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Original Article

Clinical significance of corneal confocal microscopy characteristics and inflammatory cytokines in Sjögren's syndrome-related dry eye disease

Kan Yang[1], Lei Jiang[1], Nan Su[1], Jialiang Chang[2], Wei Liang[1], Songquan Fu[3], Xingpan Du[4]

[1]Department of Ophthalmology, Lanzhou First People's Hospital, Lanzhou 730050, Gansu, China; [2]President's Office, Lanzhou First People's Hospital, Lanzhou 730050, Gansu, China; [3]Department of Geriatrics, Lanzhou First People's Hospital, Lanzhou 730050, Gansu, China; [4]Department of Ophthalmology, Gansu University of Chinese Medicine, Lanzhou 730000, Gansu, China

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Abstract: Objective: To characterize the relationships between *in vivo* confocal microscopy (IVCM) - derived corneal immune - neural metrics, and systemic cytokines and ocular surface findings in Sjögren's syndrome-associated dry eye disease (SS-DED), and to identify variables independently associated with SS-DED status. Methods: A retrospective case - control study was conducted (Jan 2019 - Jan 2022), including 120 SS-DED patients and 88 healthy controls. Corneal dendritic cell density (DCD), inflammatory cell density (ICD), and nerve fiber density (NFD) were quantified by IVCM. Tear break-up time (BUT), corneal fluorescein staining (FL), Schirmer test, and Ocular Surface Disease Index (OSDI) were assessed using standard protocols. Variables independently associated with SS-DED were identified using multivariable logistic regression, and their discrimination performance was evaluated using receiver operating characteristic (ROC) analysis. Results: Compared to controls, SS-DED patients showed significantly higher levels of IL-1β, IL-6, TNF-α, DCD, and ICD, and lower NFD levels (all P<0.001). DCD correlated positively with IL-1β, BUT, FL, and OSDI (P<0.05), while NFD correlated negatively with BUT and FL (P<0.001). ICD positively correlated with IL-1β, IL-6, TNF-α, and DCD (all P<0.001). Multivariate regression analysis identified IL-1β (OR=1.249, P<0.001), OSDI (OR=1.074, P=0.033), DCD (OR=1.411, P=0.002), and ICD (OR=1.006, P=0.018) as independent factors associated with SS-DED. Among them, DCD demonstrated the highest discriminative power (AUC=0.886; specificity 98.75%; sensitivity 65.00%). Conclusion: Elevated IL-1β, OSDI, DCD, and ICD levels are independent risk factors for SS-DED. Among these factors, DCD exhibited superior predictive performance over the others and may be a biomarker for SS-DED diagnosis and disease monitoring.

Keywords: Sjögren's syndrome, dry eye disease, in vivo confocal microscopy, inflammatory factors, dendritic cell density

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease that primarily targets the exocrine glands and commonly manifests as dry eye disease (DED) [1]. Immune pathways involving interferons, the T helper 17 (Th17) axis - interleukin-17 (IL-17) and IL-23 - and B-cell-mediated signaling, including tumor necrosis factor-alpha (TNF-α) and B-cell activating factor (BAFF), contribute to tissue injury in SS [2]. In the eye, reduced tear secretion, tear film instability, and ocular surface inflammation damage the corneal epithelium and impair vision-related quality of life [3, 4].

DED is not only a complication of SS but also an inflammatory ocular surface disorder driven by local and systemic drivers. Meta-analyses have demonstrated elevated tear concentrations of interferon-gamma (IFN-γ), IL-17, IL-1β, IL-6, and IL-8 in SS-DED compared to healthy controls [5]. These mediators present in the tears, cornea, and conjunctiva, and are linked to epithelial apoptosis, barrier disruption, and immune-cell recruitment [5]. Corneal innervation is altered in SS-DED: *in vivo* confocal microscopy (IVCM) shows reduced density and abnormal morphology of the sub-basal nerve plexus, accompanied by reduced corneal sensitivity that may amplify ocular-surface

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inflammation through neuroimmune crosstalk [6].

In vivo confocal microscopy (IVCM) offers unprecedented insights into DED investigation. This technology enables real-time visualization of corneal cellular and neural microarchitecture, allowing quantitative assessment of dendritic cell density, inflammatory cell populations, and nerve fiber density [7]. SS patients exhibit decreased epithelial and corneal nerve fiber densities together with increased inflammatory cell infiltration - changes that correlate with shorter tear film break-up time (TFBUT) and lower Schirmer values [8]. Across cohorts, corneal nerve metrics often show inverse associations with disease activity, although findings are not fully consistent [9]. Elevated corneal dendritic cell density is linked to greater systemic activity and more severe ocular surface staining [10]. The independent value of IVCM features for classifying SS-DED or forecasting outcomes remains uncertain.

Clinically, TFBUT, corneal staining, Schirmer test, and Ocular Surface Disease Index (OSDI) scores are used to evaluate EDE; however, these measures vary depending on the test performers, strip placement, room humidity, and even the patient's sleep quality, substantially limiting the comparability across studies [11]. Tear proteomics and multiplex cytokine panels offer greater stability across visits and may help to objectify SS-DED, yet current platforms are not harmonized and are still expensive for clinical use [12]. For example, tear mucin 5AC and IL-8 have shown discriminatory potential between SS and non-SS DED in selected cohorts [13]. Nevertheless, the associations between cytokine profiles and corneal microstructure changes are not well defined, and few studies have evaluated both domains together to explore prognostic implications [14].

We therefore examined SS-related DED using IVCM to quantify corneal dendritic and other inflammatory cells and subbasal nerve fiber density. In parallel, tear inflammatory cytokines were measured, and standard clinical assessments were performed. By integrating cellular, neural, and cytokine data, this study seeks to clarify how the ocular surface inflammatory - neural milieu interplay underlying SS-related DED and to provide practical reference for individualized care.

Materials and methods

Sample size calculation

The sample size was estimated following the two-sample design described by Yoon et al. [15] for SS-DED. The primary endpoint was TBUT. A two-sided comparison of means was planned with a power 0.80 and an significance level of 0.05. Pilot data indicated a mean TBUT of 9.4±1.8 seconds in healthy controls and 3.1±1.2 seconds in SS, with an average difference of 6.3 seconds. The pooled standard deviation was about 1.55 seconds, which yielded a standardized effect size of about 4.1. Under these assumptions, the minimum sample size was about 11 participants per group based on the standard two sample t-test. To account for potential attrition, between-subject heterogeneity, and the need to support multivariable logistic regression, the target sample size was expanded 30 participants per group. This margin accommodates missing data and keeps the primary TBUT comparison at ≥80% power, while improving the precision of secondary analyses.

