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ARTICLE

Serum-soluble (pro)renin receptor concentration as a biomarker for organ damage in primary aldosteronism

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Abstract

Primary aldosteronism is characterized by inappropriate overproduction of aldosterone by adrenal lesions and leads to hypertension. Excess aldosterone causes organ damage; therefore, finding a biomarker for organ damage risk is vital. The (pro)renin receptor regulates the tissue renin–angiotensin–aldosterone system. The blood soluble (pro)renin receptor concentration is a candidate biomarker that reflects the activity of the tissue renin–angiotensin–aldosterone system. This study investigated the relationships between serum soluble (pro)renin receptor concentrations and indices of organ damage in patients with primary aldosteronism. We examined plasma aldosterone and serum soluble (pro)renin receptor concentrations in patients with primary aldosteronism and evaluated the relationships between these values and organ damage indices, such as the cardio-ankle vascular index, urinary albumin excretion, estimated glomerular filtration rate, and high-sensitivity C-reactive protein levels. We enrolled 121 patients with primary aldosteronism (46 males, 54.9 ± 12.2 years of age). Serum soluble (pro)renin receptor concentrations were significantly positively correlated with the cardio-ankle vascular index, urinary albumin excretion, and high-sensitivity C-reactive protein levels and negatively associated with estimated glomerular filtration rates, independent of other factors. Plasma aldosterone concentrations showed no significant relationships with these indices. In patients with primary aldosteronism, serum soluble (pro)renin receptor concentrations, but not plasma aldosterone concentrations, showed significant associations with organ damage, suggesting that the serum soluble (pro)renin receptor level could be a high-risk biomarker of organ damage.

Keywords Arteriosclerosis · cardio-ankle vascular index · high-sensitivity C-reactive protein · tissue renin–angiotensin–aldosterone system · urinary albumin secretion

Introduction

Primary aldosteronism (PA) refers to autonomous production of aldosterone by an adrenal lesion. PA is the most frequent secondary cause of hypertension, and hypertension in 5.9% of affected patients can be the result of PA [1]. The excess aldosterone causes not only hypertension but also arteriosclerosis, kidney injury, and cardiac fibrosis [2–4]. In addition, patients with PA are at a higher risk of developing cardiovascular disease (CVD) than those with essential hypertension (EH) [5, 6]. Hypokalemia and unilaterality of aldosterone overproduction have been shown to be associated with CVD development in patients with PA [7], suggesting that unilateral aldosterone-producing adenoma (APA) may be associated with increased risk of organ damage in PA. However, adrenal venous sampling (AVS) is required for diagnosis of unilateral APA. Plasma aldosterone concentrations (PACs) do not show associations with CVD risk in patients with PA [8, 9]. Therefore, identifying patients with PA at increased risk of organ damage is difficult, and identification of a simple biomarker that can predict the risk of organ damage and as a result can indicate patients who need intensive care would be beneficial. Discovered by Nguyen and Muller [10], (pro)renin receptor ((P)RR) binds renin and/or prorenin, a nonactivated precursor of renin, to activate the tissue renin–angiotensin–aldosterone system (RAAS) [11] and mitogen-activated protein kinase pathways [10]. This activation leads to the expression of profibrotic and inflammatory genes in an angiotensin-independent manner [10]. (P)

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RR is expressed in vital organs throughout the body, including the brain, heart, kidneys, and adrenal glands [10, 12]. (P)RR is cleaved into ATP6AP2, an accessory protein of vacuolar adenosine triphosphatase, and soluble (P)RR (s(P)RR) [13]. S(P)RR is secreted into the extracellular space and is ultimately found in blood [14]. These findings suggest that s(P)RR can serve as a biomarker that reflects the activity of tissue RAAS and the activity of (P)RR.

Serum s(P)RR levels are reportedly associated with atherosclerosis [15], renal dysfunction [16], and progressive renal dysfunction [17], which are frequently observed in patients with PA. Therefore, s(P)RR levels may be associated with organ damage in patients with PA. The present study aimed to determine whether serum s(P)RR levels are associated with organ damage in patients with PA and to investigate whether any observed associations are independent of other factors.

Methods

Patients

This study enrolled patients who visited the Department of Endocrinology and Hypertension at the Tokyo Women's Medical University Hospital between October 2011 and February 2016 and were diagnosed with PA. All patients were Japanese.

