

# Neutrophil Count as a Reliable Marker of Diabetic Kidney Disease in Autoimmune Diabetes

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## Research article

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## Abstract

**Background** A growing body of evidence supports neutrophils as an active player in the development of diabetic kidney disease (DKD). However, the clinical relevance of neutrophils and DKD in autoimmune diabetes remains unknown. This study aims to investigate the relationship between circulating neutrophils and DKD in autoimmune diabetes.

**Methods** Patients with type 1 diabetes (T1D, n=226) and latent autoimmune diabetes in adults (LADA, n=79) were enrolled and stratified according to the urinary albumin to creatinine ratio (ACR). Circulating levels of white blood cells (WBCs), including neutrophils, were measured in a central laboratory, and the neutrophil-to-lymphocyte ratio (NLR) was calculated. The risk factors associated with DKD were analysed by logistic regression.

**Results** In T1D and LADA patients, the peripheral neutrophil counts increased in parallel with DKD advancement. The neutrophil counts in the patients with macroalbuminuria were significantly higher than those in the patients with normoalbuminuria for each type of diabetes. Furthermore, neutrophil counts positively correlated with the ACR. In addition, neutrophils were independently associated with DKD in T1D in the logistic regression analysis, when various well-known risk factors, including disease duration, sex, and smoking status, were adjusted as continuous variables.

**Conclusions** Neutrophil counts are closely associated with DKD in patients with autoimmune diabetes, suggesting neutrophil-mediated inflammation may be involved in the pathogenesis of DKD in patients with autoimmune diabetes.

## Introduction

Diabetic kidney disease (DKD) is one of the most common chronic complications of diabetes. In recent years, as the prevalence of diabetes has increased year by year, the prevalence of DKD has also increased significantly. More than one-third of diabetes patients suffer from DKD. DKD is the main cause of end-stage renal disease (ESRD), and it is also a high-risk factor for cardiovascular events such as coronary heart disease and stroke. In recent years, in addition to glucose and lipid metabolism disorders and haemodynamic abnormality, studies have shown that multiple pathological processes, such as chronic inflammation, oxidative stress and fibrosis, are also involved in the development of DKD.

The hyperglycaemia status of diabetic patients has long been considered to be the initiating factor of DKD. Glucose and lipid metabolism disorders and renal haemodynamic abnormality caused by hyperglycaemia are the two major pathological basis of DKD. Based on these factors, the current treatment of DKD mainly aims at control of blood glucose, blood lipids, and blood pressure and improvement of renal haemodynamics.

However, comprehensive managements that make blood glucose, blood lipids and blood pressure meet the criteria cannot completely prevent the development of DKD. Therefore, in addition to the above two aspects, there are other pathological processes involved in the development of DKD. In recent years, studies have demonstrated that a large number of lymphocytes, macrophages and mast cells accumulate in the kidney tissue of patients with DKD, which secrete large amounts of inflammatory mediators, cytokines and oxygen free radicals, directly or indirectly leading to kidney damage and increased renal fibrosis. The inflammatory hypothesis suggests that metabolic disorders in DKD patients activate inflammatory signals in the body, which in turn causes deposition of extracellular matrix in the kidney and promotes fibrosis. Therefore, inflammation plays an important role in the pathogenesis of DKD.<sup>1</sup> In the circulation, neutrophils are the main immune cell type of the inflammatory response, and increasing numbers of studies suggest that the inflammation they mediate plays a role in DKD.

Neutrophils may migrate to the kidney by increasing spontaneous adhesion,<sup>2</sup> followed by abnormal activation of the secretion of proinflammatory cytokines, degranulated products, and reactive oxygen species (ROS),<sup>3</sup> further damaging the kidneys. Clinical studies have found that peripheral total WBCs, neutrophils, and the neutrophil-to-lymphocyte ratio (NLR) are independently associated with DKD in T2D.<sup>3-11</sup> However, the association between these factors and autoimmune diabetes such as type 1 diabetes (T1D) and latent autoimmune diabetes in adults (LADA) patients has not yet been explored.

