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CLINICAL RESEARCH

Correlation of Urinary Soluble CD163 Levels With Disease Activity and Treatment Response in IgA Nephropathy

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Introduction: The TESTING trial demonstrated that corticosteroids reduce the risk of kidney failure in patients with IgA nephropathy (IgAN) but increase the risk of serious adverse events. Reliable noninvasive biomarkers are needed to identify patients who would benefit most from corticosteroid therapy. Previous studies suggest glomerular macrophage infiltration is associated with response to immunosuppressive therapy in IgAN and urinary soluble CD163 ([u-sCD163], a marker of alternatively activated macrophages [M2]c macrophage) is correlated with clinical remission in vasculitis. This study aims to investigate the association between u-sCD163 and response of steroids therapy in IgAN.

Methods: We measured u-sCD163 in patients from a large IgAN cohort and Chinese participants of the TESTING trial. The correlation of baseline or serial u-sCD163 and their response of corticosteroids therapy or kidney outcomes were investigated.

Results: In cross-sectional analysis, u-sCD163 levels correlated with kidney macrophage infiltration, especially in crescentic areas, and with active lesions. Subgroup analysis of the TESTING cohort showed higher levels u-sCD163 were associated with greater benefits from corticosteroids therapy in proteinuria remission (odds ratio, 35.56 [95% confidence interval, CI: 7.62–292.34] vs. 3.94 [95% CI: 1.39–12.93], P for interaction: 0.036). Corticosteroids therapy significantly reduced u-sCD163 levels at 6 months compared to placebo group (79% [interquartile range: 58%–91%] vs. 37% [−11% to 58%], $P < 0.001$). There was no difference in the suppressive effects on u-sCD163 by either dosage of corticosteroids (full and reduced-dose). The suppression of u-sCD163 was significantly associated with a reduced risk of kidney progression events (adjusted hazard ratio: 0.52, 95% CI: 0.30–0.93, $P = 0.027$).

Conclusion: u-sCD163 is a reliable noninvasive biomarker associated with active pathological lesions in IgAN and can guide glucocorticoid therapy.

Kidney Int Rep (2024) **9,** 3016–3026; https://doi.org/10.1016/j.ekir.2024.07.031
KEYWORDS: crescentic lesions; IgA nephropathy; treatment; u-sCD163

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IgAN is the most common primary glomerular disease worldwide.[1] More than half of the patients will eventually progress to kidney failure requiring dialysis and/or transplantation.[2] Supportive therapy in particularly optimal blood pressure control with a maximum tolerated dose of renin-angiotensin system inhibitors are guidelines recommended standard of care. Recently, the sodium-glucose cotransporter-2 inhibitors and endothelin A receptor antagonist have shown to be promising agents that are increasingly adopted in addition to renin-angiotensin-aldosterone system inhibition.[3–5] The completed trials of corticosteroids have demonstrated that these agents play a role in proteinuria reduction, preserving kidney function, and reducing the future risk of kidney failure.[6] However, this protective effect

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Received 21 January 2024; revised 1 July 2024; accepted 29 July 2024; published online 3 August 2024

comes at the expense of an elevated risk of serious infections that warrants careful risk-benefit discussion between the patients and clinicians. Although Kidney Disease: Improving Global Outcomes guidelines suggest the use of the international IgAN prediction tool for risk stratification of people with IgAN, this tool does not provide insights on the likelihood of responsiveness to a specific treatment. Therefore, it is highly clinically relevant to identify biomarkers that can guide clinicians to identify who are more likely to benefit from corticosteroid therapy to guide therapeutic decision making. Currently, there are no validated prognostic serum or urine biomarkers for IgAN to predict response of corticosteroids therapy.

Increasing evidence suggests that the mononuclear or macrophage system plays important roles in the progression of IgAN.[7] Macrophages can be broadly categorized into classically activated macrophages and M2 macrophages.[8] M2 macrophages can be further subdivided into 4 subgroups, namely M2a, M2b, M2c, and M2d. CD163, a transmembrane protein with a molecular weight of 130 kDa, is a surface marker expressed by M2c macrophages that infiltrate tissues during the healing phase of inflammation.[9] In a prospective cohort study, the intensity of glomerular macrophage infiltration, alone or combined with clinical and histologic data, are predictive of the response to immunosuppression. However, such evaluation necessitates kidney biopsy.[10] Studies have shown that the level of urinary soluble CD163 (u-sCD163) is associated with active lupus nephritis or renal vasculitis,[11-14] and is linked with clinical remission. In IgAN, it was found that kidney macrophage infiltration was mainly composed of CD163-positive M2c macrophages.[15] CD163-positive macrophages in glomeruli were associated with lower estimated glomerular filtration rate (eGFR) and presence of crescents.[16] Moreover, u-sCD163 levels are an independent and strong predictor of clinical remission in IgAN.[17] The TESTING study ($n = 503$) was the largest multicenter, randomize, placebo-controlled trial to-date that evaluated the safety and efficacy of corticosteroids (methylprednisolone) on proteinuria reduction and kidney progression in IgAN.[6] Baseline and series urine samples of participants from China were stored which will be used for this analysis. We aimed to the evaluate the reliability of u-sCD163 as a biomarker to predict treatment responsiveness to corticosteroid therapy in IgAN. In addition, we evaluated u-sCD163 in a large cohort of 517 patients with IgAN.

METHODS

Patients

This study consisted of a cross-sectional and a longitudinal evaluation to determine the role of u-sCD163 in IgAN. The cross-sectional study consists of 517 patients recruited from Peking University First Hospital between January 2012 and December 2019. Recruited participants had a diagnosis of primary IgA confirmed by a kidney biopsy with a baseline proteinuria of >1 g/d. Kidney biopsy specimen with at least 8 or more glomeruli were included in the analysis (Supplementary Figure S1A). Clinical characteristics, including age at the time of kidney biopsy, sex, eGFR, 24-hour protein excretion, blood pressure, presence of microscopic hematuria, and plasma albumin were collected at the time of renal biopsy. Among them, examination of microscopic hematuria in urine was performed using a fully automated urine particle analyzer (UF-1000; sysmex; automated method), and results were recorded as red blood cells/μl. Pathologic lesions were evaluated according to the Oxford's MEST-C classification.[18] All kidney biopsy specimens were reviewed and graded by an independent pathologist (SS) who was blinded to the participants' clinical data. For CD163 staining, 20 patients were selected from our IgAN cohort through simple random sampling. Three patients were excluded due to having fewer than 8 glomeruli in the biopsy section.

