• Clinical Research •

Distinct tear cytokine profile and ocular surface phenotype in primary biliary cholangitis patients with Sjögren's syndrome

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Abstract

● **AIM:** To compare the ocular surface phenotype and tear cytokine profile of primary biliary cholangitis (PBC) patients with and without concomitant Sjögren's syndrome (SS).

● **METHODS:** This case-control study enrolled 21 PBC patients with SS (PBC-SS group), 23 PBC patients without SS (PBC-only group), and 20 age- and sex-matched healthy controls. All participants underwent standardized ocular surface assessments including Ocular Surface Disease Index (OSDI) questionnaire, lipid layer thickness (LLT), incomplete blink rate, tear meniscus height (TMH), non-invasive tear film breakup time (NIBUT), meibomian gland loss (MGL), Schirmer I test, and corneal fluorescein staining (CFS). Tear concentrations of six cytokines [interleukin (IL)-6, IL-8, IL-10, vascular cell adhesion molecule-1 (VCAM-1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF)] were measured using a customized ultra-sensitive quantitative antibody array (QAA-CUST) platform.

● **RESULTS:** The three groups were comparable in terms of age (54.2±8.6, 52.8±9.3, and 53.5±8.9y, respectively; *P*=0.873) and gender distribution (female predominance: 100%, 91.3%, and 95%; *P*=0.412). Compared to PBC-only patients, the PBC-SS group exhibited significantly worse ocular surface parameters, including higher OSDI scores (45.6±12.3 *vs* 28.4±9.7), lower Schirmer I values (3.8±2.1 *vs* 7.6±3.2 mm/5min), reduced TMH (0.18±0.06 *vs* 0.22±0.05 mm), and shorter NIBUT (4.2±1.8 *vs* 6.2±2.0s), all *P*<0.001. Both PBC groups showed reduced LLT and increased MGL compared to healthy controls (*P*<0.05), while CFS scores were similarly elevated in both PBC groups versus controls (*P*<0.001). Tear cytokine analysis revealed significantly higher levels of IL-6, IL-8, and VCAM-1, and lower levels of IL-10 in the PBC-SS group compared to the PBC-only group (all *P*<0.05). VEGF was elevated in both PBC groups versus controls (*P*<0.05). In PBC-SS patients, VCAM-1 demonstrated the strongest correlations with Schirmer I values (*ρ*=-0.71, *P*<0.001) and CFS scores (*ρ*=0.68, *P*<0.001).

● **CONCLUSION:** Compared to PBC-only patients, PBC patients with SS exhibit a distinct and more severe ocular surface phenotype characterized by pronounced aqueous tear deficiency and a unique tear inflammatory signature with elevated VCAM-1, IL-6, IL-8 and reduced IL-10. Tear cytokine profiling, particularly VCAM-1, may serve as a valuable non-invasive tool for identifying SS comorbidity in PBC patients and monitoring disease severity.

● **KEYWORDS:** primary biliary cholangitis; Sjögren's syndrome; dry eye disease; tear cytokines; VCAM-1; ocular surface

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INTRODUCTION

Dry eye disease (DED) is a multifactorial ocular surface disorder characterized by loss of tear film homeostasis, leading to symptoms of discomfort, visual disturbance, and

potential damage to the ocular surface[1]. Emerging evidence indicates a significantly higher prevalence of DED in patients with chronic liver diseases (CLDs)[2], which extends beyond the classical association with vitamin A deficiency in cholestatic conditions. Recent studies suggest that the ocular surface may serve as a mirror reflecting systemic inflammatory and immune dysregulation[3-5]. Among CLDs, primary biliary cholangitis (PBC), an autoimmune liver disease targeting intrahepatic bile ducts, presents a particularly compelling model for studying gut-eye-liver axis interactions due to its strong predisposition for extrahepatic autoimmune comorbidities[6].

PBC is notably associated with a high frequency of concurrent autoimmune disorders, with Sjögren's syndrome (SS) being one of the most prevalent. SS is a systemic autoimmune disease characterized by lymphocytic infiltration of exocrine glands, primarily leading to dry mouth and dry eyes. The reported co-prevalence of SS in PBC patients varies widely (3.5%–73%)[7], a discrepancy largely attributed to diagnostic challenges. In clinical practice, hepatologists frequently request ophthalmological consultation for PBC patients suspected of having SS. However, the current diagnostic paradigm for SS relies heavily on invasive minor salivary gland biopsy and specific serology (anti-SSA/SSB antibodies), which may be uncomfortable for patients and lack sensitivity in early or seronegative cases[8]. Consequently, a significant proportion of PBC patients undergo comprehensive ocular surface evaluation (*e.g.*, Schirmer's test, tear film breakup time, corneal staining) yet still require a definitive, invasive biopsy. This clinical gap underscores the urgent need for non-invasive, accessible, and reliable biomarkers to aid in the early screening and differential diagnosis of SS in the PBC population[9].

