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Seronegative patients with primary Sjögren's syndrome and non-pSS sicca test positive for anti-SSA/Ro52 and -Ro60 in saliva

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ABSTRACT

Objectives: Testing for anti-SSA/Ro antibodies in serum is essential in the diagnostic work-up for primary Sjögren's syndrome (pSS). In this study, we aimed to validate our previous assay for detection of salivary anti-SSA/Ro52, and to develop assays for detection of salivary anti-SSA/Ro60 and for detection of anti-Ro52 and -Ro60 in plasma using the electric field-induced release and measurement (EFIRM) platform.
Methods: Whole saliva samples from two independent Danish cohorts (DN1 and DN2) including 49 patients with pSS, 73 patients with sicca symptoms, but not fulfilling the classification criteria for pSS (non-pSS sicca), and 51 healthy controls (HC), as well as plasma samples from the DN1 cohort were analyzed using EFIRM to detect anti-SSA/Ro52 and -Ro60.
Results: In the DN1 cohort, 100 % in the pSS group and 16 % in the non-pSS sicca group were serum anti-SSA/Ro positive by ELISA. EFIRM detected anti-SSA (Ro52 and/or -Ro60) in plasma and saliva in 100 % and 96 % patients with pSS, and 16 % and 29 % with non-pSS sicca. In the DN2 cohort, 80 % patients with pSS and 26 % with non-pSS sicca were serum anti-SSA/Ro positive. Salivary anti-SSA discriminated patients with pSS from HC and non-pSS sicca with an AUC range of 0.74–0.96 in the DN1 and DN2 cohorts. EFIRM discriminated pSS from non-pSS sicca with an AUC of 0.98 in plasma.
Conclusion: Our findings suggest that salivary anti-SSA/Ro antibodies are potential discriminatory biomarkers for pSS, which may also identify seronegative patients, addressing the unmet clinical need of early detection and stratification of pSS.

1. Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by the presence of lymphocytic infiltration of the exocrine glands and circulating autoantibodies, anti-SSA/Ro and/or anti-SSB/La [1–3]. The salivary and lacrimal glands are the main targets of the disease leading to oral and ocular dryness due to immune-mediated degeneration of the glandular tissue resulting in gland dysfunction. The pathophysiological mechanism of pSS remains to be unclear, but lymphocytic infiltration of the glands induced by an abnormal immunological response to autoantigens, SSA/SSB, is believed to be playing an important role in the development and maintenance of the disease [2]. The salivary gland epithelial cells (SGEC) have been reported to actively participate in the autoimmune response as they express epithelial adhesion molecules, receptors, chemokines and proinflammatory cytokines that are involved in recruitment, differentiation, activation as well as proliferation of immune cells. [4–7].

The presence of circulating autoantibodies, anti-SSA/Ro, and/or focal lymphocytic sialadenitis with a focal score of ≥1 in labial salivary gland tissue is essential for fulfilling the criteria for the diagnosis of pSS

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[8–10]. The prevalence of anti-SSA/Ro has been reported to be up to 70 % in patients with pSS, and presence of anti-SSA is also associated with more severe clinical manifestations [2,11]. The presence of salivary anti-SSA/Ro may originate from either circulating autoantibodies in serum and/or from a local production in the salivary glands by infiltrating B cells.

SSA/Ro consists of two different proteins, Ro52 and Ro60 [12]. Ro52/TRIM21 belongs to the Tripartite Motif Protein (TRIM) family, and is involved in protein ubiquitination, proinflammatory and apoptotic mechanisms. Of note, we have previously reported that simultaneous presence of serum anti-SSA/Ro antibodies and upregulated salivary TRIM29 yielded an AUC of 0.995 [13]. Ro60 is a protein that is a part of small cytoplasmic ribonucleoprotein complexes (hY-RNA complexes).

None of the current classification criteria sets discriminate between circulating antibodies against these two proteins as an objective criterion [8–10]. Nevertheless, several studies have reported that monoreactivity against Ro52 or Ro60 is associated with different phenotypes [14–17]. Thus, isolated testing of anti-Ro52 and anti-Ro60 may aid in patient stratification, although further validation is needed. We have previously described a new platform, electrical field-induced release and measurement (EFIRM), to detect anti-SSA/Ro52 autoantibodies in saliva in patients with pSS and patients with sicca symptoms who did not fulfill the criteria for the diagnosis of pSS (non-pSS sicca) [18]. In this study, we aimed to validate our previous assay for the detection of salivary anti-SSA/Ro52 and to develop a novel assay to detect salivary anti-SSA/Ro60 autoantibodies in two independent Danish cohorts. Furthermore, we aimed to develop two new assays on EFIRM to detect anti-SSA/Ro52 and -Ro60 antibodies in plasma, respectively, to determine the correlation between serological values measured by ELISA and EFIRM.

2. Material and methods

2.1. Patients

This cross-sectional study included two independent cohorts from Copenhagen, Denmark. All patients were referred by rheumatologists, ophthalmologists or dentists when the pSS diagnosis was suspected. The diagnosis of pSS was based on the American College of Rheumatology/European League Against Rheumatism (ACR-EULAR) classification criteria [9]. Inclusion criteria were age between 18 and 75 years and the presence of oral and/or oral dryness. Patients who were pregnant, breastfeeding, in treatment with immunosuppressant and/or had presence of any other autoimmune inflammatory connective tissue disease were excluded from the study.

The first Danish cohort 1 (DN1) included 26 patients fulfilling the ACR-EULAR classification criteria for pSS [9]; 37 patients not fulfilling the ACR-EULAR classification criteria for pSS, but having symptoms of ocular and oral dryness, and 24 gender- and age-matched healthy control subjects with no history of autoimmune disease, no oral or systemic diseases and no intake of xerogenic medication.

The second Danish cohort (DN2) included 25 patients fulfilling the ACR-EULAR classification criteria [9], 38 patients not fulfilling the ACR-EULAR classification criteria for pSS, and 23 gender- and age-matched healthy control subjects with no history of autoimmune disease, no oral or systemic diseases and no intake of xerogenic medication.

The study was performed according to the Declaration of Helsinki and approved by the Ethical Committees for the Region of Copenhagen, Denmark (H-16035289 and H-18042385) and the General Data Protection Regulation. Written informed consent was obtained from all participants.

2.2. Collection of samples

All samples and biopsies were collected at the clinic for Oral Medicine, Department of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen. A standardized interview was performed with questionnaires regarding general and oral health, medical history, alcohol intake, tobacco use as well as assessment of oral and ocular dryness. Samples from the DN1 were collected by MLSM between October 2016 and December 2017. In two patients with pSS and two patients with non-pSS sicca the amount of saliva was insufficient for further analysis, but these four subjects were included in the plasma analysis. Samples from the DN2 cohort were collected by SK between October 2019 and September 2020. All samples were analyzed by SK using the EFIRM platform at the Center of Oral/Head & Neck Oncology Research, University of California, Los Angeles.

2.2.1. Saliva collection

All patients underwent sialometry including collection of unstimulated whole saliva secretion for 15 min, and chewing-stimulated whole saliva secretion for 5 min, as previously described in details [19]. Patients were instructed not to eat, drink, smoke nor brush their teeth 2 h prior to the sialometry. The saliva was collected around 11 am for all patients to avoid any variation in the salivary flow rates. The collected saliva was kept on ice immediately upon collection and measurement and then stored at −80 °C until further analysis.

