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Anti-albuminuric effects of spironolactone in patients with type 2 diabetic nephropathy: a multicenter, randomized clinical trial

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Abstract

Background—Several studies have demonstrated that spironolactone has an anti-albuminuric property in diabetic nephropathy. As an adverse event, spironolactone often induces the elevation of creatinine levels with hypotension and hyperkalemia. Therefore, we aimed to evaluate the efficacy and safety of spironolactone in Japanese patients with type 2 diabetes treated with either angiotensin-converting enzyme inhibitors or angiotensin receptor blockers.

Methods—Fifty-two Japanese patients with diabetic nephropathy and albuminuria (100 mg/gCr–2000 mg/gCr) treated with renin–angiotensin system (RAS) blockade were enrolled in a prospective, randomized, open-label study. The patients were subjected to add-on treatment with spironolactone 25 mg once daily and compared with matched controls for 8 weeks. The primary outcome was a reduction in the rate of albuminuria at 8 weeks compared with the baseline value. This study was registered with UMIN Clinical Trials Registry (000008016).

Results—Albuminuria was reduced by 33 % (95 % confidence interval: 22–54; $P = 0.0002$) at 8 weeks with spironolactone. In the spironolactone group, blood pressure tended to lower and the estimated glomerular filtration rate (eGFR) was significantly decreased compared to those in the control group. When adjusted by systolic blood pressure and eGFR, spironolactone treatment still showed a significant effect on albuminuria reduction in a linear mixed model (coefficient $\pm$ standard error; 514.4 ± 137.6 mg/gCr, $P < 0.0005$). No patient was excluded from the study because of hyperkalemia.

Conclusions—Spironolactone reduced albuminuria along with conventional RAS inhibitors in patients with diabetic nephropathy. Our study suggests that spironolactone exerts anti-albuminuric effects independent of systemic hemodynamic alterations.

Keywords

Albuminuria; Diabetic nephropathy; Randomized study; Spironolactone

Introduction

The diabetic population will continuously increase 155 % from 382 million to 592 million worldwide by 2035 [1]. Diabetes causes burdensome complications including small vessel diseases (retinopathy, neuropathy and nephropathy) and large vessel diseases (myocardial infarction, stroke, peripheral artery disease and congestive heart failure) [2]. The complications caused by systemic vascular disease result in premature death and disabilities with reduced productivity and high health-care costs [3]. Type 2 diabetes is the leading cause of end-stage renal disease (ESRD) in Japan [4], and annually, 16000 Japanese people develop ESRD caused by diabetes [5].

Early detection and treatment of diabetic nephropathy will reduce the progression to ESRD. Microalbuminuria is seldom reversible, but remission and even regression of nephropathy are reported in patients with microalbuminuria [6]. ESRD prevention and cardiovascular comorbidity reduction were observed in patients with regression [7]. Intensive glucose and blood pressure control reduces proteinuria, slows renal dysfunction and protects against microvascular complications [8, 9]. IRMA-2 [10] and INNOVATION [11] provided evidence that angiotensin receptor blockers (ARBs) prevent the progression of microalbuminuria to macroalbuminuria in diabetic nephropathy.

Renin–angiotensin system (RAS) blockers, such as angiotensin-converting enzyme inhibitor (ACEi) and ARB, are well established as the first-line drugs for diabetic nephropathy, and thus far either calcium channel blockers or diuretics have been suggested as second-line drugs. Aldosterone antagonists inhibit the renin–angiotensin aldosterone system and act as diuretics. Spironolactone reduces proteinuria in diabetic nephropathy [12–15] and in non-diabetic renal disease [16, 17] along with RAS blockers. However, because spironolactone use is frequently associated with the elevation of serum creatinine levels and reduction in the blood pressure due to the diuretic action, its anti-albuminuric effects may be secondary phenomena via modulation of renal hemodynamics. Thus, the aldosterone antagonist has not been fully evaluated for treatment for diabetic nephropathy. In this study, we evaluated the efficacy and safety of spironolactone on the progression of albuminuria in Japanese patients with type 2 diabetes.

