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Homocysteine predicts vascular target organ damage in hypertension and may serve as guidance for first-line antihypertensive therapy

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Abstract

Homocysteine is an independent risk factor for cardiovascular and cerebrovascular disease and has been proposed to contribute to vascular dysfunction. We sought to determine in a real-world clinical setting whether homocysteine levels were associated with hypertension mediated organ damage (HMOD) and could guide treatment choices in hypertension. We performed a cross-sectional analysis of prospectively collected data in 145 hypertensive patients referred to our tertiary hypertension clinic at Royal Perth Hospital and analyzed the association of homocysteine with HMOD, renin-angiotensin-aldosterone system (RAAS), and RAAS blockade. The average age of participants was 56 ± 17 years, and there was a greater proportion of males than females (89 vs. 56). Regression analysis showed that homocysteine was significantly associated with PWV (β = 1.99; 95% CI 0.99-3.0; p < .001), albumin-creatinine ratio (lnACR: β = 1.14; 95% CI 0.47, 1.8; p < .001), 24 h urinary protein excretion (β = 0.7; 95% CI 0.48, 0.92; p < .001), and estimated glomerular filtration rate (β = −29.4; 95% CI −36.35, −22.4; p < .001), which persisted after adjusting for potential confounders such as age, sex, 24 h BP, inflammation, smoking, diabetes mellitus (DM), and dyslipidemia. A positive predictive relationship was observed between plasma homocysteine levels and PWV, with every 1.0 µmol/L increase in homocysteine associated with a 0.1 m/s increase in PWV. Homocysteine was significantly associated with elevated aldosterone concentration (β = 0.26; p < .001), and with attenuation of ACEi mediated systolic BP lowering and regression of HMOD compared to angiotensin receptor blockers in higher physiological ranges of homocysteine. Our results indicate that homocysteine is associated with hypertension mediated vascular damage and could potentially serve to guide first-line antihypertensive therapy.

1 | INTRODUCTION

Vascular compromise resulting from consistently elevated arterial pressure is a hallmark of hypertension and one of the first manifestations of target organ damage.[1] Arterial stiffness is an index of vascular health, and timely prediction of hypertension mediated organ damage (HMOD) confers additional independent predictive value for adverse cardiovascular outcomes not only in patients with essential hypertension[2,3] but also in the general population.[4] Identification of a biomarker capable of predicting vascular health in hypertension would enable stratification of the most vulnerable patient groups to guide clinical management.

Homocysteine (Hcy) is a sulfur-containing amino acid, which was discovered in 1932. Methionine derived from dietary sources is converted into Hcy and has three major fates: (a) the transsulfuration pathway where pyridoxine (vitamin B6) is an essential cofactor, (b) the remethylation pathway where folate serves as a substrate and cobalamine (vitamin B12) acts as a cofactor, and (c) release into the extracellular medium which determines the plasma concentration of Hcy.[5] Mendelian randomization studies demonstrated that the mean Hcy concentration was higher in patients with hypertension compared to the non-hypertensive population and suggested a plausible association of elevated Hcy levels with increased risk of hypertension.[6] Investigators have reported a significant association of serum Hcy with different indices of arterial stiffness such as pulse pressure and aortic stiffness in the general population[4] and in elderly patients who have multiple cardiovascular risk factors.[7] Moreover, Hcy was associated with nondipping BP pattern, a potent predictor of cardiovascular mortality and morbidity in hypertensive individuals.[8] Plasma Hcy concentrations correlated with the incidence of cardiovascular, cerebrovascular, and peripheral artery disease[9] and atherosclerotic vascular damage.[10] In addition, hyper-homocysteinemia (hHcy) was associated with peripheral microvascular endothelial dysfunction and a Hcy level of >10 μmol/L was significantly associated with all-cause mortality and atherosclerotic cardiovascular events in older obese and hypertensive patients.[11]

Several potential mechanisms may mediate the association between Hcy and HMOD. Direct vasculotoxic effects of Hcy resulting in vascular remodeling as a consequence of interactions with lipoprotein (a),[12] induction of HMG CoA reductase with subsequently enhanced cholesterol synthesis,[13] oxidative modification of low-density lipoprotein (LDL) and LDL atherogenesis,[14] and attenuation of nitric oxide mediated endothelium-dependent vasodilation in humans[15] have been suggested. Activation of the renin-angiotensin-aldosterone system is also likely to play a role. Indeed, aldosterone stimulated Hcy production has been demonstrated in rat adrenal glands,[16] highlighting the possibility of a feedforward potentiation cascade between aldosterone and Hcy. Aldosterone has been shown to play a direct role in vascular toxicity and fibrosis, independent of its effect on hemodynamics and volume homeostasis.[17] Endothelial Hcy production significantly impaired shear stress induced arterial dilation and release of nitric oxide in Wistar rats that triggered vascular superoxide mediated-angiotensin II (Ang II) signaling via the Ang II receptor 1 (AT1R).[18] In line with this, ablation of AT1R has been shown to attenuate vascular injury[19] and AT1R blockade attenuated pathological ventricular hypertrophy in hHcy rats.[20]

Here, we investigated the association between Hcy levels and vascular target organ damage in a real-world cohort of hypertensive patients. Furthermore, we explored whether Hcy is associated with BP levels and whether this differed depending on the type of renin-angiotensin-aldosterone system (RAAS) blockade.