2.2.2. Minor salivary gland biopsy

All patients also underwent a biopsy of the minor labial salivary glands for histopathological examination. Minor salivary gland biopsy was performed under local anesthesia, and included excision of 5 labial salivary gland lobules, as previously described [19]. The excised glands were then embedded in paraffin and cut in 4–6 μm sections for histopathological examination. The histopathological analysis was performed by the same oral pathologist for all patients, and the degree of focal lymphocytic infiltration was determined by calculating the focus score [20].

2.2.3. Plasma collection

Whole blood samples were collected into K2-EDTA tubes (BD Vacutainer®), and centrifuged within one hour of collection. The samples were first centrifuged for 10 min at 1800G, followed by transfer of plasma to a new tube that was then centrifuged for another 10 min at 3000G. Finally, the platelet-poor plasma was transferred to a new tube and stores at −80 °C until analysis. Plasma samples were only obtained from patients in the Danish cohort 1 (DN1).

2.2.4. Detection of anti-SSA antibodies in serum

As a part of the regular diagnostic work-up, all patients were referred for blood test for detection of anti-SSA/Ro. The detection of IgG antibodies to SSA were analyzed at authorized laboratories in Denmark using enzyme-linked immunosorbent assays (ELISA).

2.3. EFIRM immunoassay

The experimental work was performed using the EFIRM platform as previously described with only a few changes to the previously protocol outlined below [18].

For both the SSA/Ro-52 and SSA-Ro60 antibody immunoassays, saliva was diluted in a blocker casein solution (1 % *w/v* purified casein, pH 7.4; Thermo Fisher Scientific, Waltham, MA) at a volume ratio of 1:1, while plasma was diluted at a volume ratio of 1:1000. The secondary antibody, biotinylated polyclonal anti-human IgG-Fc (Thermo Fisher Scientific, Waltham, MA) was diluted in blocker casein at a volume ratio of 1:200.

2.4. Statistical analysis

Data was processed by Mann-Whitney test, Kruskal-Wallis test, Spearman correlation, multiple logistic regression and post-hoc analysis

using GraphPad Prism version 10. The discriminatory performance of salivary anti-SSA antibodies between groups was assessed by constructing ROC curves to calculate AUC and the associated 95 % confidence interval (CI) using GraphPad Prism v.10. The threshold (upper normal) cut-off was determined as mean plus 2*SD in the saliva of healthy subjects. *P*-value <0.05 was considered statistically significant.

3. Results

The demographics and characteristics of patients with pSS and non-pSS sicca in cohorts DN1 and DN2 are summarized in Table 1A and 1B, respectively.

3.1. Detection of anti-SSA/Ro52 and -Ro60 antibodies in plasma using EFIRM in DN1 cohort

In this study, we developed an EFIRM immunoassay to not only detect salivary anti-SSA/Ro52 and -Ro60 antibodies, but also to detect these antibodies in plasma to validate our findings

Levels of anti-SSA/Ro52 and -Ro60 autoantibodies in plasma were measured in 26 patients with pSS and 37 patients with non-pSS (Fig. 1A and B). A scatterplot was also constructed to show the correlation between levels of anti-SSA/Ro52 (x-axis) and -Ro60 (y-axis) demonstrating that some patients are positive for both subclasses of anti-SSA, while others are only positive for either anti-Ro52 or -Ro60 antibodies (Fig. 1C, Table 2).

Anti-SSA/Ro52 and -Ro60 antibodies were detected by EFIRM in 62 % (16/26) an 85 % (22/26) of the patients with pSS, respectively (Table 2). Furthermore, all patients with pSS (26/26), who were positive for serum anti-SSA antibodies measured by ELISA at diagnostic work-up, were also positive on EFIRM when combining the detection of anti-Ro52 and -Ro60 in plasma, which indicated great concordance between the EFIRM platform and ELISA (Table 3).

ROC curves were constructed to explore the discriminatory performance of anti-SSA/Ro52 and anti-SSA/Ro60 in plasma from patients with pSS and non-pSS sicca, using the EFIRM platform, which yielded an AUC of 0.98 (95 % CI: 0.94–1.0) (Fig. 2).

Table 1A
Demographic and clinical characteristics of the patients with pSS and non-pSS sicca and health control subjects in the Danish cohort 1 (DN1).

	pSS (n = 26)	Non-pSS (n = 37)	P-value (pSS vs non-pSS)	Healthy controls (n = 24)
Age (years)	56 ± 11	55 ± 14	0.76	59 ± 12
Female	22 (92 %)	33 (90 %)	1.00	24 (100 %)
Current smoker	6 (23 %)	7 (19 %)	1.00	1 (4 %)
No. prescribed medication,	1 (0–4)	1 (0–11)	1.00	0 (0 %)
Polypharmacy (≥5)	0 (0 %)	5 (14 %)	1.00.	0 (0 %)
Dry eyes for >3 months	21 (81 %)	? (97 %)	**<0.001**	0 (0 %)
Dry mouth for >3 months	22 (85 %)	31 (84 %)	0.70	0 (0 %)
Hyposalivation (UWS ≤0.10 ml/min)	20 (77 %)	21 (57 %)	0.70	0 (0 %)
Keratoconjunctivitis sicca	21 (81 %)	18 (50 %)	**0.016**	N.A.
Positive serum anti-SSA/Ro antibody	26 (100 %)	5 (14 %)	**<0.001**	N.A.
Focus score ≥ 1	13 (50 %)	0 (0 %)	**<0.001**	N.A.
UWS (ml/min) Median	0.09 ± 0.06 0.04	0.12 ± 0.11 0.08	0.21	0.34 ± 0.19 0.33
SWS (ml/min) Median	0.67 ± 0.59 0.47	0.86 ± 0.52 0.79	0.20	1.83 ± 0.84 1.70

Values are given in mean, SD, median, and in number of patients (%). N.A., not analyzed; SD, standard deviation; UWS, unstimulated whole saliva flow rate; SWS, chewing-stimulated whole saliva flow rate.

Table 1B
Demographic and clinical characteristics of the patients with pSS and non-pSS sicca and healthy control subjects in the Danish cohort 2 (DN2).

	pSS (n = 25)	Non-pSS (n = 38)	P-value (pSS vs non-pSS)	Healthy controls (n = 23)
Age (years)	57 ± 14	56 ± 13	0.69	59 ± 14
Female	23 (92 %)	36 (95 %)	1.00	21 (91 %)
Current smoker	6 (24 %)	12 (32 %)	0.60	1 (4 %)
Dry eyes for >3 months	22 (88 %)	32 (84 %)	1.00	0 (0 %)
Dry mouth for >3 months	21 (84 %)	32 (84 %)	0.73	0 (0 %)
Xerostomia Inventory score,	39 (16–53)	38 (11–55)	0.9	13 (11–18)
Hyposalivation (UWS ≤0.10 ml/min)	10 (40 %)	14 (37 %)	1.00	0 (0 %)
Keratoconjunctivitis sicca	22 (85 %)	25 (68 %)	**<0.001**	N.A.
Positive serum anti-SSA/Ro antibody	20 (80 %)	10 (26 %)	**<0.001**	N.A.
Focus score ≥ 1	18 (69 %)	0 (37 %)	**<0.001**	0 (0 %)
UWS (ml/min) Median	0.19 ± 0.16 0.18 (range)	0.20 ± 0.18 0.17	0.72	0.51 ± 0.34 0.41
SWS (ml/min) Median	1.19 ± 0.99 0.84 (range)	1.46 ± 0.98 1.00	0.18	2.33 ± 0.86 2.29

Values are given in mean, SD, range, median, and in numbers of patients (%), N. A, not analyzed.
SD, standard deviation; UWS, unstimulated whole saliva flow rate; SWS, chewing-stimulated whole saliva flow rate.