The TESTING study has been previously described.[6] The trial recruited 503 patients from China (including Hong Kong), Australia, Canada, India, and Malaysia. Participants were originally randomized to receive oral methylprednisolone, 0.6 to 0.8 mg/kg/d (full dose cohort), for 2 months, tapering by 8 mg/d each month for a total treatment period of 6 to 8 months or matching placebo. For participants randomized from 2017 to 2019, the study protocol was amended due to excess of infection-related serious adverse events to 0.4 mg/kg/d to a maximum daily dose of 32 mg/d. The dose was tapered after 2 months by 4 mg/d each month (reduced dose cohort), for a total of 6 to 9 months. A total of 379 participants were from China. Among them, 282 patients had urine samples at baseline (Supplementary Figure S1B). The urine biospecimens were centrifuged at 2500 rpm for 20 minutes at 4 °C and the supernatant was stored in aliquots at −80 °C until use. This study was approved by the ethics committees of each of the participating sites, and all participants provided written informed consent.

In this study, the primary end points were the composite end points of the TESTING trial. It was defined as a combined event of ≥40% reduction in eGFR, kidney failure (eGFR < 15 ml/min per 1.73 m^2, requirement for maintenance dialysis or kidney transplantation), or death due to kidney disease. For this study, complete proteinuria remission defined as 24-hour urinary protein <200 mg/d. Partial proteinuria remission is defined as proteinuria reduction less than

50% of baseline by 24-hour urinary protein, and <1 g/d. Clinical remission was defined as total proteinuria remission by the end of the 6-month therapy, which include the complete proteinuria remission and partial proteinuria remission. This study has been approved by the Ethics Committee of Peking University First Hospital (approval number 2023509, 2023531).

Detection of Urine Levels of sCD163 by Enzyme-Linked Immunosorbent Assay

The enzyme-linked immunosorbent assay kit (DuoSetDY1607; R and D systems, Minneapolis, MN) was used to assay u-sCD163. All operating procedures were performed in accordance with the manufacturer's instructions. The u-sCD163 level was normalized using urinary creatinine and expressed as ng/mg creatinine. The creatinine level was determined by an improved Jaffe method (BioAssay Systems, DICT-500). In addition, patients whose u-sCD163 decreased by more than 50% and less than the median in the sixth month from baseline were defined as responders. Otherwise, they were defined as nonresponders.

Detection of CD163, CD206 and CD68 by Immunohistochemistry

Formalin-fixed, paraffin-embedded kidney tissues were cut into 2-μm-thick sections. Two adjacent tissue sections were mounted onto a single slide. After deparaffinization in xylene and rehydration in different concentrations of alcohol, sections were treated by heating in a pressure cooker containing Tris-ethylenediaminetetraacetic acid buffer (pH, 9.0) solutions for 3 minutes. Sections were first treated in 3% H_2O_2 for 8 minutes, followed by incubation with the primary antibody for 1 hour at 37 °C, and then incubated with the secondary antibody for 20 minutes before developing the slides using 3, 3′-diaminobenzidine. Finally, 1 tissue section of all slides was counterstained with periodic acid-Schiff, and another section of all slides was stained nuclei by Mayer's hematoxylin. Then all slides were dehydrated, cleared, and permanently mounted.

The expression of CD163, CD206, and CD68 were semiquantitatively quantified as previously described.[13,19] Briefly, CD163, CD206, and CD68 expressions were assessed in 3 kidney compartments, including areas of crescents formation, glomeruli (without crescentic formation area) and tubulointerstitial area. The slides were scanned at 400× magnification using digital pathology slide scanners (Aperio AT2, Leica). Periodic acid-Schiff staining is used to assist in identifying crescents (Figure 1a and b). We evaluated all nonsclerotic glomeruli. CD163, CD206, and CD68 in the glomeruli (without crescentic formation area) and crescents area were scored on a scale of 0 to 3 as follows: 0 = no positive cells; 1 = 1 to 5 positive cells; 2 = 6 to 10 positive cells; and 3 = more than 10 positive cells. If a glomerulus has no crescentic lesions, the infiltrate score for this crescents area is 0. For tubulointerstitial area, the staining was scored on a scale of 0 to 3 (per mm^2) as follows: 0 = no positive cells; 1 = 1 to 100 positive cells; 2 = 100 to 300 positive cells; and 3 = more than 300 positive cells. The infiltrate score for glomeruli, crescents area or tubulointerstitial area is expressed as total score/the number of glomerulus or area (mm^2), respectively.

Statistics

Continuous variables that were normally distributed were expressed as the mean ± SDs or expressed as the median and interquartile range for variables that are not nonnormally distributed. Categorical variables were expressed as percentages. In the cross-sectional study, Spearman's correlation test was used to assess the correlation between u-sCD163 levels and other variables. We subcategorized all participants into 4 groups based on their u-sCD163 levels according to the quartiles. For continuous variables comparation between 4 groups, 1-way analysis of variance was used for normally distributed data, whereas Kruskal-Walli's test was employed for nonnormally distributed data. Ordinal categorical variables were compared using Kruskal-Walli's test, and positive rates were compared using the chi-square test. Bonferroni correction was used when 4 groups were compared. The linear trends in those variables according to u-sCD163 levels were tested using the linear regression analysis for continuous variables and the logistic regression analysis for proportional variables. The Kaplan-Meier curves were performed to evaluate the effect of u-sCD163 on kidney survival and differences between curves were analyzed using a log-rank test. Univariate and multivariable Cox regression analyses were applied to test the association between u-sCD163 group (responders and nonresponders) and the primary end points. In Cox model, we adjusted for age, sex, mean arterial pressure, proteinuria, eGFR, the use of methylprednisolone and Oxford classification (MEST-C scores). We tested the proportional hazards assumption for each covariate included in a Cox regression model. Time-dependent Cox regression was conducted when covariate did not satisfy the proportional hazards assumption. Comparison between groups of repeated measurements were performed using generalized estimating equation. In a subgroup's analysis of the effect of treatment on proteinuria remission, Firth logistic regression was included for analyses when the events were rare. All statistical tests were performed using SPSS version 25.0 (Chicago, IL) and R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). The figures were

