Original Article

The cardiovascular markers copeptin and high-sensitive C-reactive protein decrease following specific therapy for primary aldosteronism

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Context: Copeptin and high-sensitive C-reactive protein (hsCRP) are biomarkers associated with increased mortality in patients with cardiovascular and cerebrovascular disease as well as in the general population. No data exist regarding these markers in patients with primary aldosteronism.

Objective: To evaluate copeptin and hsCRP levels as cardiovascular risk markers in primary aldosteronism patients.

Methods: A total of 113 primary aldosteronism patients (64% male) from two centers of the prospective German Conn's Registry were identified, for whom a full data set and blood samples at baseline and follow-up (14 ± 3.4 months) after initiation of specific primary aldosteronism treatment were available. These cases were matched 1 : 3 ($n = 339$) for sex, renal function, BMI, age and SBP with participants from the Cooperative Health Research in the Region of Augsburg F4 survey. Copeptin and hsCRP were determined by sandwich fluoroimmunoassay.

Results: HsCRP was significantly higher in primary aldosteronism patients at baseline compared with matched controls. Following specific therapy, hsCRP and copeptin decreased significantly in primary aldosteronism patients [median (25th and 75th percentile): 1.6 (0.8, 3.4) to 1.2 (0.6, 2.1) mg/l, $P < 0.001$; 7.8 (4.6, 13.5) to 5.0 (3.1, 8.9) pmol/l, $P < 0.001$, respectively]. Men had higher hsCRP and copeptin levels at baseline and follow-up compared with women. The combination of sex, hypokalemia, lateralization index and blood pressure were the best predictors of outcome. However, copeptin and hsCRP had no predictive value despite the association of lower copeptin levels with better outcome regarding cure of primary aldosteronism.

Conclusion: Copeptin and hsCRP levels decrease following specific primary aldosteronism therapy reflecting successful cardiovascular risk reduction. However, they are no independent predictors regarding cure of primary aldosteronism.

Keywords: adrenalectomy, arginine vasopressin, hyperaldosteronism, mineralocorticoid receptor antagonist

Abbreviations: ADX, adrenalectomy; ANP, atrial natriuretic peptide; ARR, aldosterone-to-renin ratio; AVP, arginine vasopressin; AVS, adrenal vein sampling; BP, blood pressure; eGFR, estimated glomerular filtration rate; EH, essential hypertension; hsCRP, high-sensitive C-reactive protein; KORA, Cooperative Health Research in the Region of Augsburg; MRA, mineralocorticoid receptor antagonists; PA, primary aldosteronism

INTRODUCTION

To date, cardiovascular disease is the major cause of mortality in developed countries [1]. To identify patients with increased cardiovascular risk who would benefit from initiation of a risk-reducing intervention, extensive research on cardiovascular risk factors has been performed over the last decades. In addition to the classic risk factors such as age, sex, hypertension, smoking, dyslipidemia and diabetes mellitus [2], various biomarkers have recently come into focus. As chronic inflammation has been recognized to play an important role in the pathophysiology of atherosclerosis [3], inflammatory markers have been investigated to aid in cardiovascular risk prediction. In this context, one of the most promising biomarkers is high-sensitive C-reactive protein (hsCRP) that correlates well with the incidence of cardiovascular events and predicts cardiovascular as well as all-cause mortality [4–6].

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Another interesting biomarker in cardiovascular research is copeptin, the stable C-terminal peptide derived from the precursor molecule of arginine vasopressin (AVP), which plays an important role in fluid and electrolyte homeostasis [7]. Due to its short half-life, platelet-binding property and rapid plasma clearance, it is difficult to assess reliable AVP levels from blood samples [7]. In contrast, copeptin levels are more stable and much easier to measure [7]. Copeptin and AVP are secreted from the posterior pituitary in equimolar amounts; therefore, copeptin levels reliably reflect activation of the AVP system [8]. Several studies have underlined the advantages of copeptin as a diagnostic and prognostic marker in diseases like acute myocardial infarction (MI), heart failure, pneumonia, sepsis, diabetes mellitus and stroke [9,10]. In addition, copeptin has been shown to correlate with all-cause mortality in patients with cardiovascular and cerebrovascular diseases [11] as well as in the general population [12].

Primary aldosteronism is the most common form of secondary hypertension with a prevalence of 4.6–16.6% in hypertensive patients [13]. Despite causative treatment options like adrenalectomy (ADX) and mineralocorticoid receptor antagonists (MRA), patients with primary aldosteronism have frequently been reported to be at increased risk of cardiovascular and metabolic comorbidities as well as increased mortality in comparison with patients with essential hypertension [14,15]. So far, data regarding copeptin and hsCRP levels in primary aldosteronism are scarce [16,17]. In this study, we thus aimed at the evaluation of hsCRP and copeptin as biomarkers of cardiovascular risk with potential prognostic relevance in primary aldosteronism patients.

METHODS

Study population

Patients were recruited from the prospective cohort of the German Conn's registry. The German Conn's registry is a multicenter database that documents the diagnosis, therapy, comorbidities and long-term outcome of primary aldosteronism patients in Germany in a prospective manner since 2008 [18,19]. Inclusion in the registry requires the diagnosis of primary aldosteronism made accordingly to the Endocrine Society Clinical Practice Guideline [20]. Briefly, all patients included in this study had an increased aldosterone-to-renin ratio (ARR) and an abnormal confirmatory test namely a 0.9% saline infusion test in all the cases. Adjustment of medication prior to screening and confirmatory testing was performed whenever possible with β-blockers, central α2-blockers, angiotensin-converting enzyme inhibitors, AT1 blockers and diuretics withdrawn for at least 1 week and MRA withdrawn for at least 4 weeks. Subtype differentiation (unilateral vs. bilateral aldosterone excess) was made by adrenal imaging (MRI or computed tomography) and subsequent adrenal vein sampling (AVS), which was performed in 94.7% of the enrolled patients and was successful in 91.6% of those. AVS was considered successful when cortisol levels in both adrenal veins were at least twice as high as cortisol levels in inferior vena cava, and unilateral aldosterone secretion was defined by a lateralization index [hormone levels in the adrenal veins: $(\text{aldosterone/cortisol})_{\text{left}}/(\text{aldosterone/cortisol})_{\text{right}}$ or vice versa] of at least 4.

