Two user-friendly models and a simple cutoff value of neutrophil to lymphocyte ratio (NLR) for early diagnosis of diabetic nephropathy in middle-elderly aged patients with type 2 diabetes: A cross-sectional study

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Abstract

Background: Neutrophil to lymphocyte ratio (NLR) has been shown to predict worse outcomes of diabetic nephropathy (DN). This cross-sectional study aimed to investigate the association of NLR and DN in middle-aged patients with type 2 diabetes, and attempt to confirm an optimized cutoff value of NLR for DN prediction.

Methods: A total of 146 patients with type 2 diabetes were retrospectively included in this study. DN was defined as urine albumin to creatinine ratio (UACR) ≥30mg/g, or effective glomerular filtration rate (eGFR) ≤ 60ml/min·1.73m². Logistic regression analysis, canonical discriminant functions and receiver operating characteristics (ROC) curve analysis were used to construct the discriminant equations and identify the cutoff value of parameters.

Results: NLR, diabetes duration, systolic blood pressure (SBP) and lipo-protein A [Lp(a)] independently predicted DN diagnosis after adjusted by multi-variables. NLR value of 2.04 had a sensitivity of 48.9% and a specificity of 80.8% in predicting DN, with area under the curve (AUC) of 0.666. When the threshold of NLR was elevated to 2.50, the specificity and sensitivity were 90.9% and 29.8%, respectively. User-friendly model 1 and model 2 were constructed using the independent risk factors mentioned above, with the AUC of 0.819 and 0.817, respectively.

Conclusions: Two models of user-friendly equations were constructed for early
prediction of DN, which could be easily calculated and stored in office computer. NLR threshold of 2.50 was recommended in clinical use to locate the patients at high risk of DN, for its high specificity and remarkable convenience.

Keywords: Albumin to creatinine ratio; cardiovascular disease; inflammation; diabetic nephropathies; diabetes mellitus, type 2
Backgrounds

Diabetic nephropathy (DN) occurs in 20-40% of patients with diabetes, it has become one of the leading causes of end-stage renal disease (ESRD)\[^1\]. Typically, chronic kidney disease (CKD) develops after diabetes duration over 10 years in type 1 diabetes, but may be present at the diagnosis of type 2 diabetes mellitus. Middle-elderly patients have a relatively high prevalence of type 2 diabetes, therefore, it’s important to concern the early diagnosis of DN in this segment of population.

Albuminuria is a predictor of future renal dysfunction\[^2\], and often used as one of the criteria for clinical diagnosis of DN. In the past, urinary albumin excretion rate (UAER) of spot or 24-hour urine was usually tested in the evaluation of albumin loss through kidney\[^3\]. However, in recent years, albumin-to-creatinine ratio (UACR) of spot urine, with improved accuracy and convenience, is becoming more widely accepted in the diagnosis and monitoring of DN. In our study, UACR of spot urine was tested for the clinical diagnosis of DN, and effective glomerular filtration rate (eGFR) was calculated as well.

Inflammation plays an important role in the development of diabetes and its complications, including both macro- and micro-vascular diseases\[^4-8]\. Neutrophil to lymphocyte ratio (NLR) significantly correlates to CRP and TNF-\(\alpha\) levels\[^9, 10]\, it is accepted to be proportional to the degree of inflammation\[^11]\. Previous studies have demonstrated that NLR associated with a worse outcome in patients with diabetes,
metabolic syndrome, cancer, stroke and cardiovascular diseases (CVD)\textsuperscript{[3, 12-16]}, etc.

Several recent studies have reported the relationship between NLR and DN. However, most of them used UAER in the diagnosis of DN\textsuperscript{[10, 17-21]}, which is less efficient than UACR. More important, an adequate threshold of NLR for the prediction and evaluation of DN remains unclear. This cross-sectional study aimed to investigate the association of NLR and DN in middle-elderly aged patients with Type 2 diabetes, and attempted to confirm an optimized cutoff value of NLR in the prediction of DN diagnosis.

