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ORIGINAL ARTICLE

Serum levels of immunoglobulin G and complement 3 differentiate non-diabetic renal disease from diabetic nephropathy in patients with type 2 diabetes mellitus

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Abstract

Aims Heavy proteinuria caused by non-diabetic renal disease (NDRD) is common in type 2 diabetes mellitus (T2DM). The aim of this study was to investigate specific predictors for NDRD in addition to traditional indicators in T2DM.

Methods A total of 341 patients with T2DM who underwent renal biopsy were retrospectively included. Eligible patients were divided into a nephrotic-range group ($n=194$) and a non-nephrotic-range group ($n=147$) based on proteinuria level. Risk factors for NDRD were evaluated using logistic regression, and the diagnostic implications of these variables were assessed by subgroup.

Results Multivariate logistic regression indicated that serum IgG level (OR, 0.762; 95% CI, 0.628–0.924; $p=0.006$) was an independent predictor of NDRD in the nephrotic-range group. However, in the non-nephrotic-range group, increased C3 level was an independent risk factor for NDRD (OR, 1.313; 95% CI, 1.028–1.678; $p=0.029$). In the nephrotic-range group, the optimal cutoff value of IgG for predicting NDRD was 734.0 mg/dl, with 67.8% sensitivity and 74.8% specificity, and IgG ≤ 734.0 mg/dl was the best predictor of NDRD. In the non-nephrotic-range group, the optimal cutoff value of C3 for predicting NDRD was 122.0 mg/dl with low sensitivity (30.9%) but high specificity (97.8%).

Conclusions At different levels of proteinuria, reduced IgG and increased C3 levels were independent indicators of NDRD in T2DM. Insights into these factors will help to advance the clinical management of NDRD.

Keywords Non-diabetic renal disease · Diabetic nephropathy · Type 2 diabetes mellitus · Immunoglobulin G · Complement 3

Introduction

Non-diabetic renal disease (NDRD) is common in patients with type 2 diabetes mellitus (T2DM), with a prevalence ranging from 3.0% to 82.9% [1], and requiring a different treatment strategy to that of diabetic nephropathy (DN). Growing evidence has suggested that NDRD carries a better renal prognosis than DN, [2–4] meaning that the accurate differential diagnosis of NDRD from DN is essential in patients with T2DM. However, in clinical settings, a large proportion of T2DM patients with NDRD are misdiagnosed with DN and therefore do not receive timely and appropriate treatment, as the diagnosis of DN is, in most cases, based on clinical manifestations rather than pathological changes [5].

As the prevalence of T2DM increases worldwide, [6] the incidence of NDRD also is expected to increase in parallel. Thus, it is becoming increasingly important to distinguish NDRD from DN in T2DM patients with renal disease. Although several indicators for NDRD have been established, such as absence of diabetic retinopathy (DR), short duration of T2DM, and presence of microscopic hematuria, the predictive values of these markers have been shown to be

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highly variable in several studies [7, 8]. This discrepancy is likely derived from the marked heterogeneity of T2DM, limited sample size, and potentially confounding factors. Given the invasiveness and risks associated with renal biopsy, further insight into clinical predictors for NDRD in T2DM patients is critical to advance the clinical management of this patient population.

In clinical practice, various serological tests are typically performed on patients with diabetes, particularly those with either a sudden increase in proteinuria or an abrupt onset of proteinuria. To date, however, little has been reported on the significance of such testing in predicting NDRD. Furthermore, given that the diagnostic spectrum of NDRD varies according to proteinuria level, it may be speculated that the clinical features and predictors for NDRD might differ in different patients classified based on the level of proteinuria.

Therefore, in this study, we aimed to investigate specific predictors for NDRD in 341 T2DM patients with nephrotic- or non-nephrotic-range proteinuria via serological tests, and to further evaluate the diagnostic application of these variables.

Materials and Methods

Subjects

A total of 558 patients with DM who underwent renal biopsy in West China Hospital of Sichuan University from 2003 to 2017 were retrospectively reviewed, and 341 patients were included (Fig. 1). The indications for renal biopsy were DM patients with renal damage who lacked absolute contraindications, particularity T2DM patients without DR or with obvious glomerular hematuria and/or short duration of T2DM, or with sudden onset of overt proteinuria. Renal damage was defined as abnormal urinalysis or renal dysfunction. T2DM was diagnosed according to criteria established by the American Diabetes Association (ADA) [9].