Materials and methods

Ethics statement

The protocol of the study was approved by the ethical committee: Nagoya University Graduate School of Medicine (No. 2012-0038), Okayama University Graduate School of Medicine (No. 1432), Kanazawa Medical University (No. 253) and Shiga University of Medical Science (No. 24–49). We performed a multicenter, prospective, randomized, open-label parallel-group comparison study at Nagoya University Hospital, Shiga University of Medical Science Hospital, Kanazawa Medical University Hospital and Okayama University Hospital. All patients provided written informed consent to participate in this study. This trial was registered in Japanese University hospital Medical Information Network Clinical Trials Registry (UMIN-CTR: UMIN 000008016). The trial was started (first patient enrolled) in August 2012 and (last patient completed) completed in July 2013.

Participants

We enrolled 52 Japanese patients with type 2 diabetes and diabetic nephropathy from August 2012 to May 2013. Despite antihypertensive treatment with an RAS blocker (either ARB or ACEi), the enrolled patients had persistent albuminuria (100 mg/gCr–2000 mg/gCr). Moreover, they also met the following inclusion criteria: (1) aged 30 to 70 years; (2) estimated glomerular filtration rate (eGFR) >30 mL/min/1.73 m² calculated by serum creatinine. Diabetic nephropathy was diagnosed clinically if one or more of the following criteria were fulfilled: (1) histological diagnosis by renal biopsy; (2) presence of diabetic retinopathy; (3) history of type 2 diabetes mellitus at least 5 years before enrollment.

Exclusion criteria included the following: (1) aldosterone antagonist use; (2) diagnosis of type 1 diabetes or non-diabetic nephropathy, including chronic glomerulonephritis, polycystic kidney disease and nephrosclerosis; (3) impaired glucose tolerance secondary to exocrine pancreatic disease, endocrine disease, liver disease or infection; (4) systolic blood pressure >180 mmHg or diastolic blood pressure >110 mmHg; (5) confirmed or suspected bilateral renal artery stenosis or stenosis of the solitary renal artery in patients with one kidney; (6) cerebrovascular or cardiovascular disease within 3 months and New York Heart Association functional class III and IV heart failure; (7) malignancy; (8) rapid progression of kidney disease; (9) history of orthostatic hypotension; (10) liver dysfunction as indicated by aspartate transaminase and alanine transaminase levels >100 IU/L; (11) serum potassium level >5.0 mEq/L; (12) history of serious adverse events caused by aldosterone blockers; (13) history of rapidly declining renal function after aldosterone antagonist; and (14) women who were pregnant, possibly pregnant, breast-feeding, or planning to become pregnant during the study period.

Design

We randomly assigned the patients to two groups, one receiving additive treatment with spironolactone (25 mg/day) and the other receiving conservative therapy. Allocation ratio was 1:1. Randomization was performed by minimization methods at the Center for Advanced Medical and Clinical Research of Nagoya University Hospital. The stratifying factors for randomization were eGFR (>50 mL/min/1.73 m² or ≤50 mL/min/1.73 m²), proteinuria (>1.0 g/gCr or ≤1.0 g/gCr) and the hospital to which the patients belonged.

To the patients of the spironolactone group (Group S), the study medication was given in the morning and was added to the patients' usual antihypertensive treatment including RAS blockers for 8 weeks. The patients of the control group (Group C) continued their usual antihypertensive treatment for 8 weeks. A change in the type or dose of prior antihypertensive treatment of RAS blockers was prohibited throughout the study. The RAS blockers used in this study included telmisartan (*n* = 11), olmesartan (*n* = 11), irbesartan (*n* = 1), valsartan (*n* = 10), losartan (*n* = 10), candesartan (*n* = 6), imidapril (*n* = 3), perindopril (*n* = 1), lisinopril (*n* = 1) and temocapril (*n* = 2).

The primary end point was change in albuminuria indicated by urine albumin-to-creatinine ratio (uACR) after 8 treated weeks relative to the baseline values, and the secondary end points were change in serum potassium, eGFR calculated by serum creatinine and cystatin C and high-sensitive C-reactive protein (hs-CRP). To investigate factors in reducing albuminuria, we additionally performed an exploratory check on change in serum aldosterone, urinary angiotensinogen (AGT) and N-acetyl-beta-D-glucosaminidase (NAG), beta2-microglobulin (β2MG), L-type fatty acid-binding protein (L-FABP), neutrophil gelatinase-associated lipocalin (N-GAL) and monocyte chemoattractant protein-1 (MCP-1).

