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Clinical diagnostic performance of common laboratory indicators in primary glomerulonephritis

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Abstract. – OBJECTIVE: Immunoglobulin A nephropathy (IgAN) and membranous nephropathy (MN) are common types of primary glomerulonephritis (PGD). A lack of specific clinical features makes diagnosis difficult. Kidney function indicators have been used for their diagnosis. However, the diagnostic performance of these indicators is undetermined. The purpose of this paper is to evaluate their diagnostic potential.

PATIENTS AND METHODS: 101 patients with PGD were enrolled, including 50 with MN and 51 with IgAN. The healthy controls included 110 volunteers. The indicators related to kidney function, including TP, ALB, Cre, CysC, eGFR, C1q, Ure, Anti-PLA2R, complement C3, and complement C4 in serum, ACR in urine, and antinuclear antibody profile, IgG staining, IgA staining, IgM staining, C3 staining and C1q staining in tissue samples were evaluated.

RESULTS: Statistical differences were found in TP, ALB, Ure, CysC, eGFR, C1q, Anti-PLA2R, complement C3, complement C4 and ACR among the three groups of subjects. ROC analysis showed that Anti-PLA2R and ACR had the highest specificity for identifying IgAN and/or MN from the healthy controls, ACR had the highest sensitivity. The Sp and Se of IgA and IgG in tissue samples for the identification of IgAN and MN were both high. Both IgAN and MN were predicted by anti-PLA2R, especially MN. In tissue samples, MN patients were more likely to be IgG positive and IgAN patients were more likely to be IgA positive.

CONCLUSIONS: IgAN and MN may be differentiated using serum Anti-PLA2R, tissue IgG, and tissue IgA. Cre is only useful in middle and late stages of GPDs, ACR is an exclusion marker, and CysC and C1q cannot be used to identify MN.

Key Words:
Membranous nephropathy, IgA nephropathy, Anti-PLA2R, Kidney function indicator, Immunohistochemical staining.

Introduction

Primary glomerulonephritis (PGD) is a common hypersensitivity disease of the kidney and is characterized by glomerular injury. There are two major pathological types of glomerulonephritis: immunoglobulin A nephropathy (IgAN) and membranous nephropathy (MN)[1,2]. Each accounts for approximately 40% of PGDs. The prevalence of both IgAN and MN has been increasing year by year, and the occurrence of comorbidities, shortened life expectancy and the use of immunosuppressive drugs have posed serious challenges for clinicians to make diagnosis and treatment decisions.

Up to 50% of patients with IgAN may progress to end-stage kidney disease in the next 20 years or so. End-stage kidney disease can only be treated with dialysis or transplantation, which may result in a reduced quality of life and a significantly higher risk of death[3]. IgAN is diagnosed by kidney tissue biopsy, which shows IgA deposits in the thylakoid membrane.

Similar to IgAN, MN is also diagnosed by kidney biopsy, demonstrating diffuse immune complex deposits under the basement membrane epithelium of the glomeruli. The KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases (KDIGO) recommends antibody against the M-type phospholipase-A2-receptor (Anti-PLA2R) as a biomarker for MN[4]. In addition to its high sensitivity in detecting MN in subjects with normal kidney function, Anti-PLA2R also has a high detection rate in non-MN patients, such as 44.4% in lupus nephropathy and 29.0% in IgAN[5].

The application of kidney biopsy in clinical practice is limited due to its invasive nature.

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Traditional kidney function indicators, such as serum creatinine (Cre), estimated glomerular filtration rate (eGFR), and urinary albumin (UAlb), have been commonly used to guide treatment and monitor disease progression[6]. However, the diagnosis of IgAN and MN has been controversial. In this paper, we evaluated the performance of some kidney function indicators commonly used in clinical practice and those identified recently in the diagnosis of IgAN and MN.

Patients and Methods

Subjects

To ensure the validity of the results, the number of samples in IgAN group, MN group and healthy control (HC) group should not be less than 46, as estimated by PASS 11.0.7 v. software (NCSS, USA). In this way, 101 patients with PGD admitted to Mianyang Central Hospital from June 2021 to November 2021 were selected, including 51 cases of IgAN and 50 cases of MN. There were 44 women aged 15-72 years (mean age 40.4±15.3) and 57 men aged 9-74 years (mean age 48.4±14.4).