The ocular surface and its tear film constitute a unique biological interface. Tear fluid is an ideal medium for biomarker discovery as it can directly reflect the local inflammatory microenvironment of the ocular surface and is obtained non-invasively[10-11]. In SS-related DED, the lacrimal gland is a direct target of autoimmune attack, leading to a distinct qualitative and quantitative change in tear composition. Studies have shown that tear levels of specific cytokines and chemokines [*e.g.*, interleukin (IL)-6, IL-8, interferon (IFN)-γ] are altered in primary SS[12]. While the systemic immunological interplay between PBC and SS has been studied[6-7], a detailed comparative analysis of their ocular surface phenotypes and local tear film inflammatory milieu is lacking. Understanding these differences is crucial for two reasons: First, it may reveal specific ocular signs that could alert clinicians to a higher probability of SS in a PBC patient, streamlining referrals and diagnostic workups. Second, identifying a unique tear cytokine profile associated with PBC-SS overlap could pave the way for developing a non-invasive diagnostic or prognostic tool.

This study aims to address this critical knowledge gap. First, to systematically compare the clinical signs and symptoms of DED between PBC patients with and without concomitant SS using a standardized ocular surface assessment battery. Second, to analyze and compare the expression levels of key cytokines in the tear fluid of these two patient groups and healthy controls, using a customized ultra-sensitive quantitative antibody array (QAA-CUST) technology.

PARTICIPANTS AND METHODS

Ethical Approval This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Beijing You'An Hospital, Capital Medical University (No.2025139). Prior to any study procedure, written informed consent was obtained from all participants. The consent process included a detailed explanation of the study's purpose, the nature of non-invasive ocular surface examinations and tear collection, and the use of anonymized clinical data. Participants were informed of their right to withdraw at any time. All collected data were de-identified and analyzed anonymously to ensure participant confidentiality.

Participants This case-control study consecutively recruited participants from the inpatient services of Department of Hepatology and Immunology and Department of Hepatology and Gastroenterology, Beijing You'An Hospital between July 2025 and December 2025. The study population comprised three distinct groups: 1) PBC patients with comorbid SS (PBC-SS group), 2) PBC patients without SS (PBC-only group), and 3) age- and sex-matched healthy controls with no history of liver disease or DED.

Inclusion Criteria For the PBC-SS group: Diagnosis of PBC established according to the European Association for the Study of the Liver (EASL) Clinical Practice Guidelines[13]. Clinically stable PBC, defined as being on a stable, optimized dose of ursodeoxycholic acid (UDCA) for at least 6mo, with no evidence of hepatic decompensation (*e.g.*, ascites, variceal bleeding, hepatic encephalopathy) or plans for liver transplantation within the study period[14]. Diagnosis of SS fulfilling the 2016 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria[15], confirmed by a rheumatologist. For the PBC-only group: Explicit absence of SS was confirmed by a rheumatologist based on the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for primary SS (total score <4). This evaluation included systematic assessment of sicca symptoms, serological testing (negative anti-SSA/SSB antibodies on two separate occasions), objective ocular tests (Schirmer I test, corneal fluorescein staining), and unstimulated whole salivary flow rate. In four patients with borderline findings,

labial salivary gland biopsy was performed and showed a focus score <1, effectively excluding SS. For the healthy control group: No known history of any liver disease or autoimmune disorder, no subjective symptoms of DED, and normal ocular surface examination.

None of the enrolled PBC patients were receiving systemic immunosuppressive or immunomodulatory therapy (including corticosteroids, methotrexate, azathioprine, mycophenolate mofetil, or biologic agents) at the time of tear collection or within the preceding 3mo. All PBC patients had been on a stable, optimized dose of ursodeoxycholic acid (UDCA) for at least 6mo.

Exclusion Criteria Presence of other active ocular surface diseases that could confound tear film assessment, use of topical ophthalmic medications or systemic medications known to significantly affect tear secretion within 1mo, history of ocular surgery, diabetes mellitus, and severe systemic comorbidities.

