Original Article

Peripheral white blood cell subtypes and the development/progression of diabetic macular edema in type 2 diabetic patients: a comparative study

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Background: Inflammation and immune dysregulation are involved in the pathogenesis of diabetic macular edema (DME). The progressive increase of neutrophils in peripheral blood can lead to the increase of the number of neutrophils in the retina, thus leading to the sustained damage of the retinal vascular system and the destruction of the blood retinal barrier (BRB); lymphocytes play a protective role in vascular diseases caused by type 2 diabetes mellitus (T2DM). The purpose of this study was to study the relationship between the changes of leukocytes and their classification in peripheral blood and the occurrence and progression of DME in patients with T2DM.

Methods: A retrospective analysis was made on 81 patients with T2DM with DME (DME group) hospitalized in our hospital from January 2019 to December 2020. According to the morphological characteristics of macular edema in optical coherence tomography (OCT), they were divided into early DME group (n=33) and late DME group (n=48); 33 patients with diabetes retinopathy (DR) but without DME matched in age and course of disease served as the control group (NO-DME group). The clinical parameters assessed included eye examination, OCT results, WBCs and subtypes, blood glucose, and glycosylated hemoglobin.

Results: Compared with NO-DME group (n=33), Neutrophils% in DME group (n=81) was higher (57.37±9.52 *vs.* 63.27±7.85; P=0.001); Monocyte% (7.63±1.77 *vs.* 6.88±1.83; P=0.047) and lymphocyte% (30.35±9.51 *vs.* 27.26±6.59; P=0.032) were decreased. The optimal model was obtained with R 4.0.5 software. With other relevant variables being the same, females had a significantly increased risk of DME (b=1.273, P=0.015), %neutrophils was significantly associated with increased risk of DME (b=0.152, P=0.0006), and %lymphocytes was significantly associated with a reduced risk of DME (b=–0.027, P=0.179). However, in the early and late DME groups, no significant differences in biological markers were found, and a high-quality model was not obtained.

Conclusions: In this preliminary study, %neutrophils is associated with increased risk of DME, whereas %lymphocytes is associated with a reduced risk of DME.

Keywords: Diabetic macular edema (DME); optical coherence tomography (OCT); %neutrophils; %lymphocytes; white blood cells (WBC)

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Introduction

Diabetic macular edema (DME) is one of the characteristic manifestations of diabetes retinopathy (DR) and an important cause of vision loss in patients with diabetes. Anti-vascular endothelial growth factor (anti-VEGF) injections are currently the first-line treatment option for improving vision in DME; however, 40% of DME patients do not respond or respond poorly to anti-VEGF therapy (1). The macula is a highly specialized area of the central retina and responsible for high-acuity vision. Chronic macular edema can lead to irreversible damage of the retinal structure, so a simple and convenient marker is urgently needed to predict the occurrence and development of DME. Early detection of the relevant risk factors of DME, close follow-up and strict blood glucose control, and timely symptomatic treatment are wise choices.

With the progress of DR, the progressive increase of systemic neutrophils can lead to an increase in the number of neutrophils in the retina, leading to sustained damage to the retinal vascular system and destruction of the blood retinal barrier (BRB), and DME is the direct consequence of BRB destruction (2). Lymphocytes play a key role in host defense against non-specific injury. A study has shown that lymphocytes play a protective role in vascular lesions caused by type 2 diabetes (3). Our previous study of patients with severe DR showed that lymphocyte percentage (%lymphocytes) was closely related to the development of DME (4).

White blood cells (WBC) and their subtypes have long been used as biomarkers (5-7) and in the field of ophthalmology, the neutrophil-to-lymphocyte ratio (NLR) is a potential marker of neovascular glaucoma (8), which is closely related to the progression of DR (9) and can predict the effectiveness of anti-VEGF therapy for DME (10). However, there is no report on the correlation between leukocytes and their classification and DME.

The purpose of this study is to determine the relevant factors of the occurrence and development of DME through the detection of cheap WBCs and their classification, and apply them to the future clinical work to minimize the visual loss caused by DME. We present the following article in accordance with the STARD reporting checklist (available at https://apm.amegroups.com/article/view/10.21037/apm-22-962/rc).

Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of Nantong First People's Hospital (No. 2022KT108). Individual consent for this retrospective analysis was waived.

