

# A non-invasive diagnostic model of immunoglobulin A nephropathy and serological markers for evaluating disease severity

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## Abstract

**Background:** Immunoglobulin A nephropathy (IgAN) is the most common pathological type of glomerular disease. Kidney biopsy, the gold standard for IgAN diagnosis, has not been routinely applied in hospitals worldwide due to its invasion nature. Thus, we aim to establish a non-invasive diagnostic model and determine markers to evaluate disease severity by analyzing the serological parameters and pathological stages of patients with IgAN.

**Methods:** A total of 272 biopsy-diagnosed IgAN inpatients and 518 non-IgA nephropathy inpatients from the Department of Nephrology of Chinese People's Liberation Army General Hospital were recruited for this study. Routine blood examination, blood coagulation testing, immunoglobulin-complement testing, and clinical biochemistry testing were conducted and pathological stages were analyzed according to Lee grading system. The serological parameters and pathological stages were analyzed. The receiver operating characteristic (ROC) analysis was performed to estimate the diagnostic value of the clinical factors. Logistic regression was used to establish the diagnostic model.

**Results:** There were 15 significantly different serological parameters between the IgAN and non-IgAN groups (all  $P < 0.05$ ). The ROC analysis was performed to measure the diagnostic value for IgAN of these parameters and the results showed that the area under the ROC curve (AUC) of total protein (TP), total cholesterol (TC), fibrinogen (FIB), D-dimer (D2), immunoglobulin A (IgA), and immunoglobulin G (IgG) were more than 0.70. The AUC of the "TC + FIB + D2 + IgA + age" combination was 0.86, with a sensitivity of 85.98% and a specificity of 73.85%. Pathological grades of I, II, III, IV, and V accounted for 2.21%, 17.65%, 62.50%, 11.76%, and 5.88%, respectively, with grade III being the most prevalent. The levels of urea nitrogen (UN) ( $13.57 \pm 5.95$  vs.  $6.06 \pm 3.63$ ,  $5.92 \pm 2.97$ ,  $5.41 \pm 1.73$ , and  $8.41 \pm 3.72$  mmol/L, respectively) and creatinine (Cr) ( $292.19 \pm 162.21$  vs.  $80.42 \pm 24.75$ ,  $103.79 \pm 72.72$ ,  $96.41 \pm 33.79$ , and  $163.04 \pm 47.51$   $\mu$ mol/L, respectively) were significantly higher in grade V than in the other grades, and the levels of TP ( $64.45 \pm 7.56$ ,  $67.16 \pm 6.94$ ,  $63.22 \pm 8.56$ , and  $61.41 \pm 10.86$  vs.  $37.47 \pm 5.6$  mg/d, respectively), direct bilirubin (DB) ( $2.34 \pm 1.23$ ,  $2.58 \pm 1.40$ ,  $1.91 \pm 0.97$ , and  $1.81 \pm 1.44$  vs.  $0.74 \pm 0.57$   $\mu$ mol/L, respectively), and IgA ( $310.35 \pm 103.78$ ,  $318.48 \pm 107.54$ ,  $292.58 \pm 81.85$ , and  $323.29 \pm 181.67$  vs.  $227.17 \pm 68.12$  g/L, respectively) were significantly increased in grades II–V compared with grade I (all  $P < 0.05$ ).

**Conclusions:** The established diagnostic model that combined multiple factors (TC, FIB, D2, IgA, and age) might be used for IgAN non-invasive diagnosis. TP, DB, IgA, Cr, and UN have the potential to be used to evaluate IgAN disease severity.

**Keywords:** Immunoglobulin A nephropathy; Noninvasive; Diagnostic model; Severity

## Introduction

Immunoglobulin A nephropathy (IgAN), first discovered by Berger in 1968, is the most common pathological type of glomerular disease.<sup>[1]</sup> This disease is featured by the deposition of immunoglobulin A (IgA) in the mesangium.<sup>[2]</sup> The clinical spectrum of IgAN contains a wide

range of features, ranging from asymptomatic microscopic haematuria with/without proteinuria to gross haematuria, and few patients present with clinical signs of nephrotic or nephritic syndrome. In recent years, the proportion of IgAN in kidney disease has exceeded 50% in the world.<sup>[3-5]</sup>

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Approximately 20% to 40% patients with IgAN could develop end-stage renal disease (ESRD) within 20 years after first detection.<sup>[6]</sup> Therefore, early identification of risk factors and predicting IgAN prognosis are of great value. Nowadays, the detection of IgAN mainly depends on the microscopic examination of renal biopsies, with immunocytochemical techniques providing further confirmation of the diagnosis results.<sup>[7]</sup> Biopsy, which is essential for validating the diagnosis, may cause severe complications. Therefore, it is important to develop a non-invasive diagnostic model for IgAN and to find markers for evaluating the disease severity and progress of IgAN.