Ophthalmic Examinations A standardized and sequential ocular surface evaluation was performed on all participants by the same experienced ophthalmologist (Ruan F) under controlled environmental conditions (humidity 40%–50%, temperature 22℃–24℃). The evaluation protocol was conducted in the following strict order to prevent procedural interference, prioritizing non-invasive measurements before any instillation of dyes or physical contact. All examinations and tear collections were performed between 9:00 *a.m.* and 11:00 *a.m.* to minimize the influence of circadian variation on tear cytokine levels.

Tear Fluid Collection and Cytokine Analysis Tear samples were collected prior to any other ocular examination or manipulation to obtain basal, non-reflex secretion and avoid contamination or dilution from diagnostic dyes or procedural stimulation. A sterile, disposable glass capillary tube was gently placed at the lateral canthus of the lower eyelid without touching the ocular surface or eyelid margin. Approximately 10 μL of non-stimulated basal tear fluid was collected from each eye. Samples were immediately transferred to sterile microcentrifuge tubes and stored on dry ice. Within 2h, all samples were centrifuged at 10 000× g for 10min at 4℃ to remove cellular debris and mucins. The supernatant was aliquoted and stored at -80℃ until analysis.

Tear samples were collected from both eyes separately, and the cytokine concentrations from the two eyes were averaged for each participant for statistical analysis.

The concentrations of six target cytokines and growth factors, basic fibroblast growth factor (bFGF), IL-6, IL-8, IL-10, vascular cell adhesion molecule-1 (VCAM-1), and vascular endothelial growth factor (VEGF), were quantified using QAA-CUST. All assays were performed in duplicate according to the manufacturer's protocol, and sample positions were randomized across plates to control for batch effects. Cytokine concentrations were interpolated from a standard curve run on each plate, and values are reported in picograms per milliliter (pg/mL). Any samples with coefficient of variation (CV) >15% between duplicates were re-analyzed.

Subjective Symptom Assessment Dry eye symptom severity was quantified using the Ocular Surface Disease Index (OSDI) questionnaire[16]. Participants rated the frequency of 12 symptoms related to ocular discomfort, vision-related function, and environmental triggers over the preceding week on a scale from 0 (none of the time) to 4 (all of the time). The total score was calculated on a scale of 0 to 100, with higher scores indicating greater symptom severity. A score of ≥13 points were used to define the presence of symptomatic dry eye.

Tear Film and Ocular Surface Evaluation Dynamic assessment of the precorneal tear film lipid layer and blink pattern was performed first using the LipiView ocular surface interferometer (TearScience, Johnson & Johnson Vision, Jacksonville, FL, USA)[17]. Participants were instructed to blink naturally while focusing on a fixation target during a 20-second recording. The average tear film lipid layer thickness (LLT) was automatically calculated and expressed in nanometers (nm). The incomplete blink rate was determined as the percentage of blinks during the recording period that did not result in full eyelid closure.

Following the LipiView assessment, three key parameters were measured using the Keratograph 5M (K5M; Oculus, Optikgerate, Germany)[18]. Tear meniscus height (TMH): The height of the lower tear meniscus was measured from a high-resolution anterior segment image. Non-invasive tear film break-up time (NIBUT): The time interval from a complete blink to the first appearance of a distortion in the projected Placido disk pattern was automatically recorded. The measurement was repeated three times, and the average value was used for analysis. Meibomian gland loss (MGL): Infrared meibography images of the upper and lower eyelids were captured[19]. The percentage area of glandular dropout in the central third of each eyelid was quantified using the device's built-in analysis software.

Following the non-invasive assessments, two standard invasive tests were performed. Tear secretion was evaluated using the Schirmer I test without topical anesthesia[20]. A standardized sterile filter paper strip was placed in the lower conjunctival sac for five minutes, and the length of wetting was recorded in millimeters. Immediately thereafter, ocular surface integrity was assessed *via* corneal fluorescein staining (CFS) using a single-use fluorescein-impregnated paper strip. The strip was lightly moistened with a single drop of sterile saline and briefly touched to the inferior bulbar conjunctiva. After approximately

Table 1 Demographic and baseline clinical characteristics of participants

Characteristic	PBC-SS group (*n*=21)	PBC-only group (*n*=23)	Healthy control (*n*=20)	*P*
Gender, *n* (%)				0.412
Female	21 (100)	21 (91.3)	19 (95)	
Male	0	2 (8.7)	1 (5)	
Age (y)	54.2±8.6	52.8±9.3	53.5±8.9	0.873
BCVA (logMAR)	0.05±0.08	0.04±0.07	0.03±0.06	0.642
IOP (mm Hg)	14.2±2.3	13.5±2.1	14.6±1.7	0.351

Data are presented as mean±standard deviation or number (%). PBC: Primary biliary cholangitis; SS: Sjögren's syndrome; BCVA: Best-corrected visual acuity; IOP: Intraocular pressure; logMAR: Logarithm of the minimum angle of resolution.