Inclusion criteria: all cases underwent kidney biopsy and the diagnosis of MN or IgAN was confirmed by the pathological examination. According to the KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases[3], patients were divided into IgAN group and MN group.

Exclusion criteria: (1) patients who received kidney dialysis or nephrectomy; (2) patients with acute kidney disease or acute kidney insufficiency; (3) patients with autoimmune diseases such as systemic lupus erythematosus, dry syndrome, rheumatoid arthritis, ankylosing spondylitis, scleroderma and polyarteritis nodosa; (4) patients combined with hyperparathyroidism, hyper/hypothyroidism, abnormal liver function, malignancy, or hematological diseases; (5) patients combined with urinary tract infection, gallbladder stones, or cholecystitis; (6) patients with hypertension, diabetes, cerebrovascular accident, or cardiovascular diseases; (7) pregnant patients.

For HC group, 110 healthy individuals who received physical examinations during the same period were also included. There were 52 male cases, aged 19-67 years (mean 47.4±13.5 years); and 58 female cases, aged 25-64 years (mean 41.5±11.8 years).

All subjects signed the informed consent. The study was approved by the Human Ethics Committee of Mianyang Central Hospital (S201400048, S2018085).

Sample Collection

Blood sample: After fasting overnight, the blood samples (5.0 ml each) were collected into SST IIAdvance® serum sampling tube (containing separation gel/fibrinase procoagulant), from the elbow vein of each subject in the early morning (8:00-10:00). The serum was isolated by centrifugation at 3,000 g for 15 min within 2 h.

Urine sample: Within 30 min after collecting the blood sample, the subjects were instructed to collect the midstream specimen of urine (approximately 10.0 ml each).

Tissue samples: The patients were in prone position and the left (right) renal parenchyma was punctured with an (18G) TUR-CUT puncture needle under local anesthesia and ultrasound guidance, and 2 to 3 strips of renal tissue with a length of approximately 1.0 to 1.5 cm were aspirated.

Laboratory Examinations

Serum total protein (TP), Albumin (Alb), Urea (Ure), Cre, Complement C1q (Complement C1q) and Cystatin C (CysC) were measured on a LABOSPECT 008AS fully automated biochemistry analyzer (Hitachi, Japan). Complement C3 and complement C4 levels in serum were measured on a BN II specific protein analyzer (SSME, Shanghai, China).

The eGFR is calculated by the following eGFR formula developed by our laboratory[7]: eGFR (ml/min/1.73min) = 78.64 x CysC (mg/L)$^{-0.964}$.

Urinary albumin (UAlb) and urinary creatinine (UCr) levels were determined on the BioSystems A25 fully automated protein analyzer (BioSystems S.A., Spain). The albumin creatinine ratio (ACR) was calculated as follows: ACR (mg/g) = UAlb (mg/L)/UCr (g/L).

C1q kits were provided by Shanghai Beijia Biochemical Reagent Co. (Shanghai, China). Anti-PLA2R kits were provided by Nanjing Novozymes Medical Technology Co. (Nanjing, China). The remaining kits were provided by Sichuan Mike Biological Co. (Sichuan, China).

The commercial kits used for kidney biopsy specimens were purchased from EliVision™ plus (Maixin. Bio, Fujian, China). All operations were performed according to the kit instructions. The

semi-quantitative results were determined using a 4-level classification method[8,9]: "-" is not visible under low magnification; "+/-" is visible under high magnification; "+" is visible under low magnification; "1+" is visible under low magnification and clearly visible under high magnification; "2+" is clearly visible under low magnification and clearly visible under high magnification; "3+" is clearly visible under low magnification and dazzling under high magnification. Fluorescence intensity was determined as follows: "-" and "+/-" are scored as 0; "1+" is scored as 1; "2+" is recorded as 2 points; "3+" and above is recorded as 3 points.

Statistical Analysis

Experimental data were statistically analyzed and plotted using the software SPSS 19.0 (SPSS, IBM Corp., Armonk, NY, USA) or MedCalc v.18.2 (MedCalc Software Ltd., Ostend, Belgium). Measurement data of normal distribution were expressed as mean ± standard deviation and compared with one-way ANOVA and the LSD *t*-test if there is homogeneity of variances, or with Welch's approximate ANOVA and Dunnett's T3 test if there is no homogeneity of variances. Measurement data of non-normal distribution were expressed as median (interquartile spacing) [M (P_{25}, P_{75})], and the Kruskal-Wallis H rank sum test and the Bonferroni was used for pairwise comparisons. Categorical data were expressed as MN, IgAN and HC, which were compared with R × C columnar chi-square test. All statistical tests were two-sided, and a p-value <0.05 indicated statistical significance.

