

# Elevated Serum Chloride Level Contributes to the Poor Prognosis of IgA Nephropathy

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## Research Article

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# Abstract

## Introduction

Finding reliable prognostic factors is crucial for IgA nephropathy (IgAN). Here, we determined the relationship between prognosis of IgAN with serum chloride.

## Methods

Primary IgAN diagnosed by renal biopsy from January 1, 2015 to April 1, 2019 were recruited. Patients were divided into lower group and higher group based on the best cut-off value of survival receiver operating characteristics (ROC) curves. The baseline clinicopathological characteristics were retrospectively compared. Cox proportional hazard models were used to demonstrate the prognostic value of serum chloride in IgAN. Prognosis prediction model was built by multivariate Cox regression.

## Results

Compared to higher group, age, 24-hour urinary protein and serum creatinine (Cr) in the lower group were significantly lower, hemoglobin (Hb) and albumin were significantly higher (all  $P < 0.05$ ), the degree of endothelial cell proliferation (E) and renal tubule atrophy or renal interstitial fibrosis (T) were significantly lighter (all  $P < 0.05$ ). Univariate and multivariate Cox analysis revealed that serum chloride  $\geq 105.4$  mmol/l was an independent risk factor for the prognosis of IgAN ( $P < 0.05$ ). Serum chloride, Cr, T, hypertension, and Hb were screened out as features in predictive prognosis model. The c-index of the model was 0.85–0.82 and 0.77 for 1–2 and 3 years respectively, and brier scores were 0.06–0.09 and 0.16 respectively.

## Conclusions

Serum chloride  $\geq 105.4$  mmol/l was an independent risk factor for the prognosis of IgAN. A predictive prognosis model including serum chloride, Cr, T, hypertension and Hb exhibited a relatively good prediction effect.

## Introduction

Primary IgA nephropathy (IgAN) is the most common type of idiopathic glomerulonephritis (GN) throughout the world and the main cause of ESRD in patients with primary glomerular disease [1]. To date, predictors of prognosis in IgAN based on clinical, pathological, genetic, and noninvasive biological markers have been gradually discovered [2]. However, these parameters either not sensitive enough, or traumatic and expensive. Thus, we are committed to identifying a more convenient and accurate predictor of prognosis in IgAN.

A variety of electrolyte and acid-base alterations predictably occur with progressive loss of kidney function [3–6]. Most of the disorders are intricately linked to morbidity and mortality of kidney disease patients [7], especially potassium imbalance, metabolic acidosis and derangement of bone mineral metabolism. Chloride ion is the highest anion in the extracellular fluid, which plays a crucial role to regulate amount of functions in human body [8] including the maintenance of osmotic pressure, acid–base balance, muscular activity, and the movement of water between fluid compartments. More and more studies have begun to recognize the importance of chloride, especially with the excavation and exploration of acid-base [9, 10] and chloride ion channels [11–13]. Little of studies have assessed the relationship between serum chloride and the renal prognosis of IgAN, but we can get some clues from the relationship between serum chloride and kidney injury. Based on previous studies, serum chloride disorder may adversely affect renal function. Compared with hypochloremia, it is currently believed that hyperchloremia is more closely related to the severity of kidney injury [14, 15]. High chloride can cause thromboxane release [16] and enhance the response of renal vasoconstrictors, such as angiotensin II [17]. In addition, high chloride can induce a glomerular feedback mechanism in dense plaques, causing contraction of afferent arterioles, contraction of the glomerular mesentery and reductions in glomerular filtration rate [18]. In addition, high chloride often accompany with metabolic acidosis [9, 10]. Perchloric acidosis increases the production of endothelin-1 and aldosterone, leading to tubulointerstitial inflammation and injury, thereby accelerating the progression of CKD [7]. Although rare, there are reports revealing that low chloride is also associated with kidney damage [15]. Therefore, we hypothesize that serum chloride disorder will affect the clinical and pathological indicators and prognosis of IgAN. The main objective of this study is to determine the relationship between serum chloride and disease severity as well as prognosis in patients with IgAN.

## Materials And Methods

### Study Population

Patients were identified from a retrospective, unit-institutional database. Patients with a biopsy-based diagnosis of primary IgAN between January 1, 2015 and April 1, 2019 by the First Affiliated Hospital of Zhengzhou University were enrolled in the study. The inclusion criteria

included the following: (1) primary IgAN was diagnosed by renal biopsy, (2) follow-up time was greater than 6 months, (3) no glucocorticoid immunosuppressant was used before renal biopsy, (4) an initial estimated glomerular filtration rate (eGFR)  $\geq 15$  mL/min/1.73 m<sup>2</sup> at the time of renal biopsy. The following exclusion criteria were employed: (1) patients with secondary IgA nephropathy, including chronic hepatitis B, Henoch-Schonlein purpura, ankylosing spondylitis, systemic lupus erythematosus, and rheumatoid arthritis; (2) the number of glomeruli in renal biopsy specimens was less than 10; (3) clinicopathological data were incomplete; (4) eGFR < 15 mL/min/1.73 m<sup>2</sup>; (5) patients with acute kidney failure at the time of renal biopsy.

### **Clinical, biochemical, and histopathological data collection**

The baseline clinicopathological data were recorded at the time of the renal biopsy. Hypertension was defined as systolic BP (SBP)  $\geq 140$  mmHg, diastolic BP (DBP)  $\geq 90$  mmHg, or use of antihypertensive drugs. Oxford classification of each patient was scored by two renal pathologists blinded to clinical data<sup>[19]</sup>. Immunofluorescence results were scored as follows: 0 represents -/+, 1 for +, 2 for ++, 3 for +++, and 4 for +++++. The degree of vascular injury (A) was assessed as follows: 0 indicates no obvious abnormality, 1 represents simple vascular wall thickening, and 2 represents vascular wall thickening and other lesions, such as fibrinoid necrosis and vitreous degeneration. Renal tubular necrosis was scored as follows: 0 indicates no necrosis, and 1 indicates necrosis. The treatment plan was based on KDIGO guidelines<sup>[20]</sup>.

In order to analyze prognosis, double serum creatinine (Cr) level from baseline data, ESRD, or greater than 30% eGFR decline were used as the definition of the composite endpoint<sup>[21]</sup>. ESRD was defined as eGFR  $\leq 15$  mL/min per 1.73 m<sup>2</sup> or need for RRT (including hemodialysis, peritoneal dialysis, or renal transplantation) for the purpose of this study.

