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Abstract

Background
The V2 receptor antagonist tolvaptan is prescribed to patients with autosomal dominant polycystic kidney disease (ADPKD) to slow disease progression. Tolvaptan may alter blood pressure (BP) via various acute and chronic effects. In the present study we investigated the magnitude and time course of these effects.

Methods
This is a post-hoc study of the TEMPO 3:4 trial, which included 1445 ADPKD patients randomized 2:1 to tolvaptan or placebo for a period of 3 years. Systolic and diastolic BP, mean arterial pressure, hypertension status and use and dosing of antihypertensive drugs were evaluated over the course of the trial period.

Results
At baseline, BP did not differ between study arms. After 3 weeks of tolvaptan use, body weight had decreased from 79.7±18.3 to 78.8±18.2 kg and plasma sodium increased from 140.4±2.1 to 142.6±2.6 mmol/L (both p<0.001), suggesting a decrease in circulating volume. In the placebo arm none of these changes was observed. Notwithstanding, blood pressure remained similar in the study arms. After three years of treatment, however, systolic and diastolic BP were significantly lower in subjects receiving tolvaptan versus placebo (126±13 versus 129±14 mmHg, p=0.002 and 81.2±9.5 versus 82.6±10 mmHg, p=0.01). These differences leveled off at follow-up three weeks after discontinuation of study medication. Use of antihypertensive drugs remained similar in both study arms during the entire study.

Conclusions
Long-term treatment with tolvaptan gradually lowered BP compared to placebo, which can be attributed to a beneficial effect on disease progression or a continued natriuretic effect.

Introduction
Vasopressin or antidiuretic hormone are names that refer to the pleiotropic effects of this peptide. Although the former name is most often used, the latter may be more appropriate because decreasing urinary output is the principal function of vasopressin. Effects of vasopressin are facilitated by three types of receptors, the V1a, V1b and V2 receptors, present on various cell types. The V2 receptor, responsible for the antidiuretic effect, is activated by a minimal change in vasopressin signal. In contrast, activation of the V1a receptor, responsible for vasoconstriction, requires considerably higher vasopressin levels.

Not all of the effects of vasopressin are beneficial. It has been recognized that activation of the V2 receptor, whilst indispensable for water homeostasis, can also be damaging in a variety of kidney disorders, including autosomal dominant polycystic kidney disease (ADPKD). The V2 receptor regulates water permeability of the tubular cell membrane via an intracellular second messenger, cyclic adenosine monophosphate (cAMP). In patients with ADPKD growth of kidney cysts is stimulated by an increase in intracellular cAMP, leading to loss of kidney function and ultimately resulting in end stage kidney disease. ADPKD patients are therefore treated with tolvaptan, a selective V2 receptor antagonist, which has been shown to slow the rate of disease progression in patients at risk of rapid kidney function decline. By preventing binding of vasopressin to the V2 receptor, tolvaptan induces nephrogenic diabetes insipidus, which causes a compensatory rise in plasma vasopressin. It is important to note that tolvaptan has a high selectivity for the V2 receptor and does not block the effect of vasopressin on the V1a receptor. Consequently, given the higher levels of vasopressin induced by treatment, the V1a receptor is activated more than usual. This could have undesired off-target effects, of which a change in blood pressure (BP) could be one.

Tolvaptan could theoretically have several effects on BP. On the short term, via increased V1a receptor activation, one could expect an increase in vascular resistance and thus in blood pressure. However as mentioned above, V1a receptor activation has a variety of effects. It also increases sodium excretion, thereby potentially decreasing BP. In addition, preventing vasopressin binding to the V2 receptor could result in a reduction of blood pressure because of loss of V2 receptor mediated ENaC channel activation and therefore sodium reabsorption, loss of RAS activation due to a decrease of V2 receptor dependent renin production and loss of circulating volume due to aquaresis. Finally, on the long term, tolvaptan ameliorates the rate of disease progression, possibly also reducing the development of secondary symptoms of kidney disease, such as hypertension.

The original publication of the TEMPO 3:4 trial disclosed only limited information on the effect of tolvaptan on blood pressure. Change in blood pressure was expressed as worsening hypertension, defined as a change in blood-pressure category, or as worsening of hypertension requiring an increase in hypertensive treatment. No effect was found. However, the use of a categorical variable as outcome measure may have
resulted in a loss of power to find differences between the two study groups. Therefore, the present study investigates the magnitude and time course of the effect of tolvaptan on BP expressed as continuous variable, not only taking into account measured values, but also use of BP lowering medication.

Materials and Methods

Study population and design
This study is a post-hoc analysis of the TEMPO 3:4 trial, a prospective, multicenter, double blinded randomized controlled trial to assess the efficacy of tolvaptan in patients with early stage ADPKD (ClinicalTrials.gov identifier NCT00428948). A detailed study protocol of this randomized controlled trial has been published previously.\(^\text{17,19}\) In summary, 1445 patients were enrolled for 2:1 randomization to either treatment with tolvaptan or placebo between 2007 and 2009. Inclusion criteria were age between 18 and 50 years, total kidney volume (TKV) measured by magnetic resonance imaging larger than 750 mL and Cockcroft-Gault calculated creatinine clearance of 60 mL/min or greater. Exclusion criteria included, but were not limited to, concomitant diseases that were likely to confound study endpoints such as poorly controlled diabetes mellitus. Participants allocated to the treatment arm were started on a dosage regimen of 45 mg in the morning and 15 mg in the late afternoon, which was increased to 60/30 mg after one week and thereafter to 90/30 mg in the third week if tolerated. Participants remained on the highest tolerated dose for 36 months. Primary outcome was the annual rate of change in TKV, and secondary outcomes included annual rate of change in eGFR. Tolvaptan efficacy was studied on top of standard clinical care, which included optimal BP control. Study recommendations defined in 2007, at the start of the trial, considered optimal control to start antihypertensive treatment at a systolic BP of 130 mmHg or a diastolic BP of 85 mmHg. Adjustments in antihypertensive therapy could be made during the entire study period, in case BP exceeded the limits on two consecutive visits. RAAS inhibitors were recommended as first-line antihypertensive drug. The choice for second-line therapy was left to the discretion of the treating physician. Long term use of diuretics was discouraged for safety reasons.