Three confirmatory tests were performed for diagnosis of PA after overnight fasting. Every patient was on a 10 g sodium diet during hospitalization. For the captopril challenge test (CCT), patients received 50 mg of captopril orally after lying flat for at least 30 min. Blood samples were drawn for measurement of PAC and plasma renin activity (PRA) before captopril administration and at 60 and 90 min after captopril administration. The CCT was considered positive if the aldosterone/renin ratio was over 200 at either 60 or 90 min after administration of captopril [18]. For the furosemide upright test (FUT), the patients were kept supine for at least 30 min before measurement of basal PRA. The patients were then administered an intravenous bolus of 40 mg of furosemide. After 2 h in an upright posture, PRA was measured. The FUT was considered positive if the post-FUT PRA was below 2 ng/mL/h [18]. For the saline infusion test (SIT), the patients were kept in a supine position for at least 30 min before measurement of the basal PAC. Then, 2 L of 0.9% NaCl was administered intravenously over 4 h, and the PAC was measured. The SIT was considered positive if the PAC after SIT was over 60 pg/mL [18]. PA diagnosis required at least two positive confirmatory tests according to the Guideline for the Diagnosis and Treatment of Primary Aldosteronism by the Japan Endocrine Society (2009) [18].

Patients were either left untreated or treated with a calcium channel blocker (CCB) alone; cilnidipine, which inhibits L-type and N-type calcium channels and could affect the RAAS, was not used [19]. Patients who had experienced hemorrhagic stroke or cardiac infarction in the previous 6 months, pregnant women, and those with peripheral vascular disease, malignant disease, or uncontrolled diabetes mellitus (hemoglobin A1c [HbA1c] > 10.0%) were excluded. All patients were enrolled after obtaining informed consent, and the procedures were approved by the ethics committee of the Tokyo Women's Medical University.

Evaluation of PA subtypes

To determine the PA subtype, AVS was performed as previously described [20]. Cannulation of the adrenal vein was confirmed to be successful by an adrenal vein-to-inferior vena cava (IVC) cortisol ratio > 5.0 after adrenocorticotropic hormone (ACTH) stimulation. The aldosterone:cortisol ratio (ACR) was calculated to correct the PAC for dilution by nonadrenal blood. The lateralized ratio (LR) was calculated to determine the dominant side of plasma aldosterone production by dividing the ACR of the dominant adrenal vein by that of the nondominant adrenal vein. In addition, we calculated the contralateral ratio (CR) to determine the suppression of plasma aldosterone production by dividing the ACR of the nondominant adrenal vein by that of the IVC. The LR and CR were calculated after ACTH stimulation. APA was diagnosed if the PAC in the dominant adrenal vein was ≥14,000 pg/mL, the LR was ≥2.6, and the CR was <1, as previously described [18].

Background factors

At enrollment, information was collected on sex, age, PA subtype, use of a CCB, and body mass index (BMI).

Blood pressure and pulse rate

Blood pressure (BP) and pulse rate (PR) were measured with the patient in a sitting position after resting for at least 5 min using an automated sphygmomanometer (Omron BP-203RVIII Oscillometer; Nippon Colin, Tokyo, Japan)

Urinary testing

Spot urine samples were obtained, and creatinine and albumin concentrations, sodium (Na) levels and potassium (K) levels were quantified using standardized assessment methods at our

clinical laboratory. Albumin excretion was determined by dividing the albumin values by the creatinine concentrations.

Blood testing

Blood samples were taken after an overnight fast. Hemoglobin, creatinine, uric acid, high-sensitivity C-reactive protein (hsCRP), brain natriuretic peptide (BNP), albumin, Na, K, HbA1c, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglyceride were measured using standard laboratory methods at our clinical laboratory. Serum s(P)RR levels were measured using an enzyme-linked immunosorbent assay kit (Takara Bio, Otsu City, Japan) consisting of a solid-phase sandwich enzyme-linked immunosorbent assay with a highly specific antibody. PRA and the PAC were measured using radioimmunoassay at an external laboratory (SRL, Tokyo, Japan). The estimated glomerular filtration rate (eGFR) was calculated using the following equation: eGFR ($mL/min/1.73\ m^2$) = $194 \times creatinine^{-1.094} \times age^{-0.287}$ (×0.739, if female).