### **Statistical methods**

SurvivalROC package in R version 3.6.3 was used to calculate the best cut-off point of serum chloride. Normally distributed quantitative variables were expressed as means  $\pm$  SDs, and based on homogeneity of variance, the t-test or corrected t-test were used for comparisons between groups. Non-normally distributed features were expressed as medians and interquartile ranges, and the Wilcoxon rank sum test was used for comparisons between groups. Quantitative data were expressed by frequency and percentage, and the chi-square test was used for the comparison between groups. Unadjusted and the multivariable-adjusted Cox proportional hazards models were used to analyze the relationship between serum chloride and the prognosis of IgAN. Age, sex, hypertension, 24-H UP, Cr, Oxford classification scores were adjusted in multivariable-adjusted Cox proportional hazards models. To further expand the applicability of the result, we built a predictive prognosis model. Clinicopathological variables associated with survival were assessed a priori based on univariate Cox regression, clinical importance, and predictors identified in previously published articles<sup>[22, 23]</sup>. And forward-backward selection with the Akaike information criterion (AIC) was used to identify variables for the models. Therefore, serum chloride, hypertension, Cr, hemoglobin (Hb), and renal tubule atrophy or renal interstitial fibrosis (T) were included in the model. The internal stability of the model was tested using the bootstrap approach. The model performance was evaluated based on the predictive accuracy for individual outcomes (discriminating ability) and the accuracy of point estimates of the survival function (calibration). SPSS 21 software (SPSS Inc., Chicago, IL, USA) and R software (version 3.6.3, <http://www.R-project.org>) were used for statistical analysis. Values of *P* less than 0.05 were considered statistically significant.

## **Results**

### **Demographic and Clinicopathological Characteristics**

From January 1, 2015 to April 1, 2019, 394 patients with primary IgAN were recorded. The clinical characteristics at baseline were summarized in Table 1. In total, 212 (54%) males and 182 (46%) females were included in the study, and the mean age at biopsy was 35  $\pm$  12 years. 154 (39%) patients had hypertension. The mean serum chloride was 104.10  $\pm$  3.47 mmol/L. 267 (68%) patients accepted steroid and immunosuppressant therapy. Patients were followed for 14 (9-24 months) months, and 46 patients reached the composite endpoint.

### **Grouping based on blood chloride levels**

According to survivalROC, The areas under the receiver operating characteristics curve (AUCs) were 0.63, 0.70, and 0.61 for 1, 2 and 3 years respectively, and the best cut-off points were 105.50 for 1 year, 105.40 mmol/L for 2 and 3 years (shown in Fig. 1). To summarize the results, the patients were divided into two groups according to the cut-off value 105.40 mmol/L because no significant difference in the subsequent statistical analysis results was noted regardless of whether 105.40 mmol/L or 105.50 mmol/L serum chloride was used as the cut-off point. Lower group had 247 patients and higher group had 159 patients.

### **Relationships between the serum chloride and demographic, clinical, and histopathological data**

The clinical characteristics of the 394 patients in the lower group and the higher group are shown in Table 1. The age of the lower group was younger compared with the higher group [34  $\pm$  12 vs 36  $\pm$  12 years old], the 24-H UP and Cr in the lower group was less than that in the higher

group [1.46 (0.84, 3.44) vs 2.64 (1.28, 4.98) g; 83.0 (67.0, 111.3) vs 94.5 (70.0, 145.0)  $\mu\text{mol/l}$ ]. Albumin and Hb levels in the lower group were increased compared with the higher group [39.10(34.30, 42.90) vs 33.80 (24.95, 38.95) g/l; 130.4 $\pm$ 19.3 vs 125.2 $\pm$ 19.1g/l]. In addition, we found that blood urea nitrogen, complement 3, sodium, calcium, magnesium, and immunoglobulin G also exhibited significant differences between the two groups. No significant difference in other parameters were noted between the two groups.

In addition, endothelial cell proliferation(E) and T were mild in the lower group [179 (77)/53 (23) vs 90 (60)/61 (40); 172 (74)/29 (13)/31 (13) vs 94 (62)/18 (12)/40 (26)]. No significant difference in other pathological results were noted between the two groups.

### **Serum chloride in predicting IgAN prognosis**

Firstly, we performed univariate survival analysis. The result showed that serum chloride $\geq$ 105.40mmol/l was associated with the composite end point(hazard ratio (HR), 3.22; 95% confidence interval (95%CI), 1.76-5.86,  $P<0.001$ )(Table 2). Then we applied three adjusted Cox proportional hazards models. When adjusted for sex, age, Cr, 24-H UP, hypertension, and Oxford classification scores, serum chloride $\geq$ 105.40mmol/l still had significant association with poor renal outcome of IgAN(Model 1: HR, 3.09; 95%CI, 1.69-5.64,  $P<0.001$ ; model 2: HR, 2.33; 95%CI, 1.20-4.55,  $P= 0.01$ ; model 3: HR, 2.05; 95%CI, 1.03-4.07,  $P= 0.04$ ). These results revealed that serum chloride $\geq$ 105.40mmol/l was an independent risk factor for the prognosis of IgA nephropathy (Table 2).

### **Predictive prognosis model based on clinical and histopathological parameters and internal validation**

We incorporated serum chloride $<$ 105.40mmol/l or  $\geq$ 105.40mmol/l, hypertension (yes or no), Cr, uric acid(UA), 24-H UP, serum phosphorus, Hb, T (T0, T1 or T3), mesangial hypercellularity [M(M0 or M1)], segmental glomerulosclerosis/adhesion [S(S0 or S1)], and the degree of vascular injury [A(A0, A1 or A2)] as prognostic features. All these parameters were reduced to the most useful 5 potential predictors (serum chloride group, Cr, T, hypertension and Hb) for survival using forward-backward selection with the AIC. A nomogram based on the prognosis model was constructed to estimate 1-3 years renal survival (shown in Fig. 2). Then, bootstrap validation with 200 resampling was employed for internal validation. The discriminative ability of the final model was assessed using C statistics. The c-index of this model was 0.82 (95% CI 0.68-0.94) for 1 year; 0.85 (95% CI 0.76-0.95) for the 2 years and 0.77 (95% CI 0.52-0.99) for the 3 years. Calibration was evaluated using calibration plots and brier scores. The Brier score of this model was 0.06 (95% CI 0.04-0.09) for the first year, 0.09 (95% CI 0.06-0.14) for the second year and 0.16(0.06, 0.30) for the third year (shown in Fig. 3).

