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ORIGINAL ARTICLE

Effects of hydroxychloroquine on proteinuria in membranous nephropathy

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Abstract

Background Many patients with primary membranous nephropathy have severe proteinuria unresponsive to optimized renin–angiotensin–aldosterone system inhibitors (RAASi). We evaluated the efficacy and safety of hydroxychloroquine as an adjunctive agent in membranous nephropathy (MN) treatments.

Methods We prospectively recruited 126 patients with biopsy-proven primary membranous nephropathy and urinary protein 1–8 g/day while receiving optimized RAASi treatment for ≥ 3 months and well-controlled blood pressure. Forty-three patients received hydroxychloroquine and RAASi (hydroxychloroquine-RAASi group), and 83 patients received RAASi alone (RAASi group). Treatment responses, including proteinuria reduction, complete and partial remission rates, and autoantibody against phospholipase A2 receptor (anti-PLA2R) levels, were compared between the two groups at 6 months and over the long term.

Results At 6 months, the effective response rate (proteinuria reduction > 30%) (57.5% vs. 28.9%, $P = 0.002$), clinical remission rate (35.0% vs. 15.7%, $P = 0.015$), and percentage change in proteinuria (− 51.7% vs. − 21.9%, $P < 0.001$) were higher, and the rate of switching to immunosuppressants (25.0% vs. 45.8%, $P = 0.027$) was lower in the hydroxychloroquine-RAASi group than in the RAASi group. Hydroxychloroquine administration was an independent protective factor with an effective response (OR 0.37, $P = 0.021$). In the long term, the clinical remission rate was higher in the HCQ-RAASi group (62.5% vs. 38.6%, $P = 0.013$). Hydroxychloroquine therapy was associated with a higher rate of anti-PLA2R reduction (< 20 U/ml) (HR 0.28, $P = 0.031$). We observed no serious adverse events associated with hydroxychloroquine.

Conclusions Hydroxychloroquine could be an option for patients with membranous nephropathy seeking to achieve proteinuria reduction and anti-PLA2R antibody reduction in addition to optimized RAASi. Randomized controlled trials are needed to confirm these findings.

Trial registration ChiCTR2100045947, 20210430, retrospectively registered.

Keywords Membranous nephropathy · Hydroxychloroquine · Proteinuria · Anti-PLA2R antibody

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Introduction

Primary membranous nephropathy (MN) is characterized by the formation of subepithelial immune deposits and proteinuria that can be nephrotic and massive [1]. It is an autoimmune kidney disease, and a growing number of target antigens have been discovered. Approximately 70% of patients are positive for antibodies against the M-type phospholipase A2 receptor (PLA2R), which is expressed on the membrane of podocytes [2, 3]. Other antigens have also been discovered, including thrombospondin type-1 domain-containing 7A (THSD7A) [4], exostosin 1/exostosin 2 (EXT1/2) [5], neural epidermal growth factor-like 1 protein (NELL-1) [6],

semaphorin 3B [7], protocadherin 7 (PCDH7) [8], neural cell adhesion molecule 1 (NCAM1) [9].

Conventional immunosuppressive agents used in the treatment of membranous nephropathy were associated with a risk of adverse effects such as life-threatening infection and cancer [10], and almost one-third of patients with membranous nephropathy undergo spontaneous remission [11]; thus, immunosuppression therapy requires a careful balance between risk and benefits [12]. Nonimmunologic treatments would be the choice for patients with a low risk of progression or who have strong contraindications to immunodepression. However, supportive therapies are sometimes insufficient for reducing proteinuria. In a large cohort treated with an optimized dose of renin-angiotensin-aldosterone system inhibitors (RAASi) and with wellcontrolled blood pressure, approximately half of the patients eventually needed immunosuppressive treatment [13]. In patients with urinary protein below the nephrotic range, the incidence of progression to nephrotic proteinuria was consistently approximately 60%, despite the increasing use of RAASi [14]. Higher doses of RAASi or dual RAAS blockade might yield further reductions in proteinuria; however, these approaches may not result in incremental benefits, and increase the risk of adverse events, including dizziness, hypotension, hyperkalemia, and kidney function impairment [15, 16]. For these reasons, adjunctive treatments are needed to improve the reduction in proteinuria or for patients who have strong contraindications to immunodepression.

The antimalarial drug hydroxychloroquine (HCQ) has been widely accepted as a safe, effective, and long-term treatment for some autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis [17–19]. It has been reported to reduce the risk of kidney damage in lupus nephritis [20–23] and the risk of chronic kidney disease in rheumatoid arthritis [24, 25]. Recently, HCQ was found to be a promising and safe antiproteinuric agent in IgA nephropathy [26–28]. Nevertheless, the use of HCQ has not been investigated in patients with membranous nephropathy.

In this study, we prospectively enrolled membranous nephropathy patients with persistent proteinuria who had received optimized RAASi treatment and whose blood pressure was well controlled. The efficacy and safety of add-on hydroxychloroquine therapy was studied, with comparisons to patients receiving RAASi alone.

Methods

Study design

In this open-label prospective study, patients with biopsy-proven primary membranous nephropathy (stage I-IV) diagnosed at Peking University First Hospital were recruited from 2016 to 2019 and follow-up was completed in 2020. A total of 126 patients were consecutively recruited who fulfilled the following criteria: (1) > 18 years old; (2) persistent proteinuria ≥ 1 g/day; (3) supportive therapy including angiotensin-converting enzyme inhibitors (ACEis) and/or angiotensin-receptor blockers (ARBs) for ≥ 3 months; and (4) blood pressure < 130/80 mmHg.

Kidney biopsy performed at the time of diagnosis for all patients revealed granular deposits of immunoglobin G (IgG) and complement 3 (C3) along glomerular capillary walls upon immunofluorescence staining, glomerular basement membrane thickening upon light microscopy and electron dense deposits on the subepithelial area upon electron microscopy.