The purpose of this study was to investigate the relationship between neutrophils and DKD in patients with autoimmune diabetes and to explore new directions for the prevention and treatment of DKD.

## Methods

### 1. Subjects

We retrospectively studied the clinical data from 305 patients with autoimmune diabetes, including 226 with T1D and 79 with LADA, who visited the Department of Endocrinology and Metabolism, the Second Xiangya Hospital of Central South University between December 2009 and May 2018. We recorded confidential information of anthropometric measurements, biochemistry parameters and history of medication. The patients who showed any signs of acute or chronic infection or who had a history of any malignancies or other diseases, including severe cardio-cerebrovascular disease, liver, and other kidney diseases, were excluded from analysis in the present study. T1D was diagnosed according to the criteria of the American Diabetes Association. LADA patients were included using the inclusion criteria as described previously.<sup>12</sup>

Age, gender, WC, BMI, blood pressure, duration of diabetes, fasting glucose, postprandial glucose, fasting C-peptide, postprandial C-peptide, haemoglobin A1C (HbA1c), lipid profiles, liver function, and renal function were collected from the electronic medical records of our department, and the measuring methods were previously published.<sup>13</sup> Estimated glomerular filtration rate (eGFR) was calculated for each patient using the Cockcroft-Gault formula equation. Patients with an estimated glomerular filtration rate (eGFR) less than 30 mL/min/1.73 m<sup>2</sup> were excluded.

Peripheral blood leukocyte analysis included total WBC counts and absolute counts of neutrophils, monocytes, lymphocytes, eosinophils, and basophils. To reduce the confounding effects of infection, the WBC counts ranging from  $4.0 \times 10^9/l$  to  $10.0 \times 10^9/l$  and neutrophil counts in the normal range were taken into account for the analysis of the included patients. All abnormal or atypical specimens of WBCs and neutrophils were excluded.

Fresh morning spot urine samples were obtained from all patients, and the microalbumin to creatinine ratio (ACR) (mg/g) was calculated by dividing microalbumin (mg/dl) by creatinine (g/dl). According to their ACRs, the diabetic patients were divided into three groups: normoalbuminuria (ACR < 30 mg/g), microalbuminuria (ACR 30 - 300 mg/g), and macroalbuminuria (ACR > 300 mg/g). Diabetic nephropathy was accepted as either micro/macroalbuminuria or eGFR < 60 ml/min/1.73 m<sup>2</sup> or both. Patients with known causes of nephropathy other than diabetes were excluded from the study.

The study was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University. The study was conducted in accordance with the principles of the Helsinki Declaration. Due to the retrospective nature of the study, the requirement to obtain informed consent was waived.

## 2. Statistical analysis

All analyses were performed using IBM SPSS statistics 23.0 software. The Kolmogorov-Smirnov test was used to test the data for normality. The normally distributed data are expressed as the means  $\pm$  standard deviations, and the non-normally distributed data are expressed as the medians and interquartile ranges. One-way ANOVA was used for comparisons between groups. Relevance descriptions were appropriately related to Pearson. The risk factors for DKD were identified by ordinal logistic regression analysis. The results are expressed as odds ratios (ORs) and 95% confidence intervals (CIs). In all statistical comparisons,  $P < 0.05$  was considered statistically significant.

## Results

Among 226 patients with T1D, 204 had normoalbuminuria, 10 had microalbuminuria, and 12 had macroalbuminuria. Among 79 patients with LADA, 61 had normoalbuminuria, 10 had microalbuminuria, and 8 had macroalbuminuria. The characteristics of these patients are shown in Table 1. Age increased as the ACR increased in the patients with T1D, and this trend exhibited a stepwise increase ( $P < 0.05$ ). However, no similar changes were observed in the patients with LADA. In the two diabetic groups, the duration of diabetes increased as the ACR increased ( $P < 0.05$ ). Diastolic blood pressure gradually increased as the ACR increased in the patients with T1D ( $P < 0.05$ ). In the patients with T1D or LADA, the uric acid level was higher in the patients with macroalbuminuria than in those with normoalbuminuria or microalbuminuria ( $P < 0.05$  and  $P < 0.01$ , respectively). In the two groups of diabetic patients, the levels of eGFR and serum albumin gradually decreased with increasing ACR values ( $P < 0.01$ ). There were no significant differences between the two groups in the levels of fasting blood glucose, 2 h blood glucose, fasting C-peptide, 2 h C-peptide or HbA1c (Table 1).