Follow-up

Follow-up visits were conducted every 4 weeks after study commencement, and an interview about adverse events, physical examination, blood pressure and serum creatinine and potassium levels were obtained. As a rule for safety, a decision was made to discontinue

the study for any patient whose serum potassium level was >6 mEq/L and eGFR calculated by serum creatinine decreased >30 % from the starting level.

Laboratory measurements

Urine samples were obtained from an aliquot of a spot urine sample and collected by Nagoya University. The measurement of urinary albumin, urinary creatinine, serum aldosterone, serum hs-CRP, serum cystatin C, urinary NAG and urinary β 2MG was entrusted to the laboratory of SRL Inc., Aichi, Japan. Serum hs-CRP levels were measured by latex-nephelometry. Blood samples for aldosterone measurement were taken after 30 min of supine rest. The urinary concentrations of L-FABP, N-GAL and MCP-1 were measured using a commercially available ELISA kit (L-FABP, CMIC, Tokyo, Japan; N-GAL, BioPorto Diagnostics A/S, Gentofte, Denmark; MCP-1, R&D Systems, Inc., Minneapolis, MN, USA). Urinary AGT levels were measured using a sandwich ELISA system in Kagawa University, as previously described [18]. The eGFR from creatinine levels was calculated as follows: $\text{eGFR}_{\text{creat}} (\text{mL}/\text{min}/1.73 \text{ m}^2) = 194 \times \text{SCr}^{-1.094} \times \text{Age}^{-0.287} \times 0.739$ (if female) [19]. Additionally, the eGFR from cystatin C levels was calculated as follows: $\text{eGFR}_{\text{cys}} (\text{mL}/\text{min}/1.73 \text{ m}^2) = [104 \times \text{Cys-C}^{-1.019} \times 0.996^{\text{Age}} \times 0.929 \text{ (if female)}] - 8$ [20]. Serum potassium levels and others were determined by routine procedures at clinical chemistry facilities of each hospital.

Statistical analysis

The primary outcome was the change in albuminuria from the baseline to 8 weeks. For power calculation, we could consider a 25 % decrease from 360 mg/day to 270 mg/day in albuminuria from the baseline in Group S according to previous reports [13, 15]. Based on this assumption, we estimated that with a minimum of 17 patients in each group, this study would have 80 % power to detect a significant between-group difference in the change of albuminuria with an effect size of 0.9 SD. Thus, we set up the target number of subjects as 50 patients in total including dropouts and unfit cases.

Continuous variables are expressed as mean $\pm$ SD. To evaluate baseline characteristics, comparisons of continuous parameters between Group S and Group C were performed with the two-sample t test. Nominal variables were tested using the Chi-square test. As a primary outcome, ANCOVA, including treatment group, albuminuria at entry and eGFR at entry, was used to compare the change in albuminuria from baseline between the groups at 8 weeks. A linear mixed model including period, an interaction term between treatment group and period, albuminuria at entry, eGFR at entry, delta eGFR, SBP at entry and delta SBP as fixed effects was used to compare the change in albuminuria from baseline between the groups at 4 and 8 weeks. The albuminuria treatment response was further examined in additional mixed models including baseline albuminuria, systolic blood pressure, $\text{eGFR}_{\text{creat}}$ and serum level of aldosterone. Additionally, to evaluate the effect of spironolactone treatment, comparisons of change in clinical parameters between Group S and Group C were performed with the two-sample t test.

A P value < 0.05 was considered to be statistically significant. Statistical analyses were performed using statistical software JMP version 8.0.1 (SAS Institute Inc., Cary, NC, USA).

Results

Fifty-two Japanese patients were randomized in the trial: 49 patients completed the study and were included in the statistical analysis. Two patients refused to participate in the study, and one patient was not able to continue in the study because of a hemorrhage in the eyeground. Baseline clinical data are shown in Table 1. Baseline characteristics were similar between Group S and Group C. The mean age of the study subjects was approximately 60 years and there was a slight male predominance. The mean of uACR was 702.1 ± 728 mg/gCr in Group S and 511 ± 450 mg/gCr in Group C. The rate of statin use was higher in Group S, while the rate of other antihypertensive drug use was similar, since all patients had taken RAS blockers. There was no significant difference in baseline blood pressure and eGFR between the two groups.

