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ORIGINAL RESEARCH

Distinctive profile of monomeric and polymeric anti-SSA/Ro52 immunoglobulin A1 isoforms in saliva of patients with primary Sjögren's syndrome and Sicca

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ABSTRACT

Objective Primary Sjögren's syndrome (pSS) is the second most common chronic autoimmune connective tissue disease. Autoantibodies, immunoglobulin (Ig) anti-SSA/Ro, in serum is a key diagnostic feature of pSS. Since pSS is a disease of the salivary gland, we investigated anti-SSA/Ro52 in saliva.

Methods Using a novel electrochemical detection platform, Electric Field-Induced Release and Measurement, we measured IgG/M/A, IgG, IgA, IgA isotypes (IgA1 and IgA2) and IgA1 subclasses (polymeric and monomeric IgA1) to anti-SSA/Ro52 in saliva supernatant of 34 pSS, 35 dry eyes and dry mouth (patients with Sicca) and 41 health controls.

Results Saliva IgG/M/A, IgG, IgA, IgA isotypes and IgA1 subclasses to anti-SSA/Ro52 differed significantly between pSS, non-pSS Sicca and healthy subjects. Elevated monomeric IgA1 was observed in patients with non-pSS Sicca while elevated polymeric IgA1 (pIgA1) was observed in patients with pSS. Salivary polymeric but not monomeric IgA1 (mIgA1) isoform correlated with focus score (r2=0.467, p=0.001)

Conclusions Salivary anti-Ro52 polymeric IgA1 isoform is associated with glandular inflammation in pSS, while salivary monomeric IgA1 is associated with Sicca. Whether IgA1 isotope switching plays a role in the progression of the Sicca to pSS warrants further investigation.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The role of anti-SSA/Ro52 in the pathogenesis of primary Sjögren's syndrome (pSS) is unclear.

WHAT THIS STUDY ADDS

⇒ Observed saliva with marked elevation of mIgA1 anti-SSA/Ro52 in patient with Sicca and marked elevation of pIgA1 in pSS.

⇒ Polymeric IgA1 and IgG anti-SSA/Ro52 correlate significantly to focus score.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Findings indicate that subclasses of IgA1 (monomeric and polymeric) may be used to discern pSS from Sicca.

⇒ IgA1 isomers can be a new approach to diagnose and monitor pSS.

⇒ Monomeric IgA1 anti-SSA/Ro52 may be a potential therapeutic agent for pSS.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a complex autoimmune disease that attacks the exocrine glands, particularly the lacrimal and salivary glands through lymphocytic infiltration, leading to symptoms of ocular and oral dryness, known as sicca symptoms.[1] It is the second most common autoimmune inflammatory rheumatic disease after rheumatoid arthritis.[2] Sicca symptoms in patients without pSS can arise from dysfunctional secretory glands in the absence of autoimmune features such as autoantibody production or lymphocytic infiltrates characteristic of pSS.[3] The production of serum IgG autoantibodies targeting SSA/Ro and lymphocytic infiltrates of salivary glands are key autoimmune features of pSS. A study examining paired serum and saliva antibody profiles in a cohort of 15 patients with SS revealed the presence of anti-SSA/Ro IgG and IgA in the saliva of patients,[4] may contribute to gland destruction in pSS.

IgA is abundant in mucosal secretions and serum. The two primary IgA subclasses, namely IgA1 and IgA2, exhibit distinct distribution

patterns; IgA1 dominates within the serum, while IgA2 predominates in secretions such as saliva.[5 6] IgA1 can further be subdivided into monomeric and polymeric isoforms. Human serum IgA1 mostly exists as monomeric form (85%–90%). Monomeric (m) and polymeric (p) IgA1 are structurally different due to the presence of galactose in the mIgA1. The presence of galactose in mIgA1 can be detected using the galactose-specific legume *lectin erythrina cristagalli* (ECL)[7] in contrast to its absence in the pIgA1.[5 6 8] Moreover, pIgA1 has a J chain, which is absent in the monomeric form.[5]