Subjects

DME patients who were treated in the hospital's ophthalmology department and type 2 diabetes mellitus (T2DM) patients hospitalized in the endocrinology department from January 2019 to December 2020 were enrolled in this retrospective study. Demographic data, ophthalmic examination results, and laboratory measurements were retrieved from the medical records.

All T2DM patients were diagnosed according to the diagnostic criteria in the Chinese Diabetes Society *Guidelines for the Prevention and Treatment of Type 2 Diabetes in China* (2013) (11). Because all the DME patients were treatment-naive and the number of patients with severe/atrophic diabetic macular degeneration was small, we divided the DR patients according to the Early Treatment Diabetic Retinopathy Study (ETDRS) classification system and the optical coherence tomography (OCT)-based grading of diabetic maculopathy proposed by the European School for Advanced Studies in 2019 (12) into a No-DME group (n=33) and DME group (n=81); the DME group was further subdivided into early DME group (n=33) and late DME group (n=48).

The inclusion criteria are as follows: (I) age ≥18 years; (II) diagnosed with T2DM; and (III) T2DM with DR.

The exclusion criteria were as follows: (I) with cancer, endstage renal failure requiring dialysis, disabling stroke, coronary heart disease, tuberculosis, liver disease, blood disease, and/or rheumatoid diseases; (II) clinical evidence of any acute or chronic inflammation or infection; (III) systemic or topical use of non-steroidal anti-inflammatory drugs and/or steroids; (IV) history of prior ophthalmic surgery or intraocular inflammation or ischemia due to conditions other than DR; (V) having received laser therapy or intravitreal injections of anti-vascular growth factor drugs in both eyes due to DR or other conditions; and (VI) refractive interstitial clouding that affected fundus examination.

Examinations

Ocular examination: All patients underwent a detailed baseline ophthalmic examination including intraocular

pressure measurement (Goldman applanation tonometry), best-corrected visual acuity (BCVA) measurement, slit-lamp biomicroscopy, and mydriatic fundus examination. Patients with T2DM and DR were selected according to the ETDRS criteria.

OCT: Spectral domain OCT (SD-OCT) was performed for all DR patients, with 6-mm OCT scans centered on the fovea (12 scans, with an interval of 15°). The following items were recorded: (I) central subfoveal thickness (CST) and macular volume (MV); the normal CST range within 1 mm of the fovea is 225–315 μm, and the normal MV range is 0.17–0.26 mm; (II) intraretinal cysts (IRCs), which are circular micro-reflective spaces within the neurosensory retina and may be located in the outer nuclear layer, inner nuclear layer, or ganglion cell layer. IRCs are graded according to height (13): mild: IRC ≤380 μm; and severe: IRC >380 μm; (III) external limiting membrane and ellipsoid zone, which are the first and second highly reflective bands on the outermost layer of OCT and can be graded according to their recognizability in the fovea: grade 0: intact, grade 1: partial destruction, grade 2: total destruction; and (IV) disorganization of the inner retinal layers (Dril), which refers to poorly defined borders between the foveal ganglion cell–inner plexiform layer complex, inner nuclear layer, and the outer plexiform layer. Dril is scored as: grade 0: without Dril, grade 1: with Dril. These values and morphological manifestations were measured and calculated in a 1-mm diameter circle centered on the fovea. If macular edema was present in both eyes, data were recorded for the one with the more severe condition and clearer OCT images. OCT images were graded by two experienced ophthalmologists in a double-blind setting. If they disagreed on the grading results, a fundus specialist made the final decision.

Collection of clinical data: Serum specimens were collected from the patients after a 12-h fast and before the administration of insulin and other drugs. Complete blood count including WBC, neutrophils, monocytes, lymphocytes, and platelets was performed with a hematology analyzer (Mindray, China), and an automatic biochemical analyzer (HITACHI, Japan) was used to measure the levels of glycosylated hemoglobin (HbA1c), fasting blood glucose (FBG), blood urea nitrogen (BUN), and creatinine. The NLR, monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR) were calculated by simple division of relevant blood parameters. NLR was calculated as neutrophil count ($\times 10^9$/L)/lymphocyte count ($\times 10^9$/L), MLR monocyte count ($\times 10^9$/L)/lymphocyte count ($\times 10^9$/L), and PLR as platelet count ($\times 10^9$/L)/lymphocyte count ($\times 10^9$/L). Age, sex, duration of type 2 diabetes, insulin use, blood pressure (BP), smoking history, whether there are other complications of diabetes, history of macrovascular disease, history of hypertension and duration of hypertension were recorded for all patients.