We excluded patients if they met any of the following conditions: (1) patients had secondary membranous nephropathy, such as hepatitis B/C virus infection, lupus, malignancy, rheumatoid arthritis, medications, and heavy metal poisoning; (2) patients had other coexisting kidney diseases; (3) patients had urinary protein > 8 g/day or any of the risk factors for disease progression according to the Kidney disease: improving global outcomes (KDIGO) guidelines [12]; (4) patients had been treated with steroids or immunosuppressive therapy within the previous 3 months; (5) patients were allergic to hydroxychloroquine or had macular degeneration; and (6) patients had uncontrolled hypertension (> 140/90 mmHg), acute infections, malignancy, or liver dysfunction or were pregnant or lactating.

The research followed the Declaration of Helsinki, was approved by the ethics committee of Peking University First Hospital, and was registered at the Chinese Clinical Trial Registry (ChiCTR2100045947). Written informed consent was obtained from each patient regarding treatment and tissue and blood sampling.

HCQ-RAASi group and RAASi group

There were 43 patients who agreed to receive hydroxychloroquine treatments in addition to RAASi (HCQ-RAASi group), while 83 patients declined (RAASi group). They all continued receiving the previously optimized RAASi treatments, while the patients in the HCQ-RAASi group received the additional hydroxychloroquine treatment. The dose of hydroxychloroquine varied from 0.1 g twice daily to 0.2 g thrice daily based on the baseline estimated glomerular filtration rate (eGFR). The dose was 0.2 g twice or thrice daily for the patients with an eGFR > 45 ml/min/1.73 m^2, and the dose was 0.1 g twice or thrice daily for the patients with an eGFR between 30 and 45 ml/min/1.73 m^2.

Clinical parameters

Clinical data were collected from medical records at the time of diagnosis as well as during follow-up. Baseline data included demographic, clinical and histologic characteristics of all patients, including age, sex, time from diagnosis, mean arterial pressure (MAP; defined as the diastolic pressure plus a third of the pulse pressure), RAASi dosage [pills/day; calculated by the defined daily dose (DDD) at https://www.whocc.no/atc_ddd_index/], anti-PLA2R antibody (cut-off value < 20 U/ml), proteinuria (g/24 h), serum albumin, and eGFR (calculated by the CKD-EPI formula [29]). All patients underwent a series of follow-up examinations every 1–3 months, and these parameters were evaluated at every visit. All patients were followed-up for at least 6 months or until ESRD or death. Follow-up ended at the last visit or when the patients reached end stage renal disease or died.

Treatment responses and endpoints

An effective response to hydroxychloroquine treatment was defined as proteinuria reduction > 30% from baseline. Complete remission (CR) was defined as urinary protein excretion < 0.3 g/day. Partial remission (PR) was defined as urinary protein of 0.3–3.5 g/day and a decrease > 50% from the baseline value. Time to remission or a response was defined as the time from study initiation to the time when remission or a response was achieved.

Patients who presented with disease progression and began immunosuppressants were defined as treatment failure and withdrew from the study but were continuously followed up until the last visit. Patients who failed to show an effective response or had treatment failure were considered to have no response. Relapse was defined as recurrence of proteinuria > 3.5 g/day and an increase in urinary protein > 50% in patients who had previously achieved clinical remission.

For the evaluation of kidney function, kidney dysfunction was defined as an eGFR reduction > 50% from baseline.

The primary endpoints were the effective response rate (proteinuria reduction > 30%) and the clinical remission rate (CR and PR) within 2 years. Secondary endpoints included changes in urinary protein and eGFR, time to remission, rate of relapse and kidney dysfunction.

Adverse effects were defined as any discomfort or laboratory abnormalities resulting from hydroxychloroquine treatment during the follow-up period.

Statistical analysis

Continuous variables were expressed as the mean ± SD or medians (Q25, Q75) based on the distribution of the variable. Nominal variables were expressed as counts and percentages. The response and remission rates between the HCQ-RAASi and RAASi groups were compared. These characteristics were compared between groups using Student's t tests or Wilcoxon signed-rank tests (for continuous variables) or χ^2 tests (for nominal variables) as appropriate. Logistic regression was performed to explore the predictors of treatment response. Cumulative remission was estimated using the Kaplan–Meier method and evaluated using the log-rank and Breslow tests. Cox proportional hazards models were used to explore predictors of remission. A *P value* < 0.05 was considered statistically significant, and the performed tests were two-tailed. Statistical analysis was performed with SPSS, version 24.0 (SPSS, Chicago, IL).

Results

Baseline characteristics

In the current prospective cohort from 2016 to 2020, all 126 patients received the maximum labeled or tolerated dose of RAASi treatments, and their blood pressure was kept under control (< 130/80 mmHg). Forty-three patients received add-on hydroxychloroquine treatment (HCQ-RAASi group). Three of them withdrew within 6 months because of side effects (pruritus or headache). The remaining 40 patients were followed up for more than 6 months. Eighty-three control patients continued optimized RAASi treatment alone (RAASi group) without hydroxychloroquine, corticosteroid, or immunosuppressive treatment over the same time period. Figure 1 shows a flowchart of the study.

Clinical data for the two groups are shown in Table 1. In the HCQ-RAASi group, time from diagnosis was 12.3 (5.4, 26.4) months, baseline level of urinary protein was 3.6 (2.6, 4.6) g/day, serum albumin was 33.0 ± 3.4 g/l, and eGFR was 93.7 ± 21.8 ml/min/1.73 m^2. Twenty-four (60%) patients had detectable anti-PLA2R antibodies at a level of 69.5 (37.3, 134.3) U/ml. All demographic, clinical and pathological features at baseline were comparable between the HCQ-RAASi group and RAASi group, except that time from diagnosis was longer (12.3 vs. 5.3 months, $P = 0.011$), MAP was lower (85.2 ± 7.4 vs. 90.6 ± 9.1 mmHg, $P = 0.002$), and immunoglobulin M (IgM) and complement C1q deposits were more frequent (55.0% vs. 34.1%, $P = 0.028$ and 45.0% vs. 22.0%, $P = 0.009$, respectively) in the HCQ-RAASi group than in the RAASi group, respectively.

