Journal of Autoimmunity 100 (2019) 114–119

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Soluble siglec-5 is a novel salivary biomarker for primary Sjogren's syndrome

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ARTICLE INFO

Keywords:
Siglec
Saliva
Biomarkers
Sjogren's syndrome

ABSTRACT

Despite advances in the understanding of the pathogenesis, disease-specific biomarkers have not been included in the classification criteria for Primary Sjogren's syndrome (pSS). Based on a microarray of peripheral blood mononuclear cells (PBMCs) from patients with primary Sjogren's syndrome (pSS), we aimed to investigate whether soluble sialic acid-binding immunoglobulin-like lectin (siglec)-5 in saliva might be a biomarker for pSS. The concentration of siglec-5 in saliva and sera was determined by ELISA. Clinical parameters related with pSS were obtained from pSS registry and correlation with salivary siglec-5 level was evaluated. Receiver operating curve (ROC) analysis was performed to determine cut off value. A separate validation cohort consisted of subjects with suspicious pSS was evaluated to determine the performance. The level of salivary siglec-5 was significantly higher in pSS patients (n = 170) compared with HCs (n = 25), non SS sicca patients (n = 78) or patients with systemic lupus erythematosus (SLE) (n = 43) (1346.8 [202.8–4280.0] pg/mL, 6.08 [0–134.0] pg/mL, 195 [0–947.5] pg/mL, and 0 [0–238.7] pg/mL, median [interquartile range], $P < 0.001$). Salivary siglec-5 level negatively correlated with salivary flow rate (spearman's rho: −0.420, $P < 0.001$), and positively correlated with ocular surface score (rho: 0.331, $P < 0.001$) and serum immunoglobulin G level (rho = 0.202, $P = 0.008$). In ROC analysis, area under the curve was 0.774[0.724–0.826]. With a cut off value of 400 pg/mL, sensitivity and specificity was 0.69 and 0.70 respectively. In validation cohort (45 pSS patients and 45 non SS sicca patients), sensitivity and specificity of siglec-5 was 64.4% and 77.8%, respectively. In conclusion, the level of soluble siglec-5 is significantly higher in the saliva from pSS patients, which reflects the severity of hyposalivation and ocular surface damage. This novel salivary biomarker may provide benefits for pSS diagnosis.

1. Introduction

Primary Sjogren's syndrome (pSS) is a systemic autoimmune disease that is characterized by lymphocytic infiltration in the exocrine glands, which results in oral and ocular dryness for the affected patients. Aberrant B-cell hyperactivity, presenting as autoantibody production augmented by T-cell activity and activation of interferon (IFN) pathways, is thought to be the immunologic pathogenic mechanism. Therefore, this mechanism involves both innate and adaptive immunity [1]. Unfortunately, current treatment strategies are focused on alleviating symptoms, rather than providing a targeted therapy.

A pSS diagnosis is generally based on sets of classification criteria [2–4]. The most recent 2016 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria [4] include five objective items that are each weighed differently, with higher weights given to positive autoantibody (*anti*-Ro) and positive biopsy results (focus score ≥1), three for each test. Other items that score one include an ocular staining score (OSS) of ≥5 (or van

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https://doi.org/10.1016/j.jaut.2019.03.008
Received 1 February 2019; Received in revised form 8 March 2019; Accepted 11 March 2019
Available online 25 March 2019
0896-8411/

Bijsterveld score [VBS] ≥4), a Schirmer's test score of ≤5 mm/5 min, and an unstimulated salivary flow rate (uSFR) ≤0.1 mL/min. A patient with a total score ≥4 is considered to have pSS. In the new criteria, a minor lip biopsy is critical especially in seronegative (*anti*-Ro (−)) subjects and should be performed more frequently. Moreover, accurate measurements of the uSFR and OSS are not readily available in the typical clinical setting. Therefore, there is a growing need for diagnostic biomarkers that can be readily measured in easily obtained specimens, such as tears or saliva. In particular, saliva is drawing attention as a biospecimen for diagnostic tests because it is easy to obtain without invasive methods. Moreover, saliva appears to reflect the local environment of secretory glands, which are the target organ of pSS. Therefore, many researchers have been searching for pSS biomarkers in saliva. Several composites of molecules were identified with proteomic approaches [5,6]. Indeed, potential single biomarkers, including cystatin S [7], α-enolase [8], and β2-microglobulin, [9,10] were suggested. However, none of these have yet been clinically validated as a diagnostic marker.

