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Original Article

ORIGINAL ARTICLE

Tolvaptan and the role of kidney aquaporin-2 abundance in managing volume overload in patients with CKD

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ABSTRACT

Objective. This retrospective study evaluated tolvaptan's efficacy, safety, and predictive indicators in managing volume overload in chronic kidney disease (CKD) patients.
Methods. CKD patients with volume overload, treated with loop diuretics alone or with tolvaptan at Zhongda Hospital, Southeast University, from 1 March 2022 to 31 December 2023, were included. Patients were divided into loop diuretic (Group C) and loop diuretic combined with tolvaptan (Group T) cohorts. Primary outcomes included volume control, changes in weight, urine output, and laboratory parameters within 1 week post-medication. Adverse events such as hypernatremia and hyperkalemia, etc., were recorded. We further conducted immunohistochemical staining of renal biopsy tissues to investigate the roles of aquaporin-2 (AQP2) in the collecting duct and plasma albumin in predicting the efficacy of tolvaptan.
Results. Of 174 CKD patients with volume overload, 108 (67.07%) were male. Group C and Group T each comprised 87 patients. At baseline, no significant differences in urine output and weight were noted. By day 3, Group T exhibited a greater increase in urine output ($P < .001$) and weight reduction ($P < .001$). At day 7, Group T maintained more significant diuretic effects ($P < .001$). More Group C patients required ultrafiltration therapy ($P = .040$). Adverse event rates did not significantly differ. Notably, AQP2 expression in the collecting duct may predict tolvaptan responsiveness, while plasma albumin did not affect efficacy.
Conclusion. Tolvaptan showed efficacy and safety in managing volume overload in CKD patients. The expression of AQP2 in the collecting duct could predict tolvaptan's efficacy.

This study protocol was approved by the Ethics Committee of Zhongda Hospital Affiliated to Southeast University (Approval No. 2023ZDSYLL180-P01, Clinical Trial Registration No. ChiCTR2300075274, Trial Registration Link: https://www.chictr.org.cn/guide.html).

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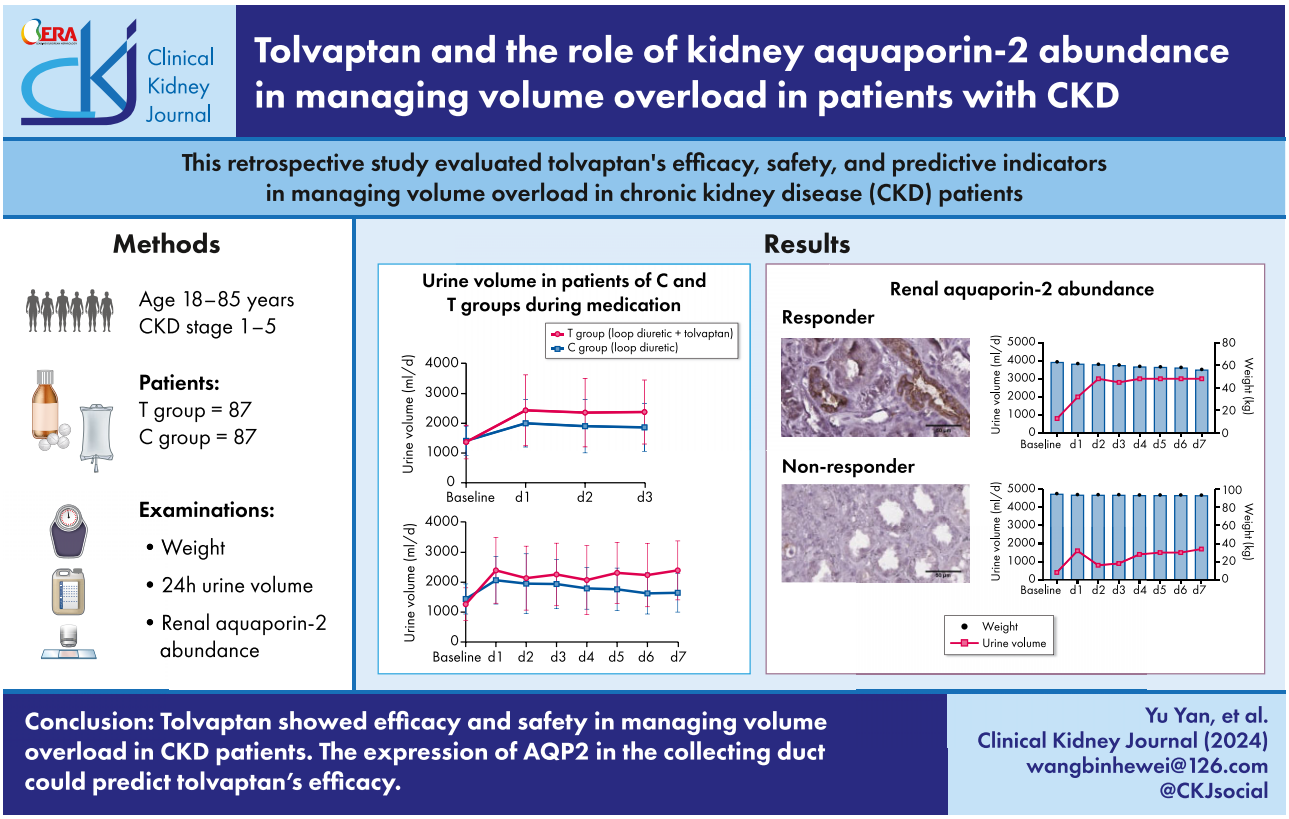
GRAPHICAL ABSTRACT



Keywords: chronic kidney disease, efficacy, precision medicine, tolvaptan, volume overload

KEY LEARNING POINTS

What was known:

- Loop diuretics may lead to adverse events such as worsening renal function. Hence, there is an urgent need to explore new drugs to address the issue of volume overload in kidney disease patients.
- Given the varying diuretic responses to tolvaptan among patients, we explored the role of renal tissue AQP2 in predicting tolvaptan responsiveness.

This study adds:

- The combination therapy of tolvaptan and loop diuretics demonstrated superior diuretic effects.
- It is safe to use tolvaptan in patients with kidney disease.
- AQP2 may be a potential predictor of responsiveness to tolvaptan.

Potential impact:

- Tolvaptan shows promising clinical application prospects for volume management in patients with kidney disease.
- Also, the abundance of renal AQP2 can be used to predict the efficacy of tolvaptan.

INTRODUCTION

Volume overload represents a prevalent and urgent clinical concern across various acute and chronic kidney diseases (CKDs). Excessive volume overload can lead to critical complications such as heart failure, acute pulmonary edema, and, in severe cases, life-threatening conditions. Additionally, it poses a risk factor for end-stage renal disease, cardiovascular diseases, and all-cause mortality [1–4]. CKD patients, owing to impaired renal function, face additional challenges in maintaining fluid balance [4], moreover, they are more prone to diuretic resistance [5]. Therefore, reducing volume overload constitutes a focal and challenging aspect of managing such patients.

Loop diuretics are the most commonly used medications for treating fluid retention in kidney disease patients.

However, these drugs may exacerbate worsening renal function (WRF) by activating the sympathetic nervous system and the renin-angiotensin system [2]. The DOSE trial demonstrated an association between high-dose loop diuretics and WRF [6], Nakano *et al.*'s research further identified high-dose loop diuretics as an independent predictor of WRF, additionally, in heart failure patients with concomitant renal impairment, there existed a dose-dependent relationship between loop diuretic use and mortality rates [7]. Hence, exploring novel interventions for volume management in kidney disease patients is crucial.