Treatment response to HCQ-RAASi over 6 months

In the HCQ-RAASi group, the effective response rate (urinary protein reduction > 30% from baseline) was 37.5% at 2 months, 42.5% at 4 months and 57.5% at 6 months. The rate of clinical remission (PR and CR) was 15% at 2 months, 25% at 4 months and 35% at 6 months.

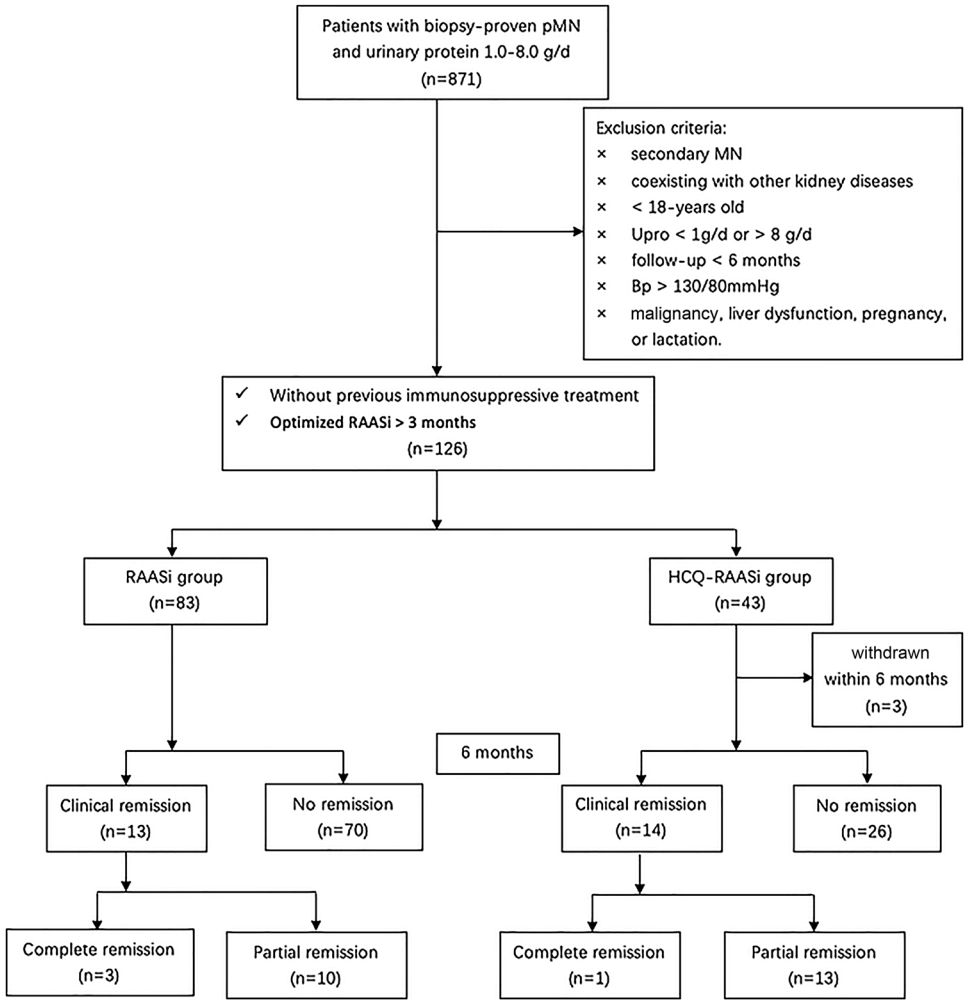

Fig. 1 Study flowchart

The levels of urinary protein decreased after hydroxychloroquine administration from 3.6 (2.6, 4.6) g/day at baseline to 2.6 (1.8, 3.7) g/day at 2 months, 2.5 (1.2, 3.5) g/day at 4 months and 1.4 (0.6, 2.9) g/day at 6 months ($P<0.001$). The change in urinary protein from baseline was − 0.8 (− 1.8, 0.2) g/day at 2 months, − 1.0 (− 2.2, − 0.3) g/day at 4 months and − 1.8 (− 3.2, − 1.0) g/day at 6 months ($P=0.013$). The percentage change in urinary protein was calculated as follows: (change in urinary protein from baseline/level of urinary protein at baseline) × 100%. The percentage change was − 25.1 (− 51.2, 4.5) % at 2 months, − 31.1 (− 60.8, − 9.9) % at 4 months and − 51.7 (− 79.5, − 33.2) % at 6 months ($P=0.013$).

Of the 24 patients with positive anti-PLA2R antibodies, 8 (33.3%) patients achieved antibody reduction (< 20 U/ml) at 3 months, and 10 (41.7%) patients achieved antibody reduction at 6 months. In patients still positive for antibodies at 6 months, the antibody levels decreased from 103.5 (56.8, 220.8) at baseline to 51.5 (37.8, 104.3) U/ml, but the difference was not statistically significant ($P=0.069$).

The rate of treatment failure (initiated immunosuppressant treatment) was 10% at 2 months, 17.5% at 4 months and 25% at 6 months.