In two participating centers (Berlin and Munich), 251 newly diagnosed primary aldosteronism patients were recruited between 2008 and 2013 in the German Conn's Registry and have been followed up since recruitment on a prospective basis. Inclusion criteria for the current study were availability of complete data sets as well as serum samples at baseline and 1-year follow-up. These prerequisites were fulfilled in 113 patients. A total of 55 patients were treated with mineralocorticoid antagonists (MRA), and 58 patients underwent ADX.

Patients were matched with controls from the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 survey which is the 7-year follow-up of the KORA S4 survey [21,22]. The KORA study sample was recruited from the population of Augsburg and 16 adjacent counties. The KORA F4 survey was conducted in 2006–2008 and included 3080 patients. All study participants underwent a standardized interview, physical examination and withdrawal of a nonfasting blood sample by trained medical staff [21,22].

The ethics committees of the University of Munich and the participating centers approved the protocol of the Conn's registry. The study design of the KORA survey was approved by the Bavarian Medical Association. Written informed consent was obtained from all participants, and data protection policies were strictly adhered to.

Laboratory measurements and definitions

In primary aldosteronism patients as well as in controls standard laboratory measurements were performed immediately and decentralized. Blood samples for hsCRP, copeptin and osmolality measurement were stored in −70 °C freezers until analysis.

Serum copeptin levels were measured by a sandwich fluoroimmunoassay (B.R.A.H.M.S., Henningsdorf/bei Berlin, Germany) on the B.R.A.H.M.S. Kryptor system in primary aldosteronism patients and controls as described recently [23]. Serum hsCRP levels were assessed using a high-sensitivity latex-enhanced nephelometric assay on the Behring BN II nephelometer (Dade Behring, Marburg, Germany) in controls and a high-sensitivity sandwich fluoroimmunoassay (B.R.A.H.M.S.) on the B.R.A.H.M.S. Kryptor system in primary aldosteronism patients.

According to recommendations of the American Heart Association [24], patients with hsCRP levels more than 10 mg were excluded from the respective analyses because of possible coexistence of infectious disease ($n = 16$). In addition, patients with copeptin levels more than 100 pmol/l, which are found in critically ill patients [9], were excluded from further analyses ($n = 2$).

We assigned a low risk of future cardiovascular disease to patients with hsCRP levels less than 1 mg/l and a high (two-fold) risk to those with hsCRP levels more than 3 mg/l, according to recommendations of the American Heart Association [24].

Hypokalemic primary aldosteronism was defined as potassium levels less than 3.5 mmol/l. Accordingly, 92 patients (81%) were hypokalemic at diagnosis. BMI was calculated as body weight (kg) per height^2 (m^2).

Glomerular filtration rate (GFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

Blood pressure (BP) was measured after 5 min of resting with standard sphygmomanometers three times on the right (KORA) or three times on each arm (German Conn's Registry) by trained medical staff in the sitting position. For statistical analysis, the average of the measurements was calculated.

Outcome categories

According to a previous study of our group [25], we defined therapy outcome categories as follows: cured (ADX-treated patients)/controlled (MRA-treated patients): potassium levels more than 3.4 mmol/l, SBP less than 140 mmHg, DBP less than 90 mmHg and no antihypertensive drugs (MRA was not counted as antihypertensive drug); improved: potassium levels more than 3.4 mmol/l and SBP less than 140 mmHg and DBP less than 90 mmHg or decrease of SBP by at least 10 mmHg or decrease of antihypertensive drugs by at least 1; and no improvement: persistent hypokalemia and/or no improvement in BP according to the above criteria.

Outcome prediction

Binary logistic regression models were set up to predict outcome in primary aldosteronism patients (cured/controlled and significantly improved vs. slightly improved and no improvement). Several confounding variables were included in the models (age, BMI, sex, duration of hypertension, baseline ARR, aldosterone levels, BP, estimated GFR (eGFR), potassium levels and lateralization index).

Statistics, matching

To identify suitable controls within the KORA cohort ($n = 1563$), we used a multivariate 1 : 3 matching. Matching was performed sex-stratified, and matching variables were BMI, renal function (expressed as eGFR), age and BP. From 3080 patients in the KORA F4 survey, patients with potassium less than 3.5 mmol/l ($n = 28$) were excluded to roughly avoid severe primary aldosteronism in controls. In addition, 1489 patients with missing measurements of hsCRP, copeptin or missing data in the matching variables were excluded. Finally, 1563 patients were available for 1 : 3 matching, resulting in 339 matching controls. To assess differences between patients and matched controls, conditional logistic regression analysis was performed.

Data are displayed as median (25th and 75th percentile) for continuous variables and percentages for categorical variables if not stated otherwise. Variables were assessed for normality using Kolmogorov–Smirnov test. Correlations were assessed using Spearman's rank correlation coefficient. Mann–Whitney *U* test was used to compare continuous data and Fisher's exact test to compare categorical variables between groups. For paired data, Wilcoxon's test was applied. Differences were considered significant when *P* value was less than 0.05. Statistical analysis was carried out in IBM SPSS Statistics 23 (IBM, Ehningen, Germany) and matching was performed using R version 3.2.1 and R-package Matching, version 4.8–3.4 (http://ftp13.frugalware.org/pub/CRAN/web/packages/matching/Matching.pdf).