Statistical analysis

Dataset cleaning and statistical analysis were performed using R 4.0.5. Continuous variables are presented as the mean ± standard deviation, and categorical variables are expressed as number (percentage). Two-sided permutation test was applied for the comparison of continuous variables among groups. Chi-square test was used to compare the categorical variables between groups. $P<0.05$ was considered statistically significant.

In order to obtain the optimal model for disease prediction, we first considered the complete model (Model 1) with the main variables including age, sex, duration of diabetes, BP, blood glucose, HbA1c, WBCs and subtypes, platelets, platelet distribution width (PDW), creatinine, and BUN. Only neutrophil percentage (%neutrophils) and PLR were significant in Model 1. Therefore, stepwise regression analysis was performed, in which the Akaike information criterion (AIC) was applied. Model 2 was obtained through the smallest AIC information statistic. Because Model 2 was found to be with collinearity, it was further optimized to remove collinearity, which yielded an optimal Model 3.

Results

No-DME group versus DME group

The proportion of female patients showed a significant difference between the two groups (33.3% *vs.* 55.6%, P=0.03). The baseline data in two groups are shown in *Table 1*. Age, duration of T2DM, insulin use, systolic BP (SBP), diastolic BP (DBP), FBG, HbA1c, creatinine, BUN, WBC count, neutrophils, monocytes, lymphocytes, platelets, PDW, smoking history, whether there are other complications of diabetes, history of macrovascular disease, history of hypertension and duration of hypertension showed no significant differences between the No-DME and DME groups. In contrast, the DME group had significantly higher %neutrophils (63.27%±7.85% *vs.* 57.37%±9.52%, P=0.001), significantly lower %monocytes

Table 1 Baseline characteristics of study patients with type 2 diabetes mellitus

Variable	No-DME (n=33)	DME (n=81)	P value
Female, n (%)	11 (33.3)	45 (55.6)	0.03
Duration of T2DM (years), mean ± SD	10.47±7.36	10.27±4.94	0.869
Family history of T2DM, n (%)	12 (36.4)	24 (29.6)	0.206
Insulin user, n (%)	8 (24.2)	20 (24.7)	N/A
Age (years), mean ± SD	59.18±14.65	58.06±8.72	0.613
BP, mean ± SD			
SBP (mmHg)	138.85±16.47	137.95±16.10	0.788
DBP (mmHg)	80.88±8.66	79.88±10.34	0.623
Laboratory tests, mean ± SD			
WBCs ($\times10^9$/L)	5.73±1.48	5.95±1.22	0.405
Neutrophils ($\times10^9$/L)	3.36±1.09	3.78±1.08	0.060
Monocytes ($\times10^9$/L)	0.42±0.15	0.41±0.15	0.665
Lymphocytes ($\times10^9$/L)	1.77±0.72	1.59±0.41	0.092
%Neutrophils	57.37±9.52	63.27±7.85	0.001
%Monocytes	7.63±1.77	6.88±1.83	0.047
%Lymphocytes	30.35±9.51	27.26±6.59	0.032
NLR	2.22±1.30	2.54±0.99	0.153
MLR	0.26±0.10	0.27±0.11	0.776
PLR	120.14±60.07	103.18±34.28	0.060
Platelets ($\times10^9$/L)	184.55±57.99	160.89±59.47	0.055
PDW (%)	16.36±6.86	16.28±3.55	0.933
Glucose (mmol/L)	9.02±4.80	9.03±3.03	0.985
HbA1c (%)	9.06±2.02	9.06±2.09	0.998
Urea nitrogen (mmol/L)	7.64±9.34	7.06±2.60	0.603
Creatinine (μmol/L)	70.71±38.89	70.53±40.71	0.982
Have other diabetic complications, n (%)	5 (15.2)	14 (17.3)	0.203
History of macrovascular disease, n (%)	3 (9.1)	8 (9.9)	0.650
Smoking history, n (%)			
Non-smoker	20 (60.6)	46 (56.8)	N/A
Current tobacco smoker	13 (39.4)	35 (43.2)	N/A
History of hypertension, n (%)	22 (66.7)	58 (71.6)	N/A
Duration of hypertension (year), median [range]	5 [0, 20]	7 [0, 26]	0.102

DME, diabetic macular edema; T2DM, type 2 diabetes; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PDW, platelet distribution width; HbA1c, glycosylated hemoglobin; N/A, not applicable.