Treatment response comparison between the two groups

At 6 months, the effective response rate (57.5% vs. 28.9%, $P=0.002$) and clinical remission rate (35.0% vs. 15.7%, $P=0.015$; Fig. 2A) were significantly higher, the level of urinary protein was significantly lower [1.4 (0.6, 2.9) vs. 2.4 (1.1, 4.2) g/day, $P=0.044$; Fig. 2B], the decrease in urinary protein [− 1.8 (− 3.2, − 1.0) vs. − 0.4 (− 1.3, 1.3) g/day, $P<0.001$; Fig. 2C] and percentage change in urinary protein [− 51.7 (− 79.5, − 33.2) vs. − 21.9 (− 56.7, 34.2) %, $P<0.001$; Fig. 2D] were significantly greater in magnitude, and the rate of treatment failure was significantly lower (25.0% vs. 45.8%, $P=0.027$) in the HCQ-RAASi group than in the RAASi group, respectively.

Among the anti-PLA2R-positive patients, the rate of anti-PLA2R antibody reduction was higher in the HCQ-RAASi

Table 1 Clinical characteristics of patients with membranous nephropathy receiving RAASi and RAASi associated with hydroxychloroquine

Characters	RAASi group (n = 83)	HCQ-RAASi group (n = 40)
Age (years)	50.5 ± 13.7	45.6 ± 12.7
Gender (male/female)	48/35	16/24
Clinical features		
Time from diagnosis (months)	5.3 (4.1, 13.4)	12.3 (5.4, 26.4)*
Urinary protein (g/24 h)	3.2 (2.1, 4.2)	3.6 (2.6, 4.6)
Serum albumin (g/l)	32.4 ± 5.9	33.0 ± 3.4
eGFR (ml/min/1.73m^2)	90.4 ± 18.7	93.7 ± 21.8
Positive rate of anti-PLA2R antibody, n (%)	49 (59.0)	24(60.0)
Level of anti-PLA2R antibodies (RU/ml)	91 (46.5, 150.5)	69.5 (37.3, 134.3)
MAP (mmHg)	90.6 ± 9.1	85.2 ± 7.4*
Histological features		
Stages of membranous injury, I/II/III + IV	34/39/9	15/20/5
Glomerular IgG deposit, –/+/++/+++/++++	3.4 ± 0.6	3.1 ± 0.9
Glomerular IgA/IgM deposit, n	23/28	10/22*
Glomerular C3/C1q deposit, n	79/18	37/18*
Chronic tubular interstitial lesions, n (%)	50 (61.0)	23 (57.5)
Previous RAASi treatments, n (%)		
ACEI alone	11 (13.3)	4 (10.0)
ARB alone	65 (78.3)	31 (77.5)
ACEI plus ARB	7 (8.4)	5 (12.5)
RAASi dosage (DDD)	1.8 ± 0.9	1.5 ± 1.3
Treatment response		
6 months		
Effective rate of proteinuria reduction > 30%, n (%)	24 (28.9)	23 (57.5)*
Clinical remission	13 (15.7)	14 (35.0)*
Complete remission	3 (3.6)	1 (2.5)
Partial remission	10 (12.0)	13 (32.5)*
No response, n (%)	59 (71.1)	17 (42.5)*
Change to/increase IS treatment, n (%)	38 (45.8)	10 (25.0)*
Anti-PLA2R antibody reduction (< 20 U/ml), n (%)	11/49 (22.4)	10/24 (41.7)
Long term		
Clinical remission in 2 years, n (%)	32 (38.6)	25 (62.5)*
Complete remission in 2 years, n (%)	22 (26.5)	13 (32.5)
Partial remission in 2 years, n (%)	10 (12.0)	12 (30.0)*
Time to remission, months	7.7 (4.9, 11.3)	6.3 (2.5, 10.3)
Relapse, n (%)	6/32 (18.8)	3/25 (12.0)
No remission in 2 years, n (%)	51 (61.4)	15 (37.5)*
Change to IS treatment in 2 years, n (%)	42 (50.6)	15 (37.5)
Anti-PLA2R antibody reduction (< 20 U/ml), n (%)	15/49 (30.6)	14/24 (58.3)*
eGFR decline ≥ 50%, n (%)	0 (0)	0 (0)
Follow-up time, months	25.0 (17.8, 35.4)	31.4 (23.6, 36.4)

HCQ, hydroxychloroquine; *eGFR,* estimated glomerular filtration rate, calculated by CKD-EPI; PLA2R, M-type phospholipase A2 receptor; *MAP,* mean arterial pressure; *IgG,* immunoglobulin G; *IgA,* immunoglobulin A; *IgM,* immunoglobulin M; *C3,* complement 3; *C1q,* complement C1q; *RAASi,* renin–angiotensin–aldosterone system inhibitors; *ACEI,* angiotensin-converting enzyme inhibitor; *ARB,* angiotensin-receptor blockers; *DDD,* defined daily dose; *IS,* immunosuppressive treatment