Inclusion and exclusion criteria

The inclusion criteria were (1) age > 18 years, (2) T2DM, (3) the diagnosis of DN and NDRD confirmed by renal biopsy, and (4) a diagnosis of NDRD mainly including various primary glomerulopathy. Exclusion criteria were

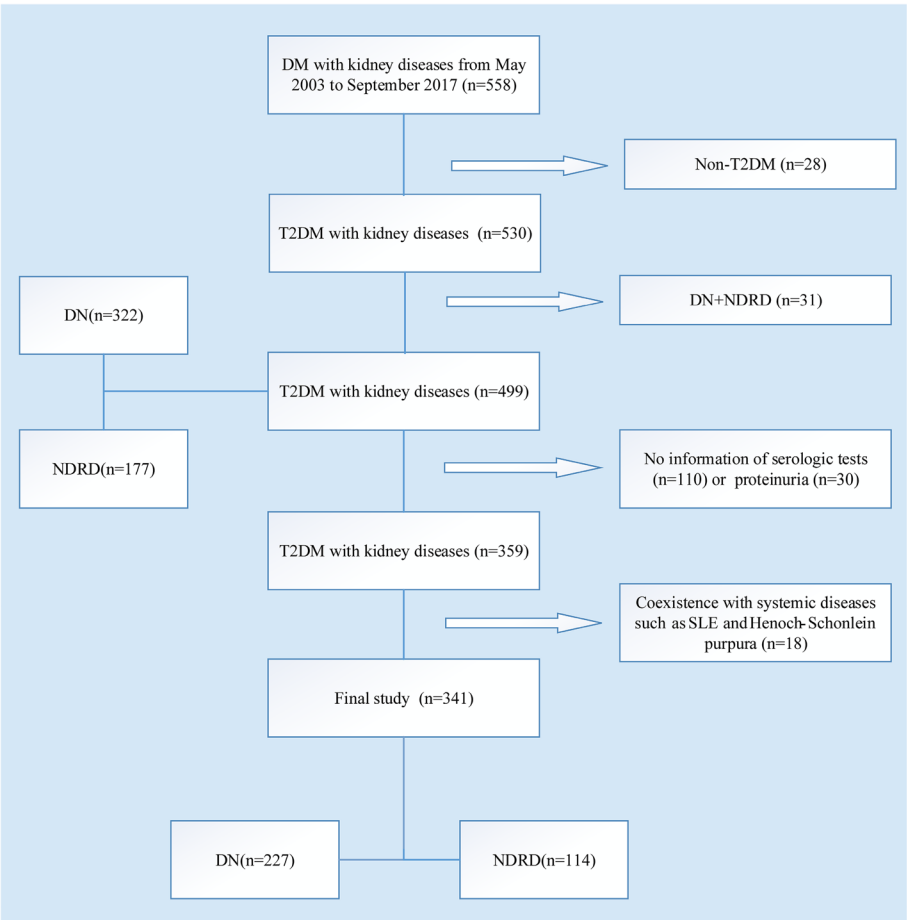

Fig. 1 Flowchart of study participants. *DN* diabetic nephropathy; *NDRD* non-diabetic renal disease. *SLE* systemic lupus erythematosus

(1) a diagnosis of DN superimposed on NDRD, (2) no data on serological tests or proteinuria, and (3) coexistence of systemic diseases such as cancer, systemic lupus erythematosus (SLE), monoclonal plasma cell disorders, or Henoch–Schonlein purpura.

Clinical characteristics

Complete clinical information was collected from the clinical database of West China Hospital at the time of renal biopsy. This information included age, gender, height, weight, duration of T2DM, blood pressure, HbA1c, 24-h urinary protein, serum creatinine (mg/dl), estimated glomerular filtration rate (e-GFR, evaluated by the CKD-EPI formula), serum albumin, immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), complement 3 (C3), and complement 4 (C4). Immunoglobulin (IgG, IgA, and IgM) and complement (C3 and C4) levels were measured by immunonephelometric assay (Beckman Coulter, Brea, CA, USA), and the following reference intervals for healthy adults were used: IgG 800–1550 mg/dl, IgA, 83.6–290 mg/dl; IgM, 70–220 mg/dl; C3, 78.5–152.0 mg/dl; and C4, 14.5–36 mg/dl. Nephrotic-range or heavy proteinuria was defined as a level of proteinuria > 3.5 g/d.

