• Clinical Research •

Combination of red blood cell distribution width and platelet-to-lymphocyte ratio for predicting severity of diabetic retinopathy

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Abstract

● **AIM:** To assess and compare the utility of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic inflammation index (SII), and red blood cell distribution width (RDW) as potential biomarkers to predict the severity of diabetic retinopathy (DR) in the United States population.

● **METHODS:** The observational study enlisted patients diagnosed with DR from the National Health and Nutrition Examination Survey (NHANES) database, spanning the period from 2005 to 2008. The severity of DR was defined according to Early Treatment for Diabetic Retinopathy Study (ETDRs). The effect of NLR, PLR, SII, and RDW on proliferative diabetic retinopathy (PDR) were explored using multivariable logistic regression analysis model. Subgroup analysis and restricted cubic splines (RCS) were conducted to assess the robustness of the correlations across subgroups and to explore nonlinear relationships between four indices and PDR. The receiver operating characteristic (ROC) analysis was employed for the purpose of assessing and evaluating the predictive efficacy of NLR, PLR, SII, and RDW in determining the severity of DR.

● **RESULTS:** After adjusting for other confounders (age, gender, race, body mass index, diabetes duration, and HbA1c) in multivariable analysis, a unit increase of PLR×0.1, SII×0.01, and RDW would raise the risk for PDR by 15.6%, 22.2%, and 33%, respectively. Particularly, there was a 2.208-fold greater risk of PDR in individuals with an elevated NLR (OR=2.208, 95%CI, 1.348-3.617, *P*<0.001). RCS analyses showed positive relationships of four indices and PDR after segmented regression based on their own turning points. The results of ROC analysis revealed that PLR+RDW [area under the curve (AUC)=0.772, 95%CI: 0.669-0.874] had the best predictive value for PDR, compared with NLR+PLR+SII (AUC=0.697, 95%CI: 0.570-0.825) or RDW alone (AUC=0.736, 95%CI: 0.646-0.826).

● **CONCLUSION:** The combination of RDW and NLR demonstrates a promising ability to predict the severity of DR across the United States population, and it could be promisingly used in clinics for monitoring the progress of DR.

● **KEYWORDS:** diabetic retinopathy; red blood cell distribution width; neutrophil-lymphocyte ratio; platelet-to-lymphocyte ratio; systemic inflammation index; National Health and Nutrition Examination Survey (NHANES) database

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INTRODUCTION

Diabetic retinopathy (DR), a significant microvascular complication of diabetic mellitus (DM), posing a substantial risk to vision, has currently been a major cause of vision impairment and blindness worldwide[1].

The observable manifestations of DR mostly consist of vascular abnormalities, which arise as a frequent consequence of toxic metabolites triggered by elevated blood glucose levels. This, in conjunction with persistent inflammation, leads to detrimental effects on the neurovascular structures of the retina during the initial phase[2]. Furthermore, the involvement of

inflammatory agents is crucial in the pathogenesis of hypoxia and ischemia in the retina. Several cytokines, including the cytokines interleukin-1 (IL-1), interleukin-1 (IL-6), interleukin-1 (IL-8), transforming growth factor β1 (TGF-β1) and tumor necrosis factor-α (TNF-α), have been associated with the development of end organ damage in individuals with DM[3-4]. Nevertheless, the actual application of these methods is constrained due to their exorbitant expense and limited accessibility within the realm of clinical practice. Additionally, the implementation of an efficient screening process may present difficulties due to the scarcity of retina specialists. Hence, there is an urgent need for the development of cost-effective and predictive methodologies to aid in the management of DR, with the aim of reducing the visual impairment associated with this condition.

The evaluation of systemic inflammation can be conducted by employing a range of biochemical or hematological markers that are routinely assessed in conventional blood tests or by calculating ratios resulting from this data[5]. In particular, previous studies have linked four indices to the morbidity of DR: complete blood cell count (CBC)-derived red blood cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic inflammation index (SII). While there have been observations made regarding the association between these ratios and the onset of DR, it ought to be noted that most of the research conducted in this area has only examined a subset of these ratios as potential biomarkers in individuals newly diagnosed with DR. Furthermore, the findings from these studies have been inconsistent. Blaslov et al. conducted a study that suggested that an elevated red cell distribution width (RDW) could potentially pose a danger for the development and advancement of DR. Conversely, researchers studied a separate study and could not find any significant association between RDW and the occurrence of DR[6-7]. Similar contradictory conclusions could also be drawn about the roles of NLR, PLR, and SII in DR[8-11].

To the best of our knowledge, the relationships among these four indices have not been studied together in the same DR population to date, let alone the comparisons of their predictive values among the proliferative DR (PDR) patients. Therefore, in this research, we intended to contrast the values of these four indices among type 2 DM (T2DM) patients with non-proliferative DR (NPDR) and with PDR. Additionally, the study also explored the potential predictive power of these index combinations in order to identify the most predictive combination model for the onset of PDR.