2min to allow for tear film mixing, corneal epithelial damage was graded under cobalt blue light using the National Eye Institute (NEI) scale (total score range: 0–15)[21].

Statistical Analysis Statistical analyses were performed using SPSS (v26.0) and R (v4.3.0). Normality was assessed with the Shapiro-Wilk test. Continuous variables are presented as mean±standard deviation (SD) or median (interquartile range; IQR) based on distribution, and categorical variables as frequencies. Intergroup comparisons were conducted using one-way analysis of variance (ANOVA) or Kruskal-Wallis test for continuous data, and Chi-square or Fisher's exact test for categorical data. Tear cytokine levels between the PBC-SS and PBC-only groups were compared using the Mann-Whitney *U* test. Spearman's rank correlation was used to assess relationships between cytokines and clinical parameters. A two-tailed $P<0.05$ was considered statistically significant.

For post hoc pairwise comparisons, Bonferroni correction was applied. For Spearman correlation analyses, no adjustment was made for multiple testing given the exploratory nature of these analyses; findings should be interpreted as hypothesis generating.

Sample Size Calculation An a priori sample size estimation was performed using G*Power software (version 3.1.9.7). Based on the effect size (Cohen's $d=1.2$) derived from our pilot study comparing Schirmer I test values between the PBC-SS and PBC-only groups, with a two-tailed α of 0.05 and a desired power of 0.80, a minimum of 18 participants per group was required. Considering the rarity of PBC and even more so of PBC-SS overlap, we enrolled all eligible patients during the study period, resulting in final sample sizes of 21, 23, and 20 in the three groups, respectively. A post-hoc power analysis for the primary comparison (Schirmer I test between PBC-SS and PBC-only) showed a power of 0.89, confirming adequate statistical power for the key outcomes.

RESULTS

Participants A total of 64 participants was enrolled, including 21 PBC patients with SS (PBC-SS group), 23 PBC patients without SS (PBC-only group), and 20 age- and sex-matched healthy controls. Demographic and baseline clinical characteristics are summarized in Table 1. The three groups were comparable in terms of age ($P=0.873$), gender distribution ($P=0.412$), best-corrected visual acuity (logMAR, $P=0.642$), and intraocular pressure ($P=0.351$). All the participants recruited were Chinese ethnicity.

Details of Tear Analysis Tear concentrations of the six targeted cytokines were successfully quantified in all participants, with significant intergroup differences observed for five of the six analytes (all $P<0.001$ except bFGF, $P=0.657$), as summarized in Table 2. The PBC-SS group exhibited a distinct pro-inflammatory tear profile characterized by significantly elevated levels of IL-6, IL-8, and VCAM-1 compared to both the PBC-only and healthy control groups (all $P<0.05$). Conversely, IL-10 concentrations were markedly lower in the PBC-SS group than in PBC-only and healthy controls ($P<0.05$), suggesting impaired local anti-inflammatory regulation in patients with SS comorbidity. VEGF levels were significantly elevated in both PBC-SS and PBC-only groups compared to healthy controls (both $P<0.05$), reflecting a potential systemic effect of PBC itself. No significant differences were observed among the three groups for bFGF ($P=0.657$).

Ocular Surface Parameters Among Study Groups The comparative analysis of ocular surface parameters revealed significant intergroup differences for most measured indicators, as summarized in Table 3. OSDI scores were significantly higher in the PBC-SS group compared to both PBC-only and healthy control groups ($P<0.001$). LLT and MGL showed significant differences only between healthy controls and both PBC groups ($P<0.001$ and $P=0.001$, respectively), with no significant differences between PBC-SS and PBC-only groups. Incomplete blink rate was similarly elevated in both PBC groups compared to healthy controls ($P=0.001$). TMH was similarly lowest in PBC-SS, intermediate in PBC-only, and highest in healthy controls ($P<0.001$). Tear film stability assessed by NIBUT was poorest in the PBC-SS group, followed by PBC-only and healthy controls ($P<0.001$ for all pairwise comparisons). Schirmer I test values demonstrated a stepwise reduction from healthy controls to PBC-only and

Table 2 Comparison of tear cytokine levels in PBC patients with or without SS and healthy controls

