deficiency
B. Wilson's disease
C. Graft versus host disease
D. Hepatitis B
E. Nonsteroid anti-inflammatory medications
F. Sarcoidosis

Correct answers: C, F
Feedback:
A. Vitamin A deficiency would be the correct answer.
B. Hemochromatosis and not Wilson's disease can cause sicca symptoms.
D. Hepatitis C would be the correct answer.
E. No relevant correlation with sicca symptoms, but commonly used to alleviate joint pain.

13. A 56-year-old woman is presenting with Raynaud's phenomenon. When asked, she also admits having dry eyes over the last year and feeling tired during the last 3 months. Routine laboratory tests are nonsignificant, ANA are positive, and anti-SSA/Ro antibodies are negative. The patient was eventually classified as SS. For which of the following tests has our patient definitely been tested positive?

A. Unstimulated salivary flow rate
B. Lissamine green ocular staining

C. Minor salivary gland biopsy
D. Schirmer's test
E. Anti-SSB/La antibodies
F. Tear breakup time

Correct answer: C
Feedback: According to the new classification criteria of 2016, SS cannot be verified unless at least anti-Ro antibodies or MSGB is positive. Since anti-Ro antibodies are negative in our case, MSGB is definitely positive (focus score ≥ 1), along with at least one other positive test among unstimulated salivary flow rate, Schirmer's test, and ocular staining.

14. Which of the following patients fulfill the American/European SS classification criteria of 2016?

A. A 43-year-old woman with total unstimulated salivary flow of 1 ml/15 min, positive ANA, positive anti-TPO/anti-TG, and Schirmer's test of 4 mm in 5 min in both eyes
B. A 42-year-old woman, regular blood donor up to recently, with new-onset xerophthalmia and arthralgias, positive anti-SSA/Ro antibodies, positive anti-CCP, and focus score of 1 in MSGB
C. A 50-year-old man with tracheostomy, complaining of sicca symptoms, fatigue, and diffuse musculoarticular pain, with unstimulated salivary flow of 0.5 ml/15 min
D. A 64-year-old woman with total unstimulated salivary flow of 0.5 ml/15 min, positive anti-SSA/Ro and anti-SSB/La antibodies, positive ACA and MALT lymphoma from minor salivary gland biopsy
E. A 60-year-old man with xerophthalmia, xerostomia, chronic dry cough, abnormal objective ocular tests, intense lymphocytic infiltration in MSGB, salivary gland enlargement, subclinical jaundice, and bilateral hydronephrosis
F. A 26-year-old woman, recently treated for Chlamydia infection, presenting with sicca symptoms and fatigue, with Schirmer's test of 3 mm and 4 mm (in 5 min) in the right and left eye, respectively, and an initial assessment of the MSGB showing lymphocytic infiltration

Correct answers: B, D
Feedback:

A. Score of 2 according to criteria. Autoimmune thyroiditis could account for sicca symptoms. Further tests needed.
B. Recently tested for hepatitis and HIV since she is a blood donor. Score of 6 according to criteria. Anti-CCP presence does not exclude SS diagnosis (anti-CCP-positive SS).
C. History on previous head and neck radiation should be recorded, as suspicion of treated laryngeal cancer is raised (presence of tracheostomy). Not fulfilling criteria.

D. Score of 7 according to criteria, since MSGB with MALT is considered positive. Lymphoma is no longer an exclusion criterion, and ACA-positive SS patients are known to be at increased risk for lymphoma.
E. Suspicion of IgG4-related disease. Need more information on MSGB and serum IgG4 levels.
F. This young woman recently had a sexually transmitted disease, so HIV and HCV infection need to be ruled out before classifying her as SS. More information on the MSGB would also be helpful, as CD8+ lymphocytes are usually the ones infiltrating salivary glands in HIV infection (in contrast to predominant CD4+ lymphocytes in SS).

15. Which of the following set of features describing different female SS patients confers the highest predicted risk for NHL?

A. Distal renal tubular acidosis, positive RF, photosensitive rash, filamentary keratitis, reduced unstimulated salivary flow rate, and dental caries
B. Raynaud's phenomenon, livedo reticularis, parotid gland enlargement, lymphadenopathy, positive anti-TPO, and positive anti-TG
C. Arthralgias, fatigue, abnormal ocular staining test, reduced unstimulated salivary flow, positive anti-SSA/Ro antibodies, and positive anti-CCP
D. Monoclonal gammopathy, positive RF, positive anti-SSA/Ro, low C4 levels, lymphadenopathy, submandibular gland enlargement, and Raynaud's phenomenon
E. Monoclonal gammopathy, positive anti-SSA/Ro and anti-SSB/La antibodies, elevated γGT, C3 and C4 hypocomplementemia, positive anti-thyroid antibodies, lymphadenopathy, and Raynaud's phenomenon

Correct answer: D
Feedback: Selection of the correct answer is based on the predictive score tool for SS-associated lymphoma, as seen in Fig. 10.8.

A. One positive factor only (3.8% probability of NHL).
B. Three positive adverse features (39.9% probability of NHL).
C. One positive factor only (3.8% probability of NHL).
D. All seven identified independent predicting factors are met, which means 100% probability of developing lymphoma.
E. Five positive predisposing factors (39.9% probability of NHL).

16. Which of the following sentences are true?

A. Ultrasound of major salivary glands has replaced sialography and scintigraphy in recently revised SS classification criteria (2016).
B. Type I interferon signature is more prominent in SS-associated lymphoma cases.
C. Epithelial cells in salivary glands have an active role in SS etiopathogenesis.
D. BAFF levels in patients' serum closely follow lymphoma flares and remissions and may therefore be used for follow-up.

E. MTHFR gene variants have been found to confer increased risk for MALT lymphomas in the context of SS.
F. MALT lymphomas localized in the salivary glands should ideally be monitored closely, without need for immediate treatment.

Correct answers: C, F
Feedback:
A. Not included in criteria yet but promising results and potential future role in diagnosis.
B. IFNγ (IFN type II) is more prominent in lymphoma.
D. BAFF levels are found elevated even years after lymphoma remission.
E. MTHFR gene variants are linked to some cases of non-MALT lymphomas.

