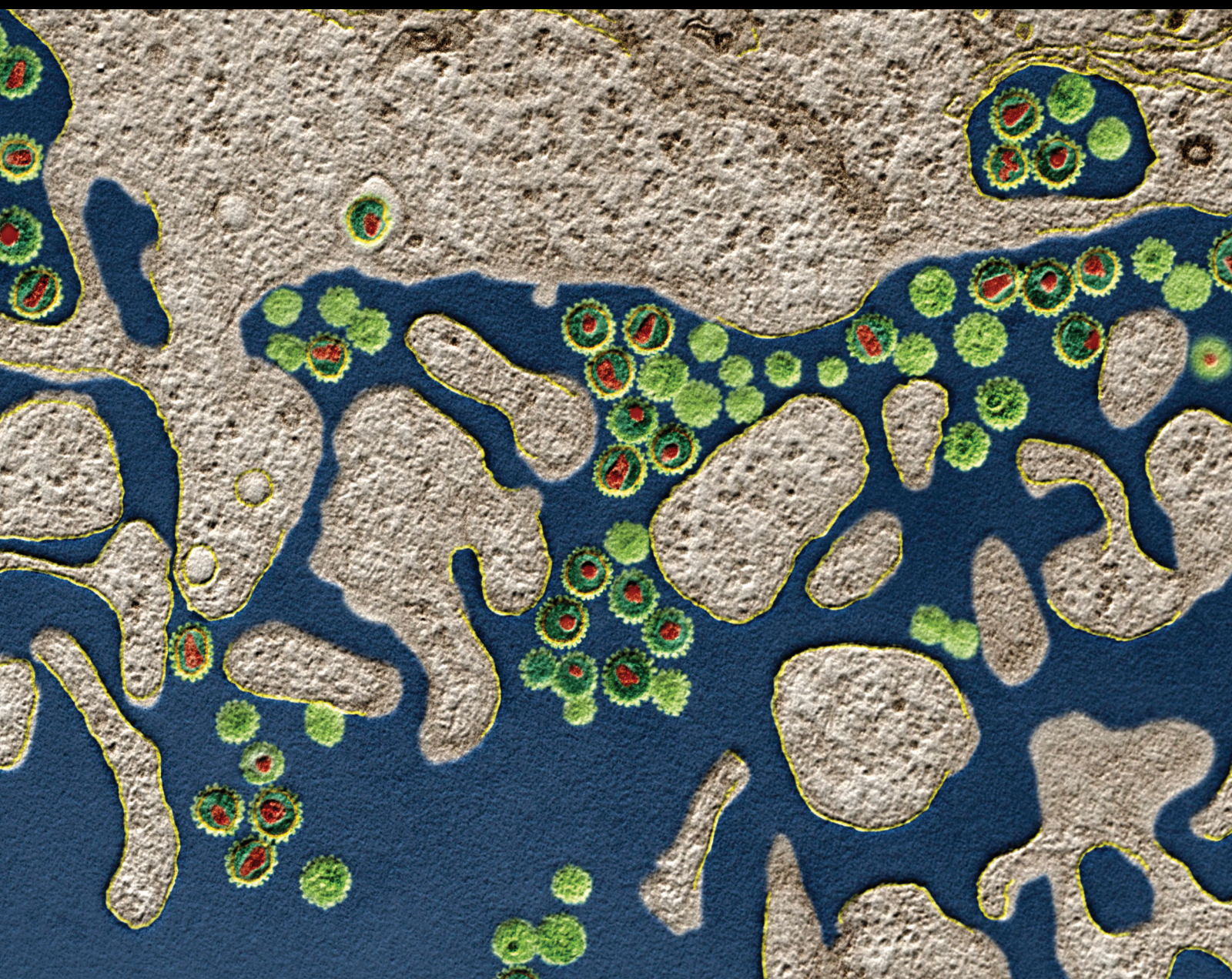
Journal of Immunology Research

Sjögren's Syndrome: Animal Models, Etiology, Pathogenesis, Clinical Subtypes, and Diagnosis

Lead Guest Editor: Long Shen
Guest Editors: Jing He, Jill M. Kramer, and Vatinee Y. Bunya



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Editorial Board

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Editorial

Sjögren's Syndrome: Animal Models, Etiology, Pathogenesis, Clinical Subtypes, and Diagnosis

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Sjögren's syndrome (SS) is a chronic autoimmune disease that classically affects the lacrimal and salivary glands resulting in dry eyes and dry mouth and can affect almost any organ system in the body including the lungs, kidney, and central nervous system [1]. Many systemic aspects of SS are described, including B cell lymphoma in addition to pulmonary and renal pathoses [2–4]. Diagnosis often takes several years once symptoms manifest [5], and even after the diagnosis is rendered, there are currently no treatments available that address the underlying disease etiology.

While the pathogenesis of SS is not well understood, both innate and adaptive immune responses are implicated in disease initiation and progression. In the innate response, an antiviral response is mounted through the recognition of viral nucleic acids by Toll-like receptors (TLRs). This recognition leads to the upregulation of the type 1 interferon (IFN) pathway. However, the means by which immune activation is initiated and maintained remain incompletely understood. Activation of TLRs is critical for the progression of numerous autoimmune diseases [6], although there are relatively few studies regarding the role of these receptors in SS. Studies in mice and humans reveal that TLRs are potent mediators of inflammation in SS. TLRs are expressed and functional in salivary tissue, and TLRs in peripheral blood cells of SS patients are also upregulated and hyperresponsive to ligation. In addition, interferon signatures have been detected in the blood and in the labial salivary gland tissues of patients with pSS [7]. However, it is unclear whether TLRs are activated by microbial products or host-derived ligands in SS [8]. Both animal models and patient studies are instrumental in understanding causes of TLR dysfunction in SS [9]. Studies that identify specific TLRs and clinically relevant ligands will likely lead to the development of novel therapeutics that will diminish chronic inflammation that is a characteristic of SS.

Several viruses have been implicated as possible environmental triggers of SS including Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes virus type 8 (HHV-8), human T-lymphotropic virus type 1 (HTLV-1), hepatitis C virus, and enterovirus [10]. In addition, paramyxovirus, which causes mumps, may persist in salivary glands and could provide the necessary trigger to initiate autoimmunity pathogenesis of SS in certain genetically susceptible individuals [11]. Cytokines, B cell growth factor, and serum B cell-activating factor (BAFF) levels also play an important role in the pathogenesis of SS. They are altered in patients with SS and correlate with antibodies to Ro and La [12, 13]. Previous studies have shown that most IL-14α transgenic mice develop gastrointestinal B cell lymphomas [14].

Most often, SS presents with polymorphic clinical presentations including the involvement of various systemic organs, which can result in delays in diagnosis. While SS is typically not associated with significant mortality, patients who demonstrate renal involvement are at risk for life-threatening complications. Renal involvement in SS is relatively rare and is seen in approximately 5-10% of SS patients [15–18]. Studies are needed to understand the mechanisms that govern kidney dysfunction in SS and to uncover the optimal diagnostic strategies for SS patients with renal complications. In addition, it is important to identify SS patients who

are at high risk of renal disease in SS in order to manage these individuals appropriately.

Non-Hodgkin lymphoma is another serious systemic manifestation of SS [19, 20]. SS is characterized by B cell hyperactivity, and the lymphomas that arise in SS patients originate exclusively from B cells [21]. There are several clinical and serological findings that are associated with lymphoma development in SS patients, including anemia, neutropenia, and thrombocytopenia [22]. In addition, BAFF polymorphisms have been identified, and BAFF levels tend to be elevated in patients who develop lymphoma [12, 23–25]. While the relationship between lymphoma development, BAFF levels, and autoantibodies is not well understood, further work is needed to identify autoantibodies that may be indicative or predictive of lymphoma development in SS [21].

The diagnosis of SS can be very challenging due to the absence of specific clinical manifestations in the early stages of the disease and the lack of noninvasive diagnostic methods with high specificity and sensitivity. This can lead to significant delays in treatment and worse clinical outcomes. Therefore, novel biomarkers and imaging methods to help recognize the disease at an early stage are needed. For example, antibodies to salivary gland protein 1 (SP1), carbonic anhydrase 6 (CA6), and parotid secretory protein autoantibodies (PSP) were first described in a mouse model for SS and have also been found in SS patients together and without anti-SSA/Ro, as well as in patients with idiopathic dry mouth and dry eye disease [26]. However, despite the discovery of new biomarkers such as these, there are continued diagnostic delays due to the limited understanding of the sequence of events that trigger the activation of the autoimmunity against specific antigenic targets. It is important to study and elucidate the pathways leading to the activation of the antigenic targets as it may lead to more precise disease diagnosis and to the development of specific targeted therapies.

The ocular manifestations of SS often cause substantial morbidity as patients experience significant decreases in quality of life and visual functioning as a result of the disease [27]. Approximately 10% of dry eye patients have underlying SS, but because dry eye is a highly prevalent condition in the general population [27], it is not possible to work-up all dry eye patients for SS. Therefore, better tests that distinguish SS from other causes of dry eye are needed. In addition, further work is required to understand the inciting events that lead to the development of the ocular manifestations of SS. While immune dysfunction is thought to underlie the ocular dysfunction observed, [28] the contribution of environmental insults and genetics to the disease remain incompletely understood [29]. Thus, further work to understand the mechanisms that govern ocular dysfunction and to develop tests for dry eye that identify patients with SS will have a significant impact.

The goal of this special issue is to highlight recent advances in the understanding of the etiopathogenesis, varying clinical presentations, and diagnosis of SS. For example, Z. Mackiewicz et al. in their article entitled "Sjögren's Syndrome: Concerted Triggering of Sicca Conditions" evaluate the role of the paramyxovirus in SS. J. Kramer and J. Kiripolsky in their paper "Current and Emerging Evidence for Toll-Like Receptor Activation in Sjögren's Syndrome" explore the role of TLRs in the pathogenesis of SS based on findings in the various mouse models. They discuss the role and significance of putative damage-associated molecular patterns in SS. J. Luo et al. discuss renal involvement of SS, its diverse clinical presentation, and the role of renal biomarkers in kidney damage assessment in their article "High-Risk Indicators of Renal Involvement in Primary Sjogren's Syndrome: A Clinical Study of 1002 Cases." Z. Xian and colleagues, in their study entitled "Association between B Cell Growth Factors and Primary Sjögren's Syndrome-Related Autoantibodies in Patients with Non-Hodgkin's Lymphoma," describe the relation between the cytokines BAFF and IL-14 with various traditional autoantibodies (anti-SSA/Ro) and newer tissue-specific autoantibodies. They also describe the role of cytokines and autoantibodies for the stratification of SS patients with gastrointestinal lymphomas. Finally, S. Karakus and colleagues conducted a cross-sectional study of dry eye patients to investigate the clinical significance of anti-salivary gland protein 1 (SP1), anti-carbonic anhydrase 6 (CA6), and anti-parotid secretory protein (PSP) autoantibodies. In their manuscript "Clinical Correlations of Novel Autoantibodies in Patients with Dry Eye," they demonstrate that disease severity stratification may be feasible using new biomarkers and conclude that anti-CA6 is seen in patients with severe aqueous-deficient dry eye. Taken together, it appears that a new era is on the horizon for a better understanding of the clinical manifestations, diagnosis, etiology, and pathogenesis of SS.

Conflicts of Interest

Drs. Long Shen, Vatinee Bunya, Jing He, and Jill M. Kramer declare that they have no conflicts of interest related to this work. Dr. Bunya receives grant funding from Bausch & Lomb/Immco Diagnostics.

Long Shen
Jing He
Jill M. Kramer
Vatinee Y. Bunya

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Research Article

High-Risk Indicators of Renal Involvement in Primary Sjogren's Syndrome: A Clinical Study of 1002 Cases

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Objective. A retrospective analysis of clinical characteristics and immunological manifestations of primary Sjogren's syndrome (pSS) patients with or without renal involvement was conducted in order to elucidate the potential risk factors of renal damage in pSS and evaluate the condition. *Methods*. A total of 1002 patients, who fulfilled the 2002 classification criteria for pSS from the Second Affiliated Hospital of Shanxi Medical University, were enrolled in the cross-sectional study. Clinical, immunological, and histological characteristics were compared between pSS patients with and without renal involvement, and potential risk factors of renal involvements in pSS patients were examined by multivariate analysis. *Results*. Among these pSS patients, there were 162 cases (16.17%) with and 840 cases (83.83%) without renal damage. Serious edema of both lower limbs, interstitial nephritis, and renal tubular acidosis were found in the pSS with renal damage group. Compared with simple pSS patients, the levels of creatinine, cystatin C, and alpha-1-microglobulin (α_1-MG) in the pSS with renal damage group were significantly increased. The difference between the two groups was statistically significant ($P < 0.05$). The AUC of the combination of creatinine and α_1-MG and creatinine, α_1-MG, and creatinine was statistically larger than that of creatinine, and the biomarker of the biggest AUC is the combination of creatinine and α_1-MG. *Conclusion*. The main clinical manifestations of pSS with renal damage were edema of the lower limbs, interstitial nephritis, and renal tubular acidosis. Creatinine and α_1-MG are effective indicators for renal function in pSS, which may provide a better understanding for clinical decision-making.

1. Introduction

Sjogren's syndrome (SS) is a chronic progressive autoimmune disorder characterized by lymphocytic infiltration of the exocrine glands, which affects the salivary and lacrimal glands, presenting dryness of the mouth and eyes. The majority of infiltrating mononuclear cells are CD4+ T cells [1]. Some patients may present diverse extraglandular impairment such as that in the lungs, kidneys, nervous system, and skin affected by this disorder [2]. The predominant serologic findings of pSS are positive anti-nuclear antibodies (ANA), anti-SSA antibodies, and anti-SSB antibodies. Renal involvement is easily ignored by the physicians because the clinical symptoms are often insidious. Growing evidence suggests that patients with pSS may have greater renal injury risk than the general population and the most common renal disease in SS is tubulointerstitial nephritis, responsible for renal tubular acidosis in 20% [3]. However, it is still challenging to diagnose renal involvement in pSS patients.

In the present study, we described the clinical presentation and serologic findings of 840 patients with pSS without renal involvement and 162 patients with renal involvement. We also analyzed whether biochemical markers were useful in identifying renal disease in pSS patients to guide further clinical work.

Table 1: Demographic, clinical, histological, immunological, and inflammatory features of primary Sjogren's syndrome with or without renal involvement.

	Without renal involvement 840	With renal involvement 162	*P* value
Seroperitoneum	0	6 (3.7%)	0.000
Dizziness	12 (1.4%)	8 (4.9%)	0.003
Palpitate	14 (1.7%)	6 (3.7%)	0.090
Breathe hard	26 (3.1%)	9 (5.6%)	0.118
Digestive tract symptoms	19 (2.3%)	31 (19.1%)	0.000
Respiratory system symptoms	30 (3.6%)	15 (9.3%)	0.001
Congestion of throat	1 (0.1%)	10 (6.2%)	0.000
Bilateral pleural effusion	0	3 (1.9%)	0.000
Lipsotrichia	53 (6.3%)	4 (2.5%)	0.053
Dry cough	4 (0.5%)	1 (0.6%)	0.815
Edema in the face	5 (0.6%)	14 (8.6%)	0.000
Edema of both lower limbs	13 (1.5%)	42 (25.9%)	0.000
Hypourocrinia	0	4 (2.5%)	0.000
Frequent micturition	8 (1.0%)	10 (6.2%)	0.000
Urgency of urine	8 (1.0%)	7 (4.3%)	0.000
Odynuria	3 (0.4%)	4 (2.5%)	0.003
Rampant caries	187 (22.3%)	34 (21%)	0.720
Erythema	149 (17.7%)	23 (14.2%)	0.274
Weak	119 (14.2%)	50 (30.9%)	0.000
Poor appetite	9 (1.0%)	25 (15.4%)	0.000
Dry mouth	669 (79.6%)	129 (79.6%)	0.997
Xerophthalmia	457 (54.4%)	94 (58%)	0.396
Arthralgia	493 (58.7%)	68 (42%)	0.000
Fever	149 (17.7%)	28 (17.3%)	0.890
Reynolds	33 (3.9%)	9 (5.6%)	0.344
Dental ulcer	69 (8.2%)	16 (9.9%)	0.487
Courpature	1 (0.1%)	1 (0.6%)	0.193
Hematuria	0	1 (0.6%)	0.023
Polydipsia	1 (0.1%)	2 (1.2%)	0.021
Diuresis	1 (0.1%)	2 (1.2%)	0.021
Nocturia	2 (0.2%)	17 (18.5%)	0.000
Parotid swelling and pain	29 (3.5%)	3 (1.9%)	0.289
Anti-scl-70	0	2 (1.2%)	0.001
Anti-Jo-1	0	2 (1.2%)	0.001
pANCA	17 (2%)	4 (2.5%)	0.717
cANCA	3 (0.4%)	1 (0.6%)	0.631
RF	144 (17.1%)	44 (27.2%)	0.003
Anti-ENA	169 (20.1%)	59 (36.4%)	0.000
Anti-ds DNA	18 (2.1%)	3 (1.9%)	0.813
Anti-SSA	579 (68.9%)	80 (49.4%)	0.000
Anti-SSB	54 (6.4%)	19 (11.7%)	0.017
Anti-Sm	4 (0.5%)	4 (2.5%)	0.009
Anti-RNP	87 (10.4%)	15 (9.3%)	0.672

Table 2: Demographic, clinical, histological, immunological and inflammatory features of primary Sjogren's syndrome with or without renal involvement.

	Without renal involvement 840	With renal involvement 162	*P* value
Age	49.46 ± 13.36	49.94 ± 15.39	0.713
Mouth disease	69.44 ± 83.05	56.76 ± 91.78	0.082
White blood cell	6.15 ± 3.035	6.34 ± 3.03	0.485
RBC	4.08 ± 0.61	3.66 ± 0.87	0.000
Hb	122.61 ± 26.56	109.75 ± 24.94	0.000
Platelet	209.61 ± 101.51	214.66 ± 100.74	0.562
Monocyte	0.43 ± 0.25	0.45 ± 0.42	0.402
Eosinophil	0.11 ± 0.17	0.13 ± 0.16	0.181
Lymphocyte%	28.73 ± 11.31	28.57 ± 11.82	0.872
Lymphocyte	1.66 ± 1.64	1.66 ± 0.84	0.973
Monocyte%	7.44 ± 3.85	6.77 ± 2.49	0.005
Eosinophil%	1.86 ± 2.35	1.97 ± 2.15	0.568
Urine RBC	5.79 ± 38.71	26.95 ± 100.88	0.009
Urine WBC	18.30 ± 57.64	11.60 ± 39.55	0.071
Urine pH	6.34 ± 0.78	6.28 ± 1.00	0.463
Proportion	1.02 ± 0.01	1.02 ± 0.01	0.074
ALT	32.81 ± 35.05	30.9 ± 77.11	0.618
AST	32.66 ± 33.19	35.79 ± 80.43	0.626
AST/ALT	1.18 ± 0.56	1.28 ± 0.47	0.039
Total bilirubin	14.26 ± 14.24	13.04 ± 33.11	0.445
Direct bilirubin	4.19 ± 7.15	4.53 ± 19.00	0.822
Indirect bilirubin	10.06 ± 8.34	8.77 ± 14.62	0.121
Prealbumin	234.65 ± 56.94	262.90 ± 66.57	0.000
Total protein	71.10 ± 10.40	68.16 ± 11.80	0.000
Albumin	37.28 ± 5.51	34.475 ± 6.87	0.004
Globulin	33.45 ± 9.10	33.70 ± 9.48	0.000
Albumin/globulin	1.19 ± 0.35	1.10 ± 0.36	0.001
Alkaline phosphatase	99.26 ± 99.52	102.35 ± 68.45	0.687
Glutamyl transpeptidase	53.52 ± 102.20	37.64 ± 69.78	0.015
Total bile acid	9.73 ± 20.71	10.21 ± 37.32	0.815
5-Nucleoglykase	8.45 ± 14.37	6.73 ± 10.15	0.147
Adenosine deaminase	19.01 ± 11.93	18.84 ± 9.07	0.858
Blood glucose (4.2-6.1)	5.31 ± 1.57	5.18 ± 1.06	0.332
Fructosamine	1.83 ± 0.65	1.81 ± 0.56	0.633
Urea nitrogen	4.50 ± 1.76	8.43 ± 6.62	0.000
Creatinine	55.70 ± 14.32	150.82 ± 150.41	0.000
CO_2 CP	25.08 ± 2.84	22.54 ± 4.60	0.000
Cystatin C	1.09 ± 0.36	2.04 ± 1.38	0.000
α_1-MG (10-30 ng/L)	20.89 ± 7.95	34.04 ± 15.93	0.000
β_2-MG (0.97-2.64 ng/L)	2.81 ± 5.19	6.02 ± 5.64	0.000
Uric acid (90-420 μmol/L)	246.66 ± 78.60	292.70 ± 115.11	0.000
Complement-C1q (159-233 mg/L)	198.06 ± 14.16	199.79 ± 15.72	0.164

Table 2: Continued.

	Without renal involvement 840	With renal involvement 162	*P* value
K (3.5-5.5 mmol/L)	3.91 ± 0.41	3.93 ± 0.63	0.631
Na (137-147 mmol/L)	139.50 ± 3.47	139.31 ± 4.08	0.579
Cl (99-110 mmol/L)	105.34 ± 3.66	107.38 ± 5.36	0.000
Ca (2.08-2.6 mmol/L)	2.24 ± 0.14	2.19 ± 0.18	0.000
P (0.83-1.48 mmol/L)	1.23 ± 0.50	1.26 ± 0.30	0.465
Mg (0.7-1.1 mmol/L)	0.91 ± 0.10	0.93 ± 0.12	0.145
Fe	14.07 ± 6.61	13.31 ± 7.20	0.190
CK	66.41 ± 124.01	82.60 ± 192.51	0.304
CK-MB	9.39 ± 8.96	8.90 ± 6.54	0.502
LDH	221.41 ± 168.21	233.16 ± 201.45	0.432
HBD	173.22 ± 141.13	180.03 ± 136.94	0.572
Total cholesterol	4.55 ± 1.25	4.53 ± 1.82	0.883
Triglyceride	1.90 ± 2.05	2.18 ± 2.36	0.156
HDL	1.21 ± 0.43	1.14 ± 0.37	0.054
LDL	2.66 ± 0.84	2.61 ± 1.25	0.607
Apolipoprotein-A_1	1.30 ± 0.39	1.23 ± 0.32	0.033
Apolipoprotein-B100	0.84 ± 0.23	0.90 ± 0.38	0.581
Apolipoprotein-E	38.84 ± 12.74	40.23 ± 22.27	0.443
Lipoprotein-α	18.41 ± 18.30	23.19 ± 21.88	0.010
HDL/cholesterol	27.13 ± 7.68	21.51 ± 9.02	0.615
Acid phosphatase	4.29 ± 2.76	5.15 ± 2.82	0.000
ESR	38.29 ± 33.68	54.84 ± 36.36	0.000
CRP	10.35 ± 20.25	10.62 ± 20.50	0.877
Complement-C3	1.01 ± 0.24	0.95 ± 0.22	0.004
Complement-C4	0.23 ± 0.25	0.25 ± 0.14	0.370
PTH	38.52 ± 17.82	220.28 ± 307.65	0.032
CA19-9 (<35 KU/L)	12.45 ± 16.20	13.12 ± 15.81	0.624
CEA < 5 ng/L	2.17 ± 1.55	2.33 ± 0.99	0.021
AFP < 20 ng/L	2.81 ± 2.06	2.73 ± 2.36	0.655
IgG	14.84 ± 6.78	14.76 ± 7.59	0.891
IgA	2.86 ± 1.46	3.02 ± 1.31	0.192
IgM	1.64 ± 1.39	1.37 ± 0.75	0.000
Light chain quantitative κ (5.74-12.8 g/L)	7.87 ± 26.02	11.96 ± 8.27	0.283
Light chain quantitative L (2.69-6.38 g/L)	2.08 ± 3.87	86.07 ± 565.90	0.294

2. Materials and Methods

2.1. Methods

2.1.1. Study Population and Clinical Data. A total of 1002 patients who fulfilled the 2002 classification criteria [4] for pSS from the Rheumatology Department of the Second Affiliated Hospital of Shanxi Medical University between September 2013 and September 2017 were enrolled in this study. The study was approved by the Ethical Committee of the Second Affiliated Hospital of Shanxi Medical University (approval # 2016KY007). The study design conformed to the current National Health and Family Planning Commission of China ethical standards, with written informed consent provided by all patients.

