Evaluation of ocular surface involvement and systemic conditions in patients with autoimmune rheumatic diseases

Yingyi Liu  
The Second Xiangya Hospital of Central South University

Mengbo Wu  
The Second Xiangya Hospital of Central South University

Yuerong Ren  
The Second Xiangya Hospital of Central South University

Jianing Feng  
The Second Xiangya Hospital of Central South University

Wen Shi  
The Second Xiangya Hospital of Central South University

Huanmin Kang  
The Second Xiangya Hospital of Central South University

Jing Tian  
The Second Xiangya Hospital of Central South University

Yan He (yanhe6416@csu.edu.cn)  
The Second Xiangya Hospital of Central South University

Research Article

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Abstract

Purpose: To evaluate and explore the ocular surface involvement and systemic conditions in autoimmune rheumatic diseases (ARDs).

Methods: 79 patients with ARDs were enrolled in our study, including 26 patients with rheumatoid arthritis (RA), 33 patients with systemic lupus erythematosus (SLE), and 20 patients with primary Sjögren's syndrome (pSS). All patients underwent ocular surface evaluation, including ocular surface symptoms and signs, conjunctival impression cytology, and tear multi-cytokine detection. The systemic conditions were also collected, including disease duration and disease activity.

Results: SLE patients have the shortest disease duration and nearly half of them have low disease activity, while RA patients and pSS patients have a relatively long disease duration and about 90% of them have moderate/high disease activity. The incidence of dry eye and the levels of pro-inflammatory tear cytokine in SLE patients is significantly lower than RA and pSS patients, while there was no significant difference between RA and pSS patients. However, pathologic squamous metaplasia on the ocular surface is more severe in SLE and pSS patients than RA patients. Dry eye severity in all ARDs patients was shown independent of disease activity, while the ocular surface Nelson's grades are positively correlated with disease duration in RA patients.

Conclusions: Dry eye and ocular surface inflammation persist in most ARDs patients, and do not occur in parallel with the disease activity. Other than pSS, dry eye and ocular surface squamous metaplasia also exist in SLE and RA. Therefore, all patients with ARDs require a regular ophthalmologic evaluation and topical medications.

1. Introduction

Autoimmune rheumatic diseases (ARDs) are a set of inflammatory diseases characterized by abnormal immune system activation involving multi-organ, and a clear female predominance in cases is reported [1–3]. Primary Sjögren's syndrome (pSS), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) are common ARDs, which share many clinical presentations and laboratory findings [4].

Although ARDs primarily affect the musculoskeletal system, ocular involvement often coexists due to destructive immune-mediated ocular inflammation that may be vision-threatening [5, 6]. A meta-analysis showed the prevalence of ocular complications was 89% in SS, 31% in SLE, and 18% in RA [7]. Antibody-mediated endothelial injury, immune complexes formation, and complement activation mediated the changes in the ocular microenvironment that play an important role [8]. The eye is known as a sensitive barometer for the occurrence or reactivation of ARDs. Ocular symptoms and signs may even be the first manifestations of ARDs, particularly in pSS, SLE and RA. The most common ocular involvement of ARDs is inflammation of various ocular tissues, which affects every part of the eye, from the eyelid and cornea to the uvea, retina, optic nerve, and sclera [9]. Among them, dry eye disease (DED) is the most common manifestation of ocular surface inflammation [10].

DED is a widespread disease occurred in patients with ARDs. The underlying pathophysiological mechanism of DED is a vicious circle that tears hyperosmolarity as the core mechanism to start up an inflammatory cascade. Inflammatory mediators and proteases were released by ocular surface epithelial cells, resulting in goblet cells, epithelial cells, and glycoalyx mucin damage and loss, which further led to tear film instability [11]. However, the current research on ARDs and DED mainly focuses on a specific disease in ARDs, and there is a lack of comparative studies between several different ARDs, such as pSS, SLE, and RA.

This study assessed the ocular surface involvement in patients with ARDs, including pSS, SLE, and RA by analyzing ocular surface symptoms and signs, conjunctival impression cytology, and tear multi-cytokine. Our study aims to investigate the differences in severity of DED and ocular surface pathophysiological mechanisms between various ARDs like pSS, SLE, and RA, as well as to explore the relationship between systemic conditions and ocular surface involvement, which may help in early diagnosis and prevent many long-term ocular sequelae in patients with ARDs.