Table 2 Complete Model (Model 1)

Variable	Estimate	SE	P value
Intercept	–3.559	16.878	0.833
Duration of T2DM (years)	0.028	0.066	0.669
Female	1.805	0.948	0.057
Age	0.004	0.036	0.900
SBP (mmHg)	–0.033	0.027	0.229
DBP (mmHg)	0.040	0.057	0.477
WBCs	4.048	3.398	0.234
Neutrophils ($\times10^9$/L)	–7.203	3.840	0.061
Monocytes ($\times10^9$/L)	13.071	17.474	0.454
Lymphocytes ($\times10^9$/L)	–7.089	3.877	0.068
%Neutrophils	0.472	0.188	0.012
%Monocytes	–0.909	1.036	0.379
%Lymphocytes	–0.037	0.072	0.611
NLR	0.055	2.352	0.982
MLR	0.340	27.989	0.990
PLR	–0.102	0.050	0.041
Platelets ($\times10^9$/L)	0.030	0.043	0.474
PDW (%)	–0.112	0.108	0.300
Glucose (mmol/L)	–0.110	0.083	0.187
HbA1c (%)	–0.017	0.178	0.924
Urea nitrogen (mmol/L)	0.275	0.203	0.174
Creatinine (μmol/L)	–0.009	0.017	0.612

SE, standard error; T2DM, type 2 diabetes; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PDW, platelet distribution width; HbA1c, glycosylated hemoglobin.

(6.88%±1.83% *vs.* 7.63%±1.77%, P=0.047) and significantly lower %lymphocytes (27.26%±6.59% *vs.* 30.35%±9.51%, P=0.032) than the No-DME group.

In order to obtain the optimal model of disease-related factors, we first considered the complete model (Model 1) (*Table 2*) with the main variables including age, sex, duration of diabetes, BP, FBG, HbA1c, WBCs and subtypes, platelets, PDW, creatinine, and BUN. Only %neutrophils and PLR were significant in Model 1. Model 2 was obtained through the smallest AIC information statistic (*Table 3*), but was found to be with collinearity and thus unable to predict diseases accurately, so it was further optimized to remove the collinearity, which yielded an optimal Model 3 (*Table 4*). With other predictors being the same, female sex had a significantly increased risk of DME (b=1.273, P=0.015) (*Figure 1*), %neutrophils was significantly associated with increased risk of DME (b=0.152, P=0.0006), and %lymphocytes was significantly associated with a reduced risk of DME (b=–0.027, P=0.179). The accuracy of the optimal model 3 can reach 82.46%. In 81 DME patients, the diagnostic accuracy of DME was 96.3% after using this model.

Early DME group versus late DME group

According to the OCT morphology of macular edema,

Table 3 Model 2

Variable	Estimate	SE	P value
Intercept	−14.260	9.471	0.132
Female	1.329	0.621	0.032
WBCs	4.154	2.148	0.053
Neutrophils (×10^9/L)	−6.118	2.875	0.033
Lymphocytes (×10^9/L)	−4.992	2.675	0.062
%Neutrophils	0.443	0.161	0.006
%Lymphocytes	−0.036	0.049	0.469
PLR	−0.079	0.028	0.005
Platelets (×10^9/L)	0.034	0.017	0.046
PDW (%)	−0.146	0.089	0.099

SE, standard error; WBC, white blood cell; PLR, platelet-to-lymphocyte ratio; PDW, platelet distribution width.

Table 4 Model 3

Variable	Estimate	SE	P value
Intercept	−6.439	3.171	0.042
Female	1.273	0.524	0.015
%Neutrophils	0.152	0.443	0.0006
%Lymphocytes	−0.027	0.020	0.179
Platelets (×10^9/L)	−0.014	0.005	0.005
PDW (%)	−0.111	0.069	0.108

SE, standard error; PDW, platelet distribution width.

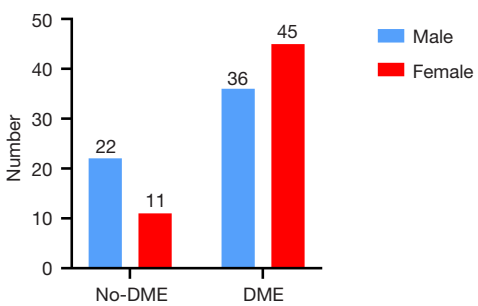

Figure 1 Sex distribution of No-DME and DME groups. DME, diabetic macular edema.

we divided the DME group into an early DME group (n=33) and a late DME group (n=48). The proportion of females was 54.5% in the early DME group and 56.3% in the late DME group (P=0.88). *Table 5* shows there were no statistically significant differences in the basic variables of these two groups. We also established the correlation model of the influencing factors of the two groups, and no significant predictor was found.