IgA receptor functions as a switch between immune activation or inhibition.[9 10] Monomeric IgA1 is a powerful anti-inflammatory effector[8 11] by binding to FcαRI, and will form ITAMi (Immunoreceptor Tyrosine-Based Activating Motif) by associating with FcRγ chain. On the other hand, when pIgA1 and/or circulating IgA-containing immune complexes bind to FcαRI and associate with FcRγ, they will form ITAM, which induce an activating signal for immune response. Thus, mIgA1 can inhibit immune response, while pIgA1 will activate immune response. As an example, IgA nephropathy is characterised by mesangial deposition of pIgA1 which may induce glomerular inflammation.[8] The lack of autoimmune disease associated with the above scheme is unfortunate.

Serum anti-SSA/Ro52 and -/60 antibody is the main diagnostic marker for pSS,[12 13] but they are two distinct antigens. Anti-Ro52 is more sensitive for pSS,while anti-Ro60 is more specific for pSS.[14] Overall, double anti-Ro52 and -Ro60 positive is more likely to be pSS, but no doubt Ro52 is an auto-antigen for pSS.

This study aimed to characterise an IgA auto-antibody anti-SSA/Ro52 subclasses and isoforms in saliva of patients with pSS and Sicca, alongside healthy controls and to investigate the association with these profiles and lymphocytic infiltrates of minor salivary glands.

MATERIALS AND METHODS

Patients

This prospective cohort included 34 patients fulfilling the American College of Rheumatology Classification Criteria for Sjögren's syndrome[15] and 35 patients who had sicca symptoms, but did not fulfil the classification criteria for pSS (designated Sicca). All patients were evaluated at the Rheumatology Clinic at Seoul National University. 41 age-matched and gender-matched healthy control subjects with no history of autoimmune disease were included as shown in table 1. The serum SSA/Ro was determined by Zeus ELISA SSA (Ro) Test System.

Saliva collection

Unstimulated whole saliva samples were collected for 15 min as previously described.[16] The samples were kept on ice and centrifuged immediately after collection at 2600 g for 15 min at 4°C. The supernatant was supplemented with 1 µL aprotinin (stock 10 mg/mL; Sigma-Aldrich Corp., St. Louis, Missouri, USA), 3 µL Na_3VO_4 (stock 400 mM; Fivephoton Biochemicals, San Diego, California, USA) and 10 µL phenylmethylsulfonyl fluoride (stock 10 mg/mL; Sigma-Aldrich Corp., St. Louis, Missouri, USA) and stored at −80°C until analysis. For analysis, the saliva samples were thawed and vortexed for 10 s.

Electric Field-Induced Release and Measurement (EFIRM) immunoassay

The EFIRM immunoassay was developed by Kamounah *et al*[17] to detect salivary anti-SSA/Ro52 autoantibodies using recombinant human SSA/Ro52 polymerised onto the gold surface of EFIRM electrodes (figure 1). The 52 kDa SSA subunit was chosen as an antigen target to capture salivary anti-SSA/Ro52 autoantibodies because anti-SSA/Ro52 has the highest positive predictive value (100%) compared with that of anti-SSA/Ro60 (25%) in serum.[18] Assay for detection of salivary SSA/Ro52 autoantibodies was optimised using human anti-SSA/Ro52 (Lifespan Biosciences, Seattle, Washington, USA) to generate an optimal calibration curve. The following secondary antibodies were used for each isotype detection: IgG/M/A (H+L) goat anti-human biotin (Invitrogen, Carlsbad, California, USA), biotinylated polyclonal anti-human IgG (H+L) (Thermo Fisher Scientific, Waltham, Massachusetts, USA), biotin anti-human IgA rabbit monoclonal antibody (RevMab Biosciences, South San Francisco, California, USA), rabbit anti-human IgA1 recombinant secondary antibody, biotin (Thermo Fisher Scientific, Waltham, Massachusetts, USA), rabbit anti-human IgA2 recombinant secondary antibody, biotin (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and ECL biotinylated (Vector Laboratories, Newark, California, USA). Polymeric IgA1 was determined by subtracting measurements of ECL from that of IgA1.