PARTICIPANTS AND METHODS

Ethical Approval The National Center for Health Statistics (NCHS) has developed the National Health and Nutrition Examination Survey (NHANES), which is a series of publicly available, cross-sectional surveys aiming to be representative of the population (https://www.cdc.gov/nchs/nhanes/). It is important to note that all individuals who participated in the survey provided informed consent prior to their inclusion. The NHANES protocols and testing procedures were all approved by the Institutional Review Board of the Centers for Disease Control and Prevention (Protocol #2005-06, Continuation of Protocol #2005-06 https://www.cdc.gov/nchs/nhanes/irba98.htm). As a matter of policy, our local Research Ethics Committee does not review secondary analyses of duly approved, publicly available data.

Study Population and Recruitment The data utilized in this study was obtained from the NHANES conducted between 2005 and 2008. The associations between DR and RDW, NLR, PLR, and SII were evaluated by utilizing two cycles of NHANES surveys. The exclusion criteria were: pregnant (n=5), without DM (n=4157), missing RDW, neutrophil, lymphocyte, or platelet data (n=48), and without DR (n=823). Finally, 404 individuals participated in the investigation.

Evaluation and Assessment of Diabetes According to the Standards of Medical Care in Diabetes[12], DM was briefly defined as follows: fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L), 2h oral glucose tolerance test (OGTT)≥200 mg/dL (11.1 mmol/L), hemoglobin A1c (HbA1c)≥6.5% (47.5 mmol/L), medication for an antidiabetic drug or insulin treatment, who replied “yes” to the question “Did the doctor tell you that you have diabetes”. The diabetes duration was calculated by the claimed age when interviewing minus the person’s age at the first time they were informed of having diabetes.

Ascertainment of DR As directed by the NHANES Digital Grading Protocol, a non-mydriatic retinal camera was applied to capture 45-degree non-mydriatic images of the ocular fundus among people aged 40 years or older. Based on the severity scale provided by the Early Treatment for Diabetic Retinopathy Study (ETDRs)[13], DR is distinguished by hemorrhages, microaneurysms, soft exudates, hard exudates, intraretinal microvascular abnormalities, venous marbling, new vessels elsewhere, fibrous proliferations elsewhere, secondary detachment of the retina, preretinal hemorrhage, and vitreous hemorrhage. In our research, when retinal photographs were available for both eyes, we chose the eye with the more severe retinopathy. The severity of DR was further classified into two subcategories: NPDR and PDR.

Calculation of NLR, PLR, SII and RDW The values of neutrophils, lymphocytes, platelets, and red blood cell distribution width were exacted from the hematology files. The following formulas were used: NLR=neutrophils/lymphocytes, PLR=platelets/lymphocytes, SII=platelets×neutrophils/lymphocytes.

Assessment of Covariates The selected demographic variables included age, gender, marital status, race, and body mass index (BMI). Anthropometric and laboratory covariates for this study included systolic blood pressure (SBP), diastolic blood pressure (DBP), high-density lipoprotein (HDL), glycosylated HbA1c, and serum creatinine (Scr). Self-reported daily behaviors and health status were also considered, including smoking, alcohol use, and medicine history.

BMI was divided into four groups based on the World Health Organization (WHO) criteria.

Dyslipidemia is frequently distinguished by three lipid abnormalities[14], namely: increased levels of triglycerides (≥150 mg/dL), increased levels of tiny low-density lipoprotein cholesterol (LDL-C) particles (LDL-C≥130 mg/dL), and decreased levels of high-density lipoprotein cholesterol (HDL-C<40 mg/dL for men; <50 mg/dL for women). Besides, people who were prescribed medication for dyslipidemia were also considered. Hypertension was determined as a blood pressure measurement over 140/ 90 mm Hg measured on three consecutive occasions, or related medicine history, or a professional diagnosis.

Smoking history was categorized based on self-report in the following manner: non-smokers: individuals who have never consumed 100 cigarettes during their lives; former smokers: individuals who previously smoked over 100 cigarettes but have quit smoking; current smokers: individuals who have a history of current smoking.

Alcohol consumption was categorized into three groups[15]: non-drinkers; drinkers: males<70 g/d in males, <56 g/d in females; severe drinkers: ≥70 g/d in males, ≥56 g/d in females.

