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The prevalence of novel candidate Sjögren's syndrome autoantibodies in the Penn Sjögren's International Collaborative Clinical Alliance (SICCA) cohort

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Abstract

Purpose: To evaluate the prevalence of novel candidate autoantibodies associated with Sjögren's syndrome (SS) and their ability to identify those with SS among participants with dry eye enrolled in the Sjögren's International Collaborative Clinical Alliance (SICCA) study at the University of Pennsylvania (Penn).

Methods: All participants previously underwent a full ocular and systemic evaluation for possible SS as part of the SICCA study. An Enzyme Linked Immunosorbent Assay (ELISA) was used to detect IgG, IgA and IgM autoantibodies to salivary protein 1 (SP-1), parotid secretory protein (PSP), and carbonic anhydrase 6 (CA-6) from previously banked baseline serum samples from SICCA study participants enrolled at Penn. The prevalence rate of each autoantibody, calculated by considering the presence of any isotype as antibody positive, was compared between dry eye participants with SS (n=81) or without SS (n=129) using the Fisher exact test.

Results: The prevalence of SP-1 IgM autoantibodies was higher in those with SS compared to those without SS (14% vs. 5%; p=0.03). Similarly, the prevalence of PSP IgA autoantibodies was higher in those with SS compared to non-SS dry eye participants (21% vs. 11%, p=0.048). There was no statistically significant difference in the prevalence of CA-6 autoantibodies between those with or without SS (15% vs. 20%, p=0.36).

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Conflicts of Interest

VYB: Bausch & Lomb/Immco Diagnostics, Inc (grant for antibody testing). Celularity (consultant).
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FBV: Consultant/investigator for Immco Diagnostics, Novartis, Biogen Idec, and Boston Pharmaceuticals
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Conclusions: In the Penn SICCA cohort, SP-1 IgM and PSP IgA autoantibodies were more prevalent in the serum of SS-related dry eye participants compared to those without SS. Further longitudinal studies are needed to determine the clinical significance of these findings.

Keywords

Sjogren's syndrome; dry eye; novel autoantibodies

INTRODUCTION

Sjögren's syndrome (SS) is a serious, potentially life-threatening autoimmune disorder that attacks the lacrimal and salivary glands, and predisposes patients to malignancies including lymphoma[1]. According to some sources, estimates of the prevalence of SS in the United States may range from 0.4 to 3.1 million[2, 3], and approximately half of SS patients remain undiagnosed due to the nonspecific nature of early clinical manifestations and challenges with diagnosis[2, 4, 5]. Early diagnosis of SS is critical to improve the probability of treatment success[6] and to identify patients who would benefit from surveillance for serious complications. Currently, the diagnosis of SS is variable and based on a constellation of signs, symptoms, histopathology, and laboratory results, none of which are definitive. Because of the heterogeneity of clinical presentations and the requirement of collaboration among multiple specialists, the diagnosis of SS is complex and is frequently delayed by 3 to 7 years from the onset of symptoms.[7, 8]

SS patients often have dry eye, which can precede the systemic findings and diagnosis of SS by an average of 10 years[9]. Because SS patients often first seek care for dry eye, ophthalmologists have the unique opportunity to screen patients for SS, thereby facilitating earlier diagnosis. However, the early diagnosis of SS is hampered by the significant limitations of sensitivity and specificity of traditional SS autoantibodies [anti-Sjögren's syndrome A (SSA), anti-Sjögren's syndrome B (SSB); anti-nuclear antibody (ANA); rheumatoid factor (RF)][10–12].