We have endeavoured to identify molecules that can be both diagnostic markers and therapeutic targets in pSS. We previously performed microarray analysis of peripheral blood mononuclear cells (PBMCs) from 26 patients with pSS and 10 healthy controls (HCs) to screen for potential pSS biomarkers [11]. Among various differentially expressed genes, we observed that the expression of sialic acid-binding immunoglobulin (Ig) like lectin (siglec) −5 was globally elevated in pSS patients.

Siglecs include a broad range of cell-surface transmembrane receptors that contain 2–17 extracellular Ig domains and are found on the surface of immune cells [12]. The extracellular Ig domains contain the sialic acid-binding site; upon ligand binding, the cytoplasmic domains recruit tyrosine phosphatases to modulate the function of immune cells [13]. Silgec-5 is expressed in a myeloid-restricted manner on the surface of neutrophils, monocytes, basophils, mast cells, and macrophages. Its cytoplasmic tail bears immunoreceptor tyrosine-based inhibitory motifs (ITIM). During group B streptococcal infection and upon ligation by β-protein, siglec-5 suppresses the neutrophil-killing function, while its paired receptor siglec-14 activates the immune response via the immunoreceptor tyrosine-based activation motif (ITAM) [14]. However, the functions of siglec-5 in autoimmune disease have not been investigated.

Based on our microarray data, we measured the concentrations of potential biomarkers in saliva and found that siglec-5 was expressed at significantly higher levels in the saliva of pSS patients than in controls. In this study, based on this previous observation and in order to determine the value of siglec-5 as a diagnostic biomarker, we investigated correlations between the clinical parameters of pSS with levels of siglec-5 in saliva.

2. Methods

2.1. Study population

2.1.1. Korean initiative of Sjogren's syndrome (KISS) cohort

The majority of the enrolled patients with pSS in this study were KISS participants. The KISS was founded in 2013 to establish a nationwide prospective cohort that provides clinical data and samples from patients with pSS for developing diagnostic and treatment tools. Informed consents were obtained from all patients according to the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC13ONMI0646). All data were collected and managed using the Clinical Research and Trial Management System (Korea National Institutes of Health, Korea Centers for Disease Control and Prevention). Recruitment began in October 2013 in Seoul St. Mary's Hospital, a tertiary care university hospital and referral center in Seoul, Korea. We enrolled patients who fulfilled either the 2002 American Europe Consensus Group criteria for pSS or the 2012 American College of Rheumatology criteria.

2.1.2. Validation cohort

We enrolled patients who visited Seoul St. Mary's Hospital with a possible case of pSS and underwent evaluation, including lower lip biopsy, in the validation cohort. Patients who fulfilled the 2016 ACR/EULAR classification criteria for SS were considered to have pSS.

2.2. Saliva preparation

We obtained saliva when subjects underwent SFR measurement. We used a protocol adapted from standard operating protocol of SICCA registry (https://sicca-online.ucsf.edu/Home/Protocols). Saliva collection was permitted to be performed at any time of the day (8:30–17:00), 3 h (at least) of fasting before the test was mandatory. However, the early morning time collection and fasting over 8 h was recommended. Therefore, the vast majority of the samples (especially those of the patients included in the KISS cohort and healthy controls) were collected in the morning after an 8-h fast. Whole saliva samples were centrifuged at 8000 rpm for 1 min at 4 °C to remove debris and cells. The resulting supernatants were stored at −80 °C until time of analysis.

2.3. Microarray analysis

We performed microarray analysis using a human HT-12 expression v.4.0 beadchip for 47,000 genes (Illumina) by Macrogen Co. (Seoul, Korea), following the manufacturer's instructions. Differentially expressed genes (DEGs) from PBMCs were compared between pSS patients (n = 26) and HCs (n = 10).