Cardio-ankle vascular index

The cardio-ankle vascular index (CAVI) of arteriosclerosis was measured using a VaSera VS-1500AN vascular screening system (Fukuda Denshi, Tokyo, Japan), as previously described [21]. The CAVI is a noninvasive marker for arterial stiffness, which is independent of BP.

Statistical analyses

Relationships between background factors and s(P)RR levels were examined using single correlation and multiple regression analyses. All data are expressed as the mean ± standard deviation or as the median value (interquartile range). Single correlation analysis was performed using Spearman's rank correlation to determine the association between background factors and s(P)RR or parameters of organ damage such as the CAVI, urinary albumin excretion (UAE), eGFR, and hsCRP. Multiple regression analysis was used to identify each possible determinant of organ damage. Backward and forward stepwise analysis with a threshold of $p < 0.2$ was applied to shorten the regression model, testing variables that were possibly associated with organ damage, such as age, systolic BP, uric acid, LDL-cholesterol, HbA1c, and s(P)RR (plus eGFR in the analysis where the CAVI or hsCRP was tested as a dependent variable). Selected variables were then entered into linear regression analysis. Significance was defined at $p < 0.05$. All statistical analyses were performed using JMP Pro version 12 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the study patients

A total of 121 patients with PA were enrolled in this study. Table 1 shows the background factors, urinary and blood data, and CAVIs of these patients.

Serum s(P)RR levels

The median serum s(P)RR level was 22.4 (interquartile range: 19.6–26.0) ng/mL, and the values were not different between males (23.1 [20.6–27.6] ng/mL, $n = 46$) and females (21.6 [19.0–24.9], $n = 75$), between patients with CCB (23.5 [20.5–26.8], $n = 37$) and without CCB use (22.0 [19.1–25.5], $n = 84$), or between patients with APA (23.1 [21.0–28.1], $n = 24$) and those not diagnosed with APA (22.2 [19.4–25.5], $n = 97$). As there were no differences in s(P)RR levels associated with sex, use of a CCB, or PA subtype, further analyses were performed without dividing the patients according to these factors.

Single correlation analysis with serum s(P)RR levels

In single correlation analysis with serum s(P)RR levels, age, UAE, creatinine, uric acid, hsCRP, Na, HbA1c, triglyceride, and the CAVI were significantly positively correlated with serum s(P)RR concentration, and eGFR and HDL-cholesterol were significantly negatively correlated with the serum s(P)RR concentration (Table 2). Neither PRA nor PACs showed correlations with serum s(P)RR levels (Table 2).

CAVI and serum s(P)RR levels

In single correlation analysis, age, UAE, urinary K, uric acid, and HbA1c were significantly positively correlated with the CAVI, a marker for arterial stiffness, and eGFR was significantly negatively correlated with the CAVI (Table 3). In terms of RAAS components, the serum s(P)RR concentration, but not PRA or the PAC, was significantly positively correlated with the CAVI (Table 3). It is known that aging, hypertension, hyperuricemia, dyslipidemia, and diabetes mellitus cause organ damage, including arteriosclerosis and kidney injury. To determine whether the correlation between the CAVI and the serum s(P)RR level was independent of these factors, stepwise multiple regression analyses that tested age, systolic BP, eGFR, uric acid, HbA1c, LDL-cholesterol, and the serum s(P)RR level as independent variables were performed. Age, uric acid, and s(P)RR were selected as possible independent variables, and age and s(P)RR were significantly positively correlated with the CAVI (Table 4).