Clinical samples (n=110) were blinded and assayed in a randomisation design to ensure that saliva samples from patients with pSS, patients with Sicca and healthy control subjects were evenly distributed across the experimental runs. The experiments were run in duplicate, and the result was obtained by taking the geometric mean across the duplicates.

Statistical analysis

Patient demographic characteristics of pSS and Sicca and study variables were summarised using mean (±SD) for continuous variables or frequency. Level of salivary immunoglobulins (IgG/M/A, IgG, IgA, IgA1 and IgA2) in patients with pSS and Sicca and health controls were assessed via Wilcoxon t-test. The discriminatory performance of anti-SSA/Ro52 autoantibodies measured in saliva was assessed using the area under the receiver operating characteristic/area under the curves (ROC/AUC). The associated 95% CI was constructed using DeLong's method to estimate the variance.[19] In order to propose an exploratory multivariable model for discriminating Sicca from patients with pSS, Classification and Regression Tree (CART) Model[20] was implemented by

Table 1 Demographic characteristics of patients with pSS and patients with Sicca

	pSS (n=34)	Sicca (n=35)	P value
Age (years)	54.0±10.5	57.8±14.5	0.207
Female	34 (100.0%)	31 (88.6%)	0.116
Symptom duration (months)	46.1±53.5	47.0±83.4	0.960
Ocular dryness	28/33 (84.8%)	32 (91.4%)	0.471
Oral dryness	29/33 (87.9%)	29/33 (87.9%)	1.000
Unstimulated whole saliva flow rate ≤0.10 mL/min	6/9 (66.7%)	–	–
Labial salivary gland focus score ≥1	22/27 (81.5%)	1/29 (3.4%)	<0.001
Keratoconjunctivitis sicca	31 (91.2%)	21 (60.0%)	0.005
Positive ANA	30 (88.2%)	12 (34.3%)	<0.001
Positive serum SSA/Ro autoantibody	29 (85.3%)	0 (0%)	<0.001
ESR, mm/hour	31.0 (18.0–47.5)	15.0 (9.0–23.0)	0.003
Rheumatoid factor	20/33 (60.6%)	5 (14.3%)	<0.001
Extra-glandular manifestations			
Rash	6 (17.6%)	2 (5.7%)	0.241
Raynaud	4 (11.8%)	1 (2.9%)	0.336
Alopecia	2 (5.9%)	2 (5.7%)	1.000
Shortness of breath	2 (5.9%)	1 (2.9%)	0.980
Tingling/neuropathy	2 (5.9%)	1 (2.9%)	0.980
Leucopenia	3 (8.8%)	0 (0)	0.228
Treatment			
Hydroxychloroquine	24 (70.6%)	2 (5.7%)	0.000
Methotrexate	1 (2.9%)	0 (0%)	0.988
Pilocarpine	11 (32.4%)	4 (11.4%)	0.070
NSAIDs	4 (11.8%)	12 (34.35)	0.054

Values are presented as mean±SD, median [IQR] or numbers (%). Statistical significance was defined as p value <0.05.
ESR erythrocyte sedimentation rate; NSAIDs nonsteroidal anti-inflammatory drugs
ANA, antinuclear antibody; pSS, primary Sjögren's syndrome.

inputting six potential markers as potential predicators (IgG, IgA, IgA1, IgA2, mIgA1 and pIgA1). Distribution of IgA1 subclasses in patients with Sicca and pSS were visualised using scatter plot of IgA1 (y-axis) and mIgA1 (x-axis) with cut-off values from the CART model. To assess the correlation between immunoglobulin isotypes and focus score, Pearson correlation coefficients were computed. Z-scores were computed by obtaining the overall mean for each marker (mIgA1 and pIgA1) and dividing by their respective SD. P values <0.05 were considered statistically

