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ORIGINAL RESEARCH

Neutrophil/Lymphocyte Ratio as an Inflammatory Predictor of Dry Eye Disease: A Case-Control Study

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Background: The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been used as indicators of inflammation, however, their roles in dry eye disease (DED) patients require advanced study

Materials and Methods: A total of 104 DED cases and 97 healthy controls from January 2020 to May 2020 were enrolled in this study. The dry eye related clinical variables, including Schirmer I test, tear break-up time (TBUT), corneal fluorescein staining (CFS) and Ocular Surface Disease Index (OSDI), were detected in all the participants. Besides, the NLR and PLR pattern in DED cases were detected and their potential value as inflammatory predictors of DED were evaluated. In advanced analyses, the correlation between NLR and DED severity was examined.

Results: The NLR and PLR were 2.59 ± 1.25 and 117.48 ± 54.68 in the DED group, respectively, while they were 2.20 ± 1.24 and 115.48 ± 54.33 in the control group, respectively. The NLR was higher in the DED group (p = 0.027), however, PLR was not significantly different compared with the control group (p = 0.951). In advanced analyses, it was found that more severe TBUT, CFS, and OSDI scores were detected in the high NLR group (NLR ≥2.145, p = 0.003, 0.013, and 0.017, respectively) compared with the low NLR group (NLR <2.145).

Conclusion: The NLR value, but not PLR, of DED patients was higher than that of healthy controls. The NLR could be used as an inflammatory predictor to estimate the severity of DED.

Keywords: dry eye disease, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, case-control study, risk factor

Background

Dry eye disease (DED), one of the most common chronic ocular surface diseases, has been reported to have affected a huge number of patients with a prevalence ranging from 5 to 50%.[1] DED is regarded as a multi-factor disease and the most common pathological progresses include tear film instability, tear hyperosmolarity, ocular surface inflammation and neurosensory abnormalities.[2] Although it is recognized that inflammation is regarded as a key factor in the development of DED as well as the cause of ocular symptoms and signs,[3–5] the exact mechanism of inflammation in DED remains unclear. It has long been considered that ocular inflammatory markers could be related with the incidence of DED,[6] however, the relatively smallamount of tear samples and high detection cost have limited their clinical application in DED cases.

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Naturally, researchers have focused their attention on the research of the potential circulating biomarkers of DED. Several previous studies demonstrated that serum biomarkers, such as epidermal fatty-acid binding protein and vitamin D,[7,8] were associated with the risk of DED. Because of the advantages of low-cost and easy operation, the diagnostic and prognostic values of NLR and PLR have been widely reported in various diseases, including diabetes, cardiovascular diseases, renal disorders, autoimmune diseases and cancers.[9–16] In addition, the potential application of NLR and PLR in ocular diseases was explored, and NLR and/or PLR were reported to be a biomarker for diabetic retinopathy (DR),[17] age-related macular degeneration (AMD),[18] and retinal vein occlusion (RVO).[19,20] One previous study reported that both NLR and PLR in DED cases were higher than in healthy controls, but their correlations with DED-related indexes were insufficient.[21] The purpose of this case-control study was to detect NLR and PLR values in Chinese DED cases and to evaluate their potential predictive value in estimating the inflammatory status in DED cases.

Materials and Methods

A prospective case-control study design was used in this research. We hypothesized that higher NLR/PLR would be detected in the DED group compared with the control group. In this current study, DED cases and age/gender matched controls were included . Based on the results of a previous report,[21] it was calculated that the minimum number of included participants of each group was 20. The study was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Changshu No. 2 Hospital. Details of the study were explained to the participants and signed informed consents were obtained.

Participants

Medical information and OSDI questionnaire results were obtained from all the participants. A detailed examination was completed of ocular symptoms and signs, oral symptoms, histopathology, salivary gland involvement, and serum autoantibody. All the participants were strictly screened according to the following criteria.

Inclusion criteria of DED group: (1) Schirmer I test (without anesthesia) <5 mm/5 minutes; and/or (2) TBUT <10s; and/or (3) Positive corneal fluorescein staining; and (4) OSDI ≥13 points.

Inclusion criteria of control group: (1) Schirmer 1 test (without anesthesia) >10 mm/5 minutes; (2) TBUT >10s; (3) Corneal fluorescein staining is negative; (4) OSDI <13 points.