Pathological examination

The pathological diagnosis of DN was based on the 2010 standards of the Renal Pathology Society [10]. The glomerular classifications were as follows: class I, glomerular basement membrane (GBM) thickening; class IIa, mild mesangial expansion; class IIb, severe mesangial expansion; class III, nodular sclerosis; and class IV, global glomerulosclerosis in > 50% of glomeruli. Interstitial fibrosis and tubular atrophy (IFTA) were scored as follows: 0, absent; 1, < 25%; 2, 25–50%; and 3, > 50% of the total area. Interstitial inflammation was scored as follows: 0, absent; 1, inflammation only in relation to IFTA; and 2, inflammation in areas without IFTA. Arteriolar hyalinosis was scored as follows: 0, absent; 1, at least one area of arteriolar hyalinosis; and 2, more than one area of arteriolar hyalinosis. NDRD was diagnosed according to characteristic changes under light microscopy, immunofluorescence, and electron microscopy examinations.

Patients were divided into four subgroups based on the level of proteinuria and biopsy findings: a nephrotic-range proteinuria group including two subgroups, DN and NDRD; and a non-nephrotic-range proteinuria group including a further two subgroups, DN-non and NDRD-non (Table 1).

Statistical analysis

All statistical tests were analyzed using SPSS for Windows (version 22.0). Continuous variables were summarized as mean ± standard deviation (SD) or median with range, and categorical data were presented as numbers and percentages. Differences in means for quantitative variables were compared using a *t* test or Mann–Whitney U test, as appropriate. Categorical variables were evaluated with a Chi-squared test. Univariate and multivariate logistic regression analyses were employed to assess risk factors for the development of NDRD in T2DM patients. The diagnostic value of variables was estimated by sensitivity, specificity, and Youden index based on receiver operating characteristic (ROC) curves. A two-sided p value < 0.05 was considered statistically significant.

Results

Demographic and clinical features

Out of a total of 558 DM patients who underwent renal biopsies from 2003 to 2017, 530 patients had T2DM and biopsy-verified kidney diseases including DN (322/530), NDRD (177/530), and DN superimposed on NDRD (31/530). As shown in Fig. 2, the number of T2DM patients undergoing renal biopsy has increased over the past 15 years, and especially in the past 2 years. A final figure of 341 T2DM patients with DN (227, 66.6%) or NDRD (114, 33.4%) was recruited (Fig. 1).

Of the 341 patients included in this study, 194 (56.9%) patients were in the nephrotic-range proteinuria group and 147 (43.1%) in the non-nephrotic-range proteinuria group. Pathological analyses revealed that NDRD was diagnosed in 59 (30.4%) patients with nephrotic-range proteinuria, and in 55 (37.4%) patients with non-nephrotic-range proteinuria. At baseline, 211 patients were male (61.9%) and the mean age was 51.37 ± 9.82 years old, while 114 (33.4%) patients had diabetic retinopathy (DR) and 250 (73.3%) had hypertension. The median duration of diabetes was 60 months (range, 0–300 months). The mean serum creatinine level was 1.36 ± 0.80 mg/dl, mean e-GFR was 68.79 ± 31.13 ml/min/1.73 m^2, mean serum IgG level was 981.35 ± 344.56 mg/dl, and mean serum C3 was 98.13 ± 20.55 mg/dl. The clinical features of patients in different groups are shown in Table 1.

Pathological findings in NDRD patients

In the 59 NDRD patients with nephrotic-range proteinuria, the most common pathological type was membranous nephropathy (32, 54.2%), followed by minimal change

Table 1 Clinical characteristics and laboratory findings of patients with DN or NDRD according to the degree of proteinuria

