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Keywords:	renal anemia, IgA nephropathy, renal tubulointerstitial lesions

Related clinical and pathological factors of renal anemia in patients with IgA nephropathy: a cross-sectional study

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Abstract

Objective: Currently there are few studies with large population on renal anemia of IgA nephropathy worldwide. This cross-sectional study is to examine the clinical and pathological characteristics and influence factors associated with renal anemia in patients with IgA nephropathy which is the most common etiology of chronic kidney disease (CKD).

Methods: A total of 462 hospitalized patients with IgA nephropathy confirmed by renal biopsy who met the inclusion criteria were consecutively recruited from January 2014 to January 2016. Their general information, routine blood test, blood chemistry, estimated glomerular filtration rate (eGFR) and renal pathology were collected. The Oxford classification was applied to characterize renal pathology. The univariable and multivariate logistic regression model were used to analyze the influence factors of anemia in IgA nephropathy.

Results*:* The incidence of renal anemia was 28.5% (132/462) in our study (21.3% in males and 38.9% in females). The anemia type was primarily normocytic and normochromic. The ratio of anemia in patients with eGFR 30-59 ml/min/1.73m^2 was higher than eGFR>60 ml/min/1.73m^2 (42.9% versus 17.8%, P<0.001), notably in the group of eGFR < 15 ml/min/1.73m^2 the anemia ratio was 100%. Logistic regression analysis showed that factors affecting the anemia of IgA nephropathy including female (OR: 3.02, CI: 1.76-5.17), albumin (OR: 0.87, CI: 0.82-0.93), eGFR (OR: 0.98, CI:0.97-0.99) and renal tubulointerstitial lesions >50% (OR: 2.57, CI: 1.22-5.40).

Conclusions: The female, hypoalbuminemia, eGFR reduction, and severe renal tubulointerstitial lesion were correlation with the renal anemia in patients with IgA nephropathy, which showed new insight in understanding of anemia in IgA nephropathy and may improve the management and treatment of clinical renal anemia.

Key words: renal anemia; IgA nephropathy; renal tubulointerstitial lesions

Strengths and limitations of this study

Existing studies on the relationship between renal anemia and rrognostic stages of IgA nephropathy in 62 IgA nephropathy patients suggested that the mean level of hemoglobin (Hb) was lower in poor prognosis group than in good prognosis group, and the fibrosis and/or inflammatory cell infiltration in the tubulointerstitial region were more obvious in the patients with poor prognosis, but there is still lack of large population studies on the morbidity, types of anemia classification, influence factors of renal anemia among patients with IgA nephropathy.

we investigated the clinical and pathological characteristics and influence factors associated with renal anemia in patients with IgA nephropathy who were diagnosed by renal biopsy.

Due to the cross-sectional design of the study and all of the patients were need to meet the criterion of renal biopsy, it was not possible to determine a causal relationship between influencing factors and anemia and there was a selection bias in our study.

Introduction

Renal anemia is one of the most common complications of chronic kidney disease (CKD). This condition can accelerate the progression of renal function injury, induce cardiovascular events, reduce the quality of life of patients, and associated with poor prognosis.[1-4] Different types of CKD demonstrated different prevalence of renal anemia and different prognosis.[5-7]

IgA nephropathy currently remains the major etiology of the progression of CKD into end-stage renal disease (ESRD).[8] Especially in Asian Pacific regions, this condition primarily occurs in young male adults. If not well-controlled, approximately 25-45% of patients will progress to chronic renal failure within 20 years; this condition requires replacement therapies such as blood purification and poses great threats to public health.[9-11] Seiki Aruga, et al [12]reported that the levels of hemoglobin （Hb）, hematocrit (Ht), and red blood cells (RBC) of 62 IgA nephropathy patients gradually decreased according to progression of renal injuries, and the fibrosis and/or

inflammatory cell infiltration in the tubulointerstitial region were more obvious in the patients with poor prognosis.

However, there is still lack of large population studies on the morbidity, types of anemia classification, influence factors of renal anemia among patients with IgA nephropathy. In order to provide a basis for better understanding of anemia in IgA nephropathy patients, and improve the efficacy of renal anemia therapy, a total of 658 patients diagnosed with IgA nephropathy by kidney biopsy at the Center of Kidney Diseases from Jan 2014 to Jan 2016 were enrolled in this study and finally 462 patients with IgA nephropathy who met the study inclusion and exclusion criteria were taken into analysis.

Methods

Study design and subjects

This cross-sectional study was performed at the Department of Nephrology of Chinese PLA General Hospital. There were consecutive inpatients 18–70 years of age enrolled who were diagnosed with IgA nephropathy by renal biopsy from January 2014 to January 2016. The exclusion criteria for enrollment were as follows: (1) secondary IgA nephropathy, such as Henoch-Schonlein purpura (HSP) nephritis, systemic lupus erythematosus (SLE), HBV-related glomerulonephritis (HBV-GN), diabetic nephropathy; (2) ≤10 glomeruli in renal biopsy; (3) acute kidney injury (AKI), nephrotic syndrome or renal replacement therapy; (4) malnutrition, body mass index(BMI) < 18.5kg/m^2; (5) acute infection, patients with liver cirrhosis, cancer, gastrointestinal bleeding, female menstrual period or blood system disease; (6) patients on treatment of anemia drugs, glucocorticoids, immunosuppressive medication and Chinese herbs without defined components within the past 6 months. Finally, 462 eligible patients were analyzed, including 132 in the anemic group and 330 in the non-anemic group. All the patients with renal biopsy had signed the research protocol of the Renal Clinical Database Establishment when hospitalized, allowing their clinical data to be used for science purpose, and this study was

approved by the Ethics Committee of the Chinese PLA General Hospital. A flow chart is shown in Figure 1.

Data collection

We collected physical and clinical information including the gender, age, body mass index (BMI), blood pressure, Hb, erythrocyte mean corpuscular volume (MCV), erythrocyte mean corpuscular hemoglobin concentration (MCHC), C-reactive protein (CRP), serum creatinine (SCr), blood urea nitrogen (BUN), serum uric acid (SUA), serum albumin (ALB), serum prealbumin, total cholesterol (TC), triglyceride (TG), and total urinary protein (UPr) within 24 h from 462 IgA nephropathy patients.

Patients were classified by the chronic kidney disease (CKD) diagnostic criteria in the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines of 2006. They were stratified into 5 stages according to eGFR values: stage 1 (≥90mL/min/1.73m^2), stage 2 (60–89mL/min/1.73m^2), stage 3 (30–59mL/min/1.73m^2), stage 4 (15–29mL/min/1.73 m^2), and stage 5 (<15mL/min/1.73m^2). The estimated glomerular filtration rate (eGFR) was calculated using the CKD-epidemiology collaboration (CKD-EPI) formula.[13]

BMI was used the formula of weight (kg)/height (m^2).

Anemia was defined as Hb < 130 g/L in males and <120g/L in females.

If the criteria of MCV 80-100fl and MCHC 320-350g/L were met simultaneously, it was diagnosed as normocytic, normochromic anemia.

Pathology of renal injury was estimated by the Oxford classification of IgA nephropathy:[14] Mesangial score <0.5 (M0) or >0.5 (M1); Endocapillary hypercellularity absent (E0) or present (E1); Segmental glomerulosclerosis absent (S0) or present (S1); presence or absence of podocyte hypertrophy/tip lesions in biopsy specimens with S1; Tubular atrophy/interstitial fibrosis <25% (T0), 26%–50% (T1), or >50% (T2); Cellular/fibrocellular crescents absent (C0), present in at least 1 glomerulus (C1), in >25% of glomeruli (C2).

Patient and Public Involvement

There were no patients involved in the recruitment to and conduct of the study.

They were consecutive inpatients enrolled who were diagnosed with IgA nephropathy by renal biopsy from January 2014 to January 2016, and no special priority and preference. All renal biopsy patients had signed the research protocol of the Renal Clinical Database Establishment when they were hospitalized, allowing their clinical data to be used for science purpose. This study was approved by the Ethics Committee of the Chinese PLA General Hospital. If possible, after the article is published, we will translate the main content into Chinese for the purpose of health education for patients.

Statistical analyses

SPSS 22.0 was used for all statistical analyses. The clinical and demographic data were compared between anemic and non-anemic subjects using Student's t-test or $\chi 2$- test as appropriate. Normally distributed variables were expressed as the mean ± standard deviation (SD), whereas non-normally distributed variables were expressed as the median (minimum-maximum). Univariate logistic regression and multivariate logistic regression were used to analyze the influence factors of anemia in IgA nephropathy. For all analyses, $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

There were 462 patients with IgA nephropathy qualified for analysis and 272 of them were males, the mean age of all the patients was 36.6 ± 11.3 years, and the mean hemoglobin level was 133 ± 19 g/L. A total of 28.5% (132/462) of patients met the diagnostic criteria for anemia. The anemia rate was 21.3% (58/272) in males and 38.9% (74/190) in females. The majority (125/132) patients with IgA nephropathy were normocytic, normochromic anemia. The clinical and demographic characteristics of patients showed in Table 1. Compared with the non-anemic group, renal anemia was more likely to occur in older and female patients with IgA nephropathy. The anemic group had lower eGFR and serum albumin, and higher 24hUPr than nonanemic group ($P < 0.05$). Blood pressure, TC, TG, and CRP were no significantly difference

between anemic and no-anemic patients (P > 0.05). As an indicator of nutritional status, the serum prealbumin level between the anemic and non-anemic group did not show a significant difference (P > 0.05).

Table 1. Comparison of the characteristics of patients with IgA nephropathy between the anemic and non-anemic group

Characteristic	Anemic n=132	Non-anemic n=330	P-value
Age(year)	39.1±12.4	35.6±10.6	0.002
gender（male/female）	58/74	214/116	0.000
BMI（kg/m^2）	24.2±3.1	25.0±3.5	0.013
Blood pressure（mmHg）			
Systolic	131.3±21.3	129.7±17.1	0.439
Diastolic	83.2±13.4	85.0±12.2	0.169
Laboratory results			
Hb，female(g/L)	106.7±11.0	129.6±8.6	0.000
Hb，male(g/L)	116.0±10.2	149.2±11.0	0.000
MCV(fl)	87.3±5.4	87.7±3.8	0.353
MCH(pg)	29.5±2.3	30.4±1.4	0.000
MCHC(g/L)	337.8±12.0	347.0±10.4	0.000
CRP(mg/dl)	0.3（0.0-2.1）	0.3（0.0-5.0）	0.361
serum albumin(g/L)	37.3±3.9	40.6±4.0	0.000
BUN(mmol/L)	6.9（1.3-31.8）	5.3（2.2-19.5）	0.000
SCr(μ mol/L)	128.2（48.3-729.3）	91.8（45.9-321.3）	0.000
UA(μmol/L)	398.8±121.7	378.4±103.7	0.091
TC(mmol/L)	4.4（2.3-7.1）	4.5（2.7-8.6）	0.787
TG(mmol/L)	1.8（0.3-6.2）	1.9（0.4-8.8）	0.055
prealbumin(g/L)	28.9（10.5-52.2）	28.9（11.8-56.2）	0.440
24hUPr(g/d)	1.7（0.1-7.9）	1.7（0.4-8.8）	0.000
eGFR(ml/min/1.73m^2）	58.8±33.4	28.7（11.8-56.2）	0.000

BMI = body mass index; Hb=hemoglobin; MCV= erythrocyte mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpusular hemoglobin concerntration; CRP= C-reactive protein; BUN= blood urea nitrogen; SCr= serum creatinine; SUA=serum uric acid; TC =total cholesterol; TG =triglyceride; UPr= urine protein; eGFR= estimated glomerular filtration rate

Data are expressed as mean ± standard deviation or median (minimum – maximum). eGFR was estimated using the CKD-epidemiology collaboration. Anemia was defined as a hemoglobin value of <130 g/L for males and <120 g/L for females.

Anemia ratio in patients with different eGFR levels

As shown in Figure 2, the ratio of anemia increased with the decline of eGFR level. The ratio of anemia in patients with eGFR 30-59 ml/min/1.73m^2 (42.9%, P < 0.001), eGFR 15-29 ml/min/1.73m^2 (87.5%, P < 0.001), eGFR < 15 ml/min/1.73m^2 (100%, P < 0.001) were higher than the patients with eGFR>60 ml/min/1.73m^2 (17.8%). Compared with the patients with eGFR 30-59 ml/min/1.73m^2, the ratio of anemia in patients with eGFR 15-29 ml/min/1.73m^2 (87.5%, P < 0.001), eGFR＜15 ml/min/1.73m^2 (100%, P=0.008) were higher, and there was no significant difference between the ratio of anemia in patients with eGFR 15-29 ml/min/1.73m^2 and eGFR < 15 ml/min/1.73m^2 (p=0.499).