\textit{Methods}

\textbf{Subjects}

Inpatients with type 2 diabetes treated in our department were included between July and December 2015. Inclusion criteria were (1) aged from 40 to 80 year-old, (2) diagnosed as type 2 diabetes over 1 year. Exclusion criteria were (1) kidney disease other than DN, use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACEI/ARB) within one month; (2) acute coronary artery disease or stroke, active inflammation, any kind of cancer; (3) eGFR < 30ml/min·1.73m\textsuperscript{2}.

Clinical, anthropometric and laboratory characteristics of the patients were extracted from medical record retrospectively. The informations of age, gender, duration of diabetes, history of smoking, past history of CVD, family history of diabetes, blood
pressure and body mass index (BMI) were included, the results of blood routine, renal
function, lipids, glycated hemoglobin A1c (HbA1c), fasting and postprandial glucose, insulin and C-peptide were also recorded.

This study was approved by the local ethical committee of the First Hospital of Qinhuangdao (201502A168), and all participants provided written informed consent.

Evaluation of DN and NLR

Fresh morning spot urine samples were obtained twice on different days for each patient, UACR (mg/g) was calculated as urine albuminuria (mg/dl) divided by urine creatinine (g/dl), and recorded as the average of the twice UACR values. Micro-albuminuria was accepted as $30 \text{mg/g} \leq \text{UACR} < 300 \text{mg/g}$, macro-albuminuria was defined as $\text{UACR} \geq 300 \text{mg/g}$.

 DN was defined as Diabetes patients combined with micro- or macro-albuminuria or eGFR $< 60 \text{ml/min} \cdot 1.73 \text{m}^2$ calculated by Cockcroft-Gault method$^{[22]}$. All included patients were assigned into DN group and non-DN group, accordingly.

Blood corpuscles were counted for each patient using the LH 780 analyzer (Beckman Coulter Inc, Miami, Florida). NLR was calculated as absolute neutrophil count ($10^9$/l) divided by lymphocyte count ($10^9$/l).
Statistics

Statistical analysis was performed using SPSS v21.0 package (IBM, Armonk, NY, USA) and MedCalc software 15.2.2 (Ostend, Belgium). Nominally distributed data were given as mean ± standard deviation (SD). Non-nominally distributed data were described as median (interquartile range, IQR). Category variables were given as number (percentage). T-test was performed to evaluate the difference between independent samples. Fisher’s exact test was carried out to analyze the distribution of category variables. Spearman’s rho test was performed to evaluate the correlation between independent variables. Binary logistic regression analysis was performed to estimate the contributions of clinical and laboratory variables to DN diagnosis. Canonical discriminant functions and Wilk’s lambda test were used to construct the discriminant equation and calculate the predicted probabilities. Receiver operating characteristics (ROC) curve analysis was used to determine the cutoff value of DN diagnosis, the comparisons of AUC were also performed. \( P<0.05 \) was considered significant.

Results

Demographic, clinical and laboratory characteristics of patients

A total of 146 Type 2 diabetes patients were included in this study, comprised 47 patients with DN and 99 patients without DN. There was no significant difference of age and gender distribution between the 2 groups. As compared to non-DN group, DN patients
had longer Diabetes duration, higher BMI and elevated systolic blood pressure (SBP) levels, while no significant difference was shown of family history of diabetes, past history of CVD and smoking between the 2 groups (table 1). 

In the view of glucose metabolism, DN patients presented with higher HbA1c and lower C-peptide levels than those of non-DN patients, meanwhile, no differences were shown of the plasma glucose and insulin concentrations between the 2 groups, irrespective of fasting or postprandial samples (table 1). As to lipids metabolism, only Lp(a) was demonstrated to be elevated in DN patients as compared with those of non-DN patients, none of the other parameters, including low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein (HDL-c), apo-lipoprotein A (apo-A) and apo-lipoprotein B (apo-B), showed significant difference between the 2 groups (table 1). Renal function tests showed a significant increase of uric acid and creatinine in DN patients as compared to those of non-DN patients, while urea nitrogen levels showed no difference between the 2 groups (table 1).