Sjogren's syndrome without other autoimmune diseases is called pSS. pSS patients were diagnosed with clinical data as oral and ocular dryness, constitutional symptoms, vasculitis, and joint, skin, pulmonary, kidney, gastrointestinal tract, and endocrine involvement. The clinical observation items

included age, gender, course of disease, glandular symptoms (xerostomia and xerophthalmia), and extraglandular symptoms (arthritis, erythema, edema, and digestive, respiratory, and renal involvement). Routine laboratory examinations were performed including routine blood test, routine urine test, liver function examination, nephric function examination, erythrocyte sedimentation rate (ESR), cystatin C, and α_1-MG. Biochemical tests were performed using standard methods in a Beckman Coulter AU 5800 chemistry analyzer, and serum creatinine measurements were used by an IDMS-traceable method. Immunologic examinations which included anti-SSA, anti-SSB, and rheumatoid factors were performed using an immunoblotting method.

2.1.2. Assessment of Renal System Involvement. We identified those with clinically significant renal involvement.

Clinically significant renal involvement in pSS, either interstitial nephritis or GN, was defined by one or more of the following criteria:

(1) Renal tubular acidosis (RTA). Subtypes of RTA were determined as follows [5]: RTA type I (distal): hyperchloremic acidosis with a minimum urine pH ≥ 5.3 and low/normal plasma potassium (<5.5 mmol/L), based on reduced H+ secretion in the distal tubule; RTA type II (proximal): hyperchloremic acidosis with a minimum urine pH < 5.3 and low/normal plasma potassium (<5.5 mmol/L), based on reduced HCO3− reabsorption in the proximal tubule; and RTA type IV: hyperchloremic acidosis with a minimum urine pH < 5.3 and high plasma potassium (≥5.5 mmol/L), based on reduced H+ and K+ excretion in the distal tubule

(2) Kidney biopsy demonstrating histologic features compatible with glomerulonephritis, interstitial nephritis, or both

(3) Fanconi syndrome not associated with any known cause

(4) Elevated serum creatinine levels

(5) Proteinuria > 500 mg/24 hours

(6) Active urine sediment (>3 red blood cells per high-power field or red blood cell casts)

2.2. Statistical Analysis. Normally distributed variables were expressed as mean ± standard deviation (SD) and compared using independent samples t-test or one-way ANOVA. Nonparametric variables were expressed as medians and interquartile range (IQR) and compared using Mann–Whitney U or Kruskal–Wallis test. Categorical variables were compared using a $\chi 2$-test. To examine correlations between risk factors and renal involvement, univariate analyses were used, firstly based on biological plausibility and literature review. Variables with $P < 0.05$ in univariate analysis were then included in a multivariate analysis using logistic regression. Statistical significance was set at $P < 0.05$. All analyses were conducted using SPSS 22.0 statistical software packages.

Table 3: Pathological types of kidney in 12 PSS patients with renal involvement.

Pathological type	Case
Mild mesangial proliferative nephritis with subacute tubulointerstitial nephropathy	1
Stage III glomerulosclerosis of nodular sclerosing diabetes mellitus	1
Mild mesangial hyperplasia	1
Atypical membranous nephropathy	1
Changes of renal tubular injury during convalescence	1
Focal proliferative sclerosing glomerulonephritis	1
Focal proliferative IgA nephropathy	1
Subacute tubulointerstitial nephropathy	1
Mild mesangial proliferative nephritis with subacute tubulointerstitial nephropathy	1
Stages I-II membranous nephropathy	1
Chronic interstitial renal damage	1
Atypical membranous nephropathy with multiple crescents and acute tubular injury	1

Table 4: Features of renal involvement in primary Sjogren's syndrome patients.

Renal involvement	Numbers	Percentage (%)
Edema in the face	14	8.6
Edema of both lower limbs	42	25.9
Hypourocrinia	4	2.5
Frequent micturition	10	6.2
Urgency of urine	7	4.3
Hematuria	1	0.6
Diuresis	2	1.2
Nocturia	17	18.5
Interstitial nephritis	6	3.7
Renal tubular acidosis	12	7.4

Receiver operating characteristic (ROC) curves were plotted to explore the significance of multiple biomarkers for renal function in pSS. The differences among the areas under the receiver operating characteristic (ROC) curves (AUC) were calculated by MedCalc Software (version 15.2.0; MedCalc Software, Belgium).

3. Results

3.1. The Characteristics of pSS Patients with or without Renal Involvement. Demographic, clinical, histological, immunological, inflammatory feature, and outcome measure data were presented in Tables 1 and 2, collected from -162 pSS patients with and 840 without renal involvement. The female to male ratio in pSS patients is 779 : 61 (92.7%). Most patients presented to the hospital at 49 years old for the first interview, and an average disease course was approximately 5 years. Compared with pSS patients without renal involvement, those with renal involvement showed much higher

Table 5: Multivariate analysis of factors associated with renal involvement in primary Sjogren's syndrome.

Independent variables	Muitivariate analysis OR (95% Cl)	P value
Arthralgia	1.32 (0.79, 2.22)	0.294
Weak	1.83 (1.01, 3.31)	0.046
Poor appetite	1.52 (0.34, 6.74)	0.580
Edema in the face	3.33 (0.58, 19.25)	0.179
Edema of both lower limbs	9.16 (3.18, 26.39)	0.000
Hypourocrinia	3768741.41 (0.00)	0.999
Frequent micturition	2.30 (0.03, 197.13)	0.714
Urgency of urine	0.51 (0.01, 27.65)	0.740
Odynuria	1.46 (0.02, 87.33)	0.856
Hematuria	97021762.92 (0.00)	1.000
Polydipsia	2521.28 (0.00)	0.999
Diuresis	0.00 (0.00)	0.999
Digestive tract symptoms	3.06 (1.02, 9.22)	0.047
Respiratory system symptoms	0.83 (0.23, 3.01)	0.779
Congestion of throat	9.02 (0.16, 507.78)	0.285
Bilateral pleural effusion	16009499.05 (0.00)	0.999
RBC (3.5-5.5 × 10^{12}/L)	1.12 (0.70, 1.81)	0.637
Hb (110-150 g/L)	1.00 (0.99, 1.01)	0.831
Urine RBC	1.01 (1.00, 1.01)	0.015
AST/ALT	1.00 (0.68, 1.49)	0.987
Prealbumin	1.01 (1.00, 1.01)	0.026
Total protein (65-85 g/L)	0.99 (0.95, 1.04)	0.778
A/G	1.37 (0.28, 6.68)	0.699
Creatinine (44-133 μmol/L)	1.03 (1.01, 1.04)	0.000
Urea nitrogen (2.8-68.2 mmol/L)	0.97 (0.85, 1.10)	0.628
CO_2 CP (22-29 mmol/L)	0.95 (0.87, 1.03)	0.220
Cystatin C (0.1-0.3 mmol/L)	1.83 (1.16, 2.87)	0.009
α_1-MG (10-30 mg/L)	1.03 (1.00, 1.05)	0.021
Uric acid (90-420 μmol/L)	1.00 (1.00, 1.00)	0.323
β_2-MG (0.97-2.64 mg/L)	1.01 (0.96, 1.06)	0.805
Cl (99-110 mmol/L)	1.10 (1.03, 1.12)	0.004
Ca (2.08-2.6 mmol/L)	3.49 (0.47, 25.83)	0.221
Apolipoprotein A_1	0.56 (0.26, 1.20)	0.134
Lipoprotein-α	1.00 (0.99, 1.02)	0.508
Acid phosphatase (1-9 U/L)	1.00 (0.91, 1.09)	0.916
ESR	1.01 (1.00, 1.02)	0.126
Complement-C3 (30.8-82.01 g/L)	0.46 (0.15, 1.37)	0.161
IgM	0.91 (0.71, 1.16)	0.434

levels of prealbumin, anti-scl-70, rheumatoid factor (RF), anti-extractable nuclear antigen (anti-ENA), anti-SSA, anti-SSB, anti-SM, globulin, urea nitrogen, cystatin C, creatinine, α_1-MG, serum β2 microglobulin (β_2-MG), uric acid, Cl, lipoprotein-a, acid phosphatase, ESR, parathyroid hormone (PTH), and carcinoembryonic antigen (CEA), but reduced level of monocyte, anti-SSA, total protein, albumin, carbon dioxide combining power (CO2CP), Ca,

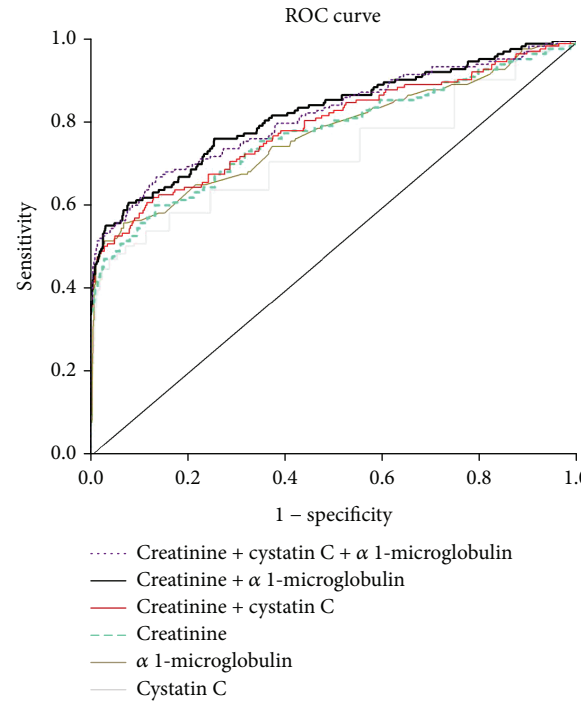

Figure 1

red blood cell (RBC), hemoglobin (Hb), apolipoprotein-A_1, immunoglobulin M (IgM), and complement-C3 ($P < 0.05$). Comparison of the two groups of clinical manifestations is shown in Tables 1 and 2.

3.2. The Characteristics of Renal Involvement in Primary Sjogren's Syndrome Patients. The SS patients with renal involvements showed glandular symptoms (xerostomia and xerophthalmia) and extraglandular symptoms (arthritis, erythema, edema, and digestive, respiratory, and renal involvement). Pathological features of patients with pSS with renal involvement are shown in Table 3. In the 12 biopsy patients with pSS with renal involvement, 6 cases had interstitial nephritis and 3 cases had mesangial glomerulonephritis. Three cases had membranous glomerulonephritis, one case diabetic nephropathy, and one case IgA nephropathy.

And the renal damage is shown in Table 4. The prevalence of edema of both lower limbs was higher than 20%. Meanwhile, the occurrences of hypourocrinia, frequent micturition, urgency of urine, hematuria, and diuresis were comparatively low.

3.3. Specific Factors Associated with Renal Involvement in pSS Patients. A series of indicators commonly used in clinical practice were selected first by univariate analysis and then logistic regression analysis as potential risk factors for renal involvement in pSS. As is shown in Tables 4 and 5, a series of variables were found to be associated with renal involvement. Compared with pSS patients without renal involvement, edema of both lower limbs and digestive tract involvement were important clinical manifestations ($P < 0.05$).

Table 6: AUC of creatinine, cystatin C, and α_1-MG.

	AUC	95% CI	P value
Creatinine	0.777	0.750-0.803	
Cystatin C	0.728	0.699-0.755	>0.05 (vs. creatinine)
α1-Microglobulin	0.775	0.748-0.801	>0.05 (vs. creatinine)
Creatinine+cystatin C	0.794	0.767-0.819	>0.05 (vs. creatinine)
Creatinine+α1-microglobulin	0.824	0.799-0.847	<0.05 (vs. creatinine)
Creatinine+cystatin C + α1-microglobulin	0.819	0.794-0.843	<0.05 (vs. creatinine)

AUC: area under the curve; CI: confidence interval.

There was a statistical significance in creatinine, cystatin C, α_1-MG, and chloridion between pSS patients with and without renal damage.

3.4. Comparison of ROC Curves and AUC of Creatinine, Cystatin C, and α_1-MG. To compare the significance of multiple indicators (creatinine, cystatin C, and α_1-MG) that had significant differences between the two groups in the identification of renal function, we have plotted ROC curves for these biomarkers (Figure 1). For the renal function biomarkers, there was no significant difference in the AUC for biomarkers (cystatin C, index: 0.728, CI 0.699-0.755; α_1-MG: 0.775, CI 0.748-0.801; and cystatin C+creatinine: 0.794, CI 0.748-0.801) compared with creatinine. The AUC of combination of creatinine+α_1-MG and creatinine+α_1-MG+creatinine were statistically larger than those of creatinine, and the biomarker of the biggest AUC is the combination of creatinine+α_1-MG (Table 6).

4. Discussion

There were 162 patients with renal involvement in this study, and the incidence rate was 16.17% (162/1002). In Goules's study, the prevalence of renal involvement was identified as 4.9% [6]. Another Chinese study also reported a relatively high incidence (33%) of renal abnormalities (based on biochemical abnormalities or kidney biopsy findings) in a study of 524 patients with PSS, 33% [7]. Because of a large number of study subjects in this work, our results suggest that the number of patients and geographical and ethnic factors might contribute to such variability.

PSS is characterized by B-cell activation with high serum IgG levels and a high frequency of autoantibodies [8]. In our study, pSS patients had multiple autoantibodies such as anti-SSA, anti-SSB, and ANA antibody, suggesting that pSS with renal abnormalities may be related to immune dysfunction. However, the pathological features of pSS with renal damage are the lymphocytic infiltration of the renal parenchyma rather than immune complex deposition and renal tubular atrophy that mainly presented interstitial nephritis mediated by an immune mechanism [9–11]. Although investigations about treatments targeting the immune factors participating in the progression of pSS show some positive outcome, more clinical trials were required before their application in human [12].

Among various manifestations of renal involvement, glomerular arterioles may be pathologically changed to glomerulonephritis, and a previous study showed that tubulointerstitial nephritis (TIN) is the most common presentation of renal involvement in the biopsy of pSS, which is consistent with our study [13].

Creatinine is primarily eliminated by glomerular filtration, and it can be used as a convenient means for estimating the glomerular filtration rate. Therefore, measurement of serum creatinine levels is the most common method used clinically for the routine monitoring of renal function [14]. Several studies have shown that serum cystatin C levels were more sensitive for detecting early and mild changes in renal function compared with the sensitivity of serum creatinine levels [15]. Serum cystatin C was produced at a constant rate by all nucleated body cells and was independent of age and gender [16–18]. Cystatin C was freely filtered at the glomerulus and was neither secreted nor reabsorbed by renal tubules [19]. Cystatin can reflect the decline of glomerular filtration rate that was the most direct indicator of renal damage, and it can be used as markers for early renal damage [20, 21]. In our study, the level of cystatin C showed a significant difference between patients with and without renal involvement and was identified as a potential risk factor for renal involvement, which was consistent with another study.

α_1-MG was described and isolated from the urine of patients with chronic cadmium poisoning in 1975 [22]. The biochemical characteristics and clinical application value of alpha-1-microglobulin have been studied by scholars. It is synthesized not only by lymphocytes in the human body [2] but also by the liver [23], and it widely exists in various body fluids and on the surface of lymphocytes. α_1-MG also is a stable urinary indicator protein which reflects acute and chronic dysfunctions of the proximal renal tubule. Our laboratory examination showed that the level of alpha-1-microglobulin in the pSS with renal damage group was significantly higher than that in the nonrenal damage group, which indicated the damage of proximal renal tubule and subsequent immune response to lymphocyte infiltration of the renal parenchyma in pSS. The combination of creatinine and α_1-MG had the best AUC, indicating that the combination of creatinine and α_1-MG was more effective in identifying renal function in pSS.

However, limitations of this study should be indicated. Firstly, the limited sample size, as well as bias caused by single-center analysis, should be considered, and secondly, as a cross-sectional study, it is limited to correlation analysis and unable to support strong causal conclusions. Therefore, to further evaluate the role of complement renal complications

in SS, more data from heterogeneous SS patients with consecutive follow-up are highly recommended.

5. Conclusions

Renal involvement is common in pSS patients. The combination of creatinine and α_1-MG is a better indicator of renal function for pSS patients, and close attention should be paid to it in clinical practice.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. There is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the manuscript.

Authors' Contributions

Jing Luo and Yu-Wei Huo contributed equally to this work.

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Research Article

Clinical Correlations of Novel Autoantibodies in Patients with Dry Eye

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Background. Diagnostic criteria for Sjögren's syndrome (SS) are continually being updated in pursuit of more precise and earlier diagnosis to prevent its complications. Owing to the high rate of false negative traditional serological markers, the need for better serological testing remains. *Objective*. To investigate the clinical significance of three recently discovered novel autoantibodies, anti-salivary gland protein 1 (SP1), anti-carbonic anhydrase 6 (CA6), and anti-parotid secretory protein (PSP), in a cohort of dry eye patients with suspected underlying inflammatory/autoimmune disease. *Methods*. Medical records of 136 patients with a primary diagnosis of dry eye who underwent laboratory testing between April 2014 and July 2017 were reviewed retrospectively. Data regarding demographic information, ocular and systemic symptoms, previous medical diagnoses, serological test results, and minor salivary gland biopsy results were collected. Dry eye evaluations included tear osmolarity, Schirmer test without anesthesia, conjunctival lissamine green staining, and corneal fluorescein staining in the order listed here. *Results*. Of the 136 patients, 9 (9/136, 6.6%) presented with a history of SS, and 9 additional patients (9/127, 7%) received a new diagnosis of SS as a result of evaluations. Fifty-six patients (56/136, 41%) tested positive for at least one of the novel autoantibodies. Fifty-four percent (6/11) of patients with primary SS who underwent the novel serological testing had a positive anti-PSP. Of those, 2 (2/11, 18%) had negative traditional serology and had to undergo minor salivary gland biopsy for definitive diagnosis. Anti-CA6 was associated with increased corneal and conjunctival staining after adjusting for age, sex, and other serologic markers (HR = 1.5, 95% CI = 1.20-1.97, and $p = 0.009$ and HR = 1.4, 95% CI = 1.04-1.76, and $p = 0.02$, respectively). *Conclusions*. This cross-sectional study demonstrated that anti-CA6 is seen in patients with severe aqueous-deficient dry eye. Whether these patients have an early stage of SS or a different type of autoimmune condition may be determined through longitudinal studies.

1. Introduction

Dry eye is a highly prevalent disease that affects up to 50% of the population worldwide [1]. Although dry eye is recognized as a multifactorial disease of the tears, inflammation has been identified as a key element in the pathogenesis [2]. About half of the patients with clinically significant dry eye have an underlying systemic inflammatory/autoimmune disease [3]. It is relevant to recognize an underlying autoimmune process, such as Sjögren's syndrome (SS), since timely diagnosis with adequate treatment can prevent possible ocular and/or systemic complications [3–6]. Approximately 1/10 patients with clinically significant dry eye has underlying SS. However, diagnosis is usually severely delayed largely due to a lack of awareness and the complexity of patient symptoms and signs [3–6]. A previous report from our center demonstrated that half of the SS patients with a vision-threatening ocular finding did not have an established diagnosis at the time of the presentation, despite having evidence of significant systemic manifestations [5]. Therefore, a high index of suspicion is necessary to recognize the disease earlier and prevent possible complications.

According to the 2012 American College of Rheumatology (ACR) classification criteria, significant dry eye must be present with either positive serology [anti-SSA and/or anti-SSB or a combination of rheumatoid factor (RF) and

antinuclear antibody (ANA) at a titer $\geq 1:320$] or a positive minor salivary gland biopsy to allow a diagnosis of SS [7]. Recently, classification criteria for SS have been updated and approved for the first time by both the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) [8]. The new set of criteria includes 5 items: (1) focal lymphocytic sialadenitis with focus score ≥ 1, (2) anti-SSA positivity, (3) ocular staining score (OSS) ≥ 5 at least in one eye, (4) Schirmer test ≤ 5 mm at least in one eye, and (5) unstimulated whole saliva flow rate ≤ 0.1 mL/min. The first two items have the highest weights, 3 points each, and the last three items a weight of 1 point each. A diagnosis of primary SS is tenable when the total score is 4 or more [8]. The combination of RF and ANA is no longer included in the criteria, and anti-SSB positivity has been removed from the criteria since positive anti-SSB in the absence of anti-SSA has no significant association with SS phenotypic features [8, 9]. Regardless of which set of criteria is used, a patient with dry eye and/or dry mouth findings but negative serology must undergo a minor salivary gland biopsy to confirm the diagnosis. Current traditional serological markers are limited in their utility with high rates of false negative results. For instance, anti-SSA antibodies are detected in only about 33-74% of patients with SS [10]. Thus, there is a need for better serological markers.

Various autoantibodies have been investigated regarding their utility in the diagnosis of SS. [11–13] Three novel autoantibodies, anti-salivary gland protein 1 (SP1), anti-carbonic anhydrase 6 (CA6), and anti-parotid secretory protein (PSP), have been suggested as useful markers to identify patients who are in the early stages of SS and perhaps with negative traditional antibodies [14, 15]. Although these novel autoantibodies were initially discovered in a mouse model, previous studies have explored their potential utility in humans [15–17]. We recently evaluated these antibodies in a small sample of dry eye patients in a prospective cross-sectional study and found that anti-CA6 was associated with severe aqueous-deficient dry eye indicating perhaps an early SS without positive traditional antibodies [17]. In this current study, a larger pool of dry eye patients with clinically significant aqueous tear deficiency who were suspected of having a systemic inflammatory/autoimmune disease and underwent a full battery of diagnostic testing was reviewed.

2. Materials and Methods

2.1. Patients. This retrospective study was approved by the Johns Hopkins University Institutional Review Board, and the study protocol adhered to the Health Insurance Portability and Accountability Act and the tenets of the Declaration of Helsinki. Patients who were examined at the Ocular Surface Diseases and Dry Eye Clinic, the Wilmer Eye Institute, the Johns Hopkins University School of Medicine, Baltimore, Maryland, with a primary diagnosis of dry eye and underwent serological testing for novel autoantibodies were considered for inclusion. The recommendation of serological testing for SS is based on the severity of dry eye (OSS ≥ 3 and/or Schirmer test ≤ 5 mm) or positive review of system items suggesting the presence of underlying systemic disease such as dry mouth, joint/muscle pain, or fatigue. In this analysis, patients with a primary diagnosis of other ocular diseases such as sterile keratitis, scleritis, or uveitis were not included. Patients having a recent history of ocular surgery, use of certain medications, or contact lens wear were not excluded. Nevertheless, none of the patients had a recent history of any ocular surgery. Six patients were on topical glaucoma medications, and none was a current contact lens wearer. A patient list was electronically generated using the test code QX206/T5307 for "Early Sjögren's Profile" (LAB26196) between April 2014 and July 2017. Medical records of patients were reviewed retrospectively. All available information was collected from each patient's chart regarding demographics; dry eye-related symptoms; SS-related systemic symptoms such as dry mouth, joint/muscle pain, fatigue, diagnosis of SS, and other associated autoimmune diseases; clinical signs of dry eye; serological test results; and minor salivary gland biopsy findings.