Kidney pathological characteristics of anemic and non-anemic patients

Table 2 shows the kidney pathological characteristics of anemic and non-anemic patients. M (M0/1), E (E0/1), S (S0/1), T (T0/1/2), C (C0/1/2) were used to represent IgA nephropathy pathological injury score. Results of χ2- test showed that the ratio of M1, T2 and C2 in anemic group were higher than that of non-anemic group(anemics versus non-anemics: M1 56.8% versus 38.5%,P<0.001; T2 52.3% versus 14.8%,P<0.001; C2 7.6% versus 2.4%,P=0.009, respectively), while the ratio of E1 and S1 were not significantly different (anemics versus non-anemics: E1 11.4% versus 16.1%, P=0.198; S1 75.8% versus 66.7%, P=0.056, respectively) between the anemics and non-anemics.

Table 2. Renal pathological injury score comparison between anemic and non -anemic

group

Renal pathology[a]	Score	Anemic, n (%)	Non-anemic, n (%)	P
M	M0	57（43.2）	203（61.5）	
	M1	75（56.8）	127（38.5）	0.000
E	E0	117（88.6）	277（83.9）	
	E1	15（11.4）	53（16.1）	0.198
S	S0	32（24.2）	110（33.3）	
	S1	100（75.8）	220（66.7）	0.056
T	T0	35（26.5）	185（56.1）	
	T1	28（21.2）	96（29.1）	
	T2	69（52.3）	49（14.8）	0.000
C	C0	72（54.5）	197(59.7)	
	C1	50（37.9）	125(37.9)	
	C2	10（7.6）	8(2.4)	0.009

[a]Renal injury was estimated by the Oxford classification of IgA nephropathy. Variables were divided into categories as follows: Mesangial score <0.5 (M0) or >0.5 (M1)；Endocapillary hypercellularity absent (E0) or present (E1)；Segmental glomerulosclerosis absent (S0) or present (S1); presence or absence of podocyte hypertrophy/tip lesions in biopsy specimens with S1； Tubular atrophy/interstitial fibrosis ＜25% (T0), 26%–50% (T1), or >50% (T2)；Cellular/fibrocellular crescents absent (C0), present in at least 1 glomerulus (C1), in >25% of glomeruli (C2).

Analysis of the influence factors associated with renal anemia in patients with IgA

nephropathy

The correlative factors of renal anemia in patients with IgA nephropathy was performed using univariable and multivariable logistic regression as shown in Table 3. Age, gender, BMI, serum albumin, eGFR, M, T, and C were used as independent variables, and anemia and non-anemia was used as the dependent variable for all analyses. After the variables were screened, the major influencing factors obtained included: gender (OR: 3.02, CI: 1.76-5.17), albumin (OR: 0.87, CI: 0.82-0.93), eGFR (OR: 0.98, CI: 0.97-0.99), T2 (OR: 2.57, CI: 1.22-5.40).

Table 3. Analysis of the influence factors associated with renal anemia in IgA nephropathy patients (logistic regression)

	Univariable Logistic		Multivariable Logistic	
	OR(95%CI)	P value	OR(95%CI)	P value
Age	1.03(1.01-1.05)	0.002	0.99(0.97-1.02)	0.510
Gender	2.35 (1.56-3.55)	0.000	3.02(1.76-5.17)	0.000
BMI	0.92(0.87-0.98)	0.013	0.95(0.88-1.03)	0.203
ALB	0.82(0.78-0.87)	0.000	0.87(0.82-0.93)	0.000
eGFR	0.97(0.96-0.98)	0.000	0.98(0.97-0.99)	0.000
Mesangial hypercellularity				
M0	1		1	
M1	2.10(1.40-3.19)	0.000	1.22(0.72-2.06)	0.468
Tubular atrophy/interstitial fibrosis				
T0	1		1	
T1	1.54(0.89-2.69)	0.126	0.81（0.42-1.57）	0.541
T2	7.44(4.45-12.45)	0.000	2.57(1.22-5.40)	0.013

Cellular/fibrocellular crescents absent				
C0	1		1	
C1	1.09(0.72-1.67)	0.677	0.81(0.48-1.37)	0.440
C2	3.42(1.30-9.01)	0.013	1.81(0.56-5.83)	0.321

OR=odds ratio, 95% CI=95% confidence interval， BMI= body mass index, ALB=serum albumin, eGFR= estimated glomerular filtration rate

Discussion

IgA nephropathy can lead to several complications including anemia, renal hypertension, vascular disease, renal osteopathy, and hyperuricemia. Anemia is one of the risks factors for kidney progression and poor prognosis.[15-17] When the renal lesion is progressing, the prognosis will be poor. The incidence of intrarenal arteriole lesions in patients with IgA nephropathy was higher than that in those with non-IgA nephropathy and membranous nephropathy;[18 19] however, there were few clinical and pathological study for renal anemia with large-papulation conducted. In this study we enrolled 462 patients for analysis, we found the mean age was 36.6 ± 11.3 years, and the male-to-female ratio was 272:190. These patients showed characteristics of the disease types of patients with IgA nephropathy, primarily occurring in young males. 28.5%patients met the diagnostic criteria for anemia, and the ratio of anemia in males (21.3%) were higher than in females (38.9%), which was the first to show that the incidence of renal anemia in female patients with IgA nephropathy is higher than that in male patients with IgA nephropathy. The results of the regression analysis also suggested that the incidence of renal anemia among female patients was higher than males. The specific reasons for this difference is still unknown and the possible reason may relate to androgen,[20 21] but still require additional study in the future. Therefore, we should pay attention to the female patients due to renal anemia differs by gender. Clinical observations and interventions for renal anemia should differ by gender.

Our study showed that renal anemia caused by IgA nephropathy had the most common presentation of normocytic normochromic anemia, which was coincide with previous study.[22] The prevalence of anemia increased with reduction of eGFR levels in all age groups .[23 24] Our study supported the hypothesis that with the eGFR is gradually reduced, the incidence of anemia gradually increases. In patients with CKD3 stage disease, the incidence of renal anemia already reaches 42.9%, which suggested that attention should be paid to the development of renal anemia in these patients and clinical intervention should be provided if necessary. All of the patients with CKD5 stage disease showed combined anemia, and active treatment was required to delay the progression of renal function injury and increase patient quality of life.

IgA nephropathy is a group of diseases characterized by renal pathological damage, especially glomerular mesangial cell proliferation/immune complex deposition.[25] The pathological characteristic of IgA nephropathy in this group showed that compared with the non-anemic group, the ratio of mesangial proliferation (M1), interstitial fibrosis, and tubular atrophy (T2) as well as the occurrence of crescent lesion scores (C2) were higher in the anemic group. These results suggest that pathological damage is associated with renal anemia. The results of multivariable logistic regression showed that the renal tubulointerstitial lesion >50% (T2) was associated with renal anemia in patients with IgA nephropathy. Mesangial proliferation, endocapillary proliferative lesions, segmental sclerosis or adhesion, and the disease severity of crescent formation were not significantly associated with renal anemia. This finding is important and consistent with our previous results, suggesting that severe renal tubulointerstitial lesions are an independent risk factor of IgA nephropathy.[26] These results suggest that patients with IgA nephropathy combined with renal anemia indicated the possibility of renal tubulointerstitial lesions. Renal tubulointerstitial lesions leads to the reduction of EPO, which is a hormone-like substance mainly secreted by renal tubulointerstitial cells that can regulate the proliferation and differentiation rates of erythrocyte precursors in bone marrow to

promote erythrocyte production.[27] Maxwell et al.[28] showed that the ability of interstitial fibroblasts to produce EPO decreased in an interstitial nephropathy experimental model. Fibroblasts are interstitial mesenchyme that structurally support epithelia by producing extracellular matrix (ECM). In chronic kidney injury, sustained inflammation accompanies the proliferation of interstitial fibroblasts and myofibroblasts,[29] leading to renal fibrosis, which is the final common pathway for all CKD and eventually leads to renal failure.[30]More importantly, the reversibility of EPO production in the fibrotic kidney raised the possibility of a therapeutic approach toward renal anemia.[31] Our study confirmed that the renal anemia was associated with the severity of renal tubulointerstitial injury, which further suggests that the major cause of renal anemia is the reduction in EPO production caused by renal tubulointerstitial injury. These results are also consistent with our clinical observation that renal anemia occurs early and severe in patients with chronic interstitial tubulointerstitial injuries. This phenomenon might be associated with the early destruction of the interstitial cells that produce EPO.

The results of the logistic regression showed that low serum albumin was a correlative factor of renal anemia in patients with IgA nephropathy. The patients selected for this study had IgA nephropathy and were first diagnosed at our center (diagnosis confirmed via renal biopsy); in other words, the enrollment had strict inclusion and exclusion criteria. Due to patients with BMI $< 18.5 kg/m^2$ and malnutrition were excluded. In addition, the results suggested that prealbumin, an important indicator used to indicate nutritional status, did not significantly differ between the anemic and non-anemic group. Therefore, although this study showed that the renal anemia in patients with IgA nephropathy was associated with hypoproteinemia, the reasons for and mechanisms behind this result remain unclear and require further exploration.

There are several important limitations to this study. Due to the study is cross-sectional design, it is not possible to determine a causal relationship between

influencing factors and anemia. In future studies, the renal anemia and the patient's prognosis will be further studied to provide a reliable basis for improving the patient's life quality and survival time, and provide the renal anemia evidence of management and treatment for clinician. In addition, since our data were all from patients who can perform renal biopsy, part of the patients were not included in renal biopsy for various reasons, therefore there was a selection bias in our study.

Conclusions

In summary, this study reported that renal anemia is a common complication in patients with IgA nephropathy with large patients' population. The anemia type was primarily normocytic and normochromic. With the aggravation of renal dysfunction, the incidence of renal anemia increased. Patients with CKD3 stage disease and above should be paid attention to renal anemia development and intervention. The female, hypoalbuminemia, eGFR reduction, and severe renal tubulointerstitial lesions were influencing factors of renal anemia in patients with IgA nephropathy. The conclusions above showed new insight in understanding of anemia in IgA nephropathy and may improve the management and treatment of clinical renal anemia.

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Competing interests None declared.

Ethics approval The Ethics Committee of the General Hospital of the Chinese People's Liberation Army.

Data sharing statement All relevant data are within the paper and its Supporting Information files.

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Legend

Figure 1. The flow chart of the study. AKI= acute kidney injury，BMI= body mass index.

Figure 2. The ratio of anemia and non-anemia in patients with different eGFR levels. Comparison of the ratio of anemia at different eGFR levels: **P*<0.05 compared with the anemic patients of eGFR>60 ml/min/1.73m^2, $^{\#}$*P*<0.05 compared with the anemic patients of eGFR 30-59ml/min/1.73m^2

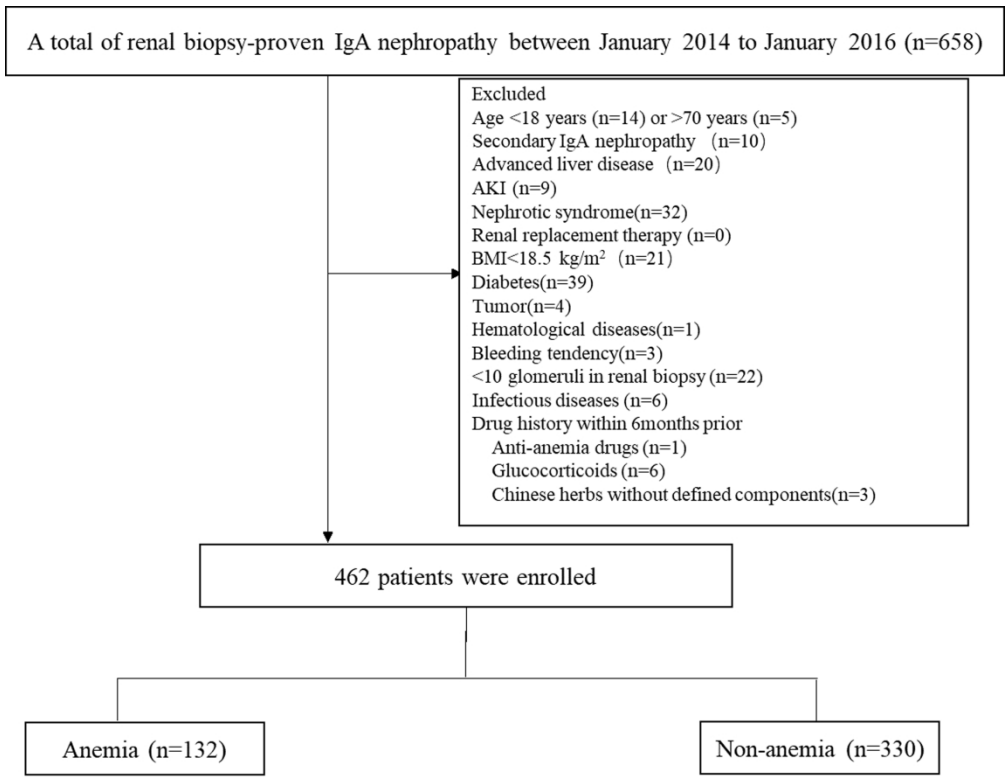

Figure 1. The flow chart of the study. AKI= acute kidney injury, BMI= body mass index.