Tolvaptan, a selective vasopressin V2 receptor antagonist, has been shown to improve fluid retention without affecting renal function and may even reduce the incidence of WRF [2, 8]. Currently, in the USA, European Union, Japan, and China, indications for tolvaptan primarily include hypervolemic and euvolemic hyponatremia, syndrome of inappropriate antidiuretic hormone secretion, congestive heart failure, and heart failure patients with inadequate response to other diuretics for volume overload[9–11]. While approval for volume overload without congestive heart failure is pending, clinical studies have partially confirmed its safety and efficacy [12–14]. What is more, patients with concomitant renal impairment have been largely excluded from most clinical studies involving tolvaptan, [12] resulting in limited evidence in evidence-based medicine for drug therapy [15, 16]. Therefore, this study focuses on patients with CKD presenting fluid retention to investigate the efficacy and safety of tolvaptan in this population.

It is worth noting that the diuretic and antiedematous effects of tolvaptan vary significantly among different patients. Responders to tolvaptan may experience effective improvement in volume overload while reducing proteinuria and enhancing myocardial injury markers [17, 18]. Currently, several potential biomarkers have been reported to predict the efficacy of tolvaptan, including aquaporin-2 (AQP2) [19], serum albumin [18], urine sodium/potassium ratio [20], urine osmolality [21], urine AQP2/plasma arginine vasopressin [22], urine AQP2 [23], urine sodium excretion [24], and urine urea nitrogen/plasma urea nitrogen ratio [25], among others. However, consensus on identifying clinical markers for tolvaptan responsiveness remains elusive. Therefore, further research is warranted to identify patients who respond effectively to tolvaptan at an early stage.

MATERIALS AND METHODS

Study participants

This study constituted a single-center, retrospective, observational investigation that compiled data from 929 patients admitted to our nephrology department for non-dialysis CKD with associated fluid retention. The patients received treatment with either tolvaptan or loop diuretics during the period spanning from 1 June 2022 to 31 December 2023. Inclusion criteria were as follows: (i) diagnosed with CKD with fluid retention (orthopnea, edema, jugular venous distension, pulmonary rales, chest X-ray or chest CT indicating signs of pulmonary congestion) and treated with loop diuretics and/or tolvaptan, (ii) aged between 18 and 85 years. Exclusion criteria were: (i) volume overload due to non-renal causes, (ii) patients undergoing renal replacement therapy simultaneously, (iii) concomitant use of spironolactone, (iv) serum sodium >147 mmol/l, and (v) incomplete data recording. Patients were divided into two groups based on their established diuretic treatment regimen: the loop diuretic treatment group (Group C) and the tolvaptan combined with loop diuretics treatment group (Group T). The flowchart of patient selection is depicted in Fig. 1.

Clinical data collection

For patients who met the inclusion and exclusion criteria, demographic data (age, sex, weight, height), concomitant medication use [sodium-glucose cotransporter 2 inhibitors (SGLT-2i), sacubitril/valsartan], baseline laboratory test results (serum sodium, serum potassium, serum creatinine, estimated glomerular filtration rate [eGFR, which was calculated using the 2021 version of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [26]: $eGFR = 142 \times (\text{serum creatinine}/A)^{B} \times 0.9938^{age} \times C$), plasma albumin, alanine aminotransferase, aspartate aminotransferase], and characteristics of volume overload (including grading of edema (Supplementary Methods), dyspnea, orthopnea, and pulmonary rales) were collected. The dose range of tolvaptan was 7.5 to 30 mg once daily, and the pharmacological equivalence between furosemide injection (40 mg), torasemide injection (20 mg), and bumetanide injection (1 mg) was considered. The doses of furosemide and torasemide were converted to bumetanide, which was used as a standardized loop diuretic and expressed as the total daily intravenous dose. If administered orally, the dose was converted to half of the intravenous dose [27]. The patients' weights and urine output were recorded before and during treatment, and the characteristics of volume overload were evaluated. Laboratory test results were collected during the treatment period.

Methods for measurement of weight and urine output, and specific procedures for immunohistochemical staining of renal biopsy specimens

Measurement methods for weight and urine output

Baseline measurements of patient weight and urine output were taken the day before initiation of either tolvaptan or loop diuretics. Throughout the treatment period, daily weight and urine output were measured by nurses in standardized hospital attire, each morning after emptying the bladder.

Immunohistochemical staining

Renal biopsy specimens underwent immunohistochemical staining for AQP2 in kidney tissues. The specific procedures for immunohistochemistry on renal biopsy specimens were as follows: kidney tissues were fixed in 20% neutral-buffered formalin, followed by embedding in paraffin to prepare 2–3-μm thick sections. Each section was incubated with primary antibody at room temperature for 2 hours, followed by three washes with PBS for 5 minutes each. Subsequently, polymer double staining reagents were applied sequentially to the sections according to the manufacturer's instructions, followed by PBS washing. Freshly prepared DAB solution was then added to each section for color development, and the reagents on the slides were rinsed off with distilled water. Finally, the samples were observed under a microscope after hematoxylin and eosin staining.

