PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Clinical and pathological factors of renal anemia in patients with IgA nephropathy in Chinese adults: a cross-sectional study
AUTHORS	Wang, Yang; Wei, Ri-bao; Su, Ting-yu; Huang, Meng-Jie; Li, Ping; Chen, Xiang-mei

VERSION 1 – REVIEW

REVIEWER	Koichi Nakanishi Graduate School of Medicine, University of the Ryukyus, Japan
REVIEW RETURNED	03-May-2018

GENERAL COMMENTS	The authors examined the incidence of anemia, and the clinical and pathological characteristics and influence factors associated with renal anemia in 462 patients with IgA nephropathy. The study is important and interesting. The manuscript is well written and easy to follow. Major concerns: 1) Renal biopsy criteria are important in the present study. They should be mentioned in the manuscript. 2) The author should describe who performed pathological diagnoses. 3) Data of Fe, UIBC and Ferritin in patients in the current study should be shown if possible. If impossible, it should be mentioned in the manuscript as a limitation. 4) Data of EPO in patients in the current study should be shown if possible. If impossible, it should be mentioned in the manuscript as a limitation. 5) The authors should discuss the comparison of incidence of anemia between the normal population and the patients with IgA nephropathy. 6) Severity of anemia is important. The authors should show the relations between severity of anemia and clinical/pathological data in the present study. 7) Even in an early stage of CKD, a considerable part of patients show anemia in the present study. But, the number of patients with anti-anemia drugs is only one (Figure 1). It is strange. The authors should explain the reason of such a low number of patients with anti-anemia drugs. Basically, the number of patients who need

	anti-anemia drugs is also important data for the incidence of anemia. The authors had better consider such a situation. Minor concern: 1) As to exclusion criteria, there is a discrepancy between the manuscript (≤10 glomeruli in renal biopsy) and Figure 1 (<10 glomeruli in renal biopsy). How did the authors deal with 10 glomeruli in renal biopsy?

REVIEWER	Jonathan Barratt University of Leicester, UK
REVIEW RETURNED	11-May-2018

GENERAL COMMENTS	This paper describes the prevalence of anaemia in a single centre cohort of patients with IgAN. It has long been established that a normochromic normocytic anaemia is associated with worsening decline in GFR and renal scarring. The authors give no rationale for why they suspect the development of anaemia may be different in IgAN compared to other causes of CKD. What would be relevant would be to determine whether the anaemia seen in patients with IgAN was disproportionate compared to a matched CKD non-IgAN population. It has been proposed that IgA-transferrin interactions may be abnormal in IgAN and therefore there may be a hypothesis to be tested with this in mind- unfortunately the authors do not address this question.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:
Reviewer: 1
Reviewer Name: Koichi Nakanishi Institution and Country: Graduate School of Medicine, University of the Ryukyus, Japan
Please state any competing interests: None declared.
Please leave your comments for the authors below.
The authors examined the incidence of anemia, and the clinical and pathological characteristics and influence factors associated with renal anemia in 462 patients with IgA nephropathy. The study is important and interesting. The manuscript is well written and easy to follow.
Major concerns:
1) Renal biopsy criteria are important in the present study. They should be mentioned in the manuscript.

Response: We acknowledge your comments very much, which are valuable in improving the preciseness of our manuscript. Following your advice, we added the renal biopsy criteria in the manuscript. It has been added to the supplementary material as Table S1 and Table S2.
The detailed information was presented on page 4, paragraph 2.
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.

2) The author should describe who performed pathological diagnoses.

Response: We appreciate and agree with this important suggestion. Based on your suggestion, we added the pathology of renal injury was estimated independently by Xiang-mei Chen and Xue-guang Zhang according to the Oxford classification of IgA nephropathy (Trimarchi H. KI 2017).
The detailed information was presented on page 5, paragraph 6.

3) Data of Fe, UIBC and Ferritin in patients in the current study should be shown if possible. If impossible, it should be mentioned in the manuscript as a limitation.