The total WBC and neutrophil counts and the NLRs of the different types of diabetic patients were compared between the different albuminuria groups (Fig. 1 A, B, C). For the total WBC counts of all patients, there were no significant differences in T1D and LADA. For the neutrophil counts, in all patients and in the normoalbuminuria patients, the patients with LADA had higher neutrophil counts than the patients with T1D ( $P < 0.05$ ). For the NLR, in all patients and in the normoalbuminuria patients, the NLR of the patients with LADA was significantly higher than that of the patients with T1D ( $P < 0.01$ ) (Fig. 1).

Within each diabetic type group, the WBC counts, neutrophil counts and the NLR were analysed between the different albuminuria groups (Fig. 2). The WBC counts in the patients with LADA gradually increased from normoalbuminuria to microalbuminuria and finally macroalbuminuria. Among the patients with LADA, WBC counts in the microalbuminuria and macroalbuminuria groups were significantly greater than that in the normoalbuminuria group (Fig. 2 a2,  $P < 0.05$ ).

The neutrophil counts of the macroalbuminuria groups among the T1D or LADA patients were significantly greater than those in the corresponding normoalbuminuria group (Fig. 2 b1,  $P < 0.01$ ; Fig. 2 b2,  $P < 0.05$ ). The NLR of the macroalbuminuria group among the patients with T1D was higher than those of the normoalbuminuria ( $P < 0.01$ ) and microalbuminuria groups ( $P < 0.05$ ).

The neutrophil counts and the NLR in peripheral blood were positively correlated with the duration, age, WHR, BMI, systolic blood pressure, diastolic blood pressure, and TG level (Table 2, both  $P < 0.01$ ) and were negatively correlated with the HDL-c level (Table T3,  $P < 0.01$ ). The ACR was positively correlated with the WBC count, neutrophil count, and NLR (Table 2,  $P < 0.001$ ). The LDL-c level was also positively correlated with the neutrophil count and NLR (Table 2,  $P < 0.01$ ). There were positive correlations between sex, the 2 h postprandial blood glucose level and the NLR (Table 2, both  $P < 0.05$ ) (Table 2).

In T1D, univariate analyses revealed that age, duration of diabetes, systolic and diastolic blood pressure (DBP), and neutrophil counts were significantly associated with DKD (Table 3). An ordinal logistic regression analysis was performed with the stages of DKD as the dependent variables; the status of DKD was predictive of the following variables: age, sex, known duration of diabetes, smoking status, BMI, systolic blood pressure (SBP) and DBP, fasting blood glucose, HbA1c, triglycerides, total cholesterol, HDL-c, and LDL-c, and neutrophil counts. Our analysis showed that HbA1c, female sex, smoking, and neutrophil counts were independent risk factors for DKD in T1D ( $P = 0.008$ ) (Table 3). In LADA, univariate analyses revealed that duration of diabetes, SBP, and neutrophil counts were associated with DKD (Table 3). Logistic regression showed that, after adjustment for the conventional risk factors mentioned above, duration of diabetes was the only independent risk factor for DKD in LADA ( $P = 0.003$ ) (Table 3).

## Discussion

Our study provides the first evidence showing that in patients with T1D or LADA, the peripheral total WBC count, neutrophil count, and NLR, even when within the normal range, are independently and significantly associated with DKD. The association between neutrophils and DKD persists even after controlling for conventional risk factors, including age, sex, smoking status, blood pressure, lipid profile, and glucose control, as well as obesity.