## **Discussion**

Our study demonstrated that serum chloride levels at the time of renal biopsy is a sensitive and convenient index to predict and identify adverse renal outcomes.

Early identification or prediction of poor prognosis in patients with IgAN is often very difficult but critical. In recent years, an increasing number of IgAN prognosis indicators have been explored[24]. Among them, hypertension, massive proteinuria, renal impairment, albumin, and severe histological findings have been accepted widely[25]. However, these indicators also have some disadvantages, such as limited sensitivity or analysis methods are traumatic and expensive. Currently, biomarkers and genetic indicators are being explored gradually[26-28]. However, they are relatively expensive, which may impose an economic burden on some patients. Therefore, we are committed to identifying a convenient, cheap, and highly sensitive and specific indicator.

MARY A demonstrated that hypertonic NaCl in dogs lead to a transient renal vasodilation that was probably related to plasma hypertonicity followed by sustained renal vasoconstriction and reduced eGFR. Thromboxane played a very important role in this progression[16]. TANAKE demonstrated that in stroke-prone spontaneously hypertensive rats (SHRSP), Cl<sup>-</sup> likely amplified microangiopathy by exacerbating hypertension and potentially increasing plasma renin activity (PRA), which potentially on account of constricting the renal afferent arteriole[17]. In addition, chloride can induce a glomerular feedback mechanism in dense plaques that involves contraction of afferent arterioles, contraction of the glomerular mesentery and reductions in the glomerular filtration rate[29]. In addition, a high level of chloride often accompanies with metabolic acidosis. Metabolic acidosis caused by the loss of carbonate in the gastrointestinal tract and kidneys often leads to hyperchloremia[8]. Evidence on the relationship between metabolic acidosis and IgAN prognosis remains limited. However, perchloric metabolic acidosis is associated with accelerated CKD progression and elevates all-cause mortality, which could provide some clues [30, 31].

We used survivalROC to divide patients into two groups. Although a range of statistical methods are available for cut-off point selection[32, 33], but disease status was considered a fixed characteristic of the study subject in classic analysis[34]. There were researches revealed that time-dependent ROC could assist in developing eligibility criteria for clinical trials[21, 34]. In this study, we considered survival time, so chose survivalROC to group.

Comparing the baseline demographic, clinical and histopathological data and treatment modalities in the two groups, we found the clinical and pathological indicators of IgAN were more serious when the Cl<sup>-</sup> level  $\geq 105.4$  mmol/L.

Subsequently, we performed a prognostic analysis. According to univariate and multivariate COX regression, the serum chloride  $\geq 105.4$  mmol/L was an independent risk factor for IgA nephropathy. This result also suggested that 105.4 mmol/L serum chloride level was a very important threshold for IgAN. Clinically, due to differences in testing techniques, the normal high limit of serum chloride is within the range of 106-107 mmol/L. In this study, considering the prognosis of patients with IgAN, the normal high limit of serum chloride level was further accurate to 105.4 mmol/L. This finding suggested that clinicians should pay close attention to the change of serum chloride level when treating IgAN, and it is ideal to ensure the level is below 105.4 mmol/L.

In addition, we also established a clinical predictive prognosis model including Cr, serum chloride  $< 105.4$  mmol/L or  $\geq 105.4$  mmol/L, hypertension, T, Hb. Our predictive prognosis model was demonstrated a good discriminative ability according to c-index. However, calibration plots did not demonstrate very good agreement.

We thought more deeply about the above results. Firstly, in clinical work, 0.9% NaCl solution is widely used, while which contains more than physiological amounts of chloride ions[35]. This can cause hyperchloremia and metabolic acidosis[36, 37]. Based on the consideration of the influence of serum chloride level on IgAN, can we replace the NaCl solution with other solutions or restrict the use of NaCl solution? There is no research in this area yet. But there are some researches that give us some inspiration[18, 38]. Malley [38] used a double-blind method to compare the results of renal transplantation patients using lactated Ringer's solution and 0.9% normal saline. The results showed that the incidence of hyperchloremia and metabolic acidosis was higher in the 0.9% saline group. And Yunos[18] proved that restricting the use of chlorine-rich fluids in tertiary ICU could decreased the incidence of acute kidney injury and renal replacement therapy requirement. Therefore, we guess that the same result will be produced in IgAN. But it still depends on further confirmation. Secondly, 99.1% of chloride ions are reabsorbed in the kidney[29]. Is it possible to improve the prognosis of IgAN patients with high blood chloride levels by reducing the kidney's reabsorption of chloride ions? This sparked our interest to find pathways to inhibit the reabsorption of chloride ions. There are many mechanisms related to the reabsorption of chloride ions in the kidney[8, 39]. Among them, the most important process occurs in the latter half of the proximal tubule, where chloride ions are transported into the cell through the Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchanger at the top membrane of the epithelial cell. The chloride ions that enter the cell are transported to the intercellular fluid through the K<sup>+</sup>-Cl<sup>-</sup> symporter of the basilar membrane, and then absorbed into the blood. In addition, chloride ions can also be reabsorbed through paracellular pathway<sup>[39]</sup>. Therefore, we speculate whether it is possible to inhibit the reabsorption of chloride ions by inhibiting the transmembrane transport of chloride ions in the proximal renal tubules or the paracellular pathway, thereby reducing the blood chloride level of IgAN, and improving the prognosis of IgAN patients. However, this speculation has yet to be further verified.

In addition, many hazards are associated with reduced blood chloride levels[8]. We modeled chloride as a continuous variable using restricted cubic splines before this research to obtain a complete picture of the relationship between blood chloride and renal outcome of IgAN (shown in Fig. 4). Interestingly, a nonlinear association was observed for IgAN progression. This curve has an asymmetrical U-shape, and patients with serum chloride  $> 105$  mmol/L exhibited distinct associations with poor renal outcome ( $P < 0.001$ ). Moreover, although IgAN patients with serum chloride  $< 101$  mmol/L tended to have less favorable renal survival, the relationship was not statistically significant ( $P = 0.70$ ) (Table 3). Thus, our study did not consider the impact of reduced blood chloride levels on the prognosis of IgAN.