*$p < 0.05$, compared between HCQ-RAASi group and RAASi group

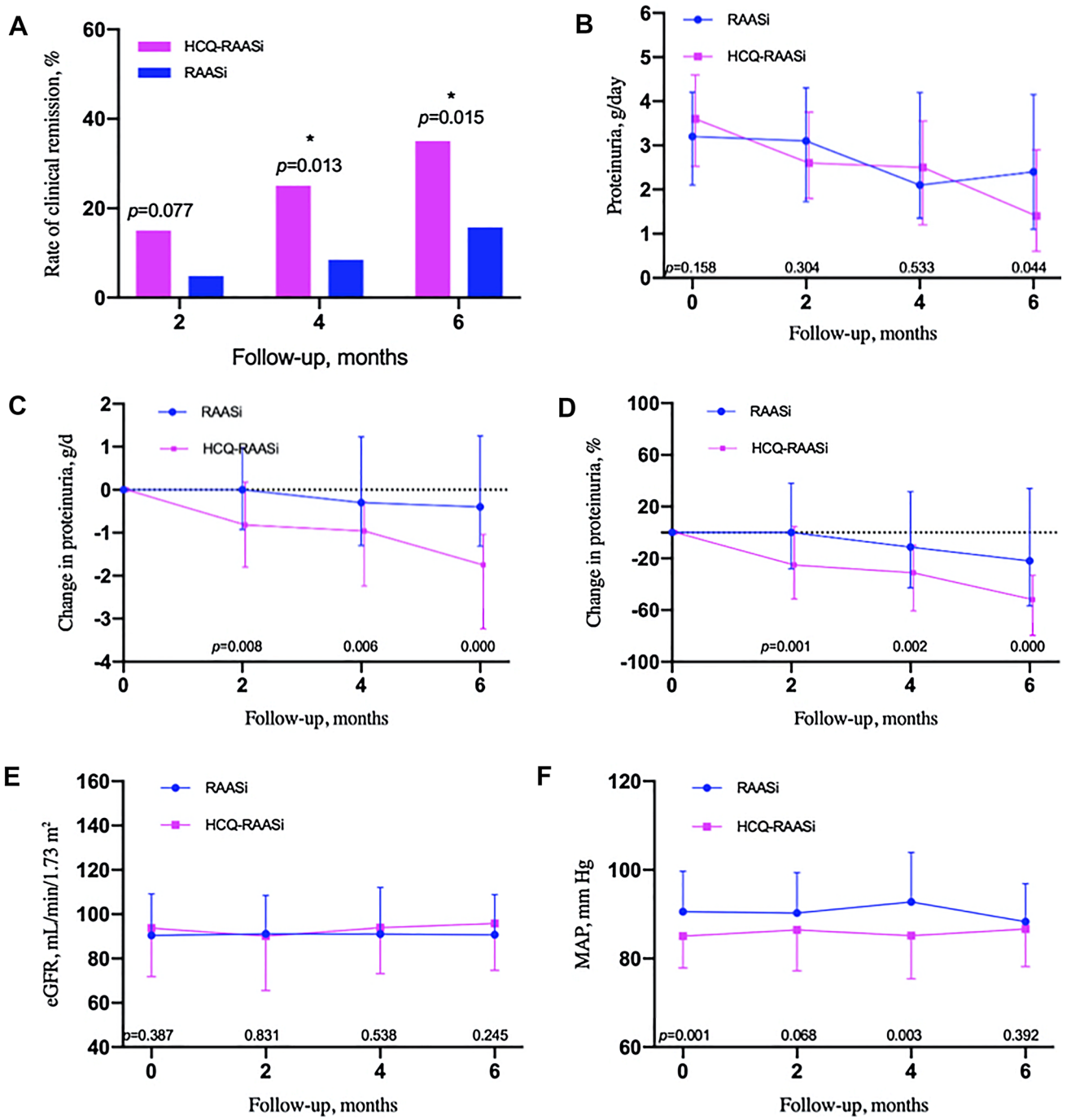

Fig. 2 Urinary protein changes in the HCQ-RAASi group and RAASi group after 6 months of treatment. **A** Rate of clinical remission. **B** Level of urinary protein. **C** Change in proteinuria. **D** Percentage change in proteinuria. **E** Level of eGFR. **F** Mean arterial pressure (MAP). Red: HCQ-RAASi group. Blue: RAASi group. The error bars represent the 25th and 75th percentiles for median values or standard deviations for mean values

group at 3 months (33.3% vs. 6.1%, $P=0.004$), but the difference was nonsignificant at 6 months (41.7% vs. 22.4%, $P=0.088$; Fig. 3).

Kidney function was stable during HCQ treatment, with an eGFR of 93.7 ± 21.8 ml/min/1.73 m^2 at baseline and 95.8 ± 21.1 ml/min/1.73 m^2 at 6 months ($P=0.690$). It was also comparable between the HCQ-RAASi and RAASi groups (95.8 ± 21.1 vs. 90.7 ± 18.1 ml/min/1.73 m^2, $P=0.245$, Fig. 2E).

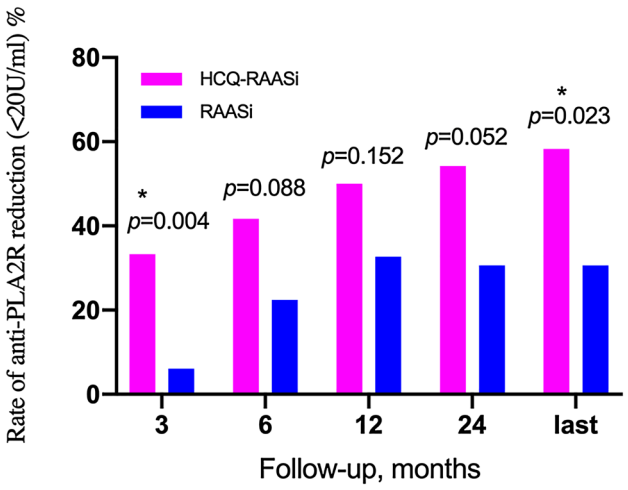

Fig. 3 Rate of anti-PLA2R antibody reduction (< 20 U/ml) in the HCQ-RAASi group and RAASi group

MAP was lower in the HCQ-RAASi group than in the RAASi group at baseline, but the difference became non-significant at 6 months (86.7 ± 8.5 vs. 88.3 ± 8.6 mmHg, $P = 0.392$, Fig. 2F).