Table 1 Characteristics of study patients

Characteristics	$n = 121$
Male sex (%)	46 (38)
Age (y)	54.9 ± 12.2
Number with aldosterone-producing adenoma (%)	24 (20)
Medication	
Calcium channel blocker (%)	37 (31)
BMI (kg/m^2)	24.2 ± 3.8
Blood pressure and pulse rate	
SBP (mmHg)	135 (124–145)
DBP (mmHg)	83 (75–90)
Pulse rate (beats/min)	68.7 ± 11.9
Urinary test	
UAE (mg/gCr)	9.3 (6.1–25.4)
Na (mEq/mgCr)	13.3 (8.53–22.2)
K (mEq/mgCr)	3.1 (2.5–4.9)
Na/K	3.7 (2.5–6.1)
Blood tests	
Hemoglobin (g/dL)	14.0 ± 1.3
Creatinine (mg/dL)	0.64 (0.58–0.78)
eGFR (mL/min/1.73 m^2)	80.2 (70.9–88.8)
Uric acid (mg/dL)	5.1 ± 1.2
hsCRP (ng/mL)	459 (227–1180)
BNP (pg/mL)	16.7 (9.9–44.2)
Albumin (g/dL)	4.3 (4.0–4.5)
Na (mEq/L)	142 ± 2
K (mEq/L)	3.9 (3.7–4.1)
Na/K	36.2 (34.6–38.9)
HbA1c (%)	5.7 (5.5–6.0)
LDL-chol (mg/dL)	129 (101–150)
HDL-chol (mg/dL)	57 (47–66)
Triglyceride (mg/dL)	118 (85–155)
RAAS components	
PRA (ng/mL/h)	0.3 (0.2–0.5)
PAC (pg/mL)	177 (123–265)
Aldosterone renin ratio	504 (330–823)
s(P)RR (ng/mL)	22.4 (19.6–26.0)
Physiological function tests	
CAVI	8.0 ± 1.2

Data are presented as mean ± standard deviation, median (interquartile range), or *n* (%)

BMI Body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *UAE* urinary albumin excretion, *eGFR* estimated glomerular filtration rate, *hsCRP* high-sensitivity C-reactive protein, *BNP* brain natriuretic peptide, *HbA1c* hemoglobin A1c, *LDL-chol* low-density lipoprotein cholesterol, *HDL-chol* high-density lipoprotein cholesterol, *RAAS* renin–angiotensin–aldosterone system, *PRA* plasma renin activity, *PAC* plasma aldosterone concentration, *s(P)RR* soluble (pro)renin receptor, *CAVI* cardio-ankle vascular index

Table 2 Single correlation analyses with serum soluble (pro)renin receptor levels

Variables	ρ	p
Age	0.229	0.014
Sex	0.156	0.095
Subtype of aldosteronism		
Aldosterone-producing adenoma	0.123	0.188
BMI	0.192	0.174
Blood pressure and pulse rate		
SBP	0.107	0.272
DBP	−0.157	0.107
Pulse rate	−0.091	0.372
Urinary test		
UAE	0.253	0.012
Na	−0.095	0.426
K	0.052	0.663
Na/K	−0.126	0.291
Blood tests		
Hemoglobin	0.045	0.643
Creatinine	0.256	0.006
eGFR	−0.249	0.008
Uric acid	0.296	0.002
hsCRP	0.349	<0.001
BNP	−0.047	0.646
Albumin	0.103	0.296
Na	0.230	0.016
K	0.107	0.267
Na/K	−0.076	0.429
HbA1c	0.247	0.012
LDL-chol	0.014	0.889
HDL-chol	−0.189	0.049
Triglyceride	0.277	0.003
RAAS components		
PRA	0.003	0.979
PAC	0.091	0.372
Physiological function tests		
CAVI	0.305	0.004

BMI Body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *UAE* urinary albumin excretion, *eGFR* estimated glomerular filtration rate, *hsCRP* high-sensitivity C-reactive protein, *BNP* brain natriuretic peptide, *HbA1c* hemoglobin A1c, *LDL-chol* low-density lipoprotein cholesterol, *HDL-chol* high-density lipoprotein cholesterol, *RAAS* renin–angiotensin–aldosterone system, *PRA* plasma renin activity, *PAC* plasma aldosterone concentration, *s(P)RR* soluble (pro)renin receptor, *CAVI* cardio-ankle vascular index

Kidney function and serum s(P)RR levels

Similar analyses were performed for two indices of kidney function: UAE and eGFR. In single correlation analyses, systolic BP, pulse rate, urinary K, and serum Na/K were