DKD is common to all types of diabetes; thus, hyperglycaemia is a major risk factor for DKD. However, hyperglycaemia does not account for all changes that are observed in the renal tissue.<sup>14</sup> The pathogenesis of DKD is complex, including genetics, haemodynamic changes, disorders of glucose and lipid metabolism, effects of cytokines and growth factors, oxidative stress, and inflammatory responses. Several lines of evidence demonstrate inflammation as a cardinal pathogenic mechanism corresponding to the development and progression of DKD, in which several types of innate immune cells are actively involved.<sup>15</sup> Neutrophils, as the most abundant and inflammation-related immune cell type in the circulation, might be involved in the pathogenesis of DKD.

The abnormal activation of blood neutrophils has been reported in diabetes patients.<sup>2,16–18</sup> Neutrophils from DKD patients exhibited faster exocytosis of primary granules than those from either normal subjects or patients without DKD. Neutrophils from DKD patients failed to remove CD11b (a subunit of Mac-1) from the cell membrane, and the adhesion molecule CD11b seems to persist at increased levels.<sup>16</sup> The elevated expression of CD11b could play a role in directing neutrophil migration in inflamed renal tissue expressing upregulated levels of the cell adhesion molecule ICAM-1. In agreement with these data, spontaneous adhesion has been shown to be increased significantly in neutrophils from diabetes patients with proteinuria compared with those from patients with normoalbuminuria or healthy control subjects.<sup>17</sup> The oxidation of serum albumin may cause neutrophil activation and further oxidation of albumin in diabetes, which are important to the severity and progression of DKD.<sup>18</sup> In addition, the metabolic disturbances accompanying the impairment of diabetes control could also induce neutrophil adherence to foreign surfaces and superoxide anion production in diabetes patients.<sup>2</sup>

The exact molecular mechanism by which neutrophils are involved in the development of DKD is unclear. However, there is some evidence that neutrophils could play a role in this pathological process. The migration of neutrophils to the kidney is a critical step in the progression of DKD. The influx of neutrophils is associated with an acute reaction to inflammation or injury. Neutrophils secrete enzymes and oxidation products that can damage the local microenvironment and induce tissue injury.<sup>19</sup> Recent studies revealed that neutrophils from T1D patients were primed to produce neutrophil extracellular traps (NETs) which are made of DNA, histones, and neutrophil proteins, and high glucose level can induce NETs *in vitro*.<sup>20,21</sup> In addition, myeloperoxidase (MPO), a well-established marker of NET formation, was observed to increase in kidney of Streptozotocin-induced diabetic rats,<sup>22</sup> suggesting neutrophil formed NETs might engage in the pathogenic mechanisms of DKD.

Furthermore, several epidemiological and clinical studies have evaluated the association of neutrophils or the NLR with DKD in T2D. Azab showed that the NLR could act as a predictor of worsening renal function in 338 American diabetic patients.<sup>4</sup> Chung *et al.* demonstrated that peripheral neutrophil counts were independently and significantly associated with DKD in 1480 Chinese patients with T2D.<sup>3</sup> In another study that recruited 253 Chinese subjects with T2D, an increased NLR was significantly associated with DKD, and the patients had a 2.088-fold increased risk of DKD for every unit increase in the NLR.<sup>5</sup> Afsar *et al.* reported that the NLR was independently associated with the 24-hour urinary protein and urinary albumin excretion in 80 Turkish patients with newly diagnosed T2D.<sup>6</sup> Ciray *et al.* also reported that the NLR was positively correlated with microalbuminuria but negatively correlated with the eGFR in 114 Turkish subjects with T2D.<sup>7</sup> Akbas *et al.* showed that in 200 Turkish patients with T2D, the albuminuria levels increased as the NLR increased, and the NLR was found to be independently associated with albuminuria.<sup>8</sup> Kahraman *et al.* also showed significant correlations among albuminuria, the glomerular filtration rate and the NLR in 112 patients with T2D.<sup>9</sup> Additionally, NLR could predict renal function loss in 108 Pima Indians and 941 Europeans with T2D, as shown in a longitudinal study.<sup>10</sup> In a recent study recruiting 247 patients with T2D and biopsy-confirmed DKD, the NLR was significantly associated with interstitial fibrosis, tubular atrophy and renal dysfunction.<sup>11</sup>