Valuables	All ($n=341$)	Patients with nephrotic-range proteinuria ($n=194$)			Patients with non-nephrotic-range proteinuria ($n=147$)		
		DN ($n=135$)	NDRD ($n=59$)	p	DN-non ($n=92$)	NDRD-non ($n=55$)	p
Age (years)	51.37 ± 9.82	51.80 ± 9.13	53.02 ± 10.65	0.418	51.72 ± 9.99	47.98 ± 9.76	0.028
Gender (Male, %)	211 (61.9)	92 (68.1)	29 (49.2)	0.012	60 (65.2)	30 (54.5)	0.199
Duration of diabetes (Months)	60 (0–300)	84 (0–252)	12 (0–120)	< 0.001	84 (0–300)	24 (0–240)	< 0.001
DM history < 5 years (%)	169 (49.6)	49 (36.3)	47 (79.7)	< 0.001	33 (35.9)	40 (72.7)	< 0.001
DR (%)	114 (33.4)	72 (53.3)	9 (15.3)	< 0.001	32 (34.8)	1 (1.8)	< 0.001
Body mass index (kg/m^2)	25.97 ± 4.23	26.42 ± 4.89	25.69 ± 3.99	0.499	25.92 ± 4.00	25.39 ± 3.45	0.554
SBP (mm Hg)	141.05 ± 23.47	151.31 ± 23.95	129.19 ± 21.69	< 0.001	139.85 ± 21.29	130.58 ± 15.67	0.003
DBP (mm Hg)	84.58 ± 13.65	89.08 ± 14.44	78.42 ± 13.07	< 0.001	82.93 ± 12.65	82.87 ± 10.15	0.975
Hypertension (%)	250 (73.3)	120 (88.9)	28 (47.5)	< 0.001	77 (83.7)	25 (45.5)	< 0.001
Hematuria (%)	178 (52.2)	84 (62.2)	38 (64.4)	0.772	33 (35.9)	23 (41.8)	0472
Initial proteinuria (g/d)	4.10 (0.02–29.60)	6.84 (3.59-27.00)	7.40 (3.59-29.60)	0.707	1.54 (0.18-3.50)	1.32 (0.02-3.46)	0.138
e-GFR (ml/min/1.73 m^2)	68.79 ± 31.13	51.15 ± 25.85	85.87 ± 29.68	< 0.001	74.47 ± 29.12	84.25 ± 25.94	0.042
e-GFR < 60 (ml/min/1.73 m^2, %)	154 (45.2)	93 (68.9)	14 (23.7)	< 0.001	34 (37.0)	13 (23.6)	0.094
Serum creatinine (mg/dl)	1.36 ± 0.80	1.79 ± 0.93	1.00 ± 0.66	< 0.001	1.18 ± 0.52	0.99 ± 0.40	0.012
Serum albumin (g/L)	33.38 ± 9.02	29.52 ± 7.06	25.91 ± 7.09	0.001	39.51 ± 6.32	40.29 ± 7.39	0.50
HbA1c (%)	7.37 ± 1.90	7.62 ± 2.12	7.22 ± 1.80	0.265	7.41 ± 1.66	6.94 ± 1.82	0.144
IgA (mg/dl)	256.86 ± 109.54	247.31 ± 113.71	265.23 ± 118.65	0.320	244.33 ± 93.01	292.25 ± 109.14	0.005
IgM (mg/dl)	130.28 ± 70.40	129.26 ± 67.27	150.10 ± 85.91	0.101	124.87 ± 69.76	120.57 ± 57.19	0.70
IgG (mg/dl)	981.35 ± 344.56	959.84 ± 322.95	704.73 ± 303.20	< 0.001	1131.10 ± 322.32	1080.36 ± 286.21	0.338
C3 (mg/dl)	98.13 ± 20.55	95.48 ± 19.34	105.24 ± 24.97	0.009	93.38 ± 16.95	104.95 ± 20.46	0.001
C4 (mg/dl)	25.11 ± 9.07	26.30 ± 10.82	27.24 ± 9.02	0.557	23.44 ± 6.94	22.69 ± 6.33	0.514

DR diabetic retinopathy; *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *e-GFR* estimated glomerular filtration rate; *HbA1c* glycosylated hemoglobin; *C*3 complement 3; *C*4 complement 4

Data are presented as the mean ± standard, the median with range or counts and percentages. A two-tailed $p < 0.05$ was considered statistically significant