Sample collection

This single-center retrospective study included patients evaluated at Lanzhou First People's Hospital between January 2019 and January 2022. A total of 120 individuals with SS-DED and 88 age- and sex-matched healthy controls were enrolled. The study protocol was approved by the Medical Ethics Committee of Lanzhou First People's Hospital, which waived the requirement for written informed consent due to the retrospective chart-review nature of the study. All data were de-identified prior to analysis.

Inclusion and exclusion criteria

Inclusion criteria: Diagnosis of Sjögren's syndrome (pSS) according to established classification criteria [16]; Diagnosis of DED per consensus diagnostic criteria [17]; Age ≥18 years, of either sex; Disease duration ≥6 months, with complete medical records containing all required variables; No recent systemic anti-inflammatory or immunosuppressive therapy,

defined as no corticosteroids or other immunosuppressants within the preceding ≥3 months.

Exclusion criteria: Recent corneal/ocular procedures within 3 months, including laser-assisted *in situ* keratomileusis (LASIK), corneal transplantation, or other invasive ocular interventions; Severe concurrent ocular surface disease, such as active bacterial or viral keratoconjunctivitis, corneal ulcer, or severe meibomian gland dysfunction; Coexisting autoimmune or systemic illness likely to confound outcomes (e.g., active systemic lupus erythematosus, uncontrolled diabetes mellitus, or end-stage hepatic/renal dysfunction); Pregnancy or lactation; Severe psychiatric or cognitive impairment precluding reliable examination or follow-up.

Clinical data collection

Study variables were extracted from electronic medical records and outpatient follow-up documentation, encompassing five categories: ① Demographics: age, sex, body mass index (BMI), marital status, education level, and household per capita monthly income; ② Lifestyle and medical history: smoking status, alcohol consumption, hypertension, and diabetes; ③ DED parameters: TFBUT, corneal fluorescein staining (FL) score, Schirmer test, and OSDI; ④ Inflammatory cytokines: IL-1β, IL-6, and TNF-α; ⑤ IVCM parameters: dendritic cell density (DCD), nerve fiber density (NFD), and inflammatory cell density (ICD).

Treatment protocol

All patients followed the same 8-week treatment regimen without randomization, using a standardized protocol and visit schedule. Systemic therapy consisted of oral prednisolone acetate and hydroxychloroquine sulfate, with dosage determined by departmental practice and adjusted as clinically indicated. Topical therapy included 0.3% sodium hyaluronate (Santen, Japan), 0.1% fluorometholone (Santen, Japan), 0.1% tacrolimus (Senju, Japan) twice daily, and 0.05% cyclosporine A (Shenyang Xingqi, China) four times daily. The schedule remained unchanged throughout the 8-week period. Follow-up visits were conducted to ensure adherence. During treatment period, no additional anti-inflammatory or immunosuppressive drugs were initiated. Any modifications or adverse events were recorded in accordance with clinic policy.

Functional scoring

Functional assessments encompassed OSDI [18], TFBUT [19], corneal FL [20], and the Schirmer test [21] to evaluate subjective symptoms and ocular surface functionality. The OSDI encompasses 12 questions assessing the frequency of dry eye symptoms, their effect on vision, and environmental triggers. The total OSDI score is calculated as the mean score of questions answered, yielding a total score from 0 to 100: Score of 0-12 indicate normal symptoms, 13-22 mild DED, 23-32 moderate DED, and 33-100 severe DED. BUT objectively assesses tear film stability by measuring time before tear film breakup (0-30 seconds): <10 seconds suggests instability, <5 seconds indicates severe DED. FL scoring follows the National Eye Institute (NEI) method (0-15 points) or Oxford method (0-V grade) to assess corneal epithelial damage; higher scores reflect greater severity. Schirmer test measures basal and reflex tear secretion in millimeters per 5 minutes (0-35 mm/5 min): <5 mm/5 min suggests moderate-to-severe DED, ≥10 mm/5 min is normal.

Laboratory indicators

Peripheral venous blood samples were collected before treatment initiation to determine inflammatory factors and biochemical indicators. Blood samples were immediately placed on ice, centrifuged for serum separation, and stored at -80°C until batch analysis. Serum concentrations of IL-1β, IL-6, and TNF-α were determined using enzyme-linked immunosorbent assay (ELISA) (Shanghai Enzyme-linked Biotechnology Co., Ltd.).

Prognostic assessment criteria

Patient prognosis was evaluated using a combination of slit-lamp microscopy, corneal FL staining, and TFBUT. Mild (good prognosis): No obvious ocular surface damage, FL staining score <5, and TFBUT ≥10 seconds. Moderate (poor prognosis): Corneal damage involving ≤2 quadrants or FL staining score of 5-29, with TFBUT between 2 and 9 seconds. Severe (poor

prognosis): Corneal damage involving ≥2 quadrants or FL staining score ≥30 (confluent coarse points, large patches, or filamentary material), and TFBUT <2 seconds.

IVCM examination and quality control

IVCM was performed using the Heidelberg Retina Tomograph III with the Rostock Cornea Module (HRT III-RCM; Heidelberg Engineering, Germany). All examinations were carried out in the same laboratory with identical equipment and imaging settings. The central cornea of each eye was scanned, and three clear, non-overlapping images were selected for analysis. IVCM was performed by a single experienced ophthalmologist trained on a unified protocol. During image acquisition, patients were instructed to fixate on a target to minimize eye movement; images with poor focus or contrast were excluded. Corneal DCD, subbasal NFD, and ICD were quantified using ImageJ (NIH, USA). Each frame measured 400 × 400 μm. Three representative frames were preselected for each participant and averaged for final analysis. Image grading was performed independently by two masked observers blinded to clinical data. Interobserver reliability was assessed in a random subset of 30 cases, yielding intraclass correlation coefficients (ICCs) >0.90 for all measures. When the two readings differed more than 10%, the graders jointly reviewed the images and reach a consensus value. These procedures were designed to minimize measurement error and ensure consistent image quality and scoring across the dataset.