2.4. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

We collected total RNA using the RNA iso plus reagent (Takara, Kusatsu, Japan). Up to 2 μg of total RNA was converted to complementary DNA using a Transcriptor First-Strand cDNA Synthesis kit (Roche, Basel, Switzerland). A LightCycler 96 instrument (Roche, Basel, Switzerland) was used for PCR amplification and analysis. We performed all reactions with SYBR Green I Master Mix (Roche, Basel, Switzerland) according to the manufacturer's instructions. Primers were designed using the web tool from Integrated DNA Technologies (http://www.idtdna.com) and were synthesized by Macrogen (Seoul, Korea). Sequences are as follows (forward and reverse, respectively): human *BETA ACTIN*, 5′-GGACTTCGAGCAAGAGATGG-3′ and 5′-TGTGTTGGG GTACAGGTCTTTG-3′; *SIGLEC5*, 5′-CTCCCCTTCCCAAAACTCTTC-3′ and 5′-GCAGGTAAAAGTGGGCAAAG-3′. All mRNA expression levels were normalized to *BETA ACTIN* expression. We calculated the relative fold induction using the equation $2^{-(\Delta Cq)}$, where ΔCq is $Cq_{(target)}$-$Cq_{(BETA\ ACTIN)}$, and Cq is the cycle at which the threshold is crossed. PCR product quality was monitored using post-PCR melting curve analysis.

2.5. Flow cytometry

PBMCs were isolated using the Ficoll-Hypaque (GE Healthcare, Chicago, IL) method followed by staining with PerCP/Cy5.5 conjugated anti-CD3 antibodies, Pacific Blue conjugated anti-CD4 antibodies, PE/Cy7 conjugated anti-CD8 antibodies, FITC conjugated anti-CD19 antibodies, BV605 conjugated anti-CD14 antibodies, and PE conjugated anti-CD170 (Siglec-5) antibodies (all from Biolegend (San Diego, CA)). We prevented nonspecific antibody binding by pre-treatment with Human Fc gamma receptor binding inhibitor (Thermo, Waltham, MA). Cells were first gated for singlets (FSC-H vs. FSC-A) then for lymphocytes (SSC-A vs. FSC-A) and live T cells or non-T cells (fixable viability dye-e780 vs. CD3-PerCP/Cy5.5-A). During analysis, we calculated the mean fluorescence intensity (MFI) of siglec-5 in cells.

2.6. Enzyme-linked immunosorbent assay (ELISA)

We used sera and saliva from the subjects to determine the level of siglec-5. A duoset of the human siglec-5 development kit was purchased from R&D systems (Minneapolis, MN); we performed measurements according to the manufacturer's instructions.

2.7. Confocal microscope

Tissues obtained from lower lip biopsies were incubated with antibodies against siglec-5 (Abcam, Cambridge, UK) at 4 °C overnight, followed by incubation with secondary antibodies conjugated to Alexa 594 (Invitrogen, Carlsbad, CA). Nuclei were stained with 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI; Invitrogen, Carlsbad, CA). Isotype control staining was conducted via probing with mouse IgG rather than primary antibodies. We acquired confocal images using an LSM 710 confocal microscope (Zeiss, Oberkochen, Germany).

2.8. Statistics

We performed statistical analyses with GraphPad Prism (Version 5, GraphPad software) and SPSS (version 24, IBM). As appropriate, continuous variables were analysed using the Mann-Whitney test, analysis of variance with the Bonferroni correction test, and analysis of covariance. We used receiver operating curve (ROC) analysis to determine the cut-off value. Correlation was analysed using the Spearman's correlation test. P values of < 0.05 were considered to be significant.