Spironolactone significantly reduced albuminuria in patients with diabetic nephropathy

During treatment with spironolactone in addition to conventional antihypertensive treatment with RAS blockers, albuminuria in Group S was significantly reduced by a mean of 297.1 ± 437 mg/gCr in 4 weeks and 374.0 ± 504 mg/gCr after 8 weeks of treatment compared to Group C. Figure 1a shows the mean of uACR. Spironolactone showed a significant reduction of uACR of -499.7 ± 130.6 mg/gCr after 8 weeks by ANCOVA ($P = 0.0004$). In a linear mixed model, spironolactone also showed a significantly persistent reduction of uACR by -256.8 ± 77.4 mg/gCr in 4 weeks and -333.8 ± 92.0 mg/gCr after 8 weeks in Group S, and by 144.8 ± 75.8 mg/gCr in 4 weeks and 185.9 ± 90.2 mg/gCr after 8 weeks in Group C. The differences between Group S and Group C after 4 weeks and 8 weeks from the start of the study were 401.7 ± 108.9 mg/gCr ($P = 0.003$) and 519.7 ± 129.4 mg/gCr ($P = 0.001$) by Tukey–Kramer multiple-comparison test, respectively.

Blood pressure and kidney function during the treatment

Albuminuria was reduced by 33 % (95 % confidence interval: 22–54; $P = 0.0002$) during treatment with spironolactone. In Group S patients, blood pressure progressively tended to decrease with systolic blood pressure by 2.48 ± 22.9 mmHg at 4 weeks and 2.68 ± 25.3 mmHg at 8 weeks, and diastolic blood pressure by 0.64 ± 13.0 mmHg at 4 weeks and 3.44 ± 14.3 mmHg at 8 weeks, although there were no statistically significant differences. There was no change in Group C patients. There was a statistically significant increase in serum creatinine of 0.056 ± 0.2 mg/dL (Fig. 1b, $P = 0.044$) with a decrease in eGFR_{creat} of 3.2 ± 9.7 mL/min/1.73 m² (Fig. 1c, $P = 0.052$) compared to Group C. Similarly, there was a significant increase in serum cystatin C of 0.083 ± 0.1 mg/L ($P = 0.004$) and a decrease in eGFR_{cys} of 5.4 ± 8.2 mL/min/1.73 m² ($P = 0.014$).

Furthermore, it was necessary to evaluate if the reduction in albuminuria was secondarily induced by the hemodynamic effect. When we individually added albuminuria at entry, systolic blood pressure and eGFR as explanatory variables in the linear mixed model, spironolactone treatment still showed a significant effect on albuminuria reduction (coefficient $\pm$ SE; 514.38 ± 137.59 mg/gCr, $P < 0.0005$, Table 2).

No impact of aldosterone levels at baseline

There was an elevated trend in serum aldosterone levels during the spironolactone treatment, but such elevation of aldosterone levels could not reach a statistically significant difference between Group S and Group C. We calculated the effect on reduction of albuminuria to add the serum concentrations of aldosterone at baseline as an explanatory variable in the same linear mixed model. We failed to find an impact of spironolactone treatment on albuminuria in patients with higher concentrations of aldosterone (coefficient $\pm$ SE; 0.78 ± 1.50 pg/mL, $P = 0.608$).

Secondary analysis of factors associated with spironolactone treatment

Next, to examine several serum and urinary biomarkers to search for additional effects of spironolactone treatment besides the hemodynamic effect, we compared the changes in clinical parameters between Group S and Group C (Table 3). There was no significant difference between the two groups in inflammatory biomarkers, serum hs-CRP levels and urinary MCP-1 levels. N-GAL was recently reported as a marker of renal injury [21] and there was also no significant difference. Increases in the urinary excretion of β 2MG and NAG suggested the presence of tubulointerstitial damage. Spironolactone treatment induced a significant decrease in urinary NAG and β 2MG excretion by 2.3 ± 6.5 U/L and 1026.9 ± 3174.6 mg/L compared to Group C ($P = 0.0304$ and 0.029), respectively. Urinary AGT levels may provide a specific index of the intrarenal RAS status, and upregulation of urinary AGT may lead to hypertension [22]. Spironolactone treatment induced a significant decrease in urinary AGT by 156.7 ± 466 mg/gCr compared to Group C ($P = 0.0004$).