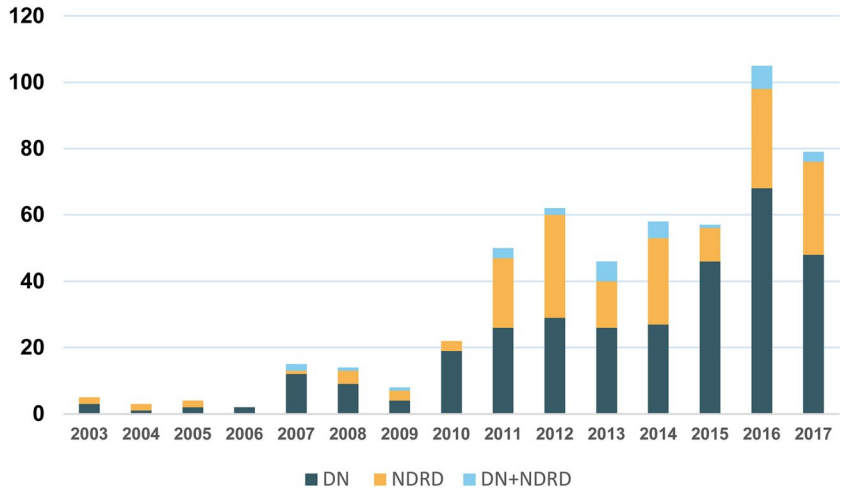

Fig. 2 Number of patients with T2DM who underwent renal biopsy between May 2003 and September 2017($n=530$). *DN* diabetic nephropathy ($n=322$), *NDRD* non-diabetic renal disease ($n=177$), DN + NDRD: DN superimposed on NDRD ($n=31$)

glomerulopathy (MCD, 11, 18.6%), IgA nephropathy (9, 15.3%), and focal segmental glomerular sclerosis (FSGS, 3, 5.1%). In contrast, in the 55 NDRD patients with non-nephrotic-range proteinuria, IgA nephropathy was the most common type (26, 47.3%), followed by MCD (14, 25.5%), membranous nephropathy (8, 14.5%), and FSGS

(6, 10.9%). The distribution of other pathological types of NDRD is presented in Table 2.

Comparison of clinical and laboratory data

As shown in Table 1 and Sup-Figure 1, in the nephrotic-range proteinuria group, the subgroup of patients with NDRD had a higher proportion of females and shorter DM duration (< 5 years); a lower incidence of DR, hypertension, and renal insufficiency (e-GFR < 60 ml/min/1.73 m^2); increased C3 level; and decreased levels of SBP, DBP, serum albumin, and IgG ($p < 0.05$) compared with the subgroup with DN. In the non-nephrotic-range proteinuria group, patients in the NDRD-non group had a higher proportion of shorter DM duration (< 5 years); a lower incidence of DR and hypertension; lower levels of SBP; higher levels of e-GFR, serum IgA; and C3; and younger age than those in the DN-non group ($p < 0.05$). The percentage of female patients and patients with renal insufficiency, and the levels of albumin and IgG, were comparable between the DN-non and NDRD-non groups. Of note, the incidence of hematuria was comparable between DN and NDRD groups in T2DM patients with nephrotic or non-nephrotic-range proteinuria.

Clinical predictors of NDRD

The results of multivariate logistic regression analysis showed that risk factors for NDRD were distinct between patients with nephrotic-range proteinuria and those with non-nephrotic-range proteinuria, although DM history < 5 years, absence of DR, and absence of hypertension were both associated with DNRD development in the two groups. In patients with nephrotic-range proteinuria, gender (male) (OR, 0.343; 95% CI, 0.122–0.960; $p = 0.042$), e-GFR < 60 ml/min/1.73 m^2 (OR, 0.198; 95% CI, 0.074–0.527; $p = 0.001$), and IgG level (OR, 0.762; 95% CI, 0.628–0.924; $p = 0.006$) were independent predictors for developing NDRD (Fig. 3). By contrast, a significant correlation between C3 level and the development of NDRD was observed in patients with non-nephrotic-range proteinuria (OR, 1.313; 95% CI, 1.028–1.678; $p = 0.029$) (Fig. 4).

Diagnostic value of variables for predicting NDRD development

In T2DM patients with nephrotic-range proteinuria, ROC curve analysis indicated predictive values of 0.750, 0.726, 0.717, 0.690, 0.707, and 0.908 for serum IgG level, e-GFR ≥ 60 ml/min/1.73 m^2, DM history < 5 years, absence of DR, absence of hypertension, and combined multifactors, respectively (Table 3). Based on the ROC curve, the optimal cutoff value of IgG for predicting NDRD was 734 mg/dl with 67.8% sensitivity and 74.8% specificity, as calculated by obtaining the best Youden index (Supplementary Fig. 2A).

In addition, in T2DM patients with non-nephrotic-range proteinuria, the predictive values for serum C3, DM history < 5 years, absence of DR, absence of hypertension, and combined multifactors were 0.649, 0.684, 0.665, 0.691, and 0.846, respectively (Table 4). The optimal cutoff value of C3 for predicting NDRD was 122 mg/dl with low sensitivity (30.9%) but high specificity (97.8%) (Supplementary Fig. 2B).