This study was approved by all local ethics committees of the participating sites and was conducted according to the International Conference of Harmonization Good Clinical Practice Guidelines. Written informed consent was obtained from all participants.

Data collection
Data were collected at baseline, during treatment at week 3, month 3 and thereafter after every 4 months for 36 months. A follow-up visit was scheduled between 1 and 3 weeks after the last dose of study medication. At every visit, a physical examination was performed including BP measurement. Systolic and diastolic BP were measured at the brachial artery after 5 minutes of rest in seated position, either manually or with a validated oscillometric device. BP was measured twice and if these values varied by more than 5 mmHg repeated two more times. Mean of these two or four values was documented. In addition, review of medication use and laboratory assessment were performed. Plasma sodium, cholesterol and glucose were measured using standard methodology. Copeptin was measured as surrogate marker for vasopressin, with an automatic immunofluorescence assay with coefficients of variation of 8.0% and 10.0% respectively (BRAHMS, Henningsdorf, Germany).\(^\text{20}\) TKV was measured by manual tracing of magnetic resonance images taken without use of a contrast agent. TKV was corrected for height (htTKV) and age to determine Mayo htTKV class, a classification system designed to predict future disease progression.\(^\text{21}\)

Blood pressure related patient characteristics
Mean arterial pressure was calculated as diastolic BP + 0.41*(systolic BP – diastolic BP).\(^\text{22}\) Hypertension was defined as either having a systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg, or use of antihypertensive medication. Antihypertensive drugs were divided into five classes: renin angiotensin aldosterone system (RAAS) inhibitors, beta-blocking agents, calcium channel blockers, diuretics and others. To be able to compare dosages between different classes of antihypertensives, these were expressed as defined daily dose (DDD). Reference values to calculate defined daily doses were obtained from the WHO Collaborating Centre for Drug statistics Methodology (https://www.whocc.no/atc_ddd_index/, accessed on 25\(^\text{th}\) of March 2020), see Supplementary Table 1.

Statistical analyses
BP and BP related patient characteristics were evaluated at baseline, after short term use of tolvaptan (3 weeks), after longer term use (36 months) and at the post-treatment follow-up visit. Change between baseline and the visit after 36 months was executed as intention to treat analysis including the last study visits of patients who ended their participation in the study prematurely. Depending on distribution, data was presented as mean ± standard deviation (SD), as median [interquartile range (IQR)] or percentage of total, unless stated otherwise. Comparisons between tolvaptan and placebo study arms were made with Student’s t-test for parametric, Mann-Whitney U test for non-parametric data and Fishers exact tests for categorical data. For comparison of the change of BP over time between the study arms in the overall study population presented in figures, mixed model repeated measures analyses was used. Within-subject comparisons between two study visits were performed with paired t-test for parametric data, Wilcoxon’s signed rank test for non-parametric data and McNemar’s test for categorical data. As secondary analysis, patients were divided into two subgroups based on hypertension status at baseline e.g. hypertension, as defined above, or normotension. To test the robustness of primary findings, effect of use of tolvaptan on mean arterial pressure was assessed in several subgroup defined by baseline disease severity related characteristics (sex, median age, Mayo htkv class, median eGFR and median copeptin value). In all these analyses, a p-value <0.05 was considered to indicate statistical significance. Statistical analyses were performed using SAS 9.4 and GraphPad Prism 8.4.2.
Table 1. Baseline characteristics of TEMPO 3:4 trial participants for the overall study population and according to hypertension status at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Normotensive at baseline</th>
<th>Hypertensive at baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tolvaptan</td>
<td>Placebo</td>
<td>Tolvaptan</td>
</tr>
<tr>
<td>Number</td>
<td>961</td>
<td>484</td>
<td>179</td>
</tr>
<tr>
<td>Male (%)</td>
<td>52</td>
<td>52</td>
<td>38</td>
</tr>
<tr>
<td>White (%)</td>
<td>84</td>
<td>84</td>
<td>78</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.6 ± 7.1</td>
<td>38.8 ± 7.1</td>
<td>36.7 ± 7.5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74 ± 0.10</td>
<td>1.74 ± 0.10</td>
<td>1.71 ± 0.10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.7 ± 18.3</td>
<td>78.6 ± 18.3</td>
<td>72.5 ± 17.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3 ± 5.1</td>
<td>25.9 ± 5.0</td>
<td>24.4 ± 4.6</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>5.0 ± 0.9</td>
<td>5.0 ± 0.9</td>
<td>5.0 ± 0.9</td>
</tr>
<tr>
<td>Glucose lowering drugs (% yes)</td>
<td>0.6</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/L)</td>
<td>5.2 ± 0.8</td>
<td>5.2 ± 0.8</td>
<td>5.1 ± 0.7</td>
</tr>
<tr>
<td>Cholesterol lowering drugs (% yes)</td>
<td>13</td>
<td>12</td>
<td>8.4</td>
</tr>
<tr>
<td>eGFR (ml/ min/1.73m²)</td>
<td>81 ± 21</td>
<td>82 ± 23</td>
<td>90 ± 19</td>
</tr>
<tr>
<td>htTKV (ml/m²)</td>
<td>979 ± 515</td>
<td>958 ± 483</td>
<td>780 ± 331</td>
</tr>
</tbody>
</table>

Mayo imaging class (%)
- tA
- tB
- tC
- tD
- tE
2
2
2
2

Differences between study arms are tested with Student’s t-test for parametric, Mann-Whitney U-test for non-parametric and Fishers’ exact test for categorical data. *signifies p<0.05, **p<0.001.

Abbreviations: eGFR, estimated glomerular filtration rate calculated with the CKD-EPI formula; htTKV, height adjusted total kidney volume; UAC ratio, urinary albumin creatinine ratio.