Recently, a group of tissue specific murine autoantibodies [SP-1 (salivary gland protein-1), CA-6 (carbonic anhydrase-6), and PSP (parotid secretory protein)] were described in the interleukin-14 α transgenic mouse model for SS and were found to appear in the blood before anti-SSA/SSB autoantibodies. These novel autoantibodies were also observed in the non-obese diabetic mouse model of SS and in humans with SS (including seronegative SS) defined according to the American -European Consensus Group classification criteria.[13] These findings suggested the possible utility of testing for the novel murine autoantibodies as a diagnostic marker for SS in humans.[13]

However, there are limited studies[14–19] regarding the prevalence of these antibodies in humans and the clinical significance of their presence is still unclear. One significant limitation of previous studies is that the true SS status of patients was often unknown as participants did not undergo complete work-ups for the disease and therefore an assessment of the true specificity and sensitivity of these antibody tests was not possible. Larger studies utilizing well-characterized cohorts with confirmed SS status are needed to understand the clinical significance of these antibodies.

The Sjögren's International Collaborative Clinical Alliance (SICCA) provides a large, well-characterized cohort. This multi-center, international study sponsored by the National Institute of Health was unique in that all participants with suspected or confirmed SS underwent systemic work-ups to establish their SS status. It was previously reported that in a small subset of serum samples from the SICCA cohort, that SSA and SSB autoantibodies were present in SS patients with higher focus scores on labial salivary gland biopsies, while the novel antibodies were found in dry eye and dry mouth patients with lower focus scores.[17] This well-characterized cohort provides a unique opportunity to further study the prevalence of the novel autoantibodies in dry eye participants with or without SS. We hypothesized that the prevalence of these autoantibodies in this cohort could be significantly different than in previous studies where the true SS status of patients was not validated. This study utilized the SICCA study database and biospecimens from participants enrolled at the University of Pennsylvania (Penn) to determine if these new candidate autoantibodies are useful in identifying dry eye patients who have SS.

METHODS

Study population

While SICCA was a multi-center, international cohort study[20, 21], the present study was a pilot study limited to those SICCA participants enrolled at Penn who had objective evidence of dry eye with an abnormal Ocular Staining Score[22] (OSS) of greater than or equal to 3. Briefly, the OSS is comprised of lissamine green staining of the conjunctiva and fluorescein staining of the cornea, with extra points for the presence of confluent or central corneal staining or filaments.[22] Permission to conduct the study was obtained from the SICCA Biorepository project directors. Previously banked blood specimens from the Penn SICCA cohort were assayed for the novel candidate SS autoantibodies (SP-1, PSP, CA-6) and traditional SS antibodies (SSA, SSB, RF, ANA) at Immco Diagnostics Laboratories (Buffalo, NY). Participants were included who had objective evidence of dry eye and were classified as either non-SS or SS, based on the 2012 American College of Rheumatology (ACR) SS classification criteria.[21] Using the 2012 ACR criteria, participants were classified as having SS if they met 2 of the following 3 criteria: 1) positive serology (positive SSA or SSB; or RF *and* ANA ≥ 1:320); 2) positive labial salivary gland biopsy with focal lymphocytic sialadenitis; or 3) OSS score of ≥ 3. 49 subjects who could not be classified as SS or non-SS and were excluded from comparative analyses of groups.

Evaluation

All participants enrolled in the SICCA study previously received a comprehensive history and physical examination, ocular surface exam (including staining with fluorescein and lissamine green, tear break-up time, unanesthetized Schirmer testing), stimulated and unstimulated sialometry, serologic testing and a labial minor salivary gland biopsy for hematoxylin and eosin staining for the calculation of a focus score.[23, 24]

Assay for traditional and novel SS antibodies

The following antibody assays were performed on each blood specimen using a standard Enzyme Linked Immunosorbent Assay (ELISA) assay: RF (IgG, IgA & IgM), SSA, SSB,

SP-1 (IgG, IgA & IgM), CA-6 (IgG, IgA & IgM), and PSP (IgG, IgA & IgM). Results were expressed in ELISA units per milliliter (EU/ml) and were reported as positive or negative. ANA antibodies by HEp-2 were assessed by indirect immunofluorescence. Calibrators, positive and negative controls and a reagent blank were run with each assay to verify the integrity and accuracy of the test. Test samples were run in duplicates and mean absorbance was used to calculate the EU/ml values.