Discussion

A large number of studies examining the incidence and predictors of NDRD have been reported, although little attention has been given to the influence of proteinuria level on NDRD. Given the potentially different pathogenesis of nephrotic- and non-nephrotic-range proteinuria, it could be speculated that predictors of NDRD in T2DM patients might differ based on the degree of proteinuria. In this study, we investigated the clinical predictors for NDRD in addition to standard markers in T2DM patients with different levels of proteinuria. The results demonstrated that DM history < 5 years, absence of DR, and

Table 2 Distribution of pathological types of NDRD

Pathological classification	All ($n = 114$)	Nephrotic-range Proteinuria ($n = 59$)	Non-nephrotic-range Proteinuria ($n = 55$)
Membranous nephropathy	40 (35.1)	32 (54.2)	8 (14.5)
IgA nephropathy	35 (30.7)	9 (15.3)	26 (47.3)
MCD	25 (21.9)	11 (18.6)	14 (25.5)
FSGS	9 (7.9)	3 (5.1)	6 (10.9)
Mesangial proliferative glomerulonephritis	3 (2.6)	2 (3.4)	1 (1.8)
Membranoproliferative glomerulonephritis	2 (1.8)	2 (3.4)	0

Values are expressed as *n* (%). Membranous nephropathy and FSGS mentioned in this study were all primary

MCD minimal change glomerulopathy; *FSGS* focal segmental glomerular sclerosis

Factors	OR	95%CI	p
Univariate			
Age (per 10 yrs)	1.143	0.828-1.576	0.416
Gender (Male)	0.452	0.242-0.845	0.013
DM history<5 years	6.874	3.331-14.186	<0.001
Absence of DR	6.349	2.893-13.936	<0.001
Hypertension (No *vs* yes)	8.857	4.221-18.584	<0.001
e-GFR<60ml/min/1.73m^2	0.141	0.070-0.283	<0.001
Serum albumin (g/dL)	0.474	0.294-0.762	0.002
IgG (g/L)	0.727	0.634-0.833	<0.001
C3 (per 10 mg/dl)	1.243	1.070-1.444	0.005
Multivariate*			
Gender (Male)	0.343	0.122-0.960	0.042
DM history<5 years	6.067	2.147-17.141	0.001
Absence of DR	3.002	1.048-8.603	0.041
Hypertension (No *vs* yes)	6.999	2.348-20.863	<0.001
e-GFR<60ml/min/1.73m^2	0.198	0.074-0.527	0.001
IgG (g/L)	0.762	0.628-0.924	0.006

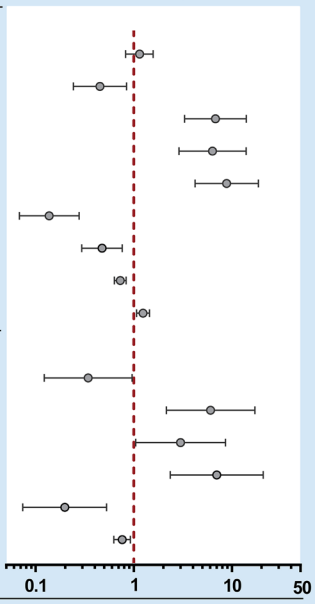

Fig. 3 Risk factors for NDRD identified by multivariate logistic regression analysis in T2DM patients with nephrotic-range proteinuria. *Multivariate analysis: adjusted for gender, DM history, DR, hypertension, e-GFR, serum albumin, and C3

Factors	OR	95%CI	p
Univariate			
Age (per 10 yrs)	0.682	0.482-0.965	0.031
Gender(male)	0.64	0.323-1.266	0.200
DM history<5 years	4.768	2.296-9.898	<0.001
Absence of DR	28.8	3.805-217.979	0.001
Hypertension (No vs yes)	6.16	2.862-13.259	<0.001
e-GFR<60ml/min/1.73m^2	0.528	0.249-1.121	0.096
IgA (g/L)	1.608	1.138-2.172	0.007
C3 (per 10 mg/dl)	1.401	1.155-1.700	0.001
Multivariate*			
DM history<5 years	3.39	1.348-8.528	0.009
Absence of DR	20.968	2.435-180.569	0.006
Hypertension (No vs yes)	5.695	2.103-15.428	0.001
C3 (per 10 mg/dl)	1.313	1.028-1.678	0.029