2. Materials And Methods

2.1 Study design

A cross-sectional study

2.2 Subjects

This study protocol was approved by the Clinical Research Ethics Committee of the Second Xiangya Hospital of Central South University (ethics number LYF2021028). It adhered to the guidelines of the Declaration of Helsinki. Prior written informed consent was obtained from all participants. We recruited study participants (n = 79) who were hospitalized from March 2020 to July 2021 at the Department of Rheumatology and Immunology of the Second Xiangya Hospital, Central South University. Study participants were divided into 3 groups: 26 RA patients, 33
SLE patients, and 20 pSS patients. All cases aged 16 years or older with RA, SLE, or pSS diagnosed by rheumatologists. Patients with RA or SLE who have secondary Sjogren syndrome were excluded. Those who had a history of intraocular surgery or ocular laser treatment, eye infection, or ocular anti-inflammatory drugs within the past 3 months were also excluded from the study. Patients met the 2010 ACR-EULAR classification criteria for RA [12], the 1997 SLICC-ACR classification criteria for SLE [13], and the 2002 American-European Consensus Group classification criteria for pSS [14], respectively. The objective disease activity of RA was based on Disease Activity Score 28 (DAS28) categorized into low (2.6 ≤ DAS28 ≤ 3.2), moderate (3.2 < DAS28 ≤ 5.1), and high (DAS28 > 5.1) [15]. The objective disease activity of SLE was based on the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) categorized into low (5 ≤ SLEDAI ≤ 9), moderate (10 ≤ SLEDAI ≤ 14), and high (SLEDAI ≥ 15) [16]. The objective disease activity of pSS was based on the EULAR Sjogren's syndrome disease activity index (ESSDAI) for systemic organ involvement that was categorized into low (ESSDAI < 5), moderate (5 ≤ ESSDAI ≤ 13), and high (ESSDAI ≥ 14). EULAR Sjogren's syndrome patient reported index (ESSPRI) was an index to measure subjective disease activity in pSS patients that < 5 scores were defined as patient-acceptable symptom state and ≥ 5 scores were defined as unsatisfactory symptom state [17].

### 2.3 Ocular surface symptoms

The Ocular Surface Disease Index (OSDI) is the most widely used questionnaire to evaluate dry eye subjective symptoms that assess the frequency of ocular symptoms, environmental triggers, and vision-related quality of life. The total OSDI scores = [(sum of severity for answered questions) × 100] / [(total number of answered questions) × 4]. Total OSDI scores ranged from 0 to 100, 0–12 scores indicate non-DED, and ≥ 13 scores indicate DED [18].

### 2.4 Ocular surface signs

#### 2.4.1 Slit-lamp microscopy of ocular surface disorder

Participants were asked to sit down with eyes open naturally, followed by an assessment of corneal transparency, corneal epithelial defect, conjunctival hyperemia, and symblepharon of both eyes with a slit lamp microscopy.

#### 2.4.2 Tear break-up time (TBUT)

TBUT is the most commonly employed test for assessing tear film stability. Fluorescein sodium was instilled in the lower eyelid conjunctival sac using fluorescein sodium ophthalmic strips (Jingming New Technological Development Co. LTD., Tianjin, China). All participants were instructed to blink naturally three times and then to stop blinking until instructed. The interval of time was measured between the last blink and the appearance of the first random black spot (first break) in the tear film under the cobalt blue light. TBUT was defined as the average of 3 consecutive measurements (normal ≥ 10 seconds) [19].

#### 2.4.3 Schirmer I test

Schirmer test without anesthesia is a standardized test to assess reflex tear secretion. Schirmer tear test strips (Jingming New Technological Development Co. LTD., Tianjin, China) were instilled on the outer 1/3 of the lower eyelid conjunctival sac. All participants were instructed to close their eyes lightly and then removed the test strips after five minutes, and the length of tear-wetting per test strip was measured [18].

#### 2.4.4 Corneal and conjunctival fluorescent staining (FL)

Corneal and conjunctival fluorescent staining (FL) is an important means to assess the integrity of ocular surface cells. We instilled fluorescein sodium in the lower eyelid conjunctival sac and observed whether the cornea and conjunctival were stained under the cobalt blue light. Oxford staining score was used to determine the severity of ocular surface staining that a scale of 0 to 5 grade depending on the intensity of punctate staining on the corneal and conjunctival surface [20]. Normal FL is a negative grade (FL = 0), and abnormal FL is positive (FL = 1–5).