3. Results

3.1. Siglec-5 expression in primary Sjogren's syndrome

As mentioned, a microarray of PBMCs from 26 pSS patients and 10 HCs revealed that siglec-5 was one of the DEGs that had elevated levels in most pSS patients (Fig. 1A). The validation PCR performed in 25 pSS patients and 4 HCs supported the microarray data (Fig. 1B). To further investigate at the protein level, we compared the frequency of siglec-5 expressing PBMCs ($CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes). As previously reported [15], the expression of siglec-5 on lymphocytes, especially on T cells, was trivial. MFI was most prominent in monocytes. However, in contrast to the PCR results, we found no difference in siglec-5 expression between the pSS patients and HCs. Next, we sought to compare the levels of soluble siglec-5 from the sera of patients with pSS, HC, and patients with systemic lupus erythematosus (SLE) as a disease control. Our results showed that the serum level of soluble siglec-5 was higher in SLE patients (Fig. 1D). Intriguingly, the concentration of soluble siglec-5 in the saliva of pSS patients was significantly higher than in saliva from non-SS subjects with sicca symptoms, SLE patients, and HCs (Fig. 1E). When we compared the SS vs. the non-SS group, the median (interquartile range) was 1303 (183.6–4160) vs. 7.967 (0–556.5) ($P < 0.001$) (Fig. 1F). As expected, the saliva reflected the local environment of the salivary glands, so we investigated siglec-5 expression in minor salivary gland biopsy samples. Using confocal microscopy, we found that siglec-5 was not expressed by epithelial cells, but it co-localized with immune cells (Fig. 1G). Collectively, the results showed that siglec-5 was highly transcribed in PBMCs of patients with pSS and the concentration of soluble siglec-5, which seems to be produced by immune cells in the salivary gland, was higher in the pSS patient group.

3.2. Clinical characteristics of SS patients and their correlation with levels of soluble salivary siglec-5

After confirming that pSS patients have higher siglec-5 expression in the saliva, we investigated if there was an association between the level of saliva siglec-5 and the clinical characteristics of pSS patients. We used samples from pSS patients who are registered in a prospective cohort; clinical data were available for most pSS patients. Table 1 shows the demographic and clinical data of the subjects. A bivariate correlation analysis found that saliva levels of siglec-5 negatively correlated with SFR but positively correlated with the xerostomia inventory (XI) score and OSS (Fig. 2). These results suggest that saliva siglec-5 level reflects the severity of secretory dysfunction. Moreover, the level of serum IgG and antinuclear antibody (ANA) titre positively correlated with saliva siglec-5 (Fig. 2). However, we found no association between the saliva level of siglec-5 and the EULAR SS Disease Activity Index