Statistical Analysis

We compared the participant characteristics between the SS and non-SS groups using the two-sample t-test for continuous measures, and the Fisher exact test for categorical measures. We performed statistical comparisons between participants with SS or without SS (non-SS) for the presence each of the novel candidate SS antibodies (SP-1, CA-6 and PSP) using the Fisher exact test. Among non-SS participants, we compared measures of dry eye severity (Schirmer score and OSS) between participants who were positive versus negative for each isotype of the novel autoantibodies. All the statistical comparisons were performed in SAS v9.4 (SAS Institute Inc., Cary, NC) and a two-sided $p<0.05$ was considered to be statistically significant.

RESULTS

Baseline characteristics

The study included 81 dry eye participants with SS (SS) and 129 participants without SS (non-SS) enrolled at Penn. The baseline characteristics of these two groups of participants are shown in Table 1. The SS group had a significantly lower mean Schirmer test score (7.2 mm versus 10.6 mm; $p=0.001$) and a significantly higher mean OSS (7.5 versus 4.8; $p<0.0001$) compared to the non-SS group.

Novel Autoantibodies

The prevalence rate of PSP IgA autoantibodies was significantly higher in SS participants than non-SS participants (21% vs. 11%, $p=0.048$)(Table 2). SS participants also had a significantly higher prevalence of SP-1 IgM antibodies compared with non-SS participants (14% versus 5%; $p=0.03$). There was no statistically significant difference between the SS and non-SS participants regarding the prevalence of CA-6 antibodies (15% vs. 20%, $p=0.36$). Among 6 SS participants who were negative for the traditional SS autoantibodies, 1 participant (17%) was positive for at least one of the novel autoantibodies.

Novel Autoantibodies and Dry Eye Severity

In order to explore the potential relationship between dry eye severity and the novel autoantibodies, we performed a subgroup analysis of the non-SS group of OSS and Schirmer scores according to positivity of the novel serologic markers (Tables 3 and 4). We found that those positive for CA-6 IgA autoantibodies had significantly lower Schirmer scores than those who were negative for these antibodies (3.5 vs. 11.1, $p=0.02$, Table 4). However, there was no significant difference in OSS scores among those who were positive or negative for the novel autoantibodies (all $p\geq0.08$, Table 3).

DISCUSSION

In the Penn SICCA study cohort, we found that in those with dry eye, the prevalence of SP-1 IgM autoantibodies was significantly higher in SS participants than in non-SS participants. Our results are consistent with a recent report from the DRy Eye Assessment and Management (DREAM©) study in which the authors found that there was a significantly higher prevalence of SP-1 antibodies (any isotype) in the SS group versus the non-SS group.[25] However, in contrast to the DREAM study, we also found that the prevalence rate of PSP IgA autoantibodies was significantly higher in SS participants compared to those with non-SS dry eye. Similarly, De Langhe and colleagues found a higher prevalence of PSP IgA antibodies in SS participants compared to those without SS.[16] In contrast to our study, Karakus et al found a higher prevalence of CA-6 autoantibodies in SS versus non-SS dry eye patients[19], whereas in our study we found no difference between those groups.

There is previous evidence that the novel antibodies may be associated with more severe ocular surface disease. For example, Karakus and colleagues found that those who were positive for CA-6 autoantibodies was associated with more severe dry eye signs and symptoms.[19] In addition, in a recent report from the DREAM study, the authors found that those who were positive for traditional SS autoantibodies, or for both traditional and novel autoantibodies, had worse corneal and conjunctival staining than those who were not positive for any of these autoantibodies.[25] Similarly, in our study we found that non-SS participants who were positive for CA-6 IgA autoantibodies had significantly lower Schirmer scores than those who were negative for those autoantibodies. However, because we performed multiple comparisons (26) to assess the association of severity and novel antibody positivity, it is possible that this finding was the result of chance. In addition, we were limited by sample size regarding our subgroup analyses. Therefore, further larger studies are needed to confirm this association.