17. Which of the following diagnostic tests does not have a place in high-risk SS patients monitoring for MALT lymphoma?

A. CT chest
B. CT abdomen
C. Colonoscopy
D. Upper GI endoscopy
E. Serum protein electrophoresis and immunofixation
F. Routine laboratory panel, including LDH and β2 microglobulin

Correct answer: C
Feedback:
A, B. CTs are commonly used for diagnosis or follow-up of MALT lymphoma in patients with severe adverse predictors or relevant history.
C. MALT lymphoma is not found in the bowel; thus, colonoscopy is an unnecessary test.
D. Stomach is a common site for the development of MALT lymphoma.
E. Protein electrophoresis and immunofixation might show hypergammaglobulinemia and monoclonality, which could mean malignant transformation in some cases.
F. Routine laboratory tests are necessary in follow-up anyway and rise in LDH or β2 microglobulin could be linked to lymphoma development or reappearance after treatment.

18. Which of the following tests would you recommend as the next diagnostic step for a patient complaining about xerostomia/xerophthalmia, who is reluctant to undergo MSGB and has a positive anti-SSA/Ro result?

A. Sialography
B. Scintigraphy
C. Major salivary gland ultrasound
D. Rose bengal ocular staining
E. Unstimulated salivary flow
F. Tear breakup time

Correct answer: E

Feedback:
A, B. Sialography and scintigraphy are no longer included in SS classification criteria (2016).
C. Ultrasound is not included in SS classification criteria, despite showing promising results.
D, E. Since anti-Ro are positive (3 points), 1 more point for SS classification is required, meaning either an abnormal, objective ocular test or unstimulated salivary flow rate measurement. The latter is the easiest and cheapest way to reach diagnosis. Furthermore, lissamine green is preferred over rose bengal for ocular staining, nowadays, since it is less irritant.
F. TBUT is not included in SS classification criteria, despite being commonly used to objectify ocular dryness.

19. Which of the following etiopathogenetic events have been proven to contribute to SS-related lymphoma development?
Multiple answers eligible

A. Persistent stimulation of autoreactive B cells
B. Chromosomal translocations
C. Coxsakievirus infection
D. Epstein-Barr virus infection
E. p53 mutations
F. BAFF polymorphisms

Correct answers: A, B, E, F
Feedback:

A. True.
B. Over-expression of Bcl-2, for example, is due to a translocation involving chromosomes 14 and 18.
C, D. These viruses have been suggested as possible triggering factors for SS etiopathogenesis, but not proven to contribute to disease onset or associated lymphoma development.
E. True.
F. True.

20. Which of the following sentences is true?

A. Secondary SS is diagnosed when sicca symptoms appear in the context of hepatitis C or HIV infection.
B. Germinal center-like structures are well-defined formations in MSGBs of SS patients, associated with late disease onset.
C. Males are not affected by SS as much as women, and even if they do, they present with milder symptoms.
D. MSGBs only have a place in SS diagnosis, and if classification criteria are met anyway, patients should not undergo this invasive procedure.
E. Ocular staining score and TBUT can be used interchangeably to objectify ocular involvement in SS, according to the latest classification criteria of 2016.

F. SS classification criteria cannot be used in patients who do not experience oral and/or ocular dryness, since sicca symptoms are considered to be the disease hallmark.
G. SS is one of the most common systemic autoimmune diseases, accompanied by the highest risk for lymphoma development among them.

Correct answer: G
Feedback:

A. Active hepatitis C and HIV infection are among exclusion criteria. The term "secondary" has previously been used to describe sicca symptoms occurring in the context of another systemic autoimmune disease and tends to be replaced nowadays by the more accurate "SS-associated disease."
B. There is lack of uniform criteria for the identification of GC-like structures in MSGBs, and their documented association with more severe disease generally leads to earlier disease diagnosis.
C. Male SS patients have been suggested to present with more severe disease phenotype, despite being fewer than women.
D. MSGBs histopathological characteristics have a major prognostic value and are therefore also used in patients' risk stratification.
E. TBUT is not included in SS classification criteria of 2016.
F. Patients scoring in at least one domain of ESSDAI (having systemic involvement) are now also considered for SS diagnosis according to the latest classification criteria, even in the absence of sicca symptoms.

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Scleroderma-specific autoantibodies: Should they be included in the diagnostic work-up for Sjögren's syndrome?

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ABSTRACT

Objectives: Sicca complaints are a frequent reason for rheumatologic consultation. Testing for specific antibodies against Ro/SSA and La/SSB antigens and minor salivary gland (MSG) biopsy are among the main tools implemented in the diagnostic work-up. Anticentromere antibodies and sicca manifestations are frequently detected in Sjögren's syndrome (SS) and systemic sclerosis (SSc), respectively. Herein, we aimed to determine the frequency and clinical associations of a wide spectrum of scleroderma (SSc)-specific autoantibodies in consecutive patients referred for evaluation of possible SS.

Methods: Demographic, clinico-pathological, and laboratory data were recorded in 216 consecutive patients with sicca complaints. All study participants were tested for SSc-specific autoantibodies (against CENP, PM/Scl, Scl-70, Ku, NOR90, RP11, RP155, fibrillarin, PDGFR, and Th/To) using a commercially available immunoblot kit. According to band intensity, the identified autoantibodies were further classified in those with strong and medium titers.

Results: SSc-specific autoantibodies were detected in 41.7% (90/216) patients evaluated (19% at strong, 22.7% at medium titers) without significant differences between anti-Ro/SSA positive and negative groups. At strong titers was significantly higher in patients with MSG biopsies fulfilling SS histopathological criteria (30% vs 12.5%, $p = 0.009$). This association remained significant after adjustment for antibodies against Ro/SSA and La/SSB autoantigens [OR 95% (CI): 4.1 (1.5–10.6)].

Conclusion: SSc-specific autoantibodies are frequently detected among patients presenting with sicca complaints and at strong but not medium titers are independently associated with MSG biopsy positivity. Taken together, these data imply a useful role of SSc antibody testing in the diagnostic work-up and possibly in the classification criteria for SS.