To the best of our knowledge, our study provided the first clinical evidence addressing the relationship between neutrophil counts and DKD in T1D and LADA patients. We showed that the patients with DKD had higher neutrophil counts than the patients without DKD in the contexts of T1D and LADA. For all patients or those with normoalbuminuria, the neutrophil counts showed a significant decrease in patients with T1D compared to those with LADA, which is consistent with findings that reduced circulating neutrophil counts associated with T1D.<sup>23</sup> However, the neutrophil counts were comparable among the albuminuria patients with T1D and LADA, indicating that the degrees of inflammation involved in DKD in different types of diabetes might be similar. Additionally, in T1D, hyperglycaemia usually starts in the first decades of life and is generally the only recognized cause of DKD. In contrast, in LADA which is also an autoimmune diabetes but frequently accompanied by metabolic syndrome,<sup>12</sup> hyperglycaemia usually develops after 30 years of age when the kidneys begin suffering from the long-term consequences of ageing and other recognized promoters of chronic renal injury, such as arterial hypertension, obesity, dyslipidaemia, and smoking. However, the association between neutrophil counts and DKD persists in T1D and LADA after adjusting for age, sex and other risk factors, suggesting the independent influence of inflammatory markers such as neutrophils on the development of DKD.

Our study has several limitations. First, due to the relatively small sample size of LADA patients and the retrospectively cross-sectional design, future large-scale, longitudinal studies are needed to clarify the alterations and dynamic changes in the circulating neutrophil counts in the patients with different types of diabetes and to further investigate the concrete roles of the neutrophil counts in the pathogenesis of DKD in each type of diabetes. Second, this study did not provide serological data on neutrophil serine proteases, such as neutrophil elastase (NE), proteinase 3 (PR3), or cathepsin G (CG). Future studies focusing on whether neutrophils are activated to secrete the neutrophil serine proteases involved in the pathogenesis of DKD should be warranted in future.

In conclusion, our study suggests that the neutrophil counts reflect DKD in subjects with autoimmune diabetes. These findings support the roles of neutrophils in the pathogenesis of the kidney complications of diabetes and provide a possible perspective for using neutrophils as a potential biomarker for the early identification of individuals at high risk of developing DKD and as potential therapeutic targets for DKD.

## Declarations

### Contributors

Yang Xiao and Zhiguang Zhou designed the study. Yao Yu and Qiuqiu Lin collected data and wrote the manuscript, and Dewei Ye and Gan Huang assisted in manuscript revision. Yao Yu, Qiuqiu Lin, Yanfei Wang, Binbin He, Yanhua Li analysed and interpreted the data. Yang Xiao took full responsibility for the integrity and accuracy of the data. All authors approved the manuscript.

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## Tables

Table 1 The characteristics of diabetic patients.