Results

Population at baseline
In this study 1445 patients were included, of which 961 were randomized to tolvaptan and 484 to placebo. There were no significant differences in baseline characteristics between both study arms (Table 1). Table 2 shows BP and BP related patient characteristics. At baseline, pressures were comparable in both study arms, with systolic and diastolic BPs of 129 ± 14 over 82.5 ± 9.9 mmHg for tolvaptan and 128 ± 14 over 82.4 ± 9.3 mmHg for placebo. Hypertension was present in 81% of the patients randomized to receive tolvaptan and 84% of the patients randomized to receive placebo. In both study arms 77% of the patients were at baseline on antihypertensive therapy, mostly RAAS inhibitors. Average dosages of these antihypertensives were similar in both study arms, namely 1.44 ± 1.38 versus 1.40 ± 1.34 defined daily doses, respectively. Plasma copeptin, as marker of vasopressin, did also not differ between the two study arms at baseline (Table 2).

Baseline characteristics of patients stratified for hypertension status can also be found in Table 1. In patients with normotension at baseline, BP at baseline did not differ between study arms, (Supplementary Table 2), even though patients randomized to receive tolvaptan (n=179) had a significantly higher BMI, used a cholesterol lowering drug more often and had a higher htTKV compared to patients that were to receive placebo (n=79) in this subpopulation (Table 1). In patients diagnosed with hypertension at baseline there were no significant differences in BP or other baseline patient characteristics between tolvaptan (n=782) and placebo (n=405) treated study arms.

Acute effect of tolvaptan
After three weeks of study treatment, copeptin was significantly higher in the tolvaptan arm compared to the placebo arm. At this time point in the study, there were no significant differences in BP related patient characteristics, such as systolic and diastolic BP or number and dose of antihypertensives, between the tolvaptan and placebo arm (Table 2). BP had decreased significantly from baseline in both arms with concurrently a slight increase in dosage of antihypertensives. Use of tolvaptan caused body weight to decrease from 79.7 ± 18.3 to 78.8 ± 18.2 kg and plasma sodium to increase from 140.4 ± 2.1 to 142.6 ± 2.6 mmol/L (both p<0.001). Changes in copeptin levels were significantly associated with the acute changes in body weight (R=0.08, p=0.02) and plasma sodium (R=0.16, p<0.001), but not with BP, in the tolvaptan arm. In the placebo arm weight increased significantly (p=0.007) and plasma sodium remained stable (p=0.85).

In the subgroup with normotension at baseline, diastolic BP after three weeks was slightly, but statistically significantly higher in the tolvaptan arm when compared to the placebo arm (79.7 ± 8.9 versus 77.1 ± 8.2 mmHg, respectively, p=0.03), despite start of antihypertensive therapy dosed at an average of 0.12 ± 0.66 defined daily doses in the tolvaptan arm versus 0.01 ± 0.06 in the placebo arm (p=0.12). No differences in systolic BP were noted. In the subgroup with hypertension at baseline no differences were found...
between the two study arms after 3 weeks in diastolic BP, in systolic BP nor in number and dose of antihypertensive drugs (Supplementary Table 2).

Table 2. Effect of tolvaptan on BP and BP related patient characteristics during the TEMPO 3:4 trial (continued)

<table>
<thead>
<tr>
<th>Table 2. Effect of tolvaptan on BP and BP related patient characteristics during the TEMPO 3:4 trial (continued)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Use of AHT (%)</td>
</tr>
<tr>
<td>Number of AHT classes per patient</td>
</tr>
<tr>
<td>Dosage of AHT per patient (DDD)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>Plasma sodium (mmol/L)</td>
</tr>
<tr>
<td>Plasma copeptin (pmol/L)</td>
</tr>
<tr>
<td><strong>Week 3</strong></td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Use of AHT (%)</td>
</tr>
<tr>
<td>Number of AHT classes per patient</td>
</tr>
<tr>
<td>Dosage of AHT per patient (DDD)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>Plasma sodium (mmol/L)</td>
</tr>
<tr>
<td>Plasma copeptin (pmol/L)</td>
</tr>
<tr>
<td><strong>Year 3 or early end of treatment</strong></td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Use of AHT (%)</td>
</tr>
<tr>
<td>Number of AHT classes per patient</td>
</tr>
<tr>
<td>Dosage of AHT per patient (DDD)</td>
</tr>
</tbody>
</table>

Differences between study arms are tested with Student’s t-test for parametric, Mann-Whitney U-test for non-parametric and Fishers’ exact test for categorical data. Year 3 or early end of treatment signifies that this is an intention to treat analysis, also including data of patients who ended their participation to this trial ahead of the final study visit. Abbreviations: AHT, anti-hypertensive therapy; BP, blood pressure; DDD, defined daily dose; Mean arterial pressure was calculated as diastolic BP + 0.4* (systolic BP – diastolic BP). Of note, copeptin was measured only in patients who completed the entire study, not in patients with early end of treatment.