Introduction

Differential diagnosis in patients presenting with dry eyes and dry mouth (sicca) symptoms is broad and often challenging for the practicing physician. As Sjögren's syndrome (SS) is the leading cause of autoimmune-related dryness symptoms, patients with sicca complaints undergo meticulous examination for the detection of objective oral and ocular dryness, serological testing for autoantibodies against Ro/SSA and La/SSB antigens and minor salivary gland (MSG) biopsy. For the exclusion of important SS mimics, such as chronic viral infections, sarcoidosis and IgG4 related sialadenitis, viral testing for hepatitis C and human immunodeficiency virus, chest X-Ray and IgG4 serum levels are also considered. Use of medications such as antidepressants and antihistamine among others are common causes of dry complaints and should be always considered in the differential diagnosis [1].

Despite the extensive evaluation and the strong suspicion for the presence of underlying SS on several occasions, the diagnosis of SS cannot be drawn, and sicca complaints cannot be always fully explained in the context of a well-defined clinical entity. These patients not fulfilling classification criteria for SS [2] were previously designated as

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suffering from dry eyes and mouth syndrome (DEMS) [3] or SAPS (sicca, asthenia, and polyalgia syndrome) [4]. This group of patients was found to have non-specific chronic musculoskeletal pain, Raynaud's phenomenon (RP), and subjective sicca symptoms at a similar rate to their age- and sex- matched SS counterparts, together with a higher prevalence of anti-thyroid antibodies. A mild interstitial infiltrate along with the presence of perivascular infiltrates in MSG biopsies were the main histopathological findings [5].

Other autoantibodies previously found to be detected in a subgroup of SS patients include those directed against centromere antigens (ACA), namely centromere proteins (CENP). Though they are classically detected in limited forms of systemic sclerosis (SSc), earlier reports revealed their presence in a subset of SS [6] sharing features with limited SSc (lcSSc) [7–13] in association with characteristic ultra-sonographic salivary gland findings such as hyperechoic bands [14].

Beyond anticentromere antibodies, many SSc-specific reactivities directed against nuclear and nucleolar antigens have been identified in recent years and included among others, antibodies against topoisomerase I (Scl-70), RNA-polymerase III (RP11, RP155), fibrillarin, nucleolar organizing region 90 (NOR90), Th/To ribonucleoprotein (Th/To) and polymyositis/scleroderma (PM/Scl) autoantigens. These antibodies were shown to be associated with distinct clinical features in the setting of SSc, such as puffy hands, RP, esophageal dysfunction, scleroderma renal crisis, interstitial lung disease (ILD) [15]. However, no data on the associations of these antibodies with sicca features have been so far reported.

In the present study we aimed to evaluate the prevalence of SSc-specific autoantibodies in a cohort of consecutive patients presenting with sicca complaints. Moreover, the association of these antibodies with clinical, serological, and histopathological features were investigated.

Patients and methods

Patients

Two hundred and seventy-nine consecutive patients with sicca complaints were referred for evaluation of possible SS between 01.01.2017 to 30.08.2021 at the Molecular Physiology-Clinical Applications Unit, Department of Physiology, School of Medicine, National and Kapodistrian University of Athens. Sixty-three patients were lost to follow up and were excluded from the study. Therefore, the final study group consisted of 216 patients, all of whom gave informed consent according to the declaration of Helsinki before they participated in the study.

Evaluation of sicca symptoms

Demographic data, clinical manifestations, medication history, as well as hematological, biochemical, and immunological parameters were recorded. In more detail, subjective and objective oral and ocular dryness measures (documented by unstimulated salivary flow rates and Schirmer's test/Lisammine Green staining, respectively) were obtained. Moreover, testing for chronic viral infections (hepatitis C, human immunodeficiency virus), as well as for several autoantibodies [(Rheumatoid factor, Antinuclear antibodies (ANA), anti-Ro/SSA and La/SSB antibodies] and complement levels was offered. Antibodies against Ro/SSA were determined by chemiluminescent immunoassays as previously described [16]. MSG biopsy was also performed, following patient consent. The latter was considered positive when focus score (FS) was higher than 1 ($\geq$50 lymphocytic infiltrates/4mm^2) of salivary gland tissue examined [17].

SSc-specific autoantibodies

Sera from all 216 patients were evaluated for the presence of SSc-specific autoantibodies by using a commercially available kit [EUROLINE Systemic Sclerosis (Nucleoli) profile (IgG) (EUROIMMUNE Medizinische Labordiagnostika AG)] against the following antigens: Scl-70, CENP A, CENP B, RP11 & RP155, Fibrillarin, NOR90, Th/To, PM/Scl against 75kd & 100kd proteins, Ku, and Platelet-Derived Growth Factor Receptor (PDGFR). According to signal intensity, the SSc-specific autoantibodies were divided into those with strong titer ($\geq$26) and those with medium titer (11–25). A band intensity below 11 was considered to denote negative autoantibody titers.

Statistics

Comparison of categorical variables was performed using a chi-square test. Numerical variables were compared with a *t*-test or the Mann-Whitney test when data did not follow a normal distribution. Backwards stepwise logistic regression analysis was implemented to explore the independent association between SSc-specific autoantibodies and MSG biopsy positivity following adjustment for antibodies to Ro/SSA and La/SSB antigens. The SPSS v.26 statistical program was used for the analysis.

Results

Prevalence of primary SS in the sicca cohort

Supp Table 1 summarizes the clinical, laboratory and histopathological characteristics of all study participants. As shown in Fig. 1, among the 216 patients of the final study sample, anti-Ro/SSA antibodies were detected in sera of 85 (39.4%) patients (seropositive), while the rest 131 (60.7%) were seronegative. MSG biopsies were available in 72 out of 85 (84.7%) in the anti-Ro/SSA positive group and 70/131 (53.4%) in the anti-Ro/SSA negative group. Classification criteria for primary SS were fulfilled in 88 out of 216 participants (40.7%) [82.4% (70/85) in the seropositive and 13.7% (18 out of 131) in the seronegative group] according to the 2016 ACR/EULAR classification criteria for primary SS [2]. In the anti-Ro/SSA- group without available MSG biopsies ($n = 61$), the diagnosis of primary SS cannot be excluded.