2.2. Evaluation of Dry Eye. Patients were evaluated by a single ophthalmologist (EKA) in a uniform manner. A complete medical history and review of systems were performed first. Dry eye was assessed using tear osmolarity, Schirmer test, and ocular surface staining, in the order mentioned here. Tear osmolarity was measured using the TearLab Osmolarity System (TearLab Corporation Inc., San Diego, CA) according to the manufacturer's recommendations [18]. The Schirmer test was performed without topical anesthesia using sterile strips (TearFlo, Sigma Pharmaceuticals, Monticello, IA), and the amount of paper wetting was measured in mm after 5 minutes. Ocular surface staining was performed using lissamine green dye for the conjunctiva (GreenGlo, HUB Pharmaceuticals LLC., Rancho Cucamonga, CA) and fluorescein for the cornea (Ful-Glo, Akorn Inc., Lake Forest, IL). Corneal staining was evaluated using a cobalt blue filter, and conjunctival staining was evaluated using a neutral density filter. Ocular surface staining score was calculated for the cornea and conjunctiva separately and then summed for a total OSS for each eye according to the Sjögren's International Collaborative Clinical Alliance (SICCA) grading system [19]. The maximum possible corneal staining score was 6 (the punctate epithelial erosions grade between 0 and 3 plus any extra points for modifiers such as central corneal staining, confluent staining, and filaments). Nasal and temporal conjunctiva staining was graded separately with a maximum score of three for each area, for a total of 6. The maximum possible OSS was 12 for each eye [19].

2.3. Laboratory Tests. Venous blood samples of patients were collected at the Johns Hopkins Medical Laboratories for serological testing. Testing for RF, ANA, and antibodies to SSA and SSB was performed at the Johns Hopkins Medical Laboratories. Additional venous blood samples were sent to the IMMCO Diagnostics Lab (Buffalo, NY) for serological testing for novel autoantibodies (SP1, CA6, and PSP). The presence of IgG, IgA, and IgM antibodies to SP1, CA6, and PSP was each reported individually. Whenever testing for any of the three isotypes was above the normal range,

the result was considered positive. The cutoff for positivity for ANA was 1 : 320.

2.4. Statistics. The worse eye values for dry eye measures (higher osmolarity, lower Schirmer's value, and higher OSS) were used for the data analysis. The *t*-test and analysis of variance (ANOVA) were used to compare the continuous variables, and the chi-square test was used to compare categorical variables between groups. Spearman's rank correlation coefficient was used to analyze the association between a continuous and binary variable, and the phi coefficient was used to analyze the association between two binary variables. Logistic regression models were used to quantify the associations between serological markers and clinical measures after adjustment for potential confounders such as age, sex, and other serological markers. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY).

3. Results

One hundred and thirty-six patients with a primary diagnosis of dry eye underwent serological testing for novel autoantibodies between April 2014 and July 2017 and were included in the analysis. Nine of these patients had a prior patient-reported history of SS at the time of the testing, 5 primary SS and 4 secondary (3 with RA and 1 with mixed connective tissue disease). Testing was repeated to confirm the diagnosis in these patients. Fourteen patients were previously tested and had negative results, and the testing was repeated based on high clinical suspicion. The remaining 113 patients had never been tested for SS before. Of the 113 patients, 12 had a known history of inflammatory systemic disease (RA = 7, psoriasis = 4, and seronegative spondyloarthropathy = 1). As a result of the initial evaluation, 9 patients (9/127, 7%) received a new diagnosis of SS, 6 primary SS and 3 secondary SS (2 with RA and 1 with psoriatic arthritis). All but one received a new diagnosis of SS based on positive serology. The only seronegative patient needed to undergo a lip biopsy to be classified as having primary SS.

The mean age of patients was 59.8 ± 11.7 years, and a greater proportion of patients were female (85%) with a female-to-male ratio of 5 : 1. The most commonly reported dry eye-related symptom was foreign body sensation/grittiness (91/136, 67%), followed by burning/tearing (70/136, 51%), light sensitivity (55/136, 40%), blurred vision (52/136, 38%), and eye pain (43/136, 32%). Fifty-six patients had at least one SS-related extraocular symptom (dry mouth, joint/muscle pain, or fatigue), with dry mouth being the most commonly reported symptom (43/136, 32%). The average value of tear osmolarity was 308 ± 18 mOsm, Schirmer test was 7.8 ± 7.2 mm, and OSS was 6.2 ± 3.4 (cornea score, 2.6 ± 1.8, and conjunctiva score, 3.6 ± 2.2). Thirty-nine patients (29%) had Schirmer test score ≤ 5 mm, 83 patients (61%) had OSS ≥ 5, and 100 patients (73%) had either Schirmer test ≤ 5 mm or OSS ≥ 5.

Fifty-six patients (56/136, 41%) tested positive for at least one of the novel autoantibodies, 21 had anti-CA6 alone, 15 had anti-PSP alone, 9 had anti-SP1 alone, 6 had both anti-SP1 and anti-CA6, 3 had both anti-SP1 and anti-PSP, 1 had both anti-CA6 and anti-PSP, and 1 had all three of them (Figure 1).

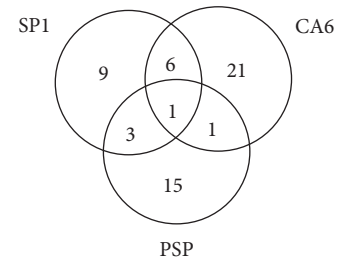

Figure 1: Venn diagram showing the number of patients with positive novel autoantibody.

With regard to demographic or clinical characteristics, no significant difference was found between patients with positive versus negative novel autoantibodies as shown in Table 1.

Table 2 displays demographic and clinical characteristics of patients according to autoimmune disease status including patients who had a known diagnosis of SS prior to the testing as well as those who received a new diagnosis of SS based on the test results.

Novel autoantibodies were detected in 8 of the 11 patients with primary SS (73%), 1 of the 7 patients with secondary SS (14%), and 10 of the 21 patients with other autoimmune diseases in the absence of SS diagnosis (48%). In addition, 37 of the 97 (38%) patients with no known autoimmune diseases at the time of the testing were positive for at least one novel autoantibody. Anti-PSP was the most frequently detected novel autoantibody in patients with primary SS (6/8, 75%), while anti-CA6 was the most commonly detected novel autoantibody in patients with other autoimmune diseases (5/10, 50%) and with no known autoimmune diseases (21/37, 57%). Two of the 11 patients with primary SS were seronegative, and both tested positive for anti-PSP.

Anti-PSP was the only novel autoantibody that correlated with having primary SS ($\phi = 0.33$, $p < 0.001$). Correlations between the antibody status and the severity of dry eye measures are shown in Table 3. Based on logistic regression analysis after adjustment for age, sex, and other serologic markers, anti-CA6 showed a significant association with both corneal and conjunctival staining scores (HR = 1.5, 95% CI = 1.20-1.97, and $p = 0.009$ and HR = 1.4, 95% CI = 1.04-1.76, and $p = 0.02$, respectively), and ANA showed a significant association with corneal staining score (HR = 1.7, 95% CI = 1.04-2.65, and $p = 0.03$). (Table 4).

4. Discussion

This retrospective study evaluated the clinical relevance of the three novel autoantibodies, anti-SP1, anti-CA6, and anti-PSP, in patients with clinically significant aqueous-deficient dry eye who were suspected of having an underlying autoimmune disease, particularly SS. Our results demonstrate that anti-PSP was the most prevalent of the novel

Table 1: Demographic and clinical characteristics of patients according to novel autoantibody status.

	Novel antibody positive ($n = 56$)	Novel antibody negative ($n = 80$)	p value
Demographics			
Age, years, mean (SD)	59.0 (10.5)	60.4 (12.4)	0.50
Female, n (%)	49 (87%)	66 (82%)	0.48
Dry eye-related symptoms, n (%)			
Light sensitivity	22 (39%)	33 (41%)	0.86
Foreign body sensation/grittiness	39 (70%)	52 (65%)	0.58
Burning/tearing	27 (48%)	43 (54%)	0.60
Eye pain	18 (32%)	25 (31%)	0.99
Blurred vision	22 (39%)	30 (37%)	0.86
Dry eye measures, mean (SD)			
Tear osmolarity (mOsm/L)	309 (22.6)	307 (14.6)	0.61
Schirmer test (mm)	6.6 (5.7)	8.5 (7.8)	0.27
Schirmer test ≤ 5 mm, n (%)	19 (63%)	20 (43%)	0.07
Total OSS (0-12)	6.4 (3.3)	6.0 (3.5)	0.56
Corneal staining (0-6)	2.7 (1.8)	2.5 (1.9)	0.57
Conjunctival staining (0-6)	3.7 (2.0)	3.6 (2.3)	0.74
OSS ≥ 5, n (%)	35 (66%)	48 (67%)	0.94
Schirmer ≤ 5 mm or OSS ≥ 5, n (%)	42 (78%)	58 (76%)	0.84
SS-Related Symptoms, n (%)			
Dry mouth	18 (32%)	24 (30%)	0.85
Joint/muscle pain	12 (21%)	12 (15%)	0.37
Fatigue	6 (11%)	10 (12%)	0.79
Autoimmune Diseases			
Sjögren's syndrome	9 (16%)	9 (11%)	0.41
Primary	8 (14%)	3 (4%)	0.051
Secondary	1 (2%)	6 (7%)	0.24
Other autoimmune diseases	10 (18%)	11 (14%)	0.57
SS diagnostic parameters			
Anti-SSA	6 (11%)	7 (9%)	0.68
Anti-SSB	2 (4%)	3 (4%)	>0.99
RF	7 (13%)	7 (9%)	0.46
ANA ≥ 1 : 320	7 (13%)	7 (9%)	0.46
Positive biopsy	1/6 (17%)	1/7 (14%)	>0.99

Results are represented as mean (standard deviation) for continuous variables and number (percentage) for binary variables. The t-test was used for comparison of continuous variables and chi-squared testing for categorical variables between groups. SD: standard deviation; OSS: ocular staining score; SSA: Sjögren's syndrome-related antigen A; SSB: Sjögren's syndrome-related antigen B; RF: rheumatoid factor; ANA: antinuclear antibody.

autoantibodies in patients with primary SS (6/11, 54%). Also, anti-PSP was detected in 2 seronegative SS patients with definitive diagnosis. On the other hand, anti-CA6 was the most prevalent of the novel autoantibodies in patients without any known autoimmune disease and the only novel autoantibody associated with severe ocular surface staining (both corneal and conjunctival). These findings are consistent with our previous report [17] and support the theory that anti-CA6 may be a marker indicating early stages of SS or another form of an autoimmune dry eye.

Inflammation regulated by both innate and adaptive immune systems plays a crucial role in ocular surface damage due to SS-related dry eye [2]. Although not fully known, an adaptive immune response to autoantigens is thought to be the triggering mechanism in SS [20]. Thus, understanding the role of autoantigens in the pathogenesis will influence the diagnosis and management of the disease. Antibodies to SSA antigens, components of a ribonucleoprotein complex, were the most commonly detected antibodies in patients with SS and currently the only serologic marker included in the most recent classification criteria for SS [8]. Other autoantibodies have been implicated in playing a role in the pathogenesis of SS, but none are currently included in the diagnostic criteria [20]. Three antigens, SP1, CA6, and PSP, are selectively expressed in salivary and lacrimal glands as opposed to the SSA antigen that can be expressed in any cell with a nucleus [15]. In fact, anti-SSA antibodies can be detected in other autoimmune diseases such as systemic

Table 2: Demographic and clinical characteristics of patients according to autoimmune disease status.

	Primary SS ($n = 11$)	Secondary SS ($n = 7$)	Other autoimmune disease ($n = 21$)	No known autoimmune disease ($n = 97$)	p value
Demographics					
Age (years), mean (SD)	58 (8.4)	61 (15.5)	58 (15.3)	60 (10.9)	0.82
Female, *n* (%)	11 (100%)	7 (100%)	18 (86%)	79 (81%)	0.26
SS-related symptoms, n (%)					
Dry mouth	3 (27%)	2 (29%)	8 (38%)	30 (31%)	0.91
Joint/muscle pain	2 (18%)	0	2 (9%)	20 (21%)	0.38
Fatigue	2 (18%)	1 (14%)	1 (5%)	13 (13%)	0.67
Diagnostic parameters, n (%)					
Anti-SSA	9 (82%)	4 (57%)	0	0	
Anti-SSB	3 (27%)	2 (29%)	0	0	
RF	5 (46%)	4 (57%)	3 (14%)	2 (2%)	
ANA ≥ 1 : 320	6 (55%)	3 (42%)	3 (14%)	2 (2%)	
Positive biopsy	2/3 (67%)	0/1 (0%)	0/2 (0%)	0/7 (0%)	
Novel autoantibodies, n (%)	8 (73%)	1 (14%)	10 (48%)	37 (38%)	0.06
Anti-SP1	3 (27%)	1 (14%)	2 (9%)	13 (13%)	0.57
Anti-CA6	3 (27%)	0	5 (24%)	21 (22%)	0.53
Anti-PSP	6 (54%)	0	4 (19%)	10 (10%)	**0.001**
Dry eye measures, mean (SD)					
Tear osmolarity (mOsm/L)	323 (33.7)	308 (17.4)	307 (17.7)	307 (14.5)	0.054
Schirmer test (mm)	9.0 (8.1)	4.8 (3.6)	7.7 (11.5)	7.9 (6.7)	0.79
Schirmer test ≤ 5 mm, *n* (%)	5 (71%)	2 (67%)	7 (78%)	25 (43%)	0.14
Total OSS (0-12)	8.3 (3.3)	8.6 (1.8)	7.2 (2.7)	5.5 (3.5)	**0.003**
Corneal staining (0-6)	3.3 (1.8)	3.4 (1.5)	2.9 (1.9)	2.3 (1.8)	0.15
Conjunctival staining (0-6)	5.0 (1.8)	5.1 (1.2)	4.4 (1.6)	3.2 (2.3)	**0.002**
OSS ≥ 5, *n* (%)	6 (60%)	4 (67%)	13 (68%)	60 (72%)	0.97
Schirmer ≤ 5 mm or OSS ≥ 5, *n* (%)	8 (73%)	5 (83%)	17 (85%)	70 (75%)	0.77

Results are represented as mean (standard deviation) for continuous variables and number (percentage) for binary variables. The one-way analysis of variance (ANOVA) was used for comparison of continuous variables and chi-squared testing for categorical variables between groups. SS: Sjögren's syndrome; SD: standard deviation; SSA: Sjögren's syndrome-related antigen A; SSB: Sjögren's syndrome-related antigen B; RF: rheumatoid factor; ANA: antinuclear antibody; SP1: salivary protein 1; CA6: carbonic anhydrase 6; PSP: parotid secretory antigen; OSS: ocular staining score.

Table 3: Correlations between positive antibody status and dry eye measures.

		Anti-SSA	Anti-SSB	RF	ANA	Anti-SP1	Anti-CA6	Anti-PSP
Tear osmolarity	Rho	0.08	-0.06	0.05	0.03	-0.01	-0.08	0.05
	p value	0.39	0.52	0.63	0.80	0.92	0.40	0.60
Schirmer test	Rho	0.06	-0.01	-0.12	0.03	0.13	-0.06	-0.08
	p value	0.62	0.91	0.30	0.83	0.26	0.62	0.49
Corneal staining	Rho	**0.18**	**0.22**	**0.27**	**0.28**	-0.10	**0.21**	0.02
	p value	**0.04**	**0.01**	**<0.001**	**<0.001**	0.26	**0.02**	0.79
Conjunctival staining	Rho	**0.24**	0.13	**0.22**	**0.18**	-0.09	**0.19**	-0.03
	p value	**0.01**	0.15	**0.01**	**0.04**	0.31	**0.03**	0.72
Total OSS	Rho	**0.25**	**0.20**	**0.29**	**0.27**	-0.10	**0.26**	0.00
	p value	**0.01**	**0.02**	**<0.001**	**<0.001**	0.29	**<0.001**	0.97

Spearman correlation coefficient (rho) was used to analyze the associations between variables. Bolded values represent $p < 0.05$. SSA: Sjögren's syndrome-related antigen A; SSB: Sjögren's syndrome-related antigen B; RF: rheumatoid factor; ANA: antinuclear antibody; SP1: salivary protein 1; CA6: carbonic anhydrase 6; PSP: parotid secretory antigen; OSS: ocular staining score.

Table 4: Multiple regression analysis demonstrating associations of antibody positivity with clinical parameters.

	Anti-SSA	Anti-SSB	RF	ANA	Anti-SP1	Anti-CA6	Anti-PSP
Tear osmolarity (mOsm/L)	1.0 (0.95-1.05)	1.6 (0.00-1.552E+83)	1.0 (0.98-1.07)	1.0 (0.95-1.03)	1.0 (0.96-1.03)	1.0 (0.96-1.03)	1.0 (0.99-1.05)
Schirmer test (mm)	1.2 (0.99-1.49)	0.3 (0.00-.)	0.7 (0.40-1.17)	1.1 (0.94-1.28)	1.1 (0.98-1.21)	0.9 (0.84-1.05)	0.9 (0.77-1.06)
Corneal staining (0-6)	0.8 (45-1.60)	2.1 (0.00-1358.86)	1.8 (0.97-3.29)	**1.7 (1.04-2.65)**	0.7 (0.49-1.02)	**1.5 (1.20-1.97)**	1.1 (0.80-1.46)
Conjunctival staining (0-6)	1.3 (0.68-2.49)	0.5 (0.06-4.63)	1.3 (0.83-1.97)	1.1 (0.78-1.63)	0.8 (0.62-1.06)	**1.4 (1.04-1.76)**	1.0 (0.77-1.29)
Total OSS (0-12)	1.0 (0.71-1.50)	0.83 (0.16-4.40)	1.3 (0.96-1.77)	1.2 (0.96-1.60)	0.8 (0.67-0.99)	**1.3 (1.08-1.53)**	1.0 (0.88-1.24)

Each clinical measure was analyzed in a separate logistic regression model including each antibody as the dependent variable and the clinical measure, age, sex, and other serologic markers as independent variables. Values represent hazard ratios and 95% confidence intervals. Bolded values represent $p < 0.05$. SSA: Sjögren's syndrome-related antigen A; SSB: Sjögren's syndrome-related antigen B; RF: rheumatoid factor; ANA: antinuclear antibody; SP1: salivary protein 1; CA6: carbonic anhydrase 6; PSP: parotid secretory antigen; OSS: ocular staining score.

lupus erythematosus or primary biliary cirrhosis without coexistent SS [21]. The animal models of SS suggest that SS starts as an organ-specific disease [14]. Therefore, detecting antibodies to salivary and/or lacrimal gland-specific antigens may, in fact, indicate early stages of the disease.

In our study, anti-PSP antibodies were detected more prominently in patients with primary SS including two patients who were seronegative at the time of the testing. PSP is one of the major secretory proteins of the parotid gland and functions as an antimicrobial agent to protect tissue surfaces exposed to the external environment [22]. Its abnormal expression has been shown in the submandibular glands as well as in lacrimal glands of an animal model of autoimmune sialadenitis [22]. To the best of our knowledge, the presence of anti-PSP antibodies alone has not been previously reported in patients with an established diagnosis of primary SS. In a previous study, novel autoantibodies were investigated in the sera of patients from the SICCA cohort and patients were grouped according to their focus score determined from minor salivary gland biopsy as the indicator of disease severity [15]. Neither anti-SP1 nor anti-PSP was detected at a level that was significantly higher in any of the study groups [15]. In our study, the biopsy information was available in one of the two seronegative patients who tested positive for anti-PSP and focus score was determined as 3.4 per 4 mm^2. Although the number of patients with SS was considerably smaller in our study ($n = 18$) and biopsy information was not available for the majority of patients, we believe that significance of anti-PSP in patients with primary SS should be further investigated in future studies with larger sample size.

Anti-CA6 deserves particular attention in the present study since it was the most prevalent of the novel autoantibodies. More importantly, anti-CA6 was more frequent in patients with no known autoimmune diseases at the time of the testing. Similarly, in our previous report, we detected anti-CA6 antibodies in 43% of the patients who had significant dry eye but negative serology and biopsy (thus not fulfilling 2012 ACR classification criteria for SS) [17]. Higher levels of anti-CA6 were also demonstrated in patients from the SICCA cohort who had significant dry eye and dry mouth but no lymphocytic focus [15]. Carbonic anhydrases are responsible for regulation of acid-base balance in both physiological and pathological states [23]. CA6 is the only secretory isoenzyme of the carbonic anhydrase enzyme family expressed by parotid and submandibular glands as well as lacrimal glands [23–25]. Cytosolic CA6 is responsible for electrolyte and water secretion by the acinar cells in both salivary and lacrimal glands. CA6 in the secretory granules, on the other hand, is discharged into the acinar lumen to maintain bicarbonate levels to regulate pH in tear fluid and protect the corneal and conjunctival epithelial cells against injuries [25]. The significant association that we found between anti-CA6 and severe ocular surface staining can be explained either by reduced secretion or by changes in pH of the tear film which make epithelial cells vulnerable. The latter makes more sense as there was no significant association between anti-CA6 and decreased tear volume. More studies are needed to explain these findings further.

Previous studies demonstrated increased levels of anti-SP1 antibodies in patients with SS, particularly in patients with secondary SS in the setting of RA [26]. Anti-SP1 antibodies were not notably prevalent in our study; however, only one in 7 patients with secondary SS tested positive for novel autoantibodies, which was anti-SP1, and this only patient had SS secondary to RA. SP1 is a murine protein expressed by both submandibular and lacrimal glands [27]. The human homolog of this protein was not known, but recently, human common SP1 was identified in the saliva of patients with periodontitis at higher levels compared to healthy individuals [23]. This protein is known to be highly expressed in stressed conditions and regulates the oral microflora through its antimicrobial activity [28]. In a recent study, anti-SP1 antibodies were predominantly detected in patients with the Schirmer test measured between 3 and 6 mm, while anti-CA6 antibodies were predominantly detected in patients with the Schirmer test less than 3 mm [29]. Since the recent classification criteria for SS require the cutoff value of 5 mm for the Schirmer test [8], we looked for correlations between the presence of certain antibodies and having the Schirmer test 5 mm or less. In our study, anti-SP1 was the only antibody correlated with having a Schirmer test ≤ 5 mm.

A recently published report on these novel autoantibodies in participants of the DREAM Study demonstrated a higher prevalence of anti-SP1 in patients with SS-related dry eye compared to patients with non-SS dry eye (33% vs. 19%) [14]. Of note, aside from using a different set of criteria for dry eye in the DREAM Study, it was not specified whether the SS group included patients with primary SS, secondary SS, or both. [14] In our study, the prevalence of anti-SP1 in patients with both primary and secondary SS was 22% while it was 13% in patients with non-SS dry eye. The most prevalent novel autoantibody in patients with both primary and secondary SS was still anti-PSP (33%).