3.2. Detection of anti-SSA/Ro52 and -Ro60 antibodies in saliva in the DN1 cohort

EFIRM quantitatively detected anti-Ro52 and -Ro60 antibodies in saliva in 54 % (13/24) and 92 % (22/24) of the patients with pSS from the DN1 cohort, respectively (Table 2). Ninety-six percent (23/24) of the patients with pSS, who were serological positive for anti-SSA/Ro at the diagnostic work-up, were also positive for salivary anti-SSA/Ro measured by EFIRM when combining salivary anti-Ro52 and -Ro60. Sixteen percent of the non-pSS sicca patients exhibited serum positivity for anti-SSA/Ro, whereas anti-SSA/Ro (anti-Ro52 and/or -Ro60) antibodies measured by EFIRM were detected in saliva in 29 % (10/35) of the patients (Fig. 3 and Table 3).

We then explored the potential of salivary anti-SSA/Ro (anti-Ro52 and -Ro60) antibodies to differentiate patients with pSS from non-pSS sicca patients and healthy control subjects (Fig. 4). The AUC of pSS versus healthy controls was 0.96 (95 % CI: 0.90–1.00), and AUC 0.87 (95 % CI: 0.77–0.97) for pSS versus non-pSS sicca.

3.3. Detection of anti-SSA/Ro52 and anti-Ro60 antibodies in saliva from cohort DN2

EFIRM quantitatively detected anti-Ro52 and -Ro60 antibodies in saliva in 65 % (16/25) and 80 % (20/25) of the patients with pSS from the DN2 cohort, respectively (Table 2). A total of 84 % (21/25) of the patients with pSS were positive for salivary anti-SSA/Ro measured by EFIRM when combining salivary anti-Ro52 and -Ro60, while 80 % (20/

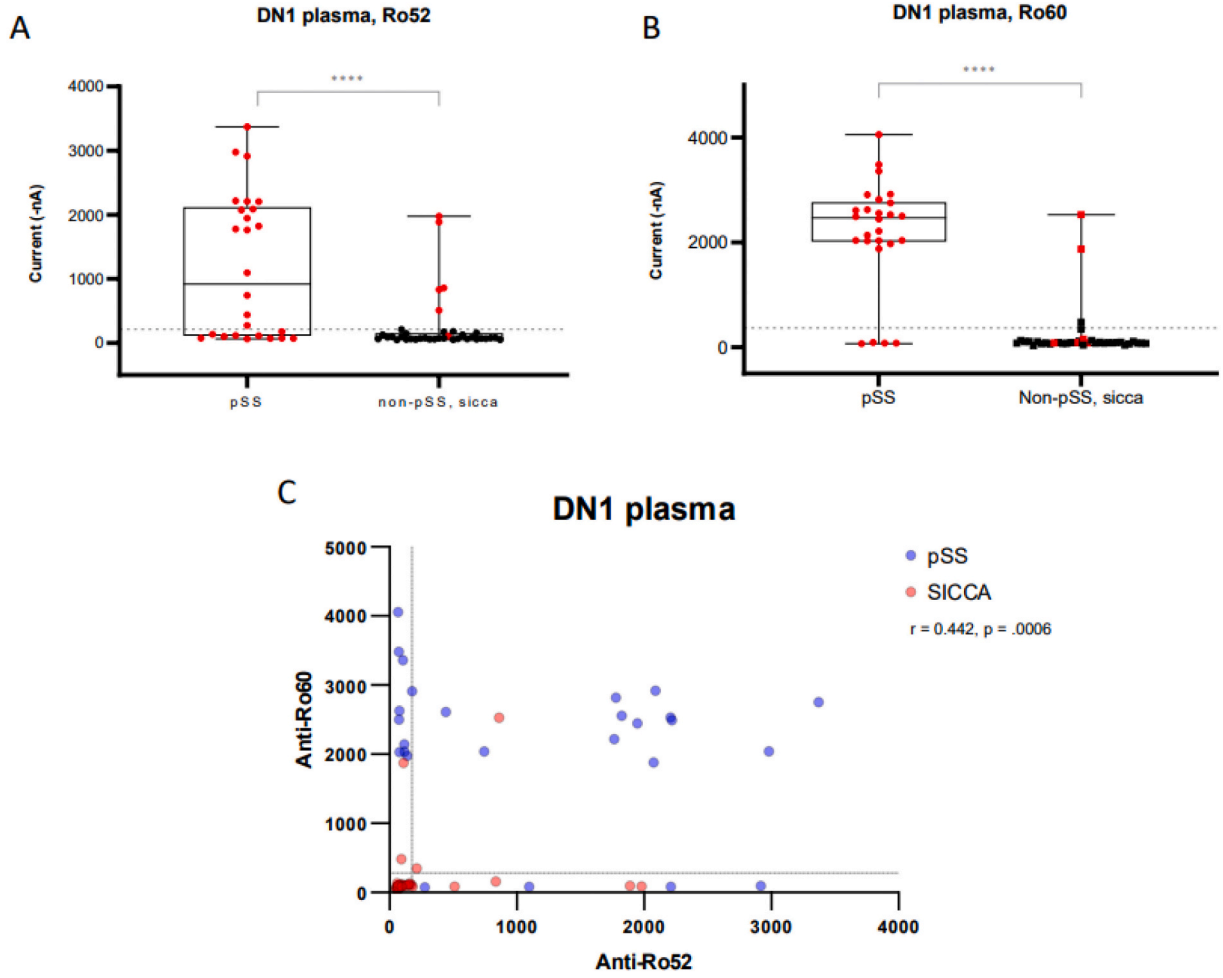

Fig. 1. EFIRM detection of anti-SSA/Ro autoantibodies in plasma of patients with pSS and non-pSS sicca in the DN1 cohort. A, B: Detection of anti-SSA/Ro52 and -Ro60 in plasma measured by EFIRM. The dashed line indicates the threshold for measurability of salivary anti-SSA/Ro-52 and -Ro60 antibodies. Serum positivity indicated by red dots and serum negativity indicated by black dots, measured by ELISA at diagnostic workup. Mann-Whitney test, **** $p \leq 0.0001$. C: Scatterplot of anti-SSA/Ro52 antibody (x-axis) and anti-SSA/Ro60 antibody (y-axis) with dashed lines on both axes indicating the threshold for each of the two autoantibodies. Spearman correlation, $r = 0.442$, $p = 0.0006$.

Table 2
Overview of positivity against anti-Ro52 and anti-Ro60 among patients positive for anti-SSA measured by EFIRM in both Danish cohorts (DN1 and DN2).

		$Ro52^+/Ro60^-$	$Ro52^-/Ro60^+$	$Ro52^+/Ro60^+$	Total
DN1 plasma	pSS	4	10	12	26
	non-pSS sicca	4	2	1	7
DN1 saliva	pSS	1	10	12	23
	non-pSS sicca	1	2	7	10
DN2 saliva	pSS	1	5	15	21
	non-pSS sicca	5	3	14	22

Values are given in number of patients.
$Ro52^+/Ro60^-$, monopositivity for anti-Ro52; $Ro52^-/Ro60^+$, monopositivity for anti-Ro60; $Ro52^+/Ro60^+$, positivity for both antibodies.