**Long term effects of tolvaptan**

Figure 1 shows systolic and diastolic BP and dose of antihypertensive drugs over the course of the trial. Over time a small difference in BP between the two study arms occurred, namely a significant decrease in systolic BP after 28 months and diastolic BP after 32 months. At the end of the treatment period both systolic and diastolic BP were lower in the tolvaptan arm compared to placebo arm, with a systolic BP of 129 ± 14 versus 126 ± 13, respectively, p=0.002 and diastolic BP of 82.6 ± 10 versus 81.2 ± 9.5 mmHg, respectively, p=0.01. Meanwhile, a similar average number of antihypertensive drugs (1.46 ± 1.01 in the tolvaptan arm versus 1.48 ± 1.07 in the placebo arm, p=0.68) was given at a similar dose (2.07 ± 1.77 versus 2.05 ± 1.84 defined daily doses, respectively, p=0.83). In Supplementary Figure 1 is shown that after 28 months of treatment, average mean arterial pressure was also significantly lower in the tolvaptan arm compared to placebo (99.7 ± 9.4 versus 100.9 ± 10.2 mmHg, respectively, p<0.04). Of note, throughout the study plasma sodium and copeptin levels of the tolvaptan treated patients were higher than those of the placebo treated patients, p<0.001. Body weight did not differ (Table 2).
In subjects with normotension at baseline, no difference in systolic BP, diastolic BP (Figure 2) and mean arterial pressure (Supplementary Figure 2) was observed between both study arms during the trial period, whereas average dose of antihypertensive drugs was slightly higher in the tolvaptan treated subjects at month 20 and 24 but not at week 3 or at the last visit. After three years, 42% and 33% of the patients who did not have an elevated BP at baseline had developed hypertension in the tolvaptan treated and placebo treated study arm, respectively (p=0.13). In the subgroup of patients diagnosed with hypertension at baseline similar observations as in the overall study population were made, with a lower systolic BP after 28 months, a lower diastolic BP after 32 months (Figure 2) and a lower mean arterial pressure after 32 months (Supplementary Figure 2).

Figure 1. BP and BP-lowering medication of participants during the TEMPO 3:4 trial. The tolvaptan study arm (n=961) is represented by solid circles, and placebo (n=484) is represented by open circles. Systolic and diastolic BP are presented in millimeters of mercury, and average dosage of antihypertensive drugs in defined daily dosages (DDDs). Error bars represent 95% confidence intervals of the mean. Treatment duration is expressed in months with the exception of w3, which indicates week 3, and FU, which indicates follow-up. *P<0.05 calculated with a mixed model repeated measures analysis.
Figure 2. BP and BP-lowering medication during the TEMPO 3:4 trial in subgroups of participants according to baseline hypertension status. (A) includes patients with normotension at baseline \((n=258)\), and (B) includes patients with hypertension at baseline \((n=1187)\). The tolvaptan study arm is represented by solid circles, and placebo is represented by open circles. Systolic and diastolic BP are presented in millimeters of mercury, and average dosage of antihypertensive drugs in defined daily dosages (DDDs). Error bars represent 95% confidence intervals of the mean. Treatment duration is expressed in months with the exception of w3, which indicates week 3, and FU, which indicates follow-up. *\(p=0.05\).

Shortly after ending long-term use of tolvaptan

Approximately 3 weeks after withdrawal of study medication, data were available for 734 patients participating in the tolvaptan arm and 407 patients participating in the placebo arm. Differences in BP that were present at month 36 had evened out between the two study arms during this follow-up visit (Table 2). In the tolvaptan arm systolic BP had increased significantly from 125 ± 12 to 127 ± 12 mmHg (\(p<0.001\)) and diastolic BP from 80.4 ± 8.9 to 81.0 ± 8.6 mmHg (\(p=0.04\)), while the number and dose of antihypertensive medication that was used in both arms remained similar. In the placebo arm, systolic BP and diastolic BP did not change. Notwithstanding that at follow-up no differences between tolvaptan and placebo arms were found, in a within study-arm comparison, systolic BP and diastolic BP measured during the follow-up visit were significantly lower compared to baseline in the tolvaptan arm (\(p=0.007\) and \(p=0.001\)), but not in the placebo arm (\(p=0.27\) and 0.10 respectively). Meanwhile, the number and dose of antihypertensives had increased equally in both study arms (Table 3). Within subclasses of RAAS inhibitors (e.g. ACE inhibitors, angiotensin II receptor blockers, renin inhibitors and mineralocorticoid receptor antagonists) and diuretics (e.g. loop diuretics, thiazide diuretics and potassium-sparing diuretics) there also were no differences in their use between the study arms (Supplementary Table 3).

After withdrawal of study medication, weight increased in the subjects previously receiving tolvaptan from 80.4 ± 18.6 to 81.0 ± 18.7 kg and plasma sodium decreased from 141.6 ± 2.6 to 139.8 ± 2.2 mmol/L (both \(p<0.001\)). In the placebo arm weight and plasma sodium did not change. Notably, plasma sodium of the tolvaptan treated subjects at this final visit was below the level in the placebo arm (140.4 ± 2.4 mmol/L, \(p<0.001\)).

Treatment effect of tolvaptan in subgroups

The placebo adjusted effect of tolvaptan on mean arterial pressure after 36 months of treatment did not differ between subgroups defined based on baseline age, sex, hypertension status, eGFR, Mayo hTKV class, plasma copeptin and use of a RAAS inhibitor (Figure 3).
Table 3. Use of antihypertensive drugs in the overall study population

<table>
<thead>
<tr>
<th></th>
<th>Tolvaptan (n=734)</th>
<th>Placebo (n=407)</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value for difference*</th>
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<tbody>
<tr>
<td>Calcium Channel blocker</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of participants</td>
<td>20.4</td>
<td>28.2</td>
<td>&lt;0.001</td>
<td>23.6</td>
<td>32.4 &lt;0.001</td>
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<tr>
<td>Defined daily dose</td>
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<td>0.5</td>
<td>0.002</td>
<td>0.4</td>
<td>0.6 0.09</td>
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<tr>
<td>RAAS inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of participants</td>
<td>72.5</td>
<td>82.0</td>
<td>&lt;0.001</td>
<td>72.5</td>
<td>80.3 &lt;0.001</td>
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<tr>
<td>Defined daily dose</td>
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<td>1.3</td>
<td>0.19</td>
<td>1.3</td>
<td>1.3 0.69</td>
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<tr>
<td>Beta-blocking agent</td>
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<tr>
<td>% of participants</td>
<td>17.8</td>
<td>23.3</td>
<td>&lt;0.001</td>
<td>19.4</td>
<td>23.8 &lt;0.001</td>
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<tr>
<td>Defined daily dose</td>
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<td>0.3</td>
<td>0.23</td>
<td>0.2</td>
<td>0.3 0.24</td>
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<tr>
<td>Diuretic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>% of participants</td>
<td>4.8</td>
<td>4.2</td>
<td>0.55</td>
<td>4.7</td>
<td>3.7 0.39</td>
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<tr>
<td>Defined daily dose</td>
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<td>0.1</td>
<td>0.21</td>
<td>0.0</td>
<td>0.1 0.02</td>
</tr>
<tr>
<td>Other antihypertensive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of participants</td>
<td>4.2</td>
<td>7.2</td>
<td>&lt;0.001</td>
<td>4.7</td>
<td>8.8 0.001</td>
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<tr>
<td>Defined daily dose</td>
<td>0.1</td>
<td>0.2</td>
<td>0.13</td>
<td>0.1</td>
<td>0.2 0.51</td>
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<tr>
<td>Total defined daily dose</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Defined daily dose</td>
<td>1.5</td>
<td>2.2</td>
<td>&lt;0.001</td>
<td>1.4</td>
<td>2.3 &lt;0.001</td>
</tr>
</tbody>
</table>