#### 2.4.5 Secretions expressed by meibomian glands

Change in secretions expressed by meibomian glands is one of the features of meibomian gland dysfunction, the main cause of evaporative dry eye disease [21]. The secretory functions of the meibomian glands are assessed by converting the upper eyelid and compressing the tarsus with the finger. The quality of expressed secretion is divided into 0–3 grades: clear liquid (grade 0), turbid liquid (grade 1), turbid and granular liquid (grade 2), and toothpaste-like consistency (grade 3) [22]. Grade 0 secretions expressed by meibomian glands are normal, and grade 1–3 is abnormal.

#### 2.4.6 Lid-parallel conjunctival folds (LIPCOF)

Lid-parallel conjunctival folds (LIPCOF) are sub-clinical folds located in the bulbar conjunctiva parallel to the lower lid margin, which may represent the initial mild stages of conjunctivochalasis and those with increased LIPCOF grades are prone to suffer from dry eye disease [23, 24]. LIPCOF was graded as 0–3 grades: no conjunctival folds (grade 0), single clear and lasting parallel fold (grade 1), two clear and lasting parallel folds (grade 2), more than two clear and lasting parallel folds (grade 3) [25]. Normal LIPCOF grade = 0, abnormal LIPCOF grade = 1–3.
2.5 Diagnosis and subtypes of DED

According to DEWS II, the diagnostic criteria of DED are (1) OSDI scores $\geq 13$; (2) TBUT $< 10$ s, or positive corneal and conjunctival FL [18]. Once the diagnosis of DED has been established, it can be further divided into three types: (1) aqueous deficient dry eye (ADDE) with decreased reflex tear secretion (TBUT $\geq 10$ s and Schirmer I test $< 10$ mm/5 min), (2) evaporative dry eye (EDE) with decreased tear film stability (TBUT $< 10$ s and Schirmer I test $\geq 10$ mm/5 min), and (3) mixed dry eye (MDE) with decreased reflex tear secretion and tear film stability (TBUT $< 10$ s and Schirmer I test $< 10$ mm/5 min) [26]. In our study, both DED and DED subtypes were analyzed using the patients’ eyes.

2.6 Conjunctival impression cytology

Conjunctival impression cytology is a non-invasive, simple, and practical technique for the assessment of DED [27]. One drop of 0.4% oxybuprocaine hydrochloride was administered to topical anesthesia, and a 10 mm diameter semicircular-shaped and sterile cellulose acetate membrane (Advantec, Tokyo, Japan) were applied for 5 seconds on the supratemporal bulbar conjunctiva of both eyes to collected samples. The specimens were then placed in 95% ethanol to fix and stained with periodic acid-Schiff (PAS) staining [28]. Three nonoverlapping regions were randomly obtained from all specimens by using an Invitrogen EVOS Auto M7000 fluorescence microscope (Thermo Fisher Scientific, Massachusetts, U.S.) with a $\times 20$ objective lens. Quantitative analysis of images was performed by ImageJ software (National Institutes of Health, Maryland, USA). The data are presented as the average goblet cell density (cells/mm$^2$) of both eyes. Evaluations of ocular surface squamous metaplasia were performed according to Nelson's classification [29], taking into account the density and morphology of conjunctival epithelial cells and goblet cells, and the nucleocytoplasmic (N/C) ratio of conjunctival epithelial cells, is the most widely used scoring currently. Grade 0 and 1 are considered to represent normal conjunctival cytology, and Grade 2 and 3 are considered to represent abnormal conjunctival cytology.

2.7 Tear collection, processing, and tear multi-cytokine measurement

Ten µl of 0.9% normal saline was dropped into the conjunctival sac of the right eye and diluted tears were collected with microcapillary fluid collectors (Seinda, Guangdong, China). Tears were immediately transferred to sterile 200 µl centrifuge tubes and stored at -80°C until use.

The concentrations of 21 cytokines in tears were determined in a 1:12.5 dilution using Milliplex Map Human High Sensitivity T-Cell Panel-Immunology Multiplex Assay (Millipore, Billerica, MA, USA) according to the manufacturer's instructions, including interleukin-1beta (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-21, IL-23, granulocyte-macrophage colony stimulating factor (GM-CSF), interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), C-X-C motif chemokine ligand 11 (CXCL11), C-X3-C motif chemokine ligand 1 (C3CL1), C-C motif chemokine ligand 3 (CCL3), C-C motif chemokine ligand 4 (CCL4), and C-C motif chemokine ligand 20 (CCL20). All the tear samples were added to the corresponding sample holes. The positive control holes consisted of cytokines in standard concentration gradients and the negative control holes consisted of an equivalent amount of solvent. Then, add microbeads and antibodies to all samples and control holes for a period of incubation and washing. The tear cytokine concentrations were measured by a MAGPIX liquid-phase chip detector (Luminex, Austin, TX, USA) with xPONENT® software (Luminex, Austin, TX, USA).