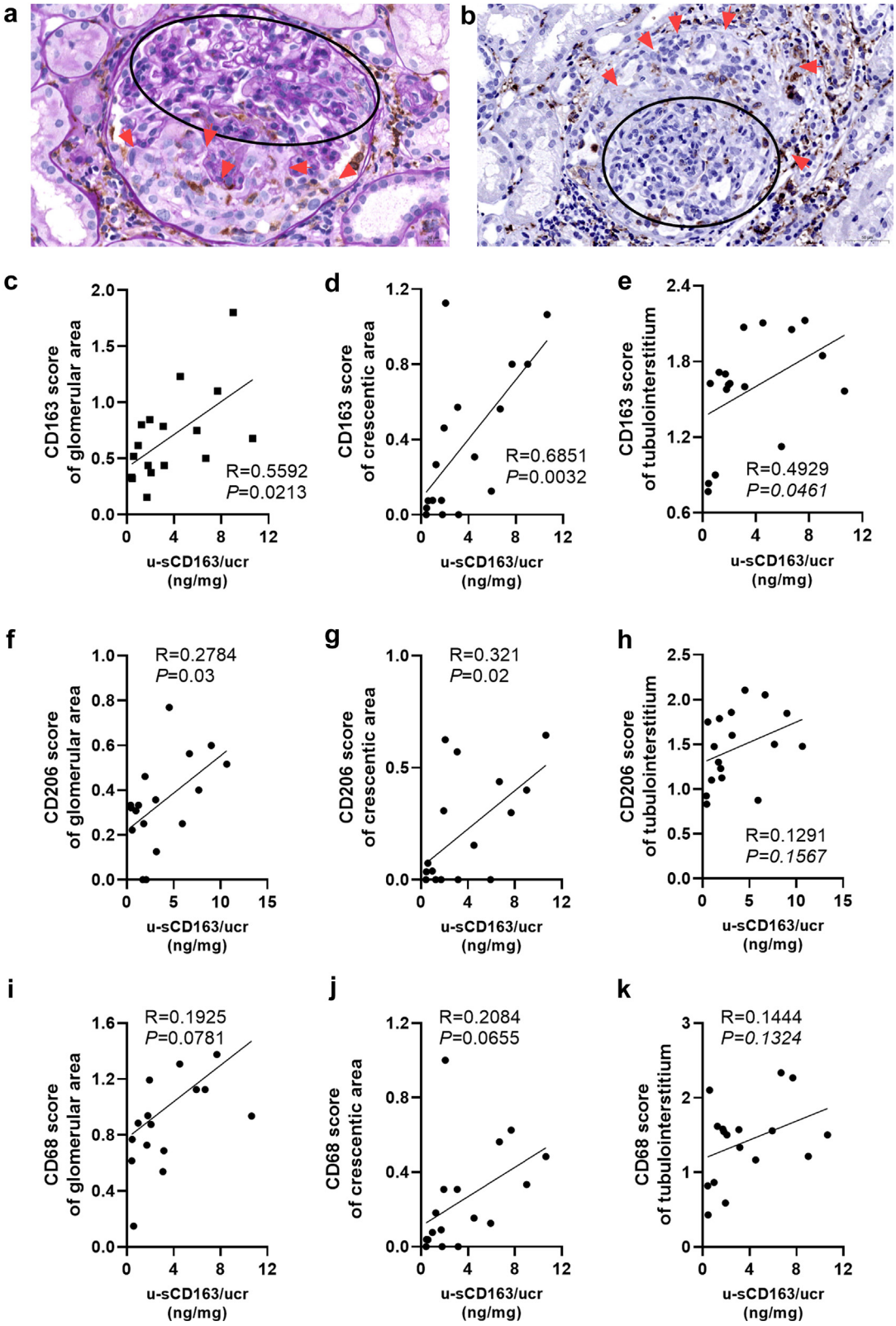

Figure 1. u-sCD163 was strongly associated with M2 macrophages infiltration on kidney biopsy. (a and b) CD163-positive cells can infiltrate the crescentic lesions (red arrow) and glomerular areas out the crescentic formation (black circle). (c–e) u-sCD163 levels were correlated with CD163-positive macrophage infiltration in glomerular, (d) in crescentic lesions and in tubulointerstitial area. (f) u-sCD163 levels were correlated with CD206 positive macrophage infiltration in glomerular, (g) in crescentic lesions, (h) but not in tubulointerstitial area. (i–k) u-sCD163 levels were not correlated with CD68 positive macrophage infiltration in kidney. Scale bar is 20 μm in a, and the scale bar is 50 μm in b. M2, alternatively activated macrophages; u-sCD163, urinary soluble CD163.