Our study has certain limitations. First, this was a retrospective single-center study, and our sample size was relatively small. In addition, the follow-up time was relatively short. Although we used rigorous statistical methods to correct confounding factors, some bias may affect the robustness of our results. In addition, it is worth noting that serum chloride levels may be affected by excessive use of saline solution during treatment. Unfortunately, our study did not delve into the causes of elevated blood chloride levels, so we could not determine whether elevated blood chloride levels were caused by the disease itself or by rehydration during treatment. In addition, our clinical predictive model does not include external validation to demonstrate its universality to a large population and other races. Although our study proves that low blood chloride levels are not a risk factor for IgA nephropathy, in general, low blood chloride levels are also harmful.

In conclusion, serum chloride levels  $\geq 105.4$  mmol/L is an independent risk factor for the prognosis of IgA nephropathy. The result suggested that we should pay more attention to blood chloride levels in IgAN.

## Declarations

### Acknowledgements

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### Author Contributions

Conceived and designed the experiments: Yaling Zhai and Xingchen Yao; Performed experiments: Xingchen Yao and Jingge Gao, Xinnian Wang; Analyzed the data: Yazhuo Chen; Contributed reagents/materials/analysis tools: Yaling Zhai and Zhazheng Zhao ; Wrote the paper: Xingchen Yao. All authors read and approved the final manuscript.

### Competing interests

The author(s) declare no competing interests.

### Data Availability Statement

Raw data used during the current study are available from the corresponding author on reasonable request for non-commercial use.

## Ethics declarations

The Medical Ethics Committee of The First Affiliated Hospital of Zhengzhou University approved the study protocol, and informed written consent was obtained from each participant. All methods reported here were carried out in accordance with relevant guidelines and regulations of The First Affiliated Hospital of Zhengzhou University.

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## Tables

Table 1 Relationships between the serum chloride ion and demographic and clinical data

Characters	total(n=394)	Lower Group (n=247)	Higher Group(n=159)	P value
<b>Demographic data</b>				
Age(years)	34±12	33±12	36.±12	0.05
Male(n%)	212(54)	125(52%)	87(57%)	0.392
<b>Clinical data</b>				
hypertension(n%)	154(39%)	89(36%)	65(41%)	0.309
BUN(mmol/l)	5.80(4.50,7.70)	5.62(4.40,7.15)	6.20(4.62,8.31)	0.043
Cr(umol/l)	85 (68,120)	83 (67,111)	95(70,145)	0.004
UA(umol/l)	356±111	350±111	367±111	0.135
ALB(g/l)	37.3(29.2,41.5)	39.1 (34.3,42.9)	33.8 (25.0,39.0)	<0.001
24-H UP(g/d)	1.86(0.95,4.04)	1.46(0.84,3.44)	2.64(1.28,4.98)	<0.001
Urine RBC(/HP)	42(9,181)	41 (10,161)	49 (8,187)	0.730
C3(g/l)	1.16(0.93,1.41)	1.20(0.98,1.48)	1.11(0.90,1.26)	0.004
C4(g/l)	0.26(0.21,0.32)	0.25(0.21,0.31)	0.28(0.21,0.33)	0.124
Hb(g/l)	128.4±19.4	130.4±19.3	125.2±19.10	0.010
K(mmol/l)	4.42±0.49	4.38±0.45	4.47±0.55	0.091
Na(mmol/l)	143±3	142±3	144±3	<0.001
Ca(mmol/l)	2.20±0.17	2.24±0.16	2.14±0.18	<0.001
Mg(mmol/l)	0.94±0.11	0.95±0.10	0.93±0.11	0.0156
P(mmol/l)	1.24±0.24	1.24±0.22	1.24±0.26	0.812
SlgA(g/l)	2.89(2.12,3.88)	2.86(2.17,3.95)	2.89(1.97,3.75)	0.459
SlgG(g/l)	9.69(6.57,11.80)	10.20(7.99,12.80)	8.63(5.32,10.97)	0.009
SlgM(g/l)	1.01(0.76,1.43)	1.06(0.83,1.40)	0.91(0.66,1.50)	0.137
<b>Histopathological data</b>				
M[M0/M1(n (%))]	295(75)/88(22)	186(80)/46(20)	109(72)/42(28)	0.069
E[E0/E1(n (%))]	269(68)/120(30)	179(77)/53(23)	90(60)/61(40)	<0.001
S[S0/S1(n (%))]	133(34)/250(63)	81(35)/151(65)	52(34)/99(66)	0.924
T[T0/T1/T2(n (%))]	266(68)/47(12)/71(18)	172(74)/29(13)/31(13)	94(62)/18(12)/40(26)	0.004
C[C0/C1/C2(n (%))]	222(56)/121(31)/11(3)	145(65)/73(33)/5(2)	77(59)/48(37)/6(5)	0.198
IgG[0/1/2(n (%))]	325(82)/50(13)/18(5)	195(82)/33(14)/11(5)	130(84)/17(11)/7(5)	0.491
IgM[0/1/2/3(n (%))]	115(29)/200(51)/66(17)/4(1)	70(30)/123(53)/38(16)/3(1)	45(30)/77(51)/28(18)/1(0.7)	0.835
IgA[0/1/2/3/4,n (%)]	4(1)/22(6)/277(70)/88(22)/1(0.25)	2(0.8)/10(4)/168(71)/57(24)/1(0.4)	2(1)/12(8)/109(71)/31(20)/0(0)	0.133
C3[0/1/2/3,n (%)]	108(27)/82(21)/165(42)/33(8)	62(26)/55(23)/100(42)/19(8)	46(31)/27(18)/65(42)/14(9)	0.909
A[0/1/2,n (%)]	133(34)/83(21)/178(45)	90(38)/46(19)/104(43)	43(28)/37(24)/74(48)	0.131
Tubular necrosis [0/1,n (%)]	375(95)/19(5)	229(95)/11(5)	146(95)/8(5)	0.782
GS%	0.96(0.00,0.27)	0.10(0.00,0.26)	0.09(0.00,0.34)	0.707
SS%	0.04(0.00,0.10)	0.03(0.00,0.10)	0.04(0.00,0.11)	0.703