Statistical Analysis The analysis of statistics was performed using Stata 16.0, R, and EmpowerStats, with a predetermined threshold of statistical significance established at $P<0.05$. To compare the disparities in baseline characteristics between the NPDR and PDR groups, continuous variables were described by mean±standard deviation (SD) when they met the normal distribution and median±quartile spacing (interquartile range) if otherwise. The categorical variable was presented as proportions and 95% confidence intervals (CI). When comparing the differences in continuous variables between patients with NPDR and PDR, if the normal distribution was satisfied, then a two-independent-samples *t*-test was chosen, otherwise using rank-sum test. The weighted Chi-square test was used in categorical variables between patients with NPDR and PDR.

A multivariate logistic regression analysis was performed to assess the relationships between PDR and NLR, PLR, SII, RDW, respectively. The odds ratio (OR) and its corresponding 95%CI were displayed for the results of the unadjusted model, minimally-adjusted and fully-adjusted. In subgroups by diabetes duration (≤15y or >15y), HbA1c (<6.5% or ≥6.5%), and gender, the correlations between the four indices and DR were separately examined. After adjusting for age, gender, race, BMI, diabetes duration, and HbA1c, logistic regression models with restricted cubic splines (RCS) were used to examine possible nonlinear relationships between four indices and PDR separately. Furthermore, the receiver operating characteristic (ROC) analysis was employed for the purpose of assessing and evaluating the predictive efficacy of NLR, PLR, SII and RDW in determining the severity of DR.

RESULTS

Characteristics of Participants A cohort of 404 patients diagnosed with DM who satisfied the specified inclusion criteria were categorized into two distinct study groups based on the severity of DR: NPDR (381 individuals) and PDR (23 individuals). Table 1 displayed the initial characteristics of the subjects.

Race, diabetic duration, hypertension presence, NLR, PLR, SII, RDW, lymphocytes, red blood cells, Scr, and HbA1c were notably distinctive between the NPDR and PDR groups ($P<0.05$). Specifically, compared with the NPDR group, PDR patients tended to have longer diabetic duration, more lymphocytes, and red blood cells, high levels of RDW distribution, Scr, and HbA1c, higher ratios of NLR, PLR, and SII, and higher proportions of non-Hispanic Black and hypertension presence. However, the proportion of non-Hispanic White was lower in PDR patients. Whereas, no difference was observed in age, gender, marital status, BMI group, drinking status, smoking status, hyperlipidemia presence, SBP, DBP, white blood cells, monocytes, neutrophils, platelets, or HDL level.

Univariate and Multivariate Logistic Regression Analysis Through conducting univariate and different multivariate logistic analyses, we confirmed that NLR, PLR×0.1, SII×0.01, and RDW were all associated with PDR, independent of other known factors ($P<0.05$; Table 2). Specifically, after several variables were adjusted (age, gender, race, BMI, diabetes duration, and HbA1c), a unit increase of PLR×0.1, SII×0.01, and RDW would raise the risk for PDR by 15.6%, 22.2%, and 33%, respectively. Particularly, from the basic model to the more intricate models, there was a 2.208-fold greater risk of PDR in individuals with an elevated NLR (OR=2.208; 95%CI, 1.348-3.617, $P<0.001$; Table 2).

Subgroup Analysis To further assess the robustness of the associations between these four indices and PDR, a subgroup analysis was conducted (Table 3). The findings from the subgroup analysis demonstrated a persistent and favorable association between the NLR, RDW and PDR occurrence across HbA1c and gender subgroups. Besides, PLR×0.1 showed a significantly higher prevalence of PDR regardless of