Outcome measurements

Primary outcomes: Independent risk factors were identified using univariate and multivariate logistic regression analyses, and their predictive performance was assessed using Receiver operating characteristic (ROC) curve analysis.

Secondary outcomes: Between-group comparisons were conducted for baseline demographics, inflammatory cytokines, ocular surface function, and IVCM indicators. Correlation analyses were performed to explore associations between IVCM indicators and other clinical variables.

Statistical analysis

All statistical analyses were performed using R version 4.3.3. Kolmogorov-Smirnov tests assessed normality for continuous variables. Normally distributed data were presented as mean ± standard deviation, non-normally distributed data as median (interquartile range [IQR]), and categorical variables as frequency (%). Between-group differences were analyzed using independent samples t-tests for normally distributed continuous variables, Mann-Whitney U tests for non-normally distributed continuous variables, and χ^2 tests for categorical variables. Pearson correlation coefficients was used to assess correlations between variables. Univariate and multivariate logistic regression analyses were used to identify independent prognostic risk factors, with odds ratios (OR) and *P* values reported. ROC curve analysis was performed to evaluate variable's predictive performance, reporting AUC, sensitivity, specificity, Youden index, and optimal cut-off values. DeLong's test was applied to compare AUCs among predictors.

Results

Baseline characteristics

Baseline demographic and clinical characteristics were compared between the SS-DED and control groups (**Table 1**). No significant differences were observed in age, sex, BMI, marital status, education, household per-capita monthly income, smoking, alcohol use, hypertension, or diabetes (all P>0.05).

Inflammatory cytokine levels

Compared to the control group, SS-DED group demonstrated significantly higher IL-1β, IL-6, and TNF-α levels (all P<0.001; **Table 2**), consistent with enhanced ocular surface inflammation in SS-related DED.

Ocular surface function indicators

Ocular surface function results are shown in **Table 3**. Compared to controls, SS-DED patients exhibited significantly shorter TBUT, higher corneal FL scores, lower Schirmer values, and higher OSDI scores (all P<0.001), indicating reduced tear film stability, greater epithelial damage, diminished tear production,

Table 1. Baseline demographics and clinical characteristics of study subjects

Variable	Total	Case Group (n=120)	Control Group (n=88)	Test Statistic	*P* Value
Age	51.10±11.76	51.02±11.89	51.22±11.65	0.12	0.904
Sex				0.756	0.384
Male	78 (37.50%)	42 (35.00%)	36 (40.91%)		
Female	130 (62.50%)	78 (65.00%)	52 (59.09%)		
BMI	24.16±2.57	24.03±2.52	24.33±2.65	0.848	0.398
Marital Status				0.364	0.546
Married	194 (93.27%)	113 (94.17%)	81 (92.05%)		
Other	14 (6.73%)	7 (5.83%)	7 (7.95%)		
Education Level				2.298	0.130
High School and Above	96 (46.15%)	50 (41.67%)	46 (52.27%)		
Below High School	112 (53.85%)	70 (58.33%)	42 (47.73%)		
Household Per Capita Monthly Income				1.854	0.173
≥4500	81 (38.94%)	42 (35.00%)	39 (44.32%)		
<4500	127 (61.06%)	78 (65.00%)	49 (55.68%)		
Smoking History				0.443	0.506
Yes	89 (42.79%)	49 (40.83%)	40 (45.45%)		
No	119 (57.21%)	71 (59.17%)	48 (54.55%)		
Alcohol Consumption History				0.434	0.51
Yes	27 (12.98%)	14 (11.67%)	13 (14.77%)		
No	181 (87.02%)	106 (88.33%)	75 (85.23%)		
Hypertension History				0.658	0.417
Yes	24 (11.54%)	12 (10.00%)	12 (13.64%)		
No	184 (88.46%)	108 (90.00%)	76 (86.36%)		
Diabetes History				0.971	0.324
Yes	21 (10.10%)	10 (8.33%)	11 (12.50%)		
No	187 (89.90%)	110 (91.67%)	77 (87.50%)		

Note: BMI: Body Mass Index, OSDI: Ocular Surface Disease Index, TNF-α: Tumor Necrosis Factor-α, IL-1β: Interleukin-1β, IL-6: Interleukin-6.

Table 2. Comparison of inflammatory factor levels between case and control groups

Variable	Total	Case Group (n=120)	Control Group (n=88)	Test Statistic	*P* Value
IL-1β (ng/L)	29.87±16.27	39.85±14.13	16.26±5.70	-14.797	<0.001
IL-6 (ng/L)	10.76±6.44	15.49±4.22	4.29±1.05	-24.309	<0.001
TNF-α (ng/L)	26.32±7.86	31.04±6.44	19.89±4.19	-14.191	<0.001

Note: IL-1β: Interleukin-1β, IL-6: Interleukin-6, TNF-α: Tumor Necrosis Factor-α.

and heavier symptom burden in SS-related DED.

Corneal immune and neural indicators

IVCM indicators are reported in **Table 4**. Compared to controls, SS-DED group demonstrated significantly higher DCD and ICD and lower NFD (all P<0.001), consistent with increased immune activity and reduced subbasal innervation in SS-related DED.

Correlations between IVCM indicators and clinical indicators

As shown in **Figures 1**, **2**, DCD exhibited significant positive correlations with IL-1β, BUT, FL, and OSDI (all P<0.05), NFD showed significant negative correlations with BUT and FL (all P<0.001), and ICD demonstrated significant positive correlations with IL-1β, IL-6, TNF-α, and DCD (all P<0.001). Other correlations were not statistically significant (P>0.05).

Table 3. Comparison of ocular surface function-related indicators between case and control groups

Variable	Total	Case Group (n=120)	Control Group (n=88)	Test Statistic	*P* Value
BUT (s)	4.65 (3.82)	3.10 (2.23)	6.95 (2.65)	10.639	<0.001
FL	4.00 (4.00)	6.00 (2.25)	2.00 (1.00)	12.467	<0.001
Schirmer Test (mm/5 min)	6.22±3.60	3.77±1.48	9.55±2.89	18.829	<0.001
OSDI	16.00 (32.25)	38.00 (37.00)	7.50 (7.00)	10.573	<0.001

Note: BUT: Break-Up Time, FL: Fluorescein Staining, OSDI: Ocular Surface Disease Index.