2.8 Statistical analysis

Measurement data were reported as mean ± standard deviation (SD). Numeration data were reported as count (n) and percent (%). The normality of measurement data was checked by Shapiro–Wilk test. Kruskal-Wallis H test and one-way ANOVA were used to compare measurement data among studied groups as appropriate. The Chi-square test was used for the comparison of numeration data among the studied groups. Correlation analysis was performed using Pearson's test for normal distributions or Spearman's test for nonnormal distributions. SPSS 20.0 software (IBM Corp, Armonk, NY, USA) was used for statistical analysis, with a P value < 0.05 considered statistically significant.

3. Results

3.1 Study Population

A sample size of 79 participants with ARDs was recruited, including 26 RA patients (52 eyes), 33 SLE patients (66 eyes), and 20 pSS patients (40 eyes). As shown in Table 1, RA patients were aged between 44 and 80 years (mean age 62.04 ± 10.28 years), consisting of 61.5% females and 38.5% males. SLE patients were aged between 16 and 59 years (mean age 36.06 ± 14.34 years), consisting of 90.9% females and 9.1% males. pSS patients were aged between 31 and 75 years (mean age 56.25 ± 12.14 years), consisting of 80% females and 20% males. The SLE patients are significantly younger than RA patients and pSS patients (all P value < 0.001). There were no statistically significant differences in age between patients with RA and pSS (P value > 0.05). The sex ratios of the three groups were similar, with a clear female bias. The basic information of all participants is shown in Table 1.
### Table 1
The basic information and systemic conditions of patients with RA, SLE, and pSS.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Mean age</th>
<th>Female</th>
<th>Male</th>
<th>Disease duration (years)</th>
<th>Objective disease activity score*</th>
<th>Subjective disease activity#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>RA</td>
<td>26</td>
<td>62.04±10.28</td>
<td>16</td>
<td>10</td>
<td>8.62±7.80</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>SLE</td>
<td>33</td>
<td>36.06±14.34</td>
<td>30</td>
<td>3</td>
<td>4.78±6.70</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>pSS</td>
<td>20</td>
<td>56.25±12.14</td>
<td>16</td>
<td>4</td>
<td>6.97±5.39</td>
<td>2</td>
<td>12</td>
</tr>
</tbody>
</table>

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; pSS, primary Sjögren's syndrome; Objective disease activity score* were DAS28 for RA, SLEDAI for SLE, and ESSDAI for pSS; Subjective disease activity# was ESSPRI for pSS.

#### 3.2 Evaluation of the systemic conditions

As shown in Table 1, the disease duration is 8.62±7.80 years in RA patients, 4.78±6.70 years in SLE patients, and 6.97±5.39 years in pSS patients, which SLE patients have the shortest course of disease compared with RA patients (P value < 0.01) and pSS patients (P value < 0.05). Regarding objective disease activity, 65.4% of RA patients with severe disease activity, 30.8% with moderate disease activity, and only 3.8% had low disease activity. 33.3% of SLE patients with severe disease activity, 18.2% with moderate disease activity, and 48.5% had low disease activity. 30% of pSS patients with severe disease activity, 60% with moderate disease activity, and only 10% had low disease activity. There were statistically significant differences in objective disease activity among the three groups (P value < 0.001), nearly half of SLE patients with low objective disease activity, while about 90% of RA and pSS patients with moderate and high objective disease activity (all P value < 0.01). In addition, 85% of pSS patients had a patient-acceptable symptom state, and 15% of pSS patients with an unsatisfactory symptom state.

#### 3.3 Ocular surface signs and DED incidence vary in patients with ARDs

As shown in Table 2, we compared ocular surface symptoms, ocular surface signs, and the incidence and subtype of DED among the RA group, SLE group, and pSS group. The results showed that there was no statistically significant difference in the ocular surface symptoms (OSDI scores) in patients with ARDs (P value > 0.05).
Table 2
Comparison of ocular surface symptoms, ocular surface signs, DED and its subtype, and conjunctival impression cytology among RA, SLE, and pSS groups.