Table 1 Baseline of participants grouped with NPDR and with PDR

Characteristics	Total	NPDR	PDR	*P*
Age (y)	63.030±11.525	63.084±11.536	61.628±11.142	0.633
Gender, % (95%CI)				0.986
Male	53.9 (47.6-60.2)	53.9 (47.4-60.3)	53.7 (29.1-76.6)	
Female	46.1 (39.8-52.4)	46.1 (39.7-52.6)	46.3 (23.4-70.9)	
Ethnicity, % (95%CI)				0.034
Mexican American	8.6 (6.7-11.1)	8.7 (6.7-11.2)	7.6 (2.4-21.9)	
Other Hispanic	4.8 (2.6-8.4)	4.5 (2.4-8.4)	10.7 (3.9-26.2)	
Non-Hispanic White	65.2 (59.7-70.4)	66.4 (60.9-71.6)	33.6 (12.2-64.9)	
Non-Hispanic Black	19.5 (15.9-23.6)	18.4 (14.9-22.5)	48.1 (24.8-72.2)	
Other-including multi-racial	1.9 (0.9-4.1)	2 (0.9-4.3)	NA	
Marital status, % (95%CI)				0.840
Never married	4.4 (2.6-7.4)	4.3 (2.5-7.4)	6.9 (1.5-26.7)	
Married	60.9 (54.7-66.8)	61.1 (54.7-67.1)	55.1 (30.9-77.1)	
Other	34.7 (29-40.8)	34.6 (28.8-40.8)	38 (18.5-62.3)	
BMI, % (95%CI)				0.738
Underweight/normal	11.7 (8.4-16)	11.4 (8.2-15.8)	17.5 (4.7-47.7)	
Overweight	32 (26.5-38)	31.7 (26.1-37.9)	39.5 (17.5-66.8)	
Obese	56.3 (50-62.4)	56.8 (50.4-63.1)	42.9 (21.6-67.3)	
Diabetic duration, y	14.663±10.185	14.444±10.254	20.365±5.745	0.027
Drinking status, % (95%CI)				0.792
Non-drinkers	28.2 (21.7-35.7)	28.4 (21.8-36.1)	19.5 (5.5-50.3)	
Drinkers	37.6 (30.2-45.6)	37.6 (30.1-45.8)	36.3 (12.6-69.3)	
Heavy-drinkers	34.3 (27.4-41.8)	34 (27-41.7)	44.2 (17-75.3)	
Smoking status, % (95%CI)				0.146
Non-smokers	52.3 (45.9-58.6)	52.6 (46.1-59.1)	42.9 (21.5-67.3)	
Current-smokers	32.5 (26.9-38.6)	31.7 (26-37.9)	53.7 (29.5-76.3)	
Former-smokers	15.2 (11.5-20)	15.7 (11.8-20.6)	3.4 (0.5-21.3)	
Hypertension, % (95%CI)				0.032
Yes	26.4 (20.8-33)	27.3 (21.5-34.1)	2.5 (0.6-10.1)	
No	73.6 (67-79.2)	72.7 (65.9-78.5)	97.5 (89.9-99.4)	
Hyperlipidemia, % (95%CI)				0.335
Yes	20.2 (15.5-25.9)	19.8 (15-25.7)	30 (12.6-56)	
No	79.8 (74.1-84.5)	80.2 (74.3-85)	70 (44-87.4)	
SBP, mm Hg	134.301±22.838	134.090±22.862	139.777±21.482	0.346
DBP, mm Hg	66.503±14.641	66.641±14.424	62.921±19.108	0.336
NLR, %	2.337±1.184	2.291±1.117	3.532±1.974	<0.001
PLR, %	133.670±65.351	131.025±62.311	202.389±97.228	<0.001
SII, 10^9/L	580.245±331.162	571.289±321.157	812.966±472.237	0.006
White blood cells, 10^9/L	7.676±3.501	7.724±3.532	6.438±2.200	0.164
Lymphocytes, 10^9/L	2.360±2.685	2.387±2.722	1.665±1.214	0.308
Monocytes, 10^9/L	0.584±0.226	0.588±0.227	0.483±0.162	0.079
Neutrophils, 10^9/L	4.437±1.599	4.451±1.598	4.083±1.597	0.384
Platelets, 10^9/L	252.095±77.233	252.240±77.184	248.317±78.404	0.848
RDW, %	13.272±1.228	13.226±1.203	14.463±1.273	<0.001
HDL, mg/dL	49.586±13.381	49.520±13.351	51.302±14.042	0.614
Scr, mg/dL	1.105±0.753	1.057±0.602	2.360±2.059	<0.001
HbA1c, %	7.372±1.803	7.317±1.757	8.821±2.307	0.001

NPDR: Non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; CI: Confidence intervals; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index; RDW: Red blood cell distribution width; HDL: High density lipoprotein; Scr: Serum creatinine; HbA1c: Glycosylated hemoglobin A1c.

Table 2 Independent associations between NLR, PLR×0.1, SII×0.01, RDW and PDR

Parameters	Model 1		Model 2		Model 3	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
NLR	1.736 (1.172-2.571)	0.006	2.005 (1.246-3.226)	0.004	2.208 (1.348-3.617)	0.002
P trend	<0.001		<0.001		<0.001	
PLR×0.1	1.115 (1.041-1.194)	0.002	1.133 (1.051-1.221)	0.001	1.156 (1.057-1.265)	0.002
P trend	<0.001		<0.001		<0.001	
SII×0.01	1.172 (1.057-1.3)	0.003	1.198 (1.079-1.331)	0.001	1.222 (1.064-1.403)	0.005
P trend	<0.001		<0.001		<0.001	
RDW	1.717 (1.294-2.278)	<0.001	1.622 (1.133-2.321)	0.008	1.33 (1.05-1.684)	0.018
P trend	<0.001		<0.001		<0.001	

Model 1: Crude model, without any adjustments; Model 2: Adjusted for age, gender, race, BMI; Model 3: Based on model 2, further adjusted for diabetes duration, HbA1c, hypertension presence, Scr. *P* trend: *P*-value for testing the trend association, which assesses whether there is a linear trend in the risk of PDR as the values of NLR, PLR×0.1, SII×0.01 and RDW change. OR: Odds ratios; CI: Confidence intervals; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index; RDW: Red blood cell distribution width; PDR: Proliferative diabetic retinopathy; BMI: Body mass index; Scr: Serum creatinine; HbA1c: Glycosylated hemoglobin A1c.