We cannot stress enough the importance of a heightened suspicion for underlying autoimmune disease in patients with significant dry eye. As a result of the initial testing, we were able to newly diagnose nine additional patients (7%), 2 of whom tested negative previously. In a study from our clinic, 12 patients received a new diagnosis of SS which corresponded to 6% of the patients who were evaluated for an underlying SS [4]. These rates are in line with previous studies [5, 16]. Furthermore, the necessity of further evaluations in patients with previous negative workup has been emphasized in earlier reports [5]. In our study, 8 in 13 patients with a previous negative workup for SS tested positive for at least one novel autoantibody while no traditional autoantibody was positive. Anti-CA6 was the most prevalent of novel autoantibodies detected in these patients. If these antibodies are indicating early stages of SS, the evolution of the disease from an organ-specific level to a systemic disease can be explained by the secondary immune response theory. Autoantigens expressed in specific tissues are targeted by the antibodies, and damage releases other autoantigens triggering secondary immune responses which may sustain the autoimmune disease [30]. First antigens, as well as the antibodies against them, could disappear in time as a result of the complete destruction of targeted tissues at the beginning [20]. This could perhaps explain why some markers disappear in time while others appear later on. Alternatively, autoimmunity towards SSA antigens can be induced by a protein that shares a sequence [20]. Further studies can shed light on this theory to understand the roles of these antigens in the pathogenesis.

One of the limitations of this study is the retrospective nature of the data collection which may have caused information bias due to missing information or measurement error. Another limitation is the small number of patients with SS, resulting in lower statistical power for the analysis in this group. Since minor salivary gland biopsy information was not available for the majority of the patients, it is not known whether seronegative patients definitively did not have SS at the time of the testing. Further, it was not possible to determine who would eventually develop SS given a longer follow-up due to the cross-sectional design of this study. Therefore, our findings should be interpreted cautiously.

5. Conclusions

Uncovering an underlying inflammatory/autoimmune disease in patients with dry eye is clinically relevant. SS is highly prevalent in a dry eye population but frequently underdiagnosed due to not only underappreciation of the disease but also the complexity of the clinical findings and difficulties with the currently available diagnostic testing. Whether patients with positive novel autoantibodies represent early stages of SS or another type of autoimmune dry eye deserves longitudinal studies with larger sample size.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper. No funding or donation in kind was received from IMMCO Lab (Buffalo, NY).

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Research Article

Association between B Cell Growth Factors and Primary Sjögren's Syndrome-Related Autoantibodies in Patients with Non-Hodgkin's Lymphoma

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Despite the overall success of using R-CHOP for the care for non-Hodgkin's lymphoma patients, it is clear that the disease is quite complex and new insight is needed to further stratify the patient for a better personized treatment. In current study, based on previous studies from animal model, new panels combining well-established cytokine (BAFF) and autoantibodies (anti-SSA/Ro) with newly identified cytokine (IL14) and autoantibodies (TSA) were used to evaluate the association between B cell growth factor and Sjögren's related autoantibodies in NHL patients. The result clearly indicates that there was a unique difference between BAFF and IL14 in association with autoantibodies. While serum BAFF was negatively associated with the presence of both traditional anti-SSA/Ro and novel TSA antibodies in GI lymphoma patient, IL14 was positively associated with the presence of both traditional anti-SSA/Ro and novel TSA antibodies in non-GI lymphoma patient. Long-term follow-ups on these patients and evaluation of their response to the R-CHOP treatment and recurrence rate will be very interesting. Our result provides a solid evidence to support using novel diagnostic panel to better stratify the NHL patients.

1. Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltrates of salivary and lacrimal glands, leading to oral and ocular dryness and increase of autoantibody secretion [1]. The term primary Sjögren's syndrome (pSS) was used to define SS without the presence of other systemic autoimmune diseases. Occurrence of lymphoma is one of the most severe complications of pSS; about 5% of patients with pSS will develop non-Hodgkin lymphomas (NHL) [2–4], with an estimated risk up to 44 times greater than normal population [5]. Investigation of the pathophysiology of lymphomagenesis associated with the development of pSS will provide a new insight for a better knowledge of the underlying mechanisms for autoimmunity and lymphomagenesis in general, which might lead to the identification of novel treatment targets for autoimmune diseases and lymphoma.

B cells play a central role to the pathogenesis of primary SS, which is characterized by polyclonal B cell hyperactivity, and later switch on monoclonal B cell expansion that results in the development of B cell lymphoma in pSS patients. The correlation of B cell growth factors with pSS has been well established. BAFF (also termed TNFSF13B) promotes B cell maturation, proliferation, and survival. BAFF transgenic mice develop features of SLE and later clinical characteristics of pSS, such as sialadenitis; 3% of these mice develop lymphoma when aged [6]. Recent studies have also demonstrated that BAFF might also be involved in the occurrence of lymphoma in pSS patients [7].

The presence of autoantibodies is one of the several hallmarks of Sjögren's syndrome; the detection of serum autoantibodies has a central role in the diagnosis and classification of Sjögren's syndrome [8]. Anti-SSA/Ro is the most common autoantibodies found in patients with pSS that directed against the autoantigens Ro/La ribonucleoprotein complex.

Previous study has demonstrated that serum BAFF levels were enhanced and correlated with levels of anti-SSA/Ro, anti-SSB/La, and RF in pSS patients [9]. Clinical trial based on the monoclonal antibody target BAFF is currently underway to evaluate the efficacy of blocking BAFF for the treatment of pSS and lymphoma.

Recently, another B cell growth factor has been shown to play an important role in the pathophysiology of pSS. IL14, also known as taxilin, was initially identified as a high molecular weight B cell growth factor which can promote B cell proliferation, especially of B cells within the GC [10]. IL14α transgenic mice present both clinical and biological characteristics of pSS [11]. When aged, 95% of IL14α transgenic mice develop B cell lymphomas in the gastrointestinal tract with histological features of a large B cell lymphoma. Novel tissue-specific autoantibodies (TSA), which include antisalivary gland protein 1 (SP1), anticarbonic anhydrase 6 (CA6), and antiparotid secretory protein autoantibodies (PSP), were first identified from IL14α transgenic mouse (IL14αTG) and have been found in patients with SS both together and without anti-SSA/Ro, as well as in patients with idiopathic dry mouth and dry eye disease [12]. It was proposed that TSA antibodies may be useful for identifying early SS, particularly among patients who are anti-SSA/Ro negative. While studies on IL14α transgenic mice model have led to interest insight about the development of pSS and lymphoma, no clinical studies has been done to evaluate the associations between IL14 and novel autoantibodies in the context of autoimmunity and lymphomagenesis.

In current study we will for the first time investigate the associations between B cell growth factors and primary Sjögren's syndrome-related autoantibodies in patients with non-Hodgkin's lymphoma.

2. Materials and Methods

2.1. Patients. A total of 139 patients with NHL were enrolled from the Department of Hematology and Department of Gastrointestinal Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan. These patients were diagnosed in accordance with WHO classification and mainly on the basis of histopathological and immunohistochemical findings of biopsy samples. Also included were 8 healthy volunteers, with informed consent obtained in writing. Sera were obtained and tested after informed consent from 118 NHL patients. Peripheral blood leukocytes were collected from the rest of 21 NHL patients and 8 health controls. The study protocol was approved by Tongji Medical College IRB committee.

2.2. Western Blot Assays. Western blot assays were run following the manufacturer's instruction. In brief, a total of 4 μl of diluted serum (1 : 100 dilution with PBS) were mixed with 20 μl PBS and 6 μl 5 × loading buffer (Thermo Fisher, Carlsbad, CA). Samples were denatured at boiled water for 8 minutes before loading to 10% dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a nitrocellulose membrane. The membrane was incubated with blocking buffer (skim milk) for 2 h at room temperature and incubated overnight at 4°C with 1 : 1000 primary antibodies in blocking buffer. After washed three times for 10 min each with TBST (0.02% Tween in TBS) (TBS; 20 mM Tris-HCl, pH 7.4, and 150 mM NaCl), the membrane was incubated with the horseradish peroxidase-labeled goat anti-mouse IgG secondary antibody (diluted 1 : 5000 in blocking buffer) for 2 h at room temperature. The membrane was washed three times for 10 min each with TBST before Pierce ECL Western blotting substrate kit containing goat anti-rabbit IgG conjugate with horseradish peroxidase (Thermo Scientific, Waltham, MA) which was used to visualized the result in a Machine (Bio-Rad, Hercules, CA).

2.3. Determine the Relative Intensity Ratio for Serum IL14α Level after WB Assay. For comparison purpose, an internal positive control was used throughout the whole study for normalization purpose between different batches of gels. Based on internal control, a ratio of mean density value can be calculated to reflect the actual relative expression level of IL14α.

2.4. ELISA. For serum BAFF level, ELISA was run following the manufacturer's instruction (R&D Systems, Minneapolis, MN). For Sjögren's related autoantibodies (Ro, La, SP1, PSP, and CA6), ELISA kits were purchased from Trinity Biotech, Buffalo, NY.

2.5. Quantitative PCR. Fresh blood was obtained from patients with NHL and normal control. Total RNAs are isolated from the blood using QIAamp RNA Blood Mini kit (Qiagen) following the manufacturer's instructions. cDNA was produced from total RNA using the SuperScript first-strand synthesis system for RT-PCR according to the manufacturer's instructions (Thermo Fisher, Carlsbad, CA). Quantitative PCR (qPCR) reaction was set up using SYBR Select Master Mix purchased from Thermo Fisher (Carlsbad, CA) following the manufacturer's instructions. qPCR was run on an StepOnePlus real-time PCR system (Life Technologies, Carlsbad, CA) with the following program: 95°C for 30 s hold, 40 cycles of 95°C, 5 s and 60°C, 31 s. Melting curve analysis was performed: 95°C, 15 s, 60°C, 60 s, 95°C, 15 s. The primers for IL14 were forward primer 5′-TCAC AGAAGCGCCTTGCTA-3′ and reverse primer 5′-CCAG TCTGGCCTGATGCTT-3′, and for 18S rRNA control were forward primer 5′-CGCGGTTCTATT TTGTTGGT-3′ and reverse primer 5′-AGTCGGCATCGTTTATGGTC-3′.

2.6. Statistical Analysis. Data were statistically analyzed with SPSS (version 16.0) or Prism (version 6.0, GraphPad software). Data are presented as mean ± standard deviation (SD). To compare the difference between two groups, unpaired two-tailed Student's test was performed. Pearson correlation coefficient was used to analyze the correlations between two variables. P values < 0.05 were considered statistically significant.

Table 1: Basic clinical characteristics of study groups.

Group	Total case number	Sex (male/female)	Age
B cell lymphoma	15	11/4	39.92 ± 17.70
Non-B cell lymphoma	6	4/2	45.00 ± 10.63
Healthy control	8	6/2	27.25 ± 3.37

3. Results

3.1. Expression of IL14α Gene in Peripheral Blood Leukocytes of Lymphoma Patients. First, peripheral blood leukocytes were collected from 3 groups of patients (Table 1), and real-time PCR assay was run to evaluate the expression of IL14α gene in the peripheral blood leukocytes of lymphoma patients compared to healthy control.

As shown in Figure 1, IL14α level in peripheral blood leukocytes of HC group was 1.21 ± 0.78, B cell lymphoma group was 6.89 ± 8.59 ($p = 0.0231$), and non-B cell lymphoma group was 2.57 ± 3.52 ($p = 0.7413$). IL14α level in peripheral blood leukocytes of B cell lymphoma group significantly increased compared to HC group ($p = 0.0231$). IL14α level in B cell lymphoma group also increased compared to non-B cell lymphoma group, but with no significant statistical difference ($p = 0.1192$).

3.2. Patient Population and Their Basic Clinical Characteristics. Next, serum from 118 NHL patients was collected to evaluate the associations between B cell growth factors and Sjögren's related autoantibodies. For analysis purpose, these NHL patients were divided into two groups using two different methods, respectively. The first method was based on the location of focus: 26 patients with gastrointestinal (GI) lymphoma, 92 patients with nongastrointestinal (non-GI) lymphoma (Table 2). The other method was based on pathologic phenotypes: 86 patients with B cell lymphoma and 32 patients with non-B cell lymphoma (Table 3). Sera were obtained and basic clinical characteristics were collected from these people to evaluate the expression of BAFF, IL14, and Sjögren's related autoantibodies.

3.3. Serum IL14α and BAFF Levels in GI Lymphoma, Non-GI Lymphoma, B Cell Lymphoma, and Non-B Cell Lymphoma Groups. The relative intensity ratio for serum IL14α level in GI lymphoma group was 1.63 ± 0.68, non-GI lymphoma group was 1.41 ± 0.69, B cell lymphoma group was 1.42 ± 0.70, and non-B cell lymphoma group was 1.49 ± 0.68. Serum BAFF level (pg/ml) in GI lymphoma group was 834.2 ± 694.3, non-GI lymphoma group was 1251 ± 976.6, B cell lymphoma group was 1206 ± 955.9, and non-B cell lymphoma group was 1113 ± 920.6. There was no difference of serum IL14 α level between GI lymphoma and non-GI group ($p = 0.1384$) as well as B cell lymphoma and non-B cell lymphoma group (Figure 2(a)). Serum BAFF level in GI lymphoma group significantly increased compared to non-GI lymphoma group, while B cell lymphoma had no difference with non-B cell lymphoma (Figure 2(b)).

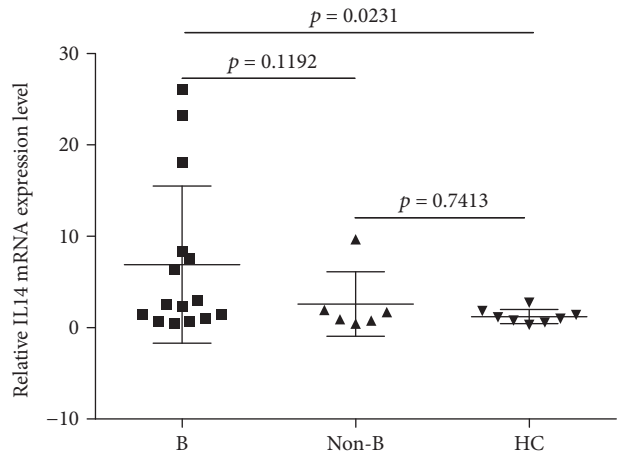

Figure 1: IL14α expression of IL14α in peripheral blood leukocytes of B cell lymphoma patients, non-B cell lymphoma patients, and HC.

Table 2: Basic clinical characteristics of study groups (basic on location of focus).

Group	Total case number	Sex (male/female)	Age
Gastrointestinal (GI) lymphoma	26	14/12	48.77 ± 15.75
Nongastrointestinal (non-GI) lymphoma	92	55/37	46.95 ± 16.57

Table 3: Basic clinical characteristics of study groups (basic on pathologic types).

Group	Total case number	Sex (male/female)	Age
B cell lymphoma	86	47/39	50.26 ± 14.88
Non-B cell lymphoma	32	22/10	39.53 ± 17.73

3.4. The Association among Serum IL14α Level, Serum BAFF Level, and Autoantibodies in Lymphoma Patients. It was shown that serum IL14α level in anti-SSA/Ro antibody positive group and TSA positive group was significantly increased compared to anti-SSA/Ro antibody negative group ($p = 0.0032$) and TSA negative group ($p = 0.0212$), while BAFF did not show a significant difference ($p = 0.2150$) ($p = 0.2329$) (Figures 3(a)–3(d)).

3.5. The Association among Serum IL14α Level, Serum BAFF Level, and Autoantibodies in GI Lymphoma and Non-GI Lymphoma Patients. In GI lymphoma patients, we found that there is no difference with serum IL14α level between autoantibodies negative and positive patients (Figures 4(a) and 4(c)); serum BAFF level was associated with the classical autoantibodies anti-SSA/Ro not TSA (Figures 4(b) and 4(d)). In non-GI lymphoma patients, the results showed serum IL14α level was associated with the classical

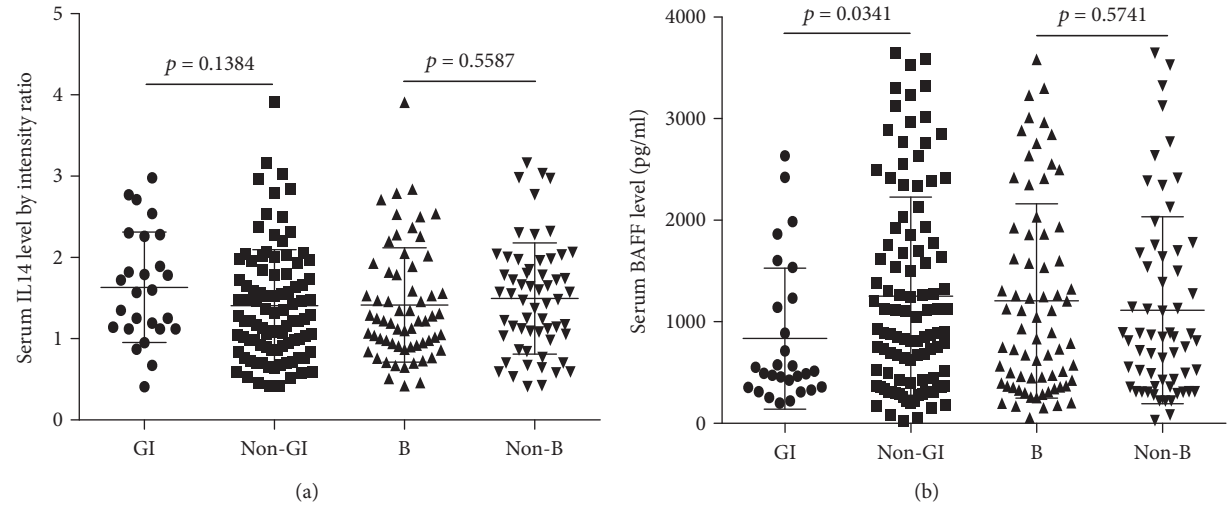

Figure 2: (a) Relative intensity ratio for serum IL14α level in GI lymphoma, non-GI lymphoma, B cell lymphoma, and non-B cell lymphoma groups. (b) Serum BAFF level in GI lymphoma, non-GI lymphoma, B cell lymphoma, and non-B cell lymphoma groups.

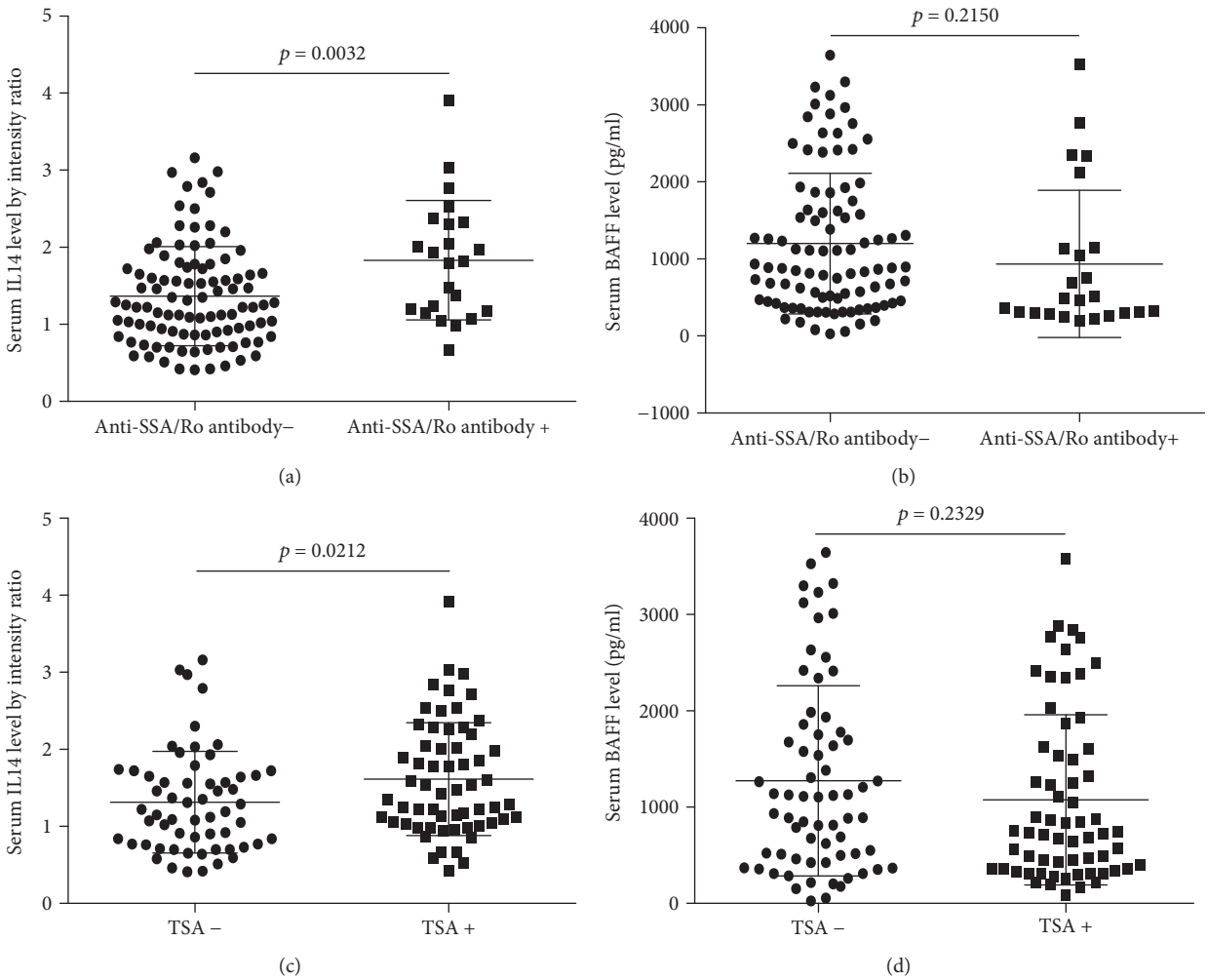

Figure 3: (a) The association between serum IL14α level and anti-SSA/Ro antibody in lymphoma patients. (b) The association between serum BAFF level and anti-SSA/Ro antibody in lymphoma patients. (c) The association between serum IL14α level and TSA in lymphoma patients. (d) The association between serum BAFF level and TSA in lymphoma patients.