RESULTS

Clinical characteristics of patients with primary aldosteronism and controls are displayed in Table 1. Matching was successful for sex, BMI and renal function. As expected,

TABLE 1. Characteristics of primary aldosteronism patients at baseline and follow-up compared with matched controls[a]

	Controls	Primary aldosteronism patients at baseline	Primary aldosteronism patients at follow-up	*P* value*	*P* value**
Sex male/female (*f*%)	216/123 (36%)	72/41 (36%)	72/41 (36%)		
GFR (CKD-EPI) (ml/min)	91.3 (77.0, 104.6)	93.8 (73.9, 105.7)	82.9 (64.6, 97.2)	0.703	*<0.001*
BMI (kg/m^2)	27.7 (25.3, 30.6)	28.3 (25.5, 31.6)	28.1 (25.2, 30.5)	0.418	0.207
Age (years)	56 (46, 67)	52 (44, 60)	53 (46, 61)	*<0.001*	*<0.001*
SBP (mmHg)	139 (121.5, 149)	149 (137, 165)	134 (124, 142)	*<0.001*	*<0.001*
DBP (mmHg)	84 (77.5, 90)	92 (85, 99)	85 (78, 92)	*<0.001*	*<0.001*
Aldosterone (pg/ml)	38 (26, 56)	204 (132, 324)	137 (37, 249)	*<0.001*	*0.005*
Renin (pg/ml)	10.7 (5.3, 21.3)	3.8 (2.0, 8.1)	15 (6.9, 32.4)	*<0.001*	*<0.001*
ARR	3.6 (1.6, 7.5)	52 (23, 109)	7.6 (2.3, 18.4)	*<0.001*	*<0.001*
Potassium (mmol/l)	4.1 (4.0, 4.3)	3.3 (3.9, 3.7)	4.1 (3.9, 4.4)	*<0.001*	*<0.001*
Sodium (mmol/l)	139 (137, 140)	140 (139, 143)	139 (137, 141)	*<0.001*	*<0.001*
Osmolality (mOsmol/kg)	Not available	304 (299, 310)	302 (296, 308)	–	*0.005*
Creatinine (mg/dl)	0.93 (0.81, 1.08)	0.81 (0.7, 1.1)	1.0 (0.8, 1.2)	*0.007*	*<0.001*
TC (mg/dl)	213 (187, 237)	195 (166, 223)	193 (169, 220)	*<0.001*	0.831
HDL cholesterol (mg/dl)	52 (43, 62)	53 (43, 68)	49 (42, 63)	0.340	*0.001*
LDL cholesterol (mg/dl)	135 (111, 158)	120 (95, 149)	121 (98, 143)	*0.001*	0.707
Triglycerides (mg/dl)	114 (80, 175)	103 (72, 141)	118 (83, 190)	*<0.001*	*<0.001*
History of MI	16 (4.7%)	2 (1.8%)	2 (1.8%)	0.175	1.0[b]
History of stroke	12 (3.5%)	1 (0.9%)	1 (0.9%)	0.161	1.0[b]

[a]Characteristics of 113 primary aldosteronism patients at baseline and follow-up after an average of 13 (11–16) months, compared with 339 matched controls. Values are median (25th and 75th percentile). ARR, aldosterone-to-renin ratio; BP, blood pressure; GFR, glomerular filtration rat; MI, myocardial infarction; TC, total cholesterol.
[b]Significance was tested with McNemar test.
*Primary aldosteronism vs. controls, differences were assessed using conditional logistic regression
**Baseline vs. follow-up, significance was tested with Wilcoxon's test.

primary aldosteronism patients had significantly higher aldosterone levels, higher ARR, lower renin and lower potassium levels than controls. Also serum sodium was significantly higher in primary aldosteronism patients at baseline compared with controls. The prevalence of history of MI and stroke in primary aldosteronism patients and controls did not differ significantly.

HsCRP levels were higher in primary aldosteronism patients at baseline compared with matched controls [1.6 (0.8, 3.4) vs. 1.5 (1.0, 2.5) mg/l, $P < 0.001$] (Fig. 1a). This difference remained significant following adjustment for age, BP and cholesterol levels. One year following initiation of specific therapy, hsCRP [1.2 (0.6, 2.1) mg/l, $P < 0.001$] decreased significantly in comparison with baseline values (Fig. 1a). A significant reduction of hsCRP levels at follow-up could be seen in the cured/controlled [1.2 (0.6, 3.1) vs. 0.8 (0.5, 1.2) mg/l, $P = 0.041$] and improved [1.6 (0.9, 3.0) vs. 1.2 (0.6, 2.3) mg/l, $P = 0.003$] patients; however, hsCRP levels did not differ significantly between outcome categories (Fig. 2). Consequently at follow–up, significantly more patients had a low cardiovascular risk (hsCRP < 1 mg/l; 46 vs. 30%, $P < 0.001$) and significantly less patients (16 vs. 26%, $P < 0.001$) had a high cardiovascular risk (hsCRP > 3 mg/l).

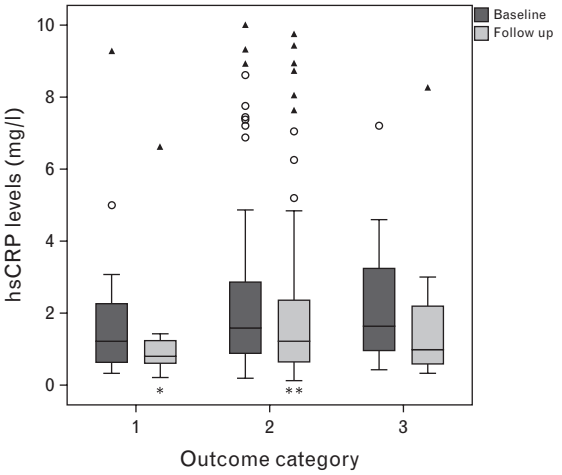

FIGURE 2 High-sensitive C-reactive protein levels in primary aldosteronism patients at baseline and follow-up, itemized for outcome categories. 1 = cured/controlled ($n = 11$), 2 = improved ($n = 74$) and 3 = no improvement ($n = 12$). For definitions of outcome categories, see the Methods section. Boxes represent median, 25th and 75th percentile. Whiskers represent the maximum and minimum inside 1.5 interquartile range. Significance was tested with Wilcoxon's test between baseline and follow-up and with Mann–Whitney *U* test between outcome categories. $^{*}P < 0.05$, $^{**}P < 0.005$ compared with baseline. hsCRP, high-sensitive C-reactive protein.