Table 3 Subgroup analysis between NLR, PLR×0.1, SII×0.01, RDW and PDR

Parameters	NLR		PLR×0.1		SII×0.01		RDW	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Stratified by diabetes duration								
≤15y	0.868 (0.183-4.115)	0.858	0.995 (0.887-1.118)	0.939	1.08 (0.550-2.121)	0.824	1.390 (0.303-6.377)	0.671
>15y	2.888 (1.640-5.086)	<0.001	1.249 (1.107-1.409)	<0.001	1.257 (1.067-1.48)	0.006	1.682 (1.164-2.430)	0.006
Stratified by HbA1c								
<6.5%	2.366 (1.274-4.393)	0.006	1.098 (0.946-1.274)	0.218	1.106 (0.814-1.502)	0.52	1.407 (0.603-3.283)	0.429
≥6.5%	2.250 (1.251-4.045)	0.007	1.187 (1.065-1.323)	0.002	1.283 (1.062-1.55)	0.010	1.234 (0.859-1.773)	0.256
Stratified by gender								
Male	2.341 (1.562-3.507)	<0.001	1.122 (1.018-1.238)	0.021	1.256 (1.086-1.453)	0.002	1.019 (0.726-1.431)	0.911
Female	2.085 (0.887-4.904)	0.092	1.149 (1.042-1.268)	0.005	1.157 (0.958-1.398)	0.130	1.334 (0.918-1.937)	0.130

Stratified by diabetes duration, HbA1c and gender. Subgroups were all adjusted for age, gender, race, BMI, diabetes duration, hypertension presence, HbA1c, Scr. PDR: Proliferative diabetic retinopathy; OR: Odds ratios; CI: Confidence intervals; BMI: Body mass index; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index; RDW: Red blood cell distribution width; Scr: Serum creatinine; HbA1c: Glycosylated hemoglobin A1c.

gender (P<0.01). In the subgroups of longer diabetic duration (>15y), higher level of HbA1c (≥6.5%), and male gender, these four indices were all positively associated with a higher risk of PDR (P<0.05).

Furthermore, models of restricted cubic splines displayed positive relationships between four indices and PDR occurrence after adjusting for the effects of covariates (Figures 1-4). Specifically, at the inflection points of NLR, PLR, SII and PDR, each group was segmented regression. On the right side, a positive association between NLR (2.05, 1.41-3.05, P<0.001), PLR (1.63, 1.09-2.51, P=0.018), SII (1.90, 1.23-2.97, P=0.004) and PDR in the turning point were found respectively, but there was no significant difference seen on the left side (P>0.05). An inverted U-shaped association was observed between RDW and PDR, only a positive connection was noticed to the left of the RDW turning point.

To assess the prognostic significance of NLR, PLR, SII, and RDW in predicting the risk of PDR, we conducted ROC analyses. The area under the curve (AUC) was calculated for each biomarker, and it was observed that all the investigated biomarkers satisfied the criterion of a minimum AUC of 0.6, indicating their predictive capability for PDR. Subsequently, these biomarkers were further compared using ROC curves (Table 4, Figure 5). Through NLR, PLR, and SII alone didn't perform better than RDW (AUC: 0.736, 95%CI: 0.646-0.826), then a combination of RDW with NLR, PLR, and SII separately indeed resulted in increased predictive performance (P<0.05), respectively (Table 4). The P-value is used to test whether the AUC is significantly better than a random guess (AUC=0.6). P<0.05 indicates that the prediction ability of the indicators is significantly better than that of random.

In order to evaluate the efficacy of a composite of these indices, we chose the main stream combination: NLR+PLR+SII, compared with the new one: RDW+PLR. As can be seen in Figure 6, model 1 representing the traditional combination (NLR+PLR+SII) showed significantly less competitive

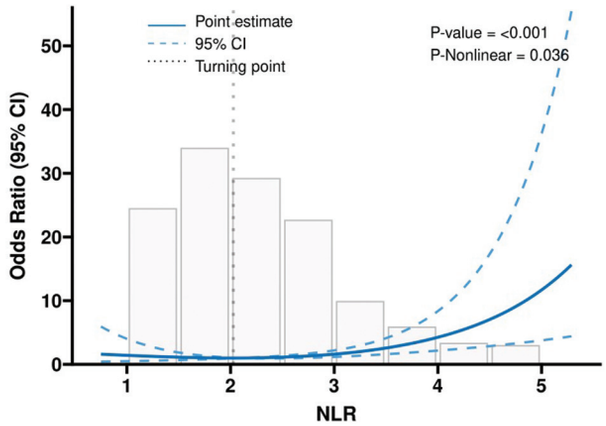

Figure 1 Relationship between NLR and PDR in restricted cubic splines PDR: Proliferative diabetic retinopathy; NLR: Neutrophil-to-lymphocyte ratio; CI: Confidence intervals.