Results

Comparison of Demographic Characteristics and laboratory Indicators

The demographic characteristics and laboratory test results of subjects are listed in Table I. Age (F=15.865, p<0.001) and gender (χ^2=13.471, p<0.001) were statistically significant among the three groups of subjects. The levels of serum CysC, C1q, eGFR, Ure, C3, C4 and Anti-PLA2R were significantly different among three groups (all p<0.05), as well as urinary ACR (p<0.05). Serum Cre (χ^2=1.502, p=0.226) was not significantly different.

There were statistical differences in age (t=5.498, p<0.001) and gender (χ^2=17.610, p<0.001) between subjects in the IgAN and MN groups. For the hematological and urinary indicators, the levels of CysC (z=-2.622, p=0.011), C1q (t=2.412, p=0.015), Cre (t=46.682, p<0.001), C4 (t=-2.012, p=0.046) and ACR (z=6.942, p<0.001) were significantly different between the IgAN and MN groups. However, no statistically significant differences were found in eGFR, Ur, Cre and C3 (all p>0.05). For the analysis of tissue samples, the IgAN and MN groups were significantly different in IgG (χ^2= 126.464, p<0.001), IgA (χ^2=108.399, p<0.001) and IgM (χ^2=12.058, p=0.001). However, there was no statistical difference between the IgAN and MN groups in C3, C1q, anti-n-RNP, anti-Sm, anti-SSA, anti-SSB, anti-Ro-52, anti-ds-DNA, anti-Nuc, anti-His, anti-Rib and anti-nuclear antibodies (all p>0.05).

These results showed that the age of IgAN patients was lower than that of MN patients; and IgAN was more prevalent in women, whereas MN was more prevalent in men. The observed indicators in blood, urine and tissue samples were different between IgAN and MN patients.

Predictive Value of Each Indicator in IgAN and MN

Multinomial logistic regression analysis was used to observe the dominance ratio of each indicator for IgAN and MN, and the results are shown in Table II. Relative to HC group, the risk of Anti-PLA2R being elevated in IgAN was OR (95% CI) = 1.249 (1.057, 1.478), and in MN was OR (95% CI) = 1.346 (1.128, 1.604). The risk of ALB reduction in IgAN was OR (95% CI) = 0.597 (0.479, 0.744) and in MN was OR (95% CI) = 0.443 (0.341, 0.577). Relative to IgAN, the risk of elevated Anti-PLA2R in MN was OR (95% CI) = 1.077 (1.019, 1.139); the risk of decreased ALB in MN was OR (95% CI) = 0.743 (0.645, 0.855); the risk of elevated IgG staining in MN was OR (95% CI) = 141.779 (10.709, 1876.963); and, the risk of elevated IgA staining in MN was OR (95% CI) = 0.016 (0.001, 0.258).

These findings revealed that elevated anti-PLA2R and decreased ALB were good predictors of both IgAN and MN, with more pronounced predictive value in MN. In tissue samples, positive IgG staining was more likely to occur in MN, while positive IgA staining was more common in IgAN.

Diagnostic Performance of Serum and Urine Indicators in IgAN and MN

To analyze the diagnostic performance of serum and urine indicators in IgAN and MN, ROC

Table I. Subject demographic characteristics and laboratory test results.