The clinical significance of various isotypes of autoantibodies in the setting of rheumatic disease varies. Of note, while for this study all 3 isotypes of RF autoantibodies were assessed, in clinical practice typically only IgM RF autoantibodies are assayed. There are reports of rheumatic diseases where the presence of various isotypes of autoantibodies is clinically significant. For example, Domingues et al found that anticardiolipin IgG, but not IgM, was associated with a greater risk of thrombosis. Nojima and colleagues recently reported a novel enzyme immunoassay for the simultaneous detection of 6 subclasses of antiphospholipid antibodies and hypothesized that the presence of specific combinations of these antibodies were associated with thromboembolic complications[26]. However, at this time it is unclear if there will be similar clinical utility to checking for all 3 isotypes of each of the novel SS autoantibodies, or if only certain isotypes will be sensitive and specific enough to be useful. Future studies in larger, well-characterized cohorts are needed to determine the sensitivity and specificity of specific isotypes of these novel autoantibodies.

In our study, the prevalence rates of the novel autoantibodies in both SS and non-SS dry eye participants differ from previously published reports.[13, 16, 18] (Table 5).

There are a few possible explanations for the differences in the prevalence in our study and previously published studies. One possible explanation is that in previous studies SS status was not assessed or was based primarily on the ocular surface exam and serological testing, and all patients did not undergo minor labial salivary gland biopsies.[15, 19, 25, 27] The ACR 2012 criteria require that at least 2 of the following 3 criteria be met: 1) positive for the traditional SS antibodies (positive for SSA or positive for SSB or (positive for rheumatoid factor and ANA ≥1:320)); 2) OSS score of 3 or more in the worse eye, and 3) labial salivary gland biopsy exhibiting focal lymphocytic sialadenitis with a focus score of 1 focus/4mm[221] Minor labial salivary gland biopsies are necessary to ascertain the true SS status of a participant if the OSS or serological results only meet 1 of the 3 ACR criteria. Therefore, in previous studies there may have been misclassification of seronegative SS participants. In contrast, in the current study, all participants underwent systematic, full work-ups for SS, including minor labial salivary gland biopsies, when indicated.

In addition, there are several different sets of classification criteria for SS and it is possible that SS in each study was classified using various sets resulting in the inclusion of heterogenous populations for each study. Finally, geographic differences and different populations being studied in various countries could lead to underlying genetic differences and may have affected the results of each study.

There is conflicting evidence in the literature regarding the natural history of dry eye disease and the development of SS. There are some reports that dry eye disease may improve over time and rarely evolves into true SS.[23, 28, 29] However, participants in those studies were being treated for dry eye so it is possible that the therapies were alleviating the disease or slowing its progression. Other reports indicate that dry eye is a progressive disease.[30] Future longitudinal studies regarding the natural history of dry eye will be helpful in elucidating this further.[31] It is possible that dry eye patients who are positive for the novel candidate SS autoantibodies should be followed more closely with repeat serological testing so that the possible evolution of SS is not missed.

Interestingly, in our study, in a small subset of participants who were negative for the traditional SS autoantibodies, 1 in 6 was positive for at least one of the novel autoantibodies. Therefore, despite the fact that the majority of SS patients in our study did not express the novel autoantibodies, it is possible that they could be helpful in identifying a subset of SS patients who would otherwise remain undiagnosed as they are negative for the traditional SS antibodies. These autoantibodies may be useful in combination with other factors for distinguishing SS from non-SS patients, as they do have some predictive ability. In addition, these autoantibodies may not play a direct role in the pathophysiology of dry eye in SS, but rather may serve as markers for early SS.

Participants who are negative for the traditional SS autoantibodies, but positive for the novel autoantibodies, would need to be followed longitudinally over time to see if they subsequently meet the classification criteria for SS in the future in order to determine if the novel antibodies can be used for predicting SS.