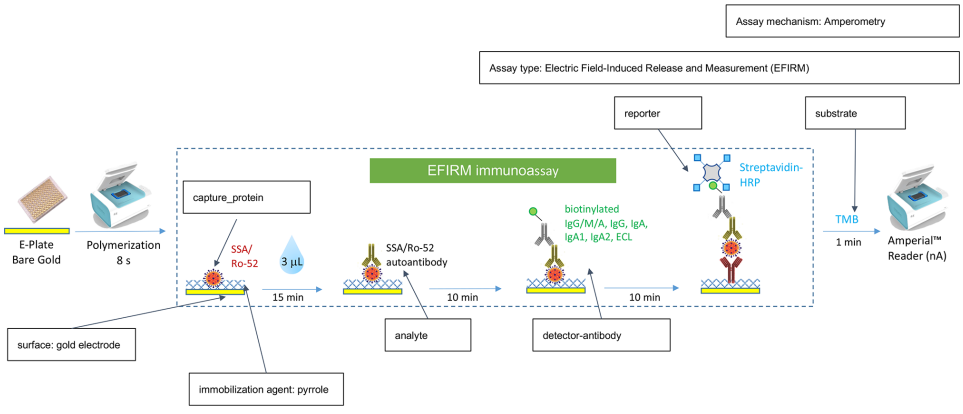

Figure 1 Schematic of the EFIRM saliva anti-SSA/Ro52 immunoassay. EFIRM, Electric Field-Induced Release and Measurement.

significant. Statistical analyses were run using Wilcoxon t-test and CART models were constructed using R V.4.1.0 (www.r-project.org, Vienna, Austria).

RESULTS

Baseline characteristics of patients

34 patients with pSS and 35 patients with Sicca were enrolled, as shown in table 1. The study population was predominantly women (94%), and the mean age (± SD) was 54.6±10.5 years in the pSS group and 57.3±14.5 years in the Sicca group. The distribution of symptoms of oral and ocular dryness did not differ between the groups. More patients with pSS had a detectable antinuclear antibody titre than did the patients with Sicca (88.2% vs 34.3%, p<0.001); Rheumatoid Factor (RF) (60.6% vs 14.3%, p<0.001); erythrocyte sedimentation rate (ESR) (31 vs 15 p<0.003), which is similar to other study.[21] Serum SSA/Ro52 and SSB/La autoantibodies were present in 85.3% (29/34) and 64.7% (22/34) of the patients with pSS, respectively, whereas none of the patients with Sicca had detectable serum SSA/Ro52 or SSB/La autoantibodies. Histological analysis of the labial salivary gland biopsies from the patients with Sicca did not reveal Sjögren's-specific histopathological changes (ie, focus score <1) .[22] Focus score ≥1 was present in 22 (81.5%) of 27 patients with pSS. Keratoconjunctivitis sicca,[23] defined as positive Schirmer test (≤5 mm of strip is wet after 5 min) and/or ocular staining score ≥5 or van Bijsterveld score ≥4, in at least one eye, was more common in the patients with pSS than the patients with Sicca (91.2% vs 60.0%, p=0.005). None of the gland and extra-glandular manifestations were significant between the two groups.

Salivary anti-SSA/Ro52 autoantibody as a biomarker to discriminate patients with pSS, patients with Sicca and healthy controls

We explored the potential of each salivary immunoglobulin isotypes to SSA/Ro52 autoantibody as a biomarker to differentiate patients with pSS, patients with Sicca and healthy control subjects. First, levels of anti-SSA/Ro52 isotypes IgG/M/A, IgG, IgA, IgA1 or IgA2 were highest in pSS, followed by patients with Sicca and health controls, with the exception of IgA, and IgA2 which were not significantly different between pSS, and Sicca (figure 2).