Statistics have been applied to determine significant predictors for the diagnosis or classification of various diseases.<sup>[8]</sup> Subsequently, different statistical algorithms, biological datasets, and parameters may result in different outputs.<sup>[9-11]</sup> More importantly, multicollinearity is always used in medical laboratory parameters, which may also cause variability and instability in a statistical model.<sup>[12]</sup> Thus, it is of great importance to choose appropriate variable for multiparameter analyses. In recent years, modeling techniques have been widely applied in medicine to help monitor disease progression and predict treatment outcomes.<sup>[13]</sup> However, no such approach exists for IgAN.<sup>[13,14]</sup> On the other hand, in the past decades, considerable efforts have been dedicated to detecting useful clinical markers for the non-invasive diagnosis of IgAN, and several markers have been proposed.<sup>[15,16]</sup> To date, several biomarkers have been suggested, but their validity has not been demonstrated in clinical practice.<sup>[17-19]</sup> Some studies have found several risk factors consistently associated with the progression of IgAN, such as proteinuria >1 g/day, arterial hypertension, reduced renal function at diagnosis and glomerular sclerosis or tubulointerstitial scarring at renal biopsy.<sup>[20-22]</sup> However, the influence of other factors such as older age, male sex, overweight, obesity, hypertriglyceridemia or hyperuricemia on IgAN development remains controversial.<sup>[23-25]</sup>

Thus, our study aims to establish a non-invasive diagnostic model and determine markers for evaluating disease severity by analyzing serological parameters and pathological stages in patients with IgAN.

## Methods

### Ethics approval

The study was approved by the Ethics Committee of the Chinese People's Liberation Army General Hospital. All patients provided informed written consent for study sample collection as well as permission for their use in research.

### Study design

A total of 790 inpatients from the Department of Nephrology of Chinese People's Liberation Army General Hospital were enrolled in our study at Chinese People's Liberation Army General Hospital from January 1, 2013, to March 15, 2016. Among them, a total of 272 patients were confirmed to have IgAN and 518 patients were confirmed to

be non-IgAN (other types of nephropathy) by renal biopsy. These patients did not undergo any treatments before they were diagnosed and the patients' complete history, clinical information, and pathological data were collected.

The inclusion criteria were as follows: (1) the patient accepted a renal biopsy during their hospitalization and (2) receiving no renal biopsy prior to pathological diagnosis at our hospital. The exclusion criteria used for the final selection of cases were as follows: (1) the renal biopsy was not conducted; (2) the patient received immunosuppression treatment or renal replacement therapy; (3) the pathological results indicated that the patient had secondary kidney disease, including diabetic nephropathy, lupus nephritis, and hepatitis-related nephropathy; (4) the clinical data were incomplete; and (5) patients with inflammatory and infectious diseases that may cause secondary IgA nephropathy. Based on the exclusion criteria, 790 cases were finally selected from the Chinese People's Liberation Army General Hospital.

### Samples and biological parameters

Blood samples of 790 patients were collected for routine blood examination, blood coagulation testing, immunoglobulin-complement testing, and clinical biochemistry testing. Pathological stages were analyzed according to Lee's grading system.<sup>[26]</sup> Serum samples were obtained by collecting venous blood into Vacutainer serum separator tubes (Greiner Bio-One, Frickenhausen, Germany), and the samples were then centrifuged at  $1500 \times g$  at  $4^{\circ}\text{C}$  for 10 min in a centrifuge (Sigma-Aldrich, St. Louis, MO, USA) within 20 min of collection. The blood biochemical and routine blood examinations were performed using enzymatic assays (Roche Products Ltd., Basel, Switzerland) on a fully automatic biochemical autoanalyzer (Cobas 8000; Roche Products Ltd., Basel, Switzerland).