Our study had certain limitations. For example, this study only included SICCA study participants enrolled at the University of Pennsylvania at baseline. Future studies that also evaluate SICCA specimens from the other 8 international SICCA centers would be helpful in increasing the generalizability of our findings. This study also utilized previously banked baseline serum samples and was cross-sectional. Longitudinal studies that include the collection of serial samples would be helpful in examining changes in autoantibody expression over time. Finally, the SICCA study did not include any normal control participants. Non-SS participants in the SICCA study were referred for study or entered the study due to some suspicion of SS and as a result this group is likely different from typical dry eye patients who are seen in the clinic. This could have potentially caused a higher than expected prevalence of the novel autoantibodies in the non-SS group. However, because we were still able to detect a difference between dry eye participants with or without SS, we hypothesize that the difference in the prevalence of the novel SS antibodies would be even greater if compared between SS and typical non-SS dry eye patients seen in the clinic. Future studies that examine the prevalence of the novel candidate SS antibodies in control patients without any suspicious signs or symptoms for SS would be useful in elucidating this relationship further.

In conclusion, in the Penn SICCA cohort, SP-1 IgM and PSP IgA murine autoantibodies were more commonly found in the serum of SS participants compared to non-SS participants. In addition, in a small subset of participants who were negative for the traditional SS autoantibodies, 1 in 6 was positive for at least one of the novel autoantibodies. Longitudinal studies in larger cohorts are needed to determine the clinical significance of these findings. To our knowledge, the present study is the largest to date examining these novel candidate SS antibodies in a well-characterized cohort whose true SS status is known after undergoing systemic work-ups. Future longitudinal studies looking at how the expression of these novel antibodies changes over time in different populations would be useful to better delineate the clinical utility of testing for these novel murine autoantibodies in SS.

Data Availability

The data and specimens used in this manuscript were obtained from the Sjögren's International Collaborative Clinical Alliance (SICCA) Biorepository, funded under contract #HHSN26S201300057C by the National Institute of Dental and Craniofacial Research (NIDCR). This manuscript was prepared using a publicly available SICCA data set and does not necessarily reflect the opinions or views of all SICCA investigators or the NIDCR.

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Table 1:

Characteristics of dry eye participants with or without Sjögren's syndrome in the Penn Sjögren's International Collaborative Clinical Alliance (SICCA) study cohort

Baseline Characteristics	**No Sjögren's syndrome* (Non-SS) (N=129)**	**Sjögren's syndrome** (SS) (N=81)**	**P-value**
Age (years): Mean (SD)	50.6 (12.2)	53.6 (14.3)	0.11
Sex: Male (%)	10 (7.8%)	8 (9.9%)	0.62
Race			
White	115 (89.8%)	70 (86.4%)	
Black	9 (7.0%)	9 (11.1%)	
Asian	3 (2.3%)	1 (1.2%)	
Hispanic/Latino	1 (0.8%)	1 (1.2%)	
Smoking status			
Never	76 (58.9%)	47 (58.0%)	
Former	44 (34.1%)	30 (37.0%)	
Current	9 (7.0%)	4 (4.9%)	
Schirmer test (mm): mean (SD)†	10.6 (8.7)	7.2 (6.1)	0.001
Total Ocular Staining Score (OSS): mean (SD)	4.8 (2.9)	7.5 (3.2)	<0.0001
Tear Break-up Time (seconds)***†			0.0005
3–4	46 (35.9%)	15 (18.5%)	
5–6	14 (10.9%)	11 (13.6%)	
7–9	20 (15.6%)	8 (9.9%)	
10 or more	14 (10.9%)	3 (3.7%)	

*Did not meet 2012 American College of Rheumatology (ACR) Sjögren's syndrome (SS) classification criteria

**Met 2012 ACR SS classification criteria

†From the worse eye of a specific ocular dry eye measure.

***Actual tear break-up time was not recorded for those with a TBUT of 10 sec or greater, so the mean value could not be calculated.