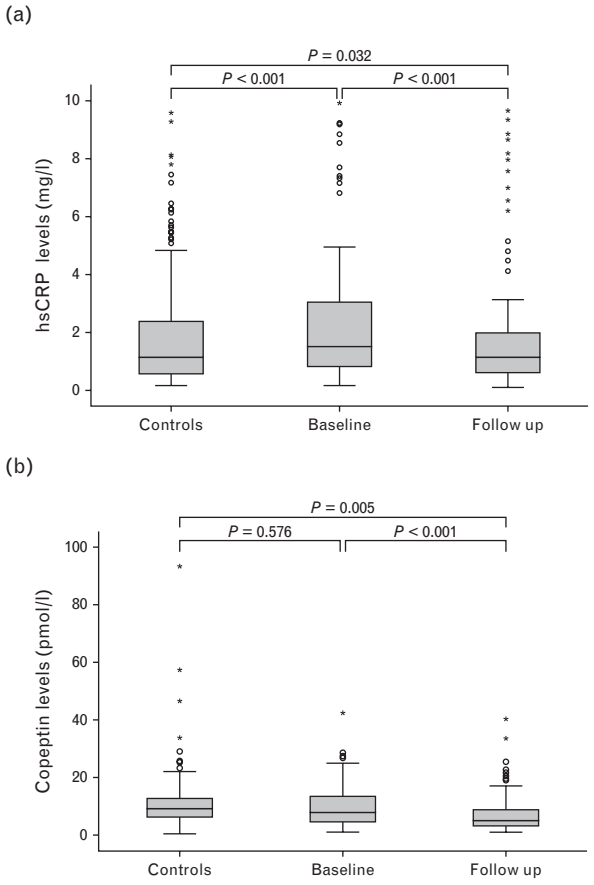

FIGURE 1 Box plots of high-sensitive C-reactive protein (a) and copeptin (b) levels in primary aldosteronism patients at baseline and follow-up and in controls, after exclusion of outliers (high-sensitive C-reactive protein >10 mg/l; copeptin >100 pmol/l). Boxes represent median, 25th and 75th percentile. Whiskers represent the maximum and minimum value inside 1.5 interquartile range. Difference between baseline and follow-up was tested for significance with Wilcoxon's test. To compare primary aldosteronism and controls, univariate conditional logistic regression analysis was applied. hsCRP, high-sensitive C-reactive protein.

Baseline hsCRP levels did not differ between patients with the hypokalemic and normokalemic form of primary aldosteronism. However, at follow–up, patients with the normokalemic form had significantly higher hsCRP levels compared with those with the hypokalemic form [1.7 (1.1, 2.4) vs. 0.9 (0.6, 1.9) mg/l, $P = 0.047$]. Furthermore, there was no difference in hsCRP levels at baseline or at follow-up between patients with the unilateral and bilateral form of primary aldosteronism. Men had higher baseline hsCRP levels than women [1.8 (1.0, 3.5) vs. 1.3 (0.7, 2.4) mg/l, $P = 0.044$].

No significant differences between primary aldosteronism patients and controls were evident in copeptin levels [7.8 (4.6, 13.5) vs. 9.1 (6.8, 11.8) pmol/l, $P = 0.576$] (Fig. 1b). Adjustment for age, BP, sodium and cholesterol levels did not change this result. One year after initiation of a specific therapy for primary aldosteronism, copeptin levels decreased significantly in comparison with baseline values [5.0 (3.1, 8.9) pmol/l, $P < 0.001$] (Fig. 1b).

Patients with unilateral primary aldosteronism had higher copeptin levels at baseline [10.6 (5.8, 14.3) pmol/l] compared with those with bilateral primary aldosteronism [6.4 (4.0, 9.3) pmol/l, $P = 0.004$]. At follow-up, copeptin levels converged between ADX-treated and MRA-treated patients [5.2 (3.2, 10.2) and 4.9 (3.0, 8.0) pmol/l, $P = 0.458$]. Furthermore, there was no difference in copeptin levels at baseline or at follow-up between patients with the hypokalemic and normokalemic form of primary aldosteronism.

Men had higher copeptin levels at baseline and follow-up than women [baseline: 9.3 (5.9, 15.3) vs. 5.5 (2.9, 10.5) pmol/l, $P = 0.001$; follow-up: 6.6 (4.5, 10.5) vs. 3.1 (2.2, 5.6) pmol/l, $P < 0.001$] (Fig. 3).

Baseline copeptin levels significantly correlated positively with baseline levels of aldosterone ($r = 0.355$) and

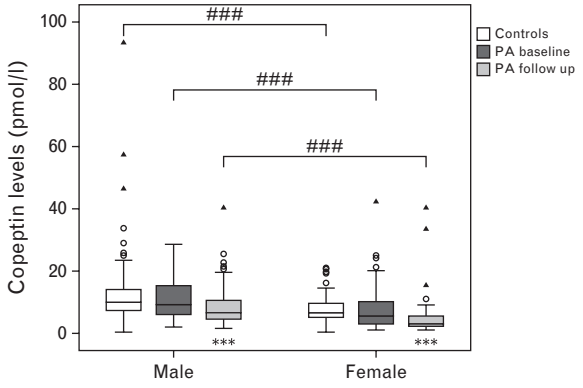

FIGURE 3 Copeptin levels in male and female primary aldosteronism patients and matched controls. Boxes represent median, 25th and 75th percentile. Whiskers represent the maximum and minimum inside 1.5 interquartile range. Significance between baseline and follow-up was tested with Wilcoxon's test, between male and female with Mann–Whitney *U* test, between primary aldosteronism and controls with conditional logistic regression. $^{***}P \leq 0.001$ compared with baseline, $^{###}P \leq 0.001$.