Prevalence of SSc-specific autoantibodies in sicca patients

Among 85 anti-Ro/SSA (+) patients by chemiluminescent immunoassays, 73 (85.9%) tested positive for anti-Ro52 according to the EUROLINE immunoassay implemented. The remaining anti-Ro/SSA positive sera were most likely reactive against Ro-60 autoantigen. Given the previously reported occurrence of sicca complaints in the context of SSc [8] we next sought to determine the prevalence of a wide

Table 1
Association of objective measures of ocular/oral dryness and antibodies against Ro/SSA and La/SSB antigens with MSG biopsy positivity. MSG: minor salivary gland biopsy; SSc Abs: Scleroderma Specific autoantibodies.

%	MSG (+) ($n = 70$)	MSG (-) ($n = 72$)	p-value	95% Confidence Interval (Odds ratio)
Abnormal ocular staining score	43.8	21.6	0.049	2.8 (1.0–8.1)
Abnormal Schirmer's test	50	58.8	0.38	0.7 (0.3–1.6)
Abnormal unstimulated whole salivary flow (<0.5 ml/5 min)	50	25	0.30	3.0 (0.4–24.9)
Anti-Ro/SSA	68.6	33.3	<0.001	4.4 (2.2 – 8.8)
Anti-La/SSB	37.3	5.9	<0.001	9.5 (3.1–29.3)
SSc-specific Abs (strong titers)	30	12.5	0.01	3.0 (1.3–7.1)
SSc-specific Abs (medium titers)	28.6	26.4	0.77	1.1 (0.5–2.3)

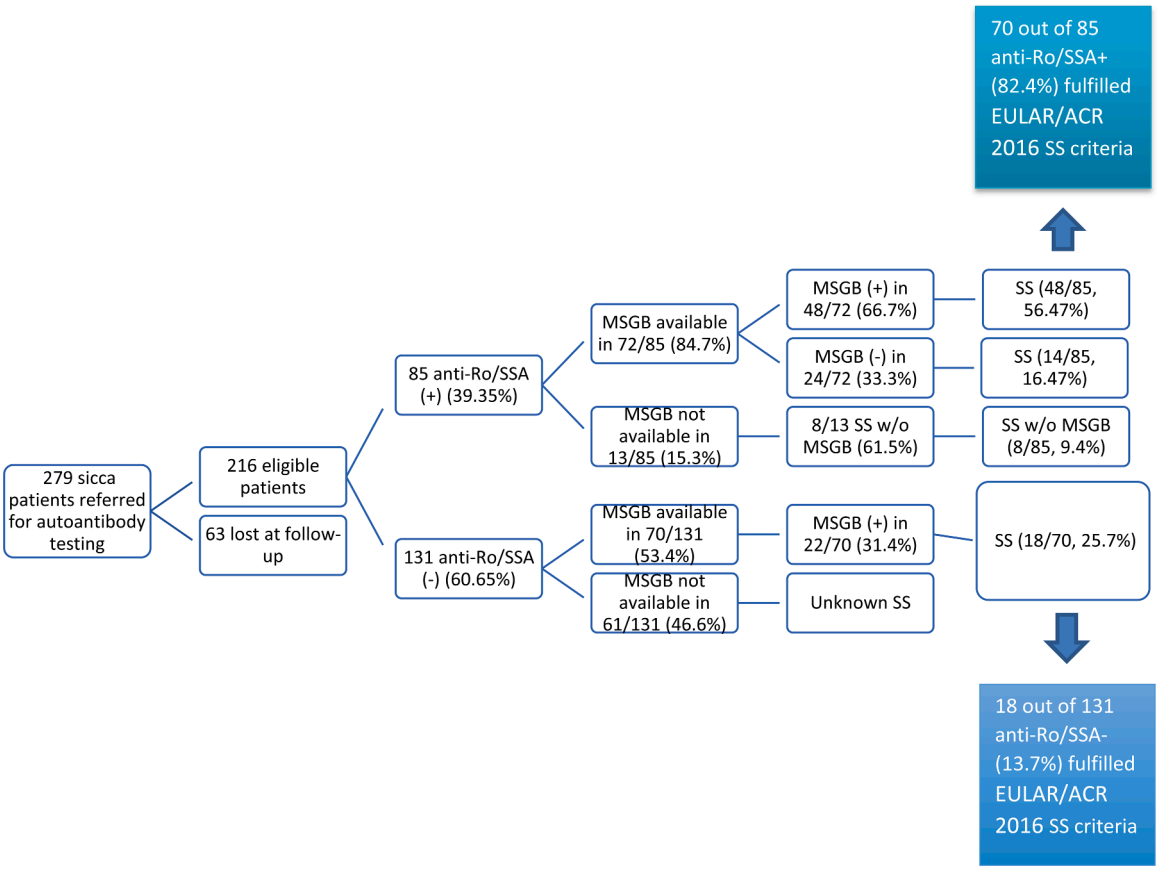

Fig. 1. Flow-chart of our consecutive sicca cohort and primary SS classification according to EULAR/ACR 2016 classification criteria (MSGB: minor salivary gland biopsy, SS: Sjögren's syndrome).