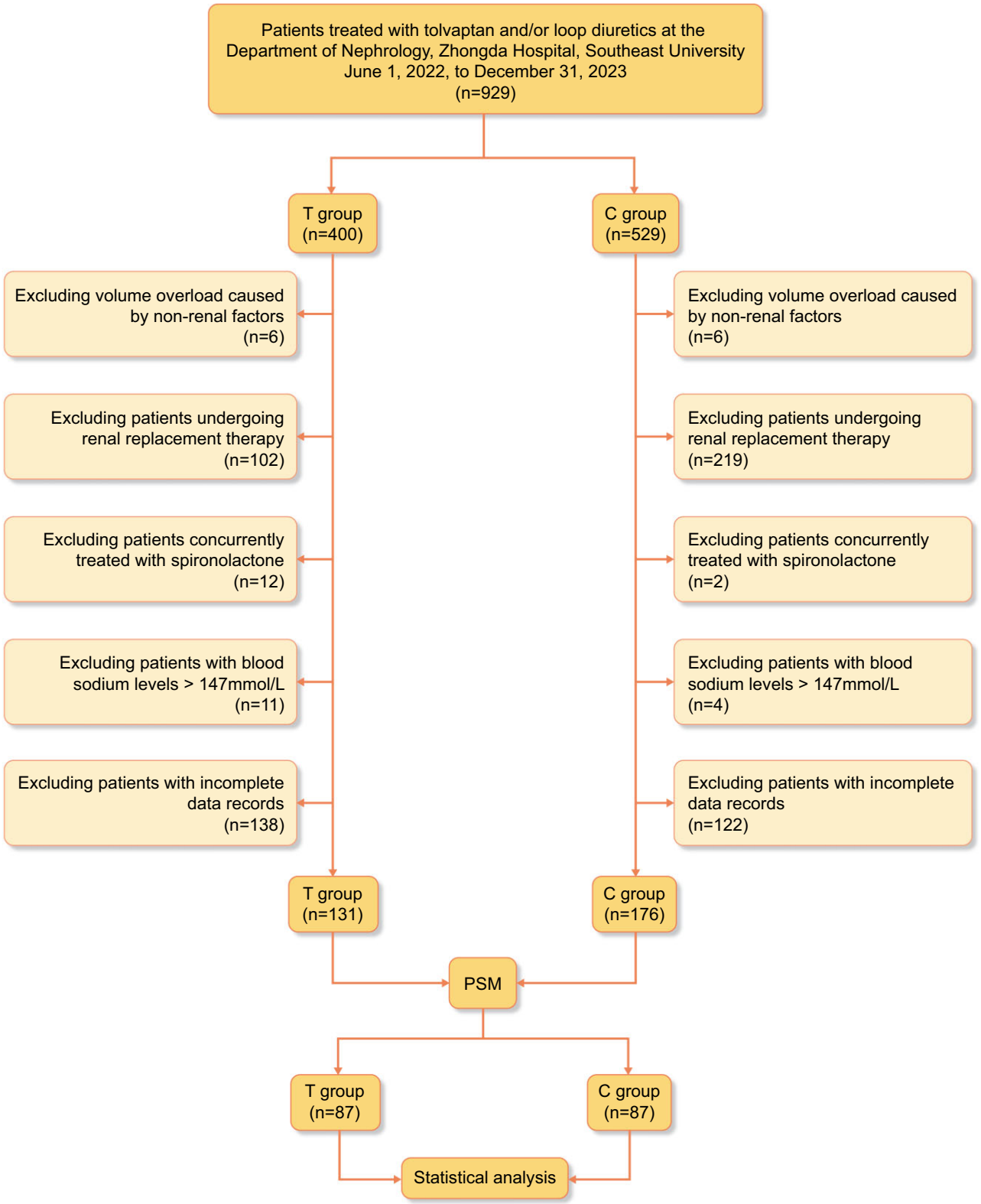


Figure 1: Flowchart of patients.

Evaluation of drug efficacy and safety

Primary endpoints

The primary objectives encompass comparing changes in urine output, weight during treatment between the T and C groups, and the incidence of patients requiring ultrafiltration due to inadequate diuretic response.

Secondary endpoints

These involve comparing alterations in volume overload characteristics after medication between the two groups. Additionally, the study aims to investigate the value of renal tissue AQP2 content and plasma albumin in identifying patients responsive to tolvaptan. Safety assessment indicators include monitoring and documenting changes in serum sodium, serum potassium, serum creatinine, and eGFR before and within one week after medication. Adverse drug reactions post-medication, such as hypernatremia, hyperkalemia, WRF, and liver function impairment, are also recorded. WRF is defined as either an increase in serum creatinine of $\geq$26.5 μmol/l or a decrease in eGFR of $\geq$20% from baseline [28].

Statistical analysis

Numerical variables were described using either mean $\pm$ standard deviation ($\bar{x} \pm s$) or median (P_{25}, P_{75}), depending on whether they followed a normal distribution. Categorical variables were presented as frequencies (percentages). The comparison of numerical variables used *t*-tests or rank-sum tests, while categorical variables were compared using chi-square tests, Fisher's exact tests, or rank-sum tests. To mitigate potential confounding factors between the tolvaptan combined with loop diuretic group and the loop diuretic group, we rigorously adjusted patients' baseline characteristics using the following algorithm: 1:1 optimal matching with a tolerance of 0.02. Potential confounding factors were selected based on clinical knowledge and relevant literature, and propensity score matching (PSM) was assessed using logistic regression models [29]. A P value $<$.05 was considered statistically significant. Linear regression analysis was conducted to evaluate the correlation between changes in urine output post-medication and serum albumin levels. The Pearson correlation coefficient or Spearman correlation coefficient (r) was used to assess this correlation. Statistical analysis and graph plotting were performed using SPSS v.26.0 software and GraphPad Prism v.9.5.0.

RESULTS

The baseline characteristics of the participants

During the period from 1 June 2022, to 31 December 2023, a total of 307 eligible patients were retrospectively enrolled (Fig. 1). Baseline characteristics of 176 patients in the C group and 131 patients in the T group were compared before PSM (Supplementary Table S1).

Baseline characteristics of patients after PSM

After PSM, 87 patients from each group were further analyzed (Fig. 1). Prior to matching, patients in the T group exhibited poorer renal function and heavier volume overload compared to the C group. As shown in Table 1, after matching, there were no significant differences in baseline characteristics between the C and T groups.

Comparison of volume overload characteristics in patients of C and T groups post-medication

We compared the volume overload characteristics between the two groups of patients post-medication (C group vs T group). Following medication, patients in both groups showed improvements in dyspnea ($P = 1.000$), orthopnea, and pulmonary rales ($P = 1.000$). We observed that, after 3 days of treatment, the mean urine output of patients in the T group was significantly higher than that of patients in the C group ($P < .001$), and the increase in urine output was more significant ($P < .001$). By contrast, there was no statistically significant difference in the mean weight between the two groups ($P = .593$), but patients in the T group showed a greater reduction in weight ($P < .001$). Furthermore, we examined the urine output and weight changes in 51 patients of the C group and 53 patients of the T group after 7 days of medication. Similarly, the mean urine output of patients in the T group was significantly higher than that of patients in the C group ($P = .002$), and the increase in urine output was more significant ($P < .001$). After 7 days of treatment, there was no statistically significant difference in the mean weight between the two groups ($P = .242$), but patients in the T group showed a greater reduction in weight loss ($P = .002$) (Table 2). In addition, as shown in Fig. 2, we compared the daily urine output and weight changes within the first 3 days of medication between the two groups. We observed that the mean urine output of patients in the T group was significantly higher than that of patients in the C group on the second and third day of treatment ($P = .006$, $P = .001$). Interestingly, we found similar results in patients who were treated for 7 days, with the mean urine output of patients in the T group remaining significantly higher than that of patients in the C group on days 5–7 ($P = .002$, $P = .002$, $P < .001$), indicating that the T group was more effective in maintaining a steady increase in urine output following medication and reducing the occurrence of diuretic resistance.

Furthermore, we conducted subgroup analyses on patients concurrently taking SGLT-2i (Supplementary Table S2). There were 14 patients in the C group and 11 patients in the T group who were using SGLT-2i. We further analyzed changes in body weight and urine volume post-medication among these patients and found that the T group exhibited superior diuretic and edema-reducing effects compared to the C group. Additionally, based on the stage of CKD, we performed subgroup analyses (Table 3). The results showed that across all stage of CKD, including stage 4–5, the T group achieved better diuretic and edema-reducing effects compared to the C group.

We subsequently analyzed the number of patients in each group who required catheter-directed ultrafiltration treatment due to poor diuretic effects post-medication, as shown in Supplementary Figure S1 We found that there were more patients in the C group who required ultrafiltration treatment due to poor diuretic effects ($P = .040$).

Safety analysis of medication

Patients in the T group exhibited higher post-medication serum sodium levels compared to those in the C group ($P = .023$). Furthermore, to minimize the influence of hyperglycemia on serum sodium levels, we corrected the patients' serum sodium

Table 1: Comparison of baseline characteristics between the C and T groups after PSM.