### Statistical analysis

The normally distributed data are expressed as the mean  $\pm$  standard deviation (SD) and were compared using unpaired Student's *t* tests. The non-normally distributed data are expressed as medians with the corresponding 25th and 75th percentiles (interquartile range) and compared using Mann-Whitney *U* tests. The categorical variables were analyzed using the Chi-square tests. Logistic regression analysis was employed to establish the diagnostic model. Receiver operating characteristic (ROC) analysis was performed to measure the diagnostic value of the clinical factors and the area under the ROC curve (AUC) more than 0.70 were considered to have a good specificity.<sup>[27]</sup> A value of  $P < 0.05$  was considered to indicate a significant difference. All statistical analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism software version 6 (GraphPad Software Inc., San Diego, CA, USA).

## Results

### Patient characteristics

Clinical characteristics of the participants in this study are shown in Table 1. The differences in the serological

parameters between the two groups are shown in Table 2. There were 15 significantly different serological parameters between the IgAN and non-IgAN groups, including total protein (TP), total bilirubin (TB), direct bilirubin (DB), creatinine (Cr), uric acid (Ua), total cholesterol (TC), triglyceride (TG), lactate dehydrogenase (LDH), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), fibrinogen (FIB), D-dimer (D2), immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin E (IgE) (all  $P < 0.05$ ). However, urea nitrogen (UN), creatine kinase (CK), immunoglobulin M (IgM), complement 3 (C3), and complement 4 (C4) were not statistically different between the 2 groups.

**ROC analysis of related characteristics**

ROC analysis was performed to measure the diagnostic value for IgAN of the 15 different serological parameters

**Table 1: Clinical characteristics of the participants in this study, n=790.**

Items	Values
Age (years), median (Q1, Q3)	41.00 (30.00, 52.00)
Gender (male/female), n	482/308
BMI (kg/m <sup>2</sup> ), median (Q1, Q3)	25.10 (22.60, 28.00)
SBP (mmHg), median (Q1, Q3)	130.00 (120.00, 144.00)
DBP (mmHg), median (Q1, Q3)	80.00 (75.00, 90.00)

1 mmHg=0.133 kPa; Q1: 25th percentiles; Q3: 75th percentiles; BMI: Body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure.

between the IgAN and non-IgAN groups. The area under the curves (AUCs) of TP (AUC=0.78), TC (AUC=0.73), FIB (AUC=0.74), D2 (AUC=0.71), IgA (AUC=0.74), and IgG (AUC=0.71) were more than 0.70 [Table 3].

**Diagnostic model based on logistic regression analysis**

Logistic regression analysis was used to establish the diagnostic model for IgAN patients. The results showed that the high level of IgA, low level of TC, FIB, D2, and young age were risk indicators for IgAN [Table 4]. The predicted probabilities (PRE) were calculated based on logistic regression analysis. The classification equation for predicting IgAN was as follows:  $PRE = 1/(1 + e^{-[2.389 - 0.237 \times TC - 0.457 \times FIB - 0.397 \times D2 + 0.008 \times IgA - 0.043 \times age]})$ .

Based on the PRE of the logistic regression analysis, the AUC of the “TC+FIB+D2+IgA+age” combination was 0.86 ( $P < 0.001$ , 95% CI: 0.83–0.89), with a sensitivity and specificity of 85.98% and 73.85%, respectively [Figure 1].

**Relationship between the pathological grades and the laboratory test results**

We collected several representative clinical parameters to analyze the relationship between the pathological grades and laboratory test results. As shown in Table 5, the levels of UN and Cr were significantly higher in grade V than in grades II–V, respectively (all  $P < 0.05$ ). And the levels of TP, DB, and IgA were significantly increased in grades II–V compared with grade I (all  $P < 0.05$ ).