Table 4. Comparison of corneal immune and neural-related indicators between case and control groups

Variable	Total	Case Group (n=120)	Control Group (n=88)	Test Statistic	*P* Value
Dendritic Cell Density (cells/mm^2)	22.53±14.08	29.78±13.87	12.63±6.07	-10.861	<0.001
Nerve Fiber Density (mm/mm^2)	14.19±3.32	12.04±2.40	17.12±1.84	-17.254	<0.001
Inflammatory Cell Density (cells/mm^2)	484.38±333.00	653.14±340.87	254.26±109.78	-10.577	<0.001

Comparison of clinical characteristics between good and poor prognosis groups

No significant differences were observed in age, sex, BMI, marital status, education level, household per capita monthly income, smoking, alcohol consumption, hypertension, diabetes, IL-6, TNF-α, Schirmer test, or NFD between the two groups (P>0.05). However, the poor prognosis group showed significantly higher IL-1β, OSDI, DCD, and ICD compared to the good prognosis group (all P<0.001). Details are provided in **Table 5**.

Logistic regression analysis for prognostic factors

Univariate and multivariate logistic regression analyses were applied to identify factors independently associated with poor prognosis (**Figure 3**). Univariate analysis revealed significant associations between patient prognosis with IL-1β, OSDI, DCD, and ICD (all P<0.05). Multivariate analysis further confirmed IL-1β (OR=1.249, P<0.001), OSDI (OR=1.074, P= 0.033), DCD (OR=1.411, P=0.002), and ICD (OR=1.006, P=0.018) as independent risk factors for poor prognosis.

ROC curve analysis for predictive performance

ROC curve analysis was conducted to evaluate the predictive value of the four independent risk factors (IL-1β, OSDI, DCD, ICD) (**Figure 4**). As shown in **Table 6**, DCD demonstrated the best discriminative performance, with an AUC of 0.886, optimal cutoff of 38.95, specificity of 98.75%, sensitivity of 65.00%, and Youden index of 63.75. IL-1β also showed strong discrimination (AUC 0.861) with a cutoff of 48.45 (specificity 92.50%; sensitivity 62.50%; Youden 55.00). OSDI and ICD provided moderate accuracy (AUC 0.775 and 0.753, respectively), with OSDI favoring sensitivity (97.50%) and ICD favoring specificity (85.00%). DeLong pairwise comparisons (**Table 7**) showed that DCD significantly outperformed OSDI (P= 0.029) and ICD (P=0.034). Differences between AUCs of other predictors were not significant (P>0.05).

Discussion

SS-related DED involves immune dysregulation with inflammatory mediator release and exocrine gland dysfunction. Conventional ocular surface tests (TBUT, FL, Schirmer) are sensitive to environmental factors and operator variability, limiting their reliability to reflect pathology and prognosis. Zhan et al. [2] highlighted the roles of IFNs, Th17-related cytokines (IL-17, IL-23), and B-cell factors (TNF, BAFF) in SS. IVCM provides a non-invasive visualization of corneal microstructural change and serves as a adjunct for investigating ocular surface pathology. Prior literature [22] reports strong associations between cytokines such as IL-6, TNF-α, IL-17, and IL-8 with DED severity, tear film metrics, and ocular surface damage. However, evidence directly linking these inflammatory mediators with IVCM-derived indicators in SS-DED remains limited. We therefore combined inflammatory cytokines, IVCM indicators,

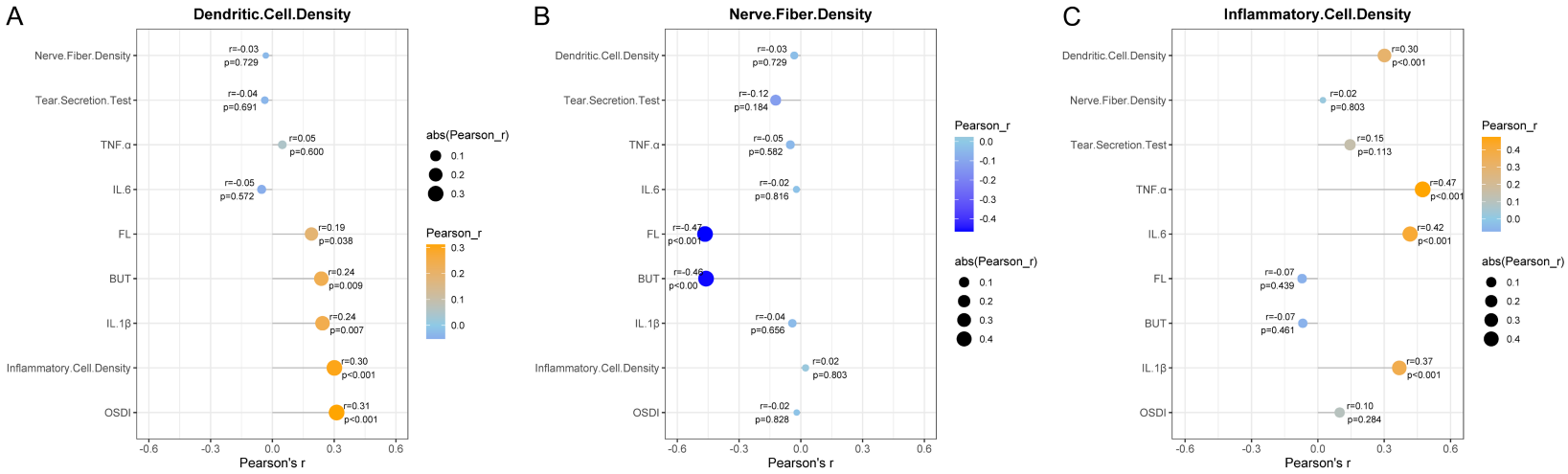

Figure 1. Correlation between dendritic cell density, nerve fiber density, inflammatory cell density, and other variables. (A-C) Pearson correlation coefficients (r) between dendritic cell density (A), nerve fiber density (B), and inflammatory cell density (C) with other variables. Point colors indicate correlation direction and strength (orange for positive correlation, blue for negative correlation), point sizes represent absolute correlation coefficient values, with correlation coefficients and *P* values annotated alongside. Note: BUT: Break-Up Time, FL: Fluorescein Staining, OSDI: Ocular Surface Disease Index, TNF-α: Tumor Necrosis Factor-α, IL-1β: Interleukin-1β, IL-6: Interleukin-6.