Observed indicators	IgAN (n = 51)	MN (n = 50)	HC (n = 110)	χ_2/F, p
Demographic characteristics				
Age	37.57 ± 13.62*	52.32 ± 13.34	44.42 ± 12.74	15.865, < 0.001
sex (M/F)	20/31*	37/13	26/29	13.471, < 0.001
Blood				
TP	68.32 ± 7.56*	48.07 ± 9.49	72.89 ± 3.09	176.324, < 0.001
ALB	40.55 ± 5.45*	24.34 ± 6.90	46.18 ± 2.60	242.772, < 0.001
CysC	1.03 (0.85, 1.18)*	1.17 (0.98, 1.40)	0.86 (0.77, 0.94)	44.747, < 0.001
C1q	204.80 ± 36.60*	220.10 ± 37.70	205.80 ± 27.10	3.217, 0.043
eGFR	75.7 (65.7, 92.5)	67.1 (56.9, 79.4)	90.9 (83.5, 100.9)	46.682, < 0.001
Ure	5.85 ± 1.76	6.52 ± 2.70	4.79 ± 1.01	10.869, < 0.001
Cre	79.0 ± 49.8	75.8 ± 21.6	68.5 ± 14.6	1.502, 0.226
Hb	132 ± 15	136 ± 17	134 ± 8	0.937, 0.394
C3	1.13 ± 0.17	1.14 ± 0.24	1.05 ± 0.15	3.501, 0.033
C4	0.26 ± 0.07*	0.29 ± 0.13	0.21 ± 0.04	11.504, < 0.001
Anti-PLA2R	16.33 ± 9.81*	81.40 ± 58.37	11.35 ± 2.02	76.277, < 0.001
Urine				
ACR	662.1 (260.6, 1267.9)*	4745.8 (1946.3, 8508.2)	4.7 (3.7, 5.7)	123.348, < 0.001
Tissue				
Anti-n-RNP	50/1/0/0	48/2/0/0	--	0.371, 0.617
Anti-Sm	51/0/0/0	49/1/0/0	--	1.416, 0.495
Anti-SSA	50/0/0/1	47/1/1/1	--	2.194, 0.317
Anti-SSB	51/0/0/0	50/0/0/0	--	0.000, 1.000
Ro-52 resistant	48/1/0/2	48/1/0/1	--	0.644, 1.000
Anti-ds-DNA	49/2/0/0	50/0/0/0	--	2.773, 0.295
Anti-Nuc	51/0/0/0	50/0/0/0	--	0.000, 1.000
Anti-His	50/0/1/0	50/0/0/0	--	1.356, 0.511
Anti-Rib	51/0/0/0	48/0/0/2	--	1.437, 0.492
Anti-nuclear antibodies	47/4/0/0	43/7/0/0	--	1.997, 0.357
IgG	49/2/0/0*	0/1/2/47	--	126.464, < 0.001
IgA	0/6/8/37*	45/4/0/1	--	108.399, < 0.001
IgM	8/39/0/4*	22/28/0/0	--	12.058, 0.001
C3	12/8/26/5	10/9/25/6	--	0.425, 0.961
C1q	51/0/0/0	50/0/0/0	--	0.000, 1.000

IgAN: IgAN group; MN, MN group; HC, healthy control; TP, total protein; ALB, albumin; CysC, cystatin C; eGFR, glomerular filtration rate; Ure, urea; Cre, serum creatinine; CysC, serum cystatin C; C1q, serum complement C1q subunit; Hb, hemoglobin; ACR, urinary albumin/creatinine ratio; Anti-PLA2R: anti-PLA2R antibody. Comparison of the three groups, $p<0.05$. Comparison with the MN group, $*p<0.05$. Number of patients with pathological grading of 0/1/2/3.

Table II. Predictive effect of serum, urine indicators and immunofluorescence index on two types of kidney disease.

Observed indicators	OR (95% CI)	Wald χ^2	p
Anti-PLA2R	1.249 (1.057, 1.478)	6.798	0.009
	1.346 (1.128, 1.604)	10.930	0.001
	1.077 (1.019, 1.139)	6.931	0.008
ALB	0.597 (0.479, 0.744)	21.081	< 0.001
	0.443 (0.341, 0.577)	36.646	< 0.001
	0.743 (0.645, 0.855)	17.088	< 0.001
IgG	--	--	--
	--	--	--
	141.779 (10.709, 1876.963)	14.131	< 0.001
IgA	--	--	--
	--	--	--
	0.016 (0.001, 0.258)	8.542	0.003

First row: IgAN *vs*. HC; second row: MN *vs*. HC; third row: IgAN *vs*. MN, "OR (95% CI)" indicates the dominance ratio and its 95% confidence interval, $p < 0.05$.