Exclusion criteria of both groups: (1) With a prescription history of tropical or systemic immunosuppressant and hormone medication in three months. (2) Those who underwent ocular surgery (cataract surgery, corneal surgery, conjunctiva surgery, etc.), lacrimal canal surgery and tear gland surgery in recent three months; (3) With a history of eye trauma, eyelid deformity, and corneal contact lens wearing within three months; (4) Those who suffered from glaucoma, DR, keratoconus (KC), RVO, thyroid-associated ophthalmopathy (TAO), AMD, inflammatory ocular diseases, and other ocular diseases related to NLR/PLR; (5) Those who suffered from diabetes, cardiovascular disease, acute/chronic infections, autoimmune diseases, hematological diseases and malignant tumors.

Finally, 104 DED cases and 97 healthy controls were included in this study.

Examinations and Measurements

Each patient underwent a basic ophthalmic examination including slit-lamp photograph, Schirmer I test, TBUT, and was required to complete the OSDI questionnaire. A folded Schirmer paper strip (5×35 mm) was placed at the outer third lower eyelid margin for 5 minutes and the wetting length of Schirmer paper was recorded as Schirmer scores.[22] The participants completed the Schirmer I test without anesthesia. TBUT was detected by recording the interval of time between complete blink and the appearance of the first break in the tear film.[22] CFS was tested by slit-lamp photograph under the cobalt blue filter, and the cornea was divided into 4 quadrants, each quadrant was scored separately: 0 (no staining), 1 (<30 points), 2 (>30 points but the staining is not fused), or 3 (clumps staining, filaments or ulcer), and the sum of the four quadrants would be used in data analyses. The OSDI was a 12-item questionnaire with a total score of 48 and this was transformed to 100 points for advanced analyses. Each answer was graded on a 5-point scale (0–4) based on the symptom frequency: (0) none of the time, (1) some of the time, (2) half of the time, (3) most of the time, and (4) all of the time.[23]

Peripheral venous blood samples were obtained from all the participants. A complete blood count (CBC) was performed with a blood cell counter (ABX Pentra DF120, Horiba, Japan) and the counts of white blood cells, neutrophils, platelets, and lymphocytes was recorded. Meanwhile, to

exclude Sjögren's syndrome cases, other laboratory indexes including C reactive protein (CRP), antinuclear antibody, anti-Ro (SSA) or anti-La (SSB) antibodies, and rheumatoid factor were also tested. The NLR/PLR was calculated by dividing the neutrophil/platelet counts by the lymphocyte counts.

Statistical Analyses

Statistical analyses were performed using Graphpad Prism 8.3 (GraphPad Software Inc.). Continuous data were presented as mean ± standard deviation (SD), and the difference between two groups were detected using non-paired *t* test. Categorical variables were presented as the number of cases and controls and tested by chi-square analyses. Receiver-operating characteristic (ROC) curve analyses were performed to evaluate the diagnostic value of NLR for DED and thus determine the cut-off value. The correlations between NLR and DED indicators were conducted with the Pearson method and the linear correlation was simulated with the linear regression method. $P<0.05$ was considered statistically significant.

Results

Demographic Data and DED Indicators

There were 104 DED cases (48 males and 56 females) and 97 healthy controls (46 males and 51 females) enrolled in this study between January 2020 and May 2020. The average age was 54.68 ± 14.49 years in the DED group and 52.05 ± 13.74 years in the control group and there was no significant difference in age or gender distributions between the two groups ($p = 0.600$ and $p = 0.888$, respectively). There were statistically significant differences in Schirmer I test, TBUT, CFS and OSDI scores between DED cases and controls ($p < 0.001$). The demographic data and DED indicators are presented in Table 1.

Table 1 Demographic Data and DED Indicators in DED and Control Group

	DED Group (n=104)	Control Group (n=97)	*P* value
Age	54.68±14.49	52.05±13.74	0.600
Gender Male Female	 48 56	 46 51	0.888
Schirmer I test (mm/5min)	4.38±1.57	16.30 ± 2.85	<0.001
TBUT(s)	5.41±1.58	12.82 ±1.93	<0.001
CFS	2.55±1.06	0.10±0.30	<0.001
OSDI	38.54±12.69	8.16±2.74	<0.001

Notes: Data are shown as mean ± standard deviation, except for gender, which is shown as counts. *p* value: non-paired *t* test.
Abbreviations: DED, dry eye disease; TBUT, tear break-up time; CFS, corneal fluorescein staining; OSDI, ocular surface disease index.