Table 1. Clinicopathological features of IgAN cohort patients

	1 n = 129	2 n = 130	3 n = 129	4 n = 129	P^a	P^b
Sex, male (n, %)	92 (71%)	73 (56%)	65 (50%)	45 (35%)	<0.001	<0.001
Age (yr)	35 ± 10	37 ± 12	38 ± 13	41 ± 16	0.024	<0.001
MAP (mm Hg)	97 ± 11	95 ± 11	95 ± 10	97 ± 13	0.258	0.877
Systolic BP (mm Hg)	128 ± 14	126 ± 16	127 ± 14	130 ± 18	0.232	0.435
Diastolic BP (mm Hg)	82 ± 10	80 ± 11	80 ± 10	81 ± 12	0.330	0.705
Proteinuria (g/24 h)	1.65 (1.28–2.32)	1.86 (1.41–2.77)	2.44 (1.62–4.10)	3.77 (2.43–5.9)	<0.001	<0.001
Scr (μmol/l)	115 (82.6–156)	107.8 (79.5–145)	108 (77.6–183.2)	113.2 (74.6–168.1)	0.889	0.212
eGFR (ml/min per 1.73 m^2)	71 (47–95)	74 (44–94)	66 (36–99)	55 (37–90)	0.154	0.058
Hematuria (RBCs/ul)	22.0 (8.3–61.8)	35 (12.5–157)	65.3 (17.5–190)	101.1 (36.9–503.8)	<0.001	<0.001
Albumin (g/l)	40.1 ± 4.4	37.7 ± 4.8	34.6 ± 6.6	31 ± 6.5	<0.001	<0.001
Oxford's classification (n, %)						
M1	69 (53%)	76 (58%)	84 (65%)	80 (62%)	0.26	0.095
E1	35 (27%)	41 (32%)	51 (40%)	90 (70%)	<0.001	<0.001
S1	92 (71%)	99 (76%)	87 (67%)	79 (61%)	0.065	0.032
T1/T2	45 (35%)/13 (10%)	52 (38%)/11 (9%)	50 (39%)/23 (18%)	52 (40%)/19 (15%)	0.088	0.025
C1/C2	64 (50%)/10 (8%)	71 (55%)/20 (15%)	65 (50%)/24 (19%)	69 (53%)/40 (31%)	<0.001	<0.001

BP, blood pressure; C, crescents; eGFR, estimated glomerular filtration rate; E, endocapillary hypercellularity; IgAN, IgA nephropathy; M, mesangial hypercellularity; MAP, mean arterial pressure; RBCs, red blood cells; Scr, creatinine; S, segmental glomerulosclerosis; T, interstitial fibrosis and tubular atrophy.
[a]Among the 3 groups.
[b]P for trend.
Unless otherwise indicated, the values represent n (%), the mean ± SD, or the median (25th–75th centiles).

generated using GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA) and R 3.6.3.

RESULTS

u-sCD163 Levels Correlate With Macrophages Infiltrations in Kidney Tissues

To evaluate the correlation between infiltration of CD163-positive macrophages in the kidney and u-sCD163 levels, 17 patients were selected for CD163 immunohistochemical staining and periodic acid-Schiff co-staining (Figure 1a and b). Their clinical pathological data were not statistically different from those of the cohort population (Supplementary Table S1). We found that the infiltrate scores for glomeruli, crescents area, and tubulointerstitial area all correlated with u-sCD163 levels (r = 0.5592, 0.6851, 0.4929, respectively; Figure 1c–e).

M2 macrophages predominantly express CD206. We also assessed the expression of CD206-positive macrophages in these 17 patients and found a correlation between u-sCD163 levels and the deposition of CD206-positive macrophages in the glomeruli (including crescents area) (Figure 1f–h). In addition, we evaluated CD68, a pan-marker for macrophages. The results indicate a trend of correlation between CD68-positive cells and u-sCD163 levels; however this did not reach statistical significance (Figure 1i–k).

Urinary-sCD163 Levels Correlate With Clinical Characteristics

In the cross-section study of 517 patients, levels of u-sCD163 at baseline were strongly correlated with eGFR ($r = -0.1083$, $P = 0.0137$; Supplementary Figure S2A), proteinuria ($r = 0.4757$, $P < 0.0001$; Supplementary Figure S2B) and serum albumin ($r = -0.5662$, $P < 0.0001$; Supplementary Figure S2C) and microscopic hematuria ($r = 0.3611$, $P < 0.0001$; Supplementary Figure S2D). We divided the participants into 4 groups based on their u-sCD163 levels according to the quartiles. In Table 1, we present the baseline characteristics according to u-sCD163 levels. Participants with higher u-sCD163 levels tended to be older and exhibited higher proteinuria, higher hematuria, and lower albumin levels (all P for trend < 0.05). The results also showed that patients with higher u-sCD163 levels were more likely to have lower eGFR levels, though this did not reach statistical significance (P for trend = 0.058). In addition, higher u-sCD163 levels were more frequently associated with E lesions and C lesions.

Association of u-sCD163 With Oxford's Classification MEST-C Scores

In Table 1, we show that patients with higher levels of u-sCD163 were more likely to exhibit C, E, and T lesions. However, we did not notice significant difference of u-sCD163 levels among patients with T0, T1, and T2 lesions when stratified by the C or E lesions. There were significant differences in u-sCD163 levels between E0 and E1 or C0 and C1-2 lesions when stratified by T lesions (Figure 2). The levels of u-sCD163 significantly increased with the increase of E and C lesions scores ($P < 0.001$) (Figure 2l).

u-sCD163 was Associated With Proteinuria Remission During Follow-up

The characteristics of the enrolled TESTING China population at randomization are shown in Supplementary Table S2. The median time of kidney biopsy to the