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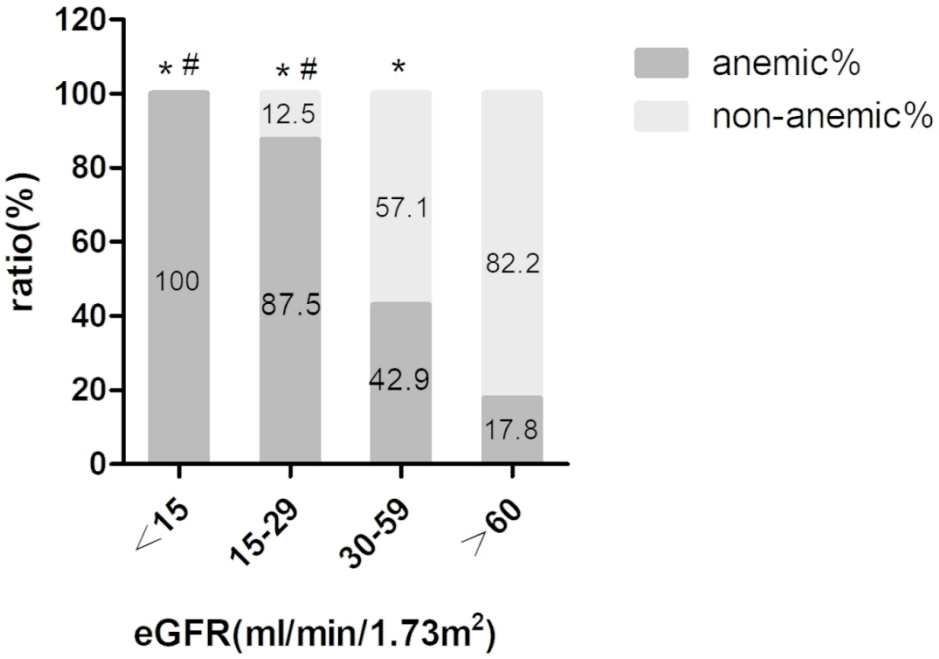

Figure 2. The ratio of anemia and non-anemia in patients with different eGFR levels. Comparison of the ratio of anemia at different eGFR levels: *P<0.05 compared with the anemic patients of eGFR>60 ml/min/1.73m2, #P<0.05 compared with the anemic patients of eGFR 30-59ml/min/1.73m2

123x90mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Page 4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 4,5
Bias	9	Describe any efforts to address potential sources of bias	Page 5,6
Study size	10	Explain how the study size was arrived at	Page 4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 4,5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 6
		(*b*) Describe any methods used to examine subgroups and interactions	Page 6
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Page 6
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 6,7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	Page 4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 6,7
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	Page 6,7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 9-11
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 9-11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 11-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 11-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Clinical and pathological factors of renal anemia in patients with IgA nephropathy in Chinese adults: a cross-sectional study

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Secondary Subject Heading:	Epidemiology, Renal medicine
Keywords:	renal anemia, IgA nephropathy, renal tubulointerstitial lesions

Clinical and pathological factors of renal anemia in patients with IgA nephropathy in Chinese adults: a cross-sectional study

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Abstract

Objective: Few studies with large sample populations concerning renal anemia and IgA nephropathy have been reported worldwide. The purpose of this cross-sectional study was to examine the clinical and pathological characteristics and influencing factors associated with renal anemia in patients with IgA nephropathy, which is the most common etiology of chronic kidney disease (CKD).

Methods: A total of 462 hospitalized patients with IgA nephropathy confirmed by renal biopsy who met the inclusion criteria were consecutively recruited from January 2014 to January 2016. Their general information, routine blood test results, blood chemistries, estimated glomerular filtration rates (eGFRs) and renal pathologies were collected. The Oxford classification was used to characterize the renal pathologies. Univariable and multivariate logistic regression models were used to analyze the influencing factors of anemia associated with IgA nephropathy.

Results: The incidence of renal anemia was 28.5% (132/462 patients) in our study (21.3% in males and 38.9% in females). The anemia type was primarily normocytic and normochromic. The rate of anemia in patients with eGFR values of 30-59 ml/min/1.73 m^2 was higher than that in patients with an eGFR >60 ml/min/1.73 m^2 (42.9% versus 17.8%, P<0.001). Notably, in the group with eGFR values < 15 ml/min/1.73 m^2, the anemia rate was 100%. Logistic regression analysis showed that factors affecting anemia in patients with IgA nephropathy included being female (OR: 3.02, CI: 1.76-5.17), low albumin levels (OR: 0.87, CI: 0.82-0.93), reduced eGFR values (OR: 0.98, CI: 0.97-0.99) and renal tubulointerstitial lesions >50% (OR: 2.57, CI: 1.22-5.40).

Conclusions: The female sex, hypoalbuminemia, reduced eGFR levels, and severe renal tubulointerstitial lesions were correlated with renal anemia in patients with IgA nephropathy. These results provide new insight into our understanding of anemia in IgA nephropathy and may improve the management and treatment of clinical renal anemia.

Keywords: renal anemia; IgA nephropathy; renal tubulointerstitial lesions

Strengths and limitations of this study

- This is the first time to study a large-scale population on the morbidity, types of anemia classification, influence factors of renal anemia among patients with IgA nephropathy who were diagnosed by renal biopsy.
- Due to the cross-sectional design of the study and the fact that all patients needed to meet the criteria of renal biopsy, a causal relationship between influencing factors and anemia could not be determined, and selection bias was present in our study.
- The missing rate of Tibc, Fe and TS were high and the EPO data was not obtained.

Introduction

Renal anemia is one of the most common complications of chronic kidney disease (CKD). This condition can accelerate the progression of renal function injury, induce cardiovascular events, reduce the quality of life of patients and is associated with a poor prognosis.[1-4] Different types of CKD have shown different prevalences of renal anemia with different prognoses.[5-7]

IgA nephropathy is currently the major etiology of the progression of CKD into end-stage renal disease (ESRD).[8] Especially in Asian-Pacific regions, this condition primarily occurs in young adult men. If not well-controlled, approximately 25-45% of patients will progress to chronic renal failure within 20 years; this condition requires replacement therapies, such as blood purification, and poses considerable threats to public health.[9-11] Therefore, we choose IgAN as the research subject. *Seiki Aruga et al*[12] reported that the levels of hemoglobin (Hb), hematocrit (Ht), and red blood cells (RBCs) of 62 IgA nephropathy patients gradually decreased according to the progression of renal injuries, and the fibrosis and/or inflammatory cell infiltration in the tubulointerstitial region was more marked in patients with a poor prognosis.

However, there remains a lack of large-scale population studies regarding the

morbidity, types of anemia classification, and influencing factors of renal anemia among patients with IgA nephropathy. To gain a better understanding of anemia in IgA nephropathy patients and improve the efficacy of renal anemia therapy, a total of 658 patients diagnosed with IgA nephropathy by kidney biopsy at the Center of Kidney Diseases between January 2014 and January 2016 were enrolled in this study. Ultimately, 462 patients with IgA nephropathy who met the study inclusion and exclusion criteria were included in the final analysis.

Methods

Study design and subjects

This cross-sectional study was performed at the Department of Nephrology of the Chinese PLA General Hospital. Consecutive inpatients aged 18-70 years who were diagnosed with IgA nephropathy by renal biopsy (renal biopsy criteria was shown in Supplementary Table S1 and Table S2) from January 2014 to January 2016 were enrolled. The exclusion criteria for enrollment were as follows: (1) secondary IgA nephropathy, such as Henoch-Schonlein purpura (HSP) nephritis, systemic lupus erythematosus (SLE), HBV-related glomerulonephritis (HBV-GN), or diabetic nephropathy; (2) <10 glomeruli in the renal biopsy; (3) acute kidney injury (AKI), nephrotic syndrome or renal replacement therapy; (4) malnutrition, body mass index (BMI) < 18.5 kg/m^2; (5) acute infection, patients with liver cirrhosis, cancer, gastrointestinal bleeding, female menstrual period or systemic blood disease; (6) patients currently being treated with anemia drugs, glucocorticoids, immunosuppressive medication or Chinese herbs without defined components within the past 6 months. Ultimately, 462 eligible patients were analyzed, including 132 in the anemic group and 330 in the non-anemic group. All patients with renal biopsies signed the research protocol of the Renal Clinical Database Establishment when hospitalized, allowing their clinical data to be used for scientific purposes, and this study was approved by the Ethics Committee of the Chinese PLA General Hospital. A flow chart of the study design is shown in Figure 1.

Data collection

We collected physical and clinical information, including patient sex, age, body mass index (BMI), blood pressure, Hb, erythrocyte mean corpuscular volume (MCV), erythrocyte mean corpuscular hemoglobin concentration (MCHC), levels of C-reactive protein (CRP), serum creatinine (SCr), blood urea nitrogen (BUN), serum uric acid (SUA), serum albumin (ALB), serum pre-albumin, total cholesterol (TC), triglyceride (TG), and total urinary protein (UPr) within 24 h from 462 IgA nephropathy patients.

Patients were classified by the chronic kidney disease (CKD) diagnostic criteria from the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines of 2006. They were stratified into 5 stages according to eGFR value: stage 1 (≥ 90 ml/min/1.73 m^2), stage 2 (60-89 ml/min/1.73 m^2), stage 3 (30-59 ml/min/1.73 m^2), stage 4 (15-29 ml/min/1.73 m^2), and stage 5 (<15 ml/min/1.73 m^2). The estimated glomerular filtration rate (eGFR) was calculated using the CKD-epidemiology collaboration (CKD-EPI) formula.[13]

BMI was calculated using the standard formula of weight (kg)/height (m^2).

Anemia was defined as Hb <130 g/L in males and <120 g/L in females.

If patients had an MCV of 80-100 fl and a MCHC of 320-350 g/L simultaneously, the anemia was diagnosed as normocytic, normochromic anemia.

Pathology of renal injury was estimated independently by Xiang-mei Chen and Xue-guang Zhang according to the Oxford classification of IgA nephropathy as follows:[14] mesangial score <0.5 (M0) or >0.5 (M1); endocapillary hypercellularity (absent (E0) or present (E1)); segmental glomerulosclerosis (absent (S0) or present (S1)); presence or absence of podocyte hypertrophy/tip lesions in biopsy specimens with S1; tubular atrophy/interstitial fibrosis <25% (T0), 26-50% (T1), or >50% (T2); and cellular/fibrocellular crescents absent (C0), present in at least 1 glomerulus (C1) or in >25% of glomeruli (C2).

Statistical analyses

SPSS software version 22.0 was used for all statistical analyses. The clinical and

demographic data were compared between anemic and non-anemic subjects using the Student's t-test or χ^2 test as appropriate. Normally distributed variables were expressed as the mean ± standard deviation (SD), whereas non-normally distributed variables were expressed as the median (minimum-maximum). Univariate logistic regression and multivariate logistic regression were used to analyze the influencing factors of anemia in IgA nephropathy. For all analyses, P-values < 0.05 were considered statistically significant.

Patient and public involvement

Patients and the public were not involved in this study.