Cytokine (pg/mL)	PBC-SS group (*n*=21)	PBC-only group (*n*=23)	Healthy control (*n*=20)	*F*	*P*
IL-6	121.22±61.09	28.70±9.67[a]	12.02±5.09[a,b]	53.913	<0.001
IL-8	766.35±239.19	164.82±107.49[a]	23.34±6.94[a,b]	135.719	<0.001
IL-10	0.74±0.27	1.32±0.62[a]	1.80±1.20[a]	8.870	<0.001
VEGF	952.40±437.23	883.69±403.87	437.51±130.32[a,b]	12.630	<0.001
VCAM-1	1881.58±1012.79	873.07±319.19[a]	385.77±103.57[a,b]	30.676	<0.001
bFGF	1.14±1.24	1.04±0.41	0.91±0.37	0.423	0.657

Data are presented as mean±standard deviation. [a]*P*<0.05 compared to PBC-SS group; [b]*P*<0.05 compared to PBC-only group (one-way ANOVA with post hoc Bonferroni correction). PBC: Primary biliary cholangitis; SS: Sjögren's syndrome; IL: Interleukin; VCAM-1: Vascular cell adhesion molecule-1; VEGF: Vascular endothelial growth factor; bFGF: Basic fibroblast growth factor; ANOVA: Analysis of variance.

Table 3 Ocular surface parameters among PBC patients with or without SS and healthy controls

Parameters	PBC-SS group (*n*=21)	PBC-only group (*n*=23)	Healthy control (*n*=20)	*F*	*P*
OSDI score	45.6±12.3	28.4±9.7[a]	8.2±3.5[a,b]	68.324	<0.001
LLT (nm)	52.3±18.7	58.6±16.2	75.4±14.8[a,b]	10.856	<0.001
Incomplete blink rate (%)	42.5±21.3	38.7±19.6	21.4±12.8[a,b]	7.892	0.001
TMH (mm)	0.18±0.06	0.22±0.05[a]	0.29±0.04[a,b]	21.345	<0.001
NIBUT (s)	4.2±1.8	6.2±2.0[a]	11.5±2.4[a,b]	58.672	<0.001
MGL (%)	28.6±12.4	26.3±11.8	15.2±7.6[a,b]	8.456	0.001
Schirmer I test (mm/5min)	3.8±2.1	7.6±3.2[a]	12.8±3.5[a,b]	45.892	<0.001
CFS score	4.8±2.1	4.2±1.9	0.5±0.7[a,b]	36.234	<0.001

Data are presented as mean±standard deviation. [a]*P*<0.05 compared to PBC-SS group; [b]*P*<0.05 compared to PBC-only group (one-way ANOVA with post hoc Bonferroni correction for normally distributed parameters. PBC: Primary biliary cholangitis; SS: Sjögren's syndrome; OSDI: Ocular Surface Disease Index; LLT: Lipid layer thickness; TMH: Tear meniscus height; NIBUT: Non-invasive tear film breakup time; MGL: Meibomian gland loss; CFS: Corneal fluorescein staining; ANOVA: Analysis of variance.

further to PBC-SS group (P<0.001 for all comparisons). CFS scores were significantly elevated in both PBC groups compared to healthy controls (P<0.001), with no significant difference between the two PBC patient groups (P>0.05).

Relationship Between Cytokines and Ocular Surface Parameters Subgroup analysis restricted to the 21 PBC-SS patients revealed distinct correlation patterns between tear cytokines and ocular surface parameters, as presented in Table 4. VCAM-1 demonstrated the strongest associations, with significant correlations observed for Schirmer I test values (ρ=-0.71, P<0.001), CFS scores (ρ=0.68, P<0.001), OSDI scores (ρ=0.62, P=0.003), NIBUT (ρ=-0.55, P=0.008), and TMH (ρ=-0.58, P=0.005). IL-6 showed significant correlations with Schirmer I values (ρ=-0.59, P=0.004), CFS scores (ρ=0.54, P=0.011), OSDI scores (ρ=0.45, P=0.038), and TMH (ρ=-0.48, P=0.026). IL-8 correlated significantly with NIBUT (ρ=-0.52, P=0.015), OSDI scores (ρ=0.49, P=0.023), and Schirmer I values (ρ=-0.46, P=0.035). IL-10 demonstrated significant positive correlations with Schirmer I values (ρ=0.55, P=0.009), TMH (ρ=0.48, P=0.026), and negative correlations with CFS scores (ρ=-0.46, P=0.034). VEGF showed significant correlations with MGL (ρ=0.47, P=0.031) and LLT (ρ=-0.43, P=0.048), while bFGF correlated significantly with CFS scores (ρ=0.44, P=0.042).