<table>
<thead>
<tr>
<th></th>
<th>RA group</th>
<th>SLE group</th>
<th>pSS group</th>
<th>P1 value</th>
<th>P2 value</th>
<th>P3 value</th>
<th>P4 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case (n)</td>
<td>26</td>
<td>33</td>
<td>20</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>OSDI scores (mean ± SD)</td>
<td>16.51 ± 16.98</td>
<td>13.32 ± 18.19</td>
<td>15.52 ± 14.06</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>OSDI level</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>&lt;13 scores n (%)</td>
<td>13 (50.0%)</td>
<td>2 (69.7%)</td>
<td>11 (55.0%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>≥13 scores n (%)</td>
<td>13 (50.0%)</td>
<td>10 (30.3%)</td>
<td>9 (45.0%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Eyes (n)</td>
<td>52</td>
<td>66</td>
<td>40</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Clear cornea</td>
<td>52 (100.0%)</td>
<td>66 (100.0%)</td>
<td>40 (100.0%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Corneal epithelial defect</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Conjunctival hyperemia</td>
<td>0</td>
<td>3 (4.5%)</td>
<td>0</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Symblepharon</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TBUT (s, mean ± SD)</td>
<td>5.98 ± 3.42</td>
<td>6.77 ± 3.19</td>
<td>3.83 ± 1.88</td>
<td>&gt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TBUT level</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&lt;10 seconds n (%)</td>
<td>33 (71.7%)</td>
<td>47 (71.2%)</td>
<td>39 (97.5%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>≥10 seconds n (%)</td>
<td>13 (28.3%)</td>
<td>19 (28.8%)</td>
<td>1 (2.5%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Schirmer test (mm, mean ± SD)</td>
<td>8.42 ± 7.65</td>
<td>13.36 ± 8.95</td>
<td>7.10 ± 6.85</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FL</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Negative n (%)</td>
<td>31 (73.8%)</td>
<td>57 (89.1%)</td>
<td>25 (62.5%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Positive n (%)</td>
<td>11 (26.2%)</td>
<td>7 (10.9%)</td>
<td>15 (37.5%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Secretions expressed by meibomian glands</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Normal n (%)</td>
<td>30 (57.7%)</td>
<td>46 (69.7%)</td>
<td>18 (45.0%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Abnormal n (%)</td>
<td>22 (42.3%)</td>
<td>20 (30.3%)</td>
<td>22 (55.0%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LIPCOF grade</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Normal n (%)</td>
<td>38 (73.1%)</td>
<td>60 (90.9%)</td>
<td>24 (60.0%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Abnormal n (%)</td>
<td>14 (26.9%)</td>
<td>6 (9.1%)</td>
<td>16 (40.0%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>DED n (%)</td>
<td>18 (34.6%)</td>
<td>5 (7.6%)</td>
<td>17 (42.5%)</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Types of DED n (%)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ADDE n (%)</td>
<td>2 (11.1%)</td>
<td>0</td>
<td>0</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>EDE n (%)</td>
<td>3 (16.7%)</td>
<td>5 (100.0%)</td>
<td>6 (35.3%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MDE n (%)</td>
<td>13 (72.2%)</td>
<td>0</td>
<td>11 (64.7%)</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Average Goblet cell density (cells/mm², mean ± SD)</td>
<td>65.72 ± 58.97</td>
<td>38.33 ± 40.06</td>
<td>41.66 ± 34.74</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; pSS, primary Sjögren's syndrome; SD, standard deviation; OSDI, Ocular Surface Disease Index; TBUT, tear break-up time; FL, corneal and conjunctival fluorescent staining; LIPCOF, lid-parallel conjunctival folds; DED, dry eye disease; ADDE, aqueous deficient dry eye; EDE, evaporative dry eye; MDE, mixed dry eye; Kruskal-Wallis H test or one-way ANOVA was used to compare measurement data as appropriate and Chi-square test was used for the comparison of numeration data. P1 value: comparison between the RA and SLE group. P2 value: comparison between the RA and pSS group. P3 value: comparison between the SLE and pSS group. P4 value: comparison among the three groups.
### Table 3.4 Abnormal Nelson's grade was more commonly seen in the SLE and pSS group compared with RA group