BUN, blood urine nitrogen; Cr, serum creatinine; UA, urine acid; ALB, albumin; 24-H UP, 24-hour urine protein; Urine RBC, urine red blood cell; C3, complement 3; C4, complement 4; Hb, hemoglobin; K, potassium; Na, sodium; Ca, calcium; Mg, magnesium; P, phosphorus; SIgA, serum immunoglobulin A; SIgG, serum immunoglobulin G; SIgM, serum immunoglobulin M; For Oxford classification, M represents Mesangial cell proliferation (M0/1); E represents endothelial cell hyperplasia (E0/1); S represents segmental sclerosis or adhesion (S0/1); T represents renal tubule atrophy or renal interstitial fibrosis (T0/1/2), T0 represents 25% renal tubule atrophy or renal interstitial fibrosis, T1 represents 26%~50% and T2 ≥ 50%; C represents crescentic lesions (C0/1/2), C0 is no crescent, C1 is < 25% globular crescent, C2 is ≥ 25% globular crescent; For immunofluorescence, IgG, immunoglobulin G:0 represents -/+, 1 for +, 2 for ++; IgM, immunoglobulin M:0 represents -/+, 1 for +, 2 for ++, 3 for +++; IgA, immunoglobulin A:0 for -/+, 1 for +, 2 for ++, 3 for +++, 4 for +++++; C3, complement 3:0 for -/+, 1 for +, 2 for ++, 3 for +++; A, the degree of vascular injury: 0 means no obvious abnormality, 1 represents simple vascular wall thickening, 2 represents not only vascular wall thickening, but also other lesions, such as fibrinoid necrosis, vitreous degeneration and so on.; Renal tubular necrosis: 0 means no necrosis, 1 means necrosis; GS, glomerular sclerosis; SS, segmental sclerosis.

Table 2 Cox regression analysis of the effect of serum chloride ion on the prognosis of IgAN patients

Models	HR(95%CI)	P value
Unadjusted	3.22(1.76-5.86)	<0.001
Model1 <sup>a</sup>	3.09(1.69-5.64)	<0.001
Model2 <sup>b</sup>	2.33(1.20-4.55)	0.01
Model3 <sup>c</sup>	2.05(1.03-4.07)	0.04

HR, hazard ratio; 95%CI, 95% confidence interval.

<sup>a</sup>Model 1 was adjusted for sex and age. Sex was analyzed as dichotomous data.

<sup>b</sup>Model 2 was adjusted for covariates in model 1 plus Cr, 24-hour urine protein, hypertension(yes or no).

<sup>c</sup>Model 3 was adjusted for covariates in model 2 plus Oxford M (mesangial hypercellularity), E (the presence of endocapillary proliferation), S (segmental glomerulosclerosis/adhesion), T (severity of tubular atrophy/interstitial fibrosis), and C (presence of crescent) scores. The latter five variables were analyzed as categorical data.

Table 3 Association between serum chorine groups and the renal outcome of IgAN.

	β	HR(95%CI)	P value
Low group(reference=normal group)	0.21	1.23(0.43,3.50)	0.70
High group(reference=normal group)	1.19	3.28 (0.31,1.67)	<0.001

low group, serum chloride <101mmol/l, normal group, serum chloride between 101-105mmol/l and high group, serum chloride ≥105mmol/l. Low group and normal group not had significant difference( $P=0.70$ ) in renal outcome of IgAN, but high group and normal group had significant difference( $P<0.001$ ).

## Figures

serum chlorine ion and IgAN, Method=KM  
year=1

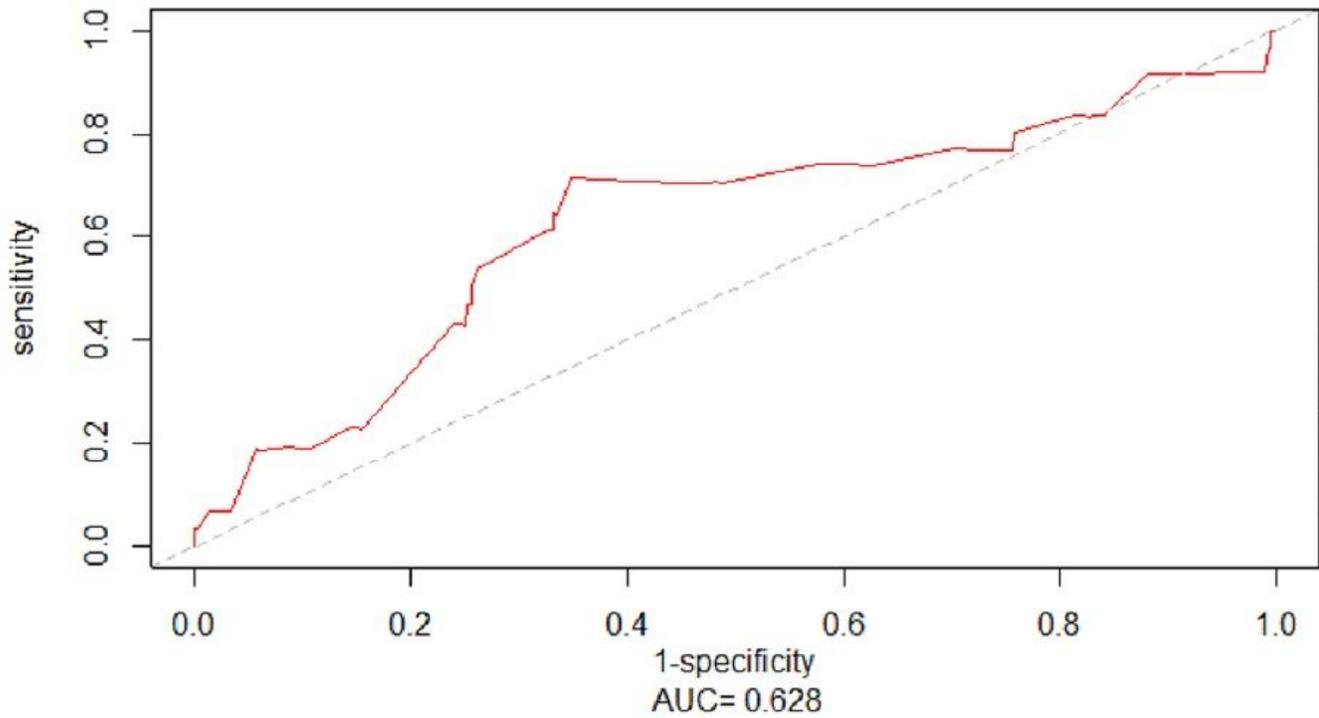
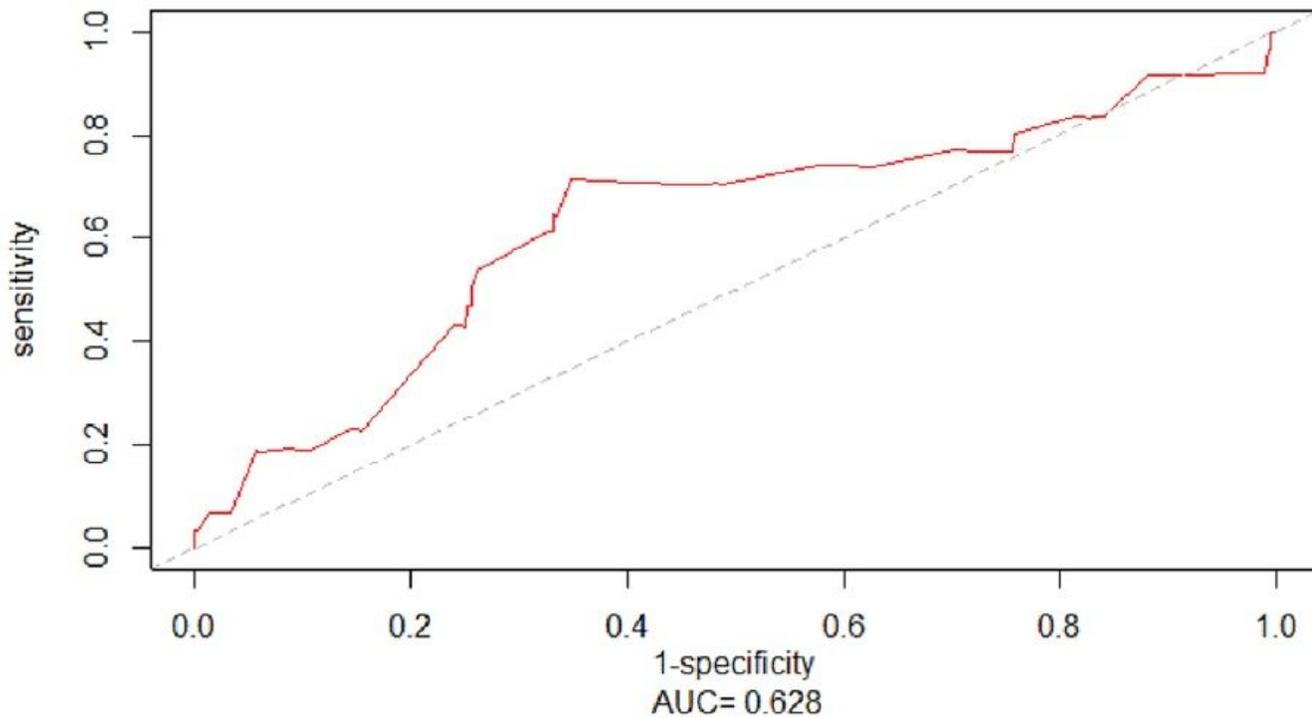