Table 4 Spearman correlation coefficients between tear cytokines and ocular surface parameters in PBC-SS patients

Parameters	IL-6	IL-8	IL-10	VCAM-1	VEGF	bFGF
OSDI score	0.45[d]	0.49[d]	-0.38	0.62[e]	0.35	0.32
LLT	-0.31	-0.36	0.29	-0.41	-0.43[d]	-0.21
Incomplete blink rate	0.34	0.30	-0.25	0.38	0.28	0.24
TMH	-0.48[d]	-0.41	0.48[d]	-0.58[e]	-0.33	-0.29
NIBUT	-0.42	-0.52[d]	0.41	-0.55[e]	-0.36	-0.31
MGL	0.36	0.33	-0.29	0.42	0.47[d]	0.23
Schirmer I test	-0.59[e]	-0.46	0.55[e]	-0.71[f]	-0.38	-0.27
CFS score	0.54[d]	0.43	-0.46[d]	0.68[f]	0.34	0.44[d]

Data are presented as Spearman's rank correlation coefficients (ρ). [d]*P*<0.05; [e]*P*<0.01; [f]*P*<0.001 (Spearman's correlation, due to non-normal distribution of cytokine data). PBC: Primary biliary cholangitis; SS: Sjögren's syndrome; OSDI: Ocular Surface Disease Index; LLT: Lipid layer thickness; TMH: Tear meniscus height; NIBUT: Non-invasive tear film breakup time; MGL: Meibomian gland loss; CFS: Corneal fluorescein staining; IL: Interleukin; VCAM-1: Vascular cell adhesion molecule-1; VEGF: Vascular endothelial growth factor; bFGF: Basic fibroblast growth factor.

Diagnostic Performance of Tear Cytokines for Identifying SS in PBC Patients To evaluate the potential of tear cytokines as non-invasive biomarkers, receiver operating characteristic (ROC) curve analysis was performed to discriminate PBC-SS

Table 5 Diagnostic performance of tear cytokines for discriminating PBC-SS from PBC-only patients

Biomarkers	AUC (95%CI)	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
VCAM-1 (pg/mL)	0.84 (0.72–0.96)	1206	71.4	91.3	88.2	77.8
IL-6 (pg/mL)	0.78 (0.66–0.90)	45	76.2	73.9	73.1	77.3
IL-10 (pg/mL)	0.82 (0.70–0.95)	1.05	90.5	65.2	70.4	88.2
Combined model[g]	0.89 (0.81–0.97)	0.5[h]	85.7	82.6	81.8	86.4

[g]Combined model based on logistic regression (VCAM-1+IL-6+IL-10); [h]Predicted probability cutoff (from logistic regression). PBC: Primary biliary cholangitis; SS: Sjögren's syndrome; IL: Interleukin; VCAM-1: Vascular cell adhesion molecule-1; AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value.

from PBC-only patients. The results are summarized in Table 5.

VCAM-1 showed an area under the curve (AUC) of 0.84. Using a cutoff value of 1206 pg/mL, the sensitivity was 71.4% and specificity 91.3%, with a positive predictive value (PPV) of 88.2% and a negative predictive value (NPV) of 77.8%. IL-6 yielded an AUC of 0.78 At a cutoff of 45 pg/mL, sensitivity was 76.2% and specificity 73.9% (PPV 73.1%, NPV 77.3%). IL-10 gave an AUC of 0.82. At a cutoff of 1.05 pg/mL, sensitivity was 90.5% and specificity 65.2% (PPV 70.4%, NPV 88.2%).

A combined model (VCAM-1+IL-6+IL-10) using logistic regression-derived predicted probability achieved an AUC of 0.89. At a predicted probability cutoff of 0.5, the sensitivity was 85.7% and specificity 82.6% (PPV 81.8%, NPV 86.4%), indicating that a panel of three cytokines outperformed any single marker in discriminating PBC-SS from PBC-only patients.

DISCUSSION

In this study, we comprehensively characterized the ocular surface phenotype and tear cytokine profile of PBC patients with and without concomitant SS. Compared to PBC-only patients, those with SS exhibit a distinct and more severe ocular surface disorder characterized by pronounced tear secretion deficiency, tear film instability, and elevated pro-inflammatory cytokines in tears, particularly VCAM-1, IL-6, and IL-8, accompanied by reduced levels of the anti-inflammatory cytokine IL-10. Notably, VCAM-1 emerged as the strongest correlate of ocular surface damage in PBC-SS patients, with robust associations with Schirmer test values, corneal staining scores, and symptom severity. These findings have important implications for understanding the pathophysiology of PBC-SS overlap and may inform the development of non-invasive diagnostic approaches.