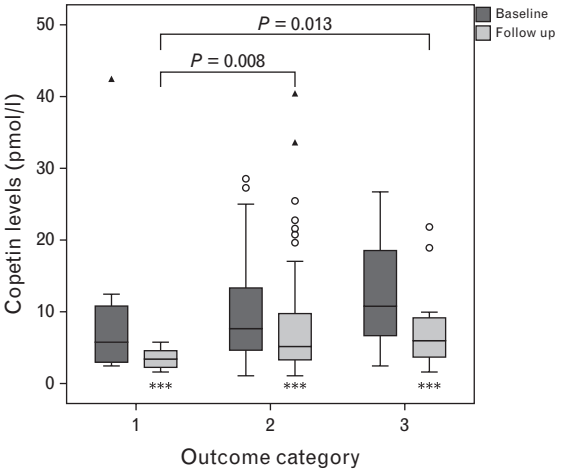

FIGURE 4 Copeptin levels in primary aldosteronism patients at baseline and follow-up, itemized for outcome categories. 1 = cured/controlled ($n = 13$), 2 = improved ($n = 83$) and 3 = no improvement ($n = 15$). For definitions of outcome categories, see the Methods section. Boxes represent median, 25th and 75th percentile. Whiskers represent the maximum and minimum inside 1.5 interquartile range. Significance was tested with Wilcoxon's test between baseline and follow-up and with Mann–Whitney *U* test between outcome categories. $^{***}P < 0.001$ compared with baseline.

sodium ($r = 0.316$), and negatively with baseline potassium levels ($r = -0.281$) and eGFR ($r = -0.419$) in primary aldosteronism patients. In addition, high baseline copeptin levels were associated with higher DBP ($r = 0.216$) and higher number of antihypertensive drugs ($r = 0.265$) at follow-up. High hsCRP levels were significantly associated with worse lipid profile (baseline HDL cholesterol: $r = -0.370$, LDL cholesterol: $r = 0.287$ and triglycerides: $r = 0.373$).

Copeptin correlated weakly ($r = -0.135$) with history of MI in the control group, however, not in primary aldosteronism patients. HsCRP did not correlate significantly with history of MI nor stroke neither in primary aldosteronism patients nor in controls.

According to our preset criteria at follow-up [after 13 (11, 16) months], 11.5% of the primary aldosteronism patients could be considered as cured or controlled, 75.2% improved and 13.3% showed no improvement. Patients who underwent ADX had a tendency toward better outcome than those patients treated with MRA (cured/controlled: 19.0 vs. 3.8%, improved: 65.5 vs. 88.5% and no improvement: 15.5 vs. 7.8%). Female patients had better outcome than males (female: 26.6% cured/controlled, 65.9% improved and 7.3% no improvement; male: 2.8% cured/controlled, 80.6% improved and 16.7% no improvement). Women were younger [47 (39, 60) vs. 54 (46, 60) years, $P = 0.02$] but there were no significant differences in frequency of hypokalemia (female 85%, male 79%, $P = 0.462$) or ADX (female 59%, male 49%, $P = 0.430$) between male and female patients. In the whole study population, better outcome was associated with lower baseline copeptin levels (Fig. 4). However, when analyzing male and female patients separately, no significant differences in copeptin levels could be found between outcome categories. Nevertheless, within each outcome category, copeptin levels decreased significantly after therapy initiation (Fig. 4).

In sex-adjusted and age-adjusted binary logistic regression models, hypokalemia and high SBP at baseline were predictive for good outcome (cured/controlled and significantly improved). Copeptin and hsCRP could not predict outcome, neither in the sex-adjusted and age-adjusted model nor when additionally adjusted for other variables (BP, BMI, eGFR, ARR, aldosterone, hypokalemia, lateralization and duration of hypertension). In stepwise logistic regression analysis, the combination of sex, hypokalemia, lateralization index and BP were parameters with the best predictive value (Nagelkerkes $R^2 = 0.456$).

DISCUSSION

Herein, we investigated the cardiovascular markers copeptin and hsCRP in a large and well defined cohort of primary aldosteronism patients and patients from the large population-representative KORA F4 survey matched for sex, renal function, BMI, age and BP. Our analysis demonstrates that primary aldosteronism patients had higher hsCRP levels at baseline compared with controls indicating enhanced inflammatory activity. From animal studies, it is well known that aldosterone excess induces inflammatory processes in several tissues such as in heart and kidney. In primary aldosteronism patients, inflammatory mechanisms that result in hypertrophy of intima-media and cardiac hypertrophy, atherosclerosis, chronic kidney disease and the metabolic syndrome are more prevalent than in patients with essential hypertension [26,27]. In addition, markers of oxidative stress, which play a key role in inflammatory processes, have been observed to be increased in primary aldosteronism patients compared with patients with essential hypertension and nonhypertensive patients [28]. Here, we corroborate these observations in a large cohort of well defined primary aldosteronism patients. By measuring hsCRP as a surrogate marker for ongoing inflammatory processes, our results suggest that inflammation might be enhanced in primary aldosteronism compared with controls. Our data contrast with two earlier studies that did not find significant differences in hsCRP levels in primary aldosteronism compared with essential hypertension,

which relied on a small sample size of only 15 and 30 patients, respectively [29,30].

HsCRP has also been described as a good marker for the metabolic syndrome [31]. In our study, the controls, who were matched for BMI, had lower BP levels and a worse lipid profile than primary aldosteronism patients at baseline. It is well documented that glucose homeostasis is disturbed in primary aldosteronism patients at diagnosis [26], which results in a higher rate of the metabolic syndrome [26]. This might also explain our finding of elevated hsCRP levels in primary aldosteronism patients at diagnosis.