Table 2 Laboratory Findings in DED and Control Group

	DED Group (n=104)	Control Group (n=97)	*P* value
Neutrophil count (10^9/L)	4.43±1.27	4.08±1.30	0.057
Lymphocyte count (10^9/L)	1.95±0.71	2.18±1.03	0.070
Platelet count (10^9/L)	202.92±56.40	210.12±49.46	0.340
NLR	2.59±1.25	2.20±1.24	0.027
PLR	117.48±54.68	115.48±54.33	0.951

Notes: Data are shown as mean ± standard deviation. *p* value: non-paired *t* test.
Abbreviations: DED, dry eye disease; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Laboratory Findings

The blood parameters are presented in Table 2. In the DED group, the neutrophil, lymphocyte, platelet counts were 4.43 ± 1.27, 1.95 ± 0.71, 202.92 ± 56.40, respectively and the NLR and PLR values were 2.59 ± 1.25 and 117.48 ± 54.68, respectively. When the control group was considered, it was found that the neutrophil, lymphocyte and platelet counts were 4.08 ± 1.30, 2.18 ± 1.03 and 210.12 ± 49.46, respectively. The average of NLR was 2.20 ± 1.24, and PLR was 115.48 ± 54.33 in control group. There were no significant differences in counts of neutrophils, lymphocytes, or platelets between the two groups ($p = 0.057$, 0.070, and 0.340, respectively), as well as PLR ($p = 0.951$). There was a significant difference in NLR value between these two groups ($p = 0.027$).

Sensitivity and Specificity Analyses of NLR as an Inflammatory Predictor of DED

The ROC curve analyses demonstrated that NLR was an inflammatory biomarker of DED, and the result is shown in Figure 1. The cut-off value would be obtained when the NLR value provided highest sensitivity plus specificity value, thus NLR = 2.145 was accepted as the cut-off value. For the patients with DED, when NLR = 2.145,

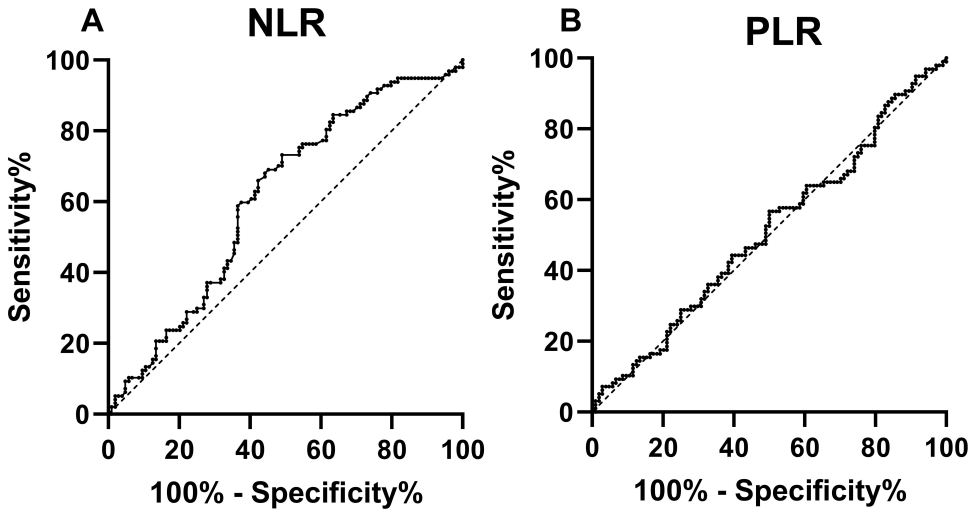

Figure 1 The diagnostic value of NLR and PLR for DED. (**A**) ROC curve for NLR with an AUC of 0.611 (95% CI, 0.533 - 0.6905, $p = 0.006$). When NLR = 2.145, sensitivity = 62.89% (52.95–71.84%) and specificity = 58.65% (49.05–67.65%). (**B**) ROC curve for PLR with an AUC of 0.508 (95% CI, 0.428– 0.589), $p = 0.839$).
Abbreviations: DED, dry eye disease; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet to lymphocyte ratio; AUC, area of under curve.

the sensitivity of NLR as a DED indicator was 62.89% (52.95–71.84%), and the specificity was 58.65% (49.05–67.65%). In the following studies, NLR = 2.145 was used to classify the high or low NLR group. However, PLR was not analyzed in this part because of a lack of significant difference between the DED cases and healthy controls.