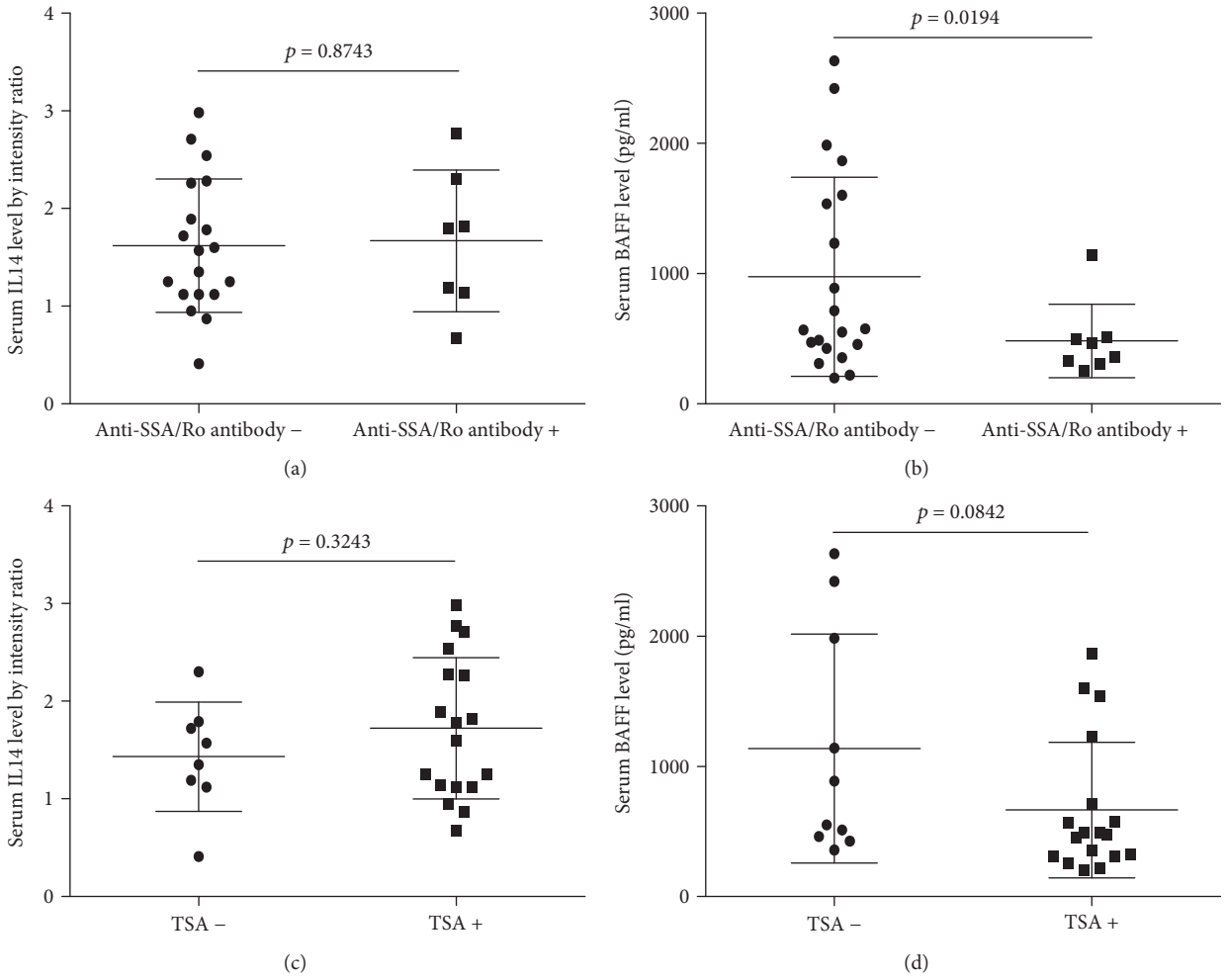

Figure 4: (a) The association between serum IL14α level and anti-SSA/Ro antibody in GI lymphoma patients. (b) The association between serum BAFF level and anti-SSA/Ro antibody in GI lymphoma patients. (c) The association between serum IL14α level and TSA in GI lymphoma patients. (d) The association between serum BAFF level and TSA in GI lymphoma patients.

autoantibodies anti-SSA/Ro, but it was not associated with TSA (Figures 5(a) and 5(c)). Whereas the serum level of BAFF (pg/ml) in non-GI lymphoma had no association with autoantibodies (Figures 5(b) and 5(d)).

4. Discussion

Despite the overall success of R-CHOP, the standard of care for non-Hodgkin's lymphoma, it is clear that many patient subsets are not cured. Based on the recognition of major genetic and biologic subtypes harboring distinct pathogenetic lesions, our understanding of non-Hodgkin's lymphoma biology evolves; it is clear that the disease is quite complex and new insight is needed to further stratify the patient for a better personized treatment.

Patients that develop autoimmune syndromes such as SLE, Sjögren's syndrome, and RA have an increased risk of developing B cell malignancies [13], which suggests there may be underlying common mechanisms between the development of both autoimmunity and lymphoid malignancies. A good example will be that excessive production of BAFF is not only associated with the development of a range of mature B cell malignancies but also play an important role in the development of pSS, indicating that BAFF may be an important molecular link between autoimmunity and cancer.

In pSS, BAFF expression is elevated and acts as a link between innate immune activation and chronic autoimmune B cell activation. Overactivation of B cells in patients is associated with the higher frequency of non-Hodgkin's B cell lymphomas found in pSS patients compared with the general population [14]. It is well established that BAFF is critical for B cell survival in the periphery, and abundant BAFF expression also contributes to the reduced levels of B cell apoptosis in SS salivary gland cells and subsequent excessive B cell activation and increased risk of lymphoma. It was also demonstrated that serum BAFF levels were enhanced and correlated with levels of autoantibodies such as anti-SSA/Ro, anti-SSB/La, and RF in pSS patients [9].

Recently, another B cell growth factor, IL14, has been shown to also play an important role in the development of pSS- and SS-associated lymphoma. It was hypothesized that IL14 can selectively act on memory B cell to enhance memory

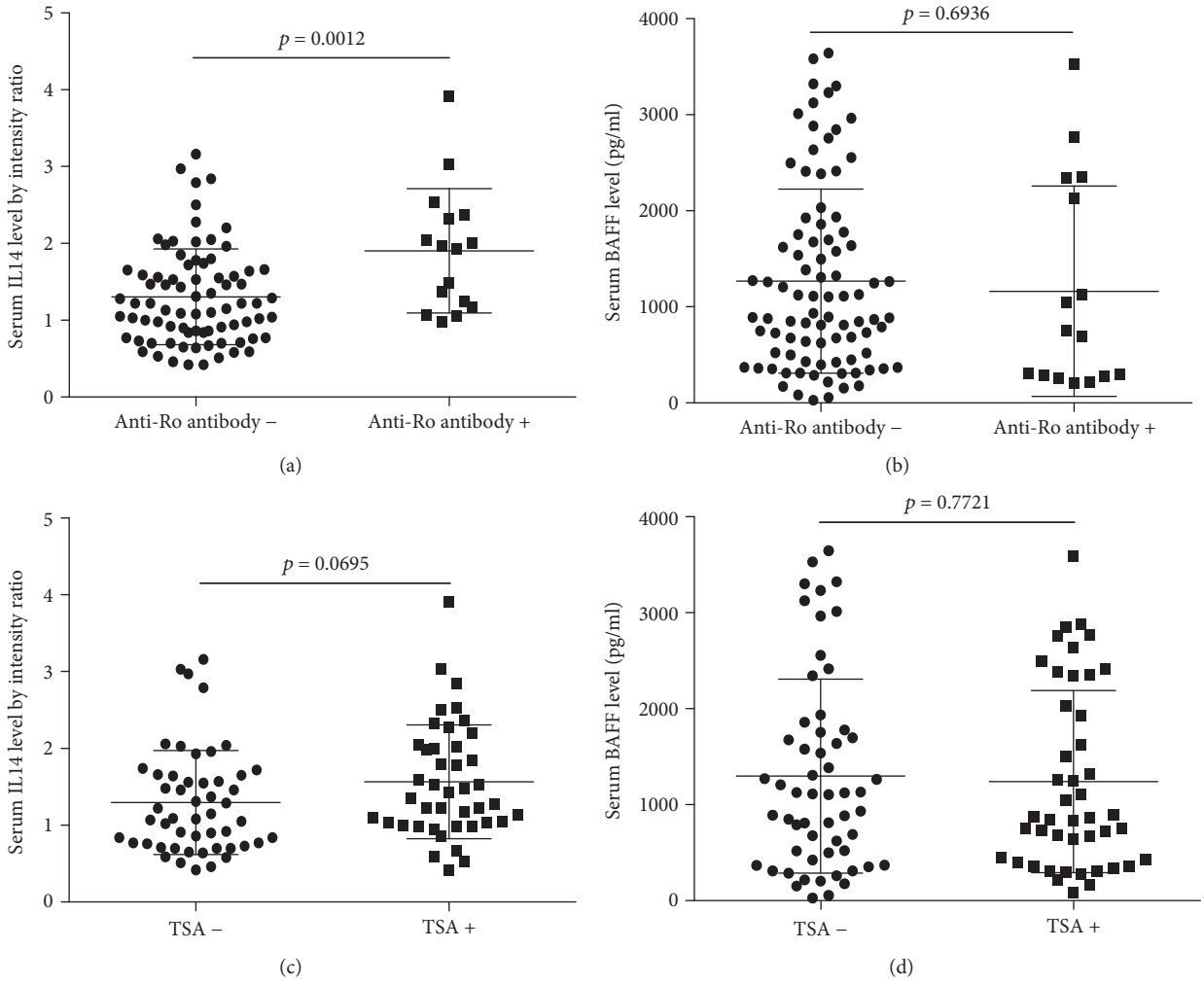

Figure 5: (a) The association between serum IL14α level and anti-SSA/Ro antibody in non-GI lymphoma patients. (b) The association between serum BAFF level and anti-SSA/Ro antibody in non-GI lymphoma patients. (c) The association between serum IL14α level and TSA in non-GI lymphoma patients. (d) The association between serum BAFF level and TSA in non-GI lymphoma patients.

B cell function and induces SS by converting low-affinity autoreactivity into high-affinity memory B cell [8, 15, 16], while both IL14 and BAFF transgenic mice shared lots of similar features as animal model for pSS, such as lymphocytic infiltration of lacrimal and submandibular gland. In the absence of MZB cell, both animal models did not develop SS phenotypes [17, 18]. There was a significant difference between this two animal models such as BAFF transgenic mice that do not spontaneously develop lymphoma, as in IL14αTG mice, and they develop more severe proliferative glomerulonephritis [19].

In current study, based on previous studies from animal model, new panels combining well-established cytokine (BAFF) and autoantibodies (anti-SSA/Ro) with newly identified cytokine (IL14) and autoantibodies (TSA) were used to evaluate the association between B cell growth factor and Sjögren's related autoantibodies in NHL patients. The result clearly indicated that there was a unique difference between BAFF and IL14 in association with autoantibodies. While serum BAFF was negatively associated with the presence of both traditional anti-SSA/Ro and novel TSA antibodies in GI lymphoma patient, IL14 was positively associated with the presence of both traditional anti-SSA/Ro and novel TSA antibodies in non-GI lymphoma patient. Long-term follow-ups on these patients and evaluation of their response to the R-CHOP treatment and recurrence rate will be very interesting. Our result provided a solid evidence to support using novel diagnostic panel to better stratify the NHL patients.

Sjögren's syndrome is one of the most common autoimmune disease in adults; however, SS is greatly under recognized in clinical practice, mostly due to diverse symptomatic expressions making the initial diagnosis difficult. It is estimated that the disease remains undiagnosed in more than half of affected adults [20, 21]. While previous long-term follow-up studies have shown about 5% of patients with pSS will develop non-Hodgkin lymphomas (NHL) later as the disease progresses, there is no study to evaluate how many NHL patients may have an underlying Sjögren's syndrome. Since all NHL patients in current study were enrolled from the Department of Hematology and Department of Gastrointestinal Surgery, there was no clinical

assessment to evaluate whether these patients meet the diagnostic criteria of SS (usually done by rheumatologist). To our surprise, as shown in current study, there were significant high number of NHL patients positive for SS-associated autoantibodies: about 20% (23 out of 118) for anti-SSA autoantibodies and 50% (59 out of 118) for TSA autoantibodies. For future clinical studies, it will be very interesting to gather additional clinical information (such as dry eye/dry mouth evaluation and minor salivary gland biopsy) for those SS-associated autoantibodies positive NHL patients to fully evaluate the potential clinical significance to stratify the NHL patients for better personized treatment.

Due to the availability of limited clinical information, we were also not able to run in-depth analysis in current study to address question such as what is the difference in the clinical manifestations and prognosis between these SS-associated antibody positive NHL patients and the others. This will be addressed in our future studies based on the interesting finding in our current study.

Data Availability

The original data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Zhenhua Xian is the first author.

Conflicts of Interest

None of the authors has conflict of interest with this submission.

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Research Article

Sjögren's Syndrome: Concerted Triggering of Sicca Conditions

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Aim. The aim of this study was to evaluate the expression of persistence of mumps virus and some cells that interact with viral infection in the focus of the autoimmune epithelitis and peripheral blood of Sjögren's syndrome patients in comparison to patients with rheumatoid arthritis (RA) and nonautoimmune sicca syndrome (nSS). *Materials and Methods*. 126 patients (119 women and 7 men) were grouped into four groups: (1) patients with primary Sjögren's syndrome (pSS), (2) patients with secondary Sjögren's syndrome due to rheumatoid arthritis (sSS), (3) patients with rheumatoid arthritis (RA), and (4) patients with nonautoimmune sicca syndrome (nSS). Immunohistochemical analysis of immune response to the suggested silent persistence of mumps virus in the minor labial salivary gland biopsies and flow cytometric analysis of blood cells was done. *Results*. Immunohistochemical signs of mumps virus persistence were found in the minor salivary glands of all study groups. Also, a significantly different immune response to virus infection (protein IFI16, interferons gamma and beta, dendritic cells, and receptor for natural killers) was revealed in the minor salivary glands of the study groups. Cytometric analysis of the blood cells revealed a dropping amount of circulating natural killers and dendritic cells in patients with SS. Significant correlations between immunohistochemical staining and serological findings were revealed. *Conclusions*. Abundant immunohistochemical signs of mumps virus protein in the salivary glands and depletion of circulating immune cells make a background for thought of presumable mumps or/and other virus participation in epithelial damage causing sicca syndrome in predisposed patients.

1. Introduction

Sjögren's syndrome is a common autoimmune disease characterised by sicca symptoms and extraglandular features. Etiology and detailed pathogenesis of Sjögren's syndrome (SS) are obscure [1]. Both innate and adaptive immune responses are implicated in the causation of SS, possibly triggered by viral infections and hormonal factors in a genetically susceptible host [2, 3].

The environmental triggers are believed to be infectious agents, which are most likely a virus [4, 5].

At the moment, conclusive evidence for a viral infection and the identity of such a virus remains elusive. Also, the wide spectrum of glandular and extraglandular manifestations in Sjögren's syndrome raises a hypothesis about the cooperation of infections agents in the mechanisms of this disease. Our earlier observations revealed a high frequency of mumps history in patients with primary and

Table 1: Clinical and serological characteristics of study patients.

Patient group features	pSS ($n = 29$)	RA ($n = 32$)	sSS ($n = 32$)	nSS ($n = 33$)
Age in years, mean (range)	57 (49–78)	54 (50–78)	59 (49–70)	59 (49–91)
Disease duration in years, mean ± SD	8 ± 5	10 ± 9	16 ± 10	8 ± 7
DAS28, mean ± SD	—	5.41 ± 1.43	5.59 ± 1.55	—
Duration of dryness in years, mean ± SD	6 ± 4	0	6 ± 5	6 ± 7
Schirmer's I test positive (≤5 mm/5 min), *n* (%)	26 (86.6)	0 (0)	23 (72)	6 (18)
Unstimulated salivary flow positive (≤1.5 mL/15 min), *n* (%)	25 (83.3)	0 (0)	13 (40.7)	2 (6)
Unstimulated salivary flow (mL/15 min), mean ± SD	1.36 ± 0.95	3.45 ± 1.36	1.80 ± 1.25	2.88 ± 1.44
Focus score positive (number of lymphocytic foci/4 mm^2), *n* (%)	29 (100)	0	32 (100)	0 (0)
Positive autoantibodies				
RF, *n* (%)	14 (48.2)	20 (62.5)	23 (71.9)	—
ACCP, *n* (%)	1 (3.4)	24 (75)	16 (50)	—
ANA, *n* (%)	21 (72.4)	1 (3.1)	7 (21.8)	—
Anti-SSA^+, *n* (%)	4 (13.8)	0 (0)	2 (2.25)	—
Anti-SSB^+, *n* (%)	0 (0)	0 (0)–	0 (0)	—
Anti-SSA/SSB^+, *n* (%)	18 (62.1)	0 (0)	3 (9.3)	—

secondary Sjögren's syndrome, and the persistence of the mumps virus was documented by PCR in their saliva and minor salivary gland tissues (the data were presented at the international conferences). Mumps is caused by the mumps virus (MuV), a member of the *Paramyxoviridae* family of enveloped, nonsegmented, and negative-sense RNA viruses. Approximately one-third to one-half of MuV infections are asymptomatic or result in only mild respiratory symptoms, sometimes accompanied by fever [6].

The participation of external factors can indicate the elevated levels of γ-globulin, autoantibodies to nonspecific antigens such as rheumatoid factor, nuclear proteins, and cellular antigens SS-A/Ro, and SS-B/La [3]. Mumps virus enhancing hemagglutinin-neuraminidase activity and accelerating attachment to the host cells' lipid membrane is supposed to play such a role [6]. Response to infection by the production of autoantibodies often preludes clinical symptoms of autoimmunity [7]. There are reports that the La/SSB antigen involved in the processing of viral RNA has been found in some Sjögren's syndrome patients [8, 9].

Recent studies have discovered a substantial role of the activation of the type I interferon (IFN) in the pathogenesis of SS. Expression of IFNs in glandular tissues could support the idea of possible viral involvement in sicca syndrome pathogenesis [10, 11].

Dendritic cells (DC) and natural killers (NK) are the cells that first fight the virus invasion directly and produce IFNs impeding viral replication.

Therefore, our goal is to evaluate the expression of the persistence of mumps virus and some cells that interact with viral infection in the focus of the autoimmune epithelitis and peripheral blood of Sjögren's syndrome patients in comparison to patients with rheumatoid arthritis (RA) and nonautoimmune sicca syndrome (nSS).

2. Patients and Methods

2.1. Patients. Patients enrolled in the study were recruited from the Rheumatology Center of Vilnius University Hospital Santara Clinics. 126 patients (119 women and 7 men) were grouped into four: (1) patients with primary Sjögren's syndrome (pSS), (2) patients with secondary Sjögren's syndrome due to rheumatoid arthritis (sSS), (3) patients with rheumatoid arthritis (RA), and (4) patients with nonautoimmune sicca syndrome (nSS). The diagnosis of pSS, sSS due to RA, and nSS was firstly established before enrolling in this study. The diagnosis of pSS and sSS was based on the inclusion and exclusion criteria as defined in the American-European Consensus Group criteria for Sjögren's syndrome [12]. RA was diagnosed according to the 2010 ACR/EULAR classification criteria for rheumatoid arthritis [13, 14]. Patients in the nonautoimmune sicca syndrome group did not fulfill the SS nor RA classification criteria. All participants of the study underwent an extensive medical examination, whole blood analysis, and serologic evaluations (Table 1). All study patients were born before 1967, prior to the introduction of the mumps-measles-rubella vaccine. No patients were on immunosuppressive medications during this study. All subjects signed informed consent. The permission for research was gained from the Vilnius Regional Biomedical Research Ethics Committee (2014-05-20, no: 158200-14-733-248).

2.2. Histological and Immunohistochemical Analysis. Minor labial salivary glands (LMSGs) were collected for differential diagnosis by the oral specialist. Five or six minor salivary gland lobules were harvested and placed into a formalin fixative. Standard paraffin preparations were prepared, sectioned, and stained with hematoxylin and eosin for histopathological evaluation, with a picrosirius solution for the estimation of fibrosis and for immunoperoxidase

immunohistochemical analysis (IHC). For IHC, 7 samples from each patient group were selected randomly.

The slides were examined for the presence of lymphocytic infiltrates by two board-certified pathologists.

For IHC analysis, the following primary antibodies were used: mouse monoclonal against mumps virus nucleoprotein (Thermo Fisher Scientific, clone 7B10, lot 1222, dilution 1 : 300), mouse monoclonal against human IFN-β (Santa Cruz, sc-73302, dilution 1 : 50), rabbit polyclonal against human IFN-γ (Abcam ab9657, dilution 1 : 200), mouse monoclonal against human IFI16 (Abcam ab55328, dilution 1 : 50), rabbit polyclonal against human NKG2D (Abcam ab203353, dilution 1 : 500), and rabbit monoclonal against human CD11c (Abcam ab52632, dilution 1 : 200).

Slides were deparaffinized in xylene, followed by rehydration in descending ethanol concentrations, from absolute to water. Endogenous peroxidase was quenched in 0.3% hydrogen peroxide for 5 min at room temperature (RT). Antigens were retrieved using citrate buffer pH 6.0 in a microwave histoprocessor *Milestone* for 20 min at 98°C. Then, sections were cooled down for 30 min at RT and incubated with primary antibody in dark humidity chamber at 4°C overnight, followed by incubation with secondary antibody from Dako detection kit (Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/-Mouse) for 30 min at RT and Dako 3,3′-diaminobenzidine staining. Finally, the sections were counterstained with Mayer hematoxylin and embedded in glycergel mounting medium. All protocol procedure steps were followed by washing with PBS. For negative staining control, primary antibodies were replaced with normal serum of animal in which a secondary antibody was raised in the same dilution as the primary antibody or with antibody diluent. Staining results were evaluated independently by two board-certified pathologists and scored by a simple score value scheme using a light microscope *Olympus BX51* equipped with Nikon digital camera dxm1200.

Score values for stain intensity and stain outspread in a high-power microscopic magnification field (HPF) were shown as follows: *stain intensity*—0 = no specific staining, 1 = moderate specific staining, 2 = strong specific staining, and 3 = very strong specific staining—and *stain outspread*—0 = no specifically stained profiles, 1 = ≤10% of specifically stained profiles in HPF, 2 = ≤30% of specifically stained profiles, 3 = up to half of specifically stained profiles, 4 = ≤70% of specifically stained profiles, and 5 = up to 100% of specifically stained examined profiles in HPF.

2.3. Flow Cytometry Analysis. For assessment of cell surface phenotypic markers, peripheral blood from the study patients was stained with fluorescein isothiocyanate- (FITC-), phycoerythrin- (PE-), allophycocyanin- (APC-), and peridinin chlorophyll protein- (PerCP-) conjugated murine monoclonal antibodies (mAbs). For dendritic cell detection, Lin cocktail CD3/14/16/19/20/56-FITC, HLA-DR-PerCP, pDC marker CD123-PE, and cDC marker CD11c-APC (BioLegend, USA) were used; for NK, CD3-FITC and CD16+CD56-PE (Simultest™, Becton Dickinson, UK). Isotype controls were obtained from BioLegend. In test tubes containing 50 μl of peripheral blood supplemented with anticoagulant, appropriate amounts of mAbs, according to the manufacturer's recommendations, were added. All samples with labeled antibodies were incubated for 30 min at RT in the dark. After incubation, red blood cells were lysed for 15 min with 160 mM NH_4Cl solution at RT in the dark, and then the samples were centrifuged (500×*g*). Precipitated cells were washed with Cell Wash Buffer (Becton Dickinson, USA) and analyzed by two- or four-color flow cytometry on a FACSCalibur flow cytometer and CELLQuest software after calibration with CaliBRITE beads (BD Biosciences, San Jose, CA, USA). Data for each sample were acquired until 100,000 cells were analyzed.

2.4. Statistics. Statistical differences were analyzed using the Mann-Whitney *U* test (unpaired samples), and correlations were assessed by Spearman's rank test using the GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA). *P* values less than 0.05 were considered significant.

3. Results

3.1. Histology and Immunohistochemistry. Analysis of the salivary gland tissue showed histopathological heterogeneity amongst SS patients, including an extent of the cellular inflammatory infiltrate and the degree of epithelial damage in acini and ducts, healing either replacement of parenchymal glandular tissue by fat or fibrosis. pSS sialadenitis revealed a focal proliferation of ductal epithelial cells combined with focal lymphocytic infiltration and atrophy of acini. LMSG specimens were recognized as pSS using hematoxylin-eosin-stained sections when scored more than one focus of 50 or more lymphocytes per 4 mm^2 of salivary tissue [12].

The typical pSS pathological picture included lymphocytic infiltrations (Figure 1(a)) between acini and ducts, occasional infiltration of plasma cells and other inflammation-related mononuclear cells, and the formation of lymphoid follicle-like structures and fibrosis (Figure 1(b)). Histological picture characteristic for chronic sialadenitis was also found in sSS and RA. In nSS patients' LMSG biopsies dominated signs of parenchymal atrophy and fat deposition.