In the complete blood counts analysis, white blood cell (WBC) and neutrophil counts were demonstrated to be higher in patients with DN than those without DN, while there was no significant difference of lymphocyte, platelet and monocyte counts between the 2 groups (table 1). Furthermore, when comparing the calculated NLR and PLR between the 2 groups, DN patients had a higher NLR level than those of non-DN patients, however, PLR showed no difference between the 2 groups (table 1).
Analysis of potential confounding factors of NLR

Since NLR could be easily affected by many other factors, we analyzed the potential confounders included in this study. As a result, weight ($r = 0.164, P = 0.049$), HbA1c ($r = 0.219, P = 0.010$), cholesterol ($r = -0.189, P = 0.023$), HDL-c ($r = -0.187, P = 0.025$) and apo-A ($r = -0.219, P = 0.013$) significantly correlated to NLR values, while no correlations were revealed between NLR and age, blood pressure, duration of diabetes, BMI, other parameters of glucose and lipids metabolism except for those mentioned above, as well as the variables of renal function ($P > 0.05$). Meanwhile, no significant difference was shown between different groups of gender, with or without past history of CVD, family history of diabetes or smoking history ($P > 0.05$, Supplemental table 1).

Predicting ability of NLR for early diagnosis of DN

To investigate the association of NLR and the 2 main elements (UACR and eGFR) for early diagnosis of DN, Spearman’s rho test was performed. As a result, there was a positive correlation of NLR and UACR levels ($r = 0.227, P = 0.006$), while no correlation was shown between NLR and the calculated eGFR ($r = 0.089, P = 0.288$).

Binary logistic regression analysis was carried out to determine the predictors of DN. After adjusted by the factors of age, gender, BMI, smoking history, HbA1c and UA, duration of diabetes, NLR, SBP and Lp(a) were shown to predict the DN diagnosis independently (table 2). This regression model had an overall prediction accuracy of
81.8%, interestingly, the sensitivity and specificity were 62.2% and 90.5%, respectively. ROC curve analysis indicated that NLR value of 2.04 had a sensitivity of 48.9% and a specificity of 80.8% in predicting DN, with area under the curve (AUC) of 0.666 [$P = 0.001$, 95%CI: 0.584-0.742, figure 1(a)]. Notably, when the threshold of NLR was elevated to 2.50, the specificity for diagnosing DN was 91.9%, even though the sensitivity decreased to 29.8% at the same point.

Furthermore, we constructed 2 models using the equations of canonical discriminant functions. Model 1 included the 4 independent risk factors of diabetes duration, NLR, Lp(a) and SBP. The factor of Lp(a) was excluded in model 2. The equations were listed as below, and the statistical characteristics were shown in supplemental table 2.

**Model 1:**

$$D = -6.230 + 0.091X_1 + 0.612X_2 + 0.026X_3 + 0.023X_4$$

**Model 2:**

$$D = -6.718 + 0.098X_1 + 0.826X_2 + 0.030X_3$$

[X₁ = Diabetes duration (year), X₂ = NLR, X₃ = SBP (mmHg), X₄ = Lp(a) (mg/dl)]

Accordingly, the ROC curve analysis was performed using the predicted probabilities
obtained from the discriminant analysis. In model 1, the predicted probability of 0.29 showed a sensitivity of 76.9% and a specificity of 75.3%, with the AUC of 0.819 [P < 0.001, 95%CI: 0.741-0.881, figure 1(b)]. In model 2, the predicted probability of 0.47 had a sensitivity of 74.4% and a specificity of 78.7%, with the AUC of 0.817 [P < 0.001, 95%CI: 0.739-0.879, figure 1(c)]. Which meant, when the calculated D > 0.29 and 0.47, the individual patient could be classified as a DN patient according to model 1 and model 2, respectively. When comparing different ROC curves, AUC of Model 1 and Model 2 showed significant improvements compared to that of NLR (P < 0.05), while there was no significant difference of AUC between model 1 and model 2 (table 3).