Figure 1

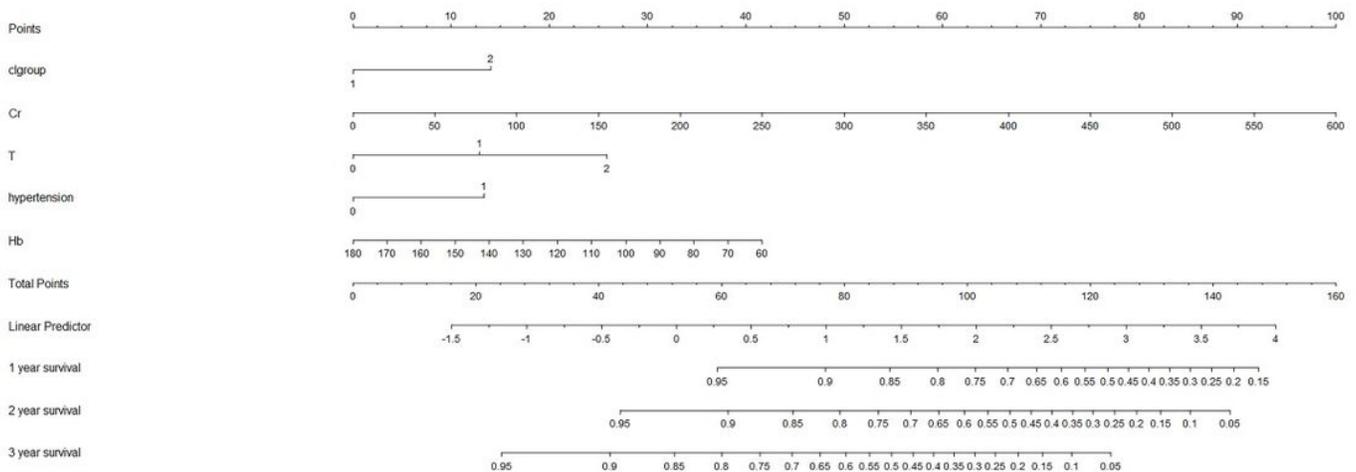
Survival receiver operating characteristics (ROC) curves for 1-3 years. The areas under the receiver operating characteristics curve(AUCs) were 0.63, 0.70 and 0.61 for 1-3 years follow-up respectively, and cut-off values were 105.50 for 1 year follow-up, and 105.40 for 2 and 3 years follow-up.