Response: We appreciate and agree with this important suggestion. We have collected the data of Total Iron Binding Capacity (Tibc), Fe and Transferrin saturation (TS), but because of the retrospective cross-sectional study, the missing rate of this data is more than 40%, there is no effective way to solve the problem. So, these data have lost valuable value in the current environment. If we include statistics, it will lead to serious bias, so we did not use this part of the data in statistics. Loss of data cannot be controlled, which is one of the limitations of our study.
However, we used t-test and Kolmogorov-Smirnov test to statistics the data of 258 subjects with available measurements. The results showed that there was a statistical difference between the two groups of Tibc and Fe ($p < 0.001$). There was no significant difference in the saturation of transferrin in the two groups ($p=0.093$).This result was placed in supplementary Table S3.
The detailed information was presented on page 6, paragraph 3; page 14, paragraph1.
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

4）Data of EPO in patients in the current study should be shown if possible. If impossible, it should be mentioned in the manuscript as a limitation.

Response: We acknowledge your comment and suggestion very much, which is valuable in improving the preciseness of our manuscript. This prospective study was conducted from January 2014 to January 2016, and the data of EPO was not collected, which made it impossible for us to provide this data. This is the limitation of our study.
The detailed information was added on page 14, paragraph 1.

5) The authors should discuss the comparison of incidence of anemia between the normal population and the patients with IgA nephropathy.

Response: Based on your suggestion, we retrieved relevant literatures. According to the survey of Mcfarlane SI et al（Mcfarlane SI, et al. AJKD 2008）, the anemia incidence in National Health and Nutrition Examination Survey (NHANES, which is a series of health examination surveys conducted by the National Center for Health Statistics of the US Centers for Disease Control and Prevention.) was 6.3%, and the incidence of renal anemia in chronic kidney disease was 15%. There are many causes of anemia, such as iron deficiency anemia, megaloblastic anemia, aplastic anemia and hemolytic anemia, etc. The main cause of renal anemia is related to decreased renal function and reduced EPO production in chronic kidney disease. Considering the particularity of the cause of renal anemia, we did not compare the incidence of anemia between the normal population and the patients with IgA nephropathy.

6) Severity of anemia is important. The authors should show the relations between severity of anemia and clinical/pathological data in the present study.

Response: We are very sorry for our negligence of the relations between severity of anemia and clinical/pathological data analysis.
According to Logstic regression results, the influence factors associated with renal anemia in patients with IgA nephropathy were sex, albumin, eGFR and renal tubulointerstitial lesions >50% (T2). Because eGFR, serum albumin, and hemoglobin are all in accordance with the normal distribution, we used hemoglobin with eGFR and albumin for correlation analysis. The results prompt that there is

a positive correlation between eGFR and hemoglobin (r=0.273 p <0.0001) as well as serum albumin and hemoglobin（r=0.484 p<0.0001）. In addition, we compared the hemoglobin concentrations of the T0, T1, and T2 groups. Because the homogeneity test of variance was not satisfied, we analyzed it with the Kruskal-Wallis test. The results suggest that the hemoglobin level of T2 is lower than T0 (p<0.001) and T1 (p<0.001), and there is no significant different between T0 and T1 (p=0.385). We presented the relations between severity of anemia and clinical/pathological data on Supplementary Figure S1.
The detailed information was added on page 9, paragraph 1; page 10, paragraph 1; page 11, paragraph 2; page 12, paragraph 2; page 13, paragraph 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.

7) Even in an early stage of CKD, a considerable part of patients show anemia in the present study. But, the number of patients with anti-anemia drugs is only one (Figure 1). It is strange. The authors should explain the reason of such a low number of patients with anti-anemia drugs. Basically, the number of patients who need anti-anemia drugs is also important data for the incidence of anemia.
Response: The patients selected for this study had IgA nephropathy and were first diagnosed at our center. When the disease was found, most of them came to our hospital without treatment to further clarify the cause. Therefore, there were few cases of treatment for kidney disease and anemia. In our cross-sectional study population, there is one case of compliance with anemia treatment.