Univariate logistic regression analyses showed that HCQ administration (OR 0.30; 95% CI 0.14, 0.66; $P = 0.003$) and a higher level of serum albumin (OR 0.9; 95% CI 0.83, 0.97; $P = 0.007$) were associated with a higher frequency of proteinuria reduction, while older age (OR 1.05; 95% CI 1.02, 1.08; $P = 0.001$) and male sex (OR 2.14; 95% CI 1.02, 4.49; $P = 0.044$) were risk factors. After adjustment, the multivariate logistic regression model showed that HCQ (OR 0.37; 95% CI 0.16, 0.86; $P = 0.021$) and baseline serum albumin (OR 0.91; 95% CI 0.84, 0.99; $P = 0.027$) were independent protective factors regarding proteinuria reduction, while older age (OR 1.04; 95% CI 1.01, 1.07; $P = 0.020$) was an independent risk factor (Table 2).

Treatment response to HCQ-RAASi over the long term

All patients were continuously followed up after the initial 6-month period, for a total of 31.4 (23.6, 36.4) months for the HCQ-RAASi group and 25.0 (17.8, 35.4) months for the RAASi group ($P = 0.203$).

There were 25 (62.5%) patients who achieved clinical remission in the HCQ-RAASi group within 2 years. The rates of clinical remission and PR at 2 years were higher in the HCQ-RAASi group than in the RAASi group (62.5% vs. 38.6%, $P = 0.013$; 30.0% vs. 12.0%, $P = 0.015$, respectively). The rate of CR was comparable between the two groups (32.5% vs. 26.5%, $P = 0.490$). Kaplan–Meier analysis demonstrated a higher cumulative remission rate in the HCQ-RAASi group ($P = 0.004$ by the log-rank test, $P = 0.002$ by the Breslow test; Fig. 4). The time to clinical remission was comparable between the two groups [6.3 (2.5, 10.3) vs. 7.7 (4.9, 11.3) months, $P = 0.281$; Table 1].

Three (12.0%) patients in the HCQ-RAASi group and 6 (18.8%) patients in the RAASi group relapsed ($P = 0.717$). Fifteen (37.5%) patients in the HCQ-RAASi group and 42 (50.6%) patients in the RAASi group switched to immunosuppressive therapies ($P = 0.172$).

Table 2 Factors associated with clinical response (proteinuria reduction>30% from baseline) at 6 months in patients with membranous nephropathy receiving RAASi (logistic regression)

Characters	Univariate			Multivariate		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Age (increase by 1 year)	1.05	(1.02, 1.08)	**0.001**	1.04	(1.01, 1.07)	**0.020**
Sex (male)	2.14	(1.02, 4.49)	**0.044**	2.16	(0.95, 4.93)	0.068
Time from diagnosis (increased by 1 month)	1.00	(0.99, 1.02)	0.955			
Baseline MAP (increase by 1 mmHg)	1.02	(0.98, 1.06)	0.459			
Baseline proteinuria (increase by 1 g/d)	1.04	(0.83, 1.30)	0.733			
Baseline serum albumin (increase by 1 g/l)	0.90	(0.83, 0.97)	**0.007**	0.91	(0.84, 0.99)	**0.027**
Baseline eGFR (increase by 1 ml/min/1.73 m^2)	0.98	(0.97, 1.00)	0.108			
Baseline anti-PLA2R positivity	1.31	(0.63, 2.74)	0.475			
Chronic tubular interstitial lesions	1.92	(0.91, 4.06)	0.086			
HCQ therapy	0.30	(0.14, 0.66)	**0.003**	0.37	(0.16, 0.86)	**0.021**
Average RAASi dosage (increase by 1 DDD)	1.05	(0.72, 1.52)	0.813			
Average MAP in 6 months (increase by 1 mmHg)	1.02	(0.97, 1.07)	0.553			

Statistically significant *P* values are shown in bold

MAP, mean arterial pressure; *eGFR,* estimated glomerular filtration rate, calculated by CKD-EPI; PLA2R, M-type phospholipase A2 receptor; *DDD,* defined daily dose *HCQ,* hydroxychloroquine; *RAASi,* renin–angiotensin–aldosterone system inhibitors

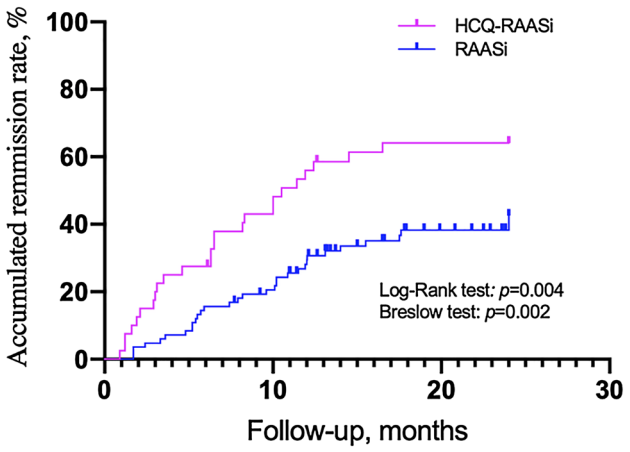

Fig. 4 Cumulative clinical remission rate in patients with membranous nephropathy in the HCQ-RAASi group and RAASi group

The univariate analyses using Cox regression showed that hydroxychloroquine therapy (HR 0.48; 95% CI 0.28, 0.80; $P=0.005$) and higher levels of serum albumin (HR 0.90; 95% CI 0.86, 0.95; $P<0.001$) were associated with a higher frequency of clinical remission; older age (HR 1.03; 95% CI 1.01, 1.05; P = 0.003), male sex (HR 1.76; 95% CI 1.04, 2.97; P = 0.036), higher baseline urinary protein levels (HR 1.25; 95% CI 1.04, 1.51; P = 0.019), anti-PLA2R positivity at baseline (HR 1.75; 95% CI 1.04, 2.94; P = 0.036), higher MAP levels at baseline (HR 1.03; 95% CI 1.00, 1.06; P = 0.034), higher average MAP levels (HR 1.04; 95% CI 1.00, 1.08; P = 0.047) and chronic tubular interstitial lesions (HR 1.99; 95% CI 1.17, 3.36; P = 0.011) were risk factors. After adjustment, the multivariate analyses showed that hydroxychloroquine was not an independent protective factor (HR 0.56; 95% CI 0.31, 1.03; $P=0.062$) (Table 3).