<table>
<thead>
<tr>
<th></th>
<th>RA group</th>
<th>SLE group</th>
<th>pSS group</th>
<th>P1 value</th>
<th>P2 value</th>
<th>P3 value</th>
<th>P4 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal n (%)</td>
<td>11 (31.4%)</td>
<td>7 (12.7%)</td>
<td>4 (10.5%)</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Abnormal n (%)</td>
<td>24 (68.6%)</td>
<td>48 (87.3%)</td>
<td>34 (89.5%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; pSS, primary Sjögren's syndrome; SD, standard deviation; OSDI, Ocular Surface Disease Index; TBUT, tear break-up time; FL, corneal and conjunctival fluorescence staining; LIPCOF, lid-parallel conjunctival folds; DED, dry eye disease; ADDE, aqueous deficient dry eye; EDE, evaporative dry eye; MDE, mixed dry eye; Kruskal-Wallis H test or one-way ANOVA was used to compare measurement data as appropriate and Chi-square test was used for the comparison of numeration data. P1 value: comparison between the RA and SLE group. P2 value: comparison between the RA and pSS group. P3 value: comparison between the SLE and pSS group. P4 value: comparison among the three groups.

All patients have clear corneas and intact corneal epithelium (P value > 0.05, Table 2). None of them have symblepharon (P value > 0.05). Conjunctival hyperemia occurred in 3 eyes of SLE patients (4.5%), while not existing in RA and pSS patients. However, the difference is not statistically significant (P value > 0.05). The average TBUT values were significantly lower in the pSS group compared with the RA group (P value < 0.01) and SLE group (P value < 0.001), while no significant difference between RA and SLE groups (P value > 0.05). The average Schirmer I values were significantly increased in the SLE group compared with RA group and pSS group (all P value < 0.001), while no significant difference between RA and pSS group (all < 10 mm/5 min, P value > 0.05).

The proportion of positive FL was lower in SLE group compared with RA group (P value < 0.05) and pSS group (P value < 0.01), while no significant difference between RA and pSS group (P value > 0.05). The proportion of abnormal meibomian gland secretion grades was higher in pSS group compared with SLE group (P value < 0.05), while no significant difference between RA group and SLE group, and between RA group and pSS group (all P value > 0.05). The proportion of abnormal LIPCOF was significantly higher in RA and pSS groups compared with SLE groups (P value < 0.05 and 0.001, respectively), while no significant difference between RA and pSS group (P value > 0.05). The proportion of DED was significantly higher in RA and pSS groups compared with SLE group (all P value < 0.001), while no significant difference between RA and pSS groups (P value > 0.05).

The type of DED (ADDE, EDE, MDE) among the three groups showed significant differences (P value < 0.01, Table 2). MDE was the most common type of DED in RA and pSS groups, while five eyes of SLE patients were diagnosed as EDE.

3.4 Abnormal Nelson's grade was more commonly seen in the SLE and pSS group compared with RA group

As presented in Table 2, we compared the goblet cell density and Nelson's grade among the three groups. The average goblet cell density was 65.72 ± 58.97 cells/mm² in RA group, 38.33 ± 40.06 cells/mm² in SLE group, and 41.66 ± 34.74 cells/mm² in pSS group. SLE patients have a lower goblet cell density compared to RA patients (P value < 0.05). Meanwhile, SLE and pSS patients have a higher proportion of patients with abnormal Nelson's grade than RA patients (all P value < 0.05). Representative images of conjunctival impression cytology in ARDs patients are shown in Fig. 1.

3.5 Most pro-inflammatory tear cytokines were relatively poorly expressed in the SLE group compared with RA and pSS group

As shown in Fig. 2, we compared the tear multi-cytokines from RA, SLE, and pSS patients. Pro-inflammatory cytokines IL-1β and IL-6 play an important role during Th17 cell differentiation. IL-6 was also one of the T-helper 2 cytokines that mediate humoral immunity. IL-7 was also pro-inflammatory and involved in peripheral T-cell expansion and survival control. IL-8, CCL4, and CCL20 are chemotactic cytokines and are chiefly involved in proinflammatory response [30, 31]. The levels of IL-1β, IL-6, IL-7, IL-8, CCL4, and CCL20 were significantly different among the three groups (P value < 0.05). Relatively poor expression of IL-6, IL-8, and CCL4 was performed in the tears of patients with SLE, compared with RA and pSS patients (P value < 0.01, P value < 0.01, P value < 0.05, respectively). The levels of IL-1β were relatively poorly expressed in the tears of SLE patients compared with RA patients (P value < 0.01). The levels of IL-7 and CCL20 were relatively poorly expressed in the tears of pSS patients compared with SLE patients (P values < 0.01 and 0.05, respectively). The levels of IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17A, IL-21, IL-23, GM-CSF, IFN-γ, TNF-α, CXCL11, CX3CL1, and CCL3 were not significantly different among the three groups (P value > 0.05).