**Table 2: Differences in the serological parameters between the IgAN and non-IgAN groups.**

Items	IgAN (n=272)	Non-IgAN (n=518)	Z	P
TP (g/L), median (Q1, Q3)	66.40 (61.63, 71.28)	53.30 (43.50, 62.80)	13.09	<0.001
TB (μmol/L), median (Q1, Q3)	8.60 (6.28, 12.10)	7.20 (5.33, 9.70)	4.98	<0.001
DB (μmol/L), median (Q1, Q3)	2.10 (1.38, 3.20)	1.40 (0.93, 2.30)	7.56	<0.001
UN (mmol/L), median (Q1, Q3)	5.43 (4.33, 7.02)	5.20 (4.04, 7.08)	1.38	0.167
Cr (μmol/L), median (Q1, Q3)	94.80 (74.58, 133.63)	79.10 (64.00, 105.60)	5.88	<0.001
Ua (μmol/L), median (Q1, Q3)	380.65 (315.25, 437.68)	348.10 (287.13, 421.75)	3.47	0.001
TC (mmol/L), median (Q1, Q3)	4.48 (3.97, 5.24)	5.76 (4.58, 7.85)	10.44	<0.001
TG (mmol/L), median (Q1, Q3)	1.66 (1.15, 2.41)	1.93 (1.31, 2.87)	3.35	0.001
CK (IU/L), median (Q1, Q3)	76.70 (60.10, 108.40)	72.90 (49.60, 115.08)	1.75	0.080
LDH (IU/L), median (Q1, Q3)	157.60 (139.27, 179.20)	179.35 (154.20, 215.08)	8.11	<0.001
HDL (mmol/L), median (Q1, Q3)	1.03 (0.88, 1.27)	1.21 (0.96, 1.58)	5.75	<0.001
LDL (mmol/L), median (Q1, Q3)	2.84 (2.41, 3.41)	3.76 (2.86, 5.49)	9.36	<0.001
FIB (g/L), median (Q1, Q3)	3.33 (2.86, 3.88)	4.43 (3.50, 5.67)	10.98	<0.001
D2 (μg/L), median (Q1, Q3)	0.33 (0.22, 0.48)	0.57 (0.33, 1.24)	9.60	<0.001
IgA (mg/dL), median (Q1, Q3)	299.00 (237.00, 372.00)	209.00 (160.75, 274.75)	11.13	<0.001
IgG (mg/dL), median (Q1, Q3)	1020.00 (801.00, 1200.00)	729.00 (455.75, 985.25)	9.56	<0.001
IgM (mg/dL), median (Q1, Q3)	95.50 (68.00, 133.00)	94.55 (65.25, 137.00)	0.14	0.893
IgE (IU/mL), median (Q1, Q3)	42.50 (16.05, 105.00)	52.10 (20.95, 164.50)	2.85	0.004
C3 (mg/dL), median (Q1, Q3)	107.00 (93.70, 117.00)	109.50 (93.05, 126.25)	1.79	0.074
C4 (mg/dL), median (Q1, Q3)	24.70 (21.50, 29.90)	26.35 (20.30, 32.10)	1.30	0.194

IgAN: Immunoglobulin A nephropathy; Q1: 25th percentiles; Q3: 75th percentiles; C3: Complement 3; C4: Complement 4; CK: Creatine kinase; Cr: Creatinine; D2: D-dimer; DB: Direct bilirubin; FIB: Fibrinogen; HDL: High density lipoprotein cholesterol; IgA: Immunoglobulin A; IgE: Immunoglobulin E; IgG: Immunoglobulin; IgM: Immunoglobulin M; LDH: Lactate dehydrogenase; LDL: Low density lipoprotein cholesterol; TB: Total bilirubin; TC: Total cholesterol; TG: Triglyceride; TP: Total protein; Ua: Uric acid; UN: Urea nitrogen.

**Discussion**

IgAN has a high incidence worldwide and a large proportion of IgAN patients will progress to ESRD.<sup>[7]</sup> Therefore, it is of great value to evaluate the disease severity and development of IgAN. Currently, although several IgAN biomarkers have been extensively researched, none have been applied for its screening in clinical practice. Moreover, a number of computational studies have been performed on many types of kidney disease, but none have focused on noninvasively diagnosing IgAN. In the present study, we used retrospective data to analyze the serological parameters and pathological stages of patients with IgAN and to establish a noninvasive diagnostic model for IgAN.