	T1D				LADA			
	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	P	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	P
Number	204	10	12		61	10	8	
Age (yr)	21.67±14.92	28.50±28.66	33.50±17.06*	0.017	52.46±10.48	58.90±14.14	57.00±9.26	0
Gender(male/female)	100/104	3/7	5/7	0.456	35/26	7/3	4/4	0
Duration of diabetes (yr)	5.13±5.05	7.27±7.59	11.03±5.12*	0.000	5.42±5.45	10.40±5.99*	12.56±6.54*	0
BMI (kg/m <sup>2</sup> )	19.19±3.82	19.14±2.60	22.73±5.52*	0.110	21.60±2.80	22.86±3.62	21.96±2.42	0
Smoker (%)	35.3	40.0	41.7	0.870	24.6	50.0	37.5	0
WHR	0.84±0.07	0.83±0.08	0.87±0.07	0.296	0.89±0.06	0.91±0.05	0.88±0.06	0
SBP (mmHg)	112.94±15.53	114.20±20.91	139.42±34.21* $\Delta$	0.000	122.72±17.23	133.90±22.83	137.75±24.26*	0
DBP (mmHg)	70.56±12.69	75.00±16.21	77.05±9.74*	0.012	77.00±9.82	79.90±15.50	85.13±10.7	0
HbA1c (%)	8.37±2.19	7.63±1.85	7.48±1.40	0.228	9.45±2.35	9.69±2.53	9.89±2.17	0
FBG (mmol/l)	9.17±4.56	7.60±3.08	10.63±4.53	0.315	8.31±4.31	8.07±3.35	6.56±3.73	0
PBG (mmol/l)	16.01±7.17	14.88±6.84	13.27±6.56	0.409	13.50±6.18	12.66±4.92	11.81±5.81	0
FCP (pmol/l) ¶	25.05(5.5, 89.58)	10.05(5.50,44.18)	5.50(5.50,157.50)	0.405	90.00(30.85,163.75)	136.75(37.53,224.88)	70.55(9.48,188.48)	0
PCP (pmol/l) ¶	41.00(5.70,215.08)	30.60(5.85,86.50)	17.1(5.50,126.30)	0.512	179.90(56.50,312.78)	251.65(76.80,593.80)	202.30(13.80,255.23)	0
TG (mmol/l) ¶	0.74(0.58,1.11)	0.86(0.49,1.23)	0.84(0.65,1.25)	0.422	0.93(0.68,1.17)	1.31(0.86,1.70)	1.30(0.91,1.54)	0
TC (mmol/l)	4.29±0.90	4.15±0.83	4.63±1.26	0.415	4.19±0.72	4.07±0.82	3.94±0.98	0
LDL-c (mmol/l)	2.58±2.39	2.24±0.83	2.72±1.04	0.357	2.43±0.64	2.38±0.78	2.37±0.94	0
HDL-c (mmol/l) ¶	1.45(1.20,1.67)	1.55(1.35,1.75)	1.48(1.30,1.64)	0.574	1.29(1.13,1.59)	1.34(0.91,1.42)	1.18(0.94,1.45)	0
Albumin(g/l)	42.38±4.08	41.72±3.65	36.02±7.88* $\Delta$	0.000	37.44±2.96	36.38±2.91	33.21±3.92* $\Delta$	0
TBIL(umol/l)	12.26±5.35	11.68±3.62	9.46±4.00	0.194	12.14±4.40	10.78±5.84	7.92±2.74	0
DBIL(umol/l)	3.92±1.94	3.87±1.08	3.13±1.66	0.382	3.88±1.44	3.28±1.65	2.64±1.18	0
UA(umol/l)	255.92±69.79	289.92±78.39	312.02±95.63*	0.018	230.89±53.26	303.57±63.54*	351.35±92.66*	0
EGFR(ml/(min*1.73m <sup>2</sup> ))	143.57±43.98	121.58±72.97	102.029±88.88*	0.011	105.06±33.03	95.01±61.97	69.27±30.81*	0
No medication	0	0	0		0	0	0	
Oral hypoglycemic agent	0	0	0		4	1	0	
Insulin	204	10	12		29	4	4	
Oral hypoglycemic agent + insulin	0	0	0		28	5	4	

Abbreviations: T1D, type 1 diabetes; LADA, latent autoimmune diabetes in adults; BMI, body mass index; WHR, waist to hip ratio; TG, triglycerides; TC, total cholesterol; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; TBIL, total bilirubin; DBIL, direct bilirubin; EGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; PBG, 2h postprandial blood glucose; FCP, fasting C-peptide; PCP, 2h postprandial C-peptide; UA, uric acid.

Data are expressed as mean±SD or median (interquartile range) as appropriate. Compared with the normal albuminuria group: \*p<0.05; Compared with the microalbuminuria group:  $\Delta$ p<0.05; ¶Non-parametric kruskal-wallis method was used to test the differences among the three groups.