25) of the patients were serologically positive for anti-SSA/Ro measured by ELISA at the time of diagnostic work-up. Furthermore, 26 % (10/38) of the non-pSS sicca patients were seropositive for anti-SSA/Ro at diagnostic work-up, whereas 58 % (22/38) of the patients displayed positivity for salivary anti-SSA/Ro measured by EFIRM (Fig. 5 and Table 3).

Table 3
Summary comparing the levels of anti-SSA/Ro antibody positivity in saliva and serum in patients with pSS and non-pSS sicca in both Danish cohorts (DN1 and DN2).

Anti-SSA positivity	Saliva (EFIRM)	Plasma (ELISA)	Plasma (EFIRM)
DN1 (pSS)	96 % (23/24)	100 % (26/26)	100 % (26/26)
DN1 (non-pSS sicca)	29 % (10/35)	16 % (6/37)	19 % (7/37)
DN2 (pSS)	84 % (21/25)	80 % (20/25)	
DN2 (non-pSS sicca)	58 % (22/38)	26 % (10/38)	

Fig. 6 shows the discriminatory performance of anti-SSA/Ro52 and -Ro60 antibodies between groups in the DN2 cohort with ROC curves. Patients with pSS versus healthy controls yielded an AUC of 0.89 (95 % CI: 0.78–0.99), and an AUC of 0.74 (95 % CI: 0.61–0.84) for pSS versus non-pSS sicca.

4. Discussion

PSS is a complex disease that is often diagnosed many years after its symptom onset. Thus, there is an unmet need for non-invasive early diagnostic biomarkers with high discriminatory performance for early screening and to prevent delay in diagnosis.

We have previously shown that the novel EFIRM platform detected

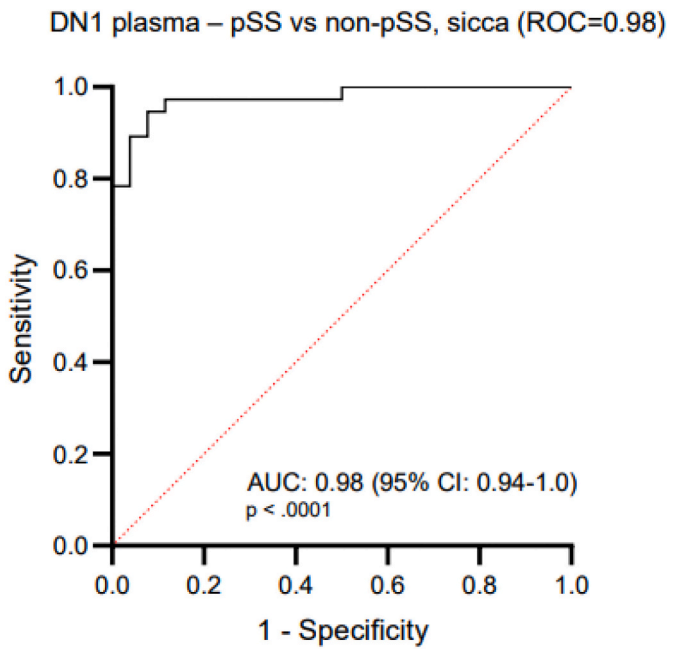

Fig. 2. Discriminatory performance of anti-SSA/Ro52 and -Ro60 EFIRM immunoassays on plasma in the DN1 cohort. ROC curves (AUC) with 95 % confidence interval in the pSS group versus non-pSS sicca group.

salivary anti-SSA/Ro52 in patients with pSS and non-pSS sicca patients, who were serologically negative for anti-SSA/Ro antibodies at diagnostic work-up measured by ELISA [18]. Inhibition studies confirmed the presence of anti-SSA/Ro52 in saliva from these patients [18]. In our previous exploratory study, we initially chose to develop the first assay to detect anti-SSA/Ro52, which has the highest predictive value in serum compared to anti-SSA/Ro60 [21]. In this study, we tested two independent cohorts to validate the salivary anti-SSA/Ro52 antibody EFIRM assay, and additionally optimized an assay for detection of anti-SSA/Ro60 antibody. Furthermore, both anti-SSA/Ro52 and -Ro60 EFIRM assays were optimized for plasma in the DN1 cohort to compare serum antibody levels measured by ELISA and EFIRM.

In the DN1 cohort, EFIRM detected salivary anti-SSA antibodies in 96 % (23/24) of the patients with pSS, while all of them were serologically positive at diagnostic work-up measured by ELISA. EFIRM also detected anti-SSA/Ro antibodies in plasma in 100 % (26/26) of the patients with pSS. Interestingly, the EFIRM assays demonstrated anti-SSA/Ro antibody positivity in saliva in 29 % (10/35) of the non-pSS sicca patients, and in plasma in 19 % (7/37) of these patients, whereas only 16 % (6/37) were serologically positive for anti-SSA/Ro measured by ELISA at diagnostic workup (Table 2). Moreover, EFIRM outperformed ELISA in the DN2 cohort as 84 % (21/25) of the patients with pSS and 58 % (22/38) of the non-pSS sicca patients were positive for salivary anti-SSA/Ro measured by EFIRM, while only 80 % and 26 % of the patients with pSS and non-pSS patients, respectively, were positive for anti-SSA/Ro antibodies measured by ELISA at diagnostic workup (Table 2). Thus, the data from these two validation cohorts support our previous findings with the presence of salivary anti-SSA/Ro antibodies in patients who are serologically negative for these autoantibodies, suggesting a local production of antibodies that might take place at the early stages of the disease. Previous studies have detected salivary anti-SSA antibodies in seropositive patients with pSS, but to our knowledge, the anti-SSA/Ro EFIRM assays are the first that have also detected salivary anti-SSA/Ro52 and -Ro60 in seronegative patients with pSS and non-pSS sicca patients [22–24].

The detection of anti-SSA/Ro antibodies in saliva in non-pSS sicca patients is consistent with the hypothesis that pSS develops from pathogenic inflammatory insults in the salivary glands, leading to the secretion of SSA autoantigens from damaged SGEC, and production of autoantibodies, anti-SSA/Ro, by local plasma cells. The presence of salivary anti-SSA/Ro antibodies in patients with non-pSS sicca suggests that a subset of non-pSS sicca patients are at early stages of the disease, and may eventually progress to develop pSS. A longitudinal study monitoring non-pSS sicca patients is needed to determine if salivary levels of anti-SSA/Ro antibodies can predict the progression of pSS disease. These capabilities will help identify sicca patients at risk of progressing to pSS for a therapeutic intervention to prevent or inhibit disease progression.

The mechanisms underlying the local production of salivary autoantibodies are currently unclear. One hypothesis is that SGEC are not silent bystanders, but actively participate in the pathogenesis by acting as antigen-presenting cells presenting SSA/Ro antigens [25]. It should also be noted that levels of serum anti-SSA/Ro antibodies at diagnostic work-up were measured using ELISA kits detecting anti-SSA/Ro (both Ro52 and Ro60), whereas the assay for detection of salivary anti-SSA/Ro on EFIRM targeted anti-SSA/Ro52 and anti-SSA/Ro60 separately. It has previously been reported that 20 % of patients who were serologically negative for anti-SSA/Ro antibodies were positive for either anti-SSA/Ro52 or -Ro60 when tested isolated [26]. It has also been suggested that anti-SSA/Ro52 and anti-SSA/Ro60 antibodies might be associated with different clinical phenotypes and should therefore be tested separately [27,28]. It is likely that the isolated detection of anti-Ro52 and -Ro60 antibodies in our study can explain why a large proportion of patients, both with pSS and non-pSS sicca, displayed positivity for anti-Ro52 and/or anti-Ro60 when measured by EFIRM despite being seronegative when tested by ELISA. These patients may represent different subtypes of pSS. In the present study, there were no significant differences in clinical nor paraclinical manifestations among the 3 subgroups ($Ro52^{+}/Ro60^{-}$ vs $Ro52^{-}/Ro60^{+}$ vs. $Ro52^{+}/Ro60^{+}$) in the 3 datasets, which may be ascribed to very few samples within each subgroup.