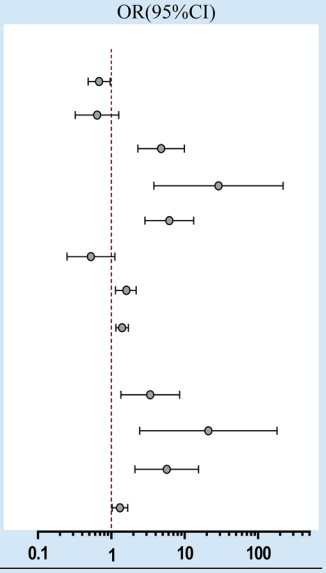

Fig. 4 Risk factors for NDRD identified by multivariate logistic regression analysis in T2DM patients with non-nephrotic-range proteinuria. *Multivariate analysis: adjusted for age, DR, DM history, hypertension, and IgA level

absence of hypertension were related to the development of DNRD in T2DM patients with nephrotic- and non-nephrotic-range proteinuria. However, serum IgG was significantly associated with NDRD only in the nephrotic-range proteinuria group, and a level of IgG ≤ 734 mg/dl emerged as the best predictor of NDRD. By contrast, serum C3 was an independent predictor of NDRD only in the non-nephrotic-range proteinuria group. These findings can be considered highly useful when considering that the patients likely underwent renal biopsy as a result of their atypical presentations.

A previous study also found that reduced serum IgG (< 919.5 mg/dl) and creatinine (< 4.1 mg/dl) were associated with NDRD development in 54 T2DM patients (24 with DN and 30 with NDRD), most of whom had heavy proteinuria [11]. In the present study, IgG level ≤ 734 mg/dl and e-GFR ≥ 60 ml/min/1.73 m^2 were good predictors for NDRD in patients with nephrotic-range proteinuria. Given

Table 3 Diagnostic performances of the variables for predicting NDRD development in T2DM patients with nephrotic-range proteinuria

	Optimal cutoff value	Sensitivity (%)	Specificity (%)	AUC (95% CI)	Youden index
IgG level (mg/dl)	734	67.8	74.8	0.750 (0.668–0.832)	0.426
e-GFR ≥ 60 ml/min/1.73 m^2	–	76.3	68.9	0.726 (0.648–0.803)	0.452
DM history < 5 years	–	79.7	63.7	0.717 (0.640–0.794)	0.434
Absence of DR	–	84.7	53.3	0.690 (0.613–0.768)	0.38
Absence of hypertension	–	52.5	88.9	0.707 (0.621–0.793)	0.414
Combined multifactors*	–	88.1	80.7	0.908 (0.866–0.951)	0.688

AUC area under the curve

**Combined multifactors* including IgG level, e-GFR, DM history, DR, and hypertension

Table 4 Diagnostic performances of the variables for predicting NDRD development in T2DM patients with non-nephrotic-range proteinuria

	Optimal cutoff value	Sensitivity (%)	Specificity (%)	AUC (95% CI)	Youden index
C3 level (mg/dl)	122.0	30.9	97.8	0.649 (0.555–0.742)	0.287
DM history < 5 years	–	72.7	64.1	0.684 (0.595–0.773)	0.368
Absence of DR	–	98.2	34.8	0.665 (0.579–0.751)	0.33
Absence of hypertension	–	54.5	83.7	0.691 (0.599–0.783)	0.382
Combined multifactors*	–	76.4	77.2	0.846 (0.784–0.907)	0.536

AUC area under the curve

**Combined multifactors* including C3 level, DM history, DR, and hypertension

the larger sample size and more accurate classification in the present study, our results might be considered more applicable. Ardawi et al. [12] reported that a significant increase in serum IgG concentration was observed in patients with diabetes versus those without diabetes. In addition, Nicoloff et al. [13] showed that elevated levels of circulating immune complexes (CIC) IgG were related to the development of early diabetic nephropathy. Kalia et al. [14] observed that IgG glycosylation was increased in patients with diabetes and DN compared with those with diabetes but without any complication, which indicated that the increased IgG might play a role in the onset of DN through altered immunoreactivity. Moreover, in this study, the IgG levels were somehow lower in NDRD patients compared to DN patients when proteinuria was in the nephrotic-range group. On the other hand, serum C3 was significantly lower in DN regardless of the proteinuria level, possibly related to selectivity of proteinuria. Nevertheless, the precise underlying mechanism for the increased levels of serum IgG in DN patients requires verification with further clinical and experimental evidence.