In this table the average defined daily dose is calculated in the subjects of whom data was available at both baseline and follow-up visit. Within study arm comparisons were made with a paired t-test or McNemar’s test as appropriate. *p value for difference between the change of antihypertensive treatment in the tolvaptan arm versus the placebo arm tested using Student’s t-test or Chi-square test as appropriate.

Figure 3. Effect of tolvaptan on change in mean arterial pressure in the overall study population and in various subgroups during the TEMPO 3:4 trial. Change in mean arterial pressure between baseline and last visit on study medication (either year 3 or early end of treatment) was studied. Bars represent mean difference in change of mean arterial pressure between tolvaptan and placebo arms (“treatment effect”) and 95% confidence interval. Mean arterial pressure was calculated as diastolic BP + 0.412×(systolic BP – diastolic BP).

Discussion

In this post-hoc study of the TEMPO 3:4 trial we investigated the magnitude and time course of the effect of tolvaptan on BP, not only taking into account measured BP as a continuous variable, but also use of BP lowering medication. We did not find an acute effect, but observed a gradually developing difference in BP between study arms in favor of tolvaptan that was not explained by a difference in antihypertensive treatment. This difference leveled off after stopping study medication.

The acute effect of a V2 receptor antagonism was studied after a three week titration period in which the highest tolerated dose of medication was reached. At this visit, none of the parameters assessed to monitor changes in BP were significantly different between tolvaptan and placebo treated subjects. In both arms, BP had decreased. This may be
explained by the higher dosage of antihypertensive drugs that were used at week 3, prescribed by physicians aiming to reach adequate BP control. Alternatively, it could also be that participants had grown more familiar with the study setting, which can have resulted in reduction of stress and consequently a lower BP. In the tolvaptan treated subjects, two additional, opposing mechanisms may have been in action, as set out in the introduction section. Use of tolvaptan led to a more than three-fold increase in copeptin, measured as surrogate marker for vasopressin, as central compensation for the loss of renal urine concentrating capacity. This hormone has regulatory functions that can increase as well as decrease BP. While V1a receptors on the vascular wall induce vasoconstriction and thus increase in BP, V1a receptors present in various segments of the kidneys have a counterbalancing effect via natriuresis.26,28 These effects, in combination with aquaresis mediated by tolvaptan blocking the V2 receptor, can lead to a reduction in total body water and in circulating volume. Indeed, we observed a decrease in body weight and increase in plasma sodium after start of study medication in the tolvaptan arm, but not in the placebo arm. This effect on total body water has been studied previously as therapeutic modality in patients with heart failure or hyponatremia, as alternative for diuretics.22,23 Notably, in our tolvaptan treated subjects, change in copeptin was associated with change in sodium concentration and body weight, but not with change in BP, possibly reflecting the opposing mechanisms that are at play. It might be suggested that a decreasing effect on BP is counteracted by activation of the RAAS system due to intravascular volume depletion. However, it has been shown in both experimental as well as human studies that tolvaptan, in contrast to other diuretics, does not increase renin or aldosterone levels.19,21,22,29 Given that markers of the RAAS were not measured, this could not be further addressed in our study. Three small-scale studies with cross-over designs have previously investigated the acute effects of tolvaptan in ADPKD patients. These studies, that included 18, 20 and 27 patients, also found no acute effect on blood pressure after a single dose or 1 to 3 weeks of tolvaptan treatment.30-32 Of note, that given in our overall population the BP effect of tolvaptan might have been obscured by the use of BP lowering medication, we also studied the normotensive subgroup separately, that did not use such medication as baseline. In this specific subgroup, there was a suggestion of an effect of tolvaptan on vascular tone, given that diastolic BP was significantly higher in the treatment arm versus the placebo arm and slightly more patients in the tolvaptan arm had started antihypertensive therapy. However, after 3 weeks of treatment no effect on systolic BP was observed in this normotensive subgroup, and in the hypertensive subgroup an effect on diastolic BP was not confirmed. The importance of this isolated finding of increase in diastolic BP in the normotensive subgroup can therefore be debated.

Next, the effect of long term use of tolvaptan was studied. During three years of treatment, a difference between systolic and diastolic BP in the subjects with tolvaptan versus placebo emerged gradually. At the end of 3 years, systolic and diastolic BP were 3 and 1.4 mmHg lower in the tolvaptan compared to the placebo arm, whereas number and dose of antihypertensive drugs did not differ between the two study arms. We interpret this slowly occurring BP lowering effect to be the result of attenuation of the rate of disease progression with tolvaptan. When the rate of kidney function decline and total kidney volume growth are reduced by therapy, development of secondary symptoms of kidney disease, such as an increase in BP, will also be reduced. Alternatively, this gradual decrease in BP could also be a reflection of tolvaptan-induced natriuresis. Given that the present study indicates that this natriuretic effect is small, it may take a longer period of sustained V2 antagonism to elicit an effect on BP. The blood pressure lowering effect of tolvaptan in the present three year lasting trial is of course relatively minor. However, it should be noted that in clinical practice tolvaptan is meant to be prescribed as a renoprotective agent for a prolonged period of time. Given that the blood pressure lowering effect of tolvaptan increases gradually over time, it may be expected that a clinically meaningful effect on blood pressure will arise over the years. Of course this is an extrapolation of our findings beyond the duration of the present study, and future studies using longer-term data are needed to prove this hypothesis.