Table 3 Single correlation analyses with organ damage indices

Variables	CAVI		UAE		eGFR		hsCRP	
	ρ	p	ρ	p	ρ	p	ρ	p
Age	0.762	<0.001	0.067	0.512	−0.542	<0.001	0.210	0.033
Sex	0.06	0.574	−0.049	0.631	−0.141	0.129	0.060	0.547
BMI	−0.245	0.17	0.114	0.472	0.113	0.417	0.463	0.003
Blood pressure and pulse rate								
SBP	−0.010	0.928	0.278	0.007	0.029	0.760	−0.036	0.728
DBP	−0.146	0.182	0.109	0.299	0.147	0.123	−0.19	0.064
Pulse rate	0.114	0.319	0.352	0.001	−0.093	0.353	−0.131	0.223
Urinary test								
UAE	0.305	0.007	—	—	−0.011	0.914	0.055	0.602
Na	0.012	0.930	0.130	0.294	0.222	0.061	0.157	0.190
K	0.281	0.030	0.290	0.017	−0.006	0.958	0.163	0.176
Na/K	−0.141	0.283	−0.005	0.969	0.251	0.034	0.041	0.733
Blood tests								
Hemoglobin	0.027	0.807	−0.166	0.109	0.020	0.839	0.036	0.728
Creatinine	0.175	0.097	−0.01	0.921	−0.737	<0.001	0.078	0.432
eGFR	−0.433	<0.001	−0.011	0.914	—	—	−0.101	0.312
Uric acid	0.224	0.034	0.045	0.662	−0.285	0.002	0.319	0.001
hsCRP	0.164	0.144	0.055	0.602	−0.101	0.312	—	—
BNP	0.063	0.574	0.027	0.798	−0.086	0.388	−0.035	0.731
Albumin	−0.185	0.093	−0.156	0.133	0.066	0.502	0.003	0.979
Na	0.175	0.105	0.177	0.084	−0.190	0.044	0.921	0.360
K	0.149	0.170	−0.312	0.002	−0.143	0.130	0.171	0.088
Na/K	−0.076	0.429	0.333	0.001	0.123	0.196	−0.164	0.102
HbA1c	0.309	0.005	0.120	0.248	−0.026	0.793	0.361	<0.001
LDL-chol	−0.001	0.993	−0.034	0.743	−0.122	0.199	0.173	0.082
HDL-chol	0.132	0.225	−0.123	0.228	−0.018	0.851	−0.381	<0.001
Triglyceride	0.072	0.504	0.049	0.633	−0.088	0.352	0.316	0.001
RAAS components								
PRA	0.106	0.377	0.128	0.255	0.006	0.953	−0.009	0.938
PAC	0.048	0.685	0.165	0.134	−0.113	0.266	0.009	0.935
s(P)RR	0.305	0.004	0.253	0.012	−0.249	0.008	0.349	<0.001

CAVI Cardio-ankle vascular index, *eGFR* estimated glomerular filtration rate, *hsCRP* high-sensitivity C-reactive protein, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *UAE* urinary albumin excretion, *BNP* brain natriuretic peptide, *HbA1c* hemoglobin A1c, *LDL-chol* low-density lipoprotein cholesterol, *HDL-chol* high-density lipoprotein cholesterol, *RAAS* renin–angiotensin–aldosterone system, *PRA* plasma renin activity, *PAC* plasma aldosterone concentration, *s(P)RR* soluble (pro)renin receptor

significantly positively correlated with UAE, and serum K was significantly negatively correlated with UAE (Table 3). In terms of RAAS components, the serum s(P)RR concentration, but not PRA or the PAC, was significantly positively correlated with UAE. In stepwise multiple regression analyses that tested age, systolic BP, uric acid, HbA1c, LDL-cholesterol, and the serum s(P)RR level as independent variables, systolic BP and s(P)RR were selected as possible independent variables, and both were significantly positively correlated with UAE (Table 4).

In single correlation analyses, age, urinary Na/K, creatinine, and uric acid were significantly negatively correlated with eGFR (Table 3). The serum s(P)RR concentration, but not PRA or the PAC, was significantly negatively correlated with eGFR (Table 3). In stepwise multiple regression analyses that tested the same factors as the analysis for UAE, age, uric acid, and s(P)RR were selected as possible independent variables, and all were significantly negatively correlated with eGFR (Table 4).