analysis was conducted. The maximum value of Youden index [YI = specificity (Sp) + sensitivity (Se)-1] was used as the cutoff value. The results are shown in Table III and Figure 1. It showed that for identifying IgAN from the healthy subjects (IgAN *vs.* HC), ACR had the highest Sp (100.0%) and Se (98.0%), while Anti-PLA2R (Sp=94.6%), Ure (Sp=83.6%), Cre (Sp=98.2%) and CysC (Sp=90.9%) had high Sp, but all had low Se (25.5%-54.9%). For identifying MN from healthy subjects (MN *vs.* HC), ACR (Sp=100.0%, Se=100.0%) and anti-PLA2R (Sp=100.0%, Se=92.0%) had the highest Sp and very high Se, but the Sp of the other indicators (CysC=90.9%, Ure=78.2% and Cre=50.9%) and their Se (Cre=74.0%, CysC=72.0% and Ure=66.0%) were poor. For identifying IgAN and MN from the healthy subjects [(IgAN+MN) *vs.* HC], the ACR had the highest Sp (100.0%) and Se (99.0%), and Ure (Sp=78.2%, Se=60.4%) and Cre (Sp=98.2%, Se=22.8%) had good Sp, but low Se. Additionally, the Sp (52.2%) and Se (72.5%) of CysC were poor. For discriminating IgAN and MN (IgAN *vs.* MN), only Anti-PLA2R (Sp=78.4%, Se=90.0%) and ACR (Sp=92.0%, Se=74.5%) had good Se and Sp. However, the diagnostic performance of Cre (Sp=74.0%, Se=47.1%), CysC (Sp=52.0%, Se=72.5%) and Ure (Sp=16.0%, Se=96.1%) were less ideal.

Collectively, only ACR is valuable in identifying IgAN and MN from the healthy subjects. Both ACR and Anti-PLA2R had good diagnostic performance in identifying IgAN and MN from other groups.

Diagnostic Performance of Indicators in Tissue Samples for IgAN and MN

ROC analysis (taking the maximum value of YI as the cutoff value) in Table IV and Figure 2 show that, for identifying IgAN and MN (IgAN *vs.* MN), both IgA (Se=100%, Sp=98.0%) and IgG (Se=100%, Sp=98.0%) had good Sp and Se. IgM and had high Se (IgM=84.3%; C3=80.0%), but low Sp (IgM=44.0%; C3=23.5%). The result of C1q was not shown because the number of positive cases was too small for ROC analysis to be valid. Thus, IgA and IgG in tissue samples were effective indicators to discriminate between IgAN and MN.

Discussion

The incidence of both IgAN and MN is increasing year by year. However, the traditional kidney function markers are still used as the main basis for their diagnosis in clinical practice, which inevitably leads to misdiagnosis and underdiagnosis of IgAN and MN[9].

Table III. Diagnostic performance of the observed indicators in serum and urine for IgAN and MN.

Observed indicators	AUC (95% CI)	Cut-off	Se (%)	Sp (%)	YI	Z	p
Anti-PLA2R	0.633 (0.534-0.725)	14.0	45.1	94.6	0.40	2.321	0.020
	0.947 (0.885-0.981)	15.3	92.0	100.0	0.92	15.120	< 0.001
	0.789 (0.716-0.850)	15.3	65.4	100.0	0.65	7.965	< 0.001
	0.898 (0.822-0.950)	19.7	90.0	78.4	0.68	12.053	< 0.001
Ure	0.669 (0.571-0.757)	5.72	51.0	83.6	0.35	3.113	< 0.001
	0.709 (0.612-0.793)	5.31	66.0	78.2	0.44	3.941	< 0.001
	0.688 (0.610-0.760)	5.31	60.4	78.2	0.39	4.529	< 0.001
	0.552 (0.450-0.651)	9.0	96.1	16.0	0.12	0.902	0.367
Cre	0.540 (0.440-0.637)	90.3	25.5	98.2	0.24	0.688	0.491
	0.580 (0.480-0.676)	65.7	74.0	50.9	0.25	1.405	0.1601
	0.560 (0.478-0.639)	90.3	22.8	98.2	0.21	1.284	0.199
	0.547 (0.445-0.647)	66.4	47.1	74.0	0.21	0.814	0.416
CysC	0.732 (0.637-0.813)	1.01	54.9	90.9	0.46	4.532	< 0.001
	0.870 (0.790-0.928)	1.0	72.0	90.9	0.63	10.400	< 0.001
	0.651 (0.550-0.744)	1.2	72.5	52.0	0.25	2.759	0.006
	0.651 (0.550-0.744)	1.2	72.5	52.0	0.25	2.759	0.006
ACR	0.980 (0.932-0.997)	11.49	98.0	100.0	0.98	24.500	< 0.001
	1.000 (0.965-1.000)	11.5	100.0	100.0	1.00	--	<0.001
	0.990 (0.959-0.999)	11.5	99.0	100.0	0.99	49.500	<0.001
	0.901 (0.825-0.951)	1127.7	74.5	92.0	0.67	13.127	<0.001