Variables	C group ($n = 87$)	T group ($n = 87$)	P value
Demographic parameters			
Gender, *n* (%)			.755
Male	53 (60.92)	55 (63.22)	
Female	34 (39.08)	32 (36.78)	
Age, years	67.00 (59.00, 73.00)	69.00 (54.00, 77.00)	.465
BMI, kg/m^2	25.94 ± 4.02	26.15 ± 4.37	.738
CKD stage, *n* (%)			.465
CKD Stage 1	13 (14.94)	11 (12.64)	
CKD Stage 2	15 (17.24)	18 (20.69)	
CKD Stage 3	18 (20.69)	10 (11.49)	
CKD Stage 4	17 (19.54)	19 (21.84)	
CKD Stage 5	24 (27.59)	29 (33.33)	
Concomitant medication, *n* (%)			
Standardized loop diuretic dose, mg			.786
1	46 (52.87)	48 (55.17)	
2	36 (41.38)	34 (39.08)	
4	5 (5.75)	4 (4.60)	
8	0 (0.00)	0 (0.00)	
10	0 (0.00)	1 (1.15)	
SGLT2i	14 (16.09)	11 (12.64)	.517
SV	15 (17.24)	11 (12.64)	.395
NYHA classification			.595
I	11 (12.64)	15 (17.24)	
II	46 (52.87)	34 (39.08)	
III	27 (31.03)	36 (41.38)	
IV	3 (3.45)	2 (2.30)	
Volume overload characteristics			
Dyspnea	19 (21.84)	17 (19.54)	.708
Orthopnea	2 (2.30)	3 (3.45)	1.000
Pulmonary rales	5 (5.75)	4 (4.60)	1.000
Edema grading			.990
Mild	25 (28.74)	27 (31.03)	
Moderate	41 (47.13)	37 (42.53)	
Severe	21 (24.14)	23 (26.44)	
Weight, kg	71.60 ± 14.34	71.36 ± 13.89	.910
Urine output, ml/day	1500.00 (1000.00, 1800.00)	1500.00 (920.00, 1800.00)	.511
Laboratory parameters			
Serum sodium, mmol/l	140.40 (138.30, 143.40)	141.50 (138.30, 143.30)	.539
Serum potassium, mmol/l	3.95 (3.54, 4.49)	3.95 (3.57, 4.47)	.920
Serum creatinine, μmol/l	161.00 (92.00, 405.00)	211.00 (100.00, 424.00)	.645
eGFR,ml·min^{-1}·(1.73 m^2)$^{-1}$	40.98 (13.25, 74.73)	25.23 (12.20, 70.53)	.646
Plasma albumin, g/l	29.10 ± 7.12	28.71 ± 7.71	.730
ALT, U/L	13.00 (10.00, 19.00)	13.00 (9.00, 21.00)	.854
AST, U/L	16.00 (13.00, 23.00)	18.00 (15.00, 26.00)	.095

Note: SV: sacubitril/valsartan, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

levels based on their blood glucose levels (glucose-corrected serum sodium, sNa) [30]. The results indicated that there was no statistically significant difference in baseline sNa levels between the T group and the C group ($P = .574$). However, after medication, the T group showed higher sNa levels compared to the C group ($P = .029$). There was no statistically significant difference between the two groups in terms of the increase of sNa levels after medication ($P = .200$) (Supplementary Table S3). Moreover, we selected patients with baseline sNa levels indicative of hyponatremia (serum sodium <135 mmol/l) before treatment: nine patients in the C group and four patients in the T group. The results showed no statistically significant differences between the two groups in terms of baseline sNa levels, post-medication sNa levels, and the increase of sNa levels after medication ($P = .165$, $P = .821$, $P = .413$) (Supplementary Table S4). However, no statistically significant differences were observed in serum potassium ($P = .483$), serum creatinine ($P = .709$), and eGFR ($P = .621$) between the two groups (Table 4). Regarding adverse events, there were no statistically significant differences between the two groups in terms of hypernatremia ($P = .231$), hyperkalemia ($P > .050$), hypokalemia ($P = .095$), and occurrence of WRF ($P = .708$) (Supplementary Figure S2).

The functions of aquaporin-2 (AQP2) in the collecting duct and plasma albumin in forecasting the effectiveness of tolvaptan

We conducted a study on the expression of AQP2 in renal biopsies from five patients in the T group. By comparing the

Table 2: Comparison of volume overload characteristics in patients of C and T groups post-medication.

Variables	C group ($n = 87$)	T group ($n = 87$)	P value
Dyspnea	1 (1.15)	1 (1.15)	1.000
Orthopnea	0 (0.00)	0 (0.00)	
Pulmonary rales	1 (1.15)	0 (0.00)	1.000
Edema grading			.787
None	63 (72.41)	60 (68.97)	
Mild	18 (20.69)	25 (28.74)	
Moderate	4 (4.60)	2 (2.30)	
Severe	2 (2.30)	0 (0.00)	
Average weight after 3 days of medication, kg	70.84 ± 14.33	69.70 ± 13.74	.593
Average weight after 7 days of medication, kg	71.76 (63.14, 81.11)	67.24 (60.55, 79.44)	.242
Weight loss after 3 days of medication, kg	0.90 (0.20, 2.00)	2.30 (1.00, 3.60)	<.001
Weight loss after 7 days of medication, kg	1.87 ± 2.75	3.69 ± 3.11	.002
Average urine output after 3 days of medication, ml/day	1800.00 (1500.00,2350.00)	2166.67 (1733.33, 3100.00)	<.001
Average urine output after 7 days of medication, ml/day	1750.00 (1457.14, 2171.43)	2214.29 (1757.14, 2678.57)	.002
Increase in average urine output after 3 days of medication, ml/day	383.33 (100.00 866.67)	933.33 (366.67, 1666.67)	<.001
Increase in average urine output after 7 days of medication, ml/day	214.29 (71.43, 714.29)	921.43 (492.86, 1525.00)	<.001

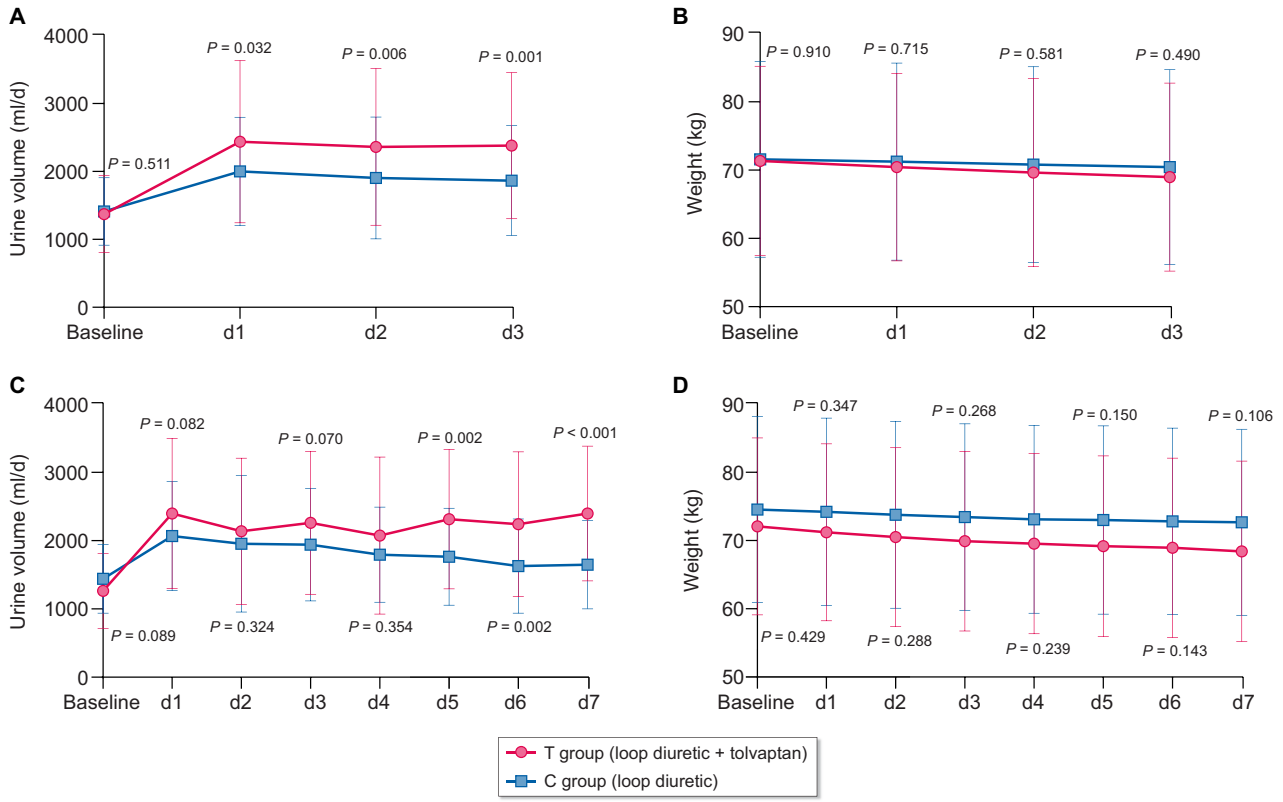


Figure 2: Weight and urine output in patients of C and T groups during medication. (a) Comparison of urine output changes in the two groups after 3 days of medication. (b) Comparison of weight changes in the two groups after 3 days of medication. (c) Comparison of urine output changes in the two groups after 7 days of medication. (d) Comparison of urine output changes in the two groups after 7 days of medication.