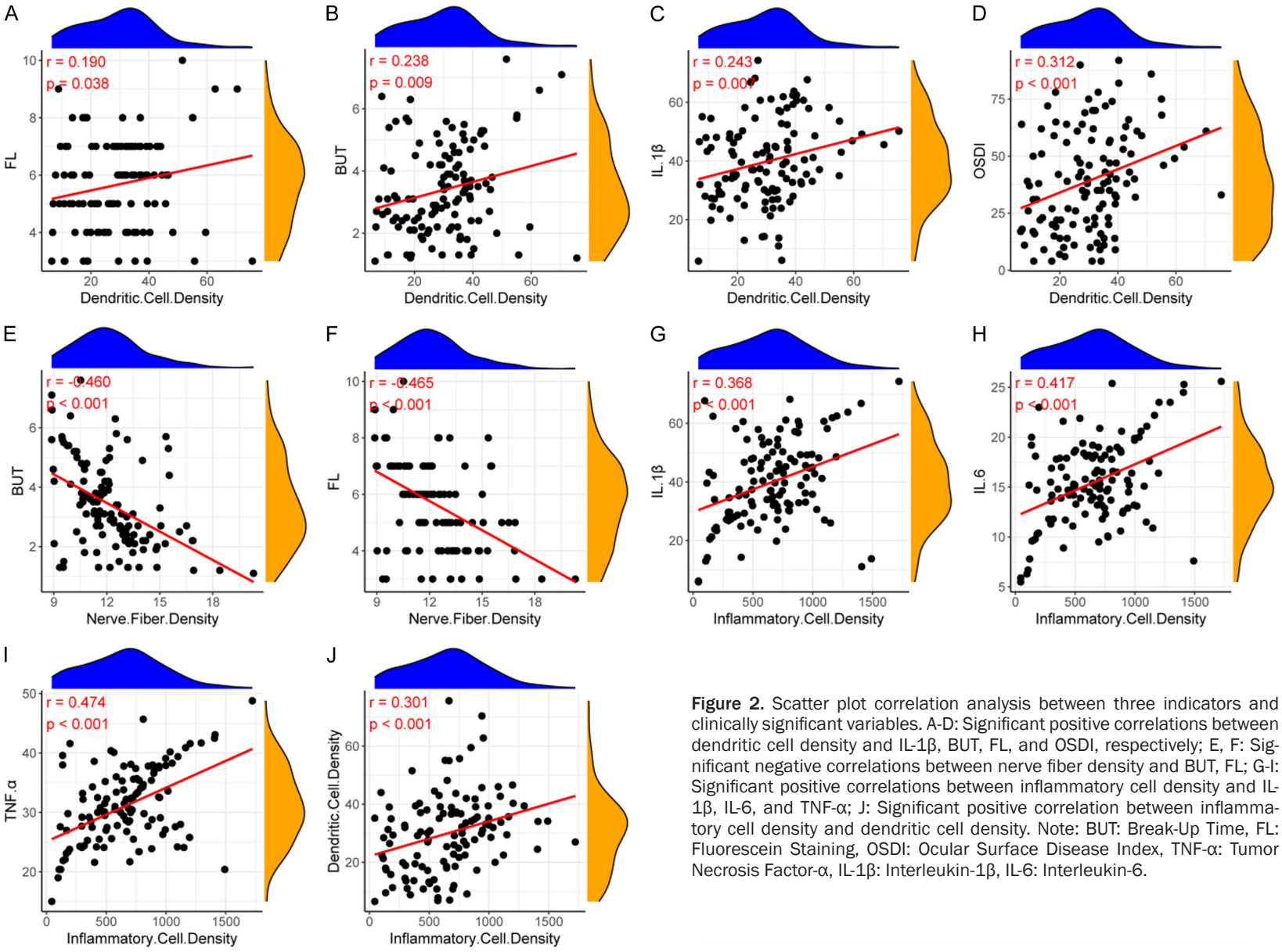

Figure 2. Scatter plot correlation analysis between three indicators and clinically significant variables. A-D: Significant positive correlations between dendritic cell density and IL-1β, BUT, FL, and OSDI, respectively; E, F: Significant negative correlations between nerve fiber density and BUT, FL; G-I: Significant positive correlations between inflammatory cell density and IL-1β, IL-6, and TNF-α; J: Significant positive correlation between inflammatory cell density and dendritic cell density. Note: BUT: Break-Up Time, FL: Fluorescein Staining, OSDI: Ocular Surface Disease Index, TNF-α: Tumor Necrosis Factor-α, IL-1β: Interleukin-1β, IL-6: Interleukin-6.

Table 5. Comparison of clinical characteristics and biological indicators between poor and good prognosis groups