Table 2:

Comparison of traditional and novel antibodies in dry eye participants by the presence of Sjögren's syndrome in the Penn Sjögren's International Collaborative Clinical Alliance (SICCA) study cohort

Antibody Test	No Sjögren's syndrome* (Non-SS) (N=129)	Sjögren's syndrome** (SS) (N=81)	Fisher's exact P-value
Traditional SS autoantibodies:			
Sjögren's Syndrome Antigen A (SSA/Ro) >25 EU/ml	13 (10.1%)	65 (80.3%)	
Sjögren's Syndrome Antigen B (SSB/La) >25 EU/ml	1 (0.8%)	19 (23.5%)	
Anti-nuclear antibody ≥1:320 *	16 (12.4%)	45 (55.6%)	
Rheumatoid Factor (RF)			
Any isotype	58 (45.0%)	53 (64.2%)	
Number of participants with positive traditional antibodies			
0	58 (45.0%)	6 (7.4%)	
1	57 (44.2%)	25 (30.9%)	
2	11 (8.5%)	11 (13.6%)	
3	3 (2.3%)	22 (27.2%)	
4	0 (0.0%)	17 (21.0%)	
Number of participants positive for SS-A(Ro) and SS-B(La)			
0	115 (89.2%)	16 (19.8%)	
1	14 (10.9%)	46 (56.8%)	
2	0 (0.0%)	19 (23.5%)	
Novel autoantibodies:			
Salivary protein 1 (SP-1)			
IgG (≥20 EU/ml)	6 (4.7%)	6 (7.4%)	0.54
IgA (≥20 EU/ml)	4 (3.1%)	3 (3.7%)	1.00
IgM (≥20 EU/ml)	6 (5%)	11 (14%)	0.03
Any positive	16 (12.4%)	17 (21.0%)	0.12
Carbonic Anhydrase 6 (CA-6)			
IgG (≥20 EU/ml)	8 (6.2%)	5 (6.2%)	1.00
IgA (≥20 EU/ml)	9 (7.0%)	1 (1.2%)	0.09
IgM (≥20 EU/ml)	12 (9.3%)	7 (8.6%)	1.00
Any positive	26 (20%)	12 (15%)	0.36
Parotid specific protein (PSP)			
IgG (≥20 EU/ml)	6 (4.7%)	5 (6.2%)	0.75
IgA (≥20 EU/ml)	14 (10.9%)	17 (21.0%)	0.048
IgM (≥20 EU/ml)	11 (8.5%)	9 (11.1%)	0.63
Any positive	27 (21%)	28 (35%)	0.04
Number of participants with positive novel autoantibody tests			0.01 (linear trend p=0.17)

Antibody Test	No Sjögren's syndrome* (Non-SS) (N=129)	Sjögren's syndrome** (SS) (N=81)	Fisher's exact P-value
0	83 (64.3%)	46 (56.8%)	
1	24 (18.6%)	20 (24.7%)	
2	21 (16.3%)	8 (9.9%)	
3	1 (0.8%)	7 (8.6%)	

* Did not meet 2012 American College of Rheumatology (ACR) Sjögren's syndrome (SS) classification criteria

** Met 2012 ACR SS classification criteria

Table 3:

Comparison of Ocular Staining Score (OSS) score by novel autoantibody status at baseline in the Penn Sjogren's International Clinical Collaborative Alliance (SICCA) Study Participants without Sjogren's Syndrome (n=129)