Inflammation and serum s(P)RR levels

An association of hsCRP, a marker for microinflammation, with progression of arteriosclerosis and kidney damage has

Table 4 Multiple regression analyses with parameters of organ dysfunctions

Variables	CAVI		UAE		eGFR		hsCRP	
	β	p	β	p	β	p	β	p
Age	0.710	<0.001	—	—	−0.449	<0.001	—	—
SBP	—	—	0.187	0.049	—	—	—	—
eGFR	—	—	n.a.	n.a.	n.a.	n.a.	—	—
Uric acid	0.097	0.174	—	—	−0.185	0.018	0.038	0.691
HbA1c	—	—	—	—	—	—	—	—
LDL-chol	—	—	—	—	—	—	−0.030	0.744
s(P)RR	0.183	0.011	0.435	<0.001	−0.426	<0.001	0.442	<0.001

$R^2 = 0.616$, $p < 0.0001$ for entire model for CAVI

$R^2 = 0.254$, $p < 0.0001$ for entire model for UAE

$R^2 = 0.405$, $p < 0.0001$ for entire model for eGFR

$R^2 = 0.195$, $p < 0.0001$ for entire model for hsCRP

CAVI Cardio-ankle vascular index, *UAE* urinary albumin excretion, *eGFR* estimated glomerular filtration rate, *hsCRP* high-sensitivity C-reactive protein, *SBP* systolic blood pressure, *HbA1c* hemoglobin A1c, *LDL-chol* low-density-lipoprotein cholesterol, *s(P)RR* soluble (pro)renin receptor, *n.a.* not applicable

been reported [22]. In single correlation analyses, age, BMI, uric acid, HbA1c, and triglyceride were significantly positively correlated with hsCRP, and HDL-cholesterol was significantly negatively correlated with hsCRP (Table 3). The serum s(P)RR concentration, but not PRA or the PAC, was significantly positively correlated with hsCRP. In stepwise multiple regression analyses that tested the same factors as the analysis for the CAVI, uric acid, LDL-cholesterol, and s(P)RR were selected as possible independent variables, and only serum s(P)RR was significantly positively correlated with hsCRP (Table 4).

Discussion

This study demonstrated that serum s(P)RR levels show significant correlations with organ damage indices, such as the CAVI, a marker for arteriosclerosis; UAE and eGFR, which are markers for renal damage; and hsCRP, a marker for microinflammation, after correction for other factors in patients with PA. In contrast, the PAC failed to show a significant association with these indices. These data suggest that the serum s(P)RR level may be independently associated with organ damage and could be a useful biomarker of risk in PA patients.

Serum s(P)RR levels are related to multiple factors

We have reported that serum s(P)RR levels are related to many factors, such as age, renal function, and lipid profiles (HDL-cholesterol and triglyceride) [16]. In terms of the mechanisms of these relationships, one may speculate that decreases in circulating renin with aging [23] inhibit downregulation of (P)RR expression through a process involving the transcription factor promyelocytic zinc-finger protein [24]. Elevated serum s(P)RR levels may reflect decreased clearance of s(P)RR from the kidneys, and increased expression of (P)RR in the kidneys may lead to both elevated serum s(P)RR levels and renal dysfunction. Patients with dyslipidemia may have increased expression of (P)RR in adipose tissues that causes elevations in serum (P)RR levels. These presumptions remain to be confirmed; however, we believe that it may be important to investigate the significance of the serum s(P)RR concentration as a biomarker in many pathological conditions.

Serum s(P)RR levels in patients with PA

In addition to a circulating RAAS, a local tissue RAAS is present in the adrenal cortex and stimulates production of aldosterone. (P)RR is expressed in normal adrenal glands in both the adrenal cortex and medulla and is predominantly expressed in the cortex in the zona glomerulosa and zona reticularis [25]. Recarti et al. [12] recently showed that (P)RR mRNA is translated into a functional protein in the subcapsular zona glomerulosa, the main site of aldosterone synthesis. (P)RR is overexpressed in APA tumor tissue [25]. Therefore, elevated serum s(P)RR levels may be due to increased expression of (P)RR in the adrenal cortex in patients with PA. However, serum s(P)RR levels in the present study were not correlated with PACs (Table 2). In addition, serum s(P)RR levels in patients with PA in this study (22.4 [interquartile range: 19.6–26.0] ng/mL, Table 1) were not significantly different from those in age- and eGFR-matched EH patients (21.8 [18.0–24.3] ng/mL), although PACs were significantly higher in patients with PA than in those with EH (data not shown). Therefore, it is unlikely that serum s(P)RR levels directly reflect the content

of (P)RR expressed in the adrenal lesions in patients with PA. S(P)RR seems to be derived from organs damaged by aldosterone, where the tissue RAAS is activated, rather than from adrenal glands generating aldosterone. However, the reasons for the increased secretion of s(P)RR from damaged organs in patients with PA remain unclear. Aldosterone may directly stimulate the expression of tissue (P)RR or (P)RR-processing enzymes. Further in vitro studies are needed.