Discussion

With rapidly increased prevalence, diabetes and its chronic complications have drawn more concerns of people[23]. DN is a common complication of diabetes, it usually starts from glomerular damage indicated by micro-albuminuria[2]. Recently, American Diabetes Association (ADA) recommended to cancel the statement of “micro-” or “macro-albuminuria” in the consideration of the continuousness of disease. On the other hand, tubulo-interstitial injury is also responsible for increased protein filtration and loss of renal function[23]. Therefore, in this study, we defined the DN as UACR > 30mg/g or eGFR < 60ml/min·1.73m² or both, but those with eGFR < 30ml/min·1.73m² was excluded for the purpose of early diagnosis.

Inflammations played an important role in the development and progression of DN. NLR
could be used as an marker of systemic inflammation. As previously reported, diabetic patients were prone to have higher NLR levels than those of healthy volunteers [1.93 IQR (1.43, 2.68) vs 1.61 IQR (1.31, 2.16), \( P < 0.001 \)]\(^{[23]}\). A longitudinal study reported that, in diabetic patients, the lowest NLR tertile included fewer patients (2.7%) with worsening renal function as compared to the middle and the highest NLR tertiles after 3-year follow-up\(^{[17]}\). Based on the results of our study, NLR positively correlated to UACR in patients with type 2 diabetes, DN patients had higher NLR levels than those without DN (2.21±0.105 vs 1.67±0.71, \( P=0.002 \)), which corresponded to the results of several previous studies\(^{[10, 18, 19, 21, 24, 25]}\). Some of the studies didn’t provide the values of NLR in DN and non-DN groups\(^{[3, 18]}\). Other clinical researches gave the mean values of NLR ranged from 1.56 to 2.20, 1.96 to 2.60 and 2.03 to 3.60 in diabetic patients with normo-, micro- and macro-alubuminuria, respectively\(^{[10, 19, 21, 26]}\). Apparently, there were overlaps among different groups, which further supported DN to be a kind of continuously progressed disease.

Moreover, NLR independently predicted DN diagnosis after adjusted by multi-variables, based on the results of both our study and previous studies\(^{[18, 19, 27]}\), however, an adequate cutoff value of NLR had never been clearly elucidated before. A recently published study of meta-analysis had focused on this point of view, but the results only provided a standardized mean difference (SMD) value of NLR (SMD = 0.63, 95%CI: 0.43-0.83, \( P < 0.001 \))\(^{[28]}\), which could scarcely help clinical doctors to make decisions. ROC curve analysis was preformed by some of the studies. Akbas et al\(^{[23]}\) reported a NLR cutoff value of 1.7 in predicting albuminuria of diabetes, with a sensitivity of 61.8% and a
specificity of 70.5% (AUC 0.660, 95%CI 0.590-0.725, P = 0.0001), which was similar to the results of our study (ROC analysis: NLR cutoff value of 2.04, sensitivity of 48.9%, specificity of 80.8%, AUC of 0.666). However, these results also revealed that NLR, as a single predictor, had a moderate efficiency in predicting DN, let alone the fact that NLR value of 1.7 and 2.04 couldn’t separate DN from diabetic patients without DN (with mean NLR value from 1.56 to 2.20\cite{10,19,21,26}) totally, a more efficient but simple model was necessary.

According to the results of the logistic regression analysis, we selected 4 independent factors [duration of diabetes, NLR, SBP and Lp(a)], as variables for the construction of discriminant equation in predicting DN, therefore, model 1 was established. Using this model, the AUC was elevated to 0.819 with a sensitivity of 76.9% and a specificity of 75.3%. However, Lp(a) might not be easily acquired in some cases, therefore, model 2 was established excluding the factor Lp(a), with only a slight loss of AUC (0.817) compared to model 1 (0.819), but an obvious improvement of sensitivity (74.4%) compared to NLR alone (48.9%).