In our large primary aldosteronism cohort, specific intervention by ADX or MRA treatment was associated with reduced hsCRP levels after 13 months and indicates a reduced cardiovascular risk. In large prospective population-based studies like the MONICA KORA research platform [4] and the EPIC-Norfolk [5] study, it has been shown that levels of hsCRP can predict coronary artery disease in apparently healthy patients and increased levels of hsCRP are associated with cardiovascular as well as all-cause mortality [6]. Also, the number of patients with two-fold cardiovascular risk (hsCRP >3 mg/l) could be reduced significantly after initiation of specific primary aldosteronism treatment.

However, hsCRP could not predict outcome of treatment success in our primary aldosteronism cohort, neither in the sex-adjusted and age-adjusted model nor when additionally adjusted for other variables including BP, BMI, eGFR, ARR, aldosterone, hypokalemia lateralization index and duration of hypertension. These findings are in contrast to a recent study from Taiwan, which stated that high hsCRP levels might be predictive for residual hypertension in unilateral primary aldosteronism after ADX, however, only when adjusted for several other parameters [16].

Recently, copeptin has been established as a reliable marker of circulating AVP levels [7]. It is secreted in equimolar amounts and thus reflects activation of the AVP system [8]. Copeptin has been shown to correlate with type 2 diabetes [23] and all-cause mortality in patients with cardiovascular and cerebrovascular disease [11]. Underlying mechanisms are not fully explained to date. However, effects of increased AVP levels are probably mediated through the three different vasopressin receptors [32]. First, the V1 receptor among others expressed in vascular smooth muscle cells, liver cells and myocardial cells mediates vasoconstriction, gluconeogenesis and influences myocardial protein synthesis. Second, the renal V2 receptor mediates the antidiuretic effect of AVP. Third, the V3 receptor mediates activation of the adrenocorticotropic axis [12,32,33]. In summary, AVP interferes with multiple pathophysiological pathways known to be associated with an increased cardiovascular risk. Therefore, it was intriguing to investigate copeptin in the context of overt aldosterone secretion, which is associated with a four-fold to 10-fold higher prevalence of cardiovascular and cerebrovascular events than those observed in patients with essential hypertension [14,15,34]. Interestingly, in our study, copeptin levels were not higher in primary aldosteronism patients at baseline compared with the matched control cohort. This is in contrast to a recent report stating elevated copeptin levels in primary aldosteronism patients compared with hypertensive controls [17]. However, the former study did not include a matching process [17], which seems to be mandatory, as copeptin is known to correlate with renal function, sex and BMI [35,36]. Bearing our findings in mind, this could be confirmed in primary aldosteronism patients as well as in the control cohort in the present study. Differences in these parameters between primary aldosteronism and essential hypertension patients might have influenced the results of the former study and might explain the different findings in our study [17].

Median copeptin levels decreased significantly by about 3 U after primary aldosteronism therapy initiation in our cohort. A meta-analysis of 28 studies with more than 14 000 patients with cardiovascular and cerebrovascular disease found mortality to be increased by 2% per unit copeptin [11]. In addition, high copeptin levels recently predicted coronary artery disease as well as cardiovascular and all-cause mortality in a large longitudinal population-based study ($n=5386$) [12]. Our data support the hypothesis that specific treatment is effectively reducing mortality in primary aldosteronism patients. This is in line with a previous study of our group that found 10-year all-cause mortality in treated primary aldosteronism patients not to be increased compared with essential hypertension controls [37].

In our whole primary aldosteronism patient cohort, we were able to demonstrate a correlation of baseline copeptin levels with outcome. However, this observation must be interpreted with caution, because this correlation did not remain significant when analyzing the data separately within each sex group. Nevertheless, this inconsistency can be due to the rather small subgroup size. In stepwise logistic regression analysis, it was not copeptin itself, but the combination of sex, hypokalemia, lateralization and BP, that predicted the outcome best. It has been reported that copeptin seems to be useful in diagnosis, rule out and outcome prediction in some acute diseases like pneumonia, sepsis, stroke, heart failure and MI [9,10]. Judging from our findings in the present study, this is rather not the case in a chronic disease such as primary aldosteronism. However, this applies only to our current definition of outcome in primary aldosteronism which is subject of controversial discussion.

So far, data regarding AVP and copeptin levels in primary aldosteronism are scarce [16,17,38]. For the interpretation of copeptin data in primary aldosteronism, it is important to appreciate regulatory mechanisms underlying AVP secretion. AVP release is stimulated by hyperosmolality, hypovolemia and hypotension and is inhibited by hypoosmolality, hypervolemia and hypertension [39]. In patients with primary aldosteronism, both stimulatory and inhibitory stimuli are coexisting and it is difficult to speculate which factor is predominant. On one hand, primary aldosteronism patients often have an increased serum sodium concentration, which might induce AVP release. On the other hand, in primary aldosteronism, extracellular volume and hypertension potentially inhibit AVP release [40], and reduced AVP levels in primary aldosteronism have been reported [41]. In addition, atrial natriuretic peptide (ANP) is a known inhibitor of AVP release [42]. In primary aldosteronism patients, ANP is elevated [43] possibly causing a

further negative effect on AVP release. This might explain that AVP levels in primary aldosteronism are not different to those of normal patients [38]. However, in primary aldosteronism patients, an acute water load did not suppress AVP release indicating a less-responsive AVP system in primary aldosteronism, which normalized after therapy [38]. In our primary aldosteronism patient cohort, slightly but significantly reduced sodium levels and reduced osmolality compared with baseline could be observed at follow-up. Unfortunately, osmolality could not be assessed in the control group. As osmolality is mainly defined by sodium levels and sodium levels (as well as potassium levels) in the control group and in primary aldosteronism patients at follow-up were similar, a similar osmolality between the two groups is probable. In addition, BP was similar in the control group and in primary aldosteronism patients at follow-up leading to the hypothesis that additional regulating factors to those discussed above might influence copeptin levels. Primary aldosteronism therapy reduced copeptin levels thus not only by reducing osmolality but probably also by influencing other regulatory parameters like volume homeostasis and BP. Consequently, in our study, we can support the finding that copeptin levels are not different between primary aldosteronism patients and control patients matched for sex, BMI and renal function.