Table 3 Factors associated with clinical remission in patients with membranous nephropathy receiving RAASi (Cox regression)

Characteristic	Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age (increase by 1 year)	1.03	(1.01, 1.05)	**0.003**	1.02	(1.00, 1.04)	0.065
Sex (male)	1.76	(1.04, 2.97)	**0.036**	1.52	(0.86, 2.70)	0.150
Time from diagnosis (increased by 1 months)	1.01	(0.99, 1.02)	0.378			
Baseline urinary protein (increase by 1 g/d)	1.25	(1.04, 1.51)	**0.019**	1.03	(0.81, 1.32)	0.792
Baseline MAP (increase by 1 mmHg)	1.03	(1.00, 1.06)	**0.034**	1.02	(0.97, 1.07)	0.404
Baseline serum albumin (increase by 1 g/l)	0.90	(0.86, 0.95)	**0.000**	0.93	(0.86, 0.99)	**0.025**
Baseline eGFR (increase by 1 ml/min/1.73 m^2)	0.99	(0.98, 1.00)	0.117			
Baseline anti-PLA2R positivity	1.75	(1.04, 2.94)	**0.036**	1.54	(0.89, 2.65)	0.122
Chronic tubular interstitial lesions	1.99	(1.17, 3.36)	**0.011**	1.36	(0.75, 2.49)	0.313
HCQ therapy	0.48	(0.28, 0.80)	**0.005**	0.56	(0.31, 1.03)	0.062
Average RAASi dosage (increase by 1 DDD)	1.05	(0.78, 1.42)	0.732			
Average MAP (increase by 1 mmHg)	1.04	(1.00, 1.08)	**0.047**	1.01	(0.95, 1.07)	0.795

Statistically significant *P* values are shown in bold

MAP, mean arterial pressure; *eGFR,* estimated glomerular filtration rate, calculated by CKD-EPI; PLA2R, M-type phospholipase A2 receptor; *DDD,* defined daily dose HCQ, hydroxychloroquine; RAASi, renin–angiotensin–aldosterone system inhibitors

Table 4 Factors associated with anti-PLA2R antibodies reduction (< 20 U/ml) in patients with membranous nephropathy receiving RAASi

Characters	Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age (increase by 1 year)	1.02	(0.98, 1.06)	0.334	1.01	(0.97, 1.05)	0.733
Sex (male)	0.97	(0.38, 2.50)	0.958	0.69	(0.23, 2.07)	0.511
Time from diagnosis (increased by 1 months)	1.01	(0.99, 1.03)	0.484			
Baseline urinary protein (increase by 1 g/day)	1.37	(1.02, 1.85)	**0.040**	1.26	(0.87, 1.82)	0.226
Baseline serum albumin (increase by 1 g/l)	0.86	(0.77, 0.97)	**0.012**	0.91	(0.79, 1.05)	0.209
Baseline eGFR (increase by 1 ml/min/1.73 m^2)	0.99	(0.97, 1.02)	0.466			
Baseline anti-PLA2R antibody levels	1.00	(1.00, 1.01)	0.147			
Chronic tubular interstitial lesions	1.37	(0.52, 3.59)	0.528			
HCQ therapy	0.32	(0.11, 0.87)	**0.026**	0.28	(0.09, 0.89)	**0.031**

Statistically significant *P* values are shown in bold

PLA2R, M-type phospholipase A2 receptor; *eGFR,* estimated glomerular filtration rate, calculated by CKD-EPI; *HCQ* hydroxychloroquine

In patients with detectable anti-PLA2R antibodies at baseline, the rate of anti-PLA2R reduction was significantly higher in the HCQ-RAASi group during the follow-up period (58.3% vs. 30.6%, $P=0.023$) (Fig. 3). In patients with undetectable anti-PLA2R antibodies at baseline, none in the HCQ-RAASi group and 6 (17.6%) patients in the RAASi group became positive for anti-PLA2R during the follow-up period ($P=0.159$).

In patients with positive anti-PLA2R antibodies, univariate logistic regression analysis showed that hydroxychloroquine administration (OR 0.32; 95% CI 0.11, 0.87; $P=0.026$) and higher serum albumin levels at baseline (OR 0.86; 95% CI 0.77, 0.97; $P=0.012$) were associated with a higher frequency of anti-PLA2R reduction; a higher urinary protein level at baseline (OR 1.37; 95% CI 1.02, 1.85; $P=0.040$) was a risk factor. After adjustment, the multivariate analyses showed that hydroxychloroquine therapy was the only independent protective factor (OR 0.28; 95% CI 0.09, 0.89; $P=0.031$) (Table 4) with regard to anti-PLA2R antibody reduction.

The eGFR values were stable in both groups, with no patient having a decrease in eGFR > 50%.

Safety and adverse events

The combination of hydroxychloroquine and RAASi was well tolerated by most patients. Table 5 details the adverse events in all patients treated with hydroxychloroquine. Three patients developed isolated cutaneous conditions; specifically, two patients had pruritus, and one had rashes and pigmentation. One patient reported headache. The two patients with pruritus and the one with headache withdrew from the hydroxychloroquine treatment, and the issues spontaneously resolved. The patient who had rashes and pigmentation received antihistamine, and the rashes resolved.