Se is sensitivity, Sp is specificity, YI = Se + Sp-1, and AUC (95% CI) is the area under the curve with 95% confidence interval. First row: IgAN *vs.* HC; second row: MN *vs.* HC; third row: (IgAN + MN) *vs.* HC; fourth row: IgAN *vs.* MN.

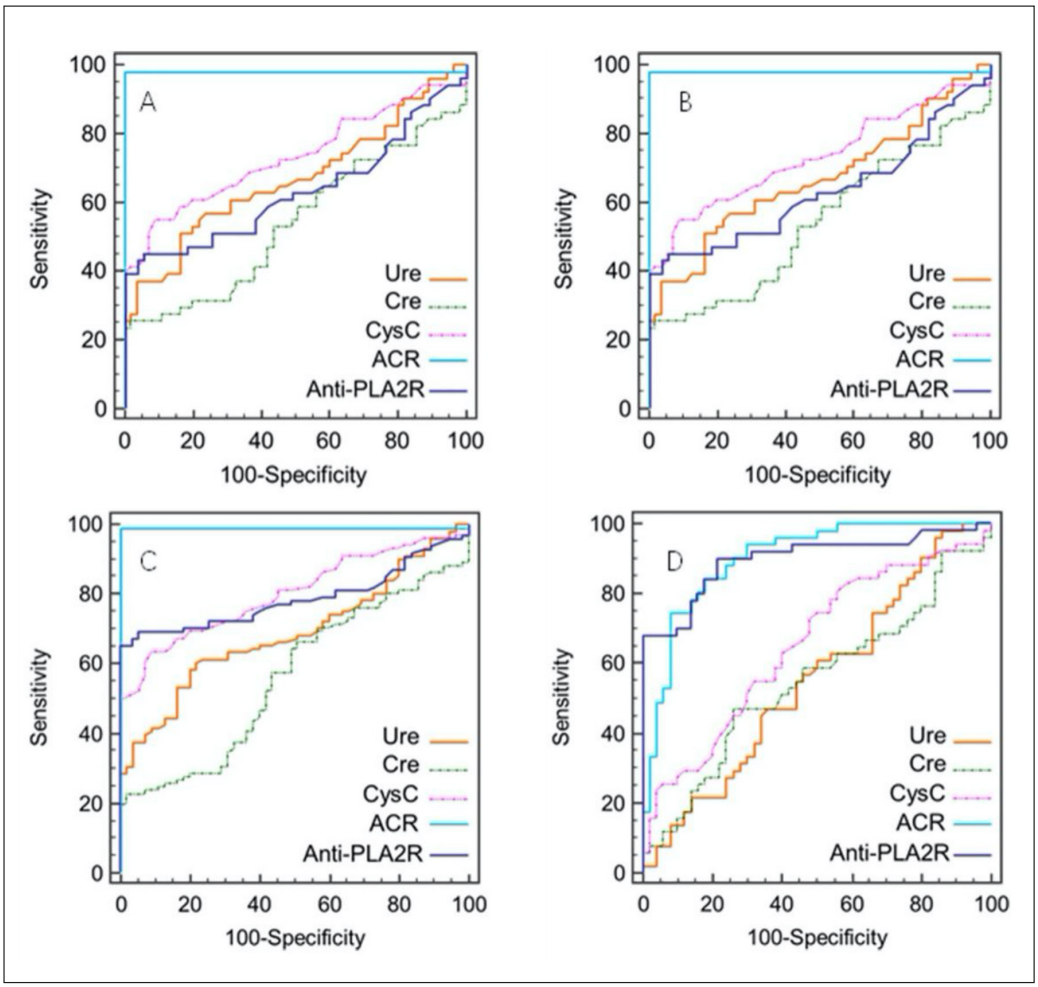

Figure 1. Laboratory serum and urine assay metrics for MN and IgAN performance evaluation. **A,** The diagnostic performance of identifying IgAN from the healthy group (IgAN *vs*. HC). **B,** Diagnostic performance of identifying MN from healthy group (MN *vs*. HC). **C,** Diagnostic performance in identifying the two nephropathies from the healthy group [(IgAN+MN) *vs*. HC]. **D,** Diagnostic performance of discriminating IgAN and MN (IgAN *vs*. MN). The evaluation of the diagnostic performance results showed that both ACR and Anti-PLA2R had good diagnostic performance in identifying IgAN and/MN.