Some limitations apply to our study. Complete data sets, including blood samples of baseline and follow-up after 1 year, were only available for 113 out of 251 (45%) prospective patients included in the two participating study centers of the German Conn Registry. Furthermore, the control cohort of the KORA F4 study is not a strictly hypertensive cohort. We thus cannot exclude that BP differences might influence some of our results. However, by matching BP as close as possible, we tried to minimize the difference in BP levels between the two groups.

In conclusion, hsCRP levels, but not copeptin levels, were higher in primary aldosteronism patients at baseline compared with matched controls. At follow-up, copeptin and hsCRP decreased significantly in primary aldosteronism patients due to therapy indicating reduced risk of cardiovascular disease and mortality. In addition, men had higher copeptin and hsCRP levels at baseline and follow-up compared with women. The combination of sex, hypokalemia, lateralization and BP predicted the outcome best, whereas copeptin and hsCRP could not predict outcome in primary aldosteronism in respect of our current definition of outcome.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, *et al.* Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2013; 380:2095–2128.
2. D'Agostino RB, Pencina MJ, Massaro JM, Coady S. Cardiovascular disease risk assessment: insights from Framingham. *Glob Heart* 2013; 8:11–23.
3. Epstein FH, Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; 340:115–126.
4. Koenig W, Sund M, Fröhlich M, Fischer H-G, Löwel H, Döring A, *et al.* C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 1999; 99:237–242.
5. Boekholdt SM, Hack CE, Sandhu MS, Luben R, Bingham SA, Wareham NJ, *et al.* C-reactive protein levels and coronary artery disease incidence and mortality in apparently healthy men and women: the EPIC-Norfolk prospective population study 1993–2003. *Atherosclerosis* 2006; 187:415–422.
6. Koenig W, Khuseyinova N, Baumert J, Meisinger C. Prospective study of high-sensitivity C-reactive protein as a determinant of mortality: results from the MONICA/KORA Augsburg Cohort Study, 1984–1998. *Clin Chem* 2008; 54:335–342.
7. Morgenthaler NG. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 2006; 52:112–119.
8. Balanescu S, Kopp P, Gaskill MB, Morgenthaler NG, Schindler C, Rutishauser J. Correlation of plasma copeptin and vasopressin concentrations in hypo-, iso-, and hyperosmolar states. *J Clin Endocrinol Metab* 2011; 96:1046–1052.
9. Dobsa L, Cullen Edozien K. Copeptin and its potential role in diagnosis and prognosis of various diseases. *Biochem Medica* 2013; 23:172–192.
10. Łukaszyk E, Małyszko J. Copeptin: pathophysiology and potential clinical impact. *Adv Med Sci* 2015; 60:335–341.
11. Sun H, Sun T, Ma B, Yang B, Zhang Y, Huang D, *et al.* Prediction of all-cause mortality with copeptin in cardio-cerebrovascular patients: A meta-analysis of prospective studies. *Peptides* 2015; 69:9–18.
12. Tasevska I, Enhörning S, Persson M, Nilsson PM, Melander O. Copeptin predicts coronary artery disease cardiovascular and total mortality. *Heart* 2016; 102:127–132.
13. Piaditis G, Markou A, Papanastasiou L, Androulakis II, Kaltsas G. Progress in aldosteronism: a review of the prevalence of primary aldosteronism in prehypertension and hypertension. *Eur J Endocrinol* 2015; 172:R191–R203.
14. Milliez P, Girerd X, Plouin P-F, Blacher J, Safar ME, Mourad J-J. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol* 2005; 45:1243–1248.
15. Born-Frontsberg E, Reincke M, Rump LC, Hahner S, Diederich S, Lorenz R, *et al.* Cardiovascular and cerebrovascular comorbidities of hypokalemic and normokalemic primary aldosteronism: results of the German Conn's Registry. *J Clin Endocrinol Metab* 2009; 94:1125–1130.
16. Wu V-C, Huang K-H, Peng K-Y, Tsai Y-C, Wu C-H, Wang S-M, *et al.* Prevalence and clinical correlates of somatic mutation in aldosterone producing adenoma-Taiwanese population. *Sci Rep* 2015; 5:11396.
17. Nogueira-Silva L, Blanchard A, Curis E, Lorthioir A, Zhygalina V, Bergerot D, *et al.* Deciphering the role of vasopressin in primary aldosteronism. *J Clin Endocrinol Metab* 2015; 100:3297–3303.
18. Reincke M, Rump LC, Quinkler M, Hahner S, Diederich S, Lorenz R, *et al.* Risk factors associated with a low glomerular filtration rate in primary aldosteronism. *J Clin Endocrinol Metab* 2009; 94:869–875.
19. Fischer E, Beuschlein F, Bidlingmaier M, Reincke M. Commentary on the Endocrine Society Practice Guidelines: consequences of