In clinical practice, invasive kidney biopsy was rarely operated in the diagnosis of DN, repeated blood drawing and expensive urine test were usually opposed too, the medical expense was another concern. Routine blood test remained more easier to be accepted by most of the patients. In this situation, when the lab data of creatine and UACR were difficult to obtain, we recommended clinical practitioners to use model 1 or model 2 for quick glimpses at DN prediction, since they were user-friendly and easily to be
calculated, with an acceptable prediction accuracy, and might be easily stored as an excel
document in the office computer. Especially the model 2, which need only 2 additional
clinical parameters of SBP and diabetes duration, except for the NLR value.

In addition, according to our study, NLR value of 2.50 with a high specificity (91.9\%) is
another user-friendly tool to identify the patients at high risk of DN, even if it had a fairly
low sensitivity of 29.8\%. It would be useful to alert doctors paying attention to the high
probability of DN, and persuade this part of patients to accept necessary blood or urine
tests further. For another important reason, as clinical doctors, we deeply understood that
there were too many data need to be remember in daily work, and too many trivial things
to distract our attentions, so a very simple but efficient tool would be more fascinated.

Above all, even the discriminant analysis provided a method of better overall accuracy in
predicting DN, we still strongly recommended the NLR threshold of 2.50 as the clinical
use to locate the patients at high risk of DN, irrespective of the low sensitivity. Because
it’s pretty simple and easy to remember, with this tool alone, we might specifically
determine around 1/3 patients with DN only, but without it, we might lose all of them.

With regards to the inner relationship between NLR and DN, in a hypothesis, excessive
nutrients could activate the pancreatic islets, liver, adipose and muscle tissues to release
chemokine and cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1)
and interleukin-8 (IL-8, also called CXCL-8), to recruit immune cells and promote
inflammation\[5\]. In particular, IL-8 was the specific chemokine of neutrophils, its binding
with CXCR-1 and CXCR-2\[29\] could induce chemotaxis, migration, aggregation and
activation of neutrophils, which participated in tissue injury and repair. A previous animal study had shown that IL-8 antagonist could reduce renal volume and UACR level, improve creatinine clearance in male mice with diabetes, attenuate high glucose induced mesangial injury, and inhibit JAK2/STAT3 and ERK1/2 pathways at molecular level\textsuperscript{[30]}. In vivo study also indicated an elevated level of urinary CXCL-8 of DN patients as compared to those of control\textsuperscript{[23]}.

Our study had several limitations. First, this was a retrospective study with relatively small sample size, leading to the restricted generalization of the results, validation of some large-scale studies remained necessary. Secondly, a validation cohort is absent, even though the results of our study were quite close to those of previously reported. We also look forward other researchers to verifying our results using their reported data set. Third, the factors impact on NLR were not completely expelled, like the most frequent complications of hypertension and dyslipidemia. Of course, we’ve tried to exclude their influence by performing multi-variable regression analysis, and they were truly existing in real world, also might share a common mechanism with diabetes and DN.

Conclusions

Two models of user-friendly equations were constructed for early prediction of DN, which could be easily calculated and stored in office computer. NLR threshold of 2.50 was strongly recommended in clinical use to identify the patients at high risk of DN, for its high specificity and remarkable convenience.
List of Abbreviations

NLR, neutrophil to lymphocyte ratio;
DN, diabetic nephropathy;
eGFR, effective glomerular filtration rate;
SBP, systolic blood pressure;
Lp(a), lipoprotein a;
AUC, area under the curve.

Declarations

Ethics approval and consent to participate
This study was approved by the Committee of Ethics in Research of the First Hospital of Qinhuangdao (201502A168). All participants provided their written informed consent to participate in this study, after approval of the process by the ethics committee.

Consent for publication
Not applicable.

Availability of data and materials
The dataset used and analysed during the current study are available from the corresponding author on reasonable request.
Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

YMT collected, analyzed data and drafted the manuscript. TL, HYG and RW collected clinical data. CMM made substantial contributions to acquisition of data. RW and FZY revised it critically for important intellectual content.