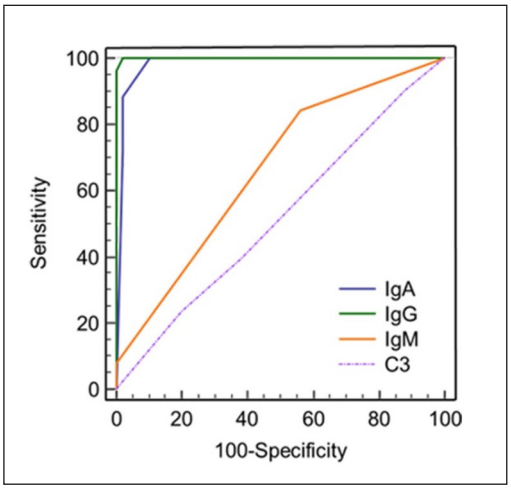

Figure 2. ROC analysis of MN and IgAN by immunofluorescence detection index. The diagnostic performance to identify IgAN and MN (IgAN *vs*. MN). The evaluation of the diagnostic performance results showed that IgA and IgG were effective indicators to discriminate between IgAN and MN in tissue samples.

In this study, there was no significant difference in serum Cre between HCs and IgAN/MN patients, suggesting that both IgAN and MN patients in this study may be in the early stage of the disease. A significant change in serum Cre will only occur when the filtration function of the kidney is reduced by 50% of its normal level, and thus serum Cre is not a sensitive indicator of kidney function[10]. Consequently, as with other chronic kidney diseases (CKDs), serum Cre is not a reliable indicator of early IgAN or MN. Otherwise, the possibility of underdiagnosis is very high, unless the disease has progressed to intermediate or advanced stages[11]. Although serum CysC (calculated value eGFR) varied significantly between the three groups and was statistically different between HCs and either IgAN or MN, there was a substantial overlap between any two of the three groups. The performance of serum CysC in diagnosing IgAN or MN, especially in the differential diagnosis, is extremely low[12]. The results of this study showed that the Se of CysC in diagnosing IgAN was only 54.9%, and in diagnosing MN was only 72.0%. It is clear that serum CysC and its calculated value eGFR are not reliable markers for the diagnosis of MN or IgAN[13]. Serum C1q cannot differentiate between healthy subjects and IgAN patients, and although it is elevated to some extent in MN patients, the results overlap mostly with those of healthy subjects. Therefore, serum C1q cannot be used to diagnose IgAN[14] or MN[15].

Several studies[16-18] have reported that Anti-PLA2R, which is identified in recent years, is a specific indicator for the diagnosis of MN. However, our study found that Anti-PLA2R was also elevated in some IgAN patients, and although the degree and extent of elevation were not as high as those in MN patients, the results overlapped with MN patients to some extent. Therefore, the presence of other CKDs, especially IgAN disease, should be excluded when using Anti-PLA2R to diagnose MN.

According to the experimental results, ACR was the best indicator for the diagnosis of IgAN or MN with a Sp of up to 100% and a Se close to 100%. But this result can only indicate that the patients must have albuminuria after the occurrence of IgAN or MN. However, there are many diseases that can cause albuminuria, such as liver cirrhosis[19], diabetes mellitus, and retinopathy induced by diabetes mellitus[20], and cardiovascular diseases (such as hypertension, atherosclerosis, and heart failure)[20-22]. In addition, the determination of UAlb is demanding. For example, the method must be sufficiently sensitive, the antibody must be polyclonal, and the traceable urine calibrator must be used instead of the plasma protein calibrator[23]. Therefore, it is correspondingly difficult to diagnose IgAN or MN by ACR, but it is indeed very useful as an exclusion criterion.