Differences of Demographic and DED Indicators in Dry Eye Patients with High NLR and Low NLR

To further evaluate the DED indicators and demographic characteristic of DED patients in different level of NLR subgroups, the DED group was divided into high NLR group (NLR ≥2.145, n = 62) and low NLR group (NLR<2.145, n = 43). Compared with the control group, a significantly higher NLR was detected in the higher NLR group (p <0.001), however, no significant difference was detected in the low NLR group (p = 0.815). The distribution of age and gender of two high and low groups are shown in Table 3, and there were no significant differences (p = 0.148 and p = 0.165, respectively). The mean results of Schirmer I test, TBUT, OSDI questionnaire, and CFS of both subgroups are demonstrated in Table 3. The TBUT, OSDI points, and CFS scores of low NLR group were better than those of high NLR group, and the differences were statistically significant (p = 0.003, p = 0.013 and p = 0.017, respectively). However, there was no significant difference among the groups in terms of Schirmer I test.

Correlation Between NLR Value and DED Indicators of DED Cases

Correlation analyses was also performed to study the relationship between NLR value and DED indicators (Figure 2). It revealed a significant negative correlation between NLR and TBUT (p = 0.003). It was also found that there was a positive correlation between NLR and CFS (p = 0.017), as well as between NLR and OSDI (p <0.001). However, no significant correlation was found between Schirmer I test and NLR (p = 0.977).

Table 3 The Demographic Data and Dry Eye Related Characteristics in DED Patients with High (≥2.145) or Low NLR (<2.145)

	High NLR Group (n=62)	Low NLR Group (n=43)	*P* value
Age	56.41±13.30	52.23±15.85	0.148
Gender Male Female	 34 28	 17 26	0.165
Schirmer I test (mm/5min)	4.34±1.46	4.42± 1.74	0.814
TBUT(s)	5.03±1.40	5.95 ±1.67	0.003
CFS	2.80±1.06	2.26±1.12	0.013
OSDI	41.89±10.25	33.80±14.34	0.017

Notes: Data are shown as mean ± standard deviation, except for gender, which is shown as counts. p value: non-paired t test.
Abbreviations: DED, dry eye disease; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; TBUT, tear break-up time; CFS, corneal fluorescein staining; OSDI, ocular surface disease index.