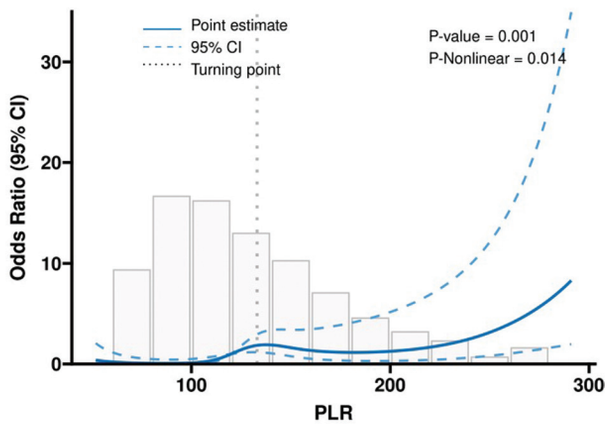

Figure 2 Relationship between PLR and PDR in restricted cubic splines PDR: Proliferative diabetic retinopathy; PLR: Platelet-to-lymphocyte ratio; CI: Confidence intervals.

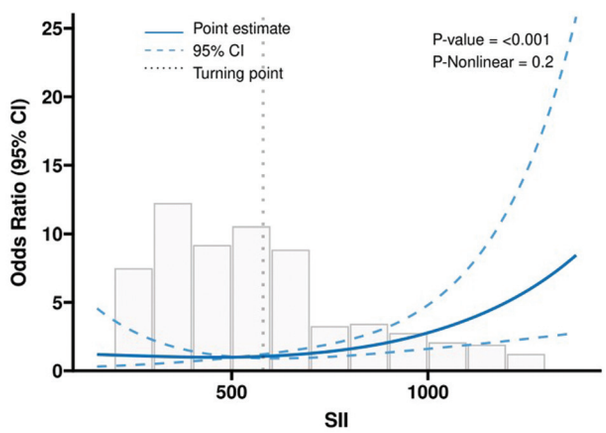

Figure 3 Relationship between SII and PDR in restricted cubic splines PDR: Proliferative diabetic retinopathy; SII: Systemic inflammation index; CI: Confidence intervals.

predictive value than model 2 (RDW+PLR; *P*=0.028; Table 5). Then, we compared these two different models for predicting PDR values after adjusting diabetes duration and HbA1c. Comparisons between these two models demonstrated in Figure 7. After adjusting these variables, these two modified models both proved a superior prediction for PDR, but no

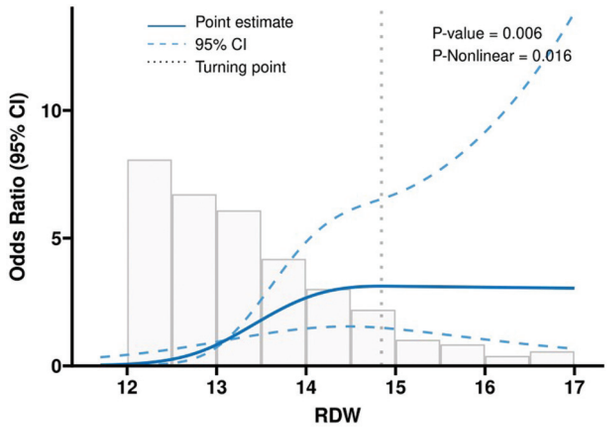

Figure 4 Relationship between RDW and PDR in restricted cubic splines PDR: Proliferative diabetic retinopathy; RDW: Red blood cell distribution width; CI: Confidence intervals.

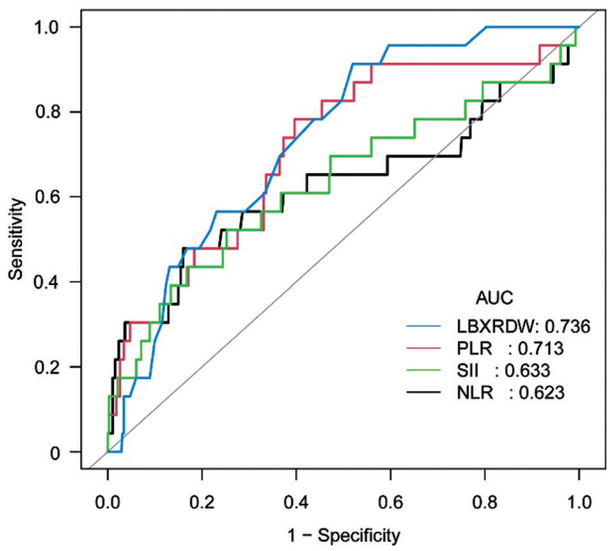

Figure 5 ROC curves ROC: Receiver operating characteristic; AUC: Area under curve; RDW: Red blood cell distribution width; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index; NLR: Neutrophil-to-lymphocyte ratio.

significant difference was observed between these two adjusted models (*P*=0.075; Table 6).