3.6 Ocular surface Nelson's grades are positively correlated with disease duration in RA patients

The correlation analysis to reveal the relationship between disease duration, disease activity scores (objective and subjective), ocular surface symptoms (OSDI score), ocular surface signs (TBUT, Schirmer I test, FL, secretions expressed by meibomian glands, and LIPCOF), and ocular
surface histopathology (goblet cell density and Nelson's grades) in ARDs patients, including RA, SLE, and pSS, were conducted while controlling for age and sex.

In all ARDs patients, no significant correlation between these indicators was found with each other. It's worth noting that only in RA patients, but not SLE or pSS patients, ocular surface Nelson's grades (the average of both eyes) are significantly positively correlated with disease duration \((r = 0.549, P < 0.05)\), indicating that although ocular surface signs do not deteriorate significantly, the degree of ocular surface squamous metaplasia increases with disease duration (Fig. 3).

4. Discussion

DED is an extremely frequent ophthalmological condition that significantly impacts vision and patients' quality of life [32]. There is a frequent association between DED and several ARDs, like pSS, SLE, and RA. Compared with dry eye patients without systemic autoimmune disease, cell injury and apoptosis on the ocular surface is more severe and treatment is trickier for dry eye patients with systemic autoimmune disease [33]. Due to the abnormal immune systems, patients with ARDs appear to have pathological autoantibodies in their bodies, which will bind to autoantigens and cause abnormal immune responses [34]. This immunological disorder can impact a variety of organs or systems, the eye can become a target organ due to its unique anatomic structure and immune privilege. Abnormal immune stimulation could influence ocular surface balance, resulting in inflammation and changes in the quantity and quality of tears, ultimately leading to the occurrence of DED [33, 35, 36].

Autoimmune-related DED is not a new topic, but there have been few studies on the comparison of dry eye pathogenesis in different ARDs. This study aims to investigate and compare the differences in ocular surface involvement and systemic conditions in ordinary 79 patients with ARDs, who visited the department of rheumatology and immunology due to rheumatic diseases, instead of visiting the department of ophthalmology due to eye symptoms, including RA, SLE, and pSS patients.

SLE is characterized by autoantibody production and immune complex deposition in the blood vessels throughout the whole body that leads to target tissue injury [37]. In our study, among all ARDs patients, SLE patients are the youngest with the shortest disease duration, most of them with low disease activity, mild ocular surface symptoms, and signs. Our study found that the prevalence of DED in SLE is not high (7.6%), and all of the types were EDE, as a previous meta-analysis showed the overall prevalence of DED in SLE patients was only 10–21% [38]. Because meibomian glands are vascular-rich tissues [39] that may become the target tissue of SLE autoimmunity, causing meibomian gland dysfunction, which is the most common cause of DED. Eyelid abnormalities may be a major cause of SLE-associated DED. Our study also found the severity of goblet cell loss and pathological ocular surface squamous metaplasia in SLE was consistent with pSS, these phenomena can even appear before the occurrence of signs and symptoms of dry eye. Our study found most pro-inflammatory cytokines, such as IL-1β, IL-6, and IL-8 were lowest in SLE patients among all ARDs patients, indicating ocular surface inflammation was relatively mild in SLE patients compared with RA and pSS patients, but still significantly worse than normal [40]. Therefore, although the incidence of DED in SLE patients is the lowest, the pathological squamous metaplasia and inflammation of the ocular surface should not be underestimated, and it can be predicted that DED will appear in the later stage. The eye needs to be closely followed up and observed, and timely pharmacological intervention for dry eyes if necessary.