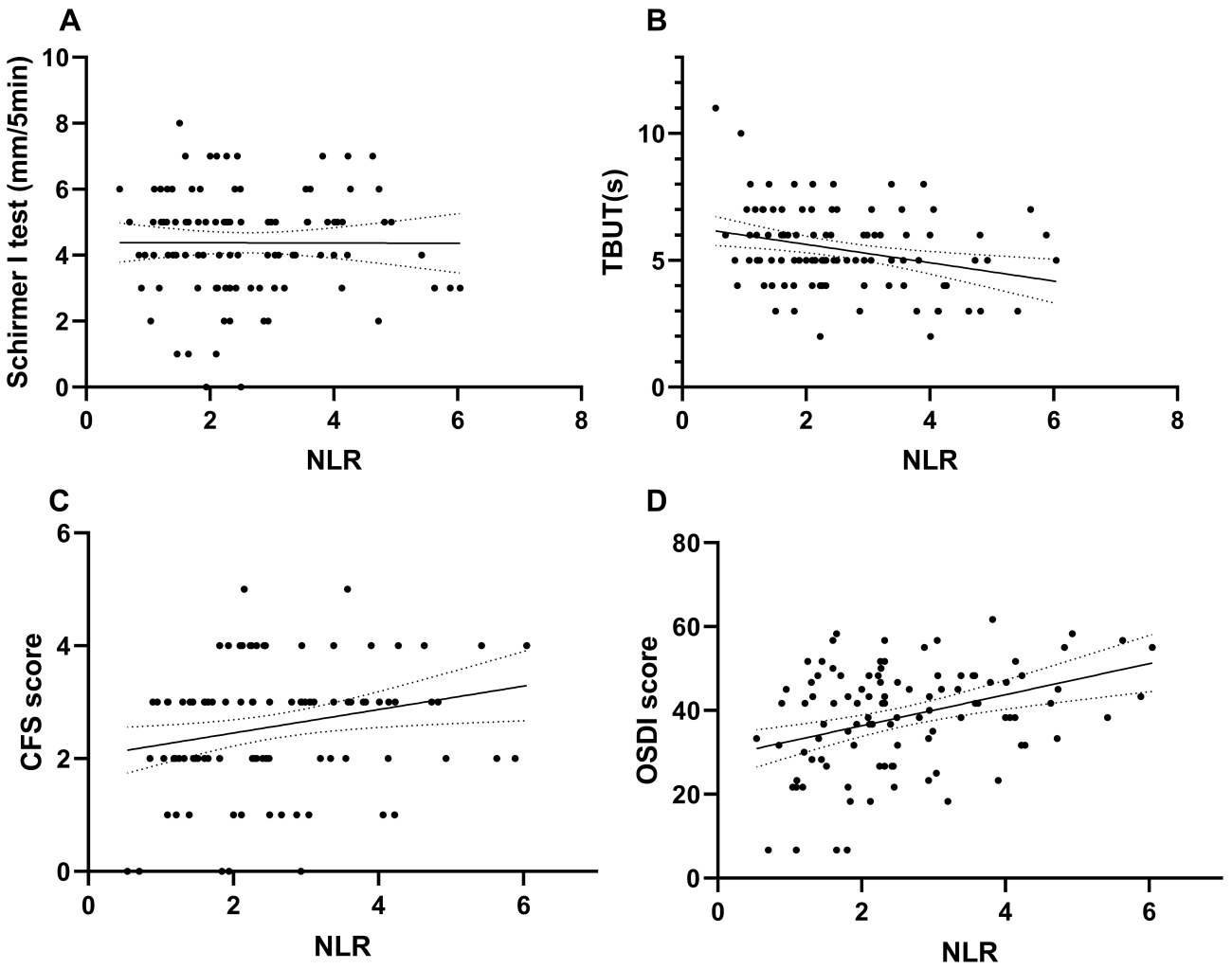

Figure 2 The association between NLR and dry eye related characteristics in dry eye disease patients. (**A**) Correlation of NLR with Schirmer I test (p = 0.977). (**B**) Correlation of NLR with TBUT (p = 0.003). (**C**) Correlation of NLR with CFS (p = 0.017). (**D**) Correlation of NLR with OSDI (p = 0.001).
Abbreviations: DED, dry eye disease; TBUT, tear break-up time; CFS, corneal fluorescein staining; OSDI, ocular surface disease index; NLR, neutrophil-to-lymphocyte ratio.

Discussion

DED is one of the most common ocular surface disorders and the burden of DED impacts the vision, life quality and work productivity considerably.[1] As inflammation is a recognized pathophysiological mechanism in the development of DED, ocular inflammatory markers were proposed to be potential indicators of DED severity.[2,24] Previous studies revealed that the levels of metalloproteinase (MMP)-9, fractalkine/CX3CL1, interleukin-1 receptor antagonist (IL-1Ra), IP-10/CXCL10, VEGF, IL-8, chemokines CCL3/MIP-1 alfa, CCL4/MIP-1 beta, CXCL9, -10, -11, and CXCR3 were all increased in DED cases.[25–30] Therefore, the concentrations of inflammatory mediators in tear and conjunctival cell could be used to estimate the severity of DED and these inflammatory markers could be regarded as potential drug targets. Even though anti-inflammatory eye drops have been used in management of severe DED, ocular inflammatory markers were not used in the diagnosis or prognosis of DED. Local markers were suitable for the research design because these reflected the disease status directly. However, it was hard for clinical researchers to use tear samples in the DED management considering the relative smaller amount of tear samples and the difficulties in tear samples collection. In addition, there were huge difficulties in implementing immunoassays of inflammatory markers and it also limited the application of these assays.