## serum chlorine ion and IgAN, Method=KM year=1



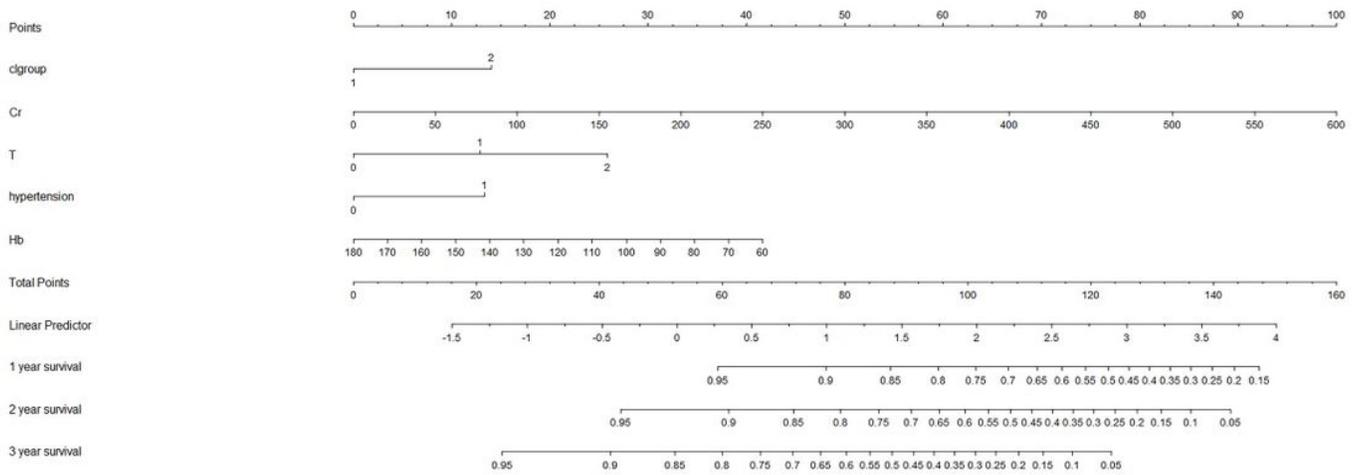
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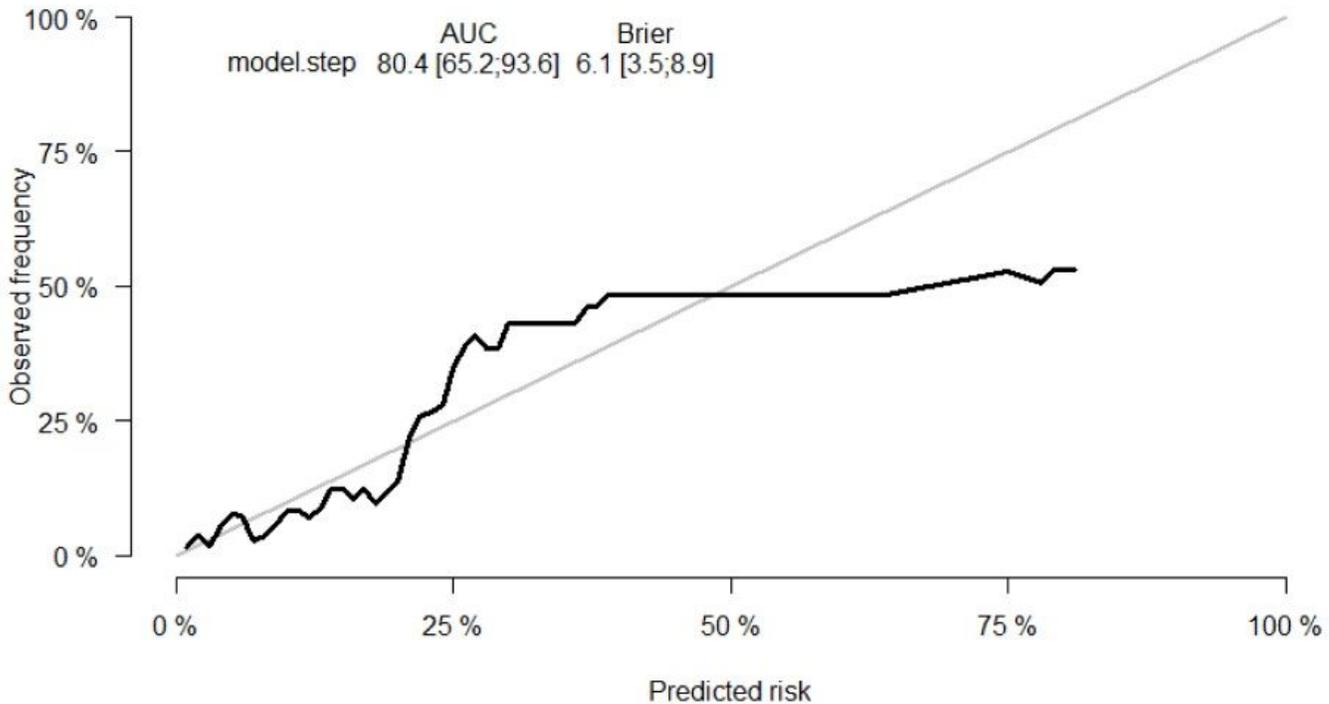
**Figure 2**

nomogram predicting the probabilities for 1, 2 and 3 years renal survival the nomogram based on the prognosis model(including Cr, hypertension, T, Hb and serum chloride( $<105.40\text{mmol/l}$  or  $\geq 105.40\text{mmol/l}$ )) were built to realize the visualization of the model. Higher total point based on the sum of the assigned number of points for each factor in the nomogram was associated with a worse prognosis.