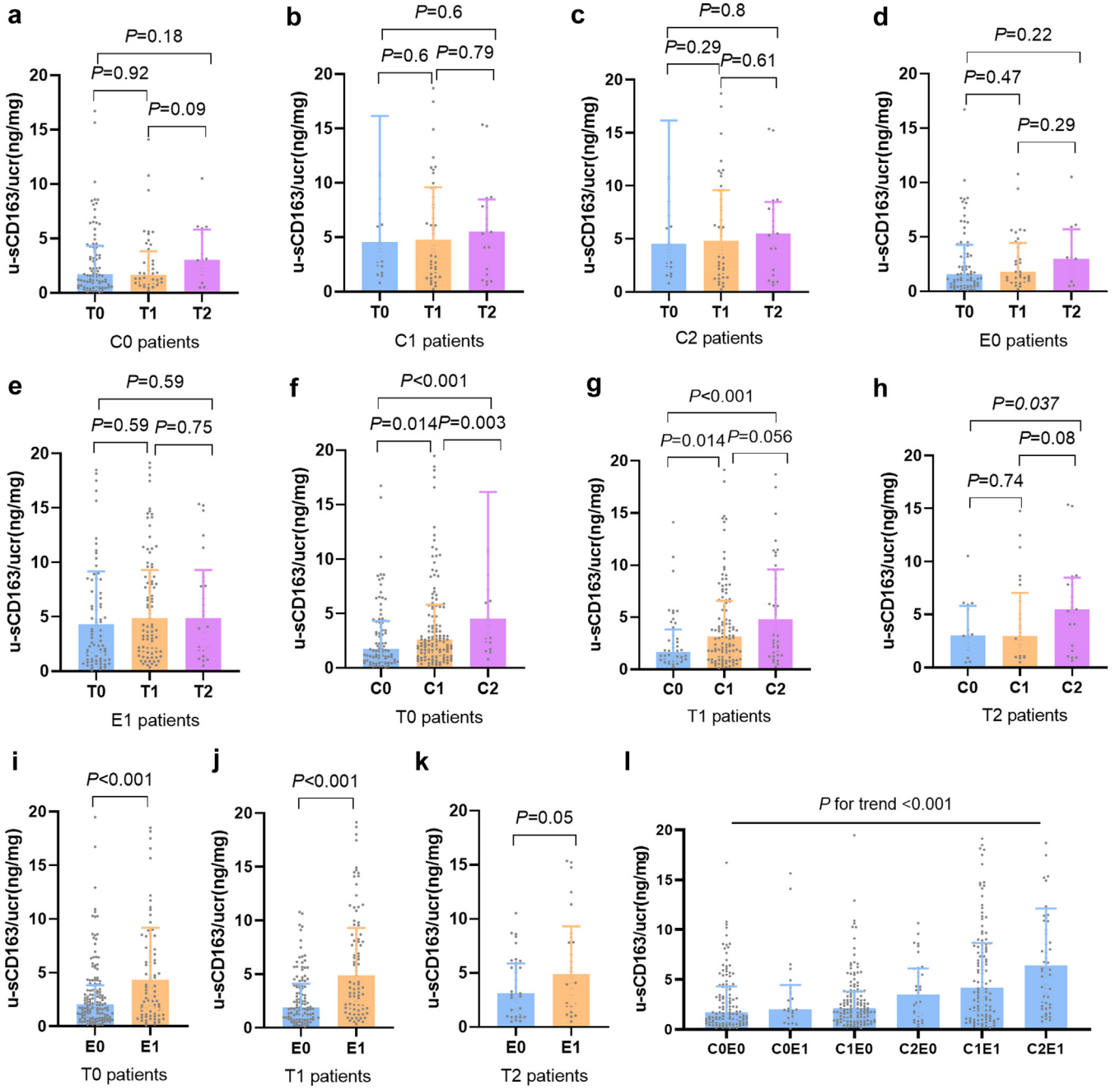

Figure 2. u-sCD163 is strongly associated with active lesions. (a–e) After stratifying by E or C lesions, there was no statistical difference in u-sCD163 levels between patients with different T lesions. (f–k) After grouping by T lesions, there were differences in u-sCD163 levels between different E lesions and between different C lesions. (l) Patients with more active lesions had higher baseline u-sCD163 levels. u-sCD163 levels in patients with different active lesions were compared, showing trends of significant increases. u-sCD163, urinary soluble CD163.

randomization was 4 months. The baseline clinical parameter, MEST-C score, and baseline u-sCD163 were comparable between participants randomized to methylprednisolone and placebo. The corticosteroid-treated group was more likely to achieve proteinuria remission at 6 months (odds ratio, 13.35; 95% CI: [5.19–34.34], $P < 0.001$) when compared to the placebo.

In a subgroup analysis based on the median of u-sCD163 at baseline, patients with high levels of u-sCD163 had a high odds ratio to reach proteinuria remission at 6 months (odds ratio, 35.56 [95% CI: 7.62–292.34] vs. 3.94 [95% CI: 1.39–12.93]) (Figure 3) and more likely to benefit from corticosteroid therapy when compared to the placebo (P for interaction = 0.0356). These benefits are mainly because in the placebo group, patients with high u-sCD163 were less likely reaching proteinuria remission (1.4% vs. 10.6%, $P = 0.03$) without glucocorticoids therapy. In addition, the excessively wide 95% CI is due to the small number of events in the placebo group without glucocorticoids therapy.