Second, the performance of IgG/M/A assay to discriminate pSS, Sicca and healthy controls from each other was evaluated by the area under the ROC curve (AUC) analyses: pSS versus Sicca, 0.81 (95% CI: 0.7070 to 0.9141) (figure 3A); Sicca versus control, 0.88 (95% CI: 0.8111 to 0.9547) (figure 3B); pSS versus control, 0.98 (95% CI: 0.9595 to 1.0) (figure 3C) and combined pSS and Sicca versus control, 0.93 (95% CI: 0.8906 to 0.9751) (figure 3D). IgG/M/A assay can distinguish between pSS, Sicca and control groups.

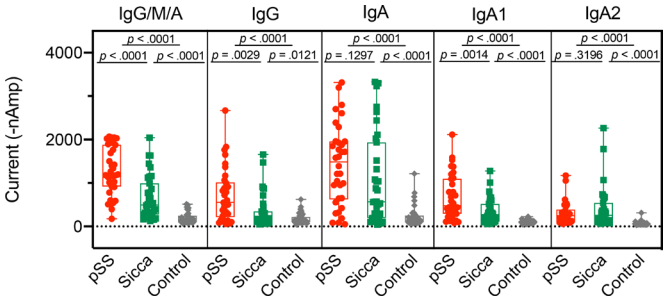

Figure 2 Performance of salivary isotypes to SSA/Ro52 autoantibody to discriminate pSS, Sicca and healthy control subjects. Groups were compared by Wilcoxon t-test. P value <0.05 indicates statistical significance. pSS, primary Sjögren's syndrome.

CART model of IgA SSA/Ro52 isotypes as potential predictors to discriminate patients with Sicca from patients with pSS

CART model that selects variables and cut-points by using recursive partitioning to discriminate healthy, pSS and Sicca groups by combining six predicators (IgG, IgA, IgA1, IgA2, mIgA1 and pIgA1) (figure 4). The overall accuracy of the cart model is 78.2% with sensitivity of 82.4% (28/34) for predicting pSS and 71.43% (25/35) for predicting Sicca. The specificity for neither pSS nor Sicca is 80.9% (33/41).

Distribution of subclasses of anti-SSA/Ro52 IgA1 (mIgA1 and pIgA1) in patients with Sicca and patients with pSS

To visualise the distribution of subclasses of IgA1, we constructed a scatter plot of two variables, mIgA1 and pIgA1, with color-coded points by Sicca and pSS groups (figure 5). To select potential cut-points in a data driven way to best separate groups, we ran a CART. The CART model selected a cut-point at 339 pIgA1 and 191 mIgA1 which split the data into three distinct clusters (figure 5). Elevated mIgA1 was observed in patients with Sicca while elevated pIgA1 was observed in patients with pSS. The classification accuracy of group status across these clusters was 80% indicating that there is an association between these markers (mIgA1 vs pIgA1) and Sicca versus pSS. This could be further demonstrated in figure 6, where Z score for pIgA1, and mIgA1 were plotted for patients with pSS, Sicca and control. It was found that pIgA1 was significantly different between pSS and Sicca (p<0.001), and mIgA1 was different in Sicca, and pSS (p<0.023). It

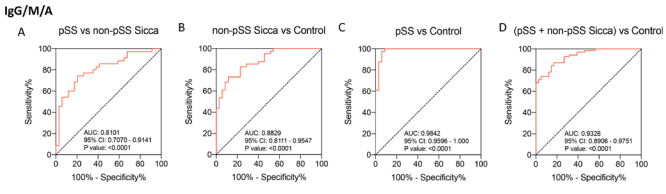

Figure 3 Performance of salivary isotypes to SSA/Ro52 autoantibody to discriminate pSS, Sicca and healthy control subjects was determined by area under the ROC curves (AUC) with 95% CI. pSS, primary Sjögren's syndrome; ROC, receiver operating characteristic.