In our study, among all ARDs patients, RA and pSS patients older with longer disease duration, moderate and high disease activity. About half of them have obvious symptoms and signs of DED. Most ocular surface pro-inflammatory cytokines are relatively high compared with SLE. Excessive pro-inflammatory cytokines in tears could lead to corneal damage [41], this also explains that the number of FL-positive patients in RA and pSS is greater than the number of FL-positive in SLE patients. The incidence of DED is relatively high (38.5% in RA and 45% in pSS) and MDE is the main type of dry eye. The ocular surface pathological squamous metaplasia was relatively mild in RA compared with pSS and SLE, but still more serious than normal according to our previous research. pSS is a chronic inflammatory autoimmune disease that is characterized by lymphocytic infiltration of the exocrine glands resulting in dry mouth and dry eyes [14]. Therefore, the high incidence of DED in pSS patients is natural and well-known. However, as same as previous research [42], DED is fairly common even in RA patients without SS in our study, with obvious dry eye symptoms and signs, and pathological squamous metaplasia on the ocular surface occurred. Therefore, a detailed ocular surface assessment of RA patients and timely dry eye treatment are very important to these patients, which contribute to the delayed progression of DED. Moreover, it should be emphasized that only in RA patients, the degree of ocular surface squamous metaplasia increases with disease duration.

In our study, dry eye was the most prominent manifestation of ocular surface involvement in all ARDs patients, none of them had other significant ocular surface disorders such as corneal epithelial defects, corneal opacity, and symblepharon. Our study also found that dry eye severity in all patients with ARDs was independent of disease activity, the deterioration of systemic condition does not necessarily result in subsequent aggravation of DED. Conversely, dry eye cannot be excluded, even in patients only experiencing the mild form of the disease. Due
to the special structure of the blood-ocular barrier [43], topical pharmacotherapy is more important than systemic immunosuppressant therapy in ARDs-related dry eye, such as artificial tears, autologous serum eye drops, corticosteroid eye drops, even immunosuppressant eye drops [44].

In conclusion, to think of ARDs as merely a disease of rheumatic diseases is misleading, ocular complications especially dry eye always should be taken into consideration, which can easily be overlooked in the early stages and seriously affect the quality of life of patients in the late stages. In addition to pSS, dry eye and ocular surface squamous metaplasia were also prevalent in SLE and RA that were without SS. Therefore, all ARDs patients should be encouraged to have regular ophthalmological examinations and supplemental topical drug therapy.

Declarations

Funding

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study design; Yuerong Ren, Jianing Feng, Wen Shi, and Huanmin Kang performed the data collection and analysis; Yingyi Liu wrote the draft of the manuscript; Mengbo Wu revised the manuscript; Yan He conducted a critical review of the manuscript; Jing Tian provided a diagnosis of disease and assessment of systemic condition; All authors read and approved the final manuscript before submission.

Ethics approval

The study followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee of The Second Xiangya Hospital of Central South University (Ethics code: LYF2021028).

Consent to participate

Written informed consent was obtained from all individual participants before study inclusion.

Data Availability Statement

The datasets are available from the corresponding author upon reasonable request.

References


Figures

Fig 1
Representative images of conjunctival impression cytology in ARDs patients (×200, PAS staining) (A) Nelson’s grade 0 (from a patient with RA). Goblet cells (green arrows) were abundant, plump and oval with an intensely PAS-positive cytoplasm. Epithelial cells (yellow arrows) are small and round and N/C ratio is 1/2. (B) Nelson’s grade 1 (from a patient with RA). Goblet cells (green arrows) were decreased, but still maintain a plump and oval shape with an intensely PAS-positive cytoplasm. Epithelial cells (yellow arrows) were slightly larger and N/C ratio is 1:3. (C) Nelson’s grade 2 (from a patient with SLE). Goblet cells (green arrows) were markedly reduced with blurred cell borders and less intensely PAS-positive cytoplasm. Epithelial cells (yellow arrows) were larger and N/C ratio is 1:4-1:5. (D) Nelson’s grade 3 (from a patient with pSS). Goblet cells were completely absent. Epithelial cells (yellow arrows) were significantly larger with small and pyknotic nuclei (N/C ratio 1:6). Red stars indicated binucleated epithelial cells. Scale bar = 50 μm.

Figure 2

The average levels of tear cytokines in ARDs patients by Luminex assay (mean ± standard deviation, pg/ml) *P value <0.05; **P value <0.01; ns, not significant; IL, interleukin; GM-CSF, granulocyte-macrophage colony stimulating factor; IFN-γ, interferon gamma; TNF-, tumor necrosis factor alpha; CXCL11, C-X-C motif chemokine ligand 11; CX3CL1, C-X3-C motif chemokine ligand 1; CCL, C-C motif chemokine ligand
Figure 3

Correlation analysis between Nelson's grades and disease duration in RA patients.

$r = 0.549$

$P$ value $= 0.042$