adjustment of antihypertensive medication in screening of primary aldosteronism. *Rev Endocr Metab Disord* 2011; 12:43–48.
20. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, *et al.* Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2008; 93:3266–3281.
21. Klüppelholz B, Thorand B, Koenig W, de las Heras Gala T, Meisinger C, Huth C, *et al.* Association of subclinical inflammation with deterioration of glycaemia before the diagnosis of type 2 diabetes: the KORA S4/F4 study. *Diabetologia* 2015; 58:2269–2277.
22. Rathmann W, Strassburger K, Heier M, Holle R, Thorand B, Giani G, *et al.* Incidence of type 2 diabetes in the elderly German population and the effect of clinical and lifestyle risk factors: KORA S4/F4 cohort study. *Diabet Med* 2009; 26:1212–1219.
23. Then C, Kowall B, Lechner A, Meisinger C, Heier M, Koenig W, *et al.* Plasma copeptin is associated with type 2 diabetes in men but not in women in the population-based KORA F4 study. *Acta Diabetol* 2015; 52:103–112.
24. Pearson TA. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; 107:499–511.
25. Weigel M, Riester A, Hanslik G, Lang K, Willenberg HS, Endres S, *et al.* Postsaline infusion test aldosterone levels indicate severity and outcome in primary aldosteronism. *Eur J Endocrinol* 2015; 172:443–450.
26. Hanslik G, Wallaschofski H, Dietz A, Riester A, Reincke M, Allolio B. Increased prevalence of diabetes mellitus and the metabolic syndrome in patients with primary aldosteronism of the German Conn Registry. *Eur J Endocrinol* 2015; 173:655–675.
27. Catena C, Colussi G, Marzano L, Sechi L. Aldosterone and the heart: from basic research to clinical evidence. *Horm Metab Res* 2012; 44:181–187.
28. Petramala L, Pignatelli P, Carnevale R, Zinnamosca L, Marinelli C, Settevendemmie A, *et al.* Oxidative stress in patients affected by primary aldosteronism. *J Hypertens* 2014; 32:2022–2029.
29. Irita J, Okura T, Manabe S, Kurata M, Miyoshi K, Watanabe S, *et al.* Plasma osteopontin levels are higher in patients with primary aldosteronism than in patients with essential hypertension. *Am J Hypertens* 2006; 19:293–297.
30. Stehr CB, Mellado R, Ocaranza MP, Carvajal CA, Mosso L, Becerra E, *et al.* Increased levels of oxidative stress, subclinical inflammation, and myocardial fibrosis markers in primary aldosteronism patients. *J Hypertens* 2010; 28:2120–2126.
31. Acevedo M, Varleta P, Kramer V, Valentino G, Quiroga T, Prieto C, *et al.* Comparison of lipoprotein-associated phospholipase A2 and high sensitive C-reactive protein as determinants of metabolic syndrome in subjects without coronary heart disease: in search of the best predictor. *Int J Endocrinol* 2015; 2015:1–7.
32. Holmes CL, Landry DW, Granton JT. Science review: vasopressin and the cardiovascular system part 1 – Receptor physiology. *Crit Care* 2003; 7:427.
33. Lanfear DE, Sabbah HN, Goldsmith SR, Greene SJ, Ambrosy AP, Fought AJ, *et al.* Association of arginine vasopressin levels with outcomes and the effect of V2 blockade in patients hospitalized for heart failure with reduced ejection fraction: insights from the EVEREST trial. *Circ Heart Fail* 2013; 6:47–52.
34. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, *et al.* Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Intern Med* 2008; 168:80–85.
35. Bhandari SS, Loke I, Davies JE, Squire IB, Struck J, Ng LL. Gender and renal function influence plasma levels of copeptin in healthy individuals. *Clin Sci* 2009; 116:257–263.
36. Bar-Shalom D, Poulsen MK, Rasmussen LM, Diederichsen AC, Sand NP, Henriksen JE, *et al.* Plasma copeptin as marker of cardiovascular disease in asymptomatic type 2 diabetes patients. *Diab Vasc Dis Res* 2014; 11:448–450.
37. Reincke M, Fischer E, Gerum S, Merkle K, Schulz S, Pallauf A, *et al.* Observational study mortality in treated primary aldosteronism the German Conn's Registry. *Hypertension* 2012; 60:618–624.
38. Kimura T, Yamamoto T, Ohta M, Ota K, Shoji M, Funyu T, *et al.* Impaired vasopressin suppression and enhanced atrial natriuretic hormone release following an acute water load in primary aldosteronism. *Eur J Endocrinol* 1997; 137:154–161.
39. McKenna K, Thompson C. Osmoregulation in clinical disorders of thirst appreciation. *Clin Endocrinol (Oxf)* 1998; 49:139–152.
40. Robertson GL, Shelton RL, Athar S. The osmoregulation of vasopressin. *Kidney Int* 1976; 10:25–37.
41. Padfield PL. Vasopressin in hypertension. *Am Heart J* 1977; 94:531–532.
42. Inoue M, Kimura T, Ota K, Iitake K, Shoji M, Sato K, *et al.* Effect of vasopressin on atrial natriuretic peptide release and renal function in dogs. *Am J Physiol Endocrinol Metab* 1988; 255:E449–E455.
43. Kato J, Etoh T, Kitamura K, Eto T. Atrial and brain natriuretic peptides as markers of cardiac load and volume retention in primary aldosteronism. *Am J Hypertens* 2005; 18:354–357.

Reviewer's Summary Evaluation

Reviewer 1

As is often the case with manuscripts emanating from the German Conn's Registry this is a well written study with a robustly phenotyped PA population. The control population is also rigorously phenotyped and followed up. Both populations have previously been utilised in well designed studies published within quality peer-reviewed journals. The study explores the relationship between two biomarkers (hs CRP and copeptin) which have been positively associated with an increased risk of CV disease and clinical features of PA as well as the effect of PA treatment on biomarker levels. The hypothesis (although not expressly stated in the introduction) is that, given PA is associated with BP-independent increase in CV risk, there will be an elevation of these biomarkers in PA vs control patients with a substantial reduction with treatment. This is indeed the case with hs CRP (and this information is largely confirmatory), but the relationship of PA and copeptin is less clear cut.

Reviewer 2

The research of Remde *et al.* is focused on evaluation of novel cardiovascular markers, as well as Copeptin and high-sensitive CRP, in a large group of patients affected by Primary Aldosteronism, revaluated after specific treatment, confirming the reduction of overall cardiovascular risk in PA patient. The strengths of this study are the significant number of patients enrolled, the high-quality of research methodology and the evaluation of further cardiovascular markers, suggesting novel knowledge of pathophysiological alterations of Primary Aldosteronism.