Circulating samples, including plasma, serum and whole blood, have been widely used in biomarker

development in various diseases. According to observational studies about the DED circulating samples, a low systemic level of omega fatty acids was a risk factor for DED[31,32] and vitamin D played potential protective roles for DED.[33,34] PLR and NLR, two new inflammatory indicators which derived from the major inflammatory cells, including neutrophils, platelets, and lymphocytes, showed extremely important clinical significance in recent studies. Both NLR and PLR have been widely used to determine the severity of inflammation in diabetes mellitus, cardiovascular disease, tumors, autoimmune diseases, and inflammatory diseases.[9–16] Meanwhile, the relationships between ocular diseases and NLR/PLR were also explored. Researchers have found that some ocular diseases, such as DR, AMD, RVO, KC, optic neuritis, and glaucoma, are relevant to NLR and PLR.[17–20,35–39] NLR was found to be higher in DR, AMD and KC cases compared with controls, and NLR values were related to the severity of diseases.[17,18,35] In terms of PLR, Ozgonul et al. have proved the beneficial effects of the value of PLR in making the diagnosis and predicting the prognosis of patients with POAG.[39]

In the present study, we evaluated the NLR and PLR levels as biomarkers of inflammation and detected the relationship between their values and severity of DED. Our results demonstrated that the NLR value of the DED group was significantly higher than that of the healthy control group, while the difference in PLR value between the two groups was not significant. Consistent with the studies of Celik and Sekeryapan et al., we found that the level of NLR increased in DED patients, and the mean results were 2.6 ± 1.2 (Celik), 2.8 ± 1.4 (Sekeryapan et al.), and 2.59±1.25 (this study), respectively.[21,40] It demonstrated that NLR value was relatively stable in DED patients, and could be a potential DED inflammatory indicator. However, we failed to detect a difference of PLR between DED subjects and healthy participants, which is inconsistent with the results of Celik's research.[21] The different conclusion on the relationship between PLR and DED risk might because of the ethnic and geographic differences as well as the relatively small amount of inclusion samples in both two studies. Besides, female DED patients accounted for 75% in Celik's study, while they accounted for 53.8% in our study, thus gender distribution difference may be another explanation of the difference. It is necessary to conduct further studies with a larger sample size to research the judgment value of PLR of the DED cases. Different from Celik and Sekeryapan et al., to further explore the correlation between the level of NLR and DED indicators, we grouped the DED patients into low and high NLR groups with a cut-off of 2.145 and it was interesting that the NLR was higher only in the DED cases with high NLR, but not low NLR cases. In advanced analyses, we found that the results of TBUT, CFS, and OSDI were better in the low NLR group, and the difference was statistically significant (p = 0.003, 0.013, and 0.017, respectively). However, the difference of Schirmer I test outcome between two groups was not statistically significant (p = 0.814). In a word, high NLR level (NLR≥2.145) was related to severe DED cases. With the advantages of easy availability, stability, and low cost, NLR shows great potential as an inflammatory predictor of DED. Rather than simply providing us with another potential biomarker for DED, the results in this study highlighted the important role of neutrophils in the development of DED. A recent review demonstrated that the majority of cells in corneal lesions were derived primarily from neutrophils that induced inflammatory events that led to tissue damage.[41] Neutrophil extracellular traps (NETs) formation, which was one of the pathological effect of neutrophils in DED, have attracted the researchers' attention. Inhibition of citrullinated proteins, a key compound of NETs, provided another advanced therapy for DED.[42] Meibomian glands dysfunction (MGD) was regarded to be concomitant with most DED cases, and the key role of neutrophil in the MGD incidence made us with increasingly interested in the role of neutrophils in DED incidence.[43] DED might be related to systemic inflammation or the NLR value. Based on the increase of peripheral blood NLR in DED patients, to lower NLR by systemic drugs or other adjuvant treatments may improve the clinical symptoms of DED patients. This might be one of the mechanisms of applying anti-inflammatory drugs and polyunsaturated fatty acids to treat severe DED. Of course, this hypothesis needs verification by further clinical studies.

One of the limitations of our study was that when 2.145 was used as the cut-off value, the sensitivity (62.89%, 52.95%-71.84%) and specificity (58.65%, 49.05% - 67.65%) of NLR as a DED diagnostic indicator were not high enough. It should therefore be approached carefully as an inflammatory predictor for DED. Another limitation is that we did not observe dynamic changes of DED indicators and NLR levels of DED patients to find a suitable NLR cut-off value to predict DED prognosis.

In conclusion, the value of NLR was related to incidence of DED, and higher NLR indicates worse ocular symptoms. We provided the evidence of application of NLR as an inflammatory predictor for DED.

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Disclosure

The authors report no conflicts of interest in this work.

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