Safety

No patients decided to discontinue the study because of hyperkalemia and kidney dysfunction. However, serum potassium levels were significantly higher in Group S ($P = 0.0003$ after 4 weeks and $P = 0.0423$ after 8 weeks). The potassium levels at baseline, after 4 weeks and 8 weeks were 4.30 ± 0.31 , 4.57 ± 0.26 and 4.51 ± 0.34 mEq/L in Group S and 4.42 ± 0.27 , 4.29 ± 0.24 and 4.27 ± 0.42 mEq/L in Group C, respectively.

Discussion

In our multicenter, prospective, randomized, open-label parallel-group comparison study, we found that the addition of spironolactone to conventional antihypertensive treatment including RAS blockers resulted in a reduction in albuminuria in Japanese patients with type 2 diabetes and nephropathy. The spironolactone treatment demonstrated a reduced trend in blood pressure and a slight, but significant decrease in eGFR. However, the reduction in albuminuria was independent of the changes in either blood pressure or eGFR and was not associated with serum aldosterone levels at baseline. Although several studies have shown that spironolactone treatment decreases albuminuria in patients with diabetic nephropathy and non-diabetic nephropathy, our study is of note in showing the anti-albuminuric effects independent of reduction in blood pressure with additional spironolactone use. Furthermore, this study also demonstrated that spironolactone significantly decreased the urinary tubulointerstitial injury markers NAG and β 2MG. Although some reports have shown the

amelioration and protection of interstitial injury by spironolactone in animal models, there have been few reports on humans.

Some previous studies have demonstrated that spironolactone treatment induced a slight deterioration in renal function [23], and we also found a similar decrease in eGFR in this study. Morales et al. demonstrated that long-term use of spironolactone should lead to recovered eGFR levels with a sustained reduction in proteinuria, although spironolactone induced an initial acute decrease in eGFR [24]. Recently, Esteghamati et al. [25] demonstrated that spironolactone use for 18 months decreased uACR superior to continuous ACEi and ARB use with a slight decrease in eGFR, but the decline rate did not differ between the two groups. van den Meiracker et al. [26] reported that the anti-albuminuric effect of spironolactone was correlated to the decrease in eGFR. In our present study, the anti-albuminuric effect was verified independent of blood pressure lowering and eGFR decline, evaluated from the adjusted calculation in the linear mixed model. Although reduction of glomerular pressure per se could still affect the changes in albuminuria because the changes in glomerular pressure might not necessarily parallel those of GFR, reductions in albuminuria was at least independent of systemic hemodynamic effects of spironolactone. Although our study was performed in a relatively short period, these results suggest that spironolactone would be a promising add-on therapy for diabetic nephropathy when the first choice RAS-blocking treatment has shown some inefficacy or “resistance”.

Previous studies have shown that serum aldosterone levels gradually increased with duration of RAS-blocking treatment in what is called the “aldosterone breakthrough” and may reverse the beneficial effect on the heart and kidneys [27]. Since 20–50 % of hypertensive patients on long-term RAS blockers use were reported to fall into “aldosterone breakthrough” and would benefit from the addition of spironolactone [28, 29], for such patients the use of spironolactone should be recommended. We failed to demonstrate greater efficacy in our patients with higher concentrations of aldosterone. Further study will be needed because of the relatively small-scale and short-period current investigation.

As well-established first-line drugs for diabetic nephropathy, RAS blockers are expected to have both anti-albuminuric and renoprotective effects. Mineralocorticoid receptor activation plays an important role in the pathogenesis of chronic kidney disease [30]. Mineralocorticoid receptor blockade exhibits renoprotective effects [31] and aldosterone blockade prevents renal injury by reducing oxidative stress [32] in animal models. Moreover, clinical evidence from a double-blind randomized study indicates that mineralocorticoid receptor blockade reduces oxidative stress [33]. These data suggest that mineralocorticoid receptor activation causes oxidative stress, leading to renal injury.