expression levels of AQP2 in these patients and the changes in urine volume and weight during medication, we found that the expression of AQP2 was localized to the apical membrane of collecting duct epithelial cells. Furthermore, we observed that higher expression of AQP2 was associated with increased urine volume in patients (Fig. 3).

Additionally, we analyzed the correlation between baseline serum albumin levels and 24-hour urine volume during medication in both groups of patients. As shown in Fig. 4, there was no correlation between baseline serum albumin levels and 24-hour urine volume in either group ($r = -.122$, $P = .262$ vs $r = -.039$, $P = .717$).

Table 3: Changes in body weight and urine volume during medication period in Groups C and T of patients in each stage of CKD.

Variables	CKD stage 1–2 C group ($n = 28$)	CKD stage 1–2 T group ($n = 29$)	P value
Average weight after 3 days of medication, kg	72.83 ± 12.92	68.56 ± 11.76	.197
Average weight after 7 days of medication, kg	71.26 ± 12.93	69.06 ± 11.55	.645
Weight loss after 3 days of medication, kg	0.90 (0.33, 2.75)	2.90 (1.50, 4.60)	.002
Weight loss after 7 days of medication, kg	2.59 ± 3.76	4.26 ± 3.87	.269
Average urine output after 3 days of medication, ml/day	2058.33 ± 723.28	2556.32 ± 996.31	.036
Average urine output after 7 days of medication, ml/day	2025.00 (1489.28, 2199.29)	2214.29 (1728.57, 2864.29)	.242
Increase in average urine output after 3 days of medication, ml/day	433.33 ± 705.55	1133.91 ± 915.57	.002
Increase in average urine output after 7 days of medication, ml/day	243.57 ± 675.14	1124.29 ± 1098.58	.023
Variables	**CKD stage 3 C Group ($n = 18$)**	**CKD stage 3 T Group ($n = 10$)**	**P value**
Average weight after 3 days of medication, kg	69.36 ± 15.61	69.12 ± 18.53	.971
Average weight after 7 days of medication, kg	75.74 ± 14.93	67.62 ± 12.35	.275
Weight loss after 3 days of medication, kg	1.00 (0.30, 2.40)	2.55 (1.03, 5.23)	.061
Weight loss after 7 days of medication, kg	1.82 ± 2.44	3.98 ± 2.67	.111
Average urine output after 3 days of medication, ml/day	2000.00 (1666.67, 2391.67)	3416.67 (1808.33, 4100.00)	.037
Average urine output after 7 days of medication, ml/day	1923.06 ± 603.29	2588.10 ± 1020.53	.109
Increase in average urine output after 3 days of medication, ml/day	391.67 (54.17, 870.84)	1583.33 (816.67, 2650.00)	.002
Increase in average urine output after 7 days of medication, ml/day	423.06 ± 468.35	1288.10 ± 764.23	.011
Variables	**CKD stage 4–5 C Group ($n = 41$)**	**CKD stage 4–5 T Group ($n = 48$)**	**P value**
Average weight after 3 days of medication, kg	70.13 ± 14.86	70.51 ± 13.99	.902
Average weight after 7 days of medication, kg	73.39 (60.73, 81.67)	71.45 (58.71, 79.54)	.534
Weight loss after 3 days of medication, kg	0.90 ± 1.34	1.92 ± 1.76	.003
Weight loss after 7 days of medication, kg	1.57 ± 2.39	3.37 ± 2.83	.011
Average urine output after 3 days of medication, ml/day	1762.60 ± 642.51	2138.61 ± 839.05	.021
Average urine output after 7 days of medication, ml/day	1729.34 ± 559.07	2130.40 ± 677.77	.016
Increase in average urine output after 3 days of medication, ml/day	433.33 (208.34, 900.00)	766.67 (179.17, 1233.33)	.088
Increase in average urine output after 7 days of medication, ml/day	432.91 ± 564.42	882.90 ± 649.76	.006

Note: CKD stage 1–2: Group C had 28 patients observed for 3 days, with 12 patients observed for 7 days. Group T had 29 patients observed for 3 days, with 15 patients observed for 7 days. CKD stage 3: Group C had 18 patients observed for 3 days, with 11 patients observed for 7 days. Group T had 10 patients observed for 3 days, with six patients observed for 7 days. CKD stage 4–5: Group C had 41 patients observed for 3 days, with 28 patients observed for 7 days. Group T had 48 patients observed for 3 days, with 32 patients observed for 7 days.

Table 4: Comparison of laboratory parameters in patients of C and T groups post-medication.

Laboratory parameters	C group ($n = 87$)	T group ($n = 87$)	P value
Serum sodium, mmol/l	141.50 (139.60 144.10)	143.00 (140.70, 145.30)	.023
Serum potassium, mmol/l	3.89 ± 0.65	3.95 ± 0.49	.483
Serum creatinine, μmol/l	160.00 (91.00 337.00)	178.00 (105.00, 382.00)	.709
eGFR, ml·min^{-1}·(1.73 m^2)$^{-1}$	33.55 (13.56,73.80)	27.69 (13.51, 65.93)	.621
Plasma albumin, g/l	29.67 ± 6.52	28.71 ± 7.10	.352
ALT, U/l	12.00 (10.00, 18.00)	14.00 (10.00, 19.00)	.609
AST, U/l	17.00 (12.00, 24.00)	18.00 (14.00, 21.00)	.528

Note: ALT: alanine aminotransferase, AST: aspartate aminotransferase

DISCUSSION

This study aimed to investigate the effectiveness, safety, and potential clinical indicators for predicting the responsiveness to tolvaptan in volume management of CKD patients when used in combination with loop diuretics. The main findings of this study are as follows: (i) the combination therapy of tolvaptan and loop diuretics demonstrated superior diuretic effects compared to the loop diuretic group, and more effectively reduced the need for ultrafiltration treatment due to diuretic resistance or inadequate diuretic effects leading to excessive volume load. (ii) Tolvaptan administration led to an increase in serum sodium levels, but there was no statistically significant difference in the incidence of hypernatremia compared to the loop diuretic group.