Cardiovascular risk in patients with PA

Patients with PA have a higher incidence of CVDs, including coronary artery disease, myocardial infarction, heart failure, arrhythmia, atrial fibrillation, and stroke, than patients with EH [5]. As the incidence is unchanged even after adjustment for age, sex, and blood pressure, the results are considered to be due to excess aldosterone production [1]. Rats fed a high-salt diet and chronically administered aldosterone develop stroke, cardiomegaly, coronary inflammatory lesions, and renal injury, and treatment with a mineralocorticoid-receptor antagonist ameliorates these effects irrespective of BP. Therefore, high aldosterone appears to elevate CVD risk. In addition, hypokalemia may cause elevated rates of CVD in patients with PA [26].

Arteriosclerosis and serum s(P)RR levels in patients with PA

One possible mechanism for the increased risk of CVD in PA is the progression of arteriosclerosis in these patients [7, 27]. Aldosterone overproduction leads to functional and structural changes in the arterial system via chronic inflammation. Animal models demonstrate that aldosterone contributes to the accumulation of different types of collagen fibers and growth factors in vascular smooth muscle cells. Aldosterone plays a profibrotic role in the endothelium and heart [28], and these effects are attributable to increased oxidative stress and endothelial inflammation [29]. Aldosterone induces a vascular inflammatory response that is independent of elevated BP and is characterized by perivascular leukocyte infiltration and fibrinoid remodeling of vascular smooth muscle [30]. In addition, mineralocorticoid-receptor antagonists may prevent or reverse these changes [31].

The CAVI is a stable metric for assessment of arterial stiffness that is not influenced by BP [32]. The CAVI increases with age and is higher in males than in females. The CAVI is also increased in patients with hypertension, diabetes mellitus, dyslipidemia, and obesity and is associated with increased risk of cardiovascular events [33]. In the present study, the serum s(P)RR level was significantly positively correlated with the CAVI, independent of other factors (Tables 3 and 4). In addition, chronic inflammation is recognized as an important factor in the pathophysiology of atherosclerosis [34]. In the present study, serum s(P)RR levels showed significant independent association with hsCRP, a marker for microinflammation (Tables 3 and 4). Therefore, serum s(P)RR levels may be associated with the progression of arteriosclerosis via increased chronic inflammation. This possibility should be addressed in future research.

Aldosterone causes organ damage in organisms fed a high-salt diet [35]. However, the PAC did not show a significant relationship with the CAVI or hsCRP (Table 3) in this study, which corroborates previous studies that have reported no correlation between PACs and arteriosclerosis markers [27]. It remains unclear why PACs fail to show associations with arteriosclerosis markers. A few possible explanations exist. First, it has been reported that a high-salt diet elevates local angiotensin II production by increasing (P)RR activity in kidneys in nephritic rats [36]. Dietary salt levels are correlated with cardiac damage in PA patients [35]. Therefore, it is possible that aldosterone has exaggerated effects on organ damage when combined with a high-salt diet that are not associated with the PAC. Second, it is possible that circulating aldosterone levels do not directly correlate with the efficacy of mineralocorticoid-receptor activation in tissues in producing damage. In any case, we consider it necessary to search for useful biomarkers other than the PAC to determine the severity of arteriosclerosis. In our previous study, the relationship between serum s(P)RR levels and the CAVI was also significant in patients with EH [16]. However, in this study, the relationship in patients with EH seemed to be relatively weak ($r = 0.22$, $p = 0.037$, $n = 122$) compared to that in patients with PA ($r = 0.305$, $p = 0.004$, $n = 121$). Furthermore, a relationship between serum s(P)RR levels and CAVI was not detected after correcting for related factors in patients with EH [16]. Thus, the serum s(P)RR concentration is a relatively useful biomarker in patients with PA compared with patients with EH.