A limitation of this study is the small sample size in both cohorts. Future studies will include larger patient cohorts in multisite settings to validate both assays for the detection of anti-Ro52 and -Ro60 antibodies. Another limitation is that the anti-SSA/Ro EFIRM values in both plasma and saliva were measured separately for anti-Ro52 and -Ro60, while antibodies against the two proteins were not tested separately by ELISA. For future studies, we aim to test anti-Ro52 and -Ro60 separately on ELISA for better comparison between ELISA and EFIRM.

In conclusion, our findings suggest that salivary anti-SSA/Ro (anti-Ro52 and Ro-60) are potential discriminatory biomarkers for pSS that may aid in patient stratification. The detection of salivary anti-SSA/Ro antibodies in seronegative non-pSS sicca patients addresses the unmet clinical need of early detection of the disease.

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CRediT authorship contribution statement

Sarah Kamounah: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Fang Wei:** Conceptualization, Methodology, Validation, Writing – review & editing. **Jin Kyun Park:** Conceptualization, Formal analysis, Writing – review & editing. **Yeong-Wook Song:** Conceptualization, Writing – review & editing. **David Chia:** Conceptualization, Supervision, Validation, Writing – review & editing. **David T.W. Wong:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Anne Marie Lynge Pedersen:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

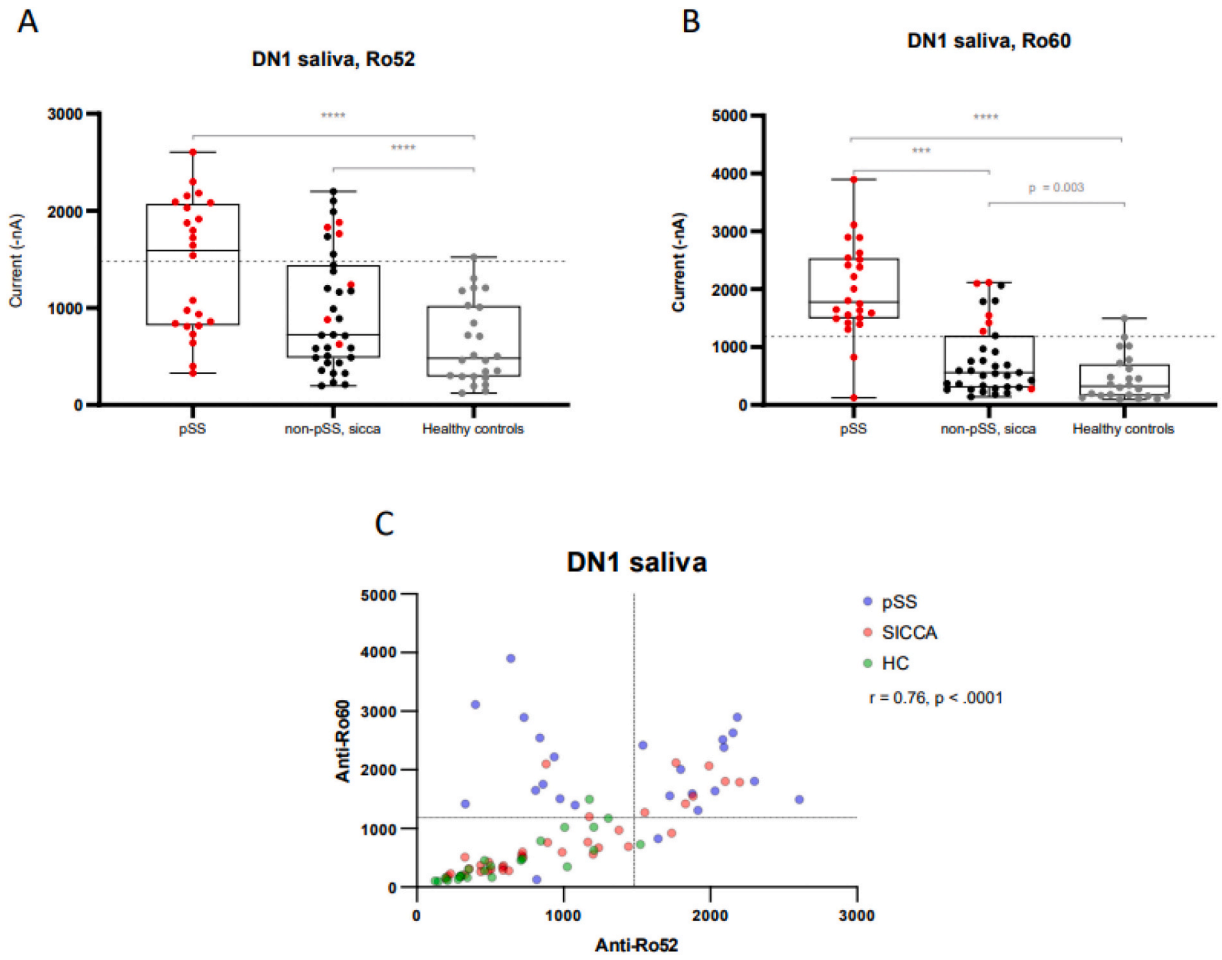

Fig. 3. EFIRM detection of SSA/Ro autoantibodies in saliva of patients with pSS, patients with non-pSS sicca, and healthy control subjects in the DN1 cohort. A, B: Detection of anti-SSA/Ro52 and -Ro60 antibodies in saliva measured by EFIRM. The dashed line indicates the threshold for measurability of salivary anti-SSA/Ro52 and -Ro60 antibodies. Serum positivity indicated by red dots and serum negativity indicated by black dots, measured by ELISA at diagnostic workup. Kruskal-Wallis test, Mann-Whitney, *** $p \leq 0.001$ **** $p \leq 0.0001$. C: Scatterplot of anti-SSA/Ro52 antibody (x-axis) and anti-SSA/Ro60 antibody (y-axis) with dashed lines on both axes indicating the threshold for each of the two autoantibodies. Spearman correlation, $r = 0.76$, $p < 0.0001$.