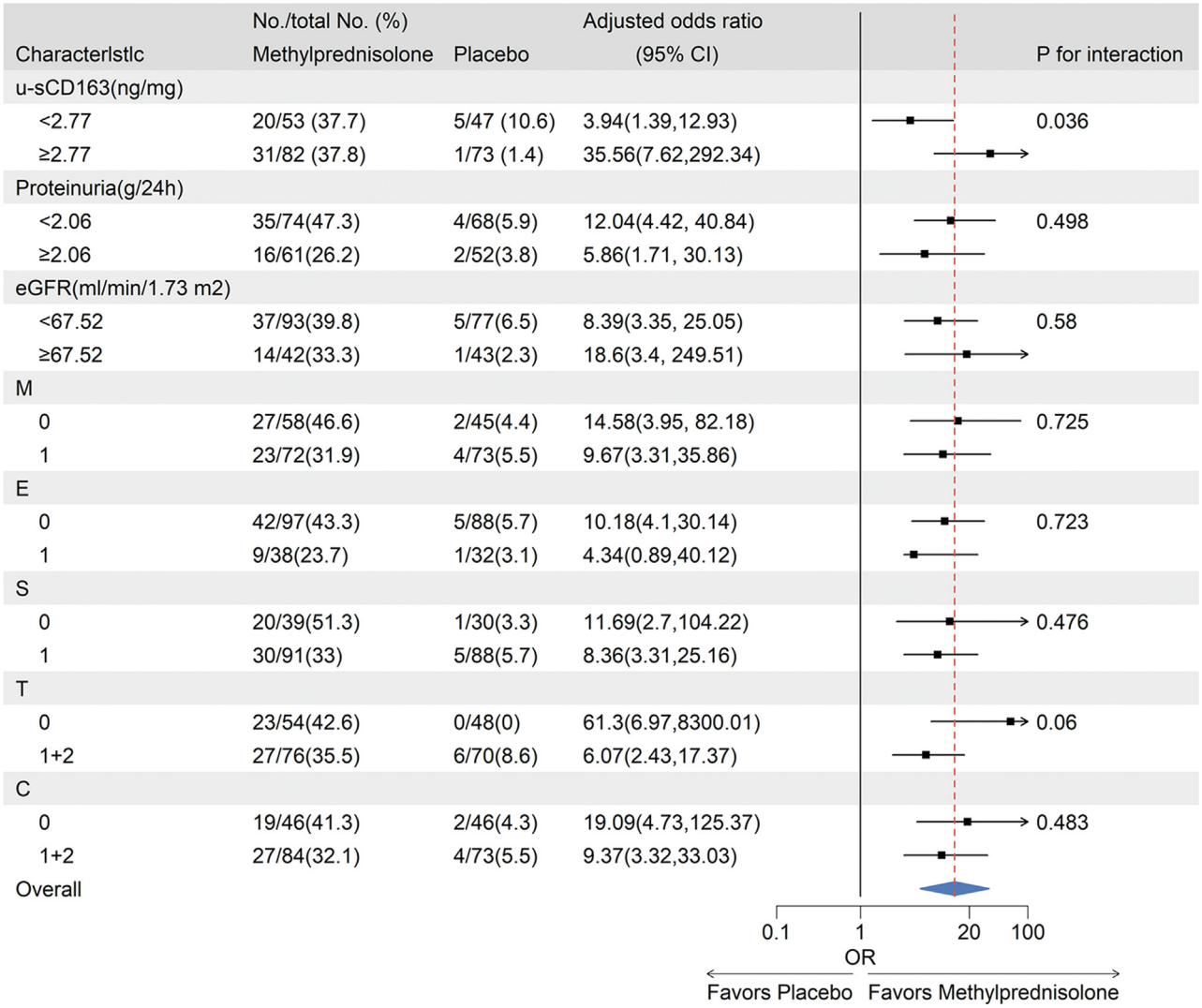

Figure 3. Associations of u-sCD163 levels, proteinuria, eGFR levels, and MESTC scores with proteinuria remission at 6 months. Patients were divided into 2 groups based on the baseline u-sCD163 levels, proteinuria levels, eGFR levels. eGFR, estimated glomerular filtration rate; u-sCD163, urinary soluble CD163.

Reduction of Levels of u-sCD163 was Significantly Associated With a Reduced Risk of Kidney Progression Events

Methylprednisolone therapy significantly reduced levels of u-sCD163 at 6 months when compared to the placebo (79% [interquartile range: 58%–91%] vs. 37% [−11% to 58%], $P < 0.001$]) and 12 months (65% [interquartile range: 5%–88%] vs. 34% [−12% to 61%], $P < 0.001$) (Figure 4a). There was no difference of the u-sCD163 levels between full dose and reduced dose corticosteroid regimen (Figure 4b).

After a median follow-up of 47 months, 39 (34.2%) responder patients (≥50% reduction of u-sCD163 from baseline) reached the study composite kidney end points as compared to 53 (51.5%) in nonresponder patients. Kaplan-Meier curve showed that the responders demonstrated a better kidney survival than the nonresponders (log-rank test $P < 0.001$; Figure 4c). As shown in Table 2, a multivariable analysis showed that reduction of u-sCD163 (responder) had a lower risk of kidney progression events (hazard ratio, 0.52; 95% CI: 0.30–0.93; $P = 0.027$) after adjusting for age, sex, mean arterial pressure, proteinuria, eGFR, the use of methylprednisolone, and MEST-C scores. Even in those without proteinuria remission at 6 months, the reduction of u-sCD163 was significantly associated with a reduced risk of kidney progression events (log-rank test $P = 0.004$; Figure 4d).

DISCUSSION

Until now, there has been no validated biomarker capable of predicting patients' prognosis or response to immunosuppressive therapy. In this cross-sectional study, we have demonstrated a positive correlation of