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Figures

Figure 1. (a) Receiver operating characteristics (ROC) curve analysis indicated that NLR value of 2.04 had a sensitivity of 48.9% and a specificity of 80.8% in predicting DN, with area under the curve (AUC) of 0.666 ($P = 0.001$, 95%CI: 0.584-0.742). (b) Model 1 had a sensitivity of 76.9% and a specificity of 75.3%, with the AUC of 0.819 ($P < 0.001$, 95%CI: 0.741-0.881), using the predicted probabilities of discriminant analysis. (c) Model 2 had a sensitivity of 74.4% and a specificity of 78.7%, with the AUC of 0.817 ($P < 0.001$, 95%CI: 0.739-0.879), using the predicted probabilities of discriminant analysis. (d) Comparisons of AUC for different ROC curves in predicting DN diagnosis.
1 **Tables**

2 **Table 1.** Demographic, clinical and laboratory characteristics of the patients in DN and non-DN groups.

|                          | DN group       | non-DN group  | *P*-value |
|--------------------------|----------------|---------------|-----------|
| gender (n of male/female)| 26/21          | 50/49         | 0.600     |
| age (year)               | 61.21±9.99     | 58.25±8.94    | 0.074     |
| diabetes duration (year) | 13(8,18)       | 9(6,13)       | 0.007*    |
| family history of Diabetes (% of yes) | 55.3 | 44.4  | 0.287     |
| past history of CVD (% of yes) | 29.8 | 27.3  | 0.844     |
| Smoking (% of yes)       | 38.3           | 27.3          | 0.186     |
| weight (kg)              | 77.11±17.17    | 72.43±11.70   | 0.057     |
| body mass index (kg/m2)  | 27.90±5.85     | 26.04±3.25    | 0.049*    |
| SBP (mmHg)               | 142.26±16.43   | 143.19±15.81  | 0.005*    |
| DBP (mmHg)               | 82.00±13.04    | 83.03±9.63    | 0.631     |
| HbA1c (%)                | 9.29±1.54      | 8.56±1.90     | 0.027*    |
| FBG (mmol/l)             | 9.41±3.51      | 9.40±3.23     | 0.982     |
| PBG2h (mmol/l)           | 14.59±4.07     | 15.34±5.19    | 0.436     |
| FBI (uIU/ml)             | 10.12±7.13     | 10.01±4.01    | 0.947     |
| PBI2h (uIU/ml)           | 29.65±16.91    | 33.03±32.69   | 0.725     |
| FBCP (ng/ml)             | 1.07±0.59      | 1.45±0.69     | 0.012*    |
| PBCP2h (ng/ml)           | 2.43±1.46      | 3.36±2.12     | 0.034*    |
| triglyceride (mmol/l)    | 2.17±2.05      | 2.02±1.35     | 0.588     |
| cholesterol (mmol/l)     | 5.22±2.17      | 4.78±1.01     | 0.188     |
| LDL-c (mmol/l)           | 3.29±1.41      | 3.12±0.85     | 0.437     |
| HDL-c (mmol/l)           | 1.25±0.48      | 1.10±0.33     | 0.064     |
| Apo-A (g/l)              | 1.09±0.23      | 1.18±1.07     | 0.589     |
| Apo-B (g/l)              | 1.01±0.42      | 0.94±0.21     | 0.321     |
| lipoprotein a (mg/dl)    | 31.25±25.67    | 18.83±14.14   | 0.007*    |
| Test                        | Normal 1 | Normal 2 | p-value |
|-----------------------------|----------|----------|---------|
| Uric acid (μmol/L)          | 331.22±96.10 | 298.59±75.65 | 0.029*  |
| Urea nitrogen (mmol/l)      | 6.38±1.84   | 5.84±1.62 | 0.075   |
| Creatinine (μmol/L)         | 69.39±25.10 | 56.21±13.30 | 0.001*  |
| WBC count (10*9/l)          | 7.09±1.65   | 6.44±1.72 | 0.033*  |
| Neutrophil count (10*9/l)   | 4.24±1.48   | 3.56±1.32 | 0.006*  |
| Lymphocyte count (10*9/l)   | 2.09±0.62   | 2.22±0.72 | 0.276   |
| Platelet count (10*9/l)     | 245.52±81.76 | 241.41±238.36 | 0.910   |
| Monocyte count (10*9/l)     | 0.54±0.14   | 0.50±0.21 | 0.256   |
| NLR                         | 2.21±1.05   | 1.67±0.71 | 0.002*  |
| PLR                         | 126.77±56.75 | 139.77±137.61 | 0.738   |

*P < 0.05.