Results

Patient characteristics

In total, 462 patients with IgA nephropathy qualified for analysis; of them, 272 were male, the mean age of all patients was 36.6 ± 11.3 years, and the mean hemoglobin level was 133 ± 19 g/L. The diagnostic criteria for anemia was met by 28.5% of the patients (132/462). The anemia rate was 21.3% (58/272) in the male patients and 38.9% (74/190) in the female patients. The majority (125/132) of patients with IgA nephropathy had normocytic, normochromic anemia. The clinical and demographic characteristics of patients are shown in Table 1. Compared with the non-anemic group, renal anemia was more likely to occur in older, female patients with IgA nephropathy. The anemic group had lower eGFR and serum albumin levels and higher 24 hr UPr levels than the non-anemic group (P < 0.05). Blood pressure, TC, TG, and CRP levels were not significantly different between anemic and non-anemic patients (P > 0.05). The serum pre-albumin level, an indicator of nutritional status, did not show any significant difference between the anemic and non-anemic group (P > 0.05). In addition, we analyzed the data of 258 subjects with available measurements of Total Iron Binding Capacity (Tibc), Fe and Transferrin saturation (TS) (the results was shown in Supplementary Table S3).

Table 1. Comparison of the characteristics of anemic and non-anemic patients with

IgA nephropathy

Characteristic	Anemic n=132	Non-anemic n=330	P value
Age (years)	39.1±12.4	35.6±10.6	0.002
Sex (male/female)	58/74	214/116	0.000
BMI (kg/m^2)	24.2±3.1	25.0±3.5	0.013
Blood pressure (mmHg)			
Systolic	131.3±21.3	129.7±17.1	0.439
Diastolic	83.2±13.4	85.0±12.2	0.169
Laboratory results			
Hb, female (g/L)	106.7±11.0	129.6±8.6	0.000
Hb, male (g/L)	116.0±10.2	149.2±11.0	0.000
MCV (fl)	87.3±5.4	87.7±3.8	0.353
MCH (pg)	29.5±2.3	30.4±1.4	0.000
MCHC (g/L)	337.8±12.0	347.0±10.4	0.000
CRP (mg/dl)	0.3 (0.0-2.1)	0.3 (0.0-5.0)	0.361
Serum albumin (g/L)	37.3±3.9	40.6±4.0	0.000
BUN (mmol/L)	6.9 (1.3-31.8)	5.3 (2.2-19.5)	0.000
SCr (μmol/L)	128.2 (48.3-729.3)	91.8 (45.9-321.3)	0.000
UA (μmol/L)	398.8±121.7	378.4±103.7	0.091
TC (mmol/L)	4.4 (2.3-7.1)	4.5 (2.7-8.6)	0.787
TG (mmol/L)	1.8 (0.3-6.2)	1.9 (0.4-8.8)	0.055
Pre-albumin (g/L)	28.9 (10.5-52.2)	28.9 (11.8-56.2)	0.440
24 hr UPr (g/d)	1.7 (0.1-7.9)	1.7 (0.4-8.8)	0.000
eGFR (ml/min/1.73 m^2)	58.8±33.4	28.7 (11.8-56.2)	0.000

BMI, body mass index; Hb, hemoglobin; MCV, erythrocyte mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; CRP, C-reactive protein; BUN, blood urea nitrogen; SCr, serum

creatinine; SUA, serum uric acid; TC, total cholesterol; TG, triglyceride; UPr, urinary protein; eGFR, estimated glomerular filtration rate

Data are expressed as the means ± standard deviation or medians (minimum – maximum). eGFR was estimated using the CKD-epidemiology standard. Anemia was defined as a hemoglobin value of <130 g/L for males and <120 g/L for females.

Anemia rate in patients with various eGFR levels

As shown in Figure 2, the rate of anemia increased with a decrease in eGFR level. The ratios of anemia in patients with an eGFR of 30-59 ml/min/1.73 m^2 (42.9%, P < 0.001), an eGFR of 15-29 ml/min/1.73 m^2 (87.5%, P < 0.001), and an eGFR < 15 ml/min/1.73 m^2 (100%, P < 0.001) were higher than in patients with an eGFR > 60 ml/min/1.73 m^2 (17.8%). Compared to patients with an eGFR of 30-59 ml/min/1.73 m^2, the ratios of anemia in patients with an eGFR of 15-29 ml/min/1.73 m^2 (87.5%, P < 0.001) and an eGFR＜15 ml/min/1.73 m^2 (100%, P=0.008) were higher, and there was no significant difference between the rate of anemia in patients with an eGFR of 15-29 ml/min/1.73 m^2 and patients with an eGFR < 15 ml/min/1.73 m^2 (p=0.499).

Kidney pathological characteristics of anemic and non-anemic patients

Table 2 shows the kidney pathological characteristics of anemic and non-anemic patients. M (M0/1), E (E0/1), S (S0/1), T (T0/1/2), and C (C0/1/2) were used to characterize the IgA nephropathy pathological injury score. χ^2 testing showed that the ratios of M1, T2 and C2 were higher in the anemic group than in the non-anemic group (anemics versus non-anemics: M1, 56.8% versus 38.5%, P<0.001; T2, 52.3% versus 14.8%, P<0.001; C2, 7.6% versus 2.4%, P=0.009, respectively), while the ratios of E1 and S1 were not significantly different (anemics versus non-anemics: E1, 11.4% versus 16.1%, P=0.198; S1, 75.8% versus 66.7%, P=0.056, respectively) between the anemic and non-anemic patients.

Table 2. Renal pathological injury score comparison between anemic and non-anemic patients

Renal pathology[a]	Score	Anemic, n (%)	Non-anemic, n (%)	P
M	M0	57 (43.2)	203 (61.5)	

	M1	75 (56.8)	127 (38.5)	0.000
E	E0	117 (88.6)	277 (83.9)	
	E1	15 (11.4)	53 (16.1)	0.198
S	S0	32 (24.2)	110 (33.3)	
	S1	100 (75.8)	220 (66.7)	0.056
T	T0	35 (26.5)	185 (56.1)	
	T1	28 (21.2)	96 (29.1)	
	T2	69 (52.3)	49 (14.8)	0.000
C	C0	72 (54.5)	197 (59.7)	
	C1	50 (37.9)	125 (37.9)	
	C2	10 (7.6)	8 (2.4)	0.009

[a]Renal injury was estimated by the Oxford classification of IgA nephropathy. Variables were divided into subcategories as follows: Mesangial score <0.5 (M0) or >0.5 (M1); Endocapillary hypercellularity absent (E0) or present (E1); Segmental glomerulosclerosis absent (S0) or present (S1); presence or absence of podocyte hypertrophy/tip lesions in biopsy specimens with S1; Tubular atrophy/interstitial fibrosis <25% (T0), 26-50% (T1), or >50% (T2); Cellular/fibrocellular crescents absent (C0), present in at least 1 glomerulus (C1), or present in >25% of glomeruli (C2).

Analysis of the influencing factors associated with renal anemia in patients with IgA nephropathy

The correlative factors for renal anemia in patients with IgA nephropathy were determined using univariable and multivariable logistic regression as shown in Table 3. Age, sex, BMI, serum albumin, eGFR, M, T, and C were used as independent variables, and anemia and non-anemia was used as the dependent variable for all analyses. After the variables were screened, the major influencing factors identified included: sex (OR: 3.02, CI: 1.76-5.17), albumin (OR: 0.87, CI: 0.82-0.93), eGFR (OR: 0.98, CI: 0.97-0.99), and T2 (OR: 2.57, CI: 1.22-5.40). According to the logstic regression results, we used eGFR and hemoglobin as well as albumin and hemoglobin

for correlation analysis. Besides, we compared the hemoglobin concentrations of the T0, T1, and T2 groups (as shown in Supplementary Figure S1).

Table 3. Analysis of the influencing factors associated with renal anemia in IgA nephropathy patients (logistic regression)

	Univariable Logistic		Multivariable Logistic	
	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.03(1.01-1.05)	0.002	0.99(0.97-1.02)	0.510
Sex	2.35(1.56-3.55)	0.000	3.02(1.76-5.17)	0.000
BMI	0.92(0.87-0.98)	0.013	0.95(0.88-1.03)	0.203
ALB	0.82(0.78-0.87)	0.000	0.87(0.82-0.93)	0.000
eGFR	0.97(0.96-0.98)	0.000	0.98(0.97-0.99)	0.000
Mesangial hypercellularity				
M0	1		1	
M1	2.10(1.40-3.19)	0.000	1.22(0.72-2.06)	0.468
Tubular atrophy/interstitial fibrosis				
T0	1		1	
T1	1.54(0.89-2.69)	0.126	0.81(0.42-1.57)	0.541
T2	7.44(4.45-12.45)	0.000	2.57(1.22-5.40)	0.013
Cellular/fibrocellular crescents absent				
C0	1		1	
C1	1.09(0.72-1.67)	0.677	0.81(0.48-1.37)	0.440
C2	3.42(1.30-9.01)	0.013	1.81(0.56-5.83)	0.321

OR, odds ratio; 95% CI, 95% confidence interval; BMI, body mass index; ALB, serum albumin; eGFR, estimated glomerular filtration rate

Discussion

IgA nephropathy can lead to several complications, including anemia, renal hypertension, vascular disease, renal osteopathy, and hyperuricemia. Anemia is one of the primary risks factors for kidney disease progression and is associated with a poor

prognosis.[15-17] When renal lesions are progressing, the prognosis is poor. The incidence of intrarenal arteriole lesions in patients with IgA nephropathy is reportedly higher than that in patients with non-IgA nephropathy and membranous nephropathy;[18 19] however, there have been few clinical and pathological studies of renal anemia with large-scale populations conducted. In this study, we enrolled 462 patients for analysis. We found that the mean patient age was 36.6 ± 11.3 years, and the male-to-female ratio was 272:190. These patients showed characteristics of the disease types of patients with IgA nephropathy, which primarily occurs in young men. In addition, 28.5% of patients met the diagnostic criteria for anemia, and the rate of anemia in males (21.3%) was lower than that in females (38.9%), making this the first study to report a higher incidence of renal anemia in female patients with IgA nephropathy than in male patients with IgA nephropathy. The results of the regression analysis also suggested that the incidence of renal anemia among female patients was higher than that in male patients. The specific reasons for this difference are still unknown; although androgen levels may play a role,[20 21] additional studies are needed in the future. Therefore, clinicians should pay attention to female patients as renal anemia rates clearly differ by sex. Clinical observations and interventions for renal anemia should also differ by sex.

Our study showed that renal anemia caused by IgA nephropathy had normocytic normochromic anemia as the most common type of presentation, which was consistent with a previous study.[22] The prevalence of anemia increased with a reduction in eGFR levels in all age groups.[23 24] Our findings support the hypothesis that as the eGFR is gradually reduced, the incidence of anemia gradually increases, and there was a positive correlation between severity of anemia and albumin. In patients with CKD stage-3 disease, the incidence of renal anemia reaches 42.9%, which suggests that clinicians must consider the development of renal anemia in these patients and that clinical intervention should be provided as necessary. All patients with CKD stage-5 disease show combined anemia, and active treatment is required to delay the progression of renal function injury and increase patient quality of life.

IgA nephropathy refers to a group of diseases characterized by renal pathological damage, especially glomerular mesangial cell proliferation/immune complex deposition.[25] The pathological characteristics of IgA nephropathy in this study showed that, compared with the non-anemic group, the rate of mesangial proliferation (M1), interstitial fibrosis, and tubular atrophy (T2) as well as the incidence of crescent lesion scores (C2), were higher in the anemic group. These results suggest that pathological damage is associated with renal anemia. The results of multivariable logistic regression analysis showed that having renal tubulointerstitial lesions >50% (T2) was associated with renal anemia in patients with IgA nephropathy, and the degree of anemia was most severe compared with T0 and T1. Mesangial proliferation, endocapillary proliferative lesions, segmental sclerosis or adhesion, and the disease severity of crescent formation were not significantly associated with renal anemia. This finding is important and consistent with our previous results, suggesting that severe renal tubulointerstitial lesions are an independent risk factor for IgA nephropathy.[26] These results suggest that patients with IgA nephropathy combined with renal anemia should be suspected of having renal tubulointerstitial lesions. Renal tubulointerstitial lesions lead to a reduction of EPO, which is a hormone-like substance primarily secreted by renal tubulointerstitial cells that can regulate the proliferation and differentiation rates of erythrocyte precursors in bone marrow to promote erythrocyte production.[27] *Maxwell et al*.[28] showed that the ability of interstitial fibroblasts to produce EPO decreased in an interstitial nephropathy experimental model. Fibroblasts are interstitial mesenchymal cells that structurally support epithelia by producing extracellular matrix (ECM). In chronic kidney injury, sustained inflammation accompanies the proliferation of interstitial fibroblasts and myofibroblasts,[29] leading to renal fibrosis, which is the final common pathway for all CKD and eventually leads to renal failure.[30] More importantly, the restoration of EPO production in the fibrotic kidney raises the possibility of a potential therapeutic approach towards treating renal anemia.[31] Our study confirmed that renal anemia is associated with the severity of renal

tubulointerstitial injury, which further suggests that the major cause of renal anemia is the reduction in EPO production caused by renal tubulointerstitial injury. These results are also consistent with our clinical observations that renal anemia occurs earlier and is more severe in patients with chronic interstitial tubulointerstitial injuries. This phenomenon might be associated with the early destruction of the interstitial cells that produce EPO.