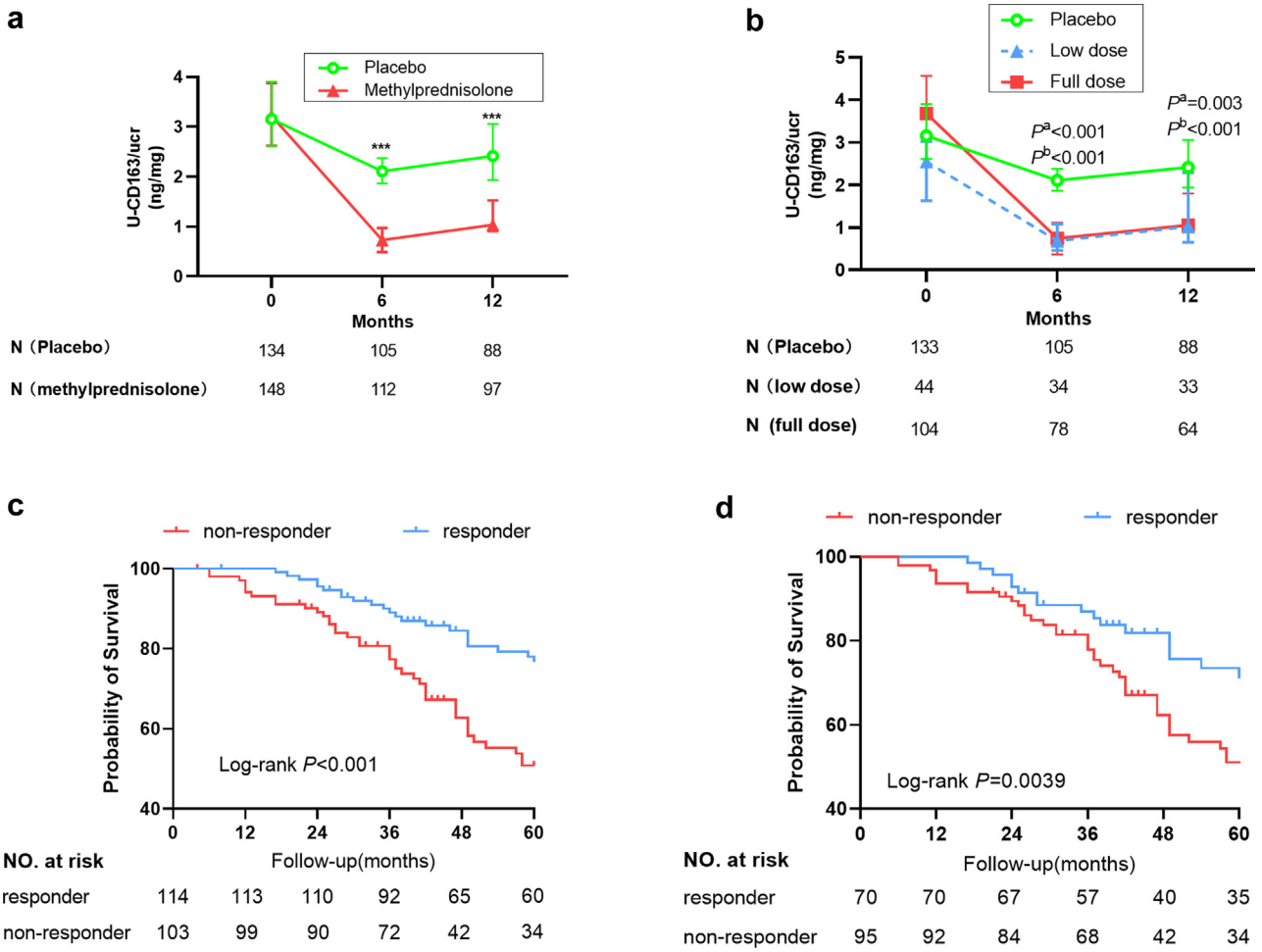

Figure 4. (a,b) Different doses of methylprednisolone both can reduce u-sCD163 levels. (a) u-sCD163 levels were lower in methylprednisolone group than placebo group. There was no statistical difference in u-sCD163 levels between groups at baseline. However, after initiating methylprednisolone treatment, u-sCD163 levels were significantly decreased compared with the placebo group in 6 months and 12 months ($P < 0.001$). (b) Whether in the low dose methylprednisolone group or the full dose methylprednisolone group, u-sCD163 levels were significantly lower than the placebo group in 6 months and 12 months. The bar shows the 95% confidence interval. (c,d) Patients without early decreased u-sCD163 (nonresponder) had poor prognosis. (c) Survival analysis of responder or nonresponder group. (d) Survival analysis of responder or nonresponder group in patients without proteinuria remission. *P* values for differences in survival were calculated using log-rank test. u-sCD163, urinary soluble CD163.

baseline u-sCD163 with active histological lesions in kidney biopsy, lower eGFR, greater degree of proteinuria, and hematuria in individuals with recently diagnosed IgAN. Importantly, using the baseline and serial biospecimen from the TESTING participants from China, we confirmed that higher baseline u-sCD163 were associated with high benefits from corticosteroids therapy for proteinuria remission. In addition, participants who showed greater decline in u-sCD163 at 6 months (defined as responders) compared to baseline is

Table 2. Cox regression model for the effect of decreased u-sCD163 (month 6 vs. month 0) on primary end points in IgAN

	Model 0[a] HR (95% CI)	Model 1[b] HR (95% CI)	Model 2[c] HR (95% CI)	Model 3[d] HR (95% CI)
Non-responder	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Responder	0.43 (0.28–0.65)[e]	0.42 (0.27–0.64)[e]	0.49 (0.32–0.75)[f]	0.52 (0.30–0.93)[g]

CI, confidence interval; HR, hazard ratio; IgAN, IgA nephropathy; M1, classically activated macrophages.
[a]Model 0 has no adjusted.
[b]Model 1 adjusted for age (<36 and ≥36) and sex (female and male). Sex and age were analyzed as dichotomous data.
[c]Model 2 adjusted for covariates in model 1 plus eGFR, proteinuria, and MAP.
[d]Model 3 adjusted for covariates in model 2 plus Oxford classification score and use of methylprednisolone. Oxford classification include mesangial hypercellularity (M1 >0.5), endocapillary hypercellularity (E1: present), segmental glomerulosclerosis (S1: present), interstitial fibrosis and tubular atrophy (T1: 26%–50%, T2: >50%), crescents (C1: 1%–24%, C2: 25%–100%). The score was analyzed as dichotomous data.
[e]represents *P* value <0.001.
[f]represents *P* value<0.01.
[g]represents *P* value <0.05.

associated with lower incidence of composite kidney end points, and the decrease of u-sCD163 was an independent predictor of future risk of kidney failure. Collectively, our data confirmed that u-sCD163 is a potential noninvasive biomarker that correlates with active pathological lesion and kidney outcomes, and more likely to benefit from corticosteroid therapy in IgAN despite maximum standard of care.