The tear film is a unique biological fluid that reflects both local ocular surface inflammation and systemic immune dysregulation[22]. In autoimmune diseases, tear cytokine profiling has emerged as a promising tool for disease assessment and monitoring. Our finding of significantly elevated IL-6 and IL-8 in PBC-SS patients aligns with previous studies in primary SS, where these cytokines have been implicated in lacrimal gland inflammation and dysfunction[12,23]. IL-6 is a pleiotropic cytokine that promotes B-cell differentiation and autoantibody production[24], while IL-8 serves as a potent chemoattractant for neutrophils, contributing to tissue damage[25]. The markedly higher levels of these cytokines in PBC-SS compared to PBC-only patients suggest that SS comorbidity amplifies the local inflammatory milieu beyond what is attributable to PBC alone.

The most striking finding of our study was the dramatic elevation of VCAM-1 in tears of PBC-SS patients and its strong correlation with ocular surface damage. VCAM-1 is a cell adhesion molecule that mediates lymphocyte adhesion and transmigration across vascular endothelium[26]. In SS, VCAM-1 expression on vascular endothelium of salivary and lacrimal glands facilitates the recruitment of lymphocytes expressing its ligand, very late antigen-4 (VLA-4), into target tissues[27]. Our observation that tears VCAM-1 levels correlated strongly with Schirmer test values (ρ=-0.71) and corneal staining scores (ρ=0.68) suggests that VCAM-1 may serve as a reliable indicator of lacrimal gland inflammation and consequent ocular surface damage. Previous studies have reported elevated soluble VCAM-1 in serum of patients with primary SS[28], but to our knowledge, this is the first demonstration of VCAM-1 elevation in tears of PBC-SS patients and its correlation with disease severity.

The significant reduction of IL-10 in PBC-SS patients compared to both PBC-only and healthy controls is particularly noteworthy. IL-10 is a key anti-inflammatory cytokine produced by regulatory T cells (Tregs) and other immune cells that suppresses pro-inflammatory responses and maintains immune homeostasis[29]. Deficiency of IL-10 has been implicated in the pathogenesis of various autoimmune diseases, including SS[30]. Our finding of reduced tear IL-10 levels in PBC-SS patients, coupled with its positive correlation with Schirmer values and negative correlation with corneal staining scores, supports the concept that impaired local immunoregulation contributes to more severe ocular surface disease in these patients.

Interestingly, we observed no significant difference in corneal fluorescein staining scores between PBC-SS and PBC-only

groups, despite marked differences in tear secretion and pro-inflammatory cytokines. Both PBC groups showed elevated CFS scores compared to healthy controls. This finding suggests that corneal epithelial damage in PBC patients may be driven predominantly by factors common to PBC itself, such as vitamin A deficiency secondary to cholestasis[31]. Vitamin A is essential for maintaining corneal epithelial integrity, and its deficiency leads to keratinization and epithelial defects[32-33]. However, because serum vitamin A levels were not measured in this study, this interpretation remains speculative and future studies incorporating serum vitamin A measurements are needed to clarify the relative contributions of nutritional deficiency, inflammation, and neural dysfunction to corneal epitheliopathy in PBC patients.

VEGF levels were significantly elevated in both PBC groups compared to healthy controls, with no difference between PBC-SS and PBC-only patients. VEGF is a key regulator of angiogenesis and vascular permeability, and its elevation in PBC may reflect underlying hepatic pathology[34]. Studies have shown that serum VEGF levels are increased in patients with chronic liver disease, including PBC, and correlate with disease severity and fibrosis[35-36]. Our finding of elevated tear VEGF in both PBC groups suggests that the ocular surface may reflect systemic VEGF dysregulation in liver disease, potentially contributing to meibomian gland dysfunction as suggested by its correlation with meibomian gland loss and lipid layer thickness in our study.

The lack of significant differences in bFGF among the three groups suggests that this growth factor may not be a major contributor to the differential ocular surface phenotype in PBC-SS patients. However, its modest correlation with CFS scores in PBC-SS patients is consistent with its role in epithelial repair and may reflect a compensatory response to chronic corneal damage[37].