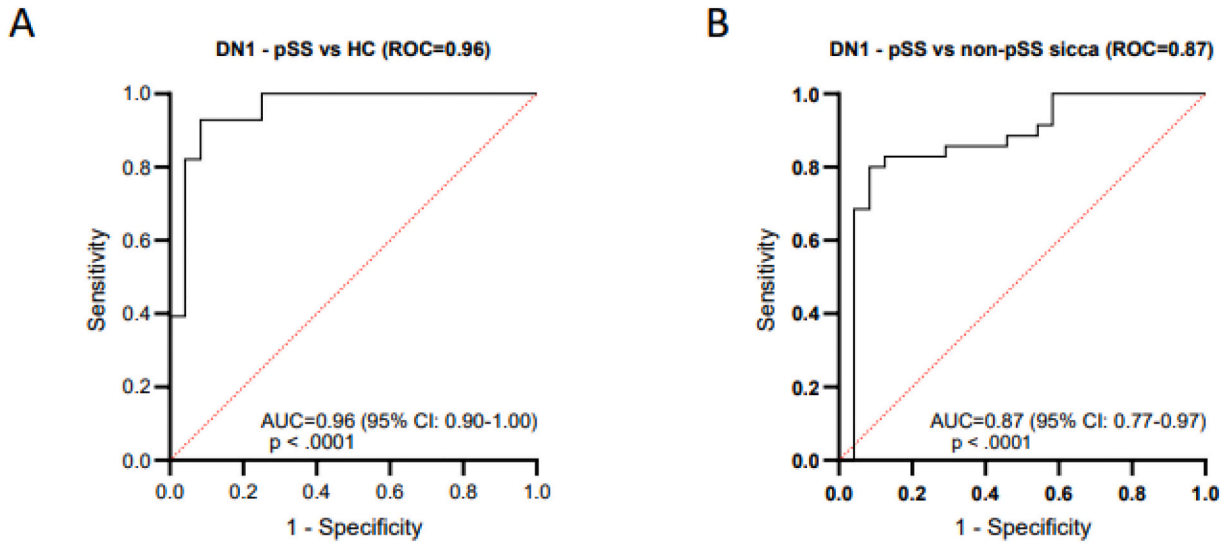

Fig. 4. Discriminatory performance of salivary anti-SSA/Ro52 and -Ro60 EFIRM immunoassays in the DN1 cohort. ROC curves (AUC) with 95 % confidence interval. A: pSS versus healthy controls (HC). B. pSS versus non-pSS sicca.

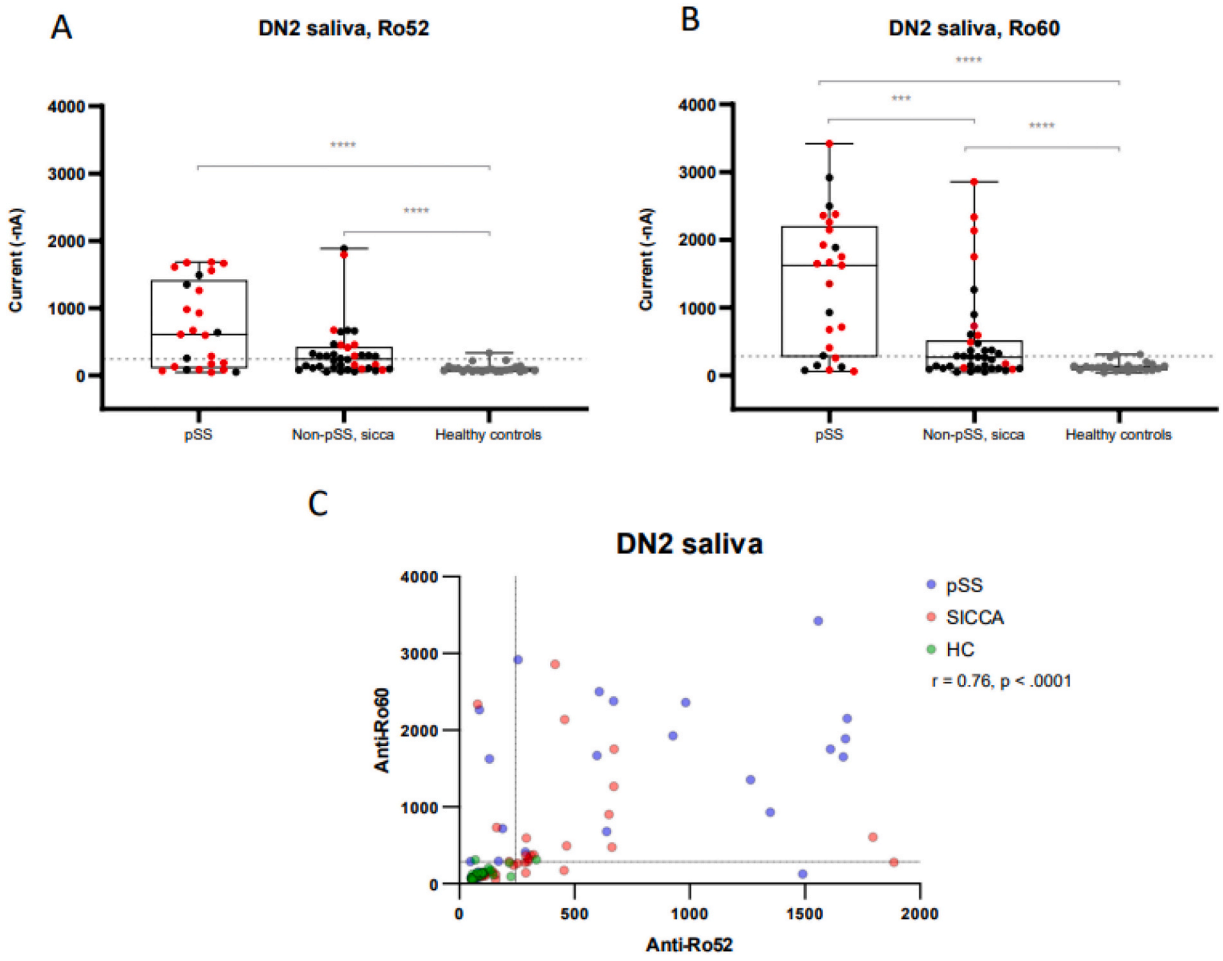

Fig. 5. EFIRM detection of SSA/Ro autoantibodies in saliva of patients with pSS, patients with non-pSS sicca, and healthy control subjects in the DN2 cohort. A, B: Detection of anti-SSA/Ro52 and -Ro60 antibodies in saliva by EFIRM. The dashed line indicates the threshold for measurability of salivary anti-SSA/Ro52 and -Ro60 antibodies. Serum positivity indicated by red dots and serum negativity indicated by black dots, measured by ELISA at diagnostic workup, and unknown serum status indicated by grey dots. Kruskal-Wallis test, Mann-Whitney, *** $p \leq 0.001$ **** $p \leq 0.0001$. C: Scatterplot of anti-SSA/Ro52 antibody (x-axis) and anti-SSA/Ro60 antibody (y-axis) with dashed lines on both axes indicating the threshold for each of the two autoantibodies. Spearman correlation, $r = 0.76$, $p < 0.0001$.