Variable	Total	Poor Prognosis Group (n=40)	Good Prognosis Group (n=80)	Test Statistic	*P* Value
Age	51.02±11.89	49.55±11.38	51.75±12.14	0.955	0.341
Sex				<0.001	>0.999
Male	42 (35.00%)	14 (35.00%)	28 (35.00%)		
Female	78 (65.00%)	26 (65.00%)	52 (65.00%)		
BMI	24.03±2.52	23.75±2.24	24.17±2.64	0.864	0.389
Marital Status				<0.001	>0.999
Married	113 (94.17%)	38 (95.00%)	75 (93.75%)		
Other	7 (5.83%)	2 (5.00%)	5 (6.25%)		
Education Level				1.097	0.295
High School and Above	50 (41.67%)	14 (35.00%)	36 (45.00%)		
Below High School	70 (58.33%)	26 (65.00%)	44 (55.00%)		
Household Per Capita Monthly Income				0.659	0.417
≥4500	42 (35.00%)	16 (40.00%)	26 (32.50%)		
<4500	78 (65.00%)	24 (60.00%)	54 (67.50%)		
Smoking History				0.017	0.896
Yes	49 (40.83%)	16 (40.00%)	33 (41.25%)		
No	71 (59.17%)	24 (60.00%)	47 (58.75%)		
Alcohol Consumption History				1.223	0.269
Yes	14 (11.67%)	7 (17.50%)	7 (8.75%)		
No	106 (88.33%)	33 (82.50%)	73 (91.25%)		
Hypertension History				0.104	0.747
Yes	12 (10.00%)	3 (7.50%)	9 (11.25%)		
No	108 (90.00%)	37 (92.50%)	71 (88.75%)		
Diabetes History				<0.001	>0.999
Yes	10 (8.33%)	3 (7.50%)	7 (8.75%)		
No	110 (91.67%)	37 (92.50%)	73 (91.25%)		
IL-1β	39.85±14.13	51.72±11.43	33.91±11.36	-8.077	<0.001
IL-6	15.49±4.22	15.91±4.51	15.28±4.09	-0.772	0.442
TNF-α	31.04±6.44	32.26±6.89	30.43±6.16	-1.469	0.145
Schirmer Test	3.77±1.48	3.80±1.35	3.76±1.54	-0.144	0.886
OSDI	38.00 (37.00)	52.00 (25.00)	27.00 (30.50)	4.897	<0.001
Dendritic Cell Density	30.30 (17.50)	40.30 (11.50)	25.15 (15.50)	6.867	<0.001
Nerve Fiber Density	11.93 (2.48)	12.33 (2.47)	11.73 (2.45)	1.269	0.204
Inflammatory Cell Density	668.95 (464.12)	864.60 (332.90)	567.95 (419.07)	4.501	<0.001

Note: BMI: Body Mass Index, OSDI: Ocular Surface Disease Index, TNF-α: Tumor Necrosis Factor-α, IL-1β: Interleukin-1β, IL-6: Interleukin-6.

and ocular surface function indices to explore their interrelationships, identify independent predictive biomarkers, and provide evidence to support early diagnosis, treatment monitoring, and prognostic assessment in SS-related DED.

Our findings revealed significantly higher serum IL-1β, IL-6, and TNF-α levels in SS-DED patients compared with healthy controls. Consistently, previous Meta-analyses [5, 23] likewise reported increased tear IFN-γ, IL-17, IL-1β, IL-4, IL-6, and IL-8 levels in SS-DED, supporting concordant systemic and ocular-surface inflammation. IL-1β activates NF-κB and amplifies downstream cytokine release [24]; tear IL-7, IL-1α, and IL-1β can help distinguish IgG4-related disease from SS [25]. IL-6 contributes to acute-

Variable	Assignment	Univariate OR (95% CI)	Univariate P value	Multivariate OR (95% CI)	Multivariate P value
Age	Continuous variables use raw data	0.984 (0.953–1.017)	0.339	—	—
BMI	Continuous variables use raw data	0.934 (0.801–1.090)	0.386	—	—
IL-1β	Continuous variables use raw data	1.148 (1.092–1.208)	<0.001	1.249 (1.131–1.471)	<0.001
IL-6	Continuous variables use raw data	1.037 (0.947–1.135)	0.439	—	—
TNF-α	Continuous variables use raw data	1.046 (0.985–1.111)	0.146	—	—
Tear Secretion Test	Continuous variables use raw data	1.019 (0.787–1.320)	0.885	—	—
OSDI	Continuous variables use raw data	1.051 (1.028–1.074)	<0.001	1.074 (1.017–1.168)	0.033
Dendritic Cell Density	Continuous variables use raw data	1.214 (1.124–1.311)	<0.001	1.411 (1.192–1.850)	0.002
Nerve Fiber Density	Continuous variables use raw data	1.129 (0.942–1.353)	0.190	—	—
Inflammatory Cell Density	Continuous variables use raw data	1.003 (1.001–1.004)	<0.001	1.006 (1.002–1.012)	0.018
Gender	1 = Male, 0 = Famale	1.000 (0.451–2.216)	1.000	—	—
Marital Status	1 = Married, 0 = Ohert	1.267 (0.235–6.835)	0.783	—	—
Education Level	1 = ≥ High School, 0 = < High School	0.658 (0.300–1.443)	0.296	—	—
Household Per Capita Monthly Income	1 = ≥4500, 0 = <4500	1.385 (0.630–3.041)	0.418	—	—
Smoking History	1 = Yes, 0 = no	0.949 (0.438–2.058)	0.896	—	—
Alcohol Consumption History	1 = Yes, 1 = no	2.212 (0.718–6.817)	0.167	—	—
Hypertension History	1 = Yes, 2 = no	0.640 (0.163–2.507)	0.521	—	—
Diabetes History	1 = Yes, 3 = no	0.846 (0.207–3.460)	0.816	—	—

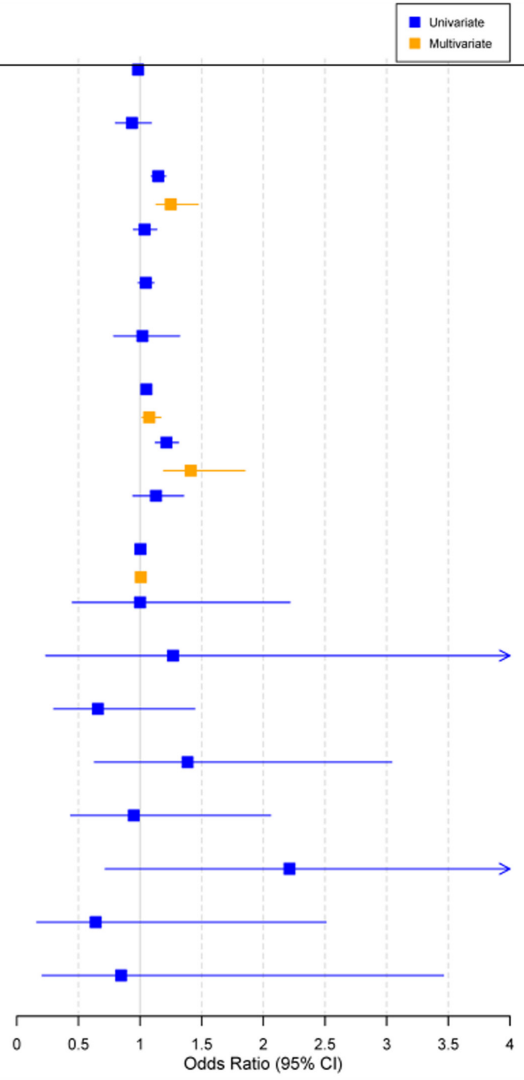

Figure 3. Logistic regression analysis for identifying independent risk factors affecting patient prognosis. Note: OR: Odds Ratio, OSDI: Ocular Surface Disease Index, IL-1β: Interleukin-1β.