The key role of complement activation in the pathogenesis of DN is well established, [15] and our previous study also found that decreased serum C3 level was associated with DN progression in T2DM patients [16]. In the present study, our results show that the serum C3 level was lower in patients with DN compared with those with NDRD, and that the increased C3 level was an independent predictor of NDRD in T2DM patients with non-nephrotic-range proteinuria. However, the results of a previous study [17] indicated that low complement (C3 and C4) levels were not related with NDRD in the multivariate logistic regression analysis (OR, 4.70; 95% CI, 0.49–45.42; $p = 0.18$). The discrepancy most likely attributable to the observation that the categories of NDRD in the previous study mainly included FSGS (22%), hypertensive nephrosclerosis (18%), and acute tubular necrosis (ATN) (17%), whereas membranous nephropathy, IgA nephropathy, and MCD were the most common types of NDRD in the present study.

In addition, we identified distinctive pathological types of NDRD in T2DM patients with different proteinuria levels. In the nephrotic-range proteinuria group, the most common pathological type of NDRD was membranous nephropathy, whereas in the non-nephrotic-range proteinuria group, IgA nephropathy was the most common type. This finding confirmed the results of a previous study [4] and might allow clinicians to evaluate T2DM patients according to the degree of proteinuria. Of note, membranous nephropathy was the most common type of NDRD in

our study (40/114), consistent with the findings of several other studies in Asian populations [3, 7, 8, 11, 18].

The results of studies by Sharma et al. [17] and Lee et al. [4] have suggested that nephrotic-range proteinuria is negatively associated with the incidence of NDRD in patients with diabetes. However, no difference in the prevalence of NDRD between the nephrotic-range proteinuria group and the non-nephrotic-range proteinuria group was found in the present study. This discrepancy most likely is derived from the limited sample size or different indications for renal biopsy in these studies among T2DM patients.

Several studies [7, 19, 20] have indicated that glomerular hematuria may be related to NDRD development in patients with diabetes. However, other studies have shown that hematuria was comparable between patients with DN and NDRD [4, 11, 18]. In the present study, no difference in the incidence of hematuria was observed between the DN and NDRD groups. Whether hematuria can predict NDRD remains controversial, and further clinical and experimental studies are required to verify the earlier findings.

Although renal biopsy in patients with T2DM probably should be more extensive to accurately diagnose NDRD, guide further treatment, and predict renal outcomes, and has also been recommended as the gold standard for the diagnosis of DN, serological examinations are noninvasive and are essential for differential diagnosis, in particular for patients in whom renal biopsy is contraindicated. As shown in this study, serum IgG and C3 were found to be beneficial in the prediction of NDRD in T2DM patients, but the combination of multiple factors achieved the overall highest diagnostic efficiency with AUC (area under the curve) of 0.908 and 0.846 in nephrotic- and non-nephrotic groups, respectively (Tables 3 and 4). In clinical practice, the differential diagnosis of NDRD from DN in patients with T2DM without renal biopsy therefore requires a comprehensive consideration of a variety of factors. Furthermore, as the global incidence of T2DM continues to rise, there is an increasing necessity for nephrologists to closely consider the risks and benefits of renal biopsy in patients with T2DM.

Several limitations of this study should be noted. First, given the retrospective study design and different renal biopsy indications for T2DM patients, selection bias was inevitable. Second, this study was conducted in a single center and the sample size was limited. This made duration of diabetes and age not comparable in both nephrotic and non-nephrotic groups at baseline. Thus, duration of diabetes and age could result in a certain bias of the data. Therefore, multivariate logistic regression analysis and combined multi-factor ROC curves were used to minimize the potential confounding factors. Of course, validation of these results in larger cohorts of such patients is required. Finally, insufficient data are available at present to precisely identity the cutoff values of serum IgG and C3 for the prediction of NDRD.

In summary, T2DM patients with nephrotic- or non-nephrotic-range proteinuria showed different patterns of clinical characteristics as well as distinctive clinical predictors of NDRD. Reduced IgG and increased C3 levels were independent indicators for NDRD in T2DM patients, and it is therefore more appropriate to consider renal biopsy in patients with T2DM presenting with these clinical features.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The ethics committee of West China Hospital approved this research. The study protocol was in compliance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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