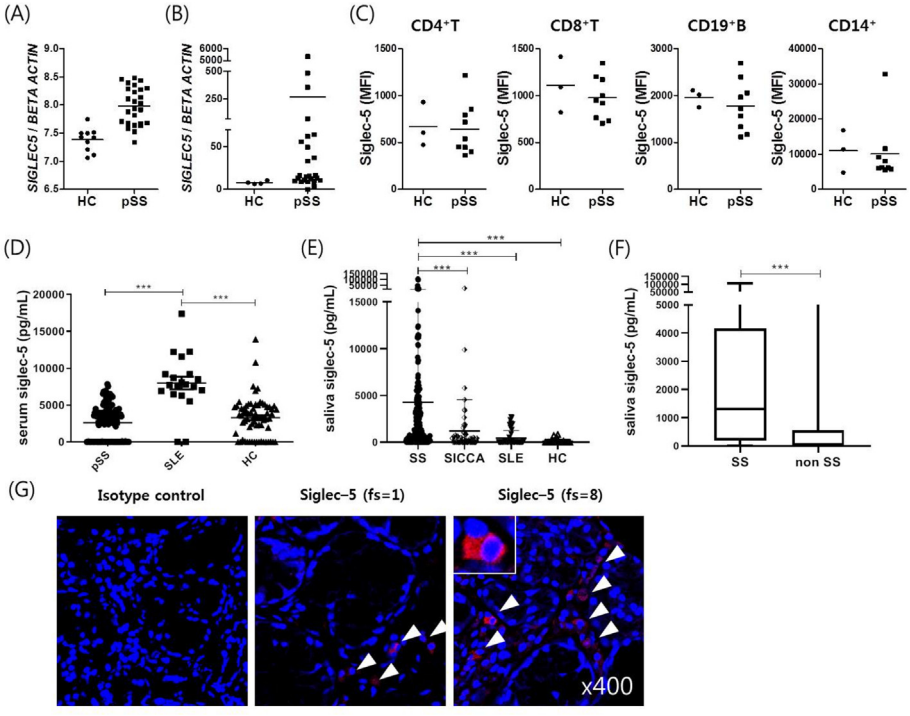

Fig. 1. Siglec-5 expression in patients with primary Sjogren's syndrome (pSS). (A) Microarray showed upregulated *SIGLEC5* gene expression in pSS patients (pSS, n = 26) compared to healthy controls (HC, n = 10). (B) The expression of *SIGLEC5* in PBMCs was confirmed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) (pSS, n = 25; HC, n = 4). (C) Flow cytometry analysis of mean fluorescence intensity (MFI) of siglec-5 on peripheral immune cells. (D–F) Levels of soluble siglec-5 were measured by enzyme-linked immunosorbent assay (ELISA) using sera (D) or saliva (E–F) from patients with systemic lupus erythematosus (SLE), sicca symptoms (SICCA), pSS, or HCs. (G) Siglec-5 (red) positive immune cells shown in minor salivary glands derived from pSS patients (focus score 1(middle) and 8 (right)). Nuclei were stained with 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) and mouse immunoglobulin G was used for an isotype control. *P < 0.05, ***P < 0.001.

Table 1
Demographics and clinical characteristics of patient cohort.

	pSS (n = 170)	non-SS SICCA (n = 78)	SLE (n = 43)	HC (n = 25)	*P*
Age (yr)	53 [45–60]	52 [41.75–61.25]	38.5 [37.05–51.25]	41 [35.5–45]	0.001
Female (%)	169 (99.4%)	70 (89.7%)	42 (100%)	24 (96%)	
Saliva Siglec-5 (pg/mL)	1346.8 [202.8–4280.0]	195 [0–947.5]	0 [0–238.7]	6.08 [0–134.0]	< 0.001
Disease duration (mo)	16.9 [1.7–48.5]				
ESSDAI	3 [1–6]				
ESSPRI	5 [4.1–6.7]				
uSFR (mL/5 min)	0.225 [0.1–0.4]	1 [0.5–1.575]			
sSFR (mL/5 min)	3.081 [1.5–6]				
Fulfilment of 2012 criteria	136 (80.0%)	0			
Fulfilment of 2002 criteria	167 (98.2%)	0			
Extraglandular manifestation	117 (68.8%)				
Treatment					
Pilocarpine	134 (78.8%)				
Hydroxychloroquine	118 (69.4%)				
Methotrexate	10 (5.9%)				
Corticosteroid	59 (34.7%)				

pSS, primary Sjogren's syndrome; HC, healthy control; ESSDAI, EULAR Sjogren's Syndrome Disease Activity Index; ESSPRI, EULAR Sjogren's Syndrome Patient Reported Index; SLE, systemic lupus erythematosus; sSFR, stimulated salivary flow rate; uSFR, unstimulated salivary flow rate. Data are presented with median [interquartile range].

(ESSDAI), EULAR SS Patient Reported Index (ESSPRI), or focus score.

3.3. Salivary siglec-5 as a single diagnostic biomarker

To determine the value of saliva siglec-5 as a diagnostic biomarker, we performed ROC analysis. The area under the curve (95% confidence interval [CI]) was 0.774 (0.724, 0.826); with cut-off level of 400 pg/mL, the sensitivity and specificity were both 0.69 and 0.70, respectively. When we compared the characteristics of siglec-5 (+) (≥400 pg/mL) and siglec-5 (−) (< 400 pg/mL) pSS patients, the siglec-5 (+) patients had a significantly higher XI score, serum IgG level, and OSS (Table 2). These patients also had a lower SFR and white blood cell (WBC) count (Table 2).

3.4. Diagnostic value of salivary siglec-5 in a separate validation cohort

We also tested the performance of saliva siglec-5 in a separate validation cohort. Among 90 individuals who underwent evaluation for pSS, 45 were diagnosed with pSS. The number of patients positive for saliva siglec-5 according to the presence of pSS is shown in Table 3. In the validation cohort, the sensitivity was 0.644 and the specificity was 0.778. As a single marker, the concentration of siglec-5 in saliva performed worse than the *anti*-Ro or focus score; however, it performed better than single uSFR, Schirmer's test, or OSS (Table 4). We found similar results in a bivariate correlation analysis: positive correlation with OSS (rho = 0.313, P = 0.015) and negative correlation with uSFR (rho = −0.357, P = 0.001) and sSFR (rho = −0.392, P < 0.001).