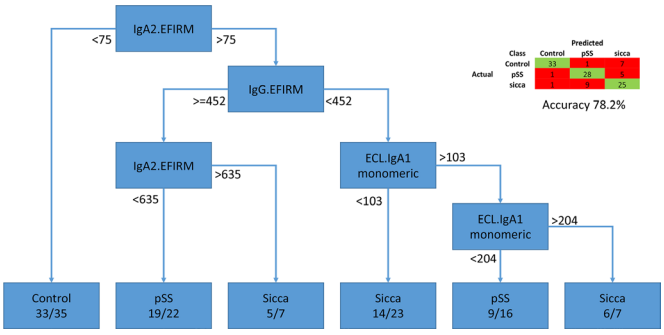

Figure 4 Classification and Regression Tree model developed to predict group status (healthy control, pSS and Sicca) from six potential predicators: IgG, IgA, IgA1, IgA2, monomeric IgA1 and polymeric IgA1. Cut-points generated from the CART model are presented in this figure. The terminal nodes (bottom boxes) show the predicted group as well as the classification rate. The correct classification rate overall was 86/110=78.2%. pSS, primary Sjögren's syndrome.

clearly demonstrated the prevalence of mIgA1 in Sicca, and pIgA1 in pSS.

Pearson correlation coefficient between immunoglobulin isotypes and focus score

Histological analysis of the labial salivary gland biopsies allowed the calculation of focus scores, determined by the number of mononuclear cell infiltrates containing at least 50 inflammatory cells in a 4 mm2 glandular section.[22] To determine the correlation of histopathological changes (ie, focus score >1) and immunoglobulin isotypes to SSA/Ro52, Pearson correlation coefficient model was used. The Pearson correlation to focus score is 0.467, p<0.001 for polymeric IgA1; 0.358 for IgG, p<0.004; 0.345

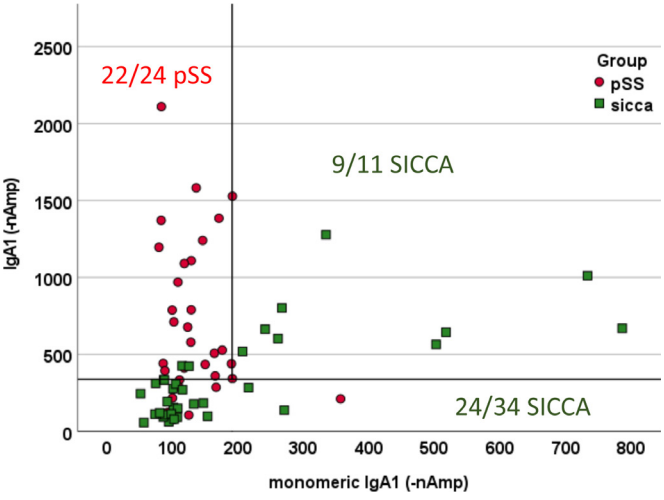

Figure 5 Two variables scatter plot of mIgA1 and pIgA1 (-nAmp) with color-coded points by group. The CART model selected potential cut-point at 339 pIgA1 and 191 mIgA1 which split the data into three distinct clusters. The classification accuracy of group status across these clusters was 80% indicating that there is an association between these markers and group. CART, Classification and Regression Tree.