Minor concern:
1)As to exclusion criteria, there is a discrepancy between the manuscript (≤10 glomeruli in renal biopsy) and Figure 1 (<10 glomeruli in renal biopsy). How did the authors deal with 10 glomeruli in renal biopsy?
Response: We are very sorry for this mistake. We corrected and changed the term<10 glomeruli in renal biopsy. According to previous studies, 10 glomeruli were the minimum required in a renal biopsy (Wang HJ, NDT 1998; Hoy WE, JASN 2006). To be more accurate in pathological results of renal biopsy, we excluded cases <10 glomeruli in renal biopsy.
We have corrected this error and marked it in red, see page 4, paragraph 2.

Reviewer: 2
Reviewer Name: Jonathan Barratt, Institution and Country: University of Leicester, UK Please state any competing interests: None declared Please leave your comments for the authors below.

1. This paper describes the prevalence of anaemia in a single centre cohort of patients with IgAN. It has long been established that a normochromic normocytic anaemia is associated with worsening decline in GFR and renal scarring. The authors give no rationale for why they suspect the development of anaemia may be different in IgAN compared to other causes of CKD.

Response: Previous studies have shown that different causes of CKD have different rates of anemia (Abramson JL, et al. Kidney Int 2003;Vlagopoulos PT, et al. JASN (2005).IgA nephropathy is the most common primary glomerular disease in the world.If not well-controlled, approximately 25-45% of IgA nephropathy patients will

progress to chronic renal failure within 20 years.Therefore, we choose IgAN as the research subject.
The detailed information was added on page 3, paragraph 5.

2.What would be relevant would be to determine whether the anaemia seen in patients with IgAN was disproportionate compared to a matched CKD non-IgAN population. It has been proposed that IgA-transferrin interactions may be abnormal in IgAN and therefore there may be a hypothesis to be tested with this in mind-unfortunately the authors do not address this question.

Response: We acknowledge your comment and suggestion very much, which is valuable in improving the preciseness of our manuscript. This study is a retrospective cross-sectional study, mainly to study the related factors of renal anemia with IgA nephropathy (IgAN), the results showed that gender, albumin, eGFR, tubulointerstitial injury were related with anemia of IgAN, but due to the limitations of the cross-sectional study, which cannot explain its causal relationship and the cause.
After searching the literature, we did not find the relevant literatures on IgA and transferrin but found some literatures on the relationship between IgA and transferrin receptor (TfR). Transferrin receptor 1 (TfR1) was identified as an IgA1 receptor can selectively bind IgA1 (Moura IC, et al. Journal of Experimental Medicine 2001). Monteiro RC, et al (Monteiro RC, et al. Trends in Molecular Medicine 2002) demonstrated that highly purified polymeric IgA1 (pIgA1) binds to TfR better than does mIgA1. Patients with IgAN often have elevated serum pIgA1 levels (Valentijn RM, KI 1984). pIgA1 positively regulates erythropoiesis through binding to TfR1 and accelerates erythropoiesis recovery in anemia (Coulon S et al. Nat Med 2011).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 (Paulson RF. Nat Med 2011). Based on these research results, we speculate that the occurrence of renal anemia in IgA nephropathy will be different from other causes of CKD.
The detailed information was added on page 13, paragraph 3.

VERSION 2 – REVIEW

REVIEWER	Koichi Nakanishi Department of Child Health and Welfare (Pediatrics), Graduate School of Medicine, University of the Ryukyus, Japan
REVIEW RETURNED	07-Aug-2018

GENERAL COMMENTS	The manuscript has been adequately improved.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author: Reviewer: 1 Reviewer Name: Koichi Nakanishi Institution and Country: Department of Child Health and Welfare (Pediatrics), Graduate School of Medicine, University of the Ryukyus, Japan

Please state any competing interests or state ‘None declared’: None declared

Response: We acknowledge your comments very much. According to your suggestion, we state ‘competing interests’ as ‘None declared’

Please leave your comments for the authors below

Response: Based on your suggestion, we listed the comments for the authors below and state it in the ‘contributors’ section:

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.