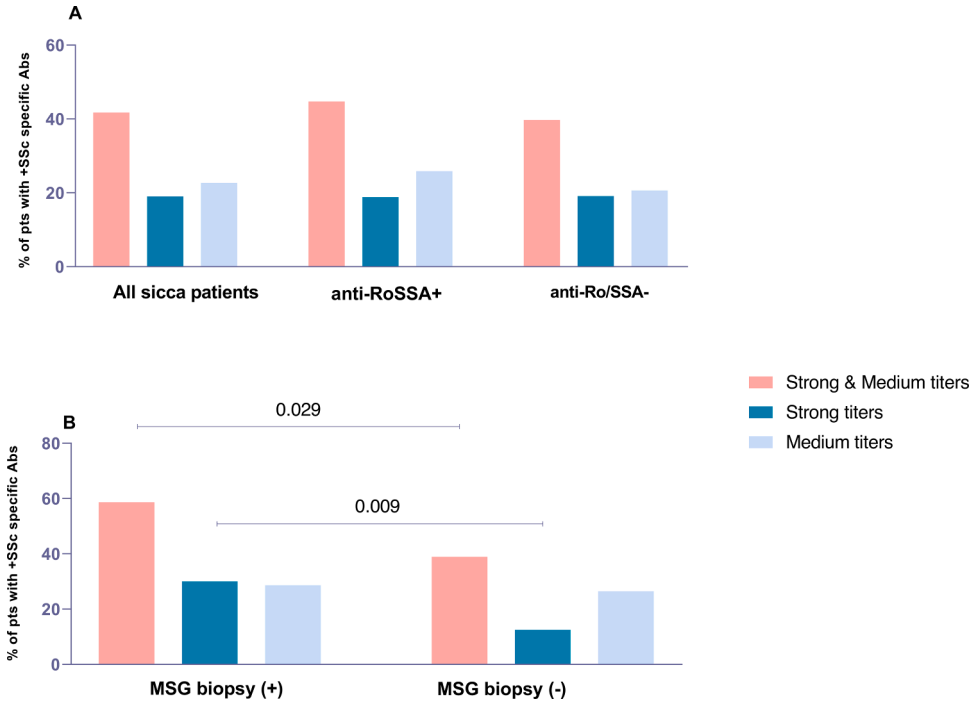

Fig. 2. High prevalence of SSc-specific antibodies at strong and medium titers in the sicca cohort (panel A) and association with MSG tissue positivity (defined by a lymphocytic focus score≥1) (panel B).

spectrum of SSc-specific antibodies in our cohort. As shown in Fig. 2A, the overall frequency of SSc-specific antibodies was 41.7% (19% at strong titers and 22.7% at medium titers). Following stratification according to the anti-Ro/SSA status, the corresponding frequencies for the seropositive vs. seronegative group were 44.7% vs. 39.7% for all SSc-specific antibodies, 18.8% vs. 19.1% for the SSc-specific antibodies at strong titers and 25.9% vs. 20.6% for those at medium titers (non-significant differences between anti-Ro/SSA+ and anti-Ro/SSA- subsets were detected). The type and frequencies of SSc specific antibodies at strong and medium titers and according to anti-Ro/SSA status are displayed in Suppl. Tables 2–4. Of note, while anticentromere staining pattern on the immunofluorescent ANA testing was detected in 11 out of 216 patients (5.1%), reactivities against CENPA and CENPB were present in 21 out of 216 sera tested by Euroline immunoblot (9.7%).

Association of SSc-specific antibodies with MSG biopsy positivity

We next sought to explore potential associations between MSG biopsy positivity and SSc-specific antibodies (strong and medium titers). As shown in Fig. 2B and Table 1, significantly increased rates of SSc-specific antibodies at strong titers were detected among patients with positive MSG biopsies compared to those without (30% vs 12.5%, p-value: 0.009), [OR 95% CI: 3.0 (1.3–7.1)]. Such differences were not detected for medium SSc-specific titers. Other features associated with MSG biopsy positivity included anti-Ro/SSA and anti-La/SSB positivity and abnormal Rose Bengal staining (Table 1). Multivariate analysis revealed an independent association of SSc-specific antibodies at strong titers with MSG biopsy positivity, following adjustment for anti-Ro/SSA and anti-La/SSB autoantibodies [OR 95% (CI): 4.1 (1.5–10.6)]. The prevalence of SSc-specific antibodies at strong and medium titers in patients according to anti-Ro/SSA and MSG biopsy positivity is displayed in Suppl Fig 1. Theoretically, if detection of SSc-specific antibodies in the sera of anti-Ro/SSA (-) individuals with negative or unavailable MSG biopsies had the same weight as that of anti-Ro/SSA, then additional 30.3% (33/109) of patients would fulfill 2016 SS classification criteria [13.8% (15/109) for strong titers and 16.5% (18/109) for medium titers, respectively). Overall, 38.9% (51/131) of anti-Ro/SSA negative individuals presenting with sicca complaints would fulfill SS classification criteria instead of 13.7% (18/131), as displayed in Fig. 1.

The type and frequency of different SSc-specific antibodies at strong titers according to MSG biopsy positivity are displayed in Fig. 3 and Suppl. Table 5. While the diversity of SSc-specific antibodies was greater in the MSG (+) group compared to MSG (-), no significant differences in frequency terms were detected.

In Figs. 4& 5, representative MSG biopsies from patients with various SSc antibodies are displayed. While histopathological criteria for SS are not fulfilled for all patients, the presence of fibrosis alone or along with lymphocytic infiltrates was detected (photos taken using Olympus Slideview VS200).

Associations of SSc-specific antibodies with distinct features

We next wished to explore whether SSc-specific autoantibodies are associated with distinct clinical and histopathological features. As shown in Supp Tables 6–8, arthralgias and myalgias occurred more frequently in patients with medium titers versus those with negative serum SSc antibody titers, while minor salivary gland histopathological focus scores ≥ 1 were more frequently detected among patients with strong SSc titers (63.3 vs 40, $p = 0.029$).

Upon clinical indication, further investigation was performed in the SSc-specific antibody-positive patient group. High resolution thoracic computed tomography (HRCT) was performed in eighteen cases of those having SSc-specific antibodies at strong titers, of which 11 (61%) had abnormal findings including micronodules, peribronchial thickening and ground glass pattern. Additionally, 16.7% (2 out of 12) patients had abnormal pulmonary function tests (PFT) consistent with restrictive disease, while pulmonary hypertension (defined as PAH>30 mm Hg measured by heart ultrasound (HUS), reflecting PAH>20mm Hg measured by right heart catheterization) [18] was detected in 3 out of 13 (23.1%). The corresponding figures for patients with SSc specific antibodies at medium titers were, abnormal findings in HRCT for 9/15 (60%), abnormal pulmonary function tests (PFTs) for 3/9 (33.3%), and pulmonary hypertension for 5/11 (45.5%) performed tests, respectively. Telangiectasias were noted in 2/41 (4.9%) of patients with SSc antibodies at strong titers and 2/49 (4.1%) of those with medium ones, respectively. Table 2 displays the final diagnosis of the study participants following extensive work-up in the anti-Ro/SSA positive and anti-Ro/SSA negative groups. Moreover, the prevalence of SSc positivity in each diagnostic group is displayed. While SS is the prevalent diagnosis among anti-Ro/SSA positive individuals (82.4%), UCTD was the commonest diagnosis among anti-Ro/SSA negative individuals (34.4%). Of interest, the prevalence of SSc specific autoantibodies in anti-Ro/SSA negative individuals with SS and UCTD was 66.7 and 71.1, respectively.