- DN, diabetic nephropathy; CVD, cardiovascular disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin A1c; FBG, fast blood glucose; PBG2h, postprandial blood pressure of 2 hour; FBI, fast blood insulin; PBI2h, postprandial blood insulin of 2 hour; FBCP, fast blood c-peptide; PBCP2h, postprandial blood c-peptide of 2 hour; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; Apo-A, apo-lipoprotein A; Apo-B, apo-lipoprotein B; WBC, white blood cell; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.
Table 2. Binary logistic regression analysis of DN with adjustments of multi-variables.

| Variables           | Wald  | P-value  | OR ( 95%CI )          |
|---------------------|-------|----------|-----------------------|
| Diabetes duration   | 12.436| <0.001*  | 1.164 ( 1.070-1.266 ) |
| NLR                 | 6.288 | 0.012*   | 2.159 ( 1.183-3.939 ) |
| SBP                 | 5.189 | 0.023*   | 1.039 ( 1.005-1.073 ) |
| Lp(a)               | 4.528 | 0.033*   | 1.027 ( 1.002-1.053 ) |
| Age                 | 0.144 | 0.704    | 0.989 ( 0.937-1.045 ) |
| Gender              | 1.729 | 0.189    | 2.170 ( 0.684-6.885 ) |
| BMI                 | 2.246 | 0.134    | 1.103 ( 0.970-1.253 ) |
| smoking history     | 0.271 | 0.603    | 1.353 ( 0.434-4.221 ) |
| HbA1c               | 1.244 | 0.265    | 1.170 ( 0.888-1.542 ) |
| UA                  | 1.139 | 0.286    | 1.004 ( 0.997-1.010 ) |
| Constant            | 15.246| <0.001*  |                       |

*P<0.05.

DN, diabetic nephropathy; NLR, neutrophil to lymphocyte ratio; SBP, systolic blood pressure; Lp(a), lipoprotein-a; BMI, body mass index; HbA1c, glycated hemoglobin A1c; UA, uric acid.
Table 3. Comparisons of area under the curve (AUC) for DN prediction.

|                                 | NLR ~ Model 1 | NLR ~ Model 2 | Model 1 ~ Model 2 |
|---------------------------------|---------------|---------------|------------------|
| Difference between the areas    | 0.169         | 0.166         | 0.00216          |
| Standard error                  | 0.0587        | 0.0604        | 0.00417          |
| 95% Confidence interval         | 0.0535 - 0.284| 0.0480 - 0.285| -0.00600 - 0.0103|
| Z statistics                    | 2.872         | 2.754         | 0.519            |
| P value                         | 0.0041*       | 0.0059*       | 0.6039           |

*P < 0.05

NLR, neutrophil to lymphocyte ratio.
Supplemental table 1. NLR values in different groups of potential confounders.

|                      | Yes     | no      | P-value |
|----------------------|---------|---------|---------|
| Gender of female     | 1.87±1.00 | 1.83±0.74 | 0.778   |
| past history of DM   | 1.81±0.78 | 1.88±0.95 | 0.600   |
| past history of CVD  | 1.96±0.98 | 1.80±0.82 | 0.313   |
| smoking history      | 1.78±0.77 | 1.88±0.92 | 0.517   |

DM, diabetes mellitus; CVD, cardiovascular disease.

Supplemental table 2. Statistical characteristics of the 2 constructed discriminant equations.

|                      | Model 1 | Model 2 |
|----------------------|---------|---------|
| Wilk’s lambda value  | 0.746   | 0.794   |
| $\chi^2$ statistics  | 36.381  | 32.958  |
| degree of freedom    | 4       | 3       |
| P-value              | < 0.001*| < 0.001*|
| centroid of DN group | 0.875   | 0.735   |
| centroid of non-DN group | -0.384 | -0.349 |

*P<0.05.