Based on the statistical analyses and clinical experience, 15 out of 20 routine and useful parameters were selected as

predictors of IgAN: TP, TB, DB, Cr, Ua, TC, TG, LDH, HDL, LDL, FIB, D2, IgA, IgG, and IgE [Table 2]. Compared with previous studies, this study included more characteristics, including fibrinogen, D-dimer, serum IgA, and C3, all of which are known biomarkers of kidney disease.<sup>[16,28]</sup> In addition, ROC analysis was performed to further assess the diagnostic value of the 15 parameters and the results showed that the AUCs of TP, TC, FIB, D2, IgA, and IgG were all more than 0.7 [Table 3]. Although serum IgA appears not to be a specific biomarker of IgAN, previous studies have reported that IgA levels are still statistically different and have differentially diagnostic value, especially when combined with other clinical parameters.<sup>[29]</sup> Berthoux *et al* reported that IgG was a biomarker for the prediction of clinicopathologic recurrence events in IgAN.<sup>[30]</sup> Our non-IgAN group consists mainly of membranous nephropathy, minimally pathological nephropathy, mesangial proliferative glomerulonephritis and other diseases characterized by nephrotic syndrome. Patients with nephrotic syndrome are often in a state of hypercoagulability, hyperfibrinolysis, hyperlipemia, and hypoproteinemia.<sup>[31]</sup> This may explain why the relevant index levels for blood clotting and blood lipids were higher in the non-IgAN group than in the IgAN group, such as FIB, D2, and TC, while the TP levels were lower in the non-IgAN group than in the IgAN group.

The multivariate logistic regression analysis requires that each explanatory variable is independent.<sup>[32]</sup> Based on our clinical experience, the level of TP is not independent of IgA and IgG levels. Therefore, we removed TP and selected the other 5 parameters for further analysis. Given that the incidence of IgAN varies from age to age, we added the age variable to the logistic regression model. The predicted probabilities were calculated based on logistic regression analysis [Table 4]. The AUC of the “TC+FIB+D2+IgA+age” combination was 0.86, with a sensitivity of 85.98% and a specificity of 73.85% [Figure 1]. The established diagnostic model that combined multiple factors (TC, FIB, D2, IgA, and age) might be used for IgAN noninvasive diagnosis.

We found that the levels of UN and Cr were significantly higher in grade V patients than those in other grades. Furthermore, apparent increases in TP, DB, and IgA were observed in grades II–V compared with grade I [Table 5]. Many studies have shown that elevated Ua, serum Cr and

**Table 3: Receiver operating characteristic analysis for the diagnostic value of the 15 clinical parameters for immunoglobulin A nephropathy.**

Items	AUC	95% CI
TP (g/L)	0.78	0.75–0.82
TB (μmol/L)	0.61	0.57–0.65
DB (μmol/L)	0.66	0.62–0.70
Cr (μmol/L)	0.58	0.54–0.61
Ua (μmol/L)	0.58	0.53–0.62
TC (mmol/L)	0.73	0.69–0.76
TG (mmol/L)	0.57	0.53–0.62
LDH (IU/L)	0.68	0.64–0.72
HDL (mmol/L)	0.63	0.59–0.67
LDL (mmol/L)	0.61	0.57–0.64
FIB (g/L)	0.74	0.70–0.77
D2 (μg/L)	0.71	0.67–0.75
IgA (mg/dL)	0.74	0.71–0.78
IgG (mg/dL)	0.71	0.67–0.74
IgE (IU/mL)	0.56	0.52–0.60

AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; Cr: Creatinine; D2: D-dimer; DB: Direct bilirubin; FIB: Fibrinogen; HDL: High density lipoprotein cholesterol; IgA: Immunoglobulin A; IgE: Immunoglobulin E; IgG: Immunoglobulin; LDH: Lactate dehydrogenase; LDL: Low density lipoprotein cholesterol; TB: Total bilirubin; TC: Total cholesterol; TG: Triglyceride; TP: Total protein; Ua: Uric acid.

**Table 4: Multivariate logistic regression analysis for immunoglobulin A nephropathy.**

Items	β	SE	Wald	P	OR	95% CI	
						Lower	Upper
TC	-0.237	0.064	13.711	<0.001	0.789	0.695	0.894
FIB	-0.457	0.095	22.979	<0.001	0.633	0.525	0.763
D2	-0.397	0.137	8.432	0.004	0.672	0.514	0.879
IgA	0.008	0.001	68.834	<0.001	1.008	1.006	1.010
Age	-0.043	0.008	32.564	<0.001	0.958	0.943	0.972
Constant	2.389	0.508	22.147	<0.001	10.901		