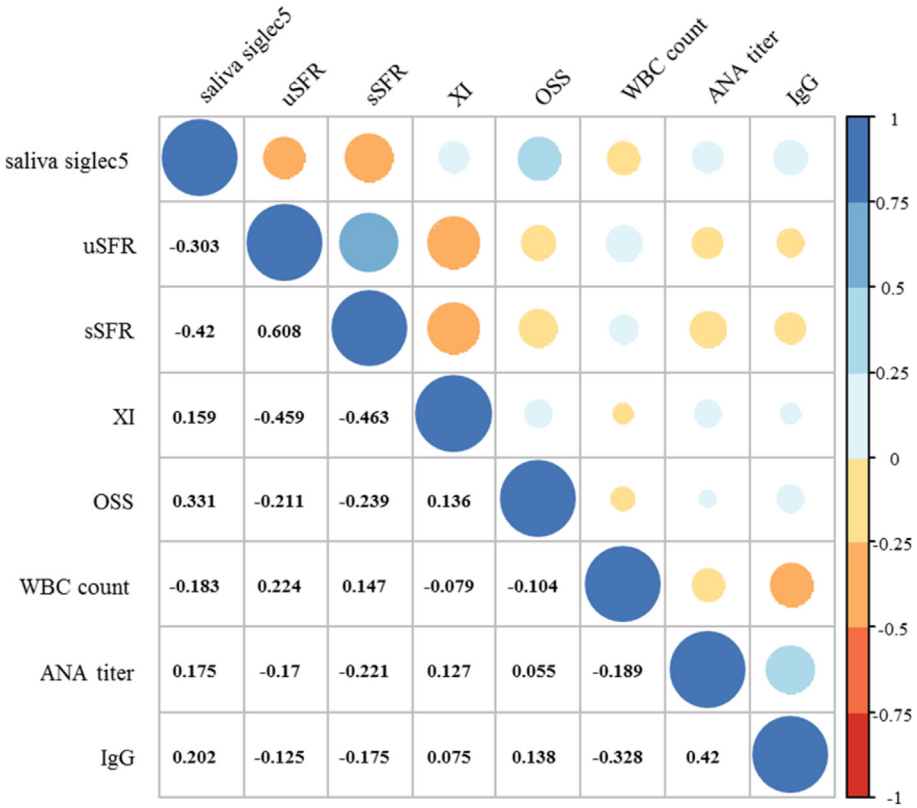

Fig. 2. Correlation between saliva siglec-5 concentration and Sjogren's syndrome-related clinical parameters. Spearman's correlation coefficient rho was calculated for the saliva siglec-5 concentration and the unstimulated salivary flow rate (uSFR), stimulated SFR (sSFR), xerostomia inventory (XI) questionnaire score, ocular staining score (OSS), serum immunoglobulin G (IgG) concentration, and antinuclear antibody (ANA) titer.

Table 2
Characteristics of primary Sjogren's syndrome patients according to siglec-5 level in saliva.

	Saliva Siglec-5		*P*
	≥400 pg/mL	< 400 pg/mL	
Number	119	51	
Saliva siglec-5 (pg/mL)	3087.2[1185.8–5695.2]	25[0–166.3]	< 0.001*
Age (yr)	54[46–60]	51[44–59]	0.209
BMI (kg/m^2)	22.5[20.5–24.2]	22.1[20.2–23.7]	0.745
Disease duration (mo)	17.4[1.7–48.3]	8.4 [0.9–51.5]	0.467
ESSDAI	3 [1–6]	4[1–7.25]	0.303
ESSPRI	5[4–6.3]	5.3 [4–7]	0.776
OSDI	39[25–52.3]	36[18.5–64]	0.991
XI	37[31–43]	31[25–41]	0.013*
uSFR (mL/5 min)	0.1[0.1–0.3]	0.3[0.1–0.75]	0.003*
sSFR (mL/5 min)	2.2[1–4.5]	5.4[2.73–9]	< 0.001*
IgG (mg/dL)	1648[1391–2058]	1489[1333–1679]	0.033*
WBC (cells/mm^3)	4460[3550–5310]	4800[4020–6070]	0.022*
Ocular staining score	4 [2–6]	2 [1–4]	0.007*
Focus score	2.5[0–5]	2[1–3.25]	0.819