Mumps virus protein was found in the part of the ductal cells, in some acinar epithelial cells (Figure 1(c)), and in the parts of connective tissue closely surrounding acini and ducts in the biopsies of the pSS patients (Figure 1(d)). The foci of lymphocyte infiltration were mostly virus protein-free, with a little amount positively stained mononuclear cells in pSS patients. In the biopsies from sSS patients' the distribution and intensity of positive mumps virus protein staining profiles were similar to pSS (Figure 1(e)). In the biopsies from RA patients (Figure 1(f)), the number of positively mumps virus protein stained acini was much smaller in comparison to pSS and sSS. In nSS patients (Figure 1(g)) virus protein was found in some acinar cells, few mononuclear cells, and some parts of the stroma situated closely to acini and ducts.

Our study revealed significant differences of the staining intensity and outspread of *CD11c antigen* in the inflammatory cells and stroma of minor salivary glands from pSS

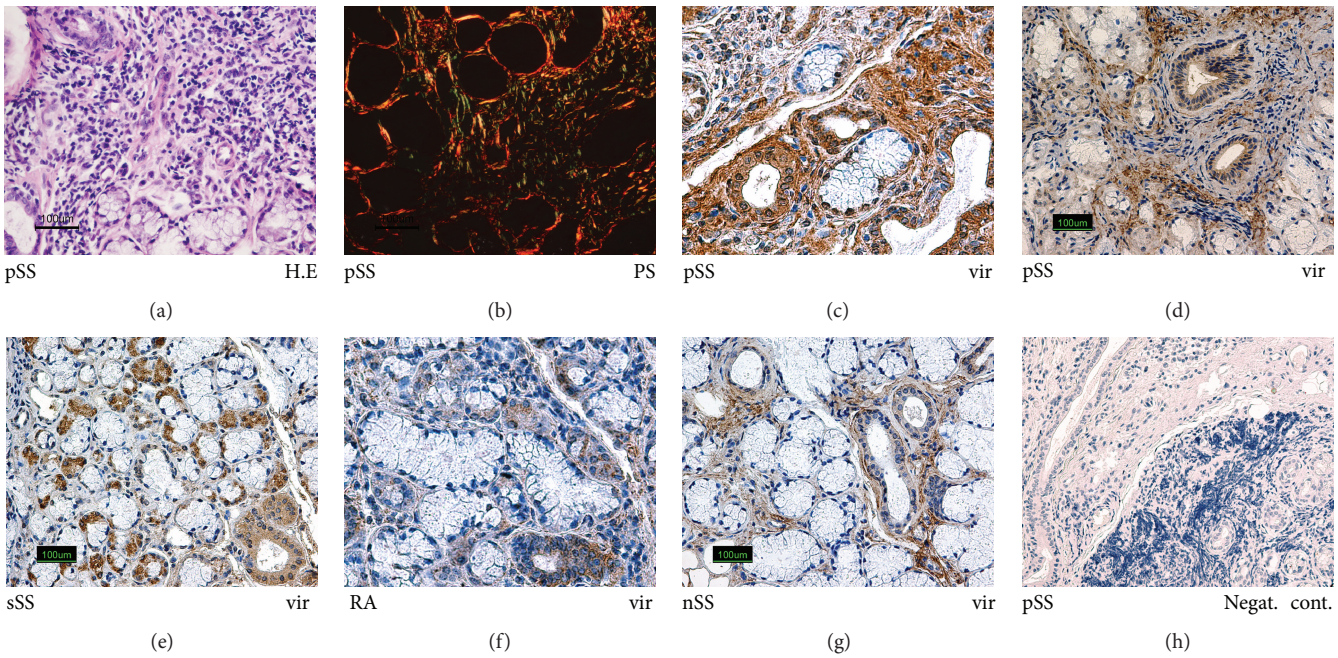

Figure 1: Expression of mumps virus protein in the minor salivary glands of patients with pSS, sSS, RA, and nSS. Histological and immunoperoxidase staining (IHC) in minor labial salivary glands: (a) H&E; (b) picrosirius-polarizing light; (c–g) mumps virus protein in the salivary glands of the study groups. Original magnification ×200. (h) Negative staining control.

patients in comparison to sSS, RA, and nSS patients (Figures 2(a), 2(b), 2(c), 2(d), 2(g), 2(h), 2(i), and 2(j)). The stain intensity and outspread of *CD11c antigen* were similar in ducts from pSS, sSS, and RA patients, but comparison between sSS and nSS patients showed a significant difference in stain intensity and outspread in ducts (Figures 2(a), 2(e), and 2(f)).

The significantly highest stain intensity of *IFN-gamma* was found in acini of pSS patients (Figures 3(a), 3(d), and 3(i)), but stain outspread of IFN-gamma was similar to nSS (Figure 3(j)). The stain intensity of IFN-gamma in ducts showed significant differences between pSS and nSS patients and between sSS and nSS patients (Figure 3(k)). On the other hand, the significantly higher stain outspread of IFN gamma in ducts was found in pSS patients in comparison to sSS (Figure 3(l)). The stain intensity and outspread of IFN-gamma in the stroma of patients with pSS and sSS were similar and significantly higher in comparison to nSS and RA (Figures 3(a), 3(b), 3(c), 3(d), 3(m), and 3(n)). The significantly lowest stain intensity and outspread were revealed in the inflammatory cells of nSS patients (Figures 3(d), 3(o), and 3(p)).

Immunohistochemical staining of *IFN-beta* was weaker than IFN-gamma and similar in all study patients groups, but a little stronger in ducts than in acini of patients with pSS (Figure 3(e)), sSS (Figure 3(f)), RA (Figure 3(g)), and nSS (Figure 3(h)).

The analysis of stain intensity and outspread of *IFI16 protein* in acini and stroma revealed significant differences between pSS and nSS patients. Also, stain outspread of IFN16 in stroma showed a significant difference between RA and nSS patients (Figures 4(a), 4(b), 4(c), 4(d), 4(l), 4(j), 4(k), and 4(l)). The significantly different stain intensity and outspread of IFN16 protein in the inflammatory cells and blood vessels were found between all groups, except that stain intensity and outspread of IFN16 protein in blood vessels were similar in RA and sSS patients (Figures 4(m), 4(n), 4(o), and 4(p)).

NKG2 receptor for natural killer was detected as mosaic distribution in the cells of ducts (Figure 4) and in some inflammatory infiltrating cells in all study groups (Figures 4(e), 4(f), 4(g), and 4(h)).

3.1.1. Analysis of DC and NK by Flow Cytometry. Distribution analysis of pDC ($CD123^+$) showed that the highest frequency was observed in nonautoimmune sicca syndrome patients' group, and this frequency was significantly higher in comparison with RA ($p < 0.0001$), pSS ($p = 0.0493$), and sSS ($p = 0.0037$) groups (Figure 5(a)). Significant differences were found between RA and pSS groups. pDC absolute count was significantly lower in RA and pSS study group compared with nSS (accordingly, $p = 0.0074$ and $p = 0.0316$) (Figure 5(b)). Significant differences in the distribution of mDC ($CD11c^+$) were found between pSS and nSS ($p = 0.0378$) and between sSS and nSS group ($p = 0.0227$) (Figure 5(c)). mDC absolute count was significantly lower in pSS study group compared with nSS ($p = 0.0269$) (Figure 5(d)).

The frequency of NK cells was similar in all study groups, and only absolute NK count was significantly reduced in sSS group compared with nSS and RA groups (accordingly, $p = 0.0272$, $p = 0.0454$) (Figures 5(e) and 5(f)).

Reliable positive correlation between immunohistochemical staining in the LMSG and serological findings was

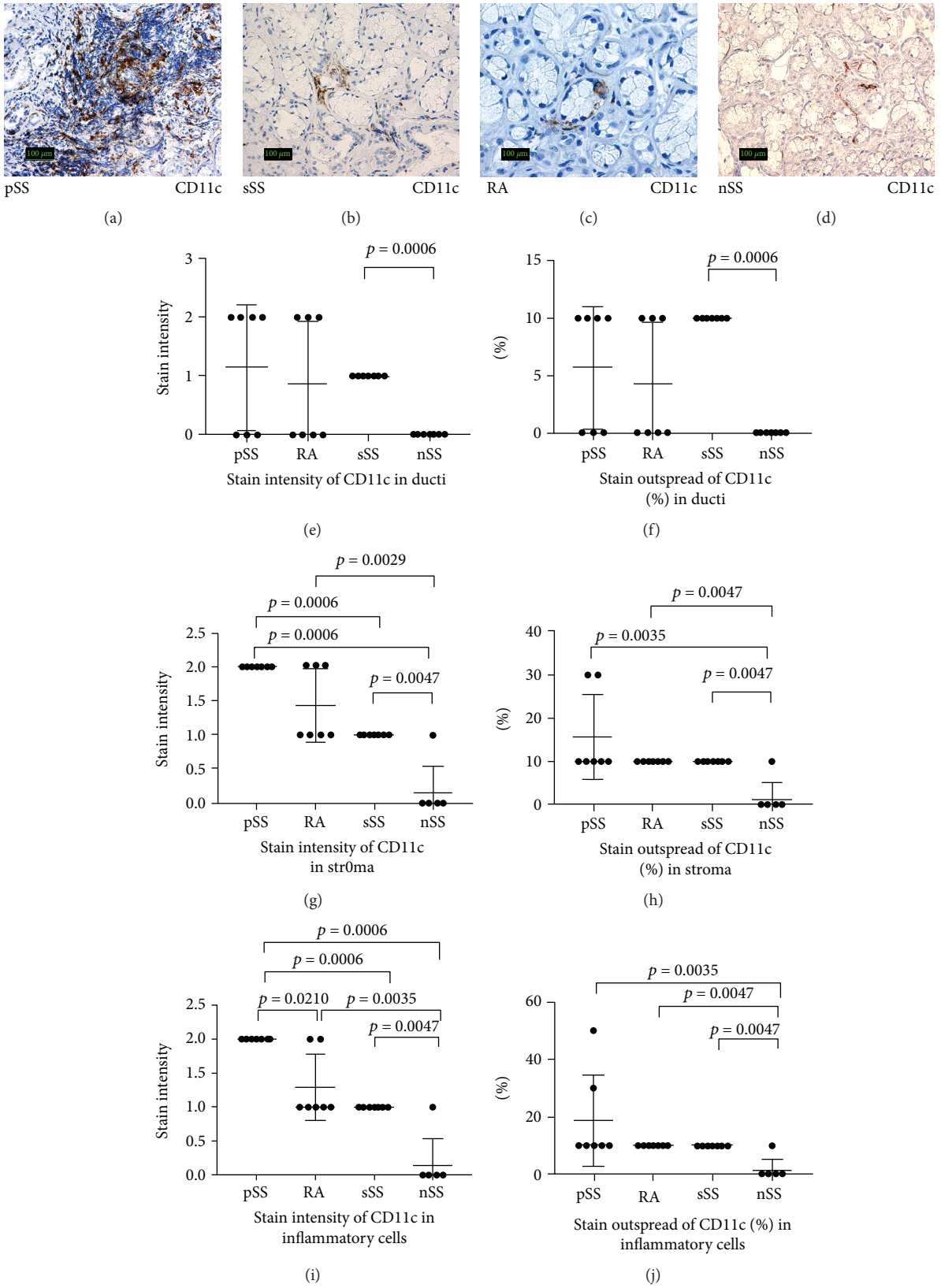

Figure 2: Expression of $CD11c^+$ in the salivary glands of patients with pSS, sSS, RA, and nSS. (a–d) Histological staining (IHC) view of $CD11c^+$ expression in the salivary glands of all study groups. Original magnification ×200. (e–j) Comparison of stain intensity and outspread of $CD11c^+$ in the salivary glands of all study groups.

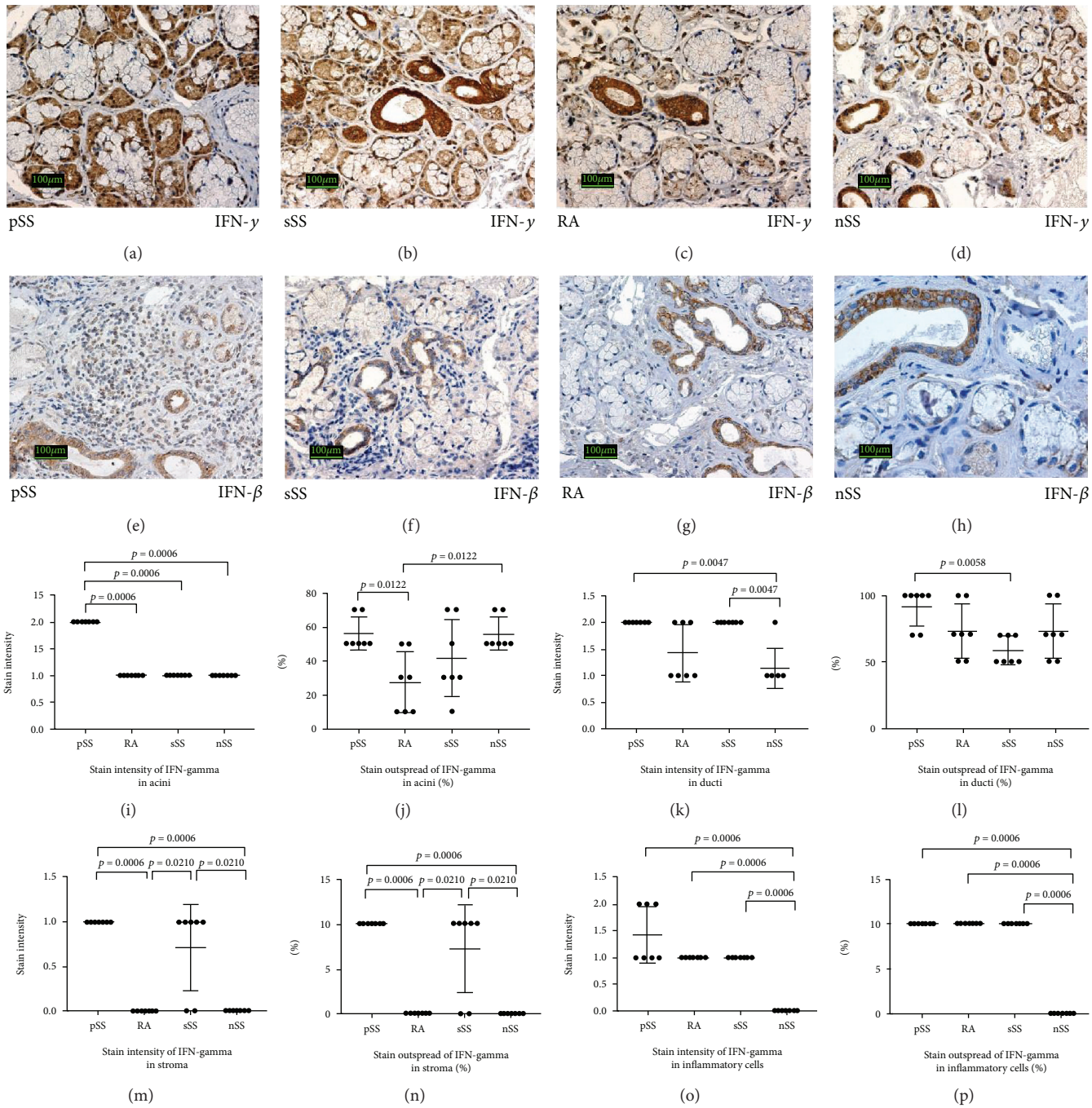

Figure 3: Expression of interferons beta and gamma in the salivary glands of patients with pSS, sSS, RA, and nSS. (a–h) Histological and immunoperoxidase staining (IHC) view of the expression of interferons beta and gamma in the salivary glands of all study groups. Original magnification ×200. (i–p) Comparison of stain intensity and outspread of interferons gamma in the salivary glands of all study groups.

established. In the RA group, the frequency of pDC in blood samples strongly correlated with the stain intensity of IFN-β in ducts ($p < 0.0001$, $r = 0.988$) and stain intensity and stain outspread of NK cells ($NKG2^+$) in LMSG ($p < 0.0001$, $r = 0.988^*$). More correlations were found in pSS group. In RA and in pSS groups, the frequency of pDC in blood samples strongly correlated with the stain intensity of IFN-β in the ducts ($p = 0.022$, $r = 0.827$), while mDC strongly correlated with the stain outspread of IFN-γ in the acini ($p = 0.016$, $r = 0.748$). Moreover, the frequency of NK cells in blood samples strongly correlated with the stain intensity and stain outspread of IFN-β in the acini ($p = 0.011$, $r = 0.915^*$) and also with the stain intensity and stain outspread of pDC in LMSG blood vessels ($p = 0.045$, $r = 0.822^*$) and stain intensity and stain outspread of mumps virus protein in ducts (accordingly, $p = 0.045$, $r = 0.822$ and $p = 0.047$, $r = 0.818$). In sSS group, only the frequency of NK cells in blood samples strongly correlated with the stain

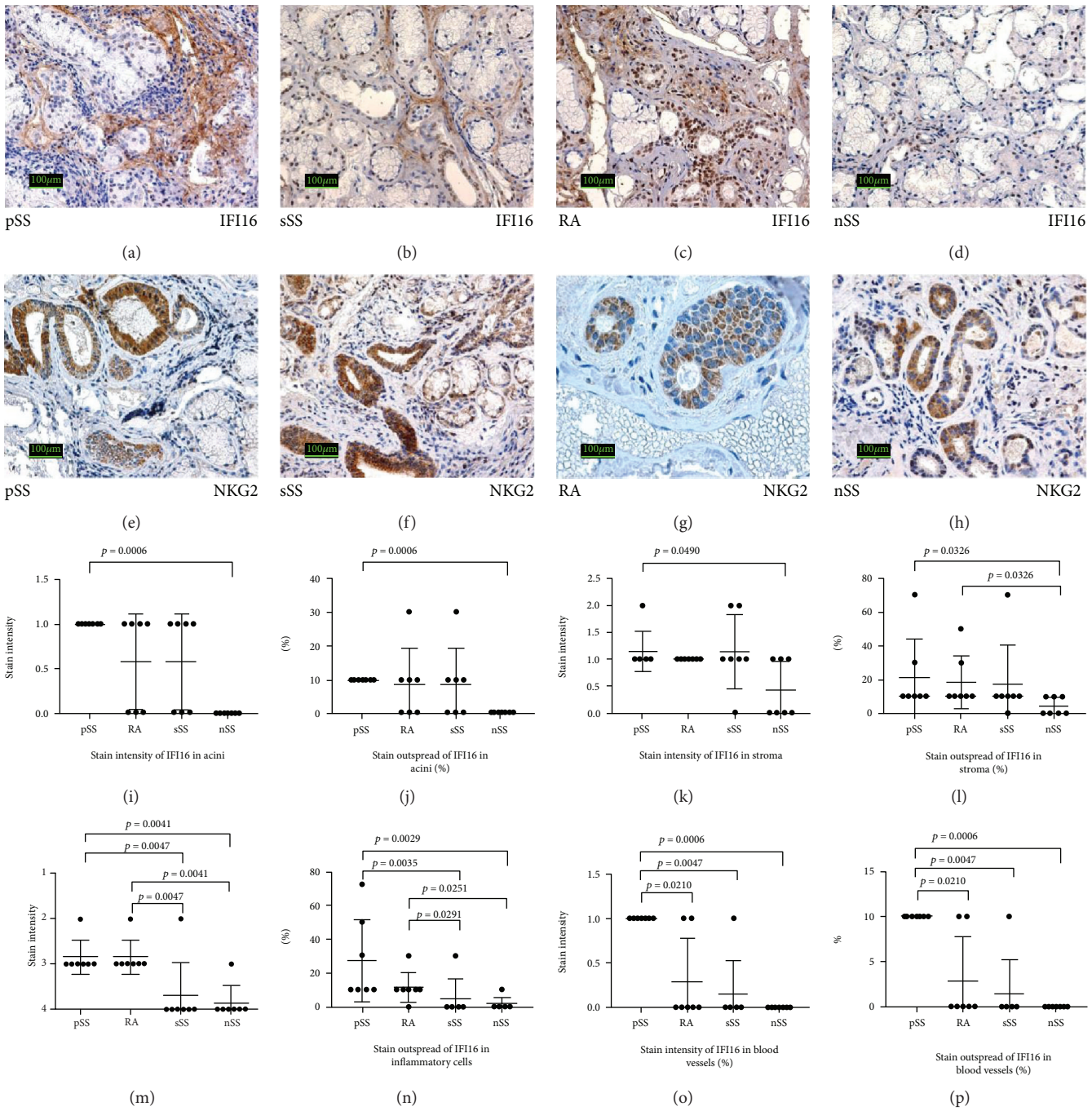

Figure 4: Expression of IFI16 and NKG2 in the salivary glands of patients with pSS, sSS, RA, and nSS. (a–h). Histological and immunoperoxidase staining (IHC) view of the expression of IFI16 and NKG2 in the salivary glands of all study groups. Original magnification ×200. (i–p) Comparison of stain intensity and outspread of IFI16 in the salivary glands of all study groups.

intensity of IFI16 in the ducts and stroma (accordingly, $p = 0.003$, $r = 0.922$ and $p = 0.001$, $r = 0.945$) and stain intensity and stain outspread of mumps virus protein in LMSG acini ($p = 0.003$, $r = 0.922^*$). In nSS group, a strong correlation was established between the frequency of mDC cells in the blood samples and stain intensity of IFN-β in LMSG ducts ($p < 0.002$, $r = 0.939$), as well as with stain intensity of IFN-γ in LMSG ducts ($p < 0.028$, $r = 0.806$).

*The score values of stain intensity and stain outspread are the same.

4. Discussion

Both innate and adaptive immune responses are implicated in the causation of SS, possibly triggered by viral infections and hormonal factors in a genetically susceptible host [2, 3]. At the moment, conclusive evidence for a viral infection and the identity of such a virus remains elusive. The wide spectrum of glandular and extraglandular manifestations in Sjögren's syndrome raises a hypothesis about the cooperation of infections agents in the mechanisms of

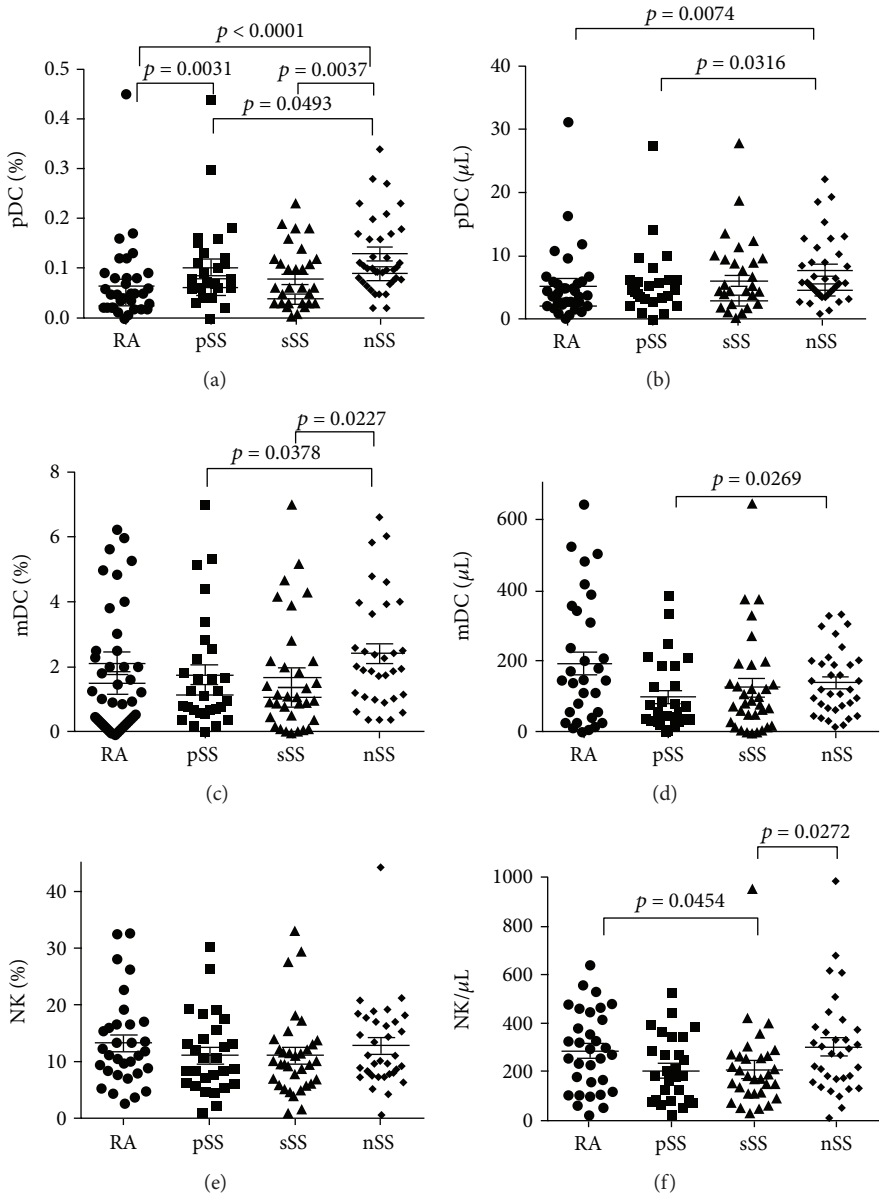

Figure 5: Expression of dendritic cells (DCs) and natural killers (NK) in the peripheral blood of pSS, sSS, RA, and nSS patients (flow cytometric analysis).

this disease. According to our earlier observations about mumps, we set tasks to evaluate the persistence of mumps virus in the salivary glands. In our study mumps virus protein was found in the minor salivary glands of pSS, sSS, RA, and nSS patients. The distribution and intensity of positive mumps virus protein in minor salivary glands were similar in pSS and sSS patients. In minor salivary glands of RA patients, the number of positively mumps virus protein stained acini was much smaller in comparison to pSS and sSS. In nSS patients virus protein was found in some acinar cells and some parts of the stroma situated closely to acini and ducts. These findings support an idea that mumps virus participate in epithelial damage causing sicca syndrome.