Table 2. Correlation between WBC-neutrophil count and NLR as well as metabolic variables

		T1D			LADA		
		WBC	N	NLR	WBC	N	NLR
Duration	r	0.097	0.229**	0.322**	0.153	0.233*	0.235*
Age	r	0.023	0.295**	0.482**	-0.060	0.099	0.362**
WHR	r	0.091	0.097	0.064	0.021	0.063	0.099
BMI	r	0.097	0.296**	0.332**	0.225*	0.200	-0.023
SBP	r	0.007	0.243**	0.406**	0.241*	0.173	-0.040
DBP	r	-0.002	0.188**	0.294**	0.241*	0.083	-0.212
FBG	r	0.058	0.090	0.084	0.057	0.062	0.102
PBG	r	-0.011	-0.088	-0.119	0.010	0.027	0.057
FCP	r	-0.053	-0.006	0.007	0.168	0.217	0.093
PCP	r	-0.060	-0.045	-0.077	0.149	0.184	0.066
HbA1C	r	0.104	0.074	-0.030	-0.100	-0.063	0.029
TC	r	0.123	0.110	0.016	0.069	0.095	0.101
TG	r	0.114	0.133*	0.052	0.187	0.218	0.081
LDL-c	r	0.190**	0.196**	0.076	0.207	0.213	0.080
HDL-c	r	-0.149*	-0.197**	-0.145*	-0.232*	-0.204	0.025
ACR	r	0.108	0.239**	0.358**	0.194	0.227*	0.080
Gender	r	-0.012	0.032	0.111	0.126	0.146	0.150
Smoking	r	-0.001	0.006	0.018	0.245*	0.189	-0.014
Cr	r	0.001	0.237**	0.392**	0.112	0.209	0.234*
Albumin	r	-0.055	-0.245**	-0.424**	-0.075	-0.179	-0.209
TBIL	r	0.003	-0.039	-0.056	-0.216	-0.236*	-0.047
DBIL	r	-0.017	-0.045	-0.042	-0.185	-0.209	-0.070
UA	r	0.020	0.061	0.047	0.337**	0.387**	0.141
EGFR	r	0.046	-0.038	-0.183**	0.098	-0.035	-0.268*

Abbreviations: WBC, white blood cell; N, neutrophil; NLR, neutrophil to lymphocyte ratio; BMI, body mass index; WHR, waist to hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FBG, fasting blood glucose; PBG, 2h postprandial blood glucose; FCP, Fasting C-peptide; PCP, postprandial C-peptide; TC, Total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; Cr, creatinine; TBIL, total bilirubin; DBIL, direct bilirubin; UA, uric acid; ACR, urinary albumin to creatinine ratio; EGFR, estimated glomerular filtration rate; \*p<0.05,\*\*p<0.01.

Table 3. Ordinal logistic regression analysis found risk factors for diabetic kidney disease.

	T1D					LADA						
	Simple		Multiple			Simple		Multiple				
	P	OR	P	95%CI	LCI	UCI	P	OR	P	95%CI	LCI	UCI
Age	0.017	1.029	0.152	-0.011	0.068	0.156	0.991	0.861	-0.108	0.090		
Duration	0.000	1.036	0.453	-0.057	0.127	0.001	1.036	0.007	0.070	0.434		
BMI	0.110	0.938	0.580	-0.293	0.164	0.435	0.773	0.138	-0.598	0.082		
SBP	0.000	1.038	0.117	-0.009	0.083	0.039	1.011	0.779	-0.065	0.087		
DBP	0.012	0.988	0.710	-0.075	0.051	0.121	1.087	0.188	-0.041	0.208		
HbA1c	0.228	0.570	0.014	-1.007	-0.116	0.864	1.201	0.455	-0.298	0.664		
FBG	0.315	1.003	0.965	-0.143	0.149	0.579	0.831	0.284	-0.524	0.154		
TG	0.422	1.670	0.522	-1.057	2.082	0.068	1.068	0.961	-2.608	2.740		
TC	0.415	3.387	0.719	-5.422	7.862	0.680	195.978	0.254	-3.794	14.350		
LDL-c	0.357	0.237	0.685	-8.402	5.523	0.965	5.651E-03	0.275	-14.479	4.127		
HDL-c	0.574	0.474	0.819	-7.127	5.635	0.241	2.587E-04	0.074	-17.322	0.803		
N	0.002	1.846	0.025	0.077	1.149	0.030	0.690	0.610	-1.795	1.054		
Gender	0.456	4.736	0.025	0.196	2.194	0.667	6.379	0.129	-0.539	4.245		
Smoking	0.870	0.279	0.044	-2.519	-0.037	0.224	0.120	0.127	-4.850	0.607		

Abbreviations: N, neutrophil; BMI, body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FBG, fasting blood glucose; TC, Total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; \* $p < 0.05$ ; \*\* $p < 0.01$ .