Moreover, spironolactone treatment demonstrated a significant decrease in the urine tubulointerstitial injury markers NAG and β 2MG in this study. In diabetic nephropathy, the renal structural alterations are characterized by renal hypertrophy in the early stage and in turn by the progressive accumulation of extracellular matrix in the glomerulus and tubulointerstitium, and finally by glomerulosclerosis and tubulointerstitial fibrosis [34]. Fujisawa et al. [35] showed that spironolactone prevented the increase in collagen deposition in the tubulointerstitial area and the degeneration of the proximal tubule with upregulated

inflammatory and fibrotic markers in diabetic model rats. Kramer et al. [36] showed with reduction in proteinuria and markers of tubular injury in experimental nephrosis that combination use of ACEi and spironolactone prevented renal damage. Although we failed to detect a reduction in urinary MCP-1 levels and serum hs-CRP levels by spironolactone administration, a significant improvement in urine tubulointerstitial injury markers suggested the inhibitory effect of spironolactone on renal damage progression in patients with diabetic nephropathy in our study.

Many previous studies have indicated caveat for hyperkalemia by combination of spironolactone with RAS inhibitors. In recent systematic review, hyperkalemia prevalence increased by up to 17 % of drop out patients [37]. Because the increase in serum potassium was reported to be less marked with ARB than with ACEi [38], the reason why this study did not show spironolactone-induced hyperkalemia was perhaps, in part, due to selection of the enrolled patients.

Some limitations of the present study should be noted. First, the study period was relatively short. An extension of several years, at least, is needed to evaluate kidney function decline such as creatinine doubling and progression to ESRD. Second, we enrolled patients who were freely treated with various RAS blockers with regard to dose and period. Additionally, as this was a multicenter study, clinical settings such as dietary education systems and others were not completely same, but may be almost similar. Third, because the range of albuminuria inclusion criteria (100 mg/gCr–2000 mg/gCr) was somewhat broad, patients with a relatively low amount of albuminuria at baseline should be prone to underestimation. Fourth, we checked the changes in urinary angiotensinogen as an exploratory investigation. However, the implication of this parameter in microalbuminuria remained unknown, because RAS inhibitors had already been given in both groups. Finally, the relatively small sample size may have influenced the results, and there may undeniably be an unrecognized factor to bias the results.

This study demonstrated that spironolactone had anti-albuminuric effects independent of systemic hemodynamic effects without adverse effects such as hyperkalemia. Moreover, the data suggested that spironolactone could improve tubulointerstitial injuries and decrease local RAS activity in the kidney. In conclusion, our study suggests that spironolactone could be recommended as a second-line treatment for patients with type 2 diabetes and nephropathy, when the control of blood pressure or albuminuria is insufficient under the RAS blocker-based therapy.

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Fig. 1. Line plot showing mean urinary albumin-to-creatinine ratio (a), mean serum creatinine (b) and mean estimated glomerular filtration rate (c). The *squares* represent the control group (Group C) and the *circles* represent the spironolactone group (Group S)

Table 1

Baseline characteristics in this study

	Spironolactone (25 mg)	Control	<i>P</i> value
Number	26	26	
Age (years)	61.0 ± 9.2	59.4 ± 10.8	0.56
Sex (male; %)	18 (69.2)	19 (73.8)	0.76
Smoking			
Current smoker ^a	7 (29)	7 (32)	0.85
History of smoking ^b	18 (72)	14 (55)	0.24
Blood pressure			
Systolic blood pressure	137.3 ± 16.3	131.3 ± 13.0	0.14
Diastolic blood pressure	76.8 ± 12.2	77.6 ± 8.8	0.79
Medication			
Calcium channel blockers (%)	9 (35)	11 (42)	0.57
Diuretics (thiazide/furosemide; %)	0/2 (0/7)	1/2 (4/8)	0.21
Statin (%)	22 (88)	16 (64)	0.043
Hemoglobin (g/dL)	13.6 ± 1.8	13.9 ± 1.6	0.61
White blood cell (/mm ³)	6624.0 ± 1683	6848.0 ± 2080	0.68
Platelet (×10 ⁴ /mm ³)	22.2 ± 6.0	22.7 ± 6.1	0.78
Serum creatinine (mg/dL)	0.86 ± 0.2	0.88 ± 0.2	0.66
BUN (mg/dL)	16.1 ± 4.2	16.8 ± 5.3	0.63
UA (mg/dL)	5.8 ± 1.1	6.0 ± 1.1	0.66
Serum sodium (mEq/L)	139.6 ± 2.9	140.0 ± 2.0	0.54
Serum potassium (mEq/L)	4.3 ± 0.3	4.4 ± 0.3	0.29
Serum chloride (mEq/L)	104.0 ± 3.7	100.5 ± 2.3	0.44
Triglyceride (mg/dL)	161.4 ± 74.0	165.5 ± 73.5	0.85
HDL cholesterol (mg/dL)	46.3 ± 11.0	47.5 ± 14.6	0.75
LDL cholesterol (mg/dL)	101.3 ± 33.0	91.5 ± 22.0	0.23
AST (IU/L)	22.7 ± 7.5	26.7 ± 17.6	0.30
ALT (IU/L)	27.0 ± 14.4	33.5 ± 19.8	0.18
Cystatin C	0.98 ± 0.2	1.06 ± 0.3	0.28
Proteinuria (g/gCr)	0.90 ± 0.9	0.94 ± 0.8	0.77
Albuminuria (mg/gCr)	702.1 ± 728.4	511.3 ± 450.1	0.28