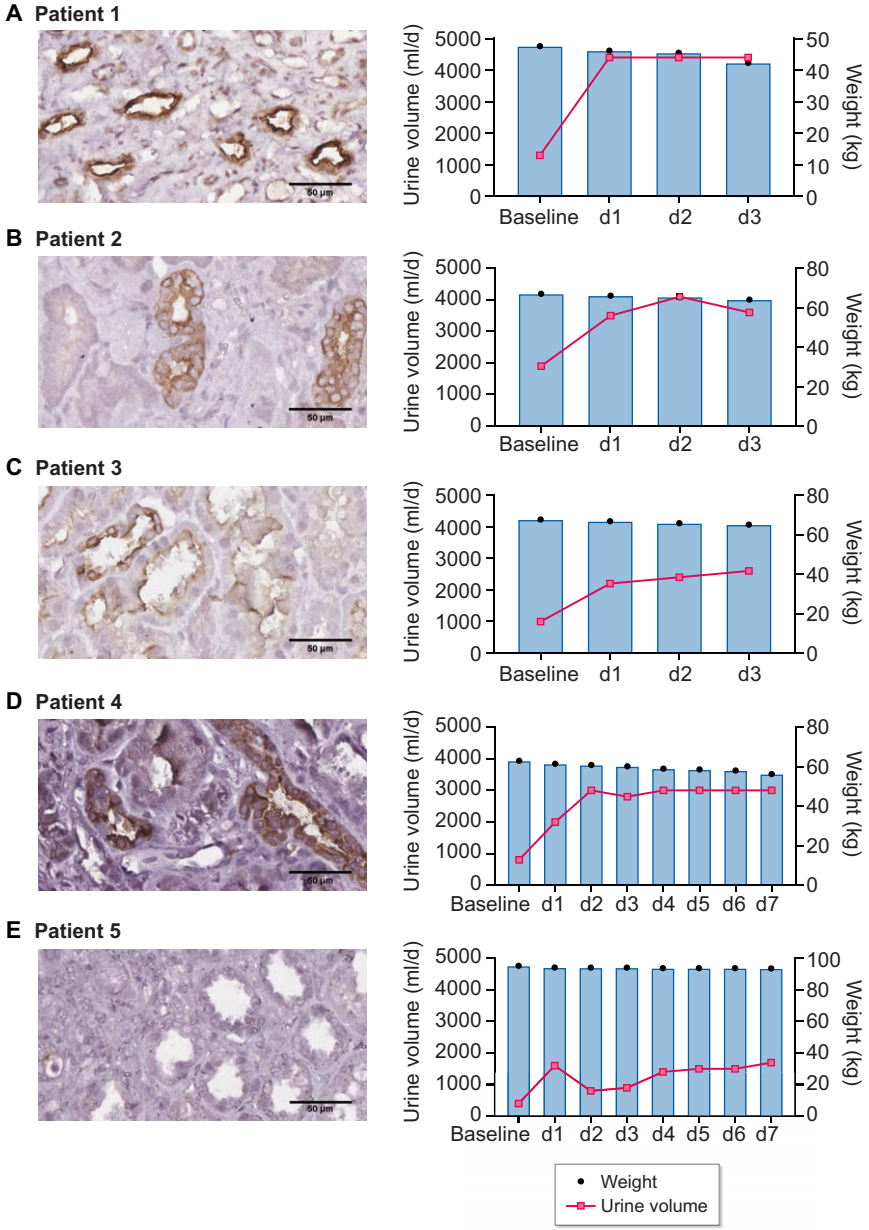


Figure 3: Immunohistochemical staining of AQP-2 in renal biopsy patients and changes in urine volume and weight during medication.

Additionally, the incidence rates of hyperkalemia, hypokalemia, and WRF were similar between the two groups. (iii) AQP2 may be a potential predictor of responsiveness to tolvaptan, while serum albumin levels may not affect the therapeutic efficacy of tolvaptan.

Volume overload is a common feature of CKD, clinically manifested as hypertension, peripheral edema, pulmonary congestion, and heart failure [2, 31]. According to reports, in 2017, there were 1.1 million hospital admissions in the USA due to volume overload, resulting in staggering hospitalization costs of $13.6 billion. This indicates that volume overload imposes a significant economic burden [31]. The kidneys are vital organs for maintaining water and salt balance in the body. As CKD progresses, there is a reduction in sodium filtration and inhibition of tubular reabsorption, ultimately leading to volume overload. Activation of the renin-angiotensin-aldosterone system in the body restricts sodium excretion, exacerbating this issue. Therefore, CKD patients typically mitigate fluid and sodium retention by reducing sodium intake and increasing sodium excretion [31, 32].

Diuretics serve as the cornerstone for managing volume overload. They are classified based on their sites and mechanisms of action into loop diuretics, thiazide diuretics, and potassium-sparing diuretics, with loop diuretics being the most commonly used [33, 34]. However, loop diuretics can lead to electrolyte imbalances and metabolic disruptions, such as hyponatremia, hypokalemia, metabolic alkalosis, and hyperuricemia [9, 34, 35]. In CKD patients, reduced bioavailability of diuretics may lead to diuretic resistance, necessitating higher doses of loop diuretics to achieve diuresis. However, high doses of loop diuretics have been associated with increased mortality rates in late-stage CKD patients with concomitant heart failure [9]. Therefore,

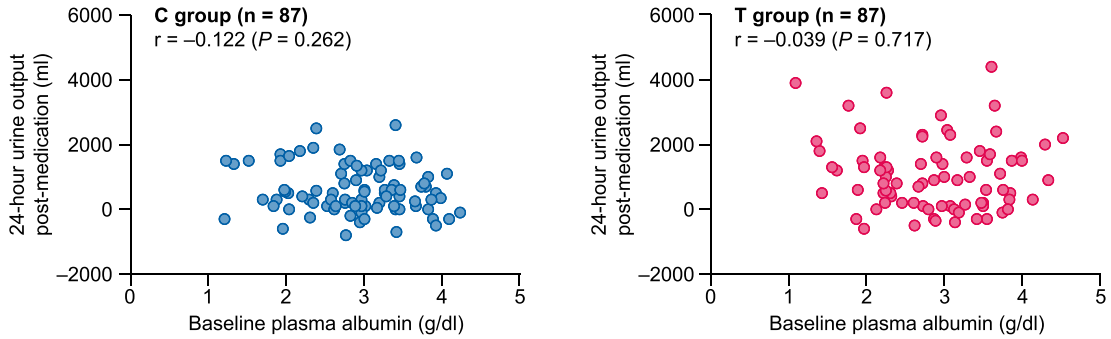


Figure 4: Correlation between baseline plasma albumin levels and 24-hour urine volume during medication in C and T groups.

there is a need to explore novel medications that can improve volume overload.