## Figures

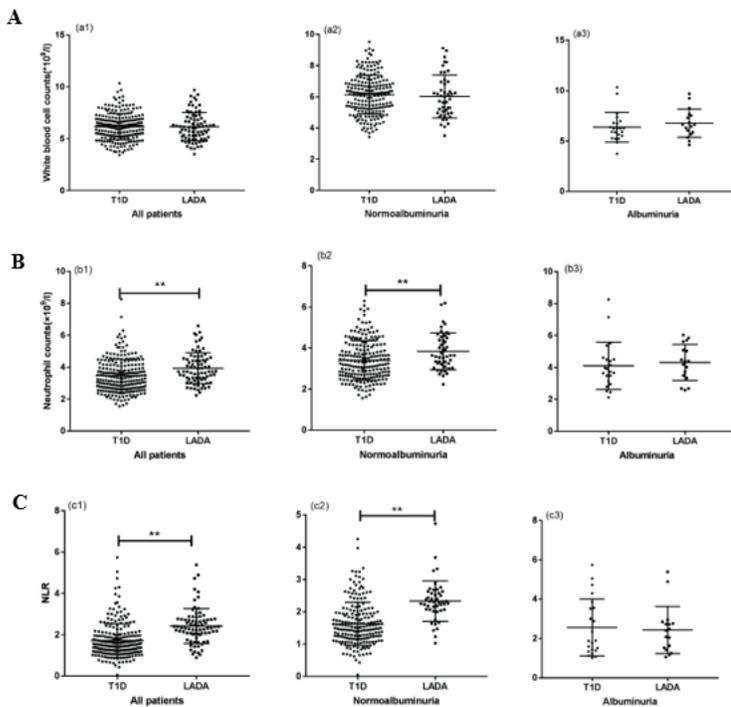
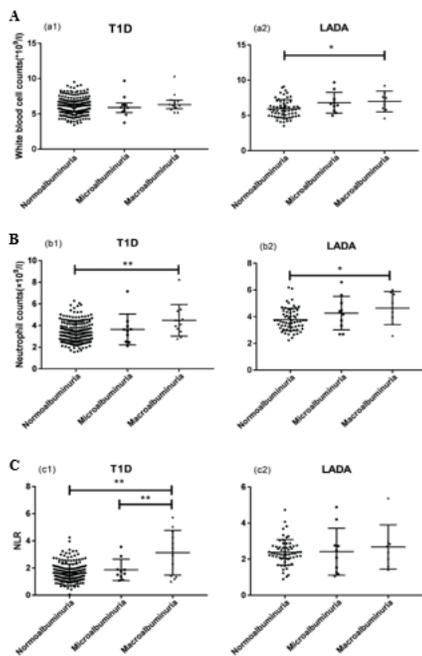


Figure 1  
White blood cell counts, neutrophil counts and neutrophil-to-lymphocyte ratio (NLR) in autoimmune patients. White blood cell counts (A), neutrophil counts (B) and NLR (C) were compared between type 1 diabetes (T1D) and latent autoimmune diabetes in adults (LADA) patients for all, with normoalbuminuria or albuminuria. The horizontal line indicates the median and quartiles. \*  $P < 0.05$ ; \*\*  $P < 0.01$ .



**Figure 2**

White blood cell counts, neutrophil counts and neutrophil-to-lymphocyte ratio (NLR) in autoimmune diabetes with different albuminuria status. White blood cell counts (A), neutrophil counts (B) and NLR (C) were compared among normoalbuminuria, microalbuminuria and macroalbuminuria group in type 1 diabetes (T1D) and latent autoimmune diabetes in adults (LADA) patients. The horizontal line indicates the median and quartiles. \* P < 0.05; \*\* P < 0.01.