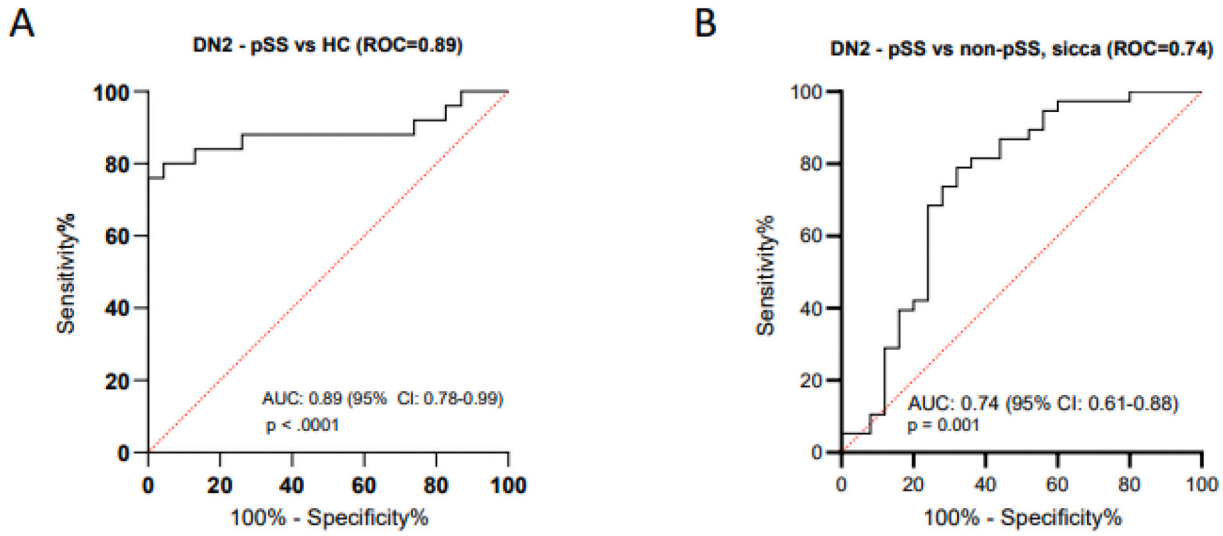

Fig. 6. Discriminatory performance of salivary anti-SSA/Ro52 and-Ro60 antibody EFIRM immunoassays in the DN2 cohort. ROC curves (AUC) with 95 % confidence interval. A: pSS versus healthy controls (HC). B. pSS versus non-pSS sicca.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: David T.W. Wong reports financial support was provided by National Institute of Dental and Craniofacial Research. David T.W. Wong reports financial support was provided by Sjogrens Syndrome Foundation. David T.W. Wong reports a relationship with GlaxoSmithKline that includes:. David T.W. Wong reports a relationship with Mars Wrigley Confectionery Ltd. that includes:. David T.W. Wong reports a relationship with Colgate Palmolive that includes:. David T.W. Wong reports a relationship with Liquid Diagnostics that includes:. Sarah Kamounah, Fang Wei, Anne Marie Lynge Pedersen, David Wong, David Chia, Yeong Wook Wong has patent pending to University of California Los Angeles, University of Copenhagen and Seoul National University. D.T.W.W. is consultant for GlaxoSmithKline, Mars Wrigley, and Colgate-Palmolive and has ownership in Liquid Diagnostics LLC. No other disclosures relevant to this article were reported. Other authors declare no conflicts of interest. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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References

[1] R.I. Fox, M. Stern, Sjögren's syndrome: mechanisms of pathogenesis involve interaction of immune and neurosecretory systems, Scand. J. Rheumatol. Suppl. 116 (2002) 3–13.
[2] P. Brito-Zerón, C. Baldini, H. Bootsma, S.J. Bowman, R. Jonsson, X. Mariette, K. Sivils, E. Theander, A. Tzioufas, M. Ramos-Casals, Sjögren syndrome, Nat. Rev. Dis. Primers 2 (2016 Jul 7) 16047.
[3] X. Mariettte, L.A. Criswell, Primary Sjögren's syndrome, N. Engl. J. Med. 378 (2018) 931–939.
[4] M.N. Manoussakis, E.K. Kapsogeorgou, The role of intrinsic epithelial activation in the pathogenesis of Sjögren's syndrome, J. Autoimmun. 35 (2010) 219–224.
[5] A. Alunno, F. Carubbi, O. Bistoni, S. Caterbi, E. Bartoloni, G. Mirabelli, F. Cannarile, P. Cipriani, R. Giacomelli, R. Gerli, T regulatory and T helper 17 cells in primary Sjögren's syndrome: facts and perspectives, Mediat. Inflamm. 2015 (2015) 243723.
[6] A.G. Tzioufas, E.K. Kapsogeorgou, H.M. Moutsopoulos, Pathogenesis of Sjögren's syndrome: what we know and what we should learn, J. Autoimmun. 39 (2012) 4–8.
[7] P. Youino, A. Saraux, J.O. Pers, B-lymphocytes govern the pathogenesis of Sjögren's syndrome, Curr. Pharm. Biotechnol. 13 (2012) 2071–2077.
[8] C. Vitali, S. Bombardieri, R. Jonsson, H.M. Moutsopoulos, E.L. Alexander, S. E. Carsons, et al., European study group on classification criteria for Sjögren's syndrome. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group, Ann. Rheum. Dis. 61 (2002) 554–558.
[9] C.H. Shiboski, S.C. Shiboski, R. Seror, L.A. Criswell, M. Labetoulle, T.M. Lietman, et al., International Sjögren's Syndrome Criteria Working Group. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts, Arthritis Rheum. 69 (2017) 35–45.
[10] S.C. Shiboski, C.H. Shiboski, L.A. Criswell, A.N. Baer, S. Challacombe, H. Lanfranchi, et al., American college of rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the SICCA cohort, Arthritis Care Res. 64 (4) (2012) 475–487.
[11] A. Fayyaz, B.T. Kurien, H. Scofield, Autoantibodies in Sjögren's Syndrome, Rheum. Dis. Clin. N. Am. 42 (2016) 419–434.
[12] R. Yoshimi, A. Ueda, K. Ozato, Y. Ishigatsubo, Clinical and pathological roles of Ro/SSA autoantibody system, Clin. Dev. Immunol. 60619512 (2012).
[13] M.L. Sembler-Møller, D. Belstrøm, H. Locht, A.M.L. Pedersen, Combined serum anti-SSA/Ro and salivary TRIM29 reveals promising high diagnostic accuracy in patients with primary Sjögren's syndrome, PLoS One 16 (10) (2021 Oct 8) e0258428.
[14] A. Robbins, M. Hentzien, S. Toquet, K. Didier, A. Servettaz, B.N. Pham, D. Giusti, Diagnostic utility of separate anti-Ro60 and anti-Ro52/TRIM21 antibody detection in autoimmune diseases, Front. Immunol. 10 (2019 Mar 12) 444.
[15] L. Deroo, H. Achten, K. De Boeck, E. Genbrugge, W. Bauters, D. Roels, F. Dochy, D. Creytens, J. Deprez, F. Van den Bosch, D. Elewaut, I. Peene, The value of separate detection of anti-Ro52, anti-Ro60 and anti-SSB/La reactivities in relation to diagnosis and phenotypes in primary Sjögren's syndrome, Clin. Exp. Rheumatol. 40 (12) (2022 Dec) 2310–2317.
[16] S. Retamozo, M. Akasbi, P. Brito-Zerón, X. Bosch, A. Bove, M. Perez-de-Lis, I. Jimenez, M.J. Soto-Cardenas, M. Gandia, C. Diaz-Lagares, O. Viñas, A. Siso, R. Perez-Alvarez, J. Yague, M. Ramos-Casals, Anti-Ro52 antibody testing influences the classification and clinical characterisation of primary Sjögren's syndrome, Clin. Exp. Rheumatol. 30 (5) (2012 Sep-Oct) 686–692.
[17] B. Armağan, S.A. Robinson, A. Bazoberry, J. Perin, T. Grader-Beck, E.K. Akpek, J. Kim, A.N. Baer, Antibodies to both Ro52 and Ro60 for identifying Sjögren's syndrome patients best suited for clinical trials of disease-modifying therapies, Arthritis Care Res. 74 (9) (2022 Sep) 1559–1565.
[18] S. Kamounah, N. Tayob, S. Chiang, F. Wei, J.K. Park, H.M. Kwon, Z. Feng, D. Chia, A.M.L. Pedersen, Y.W. Song, D.T.W. Wong, Immunoassay detects salivary anti-SSA/Ro-52 autoantibodies in seronegative patients with primary Sjögren's syndrome, Immunohorizons 7 (7) (2023 Jul 1) 554–561.
[19] A.M. Pedersen, J. Reibel, H. Nordgarden, H.O. Bergem, J.L. Jensen, B. Nauntofte, Primary Sjögren's syndrome: salivary gland function and clinical oral findings, Oral Dis. 5 (1999) 128–138.
[20] T.E. Daniels, Labial salivary gland biopsy in Sjögren's syndrome. Assessment as a diagnostic criterion in 362 suspected cases, Arthritis Rheum. 27 (2) (1984 Feb) 147–156.
[21] E. Theander, R. Jonsson, B. Sjöström, K. Brokstad, P. Olsson, G. Henriksson, Prediction of Sjögren's syndrome years before diagnosis and identification of patients with early onset and severe disease course by autoantibody profiling, Arthritis Rheum. 67 (2015) 2427–2436.
[22] E. Ben-Chetrit, R. Fischel, A. Rubinow, Anti-SSA/Ro and anti-SSB/La antibodies in serum and saliva of patients with Sjogren's syndrome, Clin. Rheumatol. 12 (4) (1993) 471–474.
[23] K.H. Ching, P.D. Burbelo, M. Gonzalez-Begne, M.E.P. Roberts, A. Coca, I. Sanz, M. J. Iadarola, Salivary anti-Ro60 and anti-Ro52 antibody profiles to diagnose Sjögren's syndrome, J. Dent. Res. 90 (2011) 445–449.
[24] A.K. Halse, M.C. Marthinussen, M. Wahren-Herlenius, R. Jonsson, Isotype distribution of anti-Ro/SS-A and anti-La/SS-B antibodies in plasma and saliva of patients with Sjögren's syndrome, Scand. J. Rheumatol. 29 (1) (2000) 13–19.
[25] P. Brito-Zerón, C. Baldini, H. Bootsma, S.J. Bowman, R. Jonsson, X. Mariette, et al., Sjögren syndrome, Nat. Rev. Dis. Primers 2 (2016) 16047.
[26] A. Mekinian, P. Nicaise-Roland, S. Chollet-Martin, O. Fain, B. Crestani, Anti-SSA Ro52/Ro60 antibody testing by immunodot could help the diagnosis of Sjögren's syndrome in the absence of anti-SSA/SSB antibodies by ELISA, Rheumatology (Oxford) 52 (2013) 2223–2228.
[27] R. Yoshimi, A. Ueda, K. Ozato, Y. Ishigatsubo, Clinical and pathological roles of Ro/SSA autoantibody system, Clin. Dev. Immunol. 606195 (2012).
[28] J. Schulte-Pelkum, M. Fritzler, M. Mahler, Latest update on the Ro/SS-A autoantibody system, Autoimmun. Rev. 8 (7) (2009) 632–637.