Novel antibody	Novel Antibody Absence		Novel Antibody Presence		
	n	Mean (SD)	n	Mean (SD)	P-value
Salivary protein 1 (SP-1)					
IgG	123	4.8 (3.0)	6	4.5 (1.4)	0.79
IgA	125	4.8 (2.9)	4	5.5 (2.9)	0.63
IgM	123	4.7 (3.0)	6	6.7 (1.6)	0.11
Any	113	4.7 (3.0)	16	5.6 (2.0)	0.27
Carbonic anhydrase 6 (CA-6)					
IgG	121	4.8 (2.9)	8	4.4 (3.3)	0.67
IgA	120	4.8 (2.9)	9	4.8 (3.5)	0.98
IgM	117	4.7 (2.9)	12	5.7 (3.3)	0.29
Any	103	4.8 (2.9)	26	4.8 (3.1)	0.94
Parotid specific protein (PSP)					
IgG	123	4.7 (2.9)	6	6.8 (2.3)	0.08
IgA	115	4.9 (2.9)	14	3.8 (2.8)	0.17
IgM	118	4.7 (2.9)	11	6.0 (3.4)	0.16
Any	102	4.8 (2.9)	27	4.8 (3.0)	0.96
Any novel antibody	83	4.9 (3.0)	46	4.7 (2.9)	0.75
Number of novel antibodies positive					
0	83	4.8 (3.0)			
1	24	4.1 (2.9)			
2–3 *	22	5.4 (2.8)			
	P=0.32 (any difference) P=0.69 for linear trend test				

* Only 1 patient had a value of 3.

Table 4:

Comparison of Schirmer test score by novel autoantibody status at baseline in the Penn Sjogren's International Clinical Collaborative Alliance (SICCA) Study Participants without Sjogren's Syndrome (n=129)

Novel antibody	Novel Antibody Absence		Novel Antibody Presence		
	n	Mean (SD)	n	Mean (SD)	P-value
Salivary protein 1 (SP-1)					
IgG	123	10.7 (8.8)	6	8.7 (6.5)	0.57
IgA	125	10.6 (8.8)	4	11.5 (7.1)	0.84
IgM	123	10.8 (8.9)	6	7.7 (3.8)	0.40
Any	113	10.9 (9.1)	16	9.0 (5.6)	0.43
Carbonic anhydrase 6 (CA-6)					
IgG	121	10.6 (8.9)	8	11.3 (6.6)	0.84
IgA	120	11.1 (8.8)	9	3.5 (2.0)	**0.02**
IgM	117	10.6 (8.8)	12	11.2 (8.7)	0.82
Any	103	11.0 (8.9)	26	9.0 (7.8)	0.31
Parotid specific protein (PSP)					
IgG	123	10.5 (8.7)	6	12.8 (10.2)	0.53
IgA	115	10.6 (8.7)	14	10.8 (9.5)	0.94
IgM	118	10.7 (8.8)	11	9.9 (7.9)	0.78
Any	102	10.5 (8.6)	27	11.2 (9.2)	0.71
Any novel antibody	83	10.6 (8.6)	46	10.7 (9.0)	0.92
Number of novel antibodies positive					
0	83	10.6 (8.6)			
1	24	13.1 (11.1)			
2–3 *	22	8.2 (5.1)			
	P=0.17 (any difference) P=0.51 for linear trend test				

* Only 1 patient had a value of 3.

Table 5:

Previous studies on the prevalence of novel autoantibodies in dry eye patients with or without Sjogren syndrome

	Sjogren Syndrome					Non-Sjogren Syndrome				
Study	**N**	**Any**	**SP-1**	**PSP**	**CA-6**	**N**	**Any**	**SP-1**	**PSP**	**CA-6**
Current Study	81	43%	21%	35%	15%	129	36%	12%	21%	20%
Karakus et al (*J Immunol Res* 2019)	11 primary SS	73%	27%	54%	27%	97	38%	13%	10%	22%
	7 secondary SS	14%	14%	0	0					
Bunya DREAM (*Cornea* 2018)	52	46%	33%	14%	21%	352	31%	19%	9%	15%
Karakus et al (*Cornea* 2018)	46		13%	11%	52%			14%	14%	43%
Everett et al (*BMC Ophthalmol* 2017)						62	60%			
Matossian (*Clin Ophthalmol* 2016)						41	21%			
Shen et al (*Clin Immunol* 2014)	123		52%							
Shen et al (*Clin Immunol* 2012)	13		54%	18%	54%					