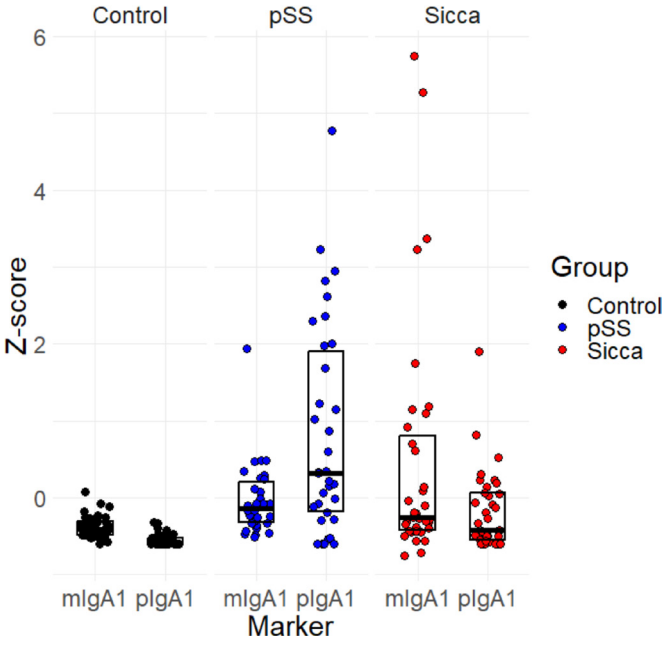

Figure 6 Plotting Z score for pIgA1, and mIgA1 for pSS, Sicca, where Z scores were computed by obtaining the overall mean for each marker (mIgA1 and pIgA1) and dividing by their respective SD.

for IgA1, p<0.010; while mIgA1 is not correlated with focus score (table 2). However, since all three of these biomarkers are also correlated with each other, they may not be providing independently significant information for predicting the degree of focal lymphocytic infiltration (foci) in the labial salivary gland tissue in a multivariable model. Also of interest, salivary IgG anti-SSA/Ro52 does not correlate with mIgA1 anti-SSA/Ro52 with a Pearson correlation of 0.089 and p<0.466, but correlate with pIgA1 at 0.589 and p<0.001.

DISCUSSION

The precise role of SSA/Ro in the pathogenesis of pSS remains elusive. Anti-SSA/Ro52 antibodies were also detected in saliva of patients with pSS,[5] yet, isotypes and subclasses of Pearson correlation in saliva have not been well characterised due to limitation in detection assay. Using the novel highly sensitive EFIRM assay, this study showed that anti-SSA/Ro52 antibodies were present not only in patients with pSS but also patients with Sicca. In saliva of healthy controls, anti-SSA/Ro52 Ig levels were consistently low. Strikingly, the distribution of IgA subclasses was disease-specific; while IgA2 levels did not exhibit any discernible difference between the patients with pSS and patients with Sicca, the polymeric IgA1 was strongly associated with pSS and correlated with lymphocytic infiltration of salivary glands.

Functional diversity of antibodies is determined by their structural features, including classes, subclasses and isotypes. Monomeric IgA1 in serum suppresses inflammatory response by binding to inhibitory FcαRI receptor, whereas pIgA1 induces inflammatory response.[11] A

Table 2 Pearson correlation coefficients between immunoglobulin isotypes and focus score pathogenesis

		Focus score -ID	IgA1 EFIRM	IgA2 EFIRM	IgA EFIRM	IgG EFIRM	Monomeric IgA1	Polymeric IgA1
Focus Score -ID	Pearson	1	0.345**	−0.076	0.192	0.358**	−0.177	0.467**
	P value		0.010	0.580	0.159	0.007	0.196	0.000
	N	55	55	55	55	55	55	55
IgA1 EFIRM	Pearson	0.345**	1	0.467**	0.800**	0.682**	0.338**	0.708**
	P value	0.010		0.000	0.000	0.000	0.004	0.000
	N	55	69	69	69	69	69	69
IgA2 EFIRM	Pearson	−0.076	0.467**	1	0.711**	0.235	0.641**	−0.032
	P value	0.580	0.000		0.000	0.052	0.000	0.797
	N	55	69	69	69	69	69	69
IgA EFIRM	Pearson	0.192	0.800**	0.711**	1	0.569**	0.472**	0.415**
	P value	0.159	0.000	0.000		0.000	0.000	0.000
	N	55	69	69	69	69	69	69
IgG EFIRM	Pearson	0.358**	0.682**	0.235	0.569**	1	0.089	0.589**
	P value	0.007	0.000	0.052	0.000		0.466	0.000
	N	55	69	69	69	69	69	69
Monomeric IgA1	Pearson	−0.177	0.338**	0.641**	0.472**	0.089	1	−0.425**
	P value	0.196	0.004	0.000	0.000	0.466		0.000
	N	55	69	69	69	69	69	69
Polymeric IgA1	Pearson	0.467**	0.708**	−0.032	0.415**	0.589**	−0.425**	1
	P value	0.000	0.000	0.797	0.000	0.000	0.000	
	N	55	69	69	69	69	69	69