Also, according to recent data, salivary gland epithelial cells (SGEC), once believed to be passive onlookers, seem to be the nidus of pathogenetic events in SS. SGEC in SS, therefore, are active players in the inflammatory and autoimmune response. Once activated, these cells orchestrate innate and adaptive immune response [15].

If Sjögren's syndrome is caused by a virus, there should be a persistent latent infection when the viral pathogen after acute infection remains present in the infected host causing sporadic reactivation events. Expression of CD11c, IFN-gamma, IFN-beta, and NKG2 receptor for natural killer in the salivary glands of our study groups demonstrated these events.

Significant differences of the staining intensity and outspread of CD11c antigen in the inflammatory cells and stroma of minor salivary glands from pSS patients in comparison to sSS, RA, and nSS patients and ducts from sSS in comparison to nSS demonstrate an activity of dendritic cells in minor salivary glands.

Both plasmacytoid dendritic cells (pDCs) and classic dendritic cells (cDCs) have a role in SS pathogenesis. Plasmacytoid DCs (pDCs) are present in the lymphocytic foci of salivary glands in patients with SS but not in healthy controls [15, 16]. Dendritic cells capture antigens and regulate reactions of adaptive immune responses [16]. Plasmacytoid DC producing CD123—the molecule consisting of three extracellular domains [17] selectively express endosomal TLR7 and TLR9 that detect virus nucleic acids. TLR7 or TLR9 triggers a downstream signaling cascade resulting in the secretion of IFN-α/β [18]. pDC—the professional IFN-α/β-producing cells were found to be reduced in the periphery and recruited preferentially in the minor salivary glands of SS patients [19]. Earlier our finding of a significant decrease of circulating pDC cells in RA and sSS due to RA patients and decrease of conventional mDC in SS patients supports the suggestion that DC populations may play a substantial role in autoimmune diseases and are exhausted in fighting viruses [11].

Principal cells that directly fight the viruses by means of IFNs are DC, cytotoxic T lymphocytes (CTL), NK, and natural killer T (NKT) cells [20]. The IFNs are a group of inflammatory cytokines that inhibit viral replication and regulate the immunity of host [21]. Like other paramyxoviruses, mumps virus initiates infection by attachment of the HN protein to sialic acid on the cell surface glycolipids and operates together with the F protein facilitating fusion with the plasma membrane [22]. IFNs disturb this process and in some cases are responsible for the initiation and perpetuation of the autoimmune disease [23–26]. Induction of IFN-α/β is based on the interactions between viral pathogen-associated molecular patterns (PAMPs) and host pattern-recognition receptors (PRRs) [27].

Though type I IFNs (IFN-α/β) were sought to be the predominant players in the pathogenesis of SS, recent data suggest an important role also of type II IFN (IFN-γ) in the immune sicca pathogenesis [10, 28]. IFN-γ is predominantly produced by T and NK cells to a lesser extent by dendritic cells, macrophages, and B cells [29]. IFN-γ promotes antimicrobial protection, apoptosis, inflammation, and also host tissue damage producing autoantigens. Both type I and type II IFNs have been thought to be implicated in the pathogenesis of Sjögren's syndrome [23].

The significantly highest stain intensity of IFN-gamma was found in the acini of pSS patients. The stain intensity of IFN-gamma in ducts showed significant differences between pSS and nSS patients and between sSS and nSS patients. The significantly higher stain outspread of IFN gamma in ducts was found in pSS patients in comparison to sSS. The significantly lowest stain intensity and outspread were revealed in the inflammatory cells of nSS patients.

Immunohistochemical staining of IFN-beta was weaker than IFN-gamma and similar in all study patients groups, but a little stronger in ducts than in acini. In accordance, recent research findings highlight a consistent ex vivo inhibitory effect of IFN-β on proinflammatory cytokine production and NO pathway in pSS patients. These data suggest that IFN-β could represent a potential candidate for targeting inflammation during pSS [30].

It is known that normal minor salivary gland cells do not constitutively express IFI16. Usually IFI16 expression is limited to the cell nuclei of hematopoietic cells, vascular endothelial cells, and keratinocytes [31]. It is also known that extracellular leakage of IFI16 breaks the tolerance to self-proteins and leads to the production of anti-IFI16 autoantibodies [32, 33]. Strong expression of the genes inducing IFNs could be a pivotal step in the development of primary Sjögren's syndrome. Our study revealed immunohistochemical evidence for the mislocation and extracellular leakage of IFI16 in the parenchymal salivary cells of patients of our study. We also found overexpressed and mislocated IFI16 in the lymphocytes of the minor salivary glands. We suppose that IFI16 may act as an infection-caused autoantigen involved in the development of autoimmunity. Also, such possibility has been postulated by other researchers [34].

NKG2 receptor for natural killer recognizing infected cell was detected as mosaic distribution in the cells of ducts and in some inflammatory infiltrating cells in all study groups.

Our study demonstrated the evidence of antiviral immune response according to the analysis of DC and NK in pSS, sSS, and RA patients' blood. Distribution analysis of pDC ($CD123^+$) showed that the highest frequency was observed in nSS patients in comparison to RA, pSS, and sSS groups. pDC absolute count was significantly lower in RA and pSS study group compared with nSS. Significant differences in the distribution of mDC ($CD11c^+$) were found between pSS and nSS and between sSS and nSS group. mDC absolute count was significantly lower in pSS study group compared with nSS.

The frequency of NK cells was similar in all study groups.

These serological findings and reliable positive correlations between immunohistochemical staining and serological findings could explain an idea that virus or viruses participate in epithelial damage causing sicca syndrome. Certainly, our study does not try to demonstrate that mumps virus is the only virus that is involved in the pathogenesis of SS. Probably mumps virus works on cooperation with another infection. One of the shortcomings of our research is that we analyzed small groups by immunohistochemistry.

Some authors suggest that rather than a specific infection, a response to self-antigens can initiate SS [35, 36].

Results of our study suggest that the pathogenesis of SS seems to be a multifactorial process leading to damage and dysfunction of the salivary glands. Possibly, environmental factors such as a viral infection affect the salivary glands and stimulate dendritic or/and glandular cells to activate the HLA-independent innate immune system, which using Toll-like receptors recognize pathogen-specific epitopes. This process leads to the upregulation of adhesion proteins and production of chemokines by the glandular epithelial cells [37].

Sjögren's syndrome is prevalent in patients of advanced age, especially in 50 years and older women [38]. Age-related disbalance in hormones, such as estrogens, prolactin, progesterone, and glucocorticoids, may affect DC differentiation, maturation, and function leading to a proinflammatory not to an anti-inflammatory or tolerogenic

phenotype. These processes can trigger autoimmune disease [1, 39, 40].

The results of our study and analysis of data of other investigators suggest that even if the expression of a viral or virus-mimicry endogenous protein in the salivary gland are not sufficient to elicit a complete SS disease phenotype, some sicca pathogenesis presumably is a response to viral infection. Certainly, Sjögren's syndrome neither any autoimmune sicca condition is considered to be infectious or contagious diseases. Multiple factors, including genetic stability, unfavorable cellular tissue environment, and/or exposure to pathogens, are required to trigger a virus-mediated development of SS [32, 41]. Disbalanced microbiome also can disturb immune homeostasis and participate in SS triggering. Probably, this can explain that nonautoimmune sicca conditions, at least partially, share similar pathogenesis with autoimmune SS.

5. Conclusion

Abundant immunohistochemical signs of mumps virus protein in the salivary glands and depletion of circulating immune cells make a background for thought of presumable mumps or/and other virus participation in epithelial damage causing sicca syndrome in predisposed patients.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflict of interests regarding this study.

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Review Article

Current and Emerging Evidence for Toll-Like Receptor Activation in Sjögren's Syndrome

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While the importance of Toll-like receptor (TLR) signaling is well established in many autoimmune diseases, the role of TLR activation in Sjögren's syndrome (SS) is poorly understood. Studies in mice and humans reveal that TLRs are potent mediators of inflammation in SS. TLRs are expressed and functional in salivary tissue, and TLRs in peripheral blood cells of SS patients are also upregulated and hyperresponsive to ligation. In this review, we will detail observations in mouse models regarding the importance of TLR activation in both local and systemic disease. We will then discuss studies in SS patients that provide evidence of the importance of TLR-mediated signaling in disease. While the ligands that activate TLRs in the context of SS are unknown, emerging data suggest that damage-associated molecular patterns (DAMPs) may be significant drivers of the chronic and unremitting inflammation that is characteristic of SS. We will discuss putative DAMPs that may be of clinical significance in disease. Therapies that target TLR signaling cascades will likely reduce both exocrine-specific and systemic manifestations of SS.

1. Introduction

Toll-like receptors (TLRs) constitute a class of pattern recognition receptors that are expressed ubiquitously [1, 2]. TLRs are ancient membrane-spanning proteins that recognize both pathogen-associated molecular patterns (PAMPs) derived from microorganisms and endogenous mediators of inflammation, termed danger-associated molecular patterns (DAMPs) [3]. TLR activation leads to recruitment of adaptor proteins within the cytosol that culminates in signal transduction. Ultimately, activation of these pathways results in the transcription of genes involved in inflammation, immune regulation, cell survival, and proliferation [3]. Although TLRs were initially thought to be important only in host defense, more recent work demonstrates a critical role for TLRs in autoimmunity [4]. While TLR signaling is required for several different autoimmune diseases, the contribution of TLR signaling to Sjögren's syndrome (SS) initiation and progression remains poorly understood [4, 5].

SS occurs in 2 forms: primary (pSS) and secondary (sSS). In pSS, SS is the sole autoimmune disease present. This is in contrast to sSS, where the disease is observed in conjunction with another autoimmune connective tissue disease [6]. In both forms of SS, salivary and lacrimal tissues are targeted by the innate and adaptive immune systems. The disease is characterized by lymphocytic infiltration of exocrine tissues along with salivary and lacrimal hypofunction [6, 7]. Loss of salivary flow results in patient discomfort, tooth decay, and difficulty in speaking and eating [8, 9]. Patients may also experience keratoconjunctivitis sicca or inflammation of the eyes as a result of dryness that often leads to ocular discomfort and impaired vision [10]. In addition, pSS patients exhibit systemic complications including hypergammaglobulinemia, fatigue, and lymphoma [6, 7]. The diagnostic criteria for SS were revised many times since the disease was initially described [11], and the current criteria include both serologic and glandular disease assessments [12]. As many as four million Americans are currently living with

SS [13]. Despite its prevalence, the disease etiology is poorly understood and there is currently no known cure for SS. Therefore, understanding the underlying pathways and networks that mediate SS is crucial in order to develop targeted treatments.

We will discuss the findings that suggest a crucial role for TLR activation in SS pathogenesis. First, we will review data from several different SS mouse models that demonstrate the importance of TLRs in SS initiation and progression. Second, we will examine evidence showing dysregulation of TLR signaling in exocrine tissue and in peripheral blood mononuclear cells (PBMCs) derived from SS patients. Third, we will discuss the potential TLR ligands that may mediate chronic inflammation in disease. Targeted therapies that modulate TLR signaling will likely be efficacious in mitigating both exocrine-specific and systemic disease manifestations.

2. Mouse Models of pSS Reveal the Importance of TLRs in Disease

While studies in SS patients are crucial to understand disease pathogenesis, the exclusive use of human patients to study SS has several challenges, as disease development is insidious and often goes undiagnosed for several years [13]. Therefore, it is difficult to study early disease events in SS patients. Moreover, there is considerable disease heterogeneity in humans [14–16]. SS mouse models are invaluable tools that facilitate the identification of underlying disease mechanisms, as these display similar disease manifestations to humans, and are well characterized in terms of the disease progression [17–19]. Moreover, SS occurs in an accelerated timeline compared to humans. Finally, use of mouse models allows testing of therapeutics that is considerably more difficult to perform in humans [17, 18]. In the following section, we will discuss findings in mouse models that illustrate the importance of TLR activation in disease.

2.1. Spontaneous Development of SS: NOD/Lt and NOD-Related Strains. One of the earliest SS models described was the nonobese diabetic strain (NOD/Lt). Initially characterized as a model of type I diabetes [20], it was later found that female NOD/Lt mice spontaneously develop lymphocytic infiltration and loss of salivary flow at about 16 weeks of age [21]. The NOD/Lt strain is one of the best characterized for the study of sSS [22, 23]. Studies in submandibular gland (SMG) tissue from female NOD/Lt animals found that *TLR1*, *TLR2*, *TLR4*, and *TLR9* were increased at pre-clinical and clinical stages when compared to a pre-disease time point [24]. In addition, myeloid differentiation primary response 88 (*MyD88*), a key adaptor used in both TLR and IL-1R family member signaling [1], was elevated at both disease time points as well. This heightened expression coincided with increasing lymphocytic infiltration in the SMG, suggesting that TLR activation may contribute to the recruitment of lymphocytes to the salivary gland [24].

To determine whether TLR inhibition may be an efficacious therapeutic, female NOD/Lt mice were given chloroquine prior to disease development until 16 weeks of age (clinical disease age). Chloroquine prevents endosomal acidification, a process that is required for TLR3, TLR7, TLR8, and TLR9 signaling [25–27]. Consistent with previous studies in lupus models, treated animals displayed reduced expression of *TLR1*, *TLR2*, *TLR3*, *TLR4*, and *TLR9* in salivary tissue [24]. In addition, chloroquine decreased sialadenitis [24]. These studies suggest that TLR-mediated signaling is important for salivary-related disease manifestations in SS and inhibition of such may be of therapeutic benefit.

Additional studies in NOD/Lt mice established the importance of TLR9 activation in SS. TLR9 ligation results in phosphorylation of p38 MAPK [28]. The kinetics of TLR9 expression and activation were examined in PBMCs isolated from NOD/Lt mice at pre-disease (4-5 weeks of age), pre-clinical (8–10 weeks), and clinical disease time points (15 weeks of age) [29]. The authors found that the percentage of cells coexpressing TLR9 and phosphorylated p38 MAPK was highest at 8 weeks of age. Moreover, expression of both proteins was higher in NOD/Lt animals as compared to controls at the 5-, 8-, and 10-week time points [29]. These data suggest that TLR9 activation may occur relatively early in disease. In a separate study, salivary flow and inflammatory mediators were evaluated in NOD mice treated with TLR9 agonists [30]. Significantly, TLR9 activation resulted in increased saliva production and decreased inflammatory mediators in salivary tissue [30]. Thus, TLR9 signaling may play a protective role in SS, similar to lupus [31], and activation of TLR9-dependent pathways may be a novel therapeutic strategy in SS.

While the NOD/Lt strain is valuable in the study of sSS, the use of pSS mouse models is important to facilitate identification of SS-specific disease mechanisms, particularly in the periphery. To this end, the C57BL/6.NOD-*Aec1Aec2* pSS strain was generated from the NOD/Lt strain. C57BL/6.NOD-*Aec1Aec2* mice have two genomic regions from NOD/Lt mice referred to as *Idd* susceptibility loci (called *Idd3* and *Idd5*) that contribute to the development of SS [32]. C57BL/6.NOD-*Aec1Aec2* mice recapitulate many aspects of human disease, including loss of salivary and tear flow, presence of autoantibodies, and glandular lymphocytic infiltrates [32]. Microarray studies performed on transcripts derived from salivary tissue of C57BL/6.NOD-*Aec1Aec2* mice at a pre-disease time point showed upregulation of genes involved in *TLR* signaling pathways [33]. Specifically, *TLR3* and *TLR7* were elevated, as well as many downstream signaling intermediates such as *TRAF6*, *interferon regulatory factor 5* (*IRF5*), and *IRF7* [33]. Thus, studies in this model provide corroborative evidence that endosomal TLR signaling cascades are dysregulated early in disease.

The NOD.B10Sn-$H2^b$ (NOD.B10) mouse model is also valuable for the study of pSS. NOD.B10 mice were derived from the NOD/Lt strain by replacing the major histocompatibility locus with that from the C57BL/10 strain [34]. The resulting congenic animals develop pSS but are resistant to type I diabetes. NOD.B10 mice share many similarities with pSS patients. They have a strong female disease predilection and exhibit spontaneous disease development. Moreover, they display antinuclear autoantibodies, lymphocytic infiltrates in salivary and lacrimal tissues, and loss of salivary flow

Table 1: Evidence for TLR activation in murine studies of SS.

Strain	Results	Refs
NOD/Lt	(i) Increased *TLR1*, *TLR2*, *TLR4*, *TLR9*, and *MyD88* expression at pre-clinical and clinical disease stages (ii) Chloroquine reduced sialadenitis and *TLR1*, *TLR2*, *TLR3*, *TLR4*, and *TLR9* expression in SMG tissue	[24]
NOD/Lt	(i) Lymphocytes expressing TLR9 are present in SMG tissue (ii) PBMCs coexpressing TLR9 and p-p38 MAPK are elevated in NOD/Lt animals at 5, 8, and 10 weeks of age compared to BALB/c controls	[29]
NOD	(i) TLR9 ligation increased salivation	[30]
C57BL/6.NOD-*Aec1Aec2*	(i) *TLR3*, *TLR7*, and downstream signaling intermediates are elevated at pre-clinical disease time point in SMG tissue	[33]
NOD.B10	(i) *MyD88*-deficient females are protected against local and systemic pSS manifestations	[36]
NZB/WF1	(i) TLR3 agonism with poly(I:C) increased *IFNβ*, *Mx-1*, *PRKR*, *IF144*, *IL-6*, *TNFα*, and *CCL5* in salivary tissue and resulted in loss of salivation	[39]
NZB/WF1	(i) Poly(I:C) upregulated *CCL2*, *CCL3*, *CCL4*, *CCL7*, *CCL11*, *CCL12*, *CXCL10*, and *Cxcl13* in SMG tissue (ii) Poly(I:C) induced robust and accelerated salivary inflammation and diminished saliva production	[40]
C57BL/6	(i) Poly(I:C) treatment caused reduced salivation and increased *IL-6*, *IL-10*, and *IL-27*p28 in SMG tissue	[41]
C57BL/6	(i) Poly(I:C) induced upregulation of chemokines in lacrimal tissue, dacryoadenitis, and reduced tear production	[43]
C57BL/6	(i) LPS treatment induced sialadenitis, increased *TNFα*, *IFNβ*, IFNγ, and *IL-6* in SMG tissue, and caused hyposalivation	[42]
C57BL/6	(i) Flagellin caused salivary inflammation, increased inflammatory cytokines and chemokines in sera, and autoantibodies	[44]

[19, 35]. Recent work demonstrates that MyD88 plays a crucial role in the development of pSS. Specifically, NOD.B10 females that lack *MyD88* (NOD.B10$^{MyD88-/-}$) were protected from loss of salivary flow and demonstrate reduced prevalence of lymphocytic infiltration in the lacrimal and submandibular glands compared to the parental strain [36]. NOD.B10$^{MyD88-/-}$ mice were also protected from extraglandular disease manifestations. These animals displayed a decrease in lymphocytic infiltrates in both the lung and kidney compared to NOD.B10 animals despite similar splenic T and B cell populations [36]. Finally, NOD.B10$^{MyD88-/-}$ mice exhibited diminished total and autoreactive antibodies as compared to the NOD.B10 parental strain [36]. Of note, since both TLRs and IL-1R family signaling are dependent on MyD88, further studies are needed to determine the specific MyD88-dependent pathways that are required for disease pathogenesis. Altogether, these studies provide compelling evidence that TLRs play a crucial role in disease in NOD mice.

2.2. TLR Agonists Induce SS in Healthy and SS-Prone Mice. Additional studies demonstrate that activation of TLRs in both animals with a genetic predisposition to develop autoimmune disease, as well as in healthy animals, results in SS-like disease. The NZB/WF1 strain is an sSS model that develops spontaneous lupus and SS [37]. Studies in the SMG of these animals revealed that TLR3 is expressed in the salivary epithelium, both in acinar tissue, ducts, and in the granular convoluted tubules (the segment of the duct system situated between the striated and intercalated ducts in rodents) [38, 39]. When NZB/WF1 mice were given a synthetic TLR3 agonist (polyinosinic:polycytidylic acid (poly(I:C)), they developed SS-like disease at an accelerated rate [39, 40]. Furthermore, TLR3 agonism upregulated type I interferon (*IFNβ*) and IFN-responsive genes and inflammatory mediators in SMG tissue, including *IL-6*, *IL-1β*, and *CCL5* [39, 40]. Interestingly, saliva production was reduced significantly one week post-treatment, despite the fact that the SMG histology remained normal in appearance [39]. Further work demonstrated that IFN and IL-6 are crucial for loss of salivary flow, as *IFNar*$^{-/-}$ and *IL-6*$^{-/-}$ mice were protected from salivary hypofunction following poly(I:C) administration [41]. Thus, poly(I:C) mediates salivary disease by induction of both type I IFN and IL-6.

Significantly, TLR activation in healthy mice also leads to the development of SS. Indeed, C57BL/6 mice given the TLR agonists lipopolysaccharide (LPS) or poly(I:C) display loss of salivary and tear flow and increased expression of proinflammatory cytokines in SMG and lacrimal tissue [41–43]. Additional studies implicate TLR5 in SS pathogenesis. C57BL/6 animals treated with the flagellar filament structural protein FliC, a TLR5 agonist, develop salivary inflammation [44]. Moreover, these mice display elevated levels of inflammatory mediators in sera and heightened IgG and anti-SSA/Ro autoantibodies [44]. Thus, these studies demonstrate a role for TLR activation in SS initiation in healthy animals and also in disease exacerbation in SS-prone mice. A summary of TLR-related studies in mouse models is provided in Table 1.