Tolvaptan is a novel oral non-peptide antagonist that selectively blocks the binding of arginine vasopressin to the V2 receptors in the collecting ducts. It induces the excretion of free water without affecting electrolyte excretion [36]. In a multicenter randomized controlled trial, the tolvaptan group showed significantly higher urine output and greater relief from dyspnea within 48 hours compared to the conventional diuretic group [37], This study demonstrated that the tolvaptan group exhibited higher urine output at days 2–3 and 5–7 compared to the loop diuretic group, suggesting that tolvaptan was more effective in reducing the occurrence of diuretic resistance and achieving stable diuretic effects. However, there was no statistically significant difference in the relief of dyspnea between the two groups, which may have been attributed to the relatively low proportion of patients experiencing dyspnea symptoms before medication in both groups. Additionally, most patients in both groups had NYHA class 1–2, indicating that dyspnea was improved in both groups after reducing volume overload through diuresis. In the large-sample international multicenter EVEREST study, heart failure patients who received tolvaptan in addition to standard therapy experienced twice the magnitude of weight reduction on the first day of treatment compared to the group without tolvaptan. The peak weight reduction was achieved by the seventh day of treatment, and this advantage was maintained throughout an average follow-up period of nearly 10 months after discharge [38], Similarly, in this study, the tolvaptan group exhibited greater reductions in body weight at 3 and 7 days of treatment compared to the loop diuretic group. Furthermore, we conducted subgroup analysis on patients concurrently using SGLT-2i. Similarly, the tolvaptan group demonstrated greater diuretic and edema-reducing effects compared to the loop diuretic group in this subgroup. However, the sample size in this subgroup was limited, and further validation of the diuretic effects of tolvaptan in combination with SGLT-2i is needed in larger populations. Additionally, we performed subgroup analysis based on CKD staging, which similarly showed that across all stages of CKD, tolvaptan exhibited superior diuretic and edema-reducing effects compared to loop diuretics. Tolvaptan is commonly used to treat patients with hypovolemic or euvolemic hyponatremia due to its ability to increase serum sodium levels [9], therefore, the serum sodium levels after tolvaptan administration were closely monitored in this study. The tolvaptan group exhibited higher serum sodium levels compared to the loop diuretic group, but there was no statistically significant difference in the occurrence of hypernatremia between the two groups, The EVEREST study also suggested a trend of increased serum sodium levels after tolvaptan administration [38]. Hence, it is important to monitor patients' serum sodium levels and adjust the dosage of tolvaptan accordingly. Several studies have reported that tolvaptan, compared to loop diuretics, can reduce the incidence of WRF in patients [7, 39]. However, in the EVEREST study, tolvaptan did not improve the elevation of serum creatinine [38]. In this study, there was also no statistically significant difference in the incidence of WRF between the two groups. This disparity may be attributed to variations in patient inclusion criteria, timing of tolvaptan initiation, dosage of tolvaptan, and underlying conditions of the patients.

The diuretic and edema-reducing effects of tolvaptan vary considerably among different patients. The EVEREST trial has demonstrated that tolvaptan can significantly improve patients' volume overload [38], However, some small-scale studies have indicated that tolvaptan may not provide adequate diuretic effects for certain patients [19, 25, 40]. Definitions of responders to tolvaptan and predictors of tolvaptan responsiveness vary among studies. Further exploration is needed to identify clinical biomarkers of tolvaptan responsiveness, facilitating clinicians in the rational and scientific use of tolvaptan. A case report indicated significantly higher renal AQP2 expression in responders to tolvaptan among patients with membranous nephropathy [17], Similarly, in another study involving 26 patients with diabetic nephropathy complicated by heart failure, non-responders to tolvaptan exhibited no expression of renal AQP-2, whereas responders to tolvaptan showed positive expression of renal AQP2 [19]. In this study, renal tissue AQP2 staining was conducted on five patients who underwent kidney biopsies. We observed that the expression of AQP2 localized to the apical membrane of the collecting duct epithelial cells, and there was a positive correlation between the expression of AQP2 and the urine output of the patients. This suggests that the expression status of renal tissue AQP2 in renal tubular epithelial cells may aid in identifying patients who respond to tolvaptan. Additionally, serum albumin levels have been shown to be associated with responsiveness to tolvaptan, with responders to tolvaptan exhibiting significantly lower serum albumin levels compared to non-responders [18]. Similar to this study, there are also literature reports indicating that serum albumin levels do not affect the efficacy of tolvaptan [41], this may be related to the mechanism of action of tolvaptan, as it exerts its diuretic effect without binding to serum albumin [42]. In conclusion, further prospective large-scale studies are needed to delve deeper into clinical biomarkers of tolvaptan responsiveness.

This study has several limitations: (i) it is retrospective in nature, with some missing patient data, and despite undergoing PSM, the sample size remains small, necessitating larger-scale

studies to validate our findings. (ii) Owing to the retrospective design, there was no uniformity in the timing of patients initiating tolvaptan and/or loop diuretics, nor in the type of loop diuretics used. (iii) The limited number of samples undergoing renal tissue AQP2 staining may introduce bias into the results. (iv) As loop diuretics still stand as the first-line treatment recommended by guidelines for volume management in patients with kidney disease, and the use of tolvaptan for volume management in kidney disease patients currently falls under off-label use, the efficacy of tolvaptan used alone cannot be definitively ascertained.

In conclusion, this study demonstrates that tolvaptan significantly and effectively improves volume overload in patients with kidney disease. Adverse reactions such as hyperkalemia and hepatic or renal impairment were not observed in the study, indicating good drug safety. Additionally, renal tissue AQP2 can be used to predict the efficacy of tolvaptan. Overall, tolvaptan shows promising clinical application prospects for volume management in patients with kidney disease.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

REFERENCES

1. Lopez T, Banerjee D. Management of fluid overload in hemodialysis patients. *Kidney Int* 2021;**100**:1170–3. https://doi.org/10.1016/j.kint.2021.09.013
2. Masuda T, Murakami T, Igarashi Y *et al*. Dual impact of Tolvaptan on intracellular and extracellular water in chronic kidney disease patients with fluid retention. *Intern Med* 2016;**55**:2759–64. https://doi.org/10.2169/internalmedicine.55.7133
3. Patil VP, Salunke BG. Fluid overload and acute kidney injury. *Indian J Crit Care Med* 2020;**24**:S94–s97.
4. Ness BM, Brown SE. Fluid overload. *Crit Care Nurs Clin North Am* 2022;**34**:409–20. https://doi.org/10.1016/j.cnc.2022.07.001
5. Hoorn EJ, Resistance EDHD. *Am J Kidney Dis* 2017;**69**:136–42. https://doi.org/10.1053/j.ajkd.2016.08.027
6. Felker GM, Lee KL, Bull DA *et al*. Diuretic strategies in patients with acute decompensated heart failure. *N Engl J Med* 2011;**364**:797–805. https://doi.org/10.1056/NEJMoa1005419
7. Nakano Y, Mizuno T, Niwa T *et al*. Impact of continuous administration of Tolvaptan on preventing medium-term worsening renal function and long-term adverse events in heart failure patients with chronic kidney disease. *Int Heart J* 2018;**59**:105–11. https://doi.org/10.1536/ihj.16-625
8. Konstam MA, Gheorghiade M, Burnett JC, Jr *et al*. Effects of oral tolvaptan in patients hospitalized for worsening heart failure: the EVEREST Outcome Trial. *JAMA* 2007;**297**:1319–31. https://doi.org/10.1001/jama.297.12.1319
9. Tagaya T, Hayashi H, Ogata S *et al*. Tolvaptan's Association with low risk of acute kidney injury in patients with advanced chronic kidney disease and acute decompensated heart failure. *Am J Nephrol* 2023;**54**:319–28. https://doi.org/10.1159/000531692
10. Schrier RW, Gross P, Gheorghiade M *et al*. Tolvaptan, a selective oral vasopressin V2-receptor antagonist, for hyponatremia. *N Engl J Med* 2006;**355**:2099–112. https://doi.org/10.1056/NEJMoa065181
11. Pan Y, Li H, Gao J *et al*. Tolvaptan for water retention in heart failure: a systematic review. *Syst Rev* 2023;**12**:130. https://doi.org/10.1186/s13643-023-02293-3
12. Tanaka A, Katsuno T, Ozaki T *et al*. The efficacy of tolvaptan as a diuretic for chronic kidney disease patients. *Acta Cardiol* 2015;**70**:217–23. https://doi.org/10.1080/AC.70.2.3073514
13. Zhou L, Xu Q, Xi X *et al*. Tolvaptan therapy for edema in patients with chronic kidney disease. *Clin Nephrol* 2024;**101**:308–16. https://doi.org/10.5414/CN111365
14. Suzuki S, Hanafusa N, Kubota K *et al*. Effects of tolvaptan on renal function in chronic kidney disease patients with volume overload. *Int J Nephrol Renovasc Dis* 2018;**11**:235–40. https://doi.org/10.2147/IJNRD.S167694
15. House AA, Wanner C, Sarnak MJ *et al*. Heart failure in chronic kidney disease: conclusions from a kidney disease: improving global outcomes (KDIGO) controversies conference. *Kidney Int* 2019;**95**:1304–17. https://doi.org/10.1016/j.kint.2019.02.022
16. Banerjee D, Rosano G, Herzog CA. Management of heart failure patient with CKD. *Clin J Am Soc Nephrol* 2021;**16**:1131–9. https://doi.org/10.2215/CJN.14180920
17. Tanaka A, Nakamura T, Sato E *et al*. Different effects of Tolvaptan in patients with idiopathic membranous nephropathy with nephrotic syndrome. *Intern Med* 2017;**56**:191–6. https://doi.org/10.2169/internalmedicine.56.7539
18. Masuda T, Ohara K, Nagayama I *et al*. Impact of serum albumin levels on the body fluid response to tolvaptan in chronic kidney disease patients. *Int Urol Nephrol* 2019;**51**:1623–9. https://doi.org/10.1007/s11255-019-02180-8