Three isotypes were significantly associated with focus score (IgA1, IgG and polymeric IgA1). Statistical significance was achieved if the p value was <0.05, *, two-tailed and <0.01, **, two-tailed.
EFIRM, Electric Field-Induced Release and Measurement.

previous study by Delacroix *et al* demonstrated increased levels of total IgA1 and pIgA1 in the serum of patients with pSS.[24] In this current study, a marked elevation of pIgA1 against SSA/Ro52 were preferentially detected in the saliva of patients with pSS, while mIgA1 targeting anti-SSA/Ro52 dominated in the saliva of patients with Sicca (figures 5 and 6). In addition, the pIgA1 levels had a strong correlation with the focus score (ie, degree of glandular inflammation) among patients with pSS (table 2), suggesting that polymeric isoform of IgA1 is tightly associated with salivary gland dysfunction and inflammation in pSS. Generation of proinflammatory pIgA1 might signify the transition from non-inflammatory Sicca to inflammatory pSS. Conversely, mIgA1 is associated with salivary dysfunction devoid of overt inflammation in patients with Sicca. Indeed, anti-SSA/Ro52 IgA1 isoform, whether monomeric or polymeric, was able to differentiate between patients with Sicca and patients with pSS. As the production pIgA1 is mediated by post-translational glycosylation, (aberrant) glycosylation of IgA1 might be an additional pathogenic hit in pSS.[25] It might be tempting to postulate that production of mIgA1 is associated with glandular dysfunction in sicca while additional generation of pIgA1 marks the transition to inflammatory pSS, as indicated by increase of IgG anti-SSA/Ro52. Furthermore, the lack of correlation between mIgA1 and salivary IgG may indicate an immune inhibition by ITAMi.

Labial salivary gland biopsy is the only reliable test (ie, gold standard) to detect glandular inflammation and, therefore, critical in diagnosis of pSS. Although this procedure is minimally invasive, it is still time consuming and uncomfortable.[26] The utilisation of EFIRM for detecting salivary anti-SSA/Ro52 antibodies, particularly pIgA1 isoform, might be a simple, non-invasive alternative to a gland biopsy, once validated in a larger prospective cohort.

This study is not exempt from limitations. First, temporal association between the antibody profiles and development of pSS remains elusive. Whether an emergency of pIgA1 in patients with Sicca predicts progression into pSS needs to be investigated in a longitudinal cohort. Second, the origin of the salivary antibodies, whether locally produced within salivary glands or introduced from systemic circulation and secreted into saliva, remains unclear. The potentially pathogenic role of an auto-antibody SSA/Ro60 IgA1 of its monomeric versus polymeric form can mediate the immune degeneration of salivary gland in pSS needs further investigation. The

information on germinal formation was not collected for this study and will be included in future studies. The role of ITAM activation by pIgA1 need to be further validated by examine its downstream gene activation (eg, cytokines, B-cell activating factor (BAFF) and so on).

In conclusion, profile of salivary anti-SSA/Ro52 antibodies is associated with glandular inflammation in patients with Sicca and patients with pSS and is useful to distinguish between patients with Sicca from patients with pSS. Further studies are required to validate current finding in a larger cohort.

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