The results of the logistic regression analysis showed that low serum albumin was a correlative factor for renal anemia in patients with IgA nephropathy. At the same time, there was a positive correlation between severity of anemia and albumin. The patients selected for this study had IgA nephropathy and were first diagnosed at our center (diagnosis confirmed via renal biopsy); in other words, the enrollment had strict inclusion and exclusion criteria. Patients with a BMI < 18.5 kg/m^2 or malnutrition were excluded. In addition, the results suggested that pre-albumin levels, an important indicator used to indicate nutritional status, did not significantly differ between the anemic and non-anemic groups. Therefore, although this study showed that the renal anemia in patients with IgA nephropathy was associated with hypoproteinemia, the reasons for and mechanisms underlying this result remain unclear and require further exploration.

At present, there have study shown that polymeric IgA1 (pIgA1) positively regulates erythropoiesis through binding to TfR1 and accelerates erythropoiesis recovery in anemia.[32] Patients with IgAN often have elevated serum pIgA1 levels.[33] Under steady-state conditions, low concentrations of pIgA1 are produced by plasma cells, and most TfR1 is bound by Fe-transferrin (Tf), with little stimulation of downstream ERK and Akt signaling pathways. Stress conditions such as hypoxia can lead to increased pIgA1 production, allowing erythroid development to be boosted via ERK and Akt signaling.[34] Based on these research results, we speculate that the occurrence of renal anemia in IgA nephropathy will be different from other causes of CKD, which need us to further research.

There are several important limitations to this study. As the study is

cross-sectional, a causal relationship between influencing factors and anemia cannot be determined. In future studies, renal anemia and patient prognosis will be further evaluated to provide a reliable basis for improving patient quality of life and survival time as well as stronger evidence for renal anemia management and treatment for clinicians. In addition, since our data were all from patients who underwent renal biopsy, some of the clinical patients initially identified did not undergo renal biopsy for various reasons; therefore, selection bias was present in our study. Loss of data cannot be controlled, which is one of the limitations of our study. There are some important data missing or no relevant data, such as the missing rate of the data of Tibc, Fe and TS was more than 40%, which led to these data lost valuable value. At the same time, the reduction of EPO generation is the major cause of renal anemia, but the data of EPO was not collected in the study. In future studies need to be more rigorous design.

Conclusions

In summary, using a large study population, we identified that renal anemia is a common complication in patients with IgA nephropathy. The anemia type was primarily normocytic and normochromic. With the aggravation of renal dysfunction, the incidence of renal anemia increased. Patients with CKD stage-3 disease and above should be monitored for renal anemia development and possible intervention. The female sex, hypoalbuminemia, eGFR reductions, and severe renal tubulointerstitial lesions were identified as influencing factors for renal anemia development in patients with IgA nephropathy. These findings provide new insight into our understanding of anemia in IgA nephropathy and may improve the management and treatment of clinical renal anemia.

Contributors WY, WRB and CXM created and designed this study. WY, STY, HMJ，LP collected and analyzed the data. WY, WRB, STY and HMJ contributed to the preparation and editing of the manuscript.

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Competing interests None declared.

Ethics approval The Ethics Committee of the General Hospital of the Chinese People's Liberation Army.

Data sharing statement All relevant data are within the paper and its Supporting Information files.

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Figure Legends

Figure 1. Flow chart showing the study design. AKI, acute kidney injury; BMI, body mass index.

Figure 2. The rate of anemia and non-anemia in patients with different eGFR levels. Comparison of the rate of anemia at different eGFR levels: **P*<0.05 compared with anemic patients with an eGFR > 60 ml/min/1.73 m^2; $^{\#}P$<0.05 compared with anemic patients with an eGFR of 30-59 ml/min/1.73 m^2

Figure S1. The relations between hemoglobin level and eGFR, Albumin and tubular atrophy/interstitial fibrosis (T). (A) Correlation of Hemoglobin with eGFR. (B) Correlation of Hemoglobin with Albumin. (C) Relationship between tubular atrophy/interstitial fibrosis score and hemoglobin level.

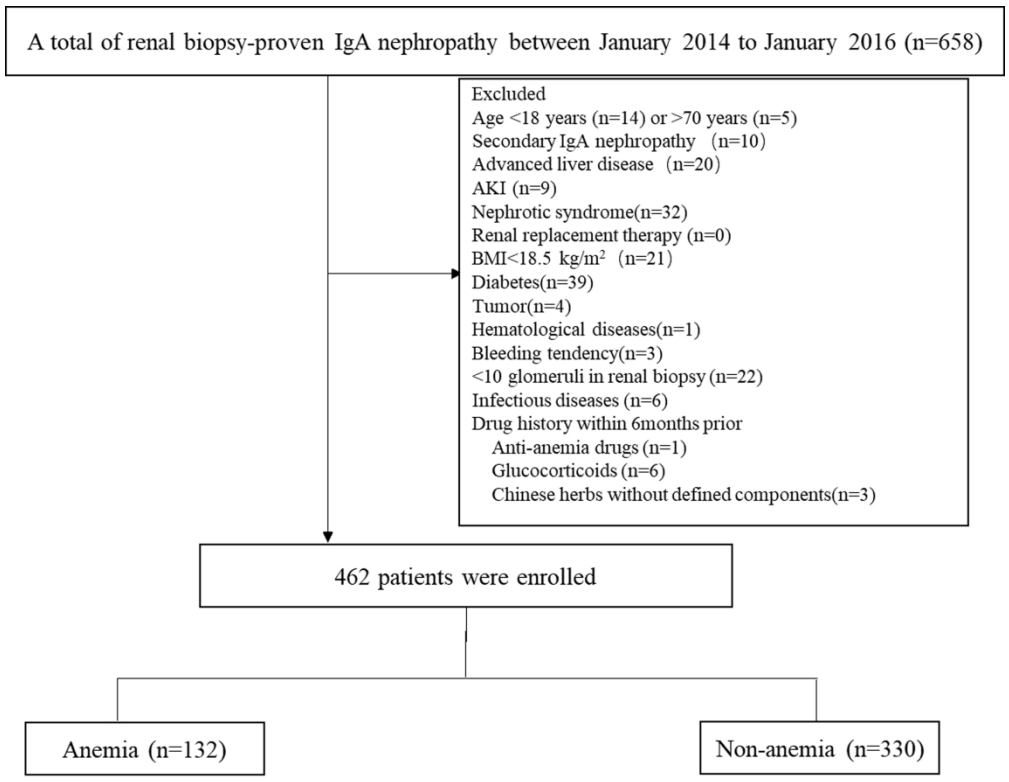

Figure 1. The flow chart of the study. AKI= acute kidney injury, BMI= body mass index.

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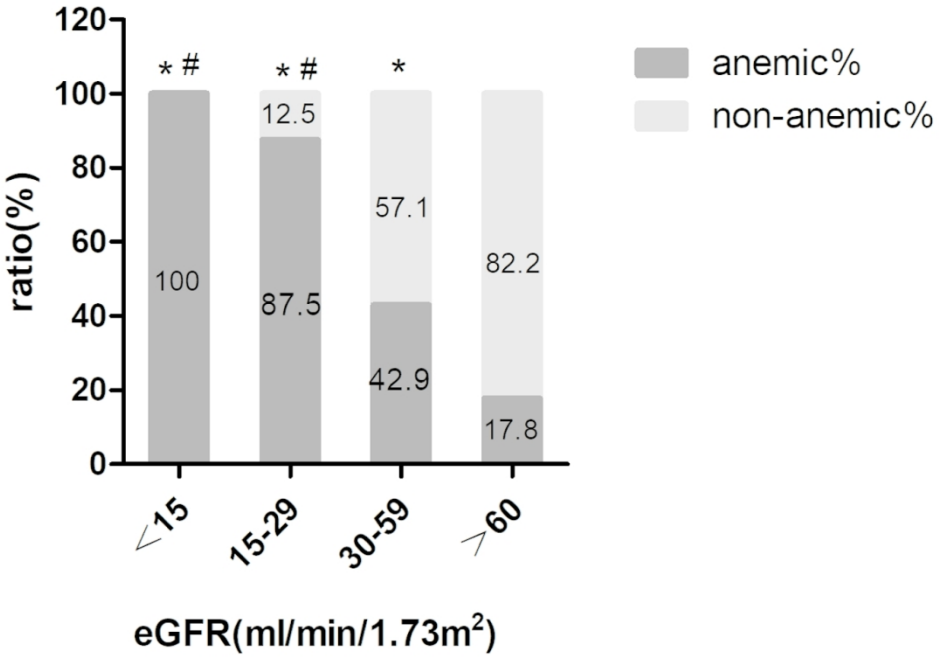

Figure 2. The ratio of anemia and non-anemia in patients with different eGFR levels. Comparison of the ratio of anemia at different eGFR levels: *P<0.05 compared with the anemic patients of eGFR>60 ml/min/1.73m2, #P<0.05 compared with the anemic patients of eGFR 30-59ml/min/1.73m2

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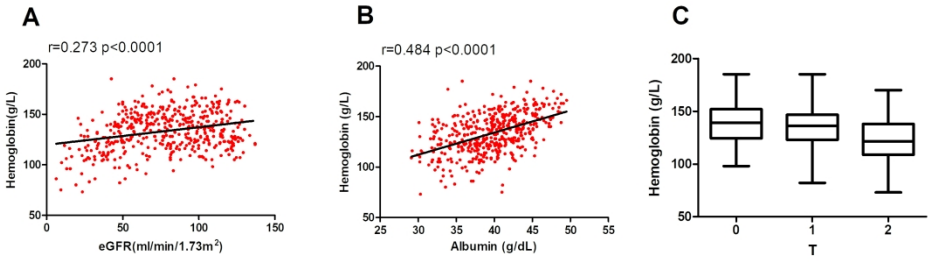

The relations between hemoglobin level and eGFR, Albumin and tubular atrophy/interstitial fibrosis (T). (A) Correlation of Hemoglobin with eGFR. (B) Correlation of Hemoglobin with Albumin. (C) Relationship between tubular atrophy/interstitial fibrosis score and hemoglobin level.

273x81mm (300 x 300 DPI)

Table S1. Possible indications for renal biopsy.

indications
Microscopic haematuria
Urologically unexplained macroscopic haematuria
Proteinuria
Nephrotic syndrome
Impaired kidney function
Hypertension
Possible renal involvement in systemic disease in:
multiple myeloma
monoclonal gammopathy of uncertain significance
systemic lupus erythematosus
antiphospholipid syndrome
diabetes
systemic vasculitis
scleroderma

Table S2. Contraindications to renal biopsy

Contraindication	Reason
Relative:	
Hypertension	Poorly controlled hypertension thought to increase risk of bleeding
Renal asymmetry	Suggestive of a process causing differential loss of renal mass (eg reflux nephropathy, atherosclerotic renal artery stenosis – although both these can cause proteinuria)
Decreased renal size (usually assessed as bipolar length on ultrasound)	Suggestive of chronic (therefore irreversible) renal damage, predictive of nonspecific fibrotic changes on biopsy Increased risk of complications reported in most series.
Single kidney	Accepted wisdom, based on the fact that the patient will be put into renal failure if there is irreversible damage to the kidney; however, if the patient appears likely to go into renal failure if left untreated, there is less to lose, and a biopsy may be justified if it might disclose a treatable condition

Unco-operative patient	Increased risk of complications if the patient cannot reliably stop breathing during needle puncture. Consider alternatives including biopsy under general anaesthetic, transvenous biopsy
Hydronephrosis	Obstructive nephropathy may be the cause of the renal disease (though seldom causes proteinuria) and should be investigated and treated first Increased risk of macroscopic haematuria due to biopsy needle penetrating renal pelvis or calyces
Suspected upper urinary tract infection	Urinary tract infection with white cell casts should be treated with antibiotics Active infection would contraindicate immunosuppressive treatment Biopsy might spread infection or be complicated by perinephric abscess formation
Absolute:	
Uncorrected coagulopathy	If biopsy is imperative, consider transvenous biopsy rather than percutaneous.