BUN blood urea nitrogen, *UA* uric acid, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

^a*N* = 46

^b*N* = 50

Table 2

Efficacy of spironolactone treatment adjusted by hemodynamic alterations

Variable	Estimate	SE	<i>t</i>	<i>P</i>
Intercept	−661.87	631.28	−1.05	0.3002
Spironolactone	−514.38	137.59	3.74	0.0005*
Albuminuria (at entry)	−0.42	0.09	−4.44	< 0.0001*
eGFR (at entry)	2.23	3.91	0.57	0.5727
eGFR [delta (8–0 week)]	2.4	6.13	0.39	0.6972
SBP (at entry)	3.24	4.09	0.79	0.4327
SBP [delta (8–0 week)]	1.87	2.57	0.73	0.4695

GFR glomerular filtration rate, *SBP* systolic blood pressure*
P < 0.05

Table 3

Changes in serum and urinary biomarkers during 8 weeks of treatment with spironolactone

	Spironolactone (25 mg)			Control		P value
	0 week	8 week	Delta (8–0 week)	0 week	8 week	
Serum						
Serum potassium (mEq/L)	4.30 ± 0.31	4.51 ± 0.34	0.20 ± 0.39	4.42 ± 0.27	4.27 ± 0.42	0.003*
Serum aldosterone (pg/mL)	84.1 ± 41.2	103.5 ± 47.6	19.4 ± 33.4	92.6 ± 39.6	94.7 ± 43.6	0.0834
hs-CRP (ng/mL)	883.5 ± 1143.8	1564.8 ± 3298.9	681.4 ± 3015.6	1200.7 ± 1586.4	1025.7 ± 1099.9	0.3254
Urine						
Angiotensinogen (µg/gCr)	206.0 ± 513.5	49.3 ± 55.1	−156.7 ± 466.0	86.1 ± 146.8	104.8 ± 117.1	0.004*
NAG (U/gCr)	9.2 ± 6.5	6.9 ± 5.5	−2.3 ± 6.5	9.0 ± 6.2	10.1 ± 7.6	0.0304*
β2MG (µg/gCr)	1361.0 ± 3887.6	334.1 ± 798.5	−1026.9 ± 3174.6	753.5 ± 2527.2	1113.1 ± 2978.4	0.029*
L-FABP (µg/gCr)	22.7 ± 56.9	8.84 ± 13.1	−13.9 ± 49.1	7.36 ± 9.3	13.0 ± 23.2	0.1637
N-GAL (µg/gCr)	35.0 ± 73	28.9 ± 77.4	−6.2 ± 37.6	22.5 ± 39.7	17.8 ± 26.0	0.8943
MCP-1 (µg/gCr)	0.18 ± 0.12	0.15 ± 0.13	−0.015 ± 0.13	0.12 ± 0.07	0.15 ± 0.11	0.4485

CRP C-reactive protein, NAG N-acetyl-beta-D-glucosaminidase, β2MG beta2-microglobulin, L-FABP L-type fatty acid-binding protein, N-GAL neutrophil gelatinase-associated lipocalin, MCP-1 monocyte chemoattractant protein-1

* $P < 0.05$