DISCUSSION

Thus far, this study was the first examination of these four indices in patients with NPDR and with PDR. Compared to NPDR patients, PDR occurrence was positively associated with high levels of RDW, NLR, PLR, and SII. As demonstrated by the multivariate logistic regression analysis, these four indices were identified as strong independent predictors of PDR, especially in subgroups (diabetic duration>15y, HbA1c≥6.5%, male). Moreover, using the RCS models, we found the correlations were distinct on the sides of their own turning points. Compared with baseline, there were significant differences among NLR, PLR, SII at the reflection points on the right side, while RDW was positively correlated with PDR on the left side. Furthermore, the AUC value for

Table 4 Specificity and sensitivity at the cut-off value predicting PDR

Parameters	PDR specificity	PDR sensitivity	Cut-off value	AUC	*P*
RDW	0.48	0.91	12.95	0.736	0.001
NLR	0.84	0.48	3.03	0.623	<0.001
PLR	0.60	0.78	129.86	0.713	<0.001
SII	0.75	0.52	688.00	0.633	<0.001
NLR+RDW	0.88	0.61	-	0.721	0.025
PLR+RDW	0.64	0.83	-	0.770	0.028
SII+RDW	0.77	0.65	-	0.734	0.011

PDR: Proliferative diabetic retinopathy; AUC: Area under curve; RDW: Red blood cell distribution width; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index.

Table 5 Comparisons between two unadjusted models predicting PDR

Models	AUC	95%CI	Specificity	Sensitivity	*P*
Model 1 (NLR+PLR+SII)	0.697	0.570-0.825	0.562	0.783	-
Model 2 (RDW+PLR)	0.772	0.669-0.874	0.638	0.826	0.028

P: Model 1 *vs* Model 2. AUC: Area under curve; CI: Confidence interval; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index; RDW: Red blood cell distribution width.

Table 6 Comparisons between two adjusted models predicting PDR

Models	AUC	95%CI	Specificity	Sensitivity	*P*
Model 1 (NLR+PLR+SII)	0.796	0.718-0.874	0.643	0.870	-
Model 2 (RDW+PLR)	0.846	0.788-0.904	0.724	0.913	0.075

Adjusted by diabetes duration and HbA1c. *P*: Model 1 *vs* Model 2. AUC: Area under curve; CI: Confidence interval; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index; RDW: Red blood cell distribution width.

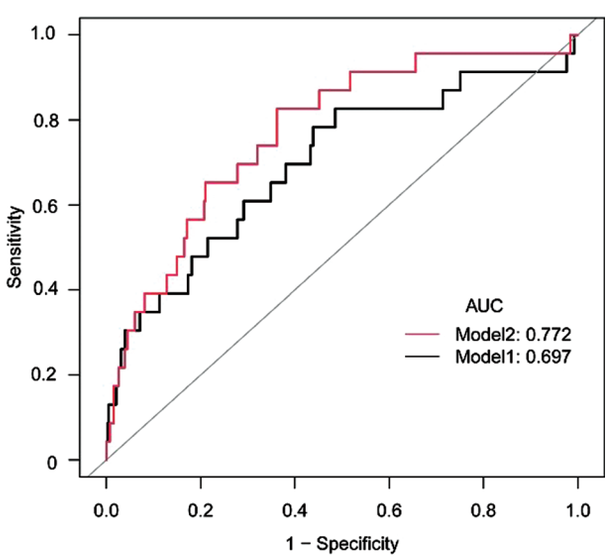

Figure 6 Comparisons ROC curves between two unadjusted models predicting PDR Model 1: NLR+PLR+SII; Model 2: RDW+PLR. ROC: Receiver operating characteristic; AUC: Area under curve; PDR: Proliferative diabetic retinopathy; RDW: Red blood cell distribution width; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index.