Another large cohort study of 621 patients from China with biopsy-confirmed IgAN showed that the intensity of glomerular CD206+ or CD68+ macrophages are predictive of the response to immunosuppressive therapy.[10] Similarly, in this study, we demonstrated a robust relationship between u-sCD163 and the glomerular infiltrate of CD163+ macrophages, and active pathological lesion by MEST-C scores. Despite being clinically meaningful, assessment of glomerular macrophage markers require a kidney biopsy, which is an invasive procedure that often precluded repeated measurement. In contrast, u-sCD163 can be easily measured from spot urine collection that enables repeated measurement at different time points. Using the TESTING cohort from China, we confirmed that higher baseline and series measurements of u-sCD163 is predictive of benefits of corticosteroid therapy, and greater reduction in u-sCD163 to an intervention is associated with favorable long-term kidney outcomes. Previous studies have indicated that u-sCD163 remains stable for at least 1 week at room temperature, for weeks at 4 °C, and for several years at −20 °C.[12] The TESTING trial commenced recruitment in 2012 and the study was reported in 2022. The majority of the biospecimen were kept at −80 °C more than 10 years. We have demonstrated that u-sCD163 is remarkably stable despite undergoing 5 times repeated freeze-thaw (data not shown). This further enhances its potential utility in a clinical setting.

u-sCD163 has been shown to correlate with histologic inflammation in lupus nephritis and antineutrophil cytoplasmic autoantibody–associated vasculitis and is an activity biomarker that varies over time with lupus nephritis activity and treatment.[11–14] However, limited research has been conducted in IgAN. Previous studies have revealed elevated u-sCD163 levels in both Chinese and Caucasian patients with IgAN.[11,17] Levels of u-sCD163 were associated with histological lesions of greater severity and high rate of proteinuria remission.[17] In the present study, we found that u-sCD163 is mainly associated with active histological lesions, a similarity shared with lupus nephritis. These studies suggest that u-sCD163, a promising biomarker, correlates with disease activity in several autoimmune renal disease. In this study, we also found a significant correlation between u-sCD163 levels and crescentic lesions. In addition to finding that patients with more severe crescentic lesions had higher u-sCD163 levels, we found the u-sCD163 levels significantly correlated with the degree of CD163-positive macrophage infiltration in crescentic lesions. This is consistent with previous studies that found that number of CD163-positive macrophages in the glomeruli were associated with crescentic lesions.[16,20] In IgAN, most previous relevant studies have focused on the staining of macrophages.[16,20–22] A previous study found that there was a moderate correlation between the extracapillary CD68 positive macrophages and crescentic lesions.[21] We also stained the CD68 (data not shown), the marker expressed by both classically activated macrophages and M2 macrophages. We found that the degree of CD68+ macrophage infiltration is closely related to the CD163+ macrophage infiltration, especially in the crescentic formation area. Therefore, we assume that macrophages, especially CD163-positive macrophages, are associated with crescentic lesions in IgAN.

One of strength of this study is that we used the TESTING randomized controlled trial with serial urine biospecimens for u-sCD163 on the response of steroids therapy and on the kidney progression. This study has provided a potential useful biomarker for IgAN. Other advantages include low number of serious adverse events, precluding correlation study between u-sCD163 and serious adverse events, and TESTING trial is not powered to determine the efficacy of full and reduced corticosteroid dose. However, this study has major limitations. It is confined to the TESTING Chinese population, and the generalizability of u-sCD163 as a biomarker to other populations remains unclear because biobanking study is optional and many sites outside China opted not to participate in the biobanking substudy. The absence of urine biospecimens at the first or third months following randomization means that the current study could not determine if u-sCD163 could be an early biomarker than proteinuria reduction in prognosis assessment and determination of responsiveness to therapy.

In conclusion, we have found that u-sCD163 is associated with greater glomeruli infiltrates and more active kidney biopsy histological scores by Oxford's classification. Importantly, we have identified that high levels of u-sCD163 are associated with substantial benefits from steroids therapy. The decrease of u-sCD163 was associated with improved kidney survival. These data strongly suggest that u-sCD163 was a potential useful biomarker guiding the steroids therapy and disease prognosis.

APPENDIX

List of the TESTING Study Biomarker Group

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DISCLOSURE

JLv reported receiving fees for advisory or scientific presentations from Chinook Therapeutics, KBP Bioscience, Alebund Pharmaceuticals, or SanReno Therapeutics outside the submitted work. MGW has received fees for advisory boards, steering committee roles, or scientific presentations from Travere, Baxter, Amgen, Abbvie, Chinook, Dimerix, Ostuka, GlaxoSmithKline, and CSL-Behring. VP is employed by UNSW Sydney and serves as a board director for St. Vincent's Health Australia and several Medical Research Institutes. He has received honoraria for steering committee roles, scientific presentations, and/or advisory board attendance from Abbvie, Amgen, Astra Zeneca, Bayer, Baxter, Boehringer Ingelheim, Chinook, Durect, Eli Lilly, Gilead, GSK, Janssen, Merck, Mitsubishi Tanabe, Mundipharma, Novartis, Novo Nordisk, Otsuka, Pharmalink, Pfizer, Reata, Travere, Relypsa, Roche, Sanofi, Servier, and Tricida.

HZ is employed by Peking University First Hospital and received consultancy from Calliditas, OMEROS, Chinook/Novartis, Ostuka, Roche, Vero, and Alexion/AZ; and has advisory or leadership role in Calliditas, OMEROS, Chinook/Novartis, Ostuka, Roche, Vero, and Alexion/AZ. All the other authors declared no competing interests.

Funding

This work was supported by the Capital Health Development Research Project of China (2024-1-4073), National Natural Science Foundation of China (81925006, 82370712, 82070733), CAMS Innovation Fund for Medical Sciences (2019-12M-5-046), National High level hospital clinical research funding (high quality clinical research project of Peking University First Hospital No. 2022CR80, 2022CX15, 2022CR81)

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Flow chart of the 2 groups of the cohort of patients with IgAN.

Figure S2. Association between u-sCD163 levels and clinicopathological parameters of the cohort of patients with IgAN.

Table S1. Comparison of baseline clinicopathological data: 17 randomly selected patients versus 517 patients with IgAN cohort.

Table S2. Clinicopathological features of TESTING cohort patients.

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