Tissue immunofluorescence staining is often used along with pathological examination for the diagnosis of diseases. In this study, we found that although the onset of both MN and IgAN is related to autoimmunity, their antinuclear antibody profiles were mostly negative, which is not meaningful for their diagnosis. Immunohistochemical fluorescence staining for IgG and IgA is a reliable method to differentiate MN from IgAN, and it has been reported that the diagnostic performance of tissue IgG staining is better than tissue anti-PLA2R staining in the early stage of MN[24]. IgA staining has a higher diagnostic value for IgAN, which is consistent with its pathogenesis[25]. Therefore, if the differential diagnosis of MN and IgAN cannot be determined, tissue immunofluorescence detection of IgG and IgA can be a valuable option. Although IgM staining showed significant differences between MN and IgAN, its results were weakly positive and thus its value for differential diagnosis was limited. The C3 and C1q staining cannot distinguish MN patients from IgAN patients and may not be meaningful until the stage of renal failure[26].

In analyzing the age of subjects, we found that IgAN patients were younger while MN patients were older. For this reason, age matched HCs were included for IgAN and MN groups, respectively. Statistical analysis revealed that except complement C1q and tissue immunofluorescence detection of IgG (**Supplementary Table**), the conclusions drawn from HCs based on age subgroups were consistent with those from HCs without grouping. Thus, the HC age subgroups were combined for analysis, and the sample size of HC group was approximately one times larger than that of IgAN and MN groups. The reason is that, in clinical practice, a patient cannot determine whether he is IgAN or MN at the first diagnosis, so he can only be diagnosed step by step after single-blind screening.

According to KDIGO guidelines[3], anti-PLA2R should be measured by ELISA and immunofluorescence. This study used a quantum dot fluorescence immunoassay to measure anti-PLA2R. Whether the test results of these

two methods are completely consistent, the performance evaluation results of the manufacturer are only borrowed in the process of this study, and our laboratory has not verified it[27].This is a retrospective study. Some new serological markers were not evaluated, such as serum IgA/C3, and IgA-Fibronectin, as well as urinary markers urinary IgG and β-2 microglobulin. Secondary nephropathy was not included in this study. Primary IgAN and secondary IgAN have the same immunological properties[28], which cannot be distinguished, while anti-PLA2R has no distinguishable value for primary and secondary MN[29]. Therefore, further studies are needed to distinguish the primary and secondary nephropathy.

Conclusions

Among the laboratory indices we selected, the serum anti-PLA2R had a good diagnostic performance in identifying both IgAN and /MN. Serum Cre is not a reliable indicator of early IgAN or MN, unless the disease has progressed to intermediate or advanced stages. Serum CysC (calculated value eGFR) has a substantial overlap between any two of the three groups. Serum C1q cannot differentiate between healthy subjects and IgAN patients, and although it is elevated to some extent in MN patients, the results overlap mostly with those of healthy subjects. Therefore, both serum CysC (used to calculate eGFR) and serum C1q cannot be used to diagnose IgAN or MN. Although urine ACR has extremely high specificity and sensitivity, it is indeed very useful as an exclusion criterion due to the excessive number of causes of elevated ACR. Immunohistochemical fluorescence staining for IgG and IgA is a reliable method to differentiate MN from IgAN, MN patients were more likely to be IgG positive and IgAN patients were more likely to be IgA positive. Only commonly used markers of the traditional kidney function were observed in this study, and some new markers recommended for renal impairment were not evaluated in this study because their clinical application had not been promoted. Therefore, new indicators of PGD with high specificity and sensitivity may be to be discovered in further studies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethics Approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Human Ethics Committee of Mianyang Central Hospital, School of Medicine, University of Electronic Science and Technology of China (protocol code: S201400048, S2018085).

Informed Consent

Informed consent was obtained from the patients, or their family involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Authors' Contribution

Data curation: X.-H. Li, W.-Y. Yang, Y.-W. Yang, C.-M. Dai and J.-F. Feng; Formal analysis: X.-H. Li, C.-M. Dai, and J.-F. Feng; Investigation: J. Wang; Methodology: X.-H. Li, Y.-W. Yang; Project administration: X.-H. Li and J.-F. Feng; Resources: J.-F. Feng; Statistical analysis: X.-H. Li, J. Wang, B. Zhang and Y.-W. Yang; Supervision: J.-F. Feng; Validation: X.-H. Li; Writing original draft: X.-H. Li and W.-Y. Yang; Writing review & editing: J.-F. Feng.

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