From a clinical perspective, our findings have several important implications. First, the distinct tear cytokine profile in PBC-SS patients suggests that a combination of elevated VCAM-1 and IL-6 with reduced IL-10 could serve as a non-invasive diagnostic tool. To evaluate this potential, we performed ROC curve analysis. VCAM-1 alone showed moderate diagnostic accuracy (AUC=0.84, 95%CI: 0.72–0.96), with sensitivity of 71.4% and specificity of 91.3% at 1206 pg/mL. Adding IL-6 and IL-10 improved performance: the combined model achieved an AUC of 0.89 (95%CI: 0.81–0.97), sensitivity 85.7%, and specificity 82.6%. These results suggest that a panel of three cytokines outperforms any single marker, potentially reducing the need for invasive minor salivary gland biopsy. Nevertheless, given the limited sample size, these findings are exploratory and require validation in larger, multi-center cohorts.

The differential patterns observed across ocular surface parameters provide insights into the distinct pathophysiological mechanisms operating in PBC-SS overlap. Parameters primarily reflecting aqueous tear deficiency (Schirmer test, TMH) showed stepwise worsening from healthy controls to PBC-only to PBC-SS patients, consistent with the additive effect of SS-related lacrimal gland inflammation on PBC-related tear abnormalities[2]. In contrast, parameters related to meibomian gland function (LLT, MGL, incomplete blink rate) were similarly affected in both PBC groups, suggesting these changes are attributable to PBC itself. This dissociation supports the concept that PBC-SS overlap represents a distinct clinical entity with unique therapeutic requirements.

Several limitations of this study should be acknowledged. First, the relatively small sample size—particularly for the PBC-SS group—reflects the inherent rarity of primary biliary cholangitis and the even lower prevalence of well-defined SS overlap meeting the 2016 ACR/EULAR criteria. Nonetheless, the limited sample size may affect the stability of the estimated diagnostic cutoff values and precludes detailed subgroup analyses. Nevertheless, the study was adequately powered for its primary comparisons (post-hoc power=0.89), as detailed in the Statistical Analysis section. The statistically significant differences observed in the key parameters (all $P<0.001$) further support the robustness of the findings despite the modest sample size. Second, all participants were of Chinese Han ethnicity and recruited from a single tertiary referral center. Therefore, our findings may not be directly generalizable to other ethnic populations or to patients managed in different healthcare settings. The tear cytokine profiles and optimal diagnostic cut off values could be influenced by genetic, environmental, or lifestyle factors. External validation in multi ethnic, multi center cohorts is essential before clinical implementation. Third, we did not measure serum cytokine levels in parallel with tear samples. This limits our ability to determine whether the observed tear cytokine elevations reflect local ocular surface inflammation, systemic immune dysregulation, or a combination of both. Future studies with paired tear and serum sampling are needed to dissect the relative contributions of local versus systemic inflammation in PBC-SS overlap dry eye. Fourth, we did not measure serum vitamin A levels, which may influence corneal epithelial integrity and confound the interpretation of corneal fluorescein staining scores. Although liver function parameters and disease severity scores were comparable between the two PBC groups, residual confounding by unmeasured nutritional or environmental factors cannot be fully excluded. Future studies should incorporate comprehensive nutritional assessments, including serum retinol and other fat-soluble vitamins. Fifth, although we made every effort to exclude

SS in the PBC-only group using clinical, serological, and objective testing according to the 2016 ACR/EULAR criteria, seronegative or subclinical SS cannot be completely ruled out without universal labial salivary gland biopsy. Such potential misclassification could theoretically dilute the differences between the two PBC groups.

Despite these limitations, our study has notable strengths, including the well-characterized patient cohorts with rigorous diagnostic criteria, standardized ocular surface assessments, and the use of a highly sensitive multiplex platform for tear cytokine analysis. To our knowledge, this is the first comprehensive characterization of both ocular surface phenotype and tear inflammatory profile in PBC patients with and without SS comorbidity.

In conclusion, this study demonstrates that PBC patients with SS exhibit a distinct and more severe ocular surface phenotype characterized by pronounced tear deficiency and a unique tear inflammatory signature with elevated VCAM-1, IL-6, IL-8 and reduced IL-10. These findings advance our understanding of the pathophysiology of PBC-SS overlap at the ocular surface and suggest that tear cytokine profiling may have value as a non-invasive diagnostic tool. Future longitudinal studies are warranted to validate these findings in larger cohorts and to explore the potential of tear biomarkers for monitoring disease progression and therapeutic response in this challenging patient population.

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