Table S3. Comparison of the results of Tibc, Fe and TS of anemic and non-anemic patients with IgA nephropathy

Laboratory results	Anemic n=177	Non-anemic n=81	P-value
Tibc (μmol/L)	50.4±8.0	45.7±8.7	0.000
Fe (μmol/L)	18.6(14.6-22.8)	14.3(10.7-17.6)	0.000
TS (%)	0.36(0.28-0.46)	0.33(0.24-0.42)	0.093

STROBE 2007 (v4) Statement - Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Page 4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 4,5
Bias	9	Describe any efforts to address potential sources of bias	*Page 5,6*
Study size	10	Explain how the study size was arrived at	Page 4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 4,5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 6
		(*b*) Describe any methods used to examine subgroups and interactions	Page 6
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Page 6
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study - eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 6,7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	Page 4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 6,7
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	Page 6,7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 9-11
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 9-11
Other analyses	17	Report other analyses done-eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 11-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 13,14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 11-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Clinical and pathological factors of renal anemia in patients with IgA nephropathy in Chinese adults: a cross-sectional study

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Primary Subject Heading:	Public health
Secondary Subject Heading:	Epidemiology, Renal medicine
Keywords:	renal anemia, IgA nephropathy, renal tubulointerstitial lesions

Clinical and pathological factors of renal anemia in patients with IgA nephropathy in Chinese adults: a cross-sectional study

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Abstract

Objective: Few studies with large sample populations concerning renal anemia and IgA nephropathy have been reported worldwide. The purpose of this cross-sectional study was to examine the clinical and pathological characteristics and influencing factors associated with renal anemia in patients with IgA nephropathy, which is the most common etiology of chronic kidney disease (CKD).

Methods: A total of 462 hospitalized patients with IgA nephropathy confirmed by renal biopsy who met the inclusion criteria were consecutively recruited from January 2014 to January 2016. Their general information, routine blood test results, blood chemistries, estimated glomerular filtration rates (eGFRs) and renal pathologies were collected. The Oxford classification was used to characterize the renal pathologies. Univariable and multivariate logistic regression models were used to analyze the influencing factors of anemia associated with IgA nephropathy.

Results: The incidence of renal anemia was 28.5% (132/462 patients) in our study (21.3% in males and 38.9% in females). The anemia type was primarily normocytic and normochromic. The rate of anemia in patients with eGFR values of 30-59 ml/min/1.73 m^2 was higher than that in patients with an eGFR >60 ml/min/1.73 m^2 (42.9% versus 17.8%, P<0.001). Notably, in the group with eGFR values < 15 ml/min/1.73 m^2, the anemia rate was 100%. Logistic regression analysis showed that factors affecting anemia in patients with IgA nephropathy included being female (OR: 3.02, CI: 1.76-5.17), low albumin levels (OR: 0.87, CI: 0.82-0.93), reduced eGFR values (OR: 0.98, CI: 0.97-0.99) and renal tubulointerstitial lesions >50% (OR: 2.57, CI: 1.22-5.40).

Conclusions: The female sex, hypoalbuminemia, reduced eGFR levels, and severe renal tubulointerstitial lesions were correlated with renal anemia in patients with IgA nephropathy. These results provide new insight into our understanding of anemia in IgA nephropathy and may improve the management and treatment of clinical renal anemia.

Keywords: renal anemia; IgA nephropathy; renal tubulointerstitial lesions

Strengths and limitations of this study

- This is the first cross-sectional study on morbidity, types of anemia classification, and influence factors of renal anemia among patients with IgA nephropathy who were diagnosed by renal biopsy with a large-scale population.
- This study is a cross-sectional design with the limitation of failure to determine the causal relationship between the influencing factors and anemia.
- We only analyzed those patients who underwent renal biopsy, but some of patients with IgA nephropathy did not receive renal biopsy for various reasons, so there was a selection bias in this study.
- The erythropoietin (EPO) data was not obtained since it is not a route examination item in daily clinical work.

Introduction

Renal anemia is one of the most common complications of chronic kidney disease (CKD). This condition can accelerate the progression of renal function injury, induce cardiovascular events, reduce the quality of life of patients and is associated with a poor prognosis.[1-4] Different types of CKD have shown different prevalences of renal anemia with different prognoses.[5-7]

IgA nephropathy is currently the major etiology of the progression of CKD into end-stage renal disease (ESRD).[8] Especially in Asian-Pacific regions, this condition primarily occurs in young adult men. If not well-controlled, approximately 25-45% of patients will progress to chronic renal failure within 20 years; this condition requires replacement therapies, such as blood purification, and poses considerable threats to public health.[9-11] Therefore, we choose IgA nephropathy as the research subject. *Seiki Aruga et al*[12] reported that the levels of hemoglobin (Hb), hematocrit (Ht), and red blood cells (RBCs) of 62 IgA nephropathy patients gradually decreased according to the progression of renal injuries, and the fibrosis and/or inflammatory cell infiltration in the tubulointerstitial region was more marked in patients with a poor prognosis.

However, there remains a lack of large-scale population studies regarding the morbidity, types of anemia classification, and influencing factors of renal anemia among patients with IgA nephropathy. To gain a better understanding of anemia in IgA nephropathy patients and improve the efficacy of renal anemia therapy, a total of 658 patients diagnosed with IgA nephropathy by kidney biopsy at the Center of Kidney Diseases between January 2014 and January 2016 were enrolled in this study. Ultimately, 462 patients with IgA nephropathy who met the study inclusion and exclusion criteria were included in the final analysis.

Methods

Study design and subjects

This cross-sectional study was performed at the Department of Nephrology of the Chinese PLA General Hospital. Consecutive inpatients aged 18-70 years who were diagnosed with IgA nephropathy by renal biopsy (renal biopsy criteria was shown in Supplementary Table S1 and Table S2) from January 2014 to January 2016 were enrolled. The exclusion criteria for enrollment were as follows: (1) secondary IgA nephropathy, such as Henoch-Schonlein purpura (HSP) nephritis, systemic lupus erythematosus (SLE), HBV-related glomerulonephritis (HBV-GN), or diabetic nephropathy; (2) <10 glomeruli in the renal biopsy; (3) acute kidney injury (AKI), nephrotic syndrome or renal replacement therapy; (4) malnutrition, body mass index (BMI) < 18.5 kg/m^2; (5) acute infection, patients with liver cirrhosis, cancer, gastrointestinal bleeding, female menstrual period or systemic blood disease; (6) patients currently being treated with anemia drugs, glucocorticoids, immunosuppressive medication or Chinese herbs without defined components within the past 6 months. Ultimately, 462 eligible patients were analyzed, including 132 in the anemic group and 330 in the non-anemic group. All patients with renal biopsies signed the research protocol of the Renal Clinical Database Establishment when hospitalized, allowing their clinical data to be used for scientific purposes, and this study was approved by the Ethics Committee of the Chinese PLA General Hospital. A flow chart

of the study design is shown in Figure 1.

Data collection

We collected physical and clinical information, including patient sex, age, body mass index (BMI), blood pressure, Hb, erythrocyte mean corpuscular volume (MCV), erythrocyte mean corpuscular hemoglobin concentration (MCHC), levels of C-reactive protein (CRP), serum creatinine (SCr), blood urea nitrogen (BUN), serum uric acid (SUA), serum albumin (ALB), serum pre-albumin, total cholesterol (TC), triglyceride (TG), and total urinary protein (UPr) within 24 h from 462 IgA nephropathy patients.

Patients were classified by the CKD diagnostic criteria from the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines of 2006. They were stratified into 5 stages according to eGFR value: stage 1 (≥ 90 ml/min/1.73 m^2), stage 2 (60-89 ml/min/1.73 m^2), stage 3 (30-59 ml/min/1.73 m^2), stage 4 (15-29 ml/min/1.73 m^2), and stage 5 (<15 ml/min/1.73 m^2). The estimated glomerular filtration rate (eGFR) was calculated using the CKD-epidemiology collaboration (CKD-EPI) formula.[13]

BMI was calculated using the standard formula of weight (kg)/height (m^2).

Anemia was defined as Hb <130 g/L in males and <120 g/L in females.

If patients had an MCV of 80-100 fl and a MCHC of 320-350 g/L simultaneously, the anemia was diagnosed as normocytic, normochromic anemia.

Pathology of renal injury was estimated independently by Xiang-mei Chen and Xue-guang Zhang according to the Oxford classification of IgA nephropathy as follows:[14] mesangial score <0.5 (M0) or >0.5 (M1); endocapillary hypercellularity (absent (E0) or present (E1)); segmental glomerulosclerosis (absent (S0) or present (S1)); presence or absence of podocyte hypertrophy/tip lesions in biopsy specimens with S1; tubular atrophy/interstitial fibrosis <25% (T0), 26-50% (T1), or >50% (T2); and cellular/fibrocellular crescents absent (C0), present in at least 1 glomerulus (C1) or in >25% of glomeruli (C2).

Statistical analyses

SPSS software version 22.0 was used for all statistical analyses. The clinical and demographic data were compared between anemic and non-anemic subjects using the

Student's t-test or χ^2 test as appropriate. Normally distributed variables were expressed as the mean ± standard deviation (SD), whereas non-normally distributed variables were expressed as the median (minimum-maximum). Univariate logistic regression and multivariate logistic regression were used to analyze the influencing factors of anemia in IgA nephropathy. For all analyses, P-values < 0.05 were considered statistically significant.

Patient and public involvement

Patients and public were not involved in the design and planning of the study.

Results

Patient characteristics

In total, 462 patients with IgA nephropathy qualified for analysis; of them, 272 were male, the mean age of all patients was 36.6 ± 11.3 years, and the mean hemoglobin level was 133 ± 19 g/L. The diagnostic criteria for anemia was met by 28.5% of the patients (132/462). The anemia rate was 21.3% (58/272) in the male patients and 38.9% (74/190) in the female patients. The majority (125/132) of patients with IgA nephropathy had normocytic, normochromic anemia. The clinical and demographic characteristics of patients are shown in Table 1. Compared with the non-anemic group, renal anemia was more likely to occur in older, female patients with IgA nephropathy. The anemic group had lower eGFR and serum albumin levels and higher 24 hr UPr levels than the non-anemic group (P < 0.05). Blood pressure, TC, TG, and CRP levels were not significantly different between anemic and non-anemic patients (P > 0.05). The serum pre-albumin level, an indicator of nutritional status, did not show any significant difference between the anemic and non-anemic group (P > 0.05). In addition, we analyzed the data of 258 subjects with available measurements of Total Iron Binding Capacity (Tibc), Fe and Transferrin saturation (TS) (the results was shown in Supplementary Table S3).