Kidney function and serum s(P)RR levels in patients with PA

Progressive kidney dysfunction may also increase the risk of CVD in patients with PA. Significant histological kidney damage has been noted in patients with PA. Increases in BP and blood volume may cause glomerular hyperfiltration and elevate urinary albumin excretion [37]. Eplerenone, a mineralocorticoid-receptor blocker, is effective in reducing the urinary albumin-to-creatinine ratio [38]. Experimental models enable more direct assessment of the effects of mineralocorticoid excess on intraglomerular hemodynamics than in vitro systems. Aldosterone has been found to exert a direct and rapid vasoconstrictive effect on the efferent renal

arteriole [39] and to abolish the vasoconstriction induced by potassium chloride at the afferent level [40]. In addition, potassium depletion by aldosterone excess is known to cause tubular dysfunction. The involvement of mineralocorticoid stimulation in renal injury is supported by the fact that renal dysfunction is partially reversible by treatment with adrenalectomy or a mineralocorticoid-receptor antagonist in patients with PA.

(P)RR is expressed in kidney cells, including mesangial cells, podocytes, and tubular cells [41]. Binding of renin or prorenin to (P)RR activates the intrarenal RAAS and induces intercellular signaling by phosphorylation of extracellular signal-regulated kinase, leading to renal damage through release of transforming growth factor-β1 and cytokines. Therefore, s(P)RR is considered to reflect intrarenal RAS, mesangial fibrosis, and matrix expansion [42].

Microalbuminuria is an important early sign of diabetic nephropathy, and a recent study showed that UAE is associated with early and late renal functional abnormalities in both diabetic and nondiabetic patients [43]. In the present study, serum s(P)RR levels were significantly positively correlated with UAE (Tables 3 and 4) and significantly negatively correlated with eGFR (Tables 3 and 4), independent of other factors, in patients with PA. Interestingly, similar to the CAVI, the PAC did not show a significant correlation with UAE (Table 3) or eGFR (Table 3), suggesting that serum s(P)RR is more strongly associated with kidney injury than the PAC in these patients.

In patients with PA, hyperfiltration due to excess aldosterone may affect UAE and eGFR. In addition, serum s(P)RR levels may be affected by increased clearance of s(P)RR, a 28-kDa protein, from the kidney, especially in the context of hyperfiltration. However, an association between serum s(P)RR levels and UAE or eGFR was observed in patients with PA, which is inconsistent with our previous findings showing that serum s(P)RR levels were significantly correlated with eGFR ($r = -0.337$, $p < 0.001$, $n = 122$) but not with UAE ($r = -0.14$, $p = 0.182$) in patients with EH [16]. Reports have shown that increased blood s(P)RR levels are associated with future progression of renal dysfunction [17]. Therefore, serum s(P)RR may be a biomarker of renal injury and a predictor of worsening renal dysfunction in patients with PA.

Mechanisms of the associations between serum s(P)RR concentrations and organ damage

The mechanism by which serum s(P)RR levels are associated with organ damage in patients with PA is undetermined and needs to be discussed. Organ damage may be partly attributable to increased (P)RR expression in tissues, eventually causing elevations in serum s(P)RR concentrations. (P)RR may consequently activate the tissue RAAS and angiotensin-independent intracellular signaling to cause fibrosis [44] and inflammation [45], which are also observed in patients with PA. Previous studies have demonstrated that s(P)RR has biological functions in vitro [46] and in vivo [47], suggesting the possibility that s(P)RR is related to the pathogeneses of some diseases. Therefore, s(P)RR may also play a role in the pathogenesis of organ damage that occurs in patients with PA. However, further in vivo and in vitro studies are needed to determine whether s(P)RR is a simple biomarker or a cause of organ damage.

Limitations

There are several limitations of this study. First, we could not determine the source of the serum s(P)RR, which showed an association with organ damage in patients with PA. As noted, the adrenal glands may not be the only sources. Alternative candidate organs may include the vasculature, kidneys, heart, and brain, which are affected by CVD and express high amounts of (P)RR in humans [10], although future studies are needed to investigate these potential source organs. Second, the number of patients was relatively small, and this was a single-center study. Third, our findings are based on the relationships between serum s(P)RR levels and background factors. Longitudinal studies may reveal the impacts of serum s(P)RR levels on the progression of organ damage and prognosis in patients with PA.

Our data demonstrated that the serum s(P)RR level, but not the PAC, is independently associated with organ damage, including arteriosclerosis and renal dysfunction, in patients with PA. The serum s(P)RR level could be used as a biomarker for increased risk of organ damage and might be useful in selecting patients who require detailed evaluation, especially for CVD. Further studies are needed to investigate the mechanism by which serum s(P)RR levels are associated with arteriosclerosis progression and renal dysfunction and to determine the clinical implications of serum s(P)RR levels.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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