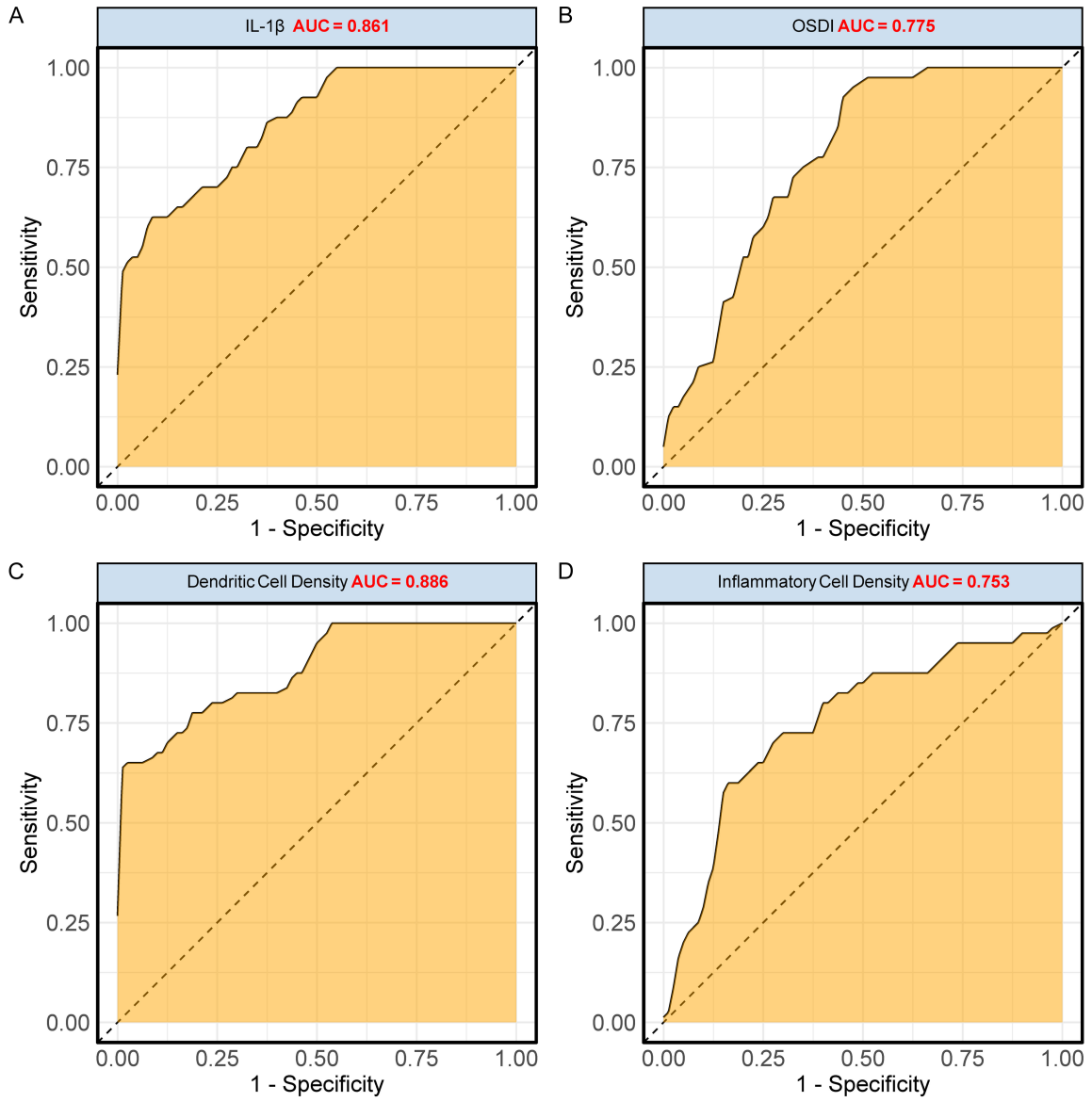

Figure 4. ROC curve analysis for four independent risk factors in predicting patient prognosis. A: ROC curve for IL-1β (AUC=0.861); B: ROC curve for OSDI (AUC=0.775); C: ROC curve for dendritic cell density (AUC=0.886); D: ROC curve for inflammatory cell density (AUC=0.753). Note: ROC: Receiver Operating Characteristic, AUC: Area Under the Curve, OSDI: Ocular Surface Disease Index, IL-1β: Interleukin-1β, DCD: Dendritic Cell Density, ICD: Inflammatory Cell Density.

phase responses and B-cell differentiation [26], and TNF-α promotes ocular-surface damage by immune-cell activation, apoptosis, and barrier disruption [27]. Together, these mediators constitute an inflammatory network driving SS-DED pathogenesis and motivate our correlation analyses between cytokine profiles, IVCM features, and clinical measures.

IVCM showed corneal microstructural changes in SS-DED patients. DCD was significantly elevated, indicating enhanced local immune activity. Prior work likewise noted higher DCD and ICD in SS-DED than in non-SS DED and healthy controls [8, 10]. Dendritic cells, serving as key antigen-presenting cells, rise with ocular-surface inflammation and can help sustain it [28]. DCD showed a positive correlation with IL-1β, suggesting correspondence between local immune activity and systemic inflammation. Higher DCD was observed alongside shorter TBUT, higher FL score, and higher OSDI scores,

Table 6. ROC curve indicators of four independent risk factors for predicting patient prognosis

Marker	AUC	95% CI	Specificity	Sensitivity	Youden Index	Cut-off
IL-1β	0.861	0.794-0.928	92.50%	62.50%	55.00%	48.45
OSDI	0.775	0.694-0.856	52.50%	97.50%	50.00%	28
Dendritic Cell Density	0.886	0.822-0.949	98.75%	65.00%	63.75%	38.95
Inflammatory Cell Density	0.753	0.658-0.847	85.00%	60.00%	45.00%	807.75

Note: AUC: Area Under the Curve, CI: Confidence Interval, OSDI: Ocular Surface Disease Index, IL-1β: Interleukin-1β.