3. TLRs Are Dysregulated in Human SS

TLR expression and function have been studied in both salivary cells and PBMCs derived from pSS patients. Studies conducted on minor salivary gland (MSG) biopsy tissue or salivary gland epithelial cells (SGECs) allow for a mechanistic understanding of salivary-specific disease events in pSS. Analysis of PBMC populations provides insight regarding systemic immune dysfunction in pSS. In the following

section, we will review the literature related to TLR expression and function both in salivary tissue and in the periphery in the context of pSS.

3.1. Cell Surface TLRs Contribute to SS Pathogenesis

3.1.1. TLR2 Mediates Inflammation in SS. TLR2 plays an important role in several different autoimmune diseases, including lupus and rheumatoid arthritis [45, 46]. TLR2 recognizes a wide range of microbial products such as peptidoglycan (PGN) from Gram-positive bacteria and bacterial lipoproteins [47]. This diverse ligand binding is due in part to the ability of TLR2 to form heterodimers with TLR1 or TLR6 in order to fine-tune its specificity [48].

TLR2 is expressed in SGECs and in MSG tissue of pSS patients [49–51], and this expression correlates with the degree of focal lymphocytic sialadenitis [49]. PGN stimulation of TLR2 results in increased expression of mediators of immune activation (ICAM-1, CD40, and MHC-1) in SGECs derived from pSS patients and controls [51]. In a corroborative study using SGECs from pSS patients, TLR2 ligation resulted in NF-κB-dependent secretion of IL-15 [52]. IL-15 mediates proliferation of activated B and T cells and is pivotal in the generation and maintenance of natural killer (NK) cells [53]. Further work using SGECs derived from pSS and control MSG biopsies found that IL-15 was upregulated in these cells [54]. These data indicate that TLR2 agonism could promote both the survival and proliferation of both innate and adaptive immune cells in salivary tissue in disease.

In addition, *TLR2* levels are elevated in PBMCs from pSS patients [49]. Interestingly, TLR2 stimulation results in upregulation of *IL-17* and *IL-23* mRNA transcripts, as well as heightened secretion of IL-17 and IL-23 in PBMCs derived from pSS patients as compared to controls [49]. Secretion of both cytokines is enhanced when cells are treated concomitantly with PGN and anti-CD3 [49]. These data suggest that TLR2 signaling may be an important mechanism leading to enhanced T cell-derived IL-17 production in pSS [55].

Although the ligands that activate TLR2 in the context of SS are unknown, it is interesting to note that both TLR1 and TLR6 are expressed at high levels in the SGECs of pSS patients [49]. Since TLR2 forms heterodimers with both TLR1 and TLR6, this suggests a diverse group of ligands may activate TLR2-dependent signals in disease. Significantly, activation of pSS PBMCs with TLR2 and TLR6 ligands (PGN and zymosan, respectively) results in additive secretion of IL-23 and IL-17 [49]. Altogether, these data indicate that TLR2-dependent pathways may lead to inflammation both in exocrine tissue and in the periphery. Therefore, therapies that target TLR2 may ameliorate both local and systemic pSS disease manifestations.

3.1.2. Emerging Data Suggest an Important Role for TLR4 in Human SS. TLR4 recognizes both pathogen-derived and endogenous molecules and plays an important role in the pathogenesis of several autoimmune diseases [45, 47, 56–58]. TLR4 is expressed in salivary glands, specifically in infiltrating mononuclear cells and in ductal and acinar cells [49, 59]. TLR4 expression is increased in the MSG of pSS patients, and receptor levels correlate with the degree of glandular inflammation [49, 60]. Importantly, TLR4 expressed by SGECs is functional, as stimulation with LPS results in increased expression of the immunoregulatory molecules ICAM-1, CD40, and MHC-1 [51]. Although additional work reported TLR4-mediated IL-6 production in human salivary gland (HSG) cells, this cell line is now recognized to be a HeLa derivative [59, 61].

However, recent work using the A253 salivary gland cell line established that TLR4 is upregulated in response to LPS stimulation in salivary cells [62]. Moreover, LPS stimulation results in secretion of numerous inflammatory mediators, including IL-6, IL-12, CCL5, and monocyte chemotactic protein-1 (MCP-1) [62]. Thus, current evidence suggests an important role for TLR4 in salivary inflammation in SS, and future work to understand the regulation and activation of TLR4 in the context of this disease may lead to novel ways to mitigate chronic inflammation in autoimmunity.

3.1.3. Hematopoietic-Derived TLR5 May Contribute to Disease. While there is a paucity of data regarding the role of TLR5 in SS (and in autoimmunity in general), TLR5-dependent signaling pathways may contribute to disease. TLR5 recognizes flagellin, a highly conserved protein found in bacteria. TLR5 expression is decreased in PBMCs from pSS patients as compared to healthy controls [63], although the significance of this finding remains to be determined. It is interesting to speculate that TLR5 activation may promote sialadenitis and autoantibody production in pSS patients, as is suggested by studies in mice (*vide supra*), although this has not been examined in humans to date [44, 64]. Further studies of TLR5 in peripheral blood samples and salivary tissue of pSS patients may reveal an important role for this poorly understood receptor in autoimmunity.

3.2. Endosomal TLRs Mediate Disease in SS Patients

3.2.1. TLR3 Mediates Cell Death and Inflammatory Cytokine Production in Salivary Gland Cells in SS. TLR3 binds dsRNA that is primarily produced by viruses, although this receptor also recognizes endogenous RNA released from necrotic cells in autoimmune disease [47, 48, 65]. TLR3 is expressed in SGECs, and treatment of these cells with poly(I:C) results in increased of expression of ICAM-1, CD40, and MHC-I [50, 51, 66]. In addition, stimulation of SGECs with poly(I:C) or reovirus-1 (a dsRNA virus) results in heightened BAFF secretion and pre-treatment of SGECs with chloroquine diminished this effect [50]. Of note, several studies in mice and humans demonstrate that BAFF plays a key role in SS pathogenesis, primarily by inducing B cell hyperactivity [67–72]. These studies suggest that TLR3 ligation of salivary cells activates both adaptive and innate immunity.

TLR3 agonism also contributes to SGEC apoptosis in SS. Accordingly, SGECs from pSS patients stimulated with poly(I:C) undergo anoikis, a process of programmed cell death in which death is triggered by loss of normal cell attachment to the extracellular matrix (ECM) [73]. Significantly, SGECs derived from a healthy individual are

Table 2: Aberrant TLR expression and activation in salivary tissue in SS.

Tissue/cell type	TLR	Results	Refs
pSS SGECs	1	(i) Increased gene expression compared to controls	[51]
Healthy and pSS SGECs	2	(i) TLR2 is expressed in SGECs (ii) Increased protein expression following treatment with PGN (iii) Upregulation of immune-activating molecules following TLR2 ligation (iv) TLR2 activation induced IL-15 secretion	[50] [51] [66] [52]
pSS MSG biopsies	2	(i) Increased protein expression compared to controls that correlates with the degree of salivary inflammation	[49]
Healthy and pSS SGECs	3	(i) TLR3 is expressed (ii) Elevated gene expression compared to controls (iii) Increased protein expression following treatment with poly(I:C) (iv) Upregulation of immune-activating molecules following TLR3 ligation	[50] [51] [66] [74]
Healthy SGECs	3	(i) Induction of BAFF mRNA and protein secretion by the TLR3 agonists poly(I:C) and a dsRNA virus	[50]
Healthy and pSS SGECs	3	(i) Poly(I:C) treatment induced expression and activation of apoptosis-related signaling intermediates and anoikis	[74] [66] [73]
Healthy and pSS MSGs	3	(i) Increased protein expression of TLR3 signaling intermediate RIPK3 kinase in pSS tissue	[74]
SGECs	3	(i) Poly(I:C) treatment upregulated *Ro52*/TRIM21 mRNA, resulting in redistribution in nucleus	[76]
pSS MSG biopsies	4	(i) Increased protein expression compared to controls that correlates with the degree of salivary inflammation	[49]
Healthy and pSS SGECs	4	(i) Elevated gene expression compared to controls (ii) Increased protein expression following LPS stimulation (iii) Upregulation of immune-activating molecules following TLR4 ligation	[51]
A253 cells	4	(i) Increased protein expression following LPS stimulation (ii) LPS-induced secretion of proinflammatory mediators	[62]
pSS MSG biopsies	6	(i) Increased protein expression compared to controls that correlates with the degree of salivary inflammation	[49]
Healthy SGECs	7	(i) TLR7 is expressed	[50]
pSS MSG biopsies	7	(i) TLR7 is expressed	[77]
Control and pSS parotid biopsies	7, 9	(i) Elevated protein expression	[78]
Healthy and pSS MSG biopsies	8, 9	(i) Elevated gene expression	[79]

protected from anoikis following TLR3 ligation, although the reason for this observation is poorly understood [73]. In a corroborative study, TLR3 agonism induced apoptosis in pSS SGECs through upregulation of RIPK3, p-FADD, and cleaved caspase-8 [74]. While apoptosis normally does not induce an immune response in healthy individuals, SS patients exhibit deficient clearance of apoptotic debris that could serve as a nidus of inflammation in disease [75].

In addition to apoptosis, TLR3 ligation in salivary tissue regulates the SS autoantigens SSA (*Ro52* and Ro60) and SSB (La) [76]. Specifically, treatment of SGECs from control and pSS patients with poly(I:C) causes redistribution of these proteins in the nucleus and increases expression of *Ro52* transcripts. Stimulation of SGECs with poly(I:C) also results in the dysregulation of interferon regulatory factors (IRFs) and elevated IFNβ levels, similar to observations in NZB/WF1 mice (*vide supra*) [39]. Significantly, neutralization of IFNβ diminished poly(I:C)-induced upregulation of *Ro52* mRNA and also inhibited autoantigen redistribution [76]. Taken together, these data suggest that TLR3 signaling cascades upregulate *Ro52* and induce apoptosis of salivary epithelium, thereby releasing autoantigens that drive immune hyperactivity in SS. Therefore, TLR3 has profound effects on salivary epithelium, as TLR3 agonists promote expression of costimulatory molecules, cytokine secretion, anoikis, and autoantigen expression within these cells.

3.2.2. Activation of TLR7, TLR8, and TLR9 Contributes to Immune Dysregulation in SS. TLR7 and TLR8 are expressed in salivary tissue and in the periphery, and several studies demonstrate that both receptors are elevated in the context of SS [50, 63, 77–79]. Significantly, data suggest that TLR7 activation contributes to the etiopathogenesis of SS. TLR7-stimulated B cells from pSS patients secrete increased levels of IFNα as compared to healthy control B cells [80]. Moreover, stimulation of naïve B cells from pSS patients with a TLR7 agonist causes increased plasma cell differentiation and class switching [81]. Finally, studies using monocyte-derived DCs (moDCs) reveal that cells isolated from pSS patients exhibit enhanced maturation following stimulation

Table 3: Systemic TLR dysregulation in human SS.

Cell type	TLR	Results	Refs
PBMCs	2	(i) PBMCs from pSS patients are more responsive to TLR2, TLR4, and TLR6 agonists than those from healthy controls (ii) TLR2, TLR4, and TLR6 agonists show additive effect in induction of IL-23 and IL-17 secretion in cultured PBMCs from pSS patients	[49]
PBMCs	5	(i) Reduced protein expression	[63]
PBMCs	7	(i) Increased gene expression	[78]
PBMCs	7	(i) Increased protein expression	[63]
B cells	7	(i) Stimulation with TLR7 agonist (CL264) causes elevated IFNα secretion	[80]
CD14+ monocytes	7	(i) Increased expression of *TLR7* in pSS patients with a positive type I IFN signature	[77]
PBMCs	8	(i) Elevated gene expression	[63]
moDCs	7/8	(i) Increased maturation in moDCs derived from pSS patients following stimulation with TLR7/8 agonist (CL097)	[82]
B cells	7, 9	(i) Stimulation of naïve B cells from pSS patients with TLR7 (imiquimod) or TLR9 (CpG) agonists causes increased plasma cell differentiation and class switching compared to controls	[81]
CD14+ monocytes	9	(i) Decreased *TLR9* expression in monocytes from both type I IFN-positive and IFN-negative pSS patients	[77]
PBMCs	9	(i) Decreased gene expression	[63]
PBMCs	9	(i) Increased gene expression	[78]
PBMCs	9	(i) Enhanced secretion of IL-8, IL-15, IL-1RA, MCP-1, and IL-2R upon stimulation with TLR9 agonist CpG (ii) TLR9 agonist (CpG) decreased CD80 and CD25 expression	[80]

Table 4: Summary of putative DAMP/TLR interactions in SS.

DAMP	TLR	Refs
(i) Biglycan and decorin are degraded by saliva from NOD.B10 mice	TLR2 and TLR4	[95]
(ii) Versican is increased in SGEC lines from SS patients	TLR2/6 heterodimer	[108]
(i) Fibronectin is dysregulated in salivary tissue from SS mice and upregulated in saliva from SS patients	TLR4	[109] [110] [95]
(ii) Calprotectin (S100A8/A9) levels are elevated in sera, salivary tissue, and saliva of pSS patients		[111] [112] [113]
(iii) MUC5B and MUC7 are mislocalized in the ECM of pSS patients		[114] [115]
(i) Long interspersed nuclear element 1 (LINE-1) is increased in MSG tissue from SS patients	TLR 7/8	[116] [117]

with a TLR7/8 agonist (CL097) as compared to those from healthy controls [82]. Taken together, these data demonstrate that pSS patients display elevated TLR7 expression and hyperresponsiveness to TLR7/8 ligands and this likely plays an important role in the chronic inflammatory landscape observed in SS patients.

While the underlying reasons for this are unknown at present, it is intriguing to speculate that the heightened TLR7 activation in SS may be due to X chromosome gene dosage effects. As mentioned previously, SS occurs more commonly in women than in men [6]. In order to compensate for the presence of two X chromosomes in females, either the maternally- or paternally-derived X chromosome is randomly silenced, a process called X chromosome inactivation (XCI). However, this epigenetic change is not 100% effective, as 15% of genes expressed by both chromosomes exhibit altered expression [83]. A recent study using gene expression datasets found upregulation of 58 X chromosome genes, including 22 genes previously shown to escape XCI, in SS patient salivary glands [84]. Of note, TLR7 is found on the X chromosome and escapes XCI in immune cells [85]. Thus, there is a potential for females to have an increased TLR7 copy number, leading to subsequent TLR7 hypersensitivity. Indeed, pSS patients exhibit increased TLR7 expression and responsiveness [77, 78, 80, 84] and this is likely mediated, at least in part, by improper gene silencing [84].

Finally, several studies have focused on TLR9 in SS, as TLR9 levels are elevated in both MSG and parotid tissues in disease [78, 79]. While one study reported increased *TLR9* expression in PBMCs [78], others report that expression is decreased in both PBMCs and monocytes from pSS patients [63, 77]. Differences are also observed in TLR9 responsiveness, as secretion of proinflammatory mediators, plasma cell differentiation, and class switch recombination were increased following TLR9 ligation in B cells derived from pSS patients as compared to those from controls [80, 81]. Of note, expression of CD80 and CD25 was diminished as compared to healthy controls [80]. As mentioned above, TLR9 plays a protective role in lupus [31] and emerging work suggests that TLR9 activation also ameliorates disease in SS [30]. Although there are few studies regarding the role of TLR9 in SS, it is possible that TLR9 upregulation in glandular tissue may promote resolution of inflammation, while the decreased expression in the periphery may lead to immune activation. Therefore,

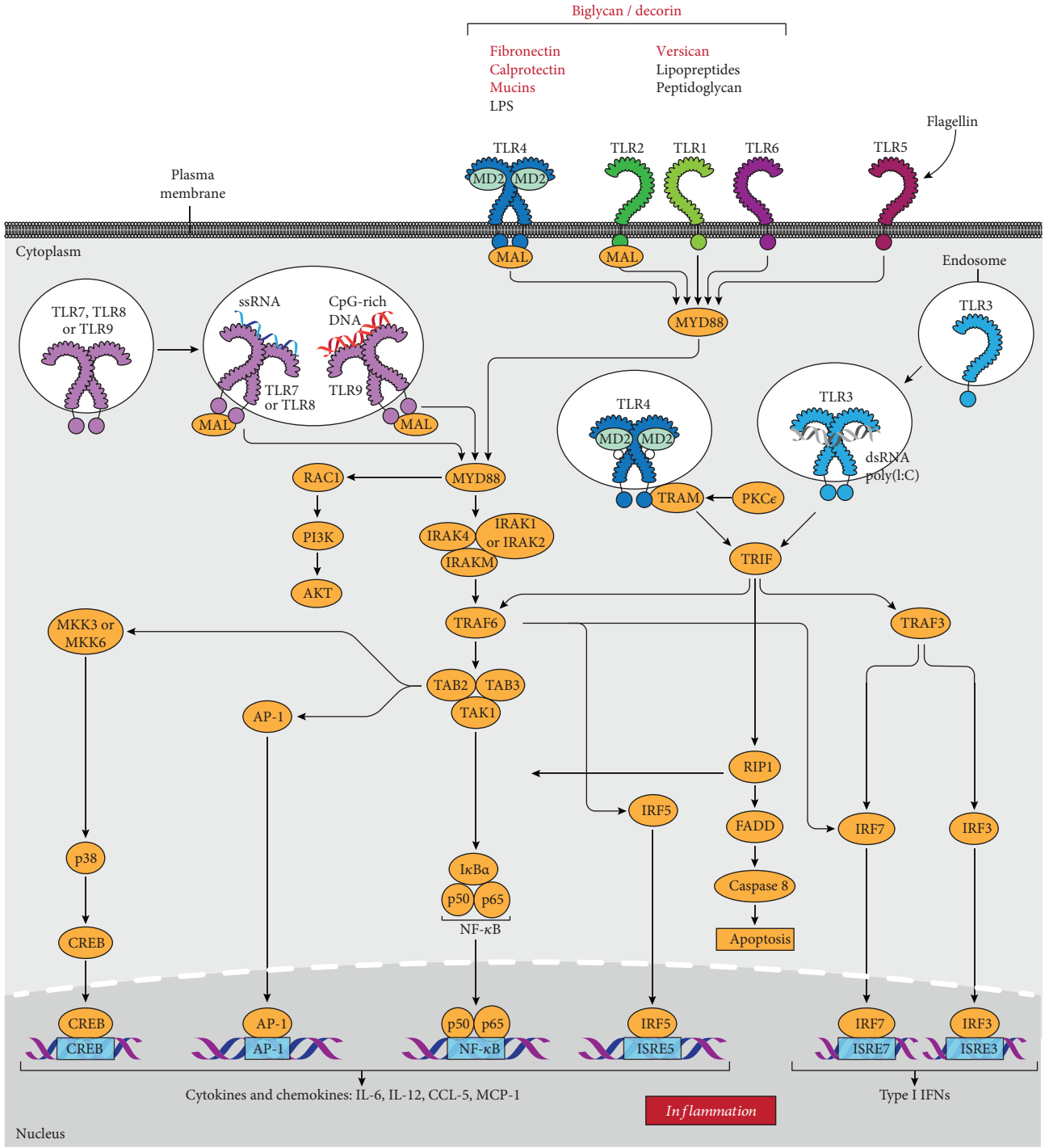

Figure 1: Overview of TLR signaling cascades in SS. TLRs activated in SS and downstream signaling cascades are shown. DAMPs implicated in SS are labeled in red.

further studies are required to understand how TLR9 is regulated in SS and how TLR9 activation modulates disease pathogenesis.

3.3. TLR-Dependent Signaling Molecules Are Dysregulated in SS. In addition to TLRs themselves, many TLR-dependent signaling molecules show altered expression in pSS. Accordingly, *MyD88* is upregulated in both monocytes and plasmacytoid DCs (pDCs) derived from pSS patients who demonstrate an IFN signature [77]. In addition, *PKR* (*EIF2AK2*), which is activated by TLR4 through a MyD88-independent pathway, is increased in monocytes from IFN-positive pSS patients as compared to those who are IFN-negative and healthy controls [77]. Moreover, *RSAD2/viperin* and *STAT1* were both increased in IFN-positive pSS patients as well [77]. STAT1 is downstream of TLR2, TLR4, TLR7,

and TLR9 [77, 82, 86], while RSAD2 is induced by TLR3 and TLR7 [77, 87, 88]. Thus, evidence points to dysregulation of both TLRs and TLR-dependent signaling intermediates in SS. A summary of studies detailing TLR dysregulation in salivary tissue (Table 2) and in the hematopoietic compartment in pSS patients (Table 3) is provided.

4. Emerging Data Identify Novel Ligands That Likely Mediate Chronic Inflammation in SS

In pSS, the ligands that activate TLRs remain unknown. While PAMPs may play a role in disease pathogenesis, it is possible that endogenous DAMPs may activate TLRs, as is the case for other autoimmune diseases [58, 89, 90]. DAMPs are a diverse group of stimuli that include ECM molecules, RNA and DNA, and saturated fatty acids [58, 90, 91]. DAMP-induced inflammation is "sterile," as it is caused by host-derived molecules that normally do not elicit an immune response. However, under conditions of tissue damage, these endogenous mediators are released in soluble form, allowing for activation of host receptors [90, 92]. Many different TLRs are activated by numerous, partially overlapping DAMPs [90]. Evidence in both SS models and patients suggests that DAMPs may contribute to disease [93–95], although more work is needed to understand the role of DAMP-derived inflammation in SS. A summary of DAMPs identified in SS that are known to activate TLRs is provided in Table 4, and the signaling cascades activated by DAMPs are shown in Figure 1.

5. Inhibition of TLR-Dependent Signaling Mitigates Human Autoimmunity

Therapeutics that target TLR signaling pathways are currently being tested for the treatment of several different autoimmune diseases [62, 89, 90, 96–100]. Small molecule inhibitors of interleukin-1 receptor-associated kinase 4 (IRAK4) were recently developed that block collagen-induced arthritis in mice [101]. In addition, a high-throughput small-molecule screening approach identified an inhibitor that blocks the binding of TRAF6 to the 2-conjugating enzyme ubiquitin-conjugating enzyme E2N, thereby preventing ubiquitination that is required for transduction of inflammatory signaling [102]. This molecule (termed C25-140) attenuated disease severity in mouse models of imiquimod-induced psoriasis and collagen-induced arthritis [102]. Moreover, a monoclonal antibody directed against TLR4 recently completed a phase 2 clinical trial for the treatment of rheumatoid arthritis [103]. Finally, IMO-8400, an inhibitor of endosomal TLRs (TLR7, TLR8, and TLR9), diminished clinical activity in a phase 2a, randomized, placebo-controlled trial in patients with plaque psoriasis [104].

Given the success of these targeted therapeutics in other autoimmune diseases, modulation of TLR signaling cascades may constitute a successful strategy to reduce local and systemic inflammation in SS. Drugs that inhibit DAMP-mediated TLR activation hold therapeutic promise, particularly if co-receptors or accessory molecules necessary for ECM binding are targeted [105, 106]. These therapies may be designed in such a way as to preserve the ability of the host to respond to pathogens while preventing pathogenic TLR activation by endogenous sources [90, 107]. Therapeutics that target TLR-dependent immune activation will likely result in improved management of SS.

6. Conclusion

In summary, the etiopathogenesis of SS remains poorly understood. While substantial evidence suggests that TLR activation is an integral part of the disease, further studies are required to elucidate the mechanisms that govern TLR regulation and activation both in exocrine tissue and in the periphery. These studies will likely lead to identification of novel mediators of inflammation and targeted therapeutic strategies to reduce the innate and adaptive immune activation that is characteristic of this debilitating disease.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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