19. Sato E, Nakamura T, Amaha M *et al*. Effect of tolvaptan in patients with chronic kidney disease due to diabetic nephropathy with heart failure. *Int Heart J* 2014;**55**:533–8. https://doi.org/10.1536/ihj.14-190
20. Komiyama Y, Kurosaki M, Nakanishi H *et al*. Prediction of diuretic response to tolvaptan by a simple, readily available spot urine Na/K ratio. *PLoS ONE* 2017;**12**:e0174649. https://doi.org/10.1371/journal.pone.0174649
21. Imamura T, Kinugawa K, Minatsuki S *et al*. Urine osmolality estimated using urine urea nitrogen, sodium and creatinine can effectively predict response to tolvaptan in decompensated heart failure patients. *Circ J* 2013;**77**:1208–13. https://doi.org/10.1253/circj.CJ-12-1328
22. Imamura T, Kinugawa K, Fujino T *et al*. Increased urine aquaporin-2 relative to plasma arginine vasopressin is a novel marker of response to tolvaptan in patients with decompensated heart failure. *Circ J* 2014;**78**:2240–9. https://doi.org/10.1253/circj.CJ-14-0244
23. Makabe S, Manabe S, Kataoka H *et al*. Urinary aquaporin 2 as a potential indicator predicting tolvaptan response in patients with ADPKD. *Kidney Int Rep* 2021;**6**:2436–44. https://doi.org/10.1016/j.ekir.2021.06.033
24. Uojima H, Kinbara T, Hidaka H *et al*. Close correlation between urinary sodium excretion and response to tolvaptan in liver cirrhosis patients with ascites. *Hepatol Res* 2017;**47**:E14–e21. https://doi.org/10.1111/hepr.12716
25. Shimizu K, Doi K, Imamura T *et al*. Ratio of urine and blood urea nitrogen concentration predicts the response of tolvaptan in congestive heart failure. *Nephrology* 2015;**20**:405–12. https://doi.org/10.1111/nep.12406
26. Inker LA, Eneanya ND, Coresh J *et al*. New creatinine- and cystatin C-based equations to estimate GFR without race. *N Engl J Med* 2021;**385**:1737–49. https://doi.org/10.1056/NEJMoa2102953
27. Committee of Exports on Rational Drug Use National Health and Family Planning Commission of The People'Republic of China; Chinese Pharmacists Association. Guidelines for pharmacotherapy rationalization in heart failure (2nd Edition). *Chinese J Med Front (Electronic Version)* 2019;**11**:1–78.
28. Jia R, Zhang X, Xu Y *et al*. Effect of Sacubitril/Valsartan on renal function in patients with chronic kidney disease and heart failure with preserved ejection fraction: a real-world 12-week study. *Eur J Pharmacol* 2022;**928**:175053. https://doi.org/10.1016/j.ejphar.2022.175053
29. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivariate Behav Res* 2011;**46**:399–424. https://doi.org/10.1080/00273171.2011.568786
30. Hillier TA, Abbott RD, Barrett EJ. Hyponatremia: evaluating the correction factor for hyperglycemia. *Am J Med* 1999;**106**:399–403. https://doi.org/10.1016/S0002-9343(99)00055-8
31. Novak JE, Ellison DH. Diuretics in states of volume overload: core curriculum 2022. *Am J Kidney Dis* 2022;**80**:264–76. https://doi.org/10.1053/j.ajkd.2021.09.029
32. Borrelli S, Provenzano M, Gagliardi I *et al*. Sodium intake and chronic kidney disease. *Int J Mol Sci* 2020;**21**:4744. https://doi.org/10.3390/ijms21134744
33. Verbrugge FH, Mullens W, Tang WH. Management of Cardio-renal syndrome and diuretic resistance. *Curr Treat Options Cardio Med* 2016;**18**:11. https://doi.org/10.1007/s11936-015-0436-4
34. Khan YH, Sarriff A, Adnan AS *et al*. Chronic kidney disease, fluid overload and diuretics: a complicated triangle. *PLoS ONE* 2016;**11**:e0159335. https://doi.org/10.1371/journal.pone.0159335
35. Ono Y, Takamatsu H, Inoue M *et al*. Clinical effect of long-term administration of tolvaptan in patients with heart failure and chronic kidney disease. *Drug Discov Ther* 2018;**12**:154–60. https://doi.org/10.5582/ddt.2018.01007
36. Hirano T, Yamamura Y, Nakamura S *et al*. Effects of the V(2)-receptor antagonist OPC-41061 and the loop diuretic furosemide alone and in combination in rats. *J Pharmacol Exp Ther* 2000;**292**:288–94.
37. Matsue Y, Suzuki M, Torii S *et al*. Clinical effectiveness of Tolvaptan in patients with acute heart failure and renal dysfunction. *J Card Fail* 2016;**22**:423–32. https://doi.org/10.1016/j.cardfail.2016.02.007
38. Gheorghiade M, Gattis WA, O'Connor CM *et al*. Effects of tolvaptan, a vasopressin antagonist, in patients hospitalized with worsening heart failure: a randomized controlled trial. *JAMA* 2004;**291**:1963–71. https://doi.org/10.1001/jama.291.16.1963
39. Tamaki S, Sato Y, Yamada T *et al*. Tolvaptan reduces the risk of worsening renal function in patients with acute decompensated heart failure and preserved left ventricular ejection fraction—prospective randomized controlled study. *Circ J* 2017;**81**:740–7. https://doi.org/10.1253/circj.CJ-16-1122
40. Kakeshita K, Koike T, Imamura T *et al*. Expression of aquaporin-2 in the collecting duct and responses to tolvaptan. *CEN Case Rep* 2021;**10**:69–73. https://doi.org/10.1007/s13730-020-00518-1
41. Sakaida I, Nakajima K, Okita K *et al*. Can serum albumin level affect the pharmacological action of tolvaptan in patients with liver cirrhosis? A post hoc analysis of previous clinical trials in Japan. *J Gastroenterol* 2015;**50**:1047–53. https://doi.org/10.1007/s00535-015-1052-5
42. Mori T, Ohsaki Y, Oba-Yabana I *et al*. Diuretic usage for protection against end-organ damage in liver cirrhosis and heart failure. *Hepatology Research* 2017;**47**:11–22. https://doi.org/10.1111/hepr.12700

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