Table 7. DeLong test results for AUC comparison of four independent risk factors

Marker 1	Marker 2	Z Value	*P* Value	AUC Difference	95% CI
IL-1β	OSDI	1.578	0.115	0.086	-0.021 - 0.193
IL-1β	Dendritic Cell Density	-0.461	0.645	-0.024	-0.128 - 0.079
IL-1β	Inflammatory Cell Density	1.831	0.067	0.108	-0.008 - 0.225
OSDI	Dendritic Cell Density	-2.178	0.029	-0.111	-0.210 - -0.011
OSDI	Inflammatory Cell Density	0.336	0.737	0.022	-0.107 - 0.152
Dendritic Cell Density	Inflammatory Cell Density	2.116	0.034	0.133	0.010 - 0.256

Note: AUC: Area Under the Curve, CI: Confidence Interval, OSDI: Ocular Surface Disease Index, IL-1β: Interleukin-1β.

suggesting that worsened ocular surface function coincides with increased immune activation.

A significant reduction in NFD was observed in this study. Earlier studies showed reduced corneal nerve fiber length and density in SS compared with controls, frequently with atypical morphology [9, 29]. Because corneal nerves are essential for maintaining sensation, epithelial health, and tear secretion, lower NFD is consistent with declining function. In our data, NFD negatively correlated with TBUT and with FL, supporting an association between neural loss, tear film instability, and epithelial damage. Independent studies also noted that tear hyperosmolarity associates with reduced nerve branching, fiber density, and length, with effects independent of DCD [30]. Possible contributors to nerve loss include direct cytokine neurotoxicity, hypoxic-ischemic injury, and immune-mediated neuroinflammation. Complementary evidence from Lanza et al. showed increased light scattering across corneal layers in SS-DED, inversely related to tear function tests [31]. Taken together, nerve fiber damage may exacerbate ocular-surface dysfunction and, by impairing lacrimal innervation, could create a self-reinforcing deterioration cycle.

Elevated ICD reflects increased inflammatory infiltration within the cornea. In our data, ICD positively correlated with IL-1β, IL-6, and TNF-α, supporting concordance between systemic inflammatory activity and local tissue involvement. Prior work has reported diagnostic utility of tear biomarkers for distinguishing SS from non-SS DED: Akpek et al. noted lower MUC5AC and higher IL-8 in SS-DED tears, and Masli et al. found that reduced TSP-1/MMP-9 ratios effectively distinguished SS-DED [13, 32]. The accumulation of inflammatory cells can both result from, and help sustain, ocular-surface inflammation by releasing cytotoxic factors. By multivariable logistic regression, IL-1β (OR=1.249), OSDI (OR=1.074), DCD (OR=1.411), and ICD (OR=1.006) emerged as independent predictors. Consistently, Wang et al. built IVCM-based models for SS serum activity, highlighting predictive value of corneal nerve density, DCD, and tear film height [33]. These patterns are pathophysiologically coherent: higher IL-1β - an initiator in inflammatory cascades - parallels disease activity; despite its subjective nature, OSDI retained independent predictive value, implying a link between symptom perception and severity. Confocal abnormalities may precede antibody positivity and overt SS manifestations, suggesting utility for early detection [34]. In ROC analyses, DCD showed the strongest single-marker discriminative performance (AUC=0.886, specificity =98.75%; sensitivity =65.00%). IL-1β also performed well, with an AUC of 0.861, sensitivity of 62.50%, and specificity of 92.50%, supporting its complementary value as an additional predictor.

Reviews have outlined the potential of proteomic and exosome-based markers for SS-DED stratification [12, 35]. In this setting, DCD and IL-1β help identify early pathologic change and can precede shifts in standard ocular surface tests, which enables earlier intervention. IVCM is useful because it is noninvasive, objective, and repeatable benefits, and capable of visualizing cellular-level corneal changes in real time [36], further supporting a personalized treatment. Patients with elevated DCD or inflammatory cytokines may benefit from intensified anti-inflammatory regimens, whereas those with reduced NFD may be candidates for neuroprotective strategies.

Topical cyclosporine has established safety and efficacy in treatment for SS-DED [11, 37]. Comparative studies suggest that 0.1% cyclosporine provides better symptom relief than 0.05% cyclosporine, with lower tolerance [11]. Additional modalities are being explored. For example, combining intense pulsed light with 0.05% cyclosporine A improved symptoms and signs in SS-DED [38]. IVCM indicators may respond earlier than routine indices during follow-up. A decline in DCD can indicate an anti-inflammatory response, and a rise in NFD may signal better long-term outcomes. Integrated risk assessment that combines cytokines, IVCM metrics, and clinical tests supports grading of disease severity, anticipation of clinical course, and identification of higher risk patients for personalized follow-up and resource allocation.

This study has several limitations. First, the cross-sectional design supports associations between inflammation and microstructural change but does not establish causality. Second, all participants were recruited from a single center in Gansu, northwestern China; therefore, regional demographics and environmental conditions may limit generalizability. Third, only primary SS-DED was included, which restricts representation of other subtypes. Fourth, IVCM outcomes are operator dependent, underscoring the need for standardized acquisition protocols and quality control. Lastly, follow-up period was short, thus, longitudinal outcomes were not available. Future work should involve large, multicenter, prospective cohorts encompassing diverse geographic and ethnic populations with extended follow-up to validate these findings and to assess the temporal stability of the proposed biomarkers.

Conclusion

This study comprehensively profiled cytokines, IVCM features, and ocular surface function in SS-related DED. IL-1β, OSDI, DCD, and ICD emerged as independent risk factors for poor prognosis, with DCD demonstrating the strongest discriminative performance. These results highlight the potential of a multidimensional assessment for prognostic prediction and enable individualized management. Confirmation in larger, longitudinal, and ethnically diverse cohorts is still warranted.

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Disclosure of conflict of interest

None.

Address correspondence to: Kan Yang, Department of Ophthalmology, Lanzhou First People's Hospital, No. 1 Wujiayuan West Street, Qilihe District, Lanzhou 730050, Gansu, China. E-mail: yangkan2024@126.com

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