After discontinuation of tolvaptan, a withdrawal effect was observed. Both systolic and diastolic BP increased to a level similar to that in the placebo arm. This was not explained by concurrent changes in antihypertensive therapy. Simultaneously, body weight increased and plasma sodium decreased significantly, indicating that the observed change in BP might be the result of an increase in circulating volume. These changes in sodium and body weight were anticipated, as they mirror the changes directly after start of tolvaptan at week 3. It should be noted that after cession of medication plasma sodium in the tolvaptan arm was significantly lower compared to the placebo arm, which may indicate there is an overcorrection. Although hypothetical, this might be the result of a change in threshold of the osmolar sensory cells that regulate plasma osmolality, caused by the three years of tolvaptan-induced aquaresis. Reset of the osmotic threshold for thirst has been observed in other states of water imbalance, such as SIADH as well as psychogenic polydipsia.32,33 We expect that over time this set point will return to its pre-study level. Follow-up data to corroborate this notion is unfortunately not available, but this line of reasoning matches our clinical experience that after stopping tolvaptan patients report that they remain drinking abundantly for some time, a habit that fades out over a couple of weeks to months.

Some limitations of our study should be acknowledged, mostly those inherent to the post-hoc design. BP would ideally have been studied over time without changing antihypertensive therapy to answer our study question irrefutably. Such a strategy is, however, not possible in clinical practice because subjecting a patient to suboptimal BP control during a 3 year period would be unethical. Second, the choice of BP therapy was dependent on the discretion of the treating physician, a given that may have introduced variability in our outcomes. Because we did not find differences between the two study arms in the number or dose of antihypertensives overall nor of specific classes of antihypertensives, this is unlikely to have induced bias. Third, BP could have been assessed more accurately with home measurements, or even 24 hour measurements, instead of measurement during an outpatient clinic visit.34 In the clinic, blood pressure was measured after 5 minutes of rest with either a manual or oscillometric device. Despite
the relative insensitivity of our BP measurement method we still observed changes in BP during long-term tolvaptan use. Given that tolvaptan induces a profound polyuria and thirst, thus possibly deblinding the study groups to the researchers, one may argue that the decrease in blood pressure is merely produced by an observer bias.\textsuperscript{35} Then again, all participants were encouraged to increase their fluid intake during the trial, thereby obscuring this potential tell-tale sign. Finally, vasopressin was not measured directly, but by its more easy and reliably to measure surrogate marker copeptin.\textsuperscript{36}

To conclude, the present study demonstrates that start of tolvaptan treatment in ADPKD patients does not have a clinically significant short-term effect on BP, perhaps due to simultaneously occurring BP increasing and BP lowering effects that cancel out. During prolonged use, however, gradually BP becomes lower in patients on tolvaptan compared to patients on placebo. This observation can likely be attributed to a sustained natriuretic effect, possibly in combination with the beneficial effect of tolvaptan on disease progression. When after 3 years of treatment tolvaptan is stopped, there is an increase in BP in tolvaptan treated patients up to a level that is again similar to that of the placebo treated subjects. This increase is likely caused by the sudden recovery of the V2 mediated antidiuretics and sodium reabsorption, resulting in an excess of circulating volume. This acute effect of stopping tolvaptan is expected to disappear on the long term.

Author contributions

The present post-hoc analysis of the TEMPO 3:4 study was conceptualized by ABC and RTG. Statistical analyses were performed by JO, JL and HI. Data interpretation was done by all co-authors. Figures were made by JEH. Draft was written by JEH and RTG and revised by all authors. All authors approved the final version of the manuscript and can be hold accountable for all aspects of the work.

Acknowledgements

We are grateful to all participants and investigators for their contribution to the TEMPO 3:4 trial.

Disclosures

ABC, VET, OD, RDP and RTG were members of the Steering Committee of the TEMPO 3:4 Trial and are consultants for Otsuka Pharmaceutical Development and Commercialization, Inc., Rockville, Maryland. JL, HL and JO are employees of Otsuka.

Funding

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Significance statement

Patients with autosomal dominant polycystic kidney disease are treated with tolvaptan, a V2 receptor antagonist, to slow progression towards end stage kidney disease. This paper describes the magnitude and time course of the effect of use of tolvaptan on blood pressure (BP) using data from the TEMPO 3:4 trial. In theory, tolvaptan could have both BP increasing and BP decreasing effects. The present study shows that directly after start of tolvaptan therapy BP does not change. On long term, however, BP gradually becomes lower in patients with tolvaptan compared to placebo. This observation might be attributed to the beneficial effect of tolvaptan on disease progression or a sustained natriuretic effect.
References


Supplementary material

**Supplementary Table 1.** Conversion table for antihypertensive medication regimens to define daily dosages. As according to the accompanying guidelines all combination tablets were counted as one defined daily dose per tablet.

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Defined daily dosage (mg)</th>
<th>Generic name</th>
<th>Defined daily dosage (mg)</th>
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<td>Benidipine</td>
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<td>Benidipine</td>
<td>10</td>
</tr>
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**Supplementary Table 2.** Effect of tolvaptan on blood pressure (BP) and BP related patient characteristics during the TEMPO 3:4 trial in subgroups according to hypertension status at baseline.