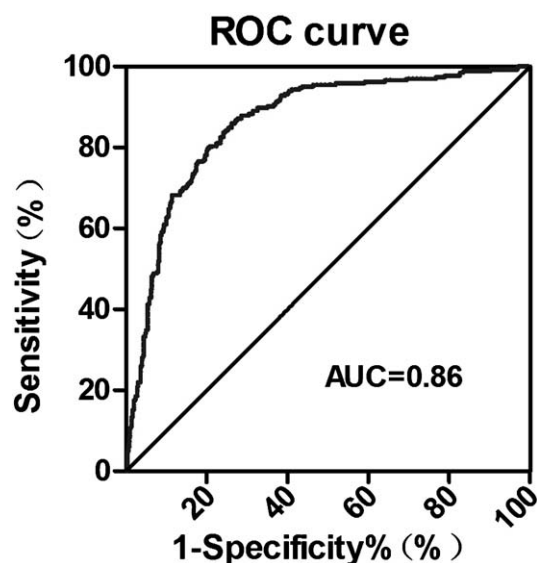
β: Logistic regression coefficient; CI: Confidence interval; D2: D-dimer; FIB: Fibrinogen; IgA: Immunoglobulin A; OR: Odds ratio; SE: Standard error; TC: Total cholesterol.



**Table 5: Pathological grades and laboratory test results for the immunoglobulin A nephropathy patients.**

Items	Pathological grades				
	Grade I	Grade II	Grade III	Grade IV	Grade V
Cases, <i>n</i> (%)	6 (2.21)	48 (17.65)	170 (62.50)	32 (11.76)	16 (5.88)
UN (mmol/L)	6.06±3.63	5.92±2.97	5.41±1.73	8.41±3.72	13.57±5.95*
Cr (μmol/L)	80.42±24.75	103.79±72.72	96.41±33.79	163.04±47.51	292.19±162.21*
TP (mg/d)	37.47±5.69 <sup>†</sup>	64.45±7.56	67.16±6.94	63.22±8.56	61.41±10.86
DB (μmol/L)	0.74±0.57 <sup>†</sup>	2.34±1.23	2.58±1.40	1.91±0.97	1.81±1.44
IgA (g/L)	227.17±68.12 <sup>†</sup>	310.35±103.78	318.48±107.54	292.58±81.85	323.29±181.67

\* Compared to grade I-IV, the level in grade V was statistically significant,  $P < 0.05$ . <sup>†</sup>Compared to grade I, the level in grade II-V was statistically significant,  $P < 0.05$ . Cr: Creatinine; DB: Direct bilirubin; IgA: Immunoglobulin A; TP: Total protein; UN: Urea nitrogen.



**Figure 1:** The diagnostic value of the “TC+FIB+D2+IgA+age” combination for immunoglobulin A nephropathy as detected using receiver operating characteristic analysis. The AUC was 0.86, with a sensitivity of 85.98% and a specificity of 73.85%. AUC: area under the receiver operating characteristic curve; D2: D-dimer; FIB: Fibrinogen; IgA: Immunoglobulin A; ROC: Receiver operating characteristic; TC: Total cholesterol.

other indicators are associated with an increased risk of IgAN.<sup>[33,34]</sup> However, there are limited reports on the relationship between these blood indicators and pathological grades or other indicators reflecting the severity of the disease.

Several strengths of our study should be stated. First, readily available clinical parameters such as patient demographics were applied. Second, all clinical characteristics were derived from biopsy-proven patients with IgAN. These patients were probably representative of patients with increased diagnostic uncertainty, which is the most challenging patients encountered in clinical practice. Lastly, our models were internally validated. However, this study has a few limitations that must be considered. First, this study was not a longitudinal investigation but rather a cross-sectional study. We are unable to determine the impact of these altered indicators on the pathogenesis of IgAN and whether this elevation is progressive or reversible. Second, because the study individuals were all

of Chinese descent, we cannot ensure that our research results are applicable to individuals of other ethnic backgrounds. Third, this was a single-center study; further multicenter studies and large cohort studies should be conducted for validation.

In conclusion, the established diagnostic model that combined multiple factors (TC, FIB, D2, IgA, and age) could effectively distinguish IgAN patients from non-IgAN patients, with high sensitivity and specificity. TP, DB, IgA, Cr, and UN could be used to evaluate IgAN disease severity.

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#### Conflicts of interest

None.

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