Update

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Corrigendum

Corrigendum to paper entitled "Seronegative patients with primary Sjogren's syndrome and non-pSS sicca test positive for anti-SSA/Ro52 and -Ro60 in saliva" [Published in: BBA – Mol. Basis Dis. Volume 1870, Issue 5, June 2024, 167168]

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The authors have regrettably found some errors in Table 1A and Table 1B.

In Table 1A, the question mark should be replaced with 36.

In Table 1B, 37 % should be replaced with 0 % (Focus score non-pSS sicca), 25 (68 %) should have been 11 (29%) (KCS non-pSS sicca) and (range) deleted.

Table 1A. Demographic and clinical characteristics of the patients with pSS and non-pSS sicca and health control subjects in the Danish cohort 1 (DN1).

	pSS (n = 26)	Non-pSS (n = 37)	P-value (pSS vs non-pSS)	Healthy controls (n = 24)
Age (years)	56 ± 11	55 ± 14	0.76	59 ± 12
Female	22 (92 %)	33 (90 %)	1.00	24 (100 %)
Current smoker	6 (23 %)	7 (19 %)	1.00	1 (4 %)
No. prescribed medication	1 (0–4)	1 (0–11)	1.00	0 (0 %)
Polypharmacy (≥5)	0 (0 %)	5 (14 %)	1.00.	0 (0 %)
Dry eyes for > 3 months	21 (81 %)	36 (97 %)	**<0.001**	0 (0 %)
Dry mouth for > 3 months	22 (85 %)	31 (84 %)	0.70	0 (0 %)
Hyposalivation (UWS ≤ 0.10 ml/min)	20 (77 %)	21 (57 %)	0.70	0 (0 %)
Keratoconjunctivitis sicca	21 (81 %)	18 (50 %)	**0.016**	N.A.
Positive serum anti-SSA/Ro antibody	26 (100 %)	5 (14 %)	**<0.001**	N.A.
Focus score ≥ 1	13 (50 %)	0 (0 %)	**<0.001**	N.A.
UWS (ml/min) Median	0.09 ± 0.06 0.04	0.12 ± 0.11 0.08	0.21	0.34 ± 0.19 0.33
SWS (ml/min) Median	0.67 ± 0.59 0.47	0.86 ± 0.52 0.79	0.20	1.83 ± 0.84 1.70

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(continued)

Table 1B Demographic and clinical characteristics of the patients with pSS and non-pSS sicca and healthy control subjects in the Danish cohort 2 (DN2).

	pSS (n = 25)	Non-pSS (n = 38)	P-value (pSS vs non-pSS)	Healthy controls (n = 23)
Age (years)	57 ± 14	56 ± 13	0.69	59 ± 14
Female	23 (92 %)	36 (95 %)	1.00	21 (91 %)
Current smoker	6 (24 %)	12 (32 %)	0.60	1 (4 %)
Dry eyes for > 3 months	22 (88 %)	32 (84 %)	1.00	0 (0 %)

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(*continued*)

	pSS (n = 25)	Non-pSS (n = 38)	P-value (pSS vs non-pSS)	Healthy controls (n = 23)
Dry mouth for > 3 months	21 (84 %)	32 (84 %)	0.73	0 (0 %)
Xerostomia Inventory score,	39 (16–53)	38 (11–55)	0.9	13 (11–18)
Hyposalivation (UWS ≤ 0.10 ml/min)	10 (40 %)	14 (37 %)	1.00	0 (0 %)
Keratoconjunctivitis sicca	22 (85 %)	11 (29 %)	**<0.001**	N.A.
Positive serum anti-SSA/Ro antibody	20 (80 %)	10 (26 %)	**<0.001**	N.A.
Focus score ≥ 1	18 (69 %)	0 (0 %)	**<0.001**	0 (0 %)
UWS (ml/min) Median	0.19 ± 0.16 0.18	0.20 ± 0.18 0.17	0.72	0.51 ± 0.34 0.41
SWS (ml/min) Median	1.19 ± 0.99 0.84	1.46 ± 0.98 1.00	0.18	2.33 ± 0.86 2.29

(*continued on next column*)

(*continued*)

Values are given in mean, SD, range, median, and in numbers of patients (%), N.A, not analyzed.

The authors would like to apologise for any inconvenience caused.

Sincerely,

Anne Marie Lynge Pedersen