BMI, body mass index; ESSDAI, EULAR Sjogren's syndrome disease activity index; ESSPRI, EULAR Sjogren's syndrome patient reported index; IgG, immunoglobulin G; OSDI, ocular surface disease index; sSFR, stimulated salivary flow rate; uSFR, unstimulated salivary flow rate; WBC, white blood cell; XI, xerostomia index. Data are presented with median[interquartile range]. P < 0.05*.

Table 3
Number of patients with Sjogren's syndrome and positive (≥400 pg/mL) for siglec-5 in saliva of the validation cohort.

	Saliva siglec-5	
	(−)	(+)
non-SS	35	10
SS	16	29

SS: Sjogren's syndrome.

4. Discussion

This study demonstrated that levels of salivary siglec-5 are higher in pSS patients and the concentration correlated with symptoms of dryness and other disease signs. To the best of our knowledge, this is the first report that siglec-5 could serve as a novel biomarker that can be measured from non-invasively obtainable samples such as saliva. Our results reflect the severity of hyposalivation, ocular damage, and immunoglobulin production in pSS. Considering that the use of a salivary marker has only been suggested for proteomics approaches, our finding of an easily measurable salivary biomarker is meaningful.

In clinical settings, it is difficult to perform the typical tests used to diagnose individuals with pSS. OSS requires experienced ophthalmologists, and salivary rate measurements require time and effort. Moreover, a lip biopsy is not readily available in many clinics, due to a lack of experienced personnel to collect samples and interpret results (pathologists). In light of these challenges, we expect our salivary marker could possibly help overcome these diagnostic difficulties.

Previously, another member of the siglec family, siglec-1, was reported to be a biomarker in pSS, which reflects the systemic disease activity of the ESSDAI and indicates an extraglandular manifestation [16]. Considering that siglec-1 is regarded as an interferon-related biomarker [17,18], siglec-1 is highly expressed in pSS patients who have high systemic activity of interferons. Similarly, an interferon signature - MxA was suggested as a biomarker of pSS which positively correlated with ESSDAI [19]. In our cohort, siglec-5 did not reflect the severity of systemic disease activity. However, the patients in our cohort had relatively low ESSDAI scores with a median of [IQR] of 3 [1–6]. Therefore, the performance of siglec-5 as an activity monitoring marker should be investigated in another cohort that enrolls patients with high disease activity.

In our validation cohort, we observed decreased diagnostic performance of salivary siglec-5. According to the newly developed 2016 ACR/EULAR classification criteria, subjects with sicca symptoms and those with one or more positive domains from the ESSDAI can be diagnosed with pSS. Therefore, we included patients in our validation cohort who had less severe oral/ocular dryness than the original cohort. The sensitivity and specificity were as low as when using a single marker. However, because this biomarker correlates with uSFR and OSS, salivary siglec-5 measurement will at a minimum provide more evidence to support patient diagnosis in clinics where the standard tests are not available.

In addition to diagnostic value, we examined whether siglec-5 had a predictive value. Among 170 pSS patients, 51 patients were negative for siglec-5. Sixteen out of 51 siglec-5 (−) patients had uSFRs of > 0.5 mL/5 min. In 9 of these 16 patients, annual follow-up up to 3 years was available, and none had a further decrease in uSFR. In contrast, 9 out of 12 patients who were siglec-5 (+) but had uSFR of > 0.5 mL/5 min showed a decrease in uSFR in annual follow-up tests. Four among 9 patients ended up with ≤0.5 mL/5 min in 3 years. As the observation period and number of patients are both small, we could not conclude that siglec-5 had value as a predictive marker. We are planning a long-term observation study to investigate the predictive value of siglec-5 in a larger patient cohort.