Discussion

DR refers to a series of lesions that occur in the ocular microvasculature of diabetic patients. DME occurs at any stage of DR and is also an important cause of vision loss in diabetic patients. A possible pathogenic mechanism may be early activation of retinal capillary endothelial cells that upregulates cell adhesion molecules, promoting WBC-endothelial cell adhesion and triggering the release of pro-inflammatory and vascular permeability factors, which together disrupt the BRB, causing increased vascular permeability, and ultimately leading to the development

Table 5 Basic characteristics of patients with early and late DME

Variable	Early DME (n=33)	Late DME (n=48)	P value
Female, n (%)	18 (54.5)	27 (56.3)	0.88
Duration of T2DM (years), mean ± SD	10.45±3.92	10.15±5.57	0.784
Age (years), mean ± SD	57.94±8.87	58.15±8.71	0.917
BP, mean ± SD			
SBP (mmHg)	138.03±16.44	137.90±16.03	0.971
DBP (mmHg)	77.82±9.03	81.29±11.03	0.139
Laboratory tests, mean ± SD			
WBCs ($\times10^9$/L)	5.75±.95	6.09±1.36	0.190
Neutrophils ($\times10^9$/L)	3.65±.89	3.88±1.20	0.354
Monocytes ($\times10^9$/L)	.39±.13	.42±.16	0.325
Lymphocytes ($\times10^9$/L)	1.56±.37	1.61±.44	0.617
%Neutrophils	63.22±7.88	63.30±7.90	0.965
%Monocytes	6.79±1.76	6.95±1.89	0.699
%Lymphocytes	27.49±6.67	27.11±6.61	0.801
NLR	2.48±0.85	2.58±1.09	0.653
MLR	0.26±0.09	0.28±0.12	0.431
PLR	103.63±34.57	102.86±34.45	0.922
Platelets ($\times10^9$/L)	159.79±57.81	161.65±61.17	0.891
PDW (%)	15.78±3.38	16.62±3.66	0.297
Glucose (mmol/L)	8.72±2.96	9.25±3.09	0.439
HbA1c (%)	9.34±2.22	8.87±1.99	0.323
Urea nitrogen (mmol/L)	7.36±3.02	6.85±2.28	0.419
Creatinine (μmol/L)	73.00±45.61	68.83±37.38	0.654

DME, diabetic macular edema; T2DM, type 2 diabetes; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PDW, platelet distribution width; HbA1c, glycosylated hemoglobin.

of DME (14). Intravitreal anti-VEGF drugs have replaced macular lasers as the first-line treatment option for DME in the past decade. In the Diabetic Retinopathy Clinical Research (15), approximately 50% of DME patients who received monthly intravitreal anti-VEGF injections still had retinal edema 1 year later, which progressed to irreversible visual impairment. According to the OCT-based grading of DME proposed by the European School for Advanced Studies in 2019 (12), it has four stages: early, advanced, chronic and atrophic maculopathy. Most OCT features associated with poor outcomes occur in the stages 2–4. Therefore, early detection and timely symptomatic treatment are essential to prevent irreversible vision loss. Unfortunately, the socioeconomic burden of diabetic complications such as DME is rising, and the detection of inflammatory factors in intraocular fluid and blood is not a practical strategy in early diagnosis and during follow-up due to its high cost and complex maneuver. Early detection of risk factors related to the occurrence of DME, close follow-up and strict blood glucose control should be carried out to delay or prevent its progression to the stage of irreversible macular structure and function.

High neutrophil and low lymphocyte counts in serum are closely associated with the macrovascular and microvascular

complications of diabetes (3). WBCs and subtypes are readily and inexpensively measured in routine blood tests and are considered to be novel inflammatory biomarkers that reflect both adaptive immune responses (mediated by lymphocytes) and innate immune responses (mediated by neutrophils and monocytes).