<table>
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<tr>
<th>Hypertension</th>
<th>Normotension</th>
<th>Tolvaptan treated</th>
<th>Placebo treated</th>
<th>p-value</th>
<th>Tolvaptan treated</th>
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<th>p-value</th>
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<tr>
<td>(mmHg)</td>
<td>122 ± 10</td>
<td>122 ± 9</td>
<td>130 ± 14</td>
<td>0.46</td>
<td>130 ± 14</td>
<td>130 ± 14</td>
<td>0.46</td>
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<tr>
<td>Diastolic BP</td>
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<tr>
<td>(mmHg)</td>
<td>77.9 ± 7.6</td>
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<td>83.5 ± 10.1</td>
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<td>Mean arterial pressure (mmHg)</td>
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<td>100</td>
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<tr>
<td>Use of AHT (%)</td>
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<td>93</td>
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<tr>
<td>(mmHg)</td>
<td>122 ± 13</td>
<td>122 ± 11</td>
<td>127 ± 13</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(mmHg)</td>
<td>79.7 ± 8.9</td>
<td>77.1 ± 8.2</td>
<td>82.0 ± 9.4</td>
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<td>Mean arterial pressure (mmHg)</td>
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<td>95.5 ± 8.4</td>
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<td>100.1 ± 9.9</td>
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<td>Heart rate (beats per minute)</td>
<td>70 ± 10</td>
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<td>70 ± 11</td>
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<td>Use of AHT (%)</td>
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<td>0.62</td>
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<tr>
<td>No. of classes of AHT per patient</td>
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<td>0.06</td>
<td>1.48 ± 0.83</td>
<td>1.54 ± 0.91</td>
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<tr>
<td>Dosage of AHT per patient (DDD)</td>
<td>0.12 ± 0.66</td>
<td>0.01 ± 0.06</td>
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<td>2.08 ± 1.58</td>
<td>2.07 ± 1.63</td>
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<tr>
<td>Body weight (kg)</td>
<td>71.0 ± 16.5</td>
<td>68.1 ± 14.5</td>
<td>80.6 ± 18.1</td>
<td>0.99</td>
<td>80.6 ± 18.0</td>
<td>0.99</td>
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</tr>
<tr>
<td>Plasma sodium (mmol/L)</td>
<td>142.5 ± 2.8</td>
<td>140.2 ± 1.9</td>
<td>&lt;0.001</td>
<td>142.6 ± 2.6</td>
<td>140.3 ± 2.3</td>
<td>&lt;0.001</td>
<td></td>
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</table>
## Supplementary Table 2. Effect of tolvaptan on blood pressure (BP) and BP related patient characteristics during the TEMPO 3:4 trial in subgroups according to hypertension status at baseline.

(continued)

<table>
<thead>
<tr>
<th></th>
<th>Normotension</th>
<th>Hypertension</th>
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<th>Tolvaptan treated</th>
<th>Placebo treated</th>
<th>p-value</th>
<th>Tolvaptan treated</th>
<th>Placebo treated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma copeptin</strong></td>
<td>19.5 [15.5 – 25.5]</td>
<td>22.3 [16.6 – 28.4]</td>
<td>&lt;0.001</td>
<td>Tolvaptan treated</td>
<td>Placebo treated</td>
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<td>Tolvaptan treated</td>
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<tr>
<td>(pmol/L)</td>
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<td>6.3 [3.8 – 10.4]</td>
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<td><strong>Year 3 or early end of treatment</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>124 ± 13</td>
<td>126 ± 12</td>
<td>0.44</td>
<td>127 ± 13</td>
<td>129 ± 14</td>
<td>0.004</td>
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<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>79.9 ± 9.0</td>
<td>80.4 ± 8.2</td>
<td>0.66</td>
<td>81.5 ± 9.6</td>
<td>83.0 ± 10.2</td>
<td>0.01</td>
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<tr>
<td><strong>Mean arterial pressure (mmHg)</strong></td>
<td>98.1 ± 9.5</td>
<td>99.0 ± 8.6</td>
<td>0.51</td>
<td>100.1 ± 10.0</td>
<td>102.0 ± 11.0</td>
<td>0.003</td>
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</tr>
<tr>
<td><strong>Heart rate (beats per minute)</strong></td>
<td>70 ± 11</td>
<td>69 ± 9</td>
<td>0.59</td>
<td>70 ± 11</td>
<td>70 ± 11</td>
<td>0.58</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Hypertension (%)</strong></td>
<td>44</td>
<td>33</td>
<td>0.13</td>
<td>97</td>
<td>96</td>
<td>0.73</td>
<td></td>
<td></td>
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<tr>
<td><strong>Use of AHT (%)</strong></td>
<td>27</td>
<td>19</td>
<td>0.21</td>
<td>95</td>
<td>95</td>
<td>0.78</td>
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<tr>
<td><strong>No. of classes of AHT per patient</strong></td>
<td>0.34 ± 0.61</td>
<td>0.22 ± 0.50</td>
<td>0.12</td>
<td>1.65 ± 0.96</td>
<td>1.73 ± 1.01</td>
<td>0.15</td>
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<tr>
<td><strong>Dosage of AHT per patient (DDD)</strong></td>
<td>0.44 ± 1.06</td>
<td>0.20 ± 0.49</td>
<td>0.06</td>
<td>2.39 ± 1.75</td>
<td>2.46 ± 1.85</td>
<td>0.53</td>
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<tr>
<td><strong>Body weight (kg)</strong></td>
<td>72.7 ± 17.6</td>
<td>69.1 ± 14.9</td>
<td>0.12</td>
<td>82.5 ± 18.7</td>
<td>82.2 ± 18.3</td>
<td>0.78</td>
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</tr>
<tr>
<td><strong>Plasma sodium (mmol/L)</strong></td>
<td>141.5 ± 2.8</td>
<td>140.4 ± 2.2</td>
<td>0.002</td>
<td>141.7 ± 2.6</td>
<td>140.3 ± 2.3</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td><strong>Plasma copeptin</strong></td>
<td>17.5 [13.5 – 21.2]</td>
<td>20.9 [15.4 – 28.4]</td>
<td>&lt;0.001</td>
<td>Tolvaptan treated</td>
<td>Placebo treated</td>
<td></td>
<td>Tolvaptan treated</td>
<td>Placebo treated</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(pmol/L)</td>
<td>5.8 [3.6 – 9.6]</td>
<td>7.6 [4.3 – 14.5]</td>
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</table>