As mentioned, siglec-5 is a receptor that is paired with siglec-14, which has an opposite function. Their extracellular domains are identical and we could not distinguish these siglecs from one another in the soluble form [20]. At the mRNA level, we verified that siglec-5 expression is elevated in the PBMCs of pSS patients. However, we unexpectedly found that siglec-5 expression was not different in flow cytometry analysis. This observation might be because we measured both siglec-5 and siglec-14 expression in the PBMCs. Indeed, mRNA expression of siglec-14 was not different between pSS patients and HCs (data not shown). We observed that soluble siglec-5 was highly expressed in the saliva of pSS patients, although we cannot tell if its original form was siglec-5, siglec-14, or both. However, considering the function of the receptor (inhibitory function with intracellular ITIM)

Table 4
Sensitivity, specificity, positive predictive value, negative predictive value of siglec-5, and other classification criteria for Sjogren's syndrome.

	N	AUC [95% CI]	Sensitivity [95% CI]	Specificity	PPV	NPV
Saliva siglec-5 ≥200	90	0.711 [0.602–0.820]	64.4% [48.78–78.13]	77.8% [62.91–88.80]	74.36% [61.69–83.93]	68.63% [58.89–76.96]
Anti Ro (+)	90	0.887 [0.811–0.964]	81.82% [67.29–91.81]	95.65% [85.16–99.47]	94.74% [82.17–98.60]	84.62% [74.55–91.17]
Focus ≥1	47	0.873 [0.761–0.985]	81.82% [64.54–93.02]	92.86% [66.13–99.82]	96.43% [80.22–99.45]	68.42% [50.87–81.93]
uSFR ≤0.5 mL/5 min	90	0.607 [0.489–0.724]	40.91% [26.34–56.75]	80.43% [66.09–90.64]	66.67% [50.20–79.87]	58.73% [51.72–65.41]
Schirmer's ≤ 5 mm/5 min	45	0.535 [0.363–0.707]	40.91% [26.34–56.75]	71.74% [56.54–84.01]	58.06% [43.63–71.24]	55.93% [48.32–63.27]
OSS ≥5	30	0.586 [0.357–0.815]	83.33% [62.62–95.26]	100.00%[54.07–100.00]	100.00%	60.00%[38.01–78.58]

AUC, area under the curve; CI, confidential interval; NPV, negative predictive value; OSS, ocular staining score; PPV, positive predictive value; uSFR, unstimulated salivary flow rate.

and the inflammatory nature of pSS, siglec-5 seems more likely. We hypothesize that siglec-5 is shed from immune cells, and its inhibitory signals are lost in pSS, which results in inflammatory condition in pSS. This issue is associated with another important remaining question: the origin of this soluble siglec-5 in the saliva. Lymphocytes are known to be the major cell population in infiltrated mononuclear cells from the salivary gland, whereas monocytes and neutrophils are rarely found. Therefore, we observed very few siglec-5 expressing cells by confocal microscopy. Because we obtained the saliva by spitting, it is possible that macrophages or neutrophils in the oral mucosa were the sources for siglec-5. Future research must be performed to determine the exact mechanism that results in a high siglec-5 concentration in pSS patient saliva. Another unanswered question concerns the association of saliva siglec-5 with secretory dysfunction. We could not perform *in vivo* studies to address the function of siglec-5 on salivation in murine model, because siglec-5 is not expressed in mice. Therefore, to determine whether soluble siglec-5 directly disturbs secretory function or if it is a just a by-product of another mechanism will require additional functional studies.

5. Conclusions

The level of soluble siglec-5 was significantly higher in the saliva of pSS patients than in controls, which reflects the severity of hyposalivation and ocular surface damage in this condition. As a single marker, soluble siglec-5 showed a relatively high diagnostic value in two separate cohorts. Although the mechanistic details of siglec-5 contribution to gland dysfunction remain unclear, this easily obtained salivary biomarker may provide benefits for the diagnosis of pSS.

Author contributions

JL, SKK and SHP directed the work, contributed to designing the study, and reviewed/interpreted the data. JL and SYB conducted all the experiments. JHK, JWK, SHC and SYK helped with data clinical data acquisition and analysis. SSC interpreted the microarray data. MLC and JHJ interpreted the data. JL and JL prepared the manuscript and figures. All authors read and approved the final manuscript.

Conflicts of interest

The authors have no conflict of interest.

Acknowledgments

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI13C0016).

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