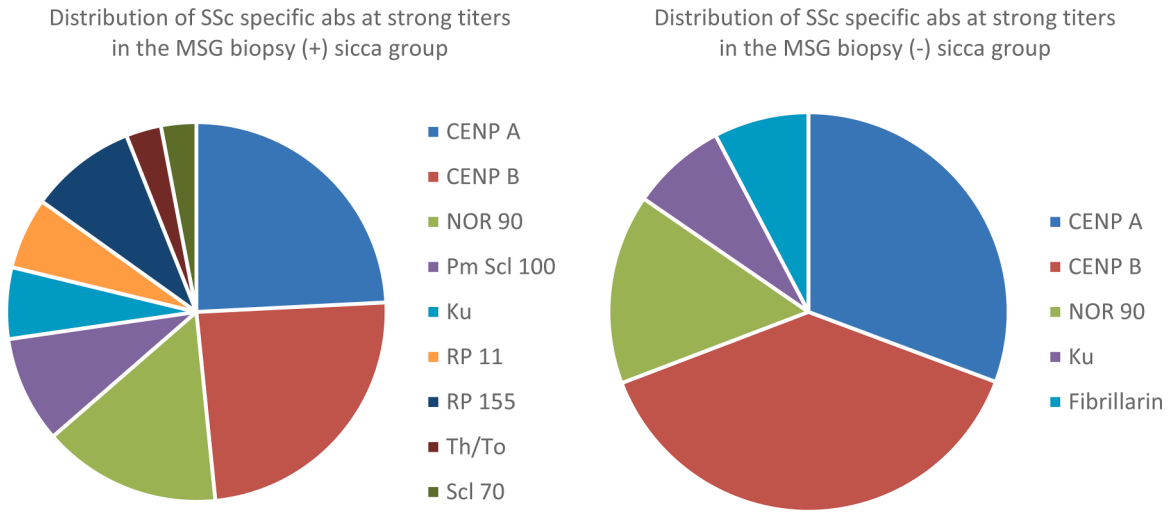

Fig. 3. Type and frequency of different SSc-specific antibodies at strong titers according to MSG biopsy positivity (SSc: systemic sclerosis; MSG: minor salivary gland; CENPA, CENPB: centromere protein A, B Scl-70: Scleroderma-70 or Topoisomerase I; RP: RNA-polymerase III; NOR 90: Nucleolar Organizing Region 90; PM/Scl: Polymyositis/Scleroderma).

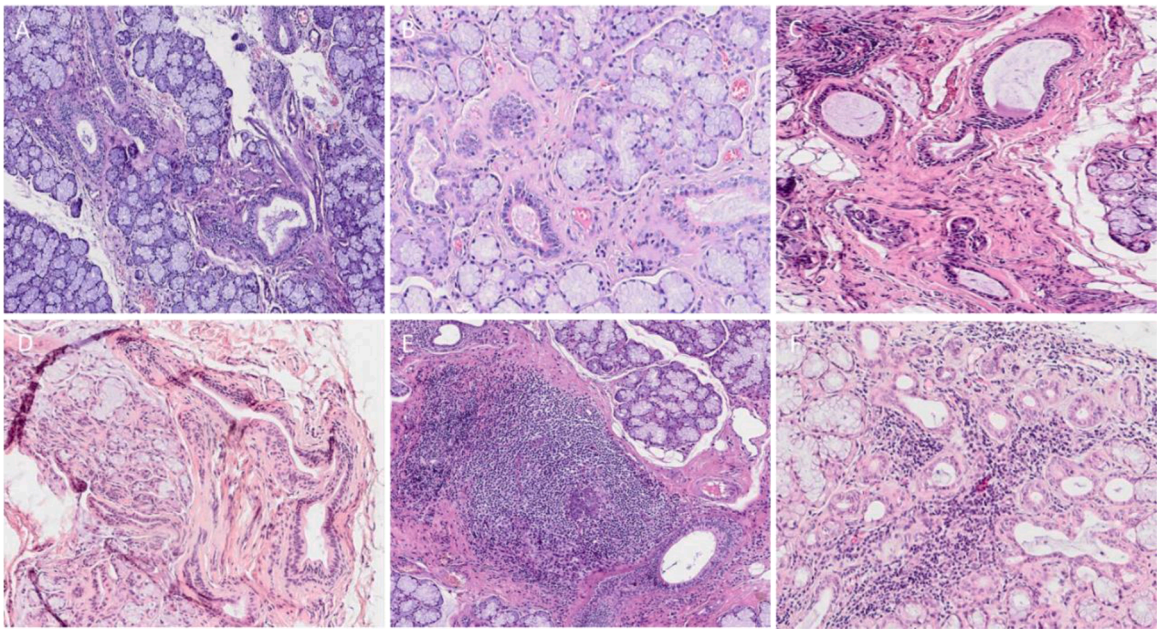

Fig. 4. Representative MSG tissue biopsies from sicca patients with positive SSc specific antibodies at strong titers (hematoxylin/eosin staining). A. anti-Ku+, anti-Ro/SSA- FS: 0.65; B. anti-CENPB+, anti-Ro/SSA-, FS:0; C. anti-fibrillarin+, anti-Ro/SSA-, FS: 0; D. anti-Scl70+, anti-Ro/SSA-, FS: 0.63; E. anti-NOR90+, anti-Ro/SSA-, FS: 1.01, F. anti-CENPA+, anti-CENPB+, anti-Ro/SSA-, FS: 1.35.
(MSG: minor salivary gland; NOR90: Nucleolar Organizing Region 90; SSc: systemic sclerosis; SS: Sjögren's syndrome; CENPA, CENPB: centromere protein A, B; FS: focus score).