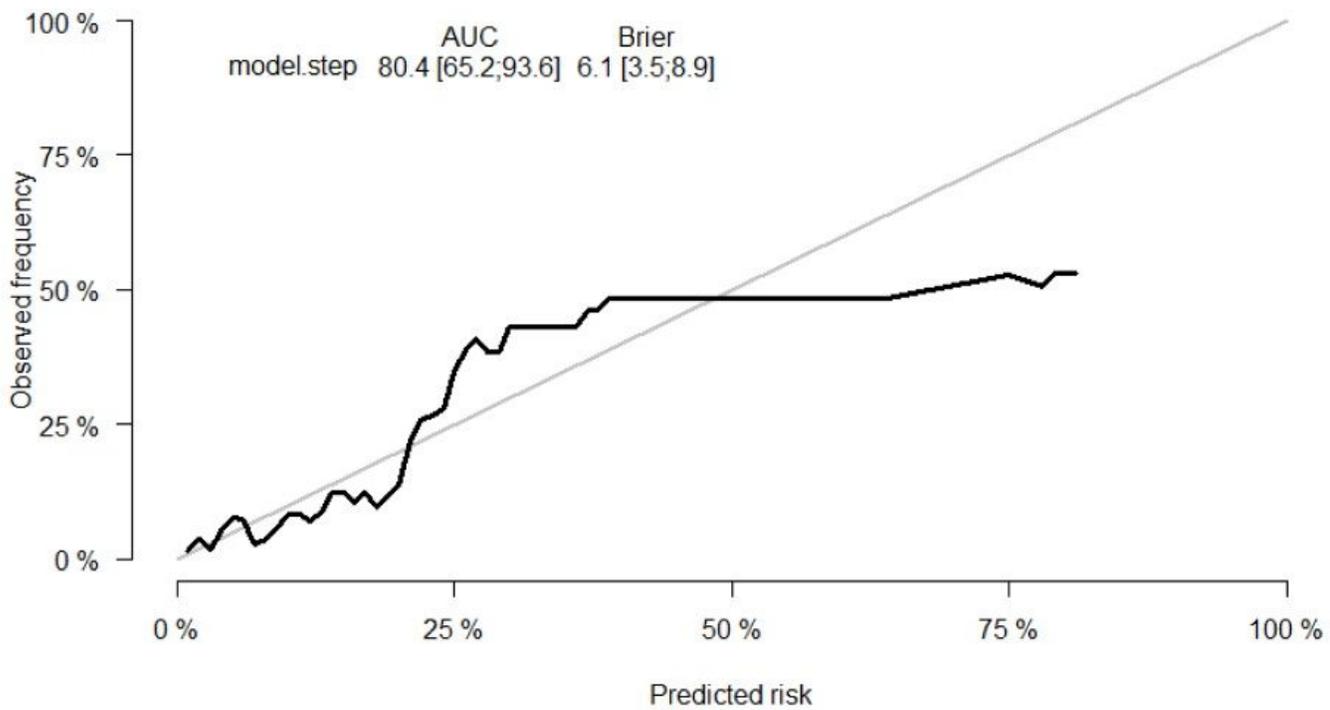
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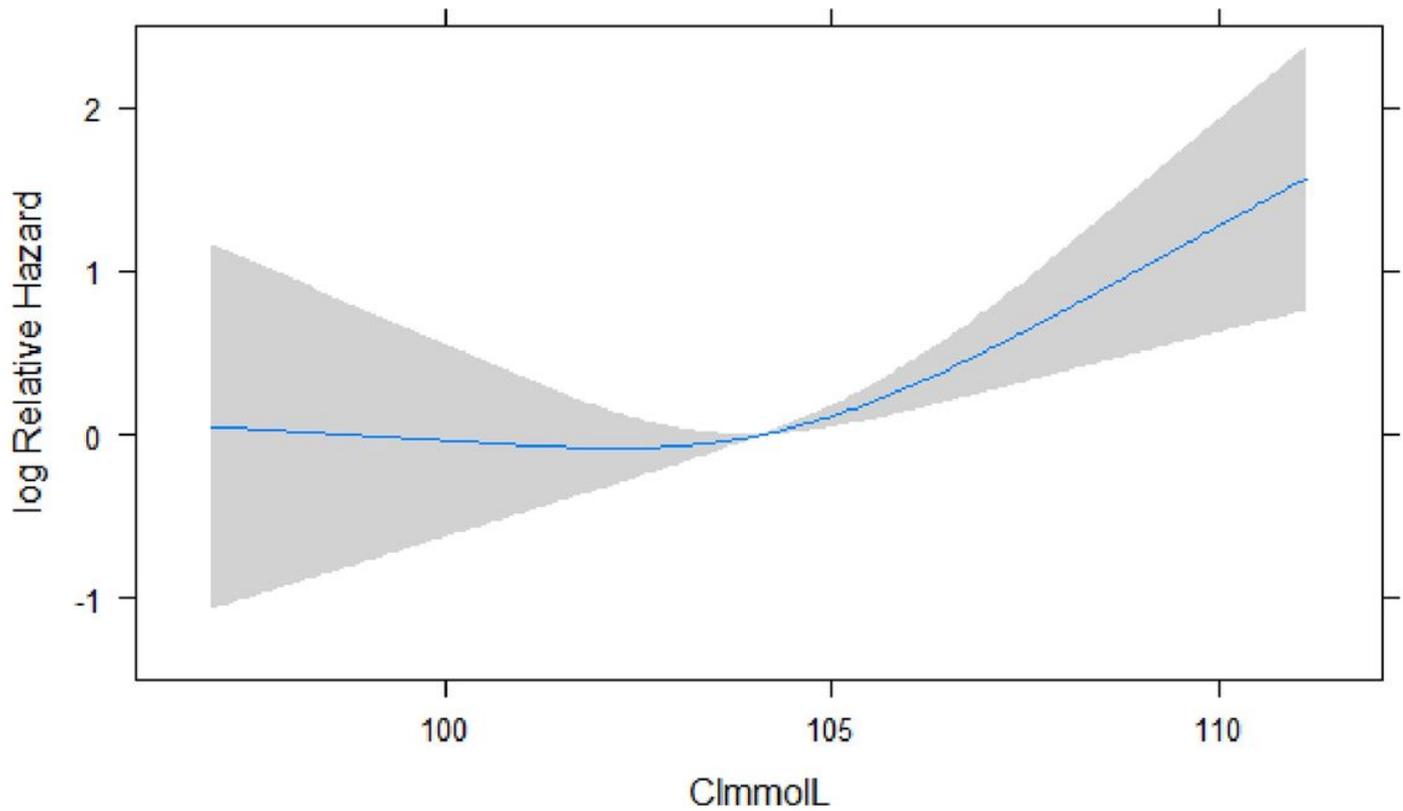
**Figure 3**

C-indexes and Calibration curves for the new model the 200-sample bootstrapped calibration plots for the prediction of 1-3 y renal survival are shown. The gray line represents the ideal fit; black line represents practical fit. C-indexes of 1-3 years were 0.80 (95% CI 0.76-0.94), 0.86 (95% CI 0.76-0.94) and 0.78 (95% CI 0.55-0.97) respectively; brier scores were 0.06 (95% CI 0.04-0.09), 0.09 (95% CI 0.05-0.13) and 0.16(0.06, 0.32) respectively.



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**Figure 4**

Restricted cubic splines to reveal the nonlinear relationship assumption between blood chloride and renal outcome of IgAN. Nonlinear association between serum chloride and renal outcome of IgAN. The solid line represents the estimated hazard ratio, the shaded area represents the 95% confidence interval. Patients with higher serum chloride(>105mmol/l) showed distinctly higher hazard ratios for the composite end point, whereas although patients with lower serum chloride(<101mmol/l) tended to have a less favorable renal survival, the relationship was not statistically significant.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalMaterial.pdf](#)
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