When the BRB is still intact in early DR, low levels of inflammation (parainflammation) maintain homeostasis and restore function. However, long-term stimulation of the retina by chronic inflammation can lead to a maladapted immune system and dysregulated parainflammation, resulting in circulating immune cells (including neutrophils and monocytes) and serum proteins infiltrating the retina, accelerating the development of DR (2). Obasanmi *et al.* (16) investigated the relationship between changes in circulating WBCs and the progression of DR and concluded that DR is a consequence rather than a cause of systemic immune changes in T1DM. Activation of inflammatory and immune pathways is increasingly recognized as key mediators in the development and progression of DR (17).

Intravitreal injection of sustained-release steroids can effectively control DME (e.g., reducing macular thickness and improving visual function) (18). Immune cells (e.g., macrophages, neutrophils, and lymphocytes) have been detected in the epiretinal membrane of patients with diffuse DME (19), suggesting inflammation and immune dysregulation play key roles in the pathogenesis of DME. We speculate that DME is associated with increased innate cellular immunity (especially neutrophils) and decreased adaptive cellular immunity (especially lymphocytes).

In our current study, we divided the research subjects into No-DME and DME groups according to a structural measure of the macular area (OCT) in DR patients. The percentages of neutrophils, lymphocytes, and monocytes significantly differed between these two groups. The percentage stability of each WBC subtype is superior to pure neutrophil and lymphocyte counts as it is not easily affected by various physiological and pathological conditions and can better reflect the inflammatory state and immune response. Through statistical analysis, we found the optimal model of DME related risk factors, with an accuracy rate of 82.42% When the model was applied in 81 DME patients, diagnostic accuracy reached 96.3%.

Ilhan *et al.* (20) found the NLR was higher in their DME group than in two other control groups and concluded that NLR was a highly sensitive and specific diagnostic indicator of DME. Hu *et al.* (10) followed up 91 DME patients treated with intravitreal injection of anti-VEGF drugs and found that a higher NLR before treatment was associated with worse BCVA prognosis. However, in our present study, we did not find such a correlation between NLR and DME in either the No-DME or DME group; rather, we found in our model that %neutrophils and %lymphocytes correlated with the risk of DME. Such differences may be explained by differences in study design, patient characteristics (e.g., duration of diabetes), and study populations (e.g., ethnicity, region, and economic level).

Growing evidence suggests that sex is an important factor in DR (21,22). The average age of female patients in our study was ≈58 years, and the incidence of DME was significantly higher in females than in males, with statistical significance. In a previous study of 42 patients with severe DR, we also found that the incidence of DME in women was significantly higher than that in men (4). Estrogen exerts its protective effect on the retina by activating the PI3K/Akt signaling cascade, serving as antioxidant, and exerting a vasodilatory effect (23). A Korean study of postmenopausal women showed that those not receiving estrogen replacement therapy had a higher incidence of eye diseases, including anterior polar cataracts and other retinal diseases (24). Among T2DM patients with a diabetic history of more than 10 years, age over 60 years, and a relatively intermediate economic status in China, females had a higher prevalence of DR than males (25). Thus, postmenopausal estrogen decline is closely related to the occurrence of DME, although prospective studies are needed to further investigate the causal relationship between these two factors.

In our current study, WBCs and subtypes did not significantly differ between the early and late DME groups, which might be due to the small sample size; however, it is also possible that there are other unknown inflammatory markers during the development of DME.

Our study has some limitations. First, as a retrospective study, it only analyzed data related to WBCs and subtypes; second, the sample size was small, and additional prospective and multicenter studies are needed; third, the role of the imbalanced immune system in the occurrence and development of DME needs to be confirmed by more evidence from clinical studies and basic research; and fourth, the development of DME from an early stage to a more advanced stage involves a series of changes in morphology, and the influential factors and mechanism of action were not elucidated in the present study.

In conclusion, WBCs and subtypes are representative markers of inflammation and the immune system, and their

associations with DR have been demonstrated; however, few have described their relationships with DME. As shown in our current study, with all other predictors being the same, %neutrophils was significantly associated with increased risk of DME and %lymphocytes was significantly associated with a reduced risk of DME. Females are more likely to develop DME. The model we established had a predictive accuracy of 82.46%. In addition, imbalance of the immune system plays a key role in the pathogenesis of DME, and it will become an important target for DME treatment in the future.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://apm.amegroups.com/article/view/10.21037/apm-22-962/rc

Data Sharing Statement: Available at https://apm.amegroups.com/article/view/10.21037/apm-22-962/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://apm.amegroups.com/article/view/10.21037/apm-22-962/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of Nantong First People's Hospital (No. 2022KT108). Individual consent for this retrospective analysis was waived.

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