Table 1. Comparison of the characteristics of anemic and non-anemic patients with IgA nephropathy

Characteristic	Anemic n=132	Non-anemic n=330	P value
Age (years)	39.1±12.4	35.6±10.6	0.002
Sex (male/female)	58/74	214/116	0.000
BMI (kg/m^2)	24.2±3.1	25.0±3.5	0.013
Blood pressure (mmHg)			
Systolic	131.3±21.3	129.7±17.1	0.439
Diastolic	83.2±13.4	85.0±12.2	0.169
Laboratory results			
Hb, female (g/L)	106.7±11.0	129.6±8.6	0.000
Hb, male (g/L)	116.0±10.2	149.2±11.0	0.000
MCV (fl)	87.3±5.4	87.7±3.8	0.353
MCH (pg)	29.5±2.3	30.4±1.4	0.000
MCHC (g/L)	337.8±12.0	347.0±10.4	0.000
CRP (mg/dl)	0.3 (0.0-2.1)	0.3 (0.0-5.0)	0.361
Serum albumin (g/L)	37.3±3.9	40.6±4.0	0.000
BUN (mmol/L)	6.9 (1.3-31.8)	5.3 (2.2-19.5)	0.000
SCr (μmol/L)	128.2 (48.3-729.3)	91.8 (45.9-321.3)	0.000
UA (μmol/L)	398.8±121.7	378.4±103.7	0.091
TC (mmol/L)	4.4 (2.3-7.1)	4.5 (2.7-8.6)	0.787
TG (mmol/L)	1.8 (0.3-6.2)	1.9 (0.4-8.8)	0.055
Pre-albumin (g/L)	28.9 (10.5-52.2)	28.9 (11.8-56.2)	0.440
24 hr UPr (g/d)	1.7 (0.1-7.9)	1.7 (0.4-8.8)	0.000
eGFR (ml/min/1.73 m^2)	58.8±33.4	28.7 (11.8-56.2)	0.000

BMI, body mass index; Hb, hemoglobin; MCV, erythrocyte mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; CRP, C-reactive protein; BUN, blood urea nitrogen; SCr, serum creatinine; SUA, serum uric acid; TC, total cholesterol; TG, triglyceride; UPr, urinary

protein; eGFR, estimated glomerular filtration rate

Data are expressed as the means ± standard deviation or medians (minimum – maximum). eGFR was estimated using the CKD-epidemiology standard. Anemia was defined as a hemoglobin value of <130 g/L for males and <120 g/L for females.

Anemia rate in patients with various eGFR levels

As shown in Figure 2, the rate of anemia increased with a decrease in eGFR level. The ratios of anemia in patients with an eGFR of 30-59 ml/min/1.73 m^2 (42.9%, P < 0.001), an eGFR of 15-29 ml/min/1.73 m^2 (87.5%, P < 0.001), and an eGFR < 15 ml/min/1.73 m^2 (100%, P < 0.001) were higher than in patients with an eGFR > 60 ml/min/1.73 m^2 (17.8%). Compared to patients with an eGFR of 30-59 ml/min/1.73 m^2, the ratios of anemia in patients with an eGFR of 15-29 ml/min/1.73 m^2 (87.5%, P < 0.001) and an eGFR＜15 ml/min/1.73 m^2 (100%, P=0.008) were higher, and there was no significant difference between the rate of anemia in patients with an eGFR of 15-29 ml/min/1.73 m^2 and patients with an eGFR < 15 ml/min/1.73 m^2 (p=0.499).

Kidney pathological characteristics of anemic and non-anemic patients

Table 2 shows the kidney pathological characteristics of anemic and non-anemic patients. M (M0/1), E (E0/1), S (S0/1), T (T0/1/2), and C (C0/1/2) were used to characterize the IgA nephropathy pathological injury score. χ^2 testing showed that the ratios of M1, T2 and C2 were higher in the anemic group than in the non-anemic group (anemics versus non-anemics: M1, 56.8% versus 38.5%, P<0.001; T2, 52.3% versus 14.8%, P<0.001; C2, 7.6% versus 2.4%, P=0.009, respectively), while the ratios of E1 and S1 were not significantly different (anemics versus non-anemics: E1, 11.4% versus 16.1%, P=0.198; S1, 75.8% versus 66.7%, P=0.056, respectively) between the anemic and non-anemic patients.

Table 2. Renal pathological injury score comparison between anemic and non-anemic patients

Renal pathology[a]	Score	Anemic, n (%)	Non-anemic, n (%)	P
M	M0	57 (43.2)	203 (61.5)	
	M1	75 (56.8)	127 (38.5)	0.000

E	E0	117 (88.6)	277 (83.9)	
	E1	15 (11.4)	53 (16.1)	0.198
S	S0	32 (24.2)	110 (33.3)	
	S1	100 (75.8)	220 (66.7)	0.056
T	T0	35 (26.5)	185 (56.1)	
	T1	28 (21.2)	96 (29.1)	
	T2	69 (52.3)	49 (14.8)	0.000
C	C0	72 (54.5)	197 (59.7)	
	C1	50 (37.9)	125 (37.9)	
	C2	10 (7.6)	8 (2.4)	0.009

[a]Renal injury was estimated by the Oxford classification of IgA nephropathy. Variables were divided into subcategories as follows: Mesangial score <0.5 (M0) or >0.5 (M1); Endocapillary hypercellularity absent (E0) or present (E1); Segmental glomerulosclerosis absent (S0) or present (S1); presence or absence of podocyte hypertrophy/tip lesions in biopsy specimens with S1; Tubular atrophy/interstitial fibrosis <25% (T0), 26-50% (T1), or >50% (T2); Cellular/fibrocellular crescents absent (C0), present in at least 1 glomerulus (C1), or present in >25% of glomeruli (C2).

Analysis of the influencing factors associated with renal anemia in patients with IgA nephropathy

The correlative factors for renal anemia in patients with IgA nephropathy were determined using univariable and multivariable logistic regression as shown in Table 3. Age, sex, BMI, serum albumin, eGFR, M, T, and C were used as independent variables, and anemia and non-anemia was used as the dependent variable for all analyses. After the variables were screened, the major influencing factors identified included: sex (OR: 3.02, CI: 1.76-5.17), albumin (OR: 0.87, CI: 0.82-0.93), eGFR (OR: 0.98, CI: 0.97-0.99), and T2 (OR: 2.57, CI: 1.22-5.40). According to the logstic regression results, we used eGFR and hemoglobin as well as albumin and hemoglobin for correlation analysis. Besides, we compared the hemoglobin concentrations of the T0, T1, and T2 groups (as

shown in Supplementary Figure S1).

Table 3. Analysis of the influencing factors associated with renal anemia in IgA nephropathy patients (logistic regression)

	Univariable Logistic		Multivariable Logistic	
	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.03(1.01-1.05)	0.002	0.99(0.97-1.02)	0.510
Sex	2.35(1.56-3.55)	0.000	3.02(1.76-5.17)	0.000
BMI	0.92(0.87-0.98)	0.013	0.95(0.88-1.03)	0.203
ALB	0.82(0.78-0.87)	0.000	0.87(0.82-0.93)	0.000
eGFR	0.97(0.96-0.98)	0.000	0.98(0.97-0.99)	0.000
Mesangial hypercellularity				
M0	1		1	
M1	2.10(1.40-3.19)	0.000	1.22(0.72-2.06)	0.468
Tubular atrophy/interstitial fibrosis				
T0	1		1	
T1	1.54(0.89-2.69)	0.126	0.81(0.42-1.57)	0.541
T2	7.44(4.45-12.45)	0.000	2.57(1.22-5.40)	0.013
Cellular/fibrocellular crescents absent				
C0	1		1	
C1	1.09(0.72-1.67)	0.677	0.81(0.48-1.37)	0.440
C2	3.42(1.30-9.01)	0.013	1.81(0.56-5.83)	0.321

OR, odds ratio; 95% CI, 95% confidence interval; BMI, body mass index; ALB, serum albumin; eGFR, estimated glomerular filtration rate

Discussion

IgA nephropathy can lead to several complications, including anemia, renal hypertension, vascular disease, renal osteopathy, and hyperuricemia. Anemia is one of the primary risks factors for kidney disease progression and is associated with a poor prognosis.[15-17] When renal lesions are progressing, the prognosis is poor. The incidence

of intrarenal arteriole lesions in patients with IgA nephropathy is reportedly higher than that in patients with non-IgA nephropathy and membranous nephropathy;[18 19] however, there have been few clinical and pathological studies of renal anemia with large-scale populations conducted. In this study, we enrolled 462 patients for analysis. We found that the mean patient age was 36.6 ± 11.3 years, and the male-to-female ratio was 272:190. These patients showed characteristics of the disease types of patients with IgA nephropathy, which primarily occurs in young men. In addition, 28.5% of patients met the diagnostic criteria for anemia, and the rate of anemia in males (21.3%) was lower than that in females (38.9%), making this the first study to report a higher incidence of renal anemia in female patients with IgA nephropathy than in male patients with IgA nephropathy. The results of the regression analysis also suggested that the incidence of renal anemia among female patients was higher than that in male patients. The specific reasons for this difference are still unknown; although androgen levels may play a role,[20 21] additional studies are needed in the future. Therefore, clinicians should pay attention to female patients as renal anemia rates clearly differ by sex. Clinical observations and interventions for renal anemia should also differ by sex.

Our study showed that renal anemia caused by IgA nephropathy had normocytic normochromic anemia as the most common type of presentation, which was consistent with a previous study.[22] The prevalence of anemia increased with a reduction in eGFR levels in all age groups.[23 24] Our findings support the hypothesis that as the eGFR is gradually reduced, the incidence of anemia gradually increases, and there was a positive correlation between severity of anemia and albumin. In patients with CKD stage-3 disease, the incidence of renal anemia reaches 42.9%, which suggests that clinicians must consider the development of renal anemia in these patients and that clinical intervention should be provided as necessary. All patients with CKD stage-5 disease show combined anemia, and active treatment is required to delay the progression of renal function injury and increase patient quality of life.

IgA nephropathy refers to a group of diseases characterized by renal pathological damage, especially glomerular mesangial cell proliferation/immune complex

deposition.[25] The pathological characteristics of IgA nephropathy in this study showed that, compared with the non-anemic group, the rate of mesangial proliferation (M1), interstitial fibrosis, and tubular atrophy (T2) as well as the incidence of crescent lesion scores (C2), were higher in the anemic group. These results suggest that pathological damage is associated with renal anemia. The results of multivariable logistic regression analysis showed that having renal tubulointerstitial lesions >50% (T2) was associated with renal anemia in patients with IgA nephropathy, and the degree of anemia was most severe compared with T0 and T1. Mesangial proliferation, endocapillary proliferative lesions, segmental sclerosis or adhesion, and the disease severity of crescent formation were not significantly associated with renal anemia. This finding is important and consistent with our previous results, suggesting that severe renal tubulointerstitial lesions are an independent risk factor for IgA nephropathy.[26] These results suggest that patients with IgA nephropathy combined with renal anemia should be suspected of having renal tubulointerstitial lesions. Renal tubulointerstitial lesions lead to a reduction of erythropoietin (EPO), which is a hormone-like substance primarily secreted by renal tubulointerstitial cells that can regulate the proliferation and differentiation rates of erythrocyte precursors in bone marrow to promote erythrocyte production.[27] *Maxwell et al*.[28] showed that the ability of interstitial fibroblasts to produce EPO decreased in an interstitial nephropathy experimental model. Fibroblasts are interstitial mesenchymal cells that structurally support epithelia by producing extracellular matrix (ECM). In chronic kidney injury, sustained inflammation accompanies the proliferation of interstitial fibroblasts and myofibroblasts,[29] leading to renal fibrosis, which is the final common pathway for all CKD and eventually leads to renal failure.[30] More importantly, the restoration of EPO production in the fibrotic kidney raises the possibility of a potential therapeutic approach towards treating renal anemia.[31] Our study confirmed that renal anemia is associated with the severity of renal tubulointerstitial injury, which further suggests that the major cause of renal anemia is the reduction in EPO production caused by renal tubulointerstitial injury. These results are also consistent with our clinical observations

that renal anemia occurs earlier and is more severe in patients with chronic interstitial tubulointerstitial injuries. This phenomenon might be associated with the early destruction of the interstitial cells that produce EPO.

The results of the logistic regression analysis showed that low serum albumin was a correlative factor for renal anemia in patients with IgA nephropathy. At the same time, there was a positive correlation between severity of anemia and albumin. The patients selected for this study had IgA nephropathy and were first diagnosed at our center (diagnosis confirmed via renal biopsy); in other words, the enrollment had strict inclusion and exclusion criteria. Patients with a BMI < 18.5 kg/m^2 or malnutrition were excluded. In addition, the results suggested that pre-albumin levels, an important indicator used to indicate nutritional status, did not significantly differ between the anemic and non-anemic groups. Therefore, although this study showed that the renal anemia in patients with IgA nephropathy was associated with hypoproteinemia, the reasons for and mechanisms underlying this result remain unclear and require further exploration.