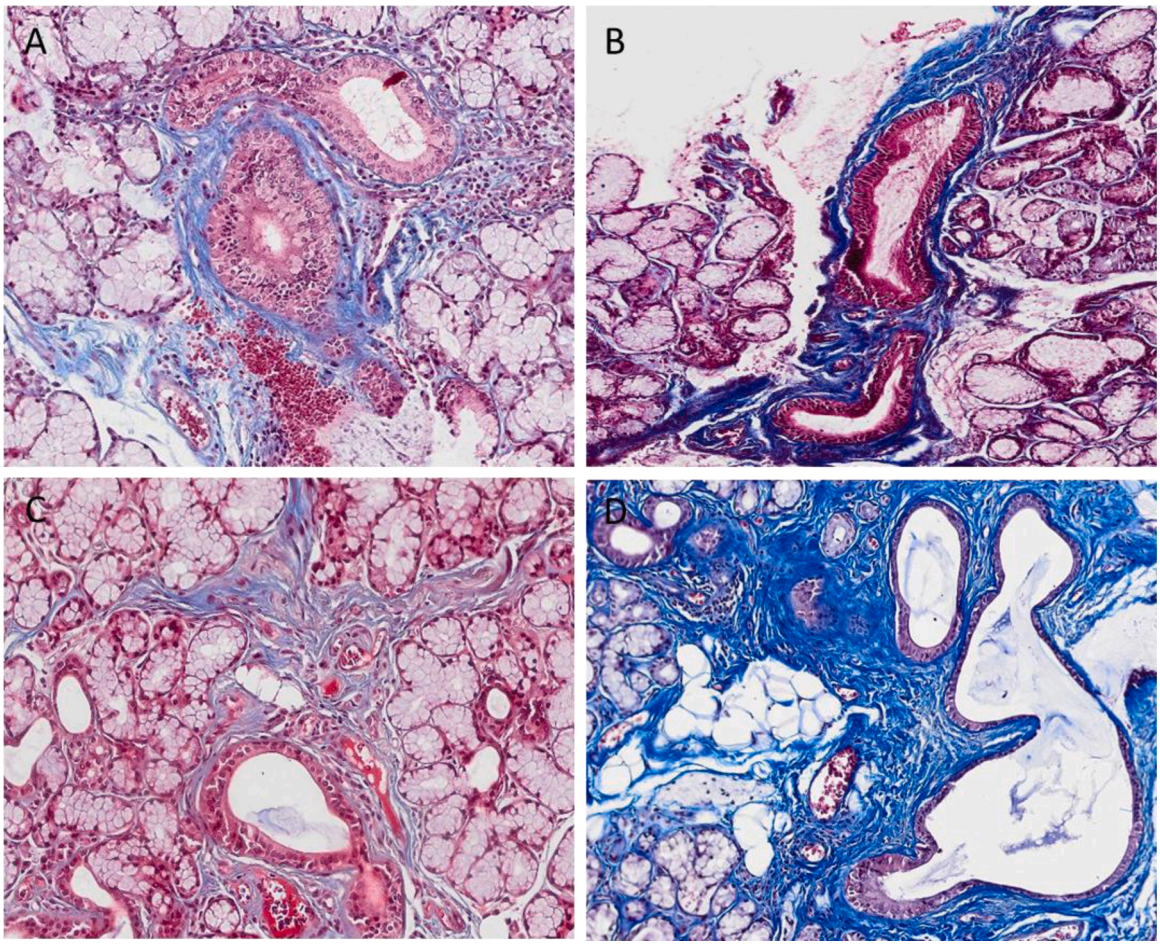

Fig. 5. Representative MSG tissue biopsies depicting the presence of fibrosis using Masson staining from the same sicca patients (A-D), displayed in Fig. 4.

Table 2
Final diagnoses in the anti-Ro/SSA+ and anti-Ro/SSA- groups and rates of SSc+ sera in each diagnosis following extensive diagnostic work up.

Final diagnosis n (%)	Sicca cohort (n = 216)	SSc+ positive sera (n = 90)
Anti-Ro/SSA (+) group (n = 85)		
SS	70 (82.4)	31/70 (44.3)
UCTD	5 (5.8)	3/5 (60)
RA	2 (2.4)	1/2 (50)
SLE	5 (5.8)	1/5 (20)
DM	1 (1.2)	1/1 (100)
PBC	1 (1.2)	0/1 (0)
SSc	1 (1.2)	1/1 (100)
Anti-Ro/SSA (-) group (n = 131)		
UCTD	45 (34.4)	32/45 (71.1)
Hashimoto	29 (22.1)	2/29 (6.9)
SS	18 (13.7)	12/18 (66.7)
SLE	13 (10)	2/13 (15.4)
RA	11 (8.4)	2/11 (18.2)
Dermatomyositis	3 (2.2)	1/3 (33.3)
Fibromyalgia	3 (2.2)	1/3 (33.3)
Non-specific sialadenitis	3 (2.2)	0/3 (0)
Sclerosing sialadenitis	1 (0.8)	0/1 (0)
Chronic blepharitis	1 (0.8)	0/1 (0)
MGUS	1 (0.8)	0/1 (0)
PBC	1 (0.8)	0/1 (0)
PsA	1 (0.8)	0/1 (0)
RP	1 (0.8)	0/1 (0)

Sjögren's syndrome, UCTD: undifferentiated connective tissue disease, SLE: systematic lupus erythematosus, MGUS: monoclonal gammopathy of unknown significance, PBC: primary biliary cirrhosis, PsA: psoriatic arthritis, RA: rheumatoid arthritis, RP: Raynaud's phenomenon.

Discussion

In the present study, we report that SSc-specific antibodies were frequently detected in a cohort of consecutive patients evaluated for sicca complaints in an outpatient rheumatology setting. Notably, SSc specific at strong -but not medium- titers were found to be independently associated with positive MSG biopsies, even after adjustment for the presence of serum antibodies towards Ro/SSA and La/SSB antigens, implying a potentially contributory role of these antibodies in SS classification. Reactivities against centromere proteins accounted for almost half of all SSc specific antibodies detected at strong titers, with the remaining being directed against other nucleolar (NOR90, fibrillarin, PM/Scl, Th/To) or nuclear antigens (Scl70/Topoisomerase I, RNA polymerase III, Ku). Periductal fibrosis was a prominent histopathological feature in minor salivary glands of these patients either alone or in association with focal or sparse lymphocytic infiltrates. Some of these patients presented with occult scleroderma features, and follow-up may reveal those who will eventually develop systemic sclerosis. Theoretically, if SSc-specific antibodies had the same weight as anti-Ro/SSA antibodies in the classification criteria for SS, almost one third of anti-Ro/SSA negative patients with either negative or unavailable MSG biopsies would be classified as SS. Whether this patient subset represents a distinct clinical entity or a subtype of SS remains to be further explored.