Follow-up

<table>
<thead>
<tr>
<th></th>
<th>Normotension</th>
<th>Hypertension</th>
<th></th>
<th>Tolvaptan treated</th>
<th>Placebo treated</th>
<th>p-value</th>
<th>Tolvaptan treated</th>
<th>Placebo treated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>126</td>
<td>67</td>
<td>608</td>
<td>340</td>
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</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>125 ± 11</td>
<td>124 ± 11</td>
<td>0.58</td>
<td>127 ± 12</td>
<td>128 ± 13</td>
<td>0.29</td>
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</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>79.3 ± 8.1</td>
<td>79.0 ± 8.3</td>
<td>0.82</td>
<td>81.4 ± 8.7</td>
<td>82.2 ± 8.8</td>
<td>0.16</td>
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</tr>
<tr>
<td><strong>Mean arterial pressure</strong></td>
<td>98.1 ± 8.4</td>
<td>97.6 ± 8.5</td>
<td>0.67</td>
<td>100.3 ± 9.2</td>
<td>101.2 ± 9.5</td>
<td>0.18</td>
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</tr>
<tr>
<td><strong>Heart rate (beats per minute)</strong></td>
<td>71 ± 10</td>
<td>68 ± 10</td>
<td>0.07</td>
<td>69 ± 10</td>
<td>69 ± 10</td>
<td>0.98</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Hypertension (%)</strong></td>
<td>46</td>
<td>31</td>
<td>0.06</td>
<td>98</td>
<td>98</td>
<td>0.81</td>
<td></td>
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</tr>
<tr>
<td><strong>Use of AHT (%)</strong></td>
<td>34</td>
<td>22</td>
<td>0.10</td>
<td>97</td>
<td>97</td>
<td>0.84</td>
<td></td>
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<tr>
<td><strong>No. of classes of AHT per patient</strong></td>
<td>0.41 ± 0.62</td>
<td>0.24 ± 0.46</td>
<td>0.05</td>
<td>1.70 ± 0.95</td>
<td>1.79 ± 0.99</td>
<td>0.17</td>
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</tr>
<tr>
<td><strong>Dosage of AHT per patient (DDD)</strong></td>
<td>0.49 ± 1.02</td>
<td>0.30 ± 0.92</td>
<td>0.19</td>
<td>2.48 ± 1.77</td>
<td>2.52 ± 1.80</td>
<td>0.76</td>
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</tr>
</tbody>
</table>

Differences between groups are tested with Student’s t-test for parametric, Mann-Whitney U-test for non-parametric and Fishers’ exact test for categorical data. Year 3 or early end of treatment signifies that this is an intention to treat analysis. Abbreviations: AHT, anti-hypertensive therapy; BP, blood pressure; DDD, defined daily dose; Mean arterial pressure was calculated as diastolic BP + 0.412*(systolic BP – diastolic BP). NB copeptin was not measured in patients with early end of treatment.
Supplementary Table 3. Distribution of the use of subclasses of RAAS inhibitors and diuretics in the overall study population

<table>
<thead>
<tr>
<th></th>
<th>Tolvaptan (n=734)</th>
<th>Placebo (n=407)</th>
<th>p-value for difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td><strong>RAAS inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ACE inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of participants</td>
<td>44.4</td>
<td>47.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Defined daily dose</td>
<td>0.76</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Angiotensin II receptor blockers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of participants</td>
<td>32.0</td>
<td>39.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Defined daily dose</td>
<td>0.47</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Renin inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of participants</td>
<td>0.1</td>
<td>0.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Defined daily dose</td>
<td>0.003</td>
<td>0.011</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Mineralocorticoid receptor antagonists</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>% of participants</td>
<td>0</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>Defined daily dose</td>
<td>0.003</td>
<td>0.16</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Diuretics</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Loop diuretics</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>% of participants</td>
<td>0.7</td>
<td>1.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Defined daily dose</td>
<td>0.005</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Thiazide diuretics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of participants</td>
<td>2.7</td>
<td>2.0</td>
<td>0.32</td>
</tr>
<tr>
<td>Defined daily dose</td>
<td>0.029</td>
<td>0.014</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Potassium-sparing diuretics</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>% of participants</td>
<td>0.1</td>
<td>0</td>
<td>0.14</td>
</tr>
<tr>
<td>Defined daily dose</td>
<td>0.001</td>
<td>0.32</td>
<td>0.0</td>
</tr>
</tbody>
</table>

In this table the average defined daily dose is calculated in the subjects of whom data was available at both baseline and follow-up visit. Within study arm comparisons were made with a paired t-test or McNemar’s test as appropriate. *p value for difference between the change of antihypertensive treatment in the tolvaptan arm versus the placebo arm tested using Student’s t-test or Chi-square test as appropriate.

Supplementary Figure 1. Mean arterial pressure and average dosage of antihypertensive drugs (in defined daily dosages, DDD) of participants during the TEMPO 3:4 trial. The tolvaptan study arm (n=961) is represented by solid circles (●) and placebo (n=484) by open circles (○). Error bars represent 95% confidence intervals of the mean, * indicates p<0.05 calculated with a mixed model repeated measures analysis. Treatment duration is expressed in months with the exception of w3, which indicates week 3, and FU which indicates follow-up. Mean arterial pressure was calculated as diastolic BP + 0.412*(systolic BP – diastolic BP).
Supplementary Figure 2. Mean arterial pressure and average dosage of antihypertensive drugs (in defined daily dosages, DDD) in subgroups of the trial participants according to baseline hypertension status. A) includes patients with normotension at baseline (n=258) and B) patients with hypertension at baseline (n=1187). The tolvaptan study arm is represented by solid circles (●) and placebo by open circles (○). Error bars represent 95% confidence intervals of the mean, * indicates p<0.05. Treatment duration is expressed in months with the exception of w3, which indicates week 3 and FU which indicates follow-up. Mean arterial pressure was calculated as diastolic BP + 0.412*(systolic BP – diastolic BP).