The main mechanism of renal anemia is related to the reduction of EPO production after kidney injury. But Coulon S et al [32]reported that polymeric IgA1 (pIgA1) positively regulates erythropoiesis through binding to Transferrin receptor 1 (TfR1) and accelerates erythropoiesis recovery in anemia. Under steady-state conditions, low concentrations of pIgA1 are produced by plasma cells, and most TfR1 combined with Fe-transferrin (Tf), with little stimulation of downstream ERK and Akt signaling pathways. Stress conditions such as hypoxia can lead to increase the pIgA1 production, allowing erythroid development to be boosted via ERK and Akt signaling.[33] Elevated serum pIgA1 levels were often observed in patients with IgA nephropathy,[34] based on the above research results, we speculate that the occurrence of renal anemia in IgA nephropathy will be different from that of other causes of CKD, and it need further research**.**

There are several important limitations to this study. As the study is cross-sectional, a causal relationship between influencing factors and anemia cannot be determined. In

future studies, renal anemia and patient prognosis will be further evaluated to provide a reliable basis for improving patient quality of life and survival time as well as stronger evidence for renal anemia management and treatment for clinicians. In addition, since our data were all from patients who underwent renal biopsy, some of the clinical patients initially identified did not undergo renal biopsy for various reasons; therefore, selection bias was present in our study. Data missing is another limitation of our study, such as Tibc, Fe and TS. There are more than 40% patients without these data (Tibc, Fe and TS) which are important indicators for distinguish iron deficiency anemia (IDA) from non-IDA. But fortunately, the routine blood test indicated the IgA nephropathy anemia is normocytic normochromic anemia. That means IDA is not the reason for IgA nephropathy anemia. At the same time, the reduction of EPO production is the major cause of renal anemia, but EPO test was not available in daily clinical work in this retrospective study. In future prospective studies we need to take EPO into observation and the design should be more scientific and stricter.

Conclusions

In summary, using a large study population, we identified that renal anemia is a common complication in patients with IgA nephropathy. The anemia type was primarily normocytic and normochromic. With the aggravation of renal dysfunction, the incidence of renal anemia increased. Patients with CKD stage-3 disease and above should be monitored for renal anemia development and possible intervention. The female sex, hypoalbuminemia, eGFR reductions, and severe renal tubulointerstitial lesions were identified as influencing factors for renal anemia development in patients with IgA nephropathy. These findings provide new insight into our understanding of anemia in IgA nephropathy and may improve the management and treatment of clinical renal anemia.

Contributors WY, WRB and CXM contributed to design this study. WY, STY, HMJ，LP collected and analyzed the data. WY, WRB, STY and HMJ contributed to the

preparation and editing of the manuscript. All authors agreed to be accountable for all aspects of the work to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

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Competing interests None declared.

Ethics approval The Ethics Committee of the General Hospital of the Chinese People's Liberation Army.

Data sharing statement All relevant data are within the paper and its Supporting Information files.

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Figure Legends

Figure 1. Flow chart showing the study design. AKI, acute kidney injury; BMI, body mass index.

Figure 2. The rate of anemia and non-anemia in patients with different eGFR levels. Comparison of the rate of anemia at different eGFR levels: *P<0.05 compared with anemic patients with an eGFR > 60 ml/min/1.73 m^2; #P<0.05 compared with anemic patients with an eGFR of 30-59 ml/min/1.73 m^2

Figure S1. The relations between hemoglobin level and eGFR, Albumin and tubular atrophy/interstitial fibrosis (T). (A) Correlation of Hemoglobin with eGFR. (B) Correlation of Hemoglobin with Albumin. (C) Relationship between tubular atrophy/interstitial fibrosis score and hemoglobin level.

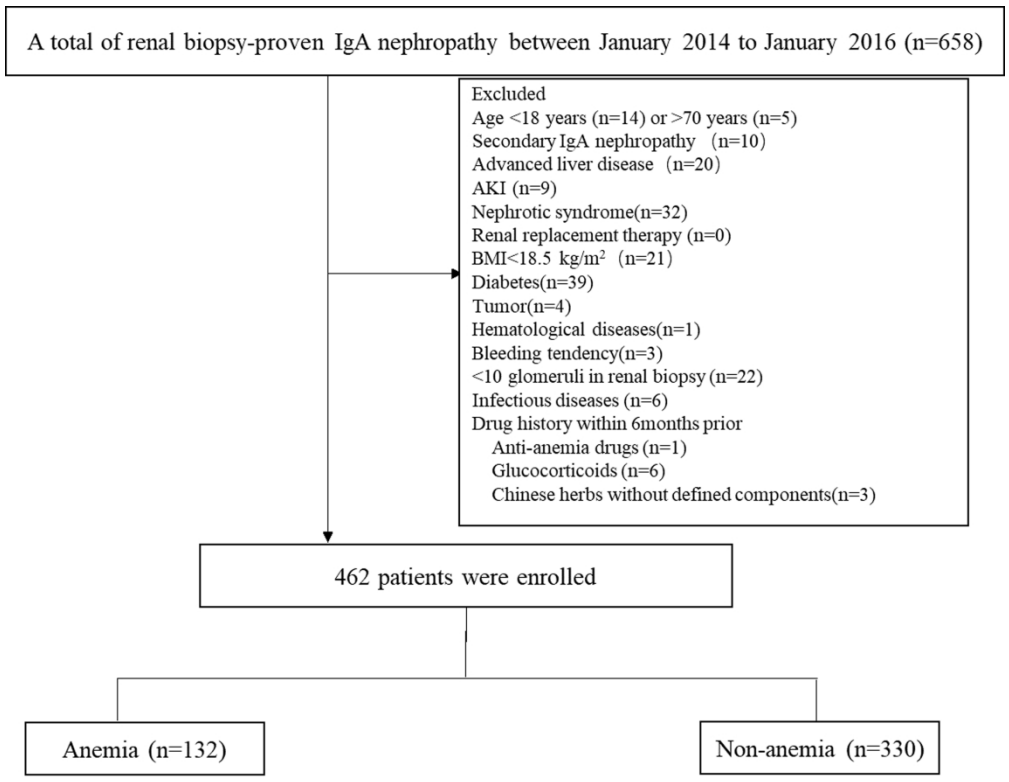


Figure 1. The flow chart of the study. AKI= acute kidney injury, BMI= body mass index.

309x238mm (300 x 300 DPI)

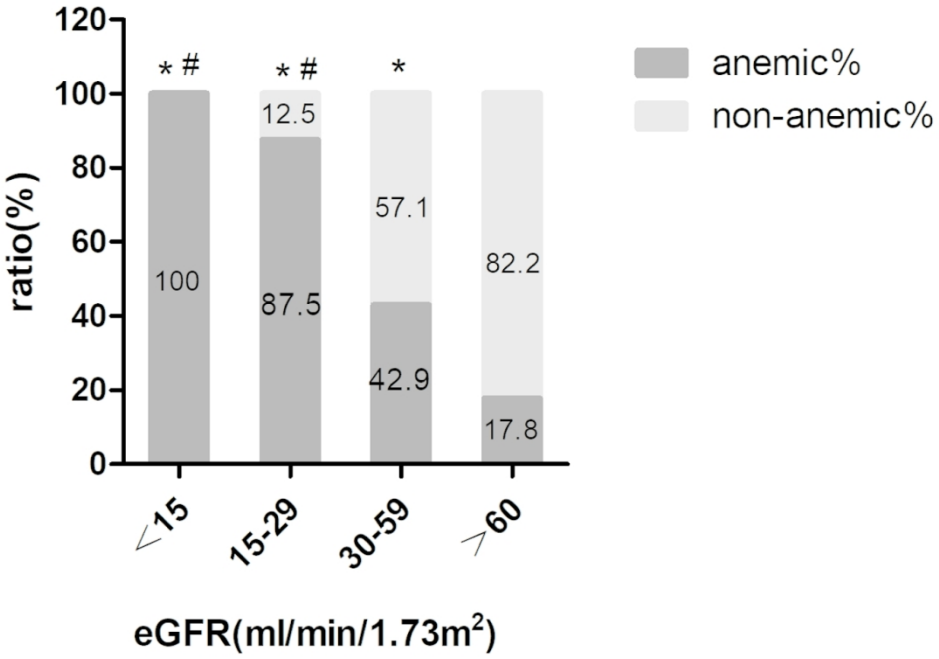
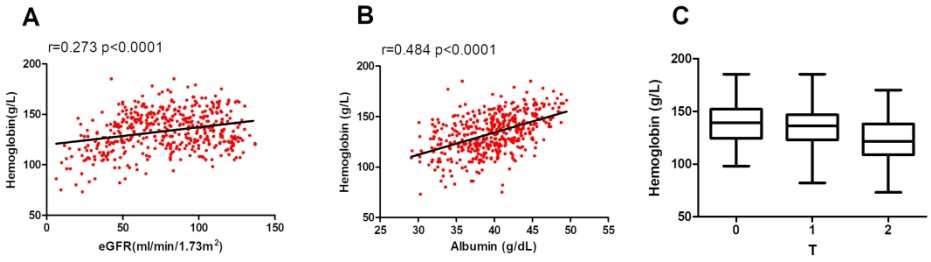


Figure 2. The ratio of anemia and non-anemia in patients with different eGFR levels. Comparison of the ratio of anemia at different eGFR levels: *P<0.05 compared with the anemic patients of eGFR>60 ml/min/1.73m2, #P<0.05 compared with the anemic patients of eGFR 30-59ml/min/1.73m2

123x90mm (300 x 300 DPI)



The relations between hemoglobin level and eGFR, Albumin and tubular atrophy/interstitial fibrosis (T). (A) Correlation of Hemoglobin with eGFR. (B) Correlation of Hemoglobin with Albumin. (C) Relationship between tubular atrophy/interstitial fibrosis score and hemoglobin level.

273x81mm (300 x 300 DPI)

Table S1. Possible indications for renal biopsy.

indications
Microscopic haematuria
Urologically unexplained macroscopic haematuria
Proteinuria
Nephrotic syndrome
Impaired kidney function
Hypertension
Possible renal involvement in systemic disease in:
multiple myeloma
monoclonal gammopathy of uncertain significance
systemic lupus erythematosus
antiphospholipid syndrome
diabetes
systemic vasculitis
scleroderma

Table S2. Contraindications to renal biopsy

Contraindication	Reason
Relative:	
Hypertension	Poorly controlled hypertension thought to increase risk of bleeding
Renal asymmetry	Suggestive of a process causing differential loss of renal mass (eg reflux nephropathy, atherosclerotic renal artery stenosis – although both these can cause proteinuria)
Decreased renal size (usually assessed as bipolar length on ultrasound)	Suggestive of chronic (therefore irreversible) renal damage, predictive of nonspecific fibrotic changes on biopsy Increased risk of complications reported in most series.
Single kidney	Accepted wisdom, based on the fact that the patient will be put into renal failure if there is irreversible damage to the kidney; however, if the patient appears likely to go into renal failure if left untreated, there is less to lose, and a biopsy may be justified if it might disclose a treatable condition

Unco-operative patient	Increased risk of complications if the patient cannot reliably stop breathing during needle puncture. Consider alternatives including biopsy under general anaesthetic, transvenous biopsy
Hydronephrosis	Obstructive nephropathy may be the cause of the renal disease (though seldom causes proteinuria) and should be investigated and treated first Increased risk of macroscopic haematuria due to biopsy needle penetrating renal pelvis or calyces
Suspected upper urinary tract infection	Urinary tract infection with white cell casts should be treated with antibiotics Active infection would contraindicate immunosuppressive treatment Biopsy might spread infection or be complicated by perinephric abscess formation
Absolute:	
Uncorrected coagulopathy	If biopsy is imperative, consider transvenous biopsy rather than percutaneous.

Table S3. Comparison of the results of Tibc, Fe and TS of anemic and non-anemic patients with IgA nephropathy

Laboratory results	Anemic n=177	Non-anemic n=81	P-value
Tibc (μmol/L)	50.4±8.0	45.7±8.7	0.000
Fe (μmol/L)	18.6(14.6-22.8)	14.3(10.7-17.6)	0.000
TS (%)	0.36(0.28-0.46)	0.33(0.24-0.42)	0.093

STROBE 2007 (v4) Statement - Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Page 4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 4,5
Bias	9	Describe any efforts to address potential sources of bias	*Page 5,6*
Study size	10	Explain how the study size was arrived at	Page 4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 4,5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 6
		(*b*) Describe any methods used to examine subgroups and interactions	Page 6
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Page 6
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study - eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 6,7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	Page 4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 6,7
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	Page 6,7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 9-11
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 9-11
Other analyses	17	Report other analyses done-eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 11-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 13,14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 11-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.