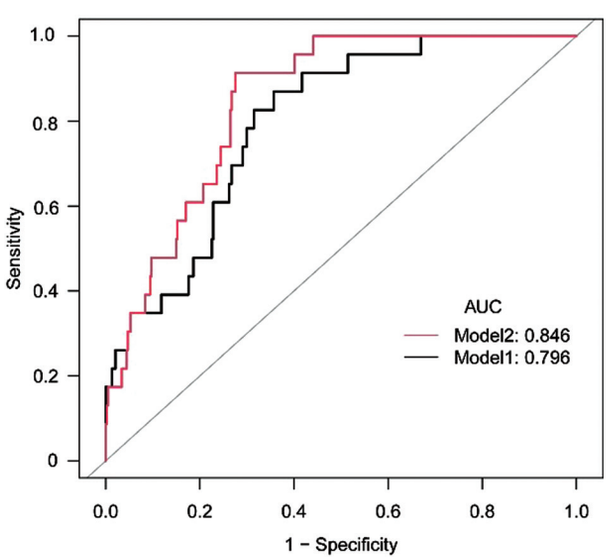

Figure 7 Comparisons ROC curves between two adjusted models predicting PDR Model 1: NLR+PLR+SII; Model 2: RDW+PLR. ROC: Receiver operating characteristic; AUC: Area under curve; PDR: Proliferative diabetic retinopathy; RDW: Red blood cell distribution width; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index.

RDW was comparatively higher when compared to NLR, PLR, and SII. Moreover, based on our current knowledge, we were also the first team to propose the new predictive combination RDW+PLR, which was then compared with the main stream model (NLR+PLR+SII) in the US population. Surprisingly, compared with the previously reported model

(NLR+PLR+SII), the novel combination (RDW+PLR) had a better predictive value with high specificity and sensitivity even after adjustments (AUC: 0.846, specificity: 72.4%, sensitivity: 91.3%).

In recent years, accumulating studies have reported that chronic inflammation contributed to the pathophysiological progression observed in DR, involving multiple mechanisms such as endothelial failure, leukocyte adhesion and infiltration, platelet activation, and neovascularization[16-18]. In an environment characterized by elevated levels of systemic inflammation, there was a positive correlation between increased RDW and the process of erythrocyte destruction and fragmentation. Additionally, this correlation was observed with endothelial activation, the formation of sludge, and the obstruction of microcapillaries[19]. The presence of leukocytes and their subgroups in peripheral blood has been found to be correlated with the occurrence of microvascular and macrovascular complications in individuals with DM[20].

Previous studies also discussed the associations regarding systemic inflammation and the progression of DR. Of all, NLR, PLR, and SII were three indices investigated mostly among diabetes patients with or without DR[8-9,21-23]. Our findings were partially similar to those of Rajendrakumar *et al*[8], high values of NLR, PLR, and SII were significantly correlated with PDR in T2DM patients instead of the incidence of DR. Contradictory to our analysis, RDW was an independent risk factor in PDR, and RDW showed more potency in reflecting the inflammatory status and prognosis in PDR compared to the markers NLR, PLR, and SII, which were not observed in Rajendrakumar *et al*[8] findings. Nevertheless, the lack of association between RDW and PDR appeared less probable given that oxidative stress, chronic inflammation, and endothelial dysfunction have also been involved in the development of PDR. An elevated RDW may serve as an indicator of reduced amounts of antioxidants in circulation, hence heightening the susceptibility of red blood cells to oxidative harm[24]. The presence of inflammation can result in altered levels of red blood cells in the bloodstream, primarily due to the impact of cytokines on the impairment of erythropoiesis[25]. In another study, RDW was shown to be significantly correlated with PDR[26], which was in line with our findings. The same conclusions proved that our results could also be found in another research[7], which indicated that RDW could be a far more promising predictive index than our estimation.

Apart from RDW, elevated PLR also had superiority for predicting PDR compared with NLR and SII in our study. This observation aligned with the conclusions made by Zeng *et al*[27] and Atlı *et al*[23]; however, it contradicts the outcomes shown by Yue *et al*[28]. They discovered that PLR did not exhibit an independent association with the incidence of DR. This observed discrepancy might perhaps be attributed to variations in the sizes of the respective samples, participant heterogeneity, and observation outcome setting.

In this study, a more robust diagnostic or predictive model has been developed, surpassing the individual use of NLR, PLR, and SII. As DR developed, RDW+PLR earned a more predictive value for PDR. Different from previous studies, our research aimed at constructing a new combination prediction model, that was easily utilized in clinics for monitoring the progress and severity of DR.

Some limitations in our study needed to be mentioned. The establishment of causality was not possible in a cross-sectional study alone; more prospective studies are necessary to address this. In addition, the data were derived from a single blood test. Serial testing offers a greater wealth of information due to the limited lifespan of blood cells. Furthermore, fundus photographs were not side-field photographs, which may have affected our misclassification bias. Considering these limitations, multi-center-controlled trials are needed to verify the clinical utility of our findings.

In conclusion, a new combination (RDW+PLR) had a more predictive value for PDR; however, prospective studies are needed to prove its reliability and clinical utility.

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