Discussion

In the current study, we investigated the effect of hydroxychloroquine combined with RAASi in membranous nephropathy patients with urinary protein 1–8 g/day and compared the effects to those in patients receiving RAASi alone. The results showed that the addition of hydroxychloroquine to RAASi was associated with a decrease in the level of proteinuria and anti-PLA2R antibodies, with

Table 5 Adverse events of associated with therapy with hydroxychloroquine in patients with membranous nephropathy

Adverse events	Values	Management	Follow-up
Participants with any adverse events	4		
Infections	0		
Gastrointestinal disorders	0		
Skin and subcutaneous tissue disorders	3		
Pruritus	2	Withdrawal	Resolved
Rashes and pigmentation	1	Antihistamine	Rashes resolved but pigmentation remained
Headache	1	Withdrawal	Resolved

an increase in remission rate, and with a reduction of need for immunosuppressive therapy at 6-months and over long-term follow-up. The drug was well tolerated and did not cause serious side effects. Thus, our data suggest that add-on hydroxychloroquine therapy has antiproteinuric effects and immune-modulating effects in patients with membranous nephropathy.

The poor tolerance of ACEIs in Asian patients [30], the ceiling effects with ARBs [31–33], and the insufficient safety of dual RAAS blockade [15, 16] have limited the use of RAASi alone to maximize proteinuria reduction in patients with membranous nephropathy. In our study, baseline MAP in the HCQ-RAASi group was < 86 mmHg and 5.4 mmHg lower ($P = 0.002$) than that in the RAASi group; however, the urinary protein level was slightly higher in the HCQ-RAASi group (3.6 vs. 3.2 g/day, $P = 0.158$).

The kidney protective effects of hydroxychloroquine in lupus nephritis treatment have been well established [20–23, 34–36]. Recently, one randomized control trial and several retrospective studies consistently reported beneficial antiproteinuric effects of hydroxychloroquine in patients with IgA nephropathy [26–28]. Thus, we hypothesized that the antiproteinuric effects of hydroxychloroquine might be a potential add-on therapy for patients with membranous nephropathy.

We administered hydroxychloroquine to patients with membranous nephropathy who had been receiving optimized RAASi treatment but still had proteinuria levels of 1–8 g/day. We found that at 6 months, the effective response rate and clinical remission rate were both significantly higher in the HCQ-RAASi group than in the RAASi group (57.5 vs. 28.9%, $P = 0.002$; 35.0 vs. 15.7%, $P = 0.015$, respectively). More importantly, the rate of switching to immunosuppressive treatment was significantly lower in the HCQ-RAASi group (25.0 vs. 45.8%, $P = 0.027$). Multivariable analysis showed that hydroxychloroquine administration was an independent protective factor related to proteinuria reduction (OR 0.37, $P = 0.021$). During long-term follow-up, the clinical remission rate was higher in the HCQ-RAASi group (62.5 vs. 38.6%, $P = 0.013$). All of these findings provide strong evidence of the antiproteinuric effects of hydroxychloroquine in patients with membranous nephropathy.

Our study also supported the immunomodulatory actions of hydroxychloroquine in patients with membranous nephropathy. The rate of anti-PLA2R antibody reduction was higher in the HCQ-RAASi group at 3 months and during follow-up (33.3 vs. 6.1%, $P = 0.004$; 58.3 vs. 30.6%, $P = 0.023$). Multivariable analysis showed that HCQ administration was an independent protective factor for anti-PLA2R antibody reduction during follow-up (OR 0.28, $P = 0.031$). In patients with PLA2R-associated membranous nephropathy, many studies have described immune remission prior to clinical remission [37–39]. Thus, we speculated that the immunomodulatory actions of hydroxychloroquine might explain its antiproteinuric effects.

As a weak diprotic base, hydroxychloroquine interferes with lysosomal activity and autophagy by increasing the pH of endosomal compartments [40], which inhibits major histocompatibility complex (MHC) II molecule expression and antigen presentation [17, 18, 41]. In autoimmune disorders involving PLA2R-associated membranous nephropathy, risk alleles at the human leukocyte antigen (HLA)-D locus expressed on antigen-presenting cells can present epitopes of PLA2R to activate CD4+ helper T cells and then drive the differentiation of activated B cells into plasma cells, which produce anti-PLA2R antibodies [42, 43]. Hydroxychloroquine can also increase regulatory T cell activity by down-regulating the costimulatory molecule CD154 on CD4+ T cells and several proinflammatory cytokines [41, 44–46]. In patients with membranous nephropathy, the percentage of Tregs within CD4+ T cells significantly increased after immunosuppressive treatment and predicted the treatment response [47, 48].

Hydroxychloroquine treatment was safe and well tolerated. Side effects occurred in 4 (9.3%) patients, which were not severe and mostly resolved by discontinuing treatment or by antiallergic therapy. Kidney function was stable during the entire follow-up period.

The major limitation of this study is the relatively small sample size and the lack of randomization. We cannot draw

a conclusion about the therapeutic effects of hydroxychloroquine in patients with membranous nephropathy, but we do provide some observational information for future studies.

In conclusion, our data support the antiproteinuric and immunomodulatory effects of hydroxychloroquine in patients with membranous nephropathy, and suggest that hydroxychloroquine can be a useful add-on treatment for patients who do not achieve satisfactory proteinuria reduction by optimized doses of RAASi alone. Future randomized controlled trials are needed to confirm our observations.

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Author contributions Research idea and study design: ZC and X-YC; data acquisition: X-YC and Y-JC; data analysis/interpretation and statistical analysis: Y-JC; supervision and mentorship: M-HZ. Each author contributed important intellectual content during the drafting and revision of the manuscript and has accepted responsibility for the overall work by ensuring that any questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. ZC takes responsibility for the following: that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Availability of data and material The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to disclose.

Ethical approval The research followed the Declaration of Helsinki and was approved by the ethics committee of Peking University First Hospital.

Consent to participate Written informed consent was obtained from all individual participants.

Consent for publication Not applicable.

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