While anticentromere antibodies have been extensively investigated in the context of SS [6,9,10–13,19,20–22], and even been proposed as a serologic marker for SS diagnosis equivalent to anti-Ro/SSA and anti-La/SSB positivity [24], this is the first attempt to evaluate the role of a wide spectrum of SSc-specific autoantibodies and their contribution to salivary gland inflammation and generation of sicca symptomatology.

Anticentromere antibodies have been previously suggested as the serological link between distinct autoimmune entities, as for PACK syndrome [PBC (primary biliary cirrhosis, ACA, CREST (Calcinosis, RP, Esophageal dysfunction, Sclerodactyly, Telangiectasias) and KCS (Keratoconjuctivitis sicca)] [25]. SS patients with anticentromere antibodies have been shown to share overlapping features with SSc such as RP [7,12,21,23,26,27], dysphagia [7,12,27], sclerodactyly [12,27], telangiectasias [12,27], in addition to sicca phenotype [13,26,27]. Compared to their SS counterparts, they have seldom hypergammaglobulinemia [21], display reduced frequency of antibodies against Ro/SSA and/or La/SSB [12,20,25,26] but share similar histopathological findings [12,25,26] in MSG tissues. While a more severe clinical phenotype [21] conferring higher lymphoma risk [23] was previously reported, this was not confirmed in other studies [28,29]. Interestingly, as much as 25% of SS patients with anticentromere antibodies may eventually be classified as limited cutaneous SSc on follow-up [10,30,31], although this was not universally detected [26].

Sicca features have been consistently reported in the setting of both limited (lc) and diffuse (dc) cutaneous SSc. In patients with lcSSc, the frequency of oral and ocular dryness is variable, ranging from 23 to 75% [26,32–34] and 21–100% [26,32–34], respectively; interestingly, 49% of these patients have been shown to display lymphocytic sialadenitis in MSG biopsy [34]. In dcSSc, sicca features were reported in 39–49% of patients [32,33,35,36,37], along with evidence of fibrotic changes in MSG biopsies [37]. Furthermore, recurrent SGE was evident in 11.4% of patients and MSG biopsy revealed distinct groups of significant fibrosis in either periductal, perilobular or intralobular regions; fibrosis was absent in the patients with mixed connective tissue disease, RP or healthy controls [37]. Another study of 44 dcSSc patients showed that SGE was present in 44.4% and one fifth had MSG findings compatible with SS, while 38.6% had mild fibrotic changes [38]. Earlier reports have estimated an SS prevalence ranging from 0- 88% in the context of SSc [35,37,39–41].

Features of objective mucosal dryness have been also reported in 57% of patients with idiopathic pulmonary fibrosis (IPF) [42]. Sicca symptomatology seems to increase disease burden in SSc patients as it shows strong association to the SF-36 quality of life score [43]. Along the same lines, sicca complaints have been suggested [44] to be included in the Scleroderma Clinical Trials Consortium-Damage Index (SCTC-DI) [45].

While sicca features in patients with positive MSG biopsies are attributed to the local inflammatory process and ensuing impaired glandular function, the underlying mechanisms accounting for the generation of sicca complaints in the absence of classical histopathological SS findings are not fully elucidated. Neurosecretory dysfunction as the result of an inflammation-related dampened neurotransmitter release, or autoantibodies against muscarinic 3 receptor [32,46], as well as fibrosis and vascular inflammation has been proposed as having a contributory role for dry features in the context of SS and SSc [47].

Given the association of abnormal findings in salivary gland ultrasound (SGUS) with positive MSG biopsy in SS patients [48], abnormal parotid gland SGUS in patients with antibodies against Ro/SSA and La/SSB antigens has been shown to have high predictive value for SS [49]. Notably, abnormal findings in SGUS were observed in one third of SSc patients, the majority of which were positive for anticentromere antibodies [43], further supporting an SS/SSc overlap phenotype [50]. Furthermore, abnormal ultrasonographic features of salivary glands were present in 51.6% of SS and 62.7% of SSc patients, respectively [51]. Nevertheless, the two modalities (US and MSG biopsy) are considered complementary in pursuit of SS diagnosis and cannot replace one another [52]. Therefore, the presence of SSc-specific autoantibodies at strong titers in sicca patients is the sole non-invasive modality so far shown to be independently associated to MSG biopsy positivity irrespective of the presence of anti-Ro/SSA and anti-La/SSB antibodies.

The major limitation of the current study is the lack of available minor salivary gland biopsies in all study participants, mainly due to reluctancy of some patients to undergo labial gland biopsies. Moreover, one could argue that MSG biopsies were more often performed in the anti-Ro/SSA positive rather than the anti-Ro/SSA negative group. However, given the increased risk for lymphoma development among anti-Ro/SSA positive individuals [19], anti-Ro/SSA patients with sicca complaints are routinely offered an MSG biopsy, despite they fulfill ACR 2016 criteria for SS. Additionally, while the presence of fibrosis in MSG tissue has been earlier shown in patients with SSc [37], and even

detected in one third of patients in another prospective study of SSc patients who underwent MSG biopsy [53], a prospective matched and blinded study quantitating the amount of fibrosis in MSG biopsies from patients with distinct serological reactivities should be performed.

In conclusion, these data suggest that SSc-specific antibodies are frequently detected in patients presenting with sicca complaints and at strong titers they are independently associated with MSG positivity. Although our observations must be confirmed in larger cohorts, we propose that testing for SSc-specific autoantibodies could be a useful tool in the diagnostic work-up and potentially in future classification criteria for SS.

Ethics

Our study complies with the Declaration of Helsinki. The locally appointed ethics committee has approved the research protocol. Written informed consent has been obtained from the subjects.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.semarthrit.2022.152026.

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