2 | METHODS

2.1 | Patient cohort

We performed a cross-sectional analysis of prospective collected data from February 2016 to October 2019 which included a cohort of 145 patients with essential hypertension referred to the Dobney Hypertension Centre at Royal Perth Hospital, Perth, Australia. Patients with secondary causes were excluded from the study. This study was performed in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice guidelines. All patients provided written, informed consent to participate in this systematic prospective data collection, which has been approved by the Royal Perth Hospital research ethics committee, clinical auditing of the data as GEKO Quality Activity Number 34724.

2.2 | Clinical workup

All patients had their medical history taken and underwent physical examination and collection of anthropometric data. All participants were fitted with ambulatory BP monitor (Spacelabs; Mobil-O-Graph IEM GmbH; OSCAR-2 SunTech) which were programmed to take 4 measurements per hour in the day-time (06:00-22:00 h) and 2 measurements per hour during the night-time (22:00-06:00 h), and the total average of the systolic and diastolic reading was used. PWV was performed using SphygmoCor Xcel system (AtCor Medical Pty Ltd) as per manufacturer's protocol at trough in a supine position. PWV was measured twice, and the average was used for analysis.

2.2.1 | Biochemistry

All biochemical analyses were performed at the Royal Perth Hospital Central Laboratory using standard methods and reagents. Full blood count, plasma lipid profile, glucose, HbA1c, renal and liver parameters, aldosterone, HsCRP, and Hcy were determined from the same fasting venous blood samples. LDL cholesterol was calculated using Friedewald formula. 24 h urine was collected as per established local protocols for the assessment of renal excretion of protein, sodium, and potassium throughout the day.

TABLE 1 Baseline characteristics of the study participants by homocysteine (Hcy) grouping

Patient characters	Group 1: Hcy <10 µmol/L (Mean ± SD)	Group 2: 10 ≥ Hcy ≤ 15 µmol/L (Mean ± SD)
Age (yrs)	52 ± 17.6	59 ± 16.0
Sex (male/female)	41/30	48/26
Weight (kg)	93.5 ± 25.8	91.9 ± 21.5
Height (cm)	169.1 ± 11.0	168.8 ± 10.4
Body mass index (kg/m^2)	30.2 ± 10.4	28.9 ± 10.7
24 h Systolic BP average (mmHg)	133 ± 18.2	136 ± 14.7
24 h diastolic BP average (mmHg)	78 ± 11.8	78 ± 11.0
Pulse Wave Velocity, PWV (m/s)	8.2 ± 2.11	9.1 ± 2.05
Glucose mmol/L	5.8 ± 2.11	6.9 ± 2.12
HbA1c (%)	5.8 ± 0.79	6.3 ± 1.3
HsCRP (mg/L)	4.1 ± 6.1	5.8 ± 9.6
eGFR (ml/min/1.73 m^2)	84.3 ± 10.7	73.6 ± 16.1
Total cholesterol (mmol/L)	4.9 ± 1.2	4.7 ± 1.2
LDL cholesterol (mmol/L)	2.9 ± 1.01	2.8 ± 0.92
Triglycerides (mmol/L)	1.54 ± 0.89	1.63 ± 0.71
HDL cholesterol (mmol/L)	1.26 ± 0.38	1.2 ± 0.29
Lipoprotein(a) (g/L)	0.45 ± 0.44	0.41 ± 0.47
Homocysteine (µmol/L)	7.6 ± 1.12	11.9 ± 1.43
Aldosterone pmol/L	296.3 ± 168.1	341.1 ± 225.5
Urine parameters		
ACR mg/mmol	4.8 ± 13.4	12.3 ± 13.3
24 h urine protein mmol/day	0.14 ± 0.2	0.26 ± 0.61
24 h urine sodium mmol/day	150.1 ± 64.7	138.86 ± 63.4
24 h urine potassium mmol/day	77.9 ± 29.9	69.8 ± 32.1
24 h urine creatinine mmol/day	11.75 ± 5.3	25.9 ± 30.64

Note: Continuous baseline characteristics of the patient cohort. Data are given as mean ± SD.
Abbreviations: BMI, body mass index; eGFR estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; PWV, carotid-femoral pulse wave velocity.

2.3 | Statistical analysis

Linear regression analysis was used to evaluate the association of homocysteine with various parameters of vascular damage in hypertension. Hcy demonstrated a skewed distribution and hence was log transformed to obtain a normal distribution for regression analysis,[21] and similar transformation was applied to the albumin-creatinine ratio. The baseline model assessed the association of the predictor variable Hcy on the outcome variables of macrovascular damage—PWV and microvascular damage: albumin-creatinine ratio, 24 h urinary protein excretion and eGFR, adjusted for potential confounders such as age, sex, 24 h systolic and diastolic BP, inflammatory status (high sensitivity C-reactive protein; hsCRP), smoking status, DM, and dyslipidemia. Predictive margin analysis was used to determine the association of Hcy with arterial stiffening, measured by PWV in an age and systolic BP adjusted model. Correlation analysis was performed to determine the association of Hcy with aldosterone and systemic salt retention detected from the 24 urinary excretion of sodium and potassium. Sub-group analysis was performed to assess the influence of angiotensin converting enzyme inhibitors (ACEi) vs angiotensin receptor blockers (ARBs) in lower and higher levels of physiological Hcy concentrations (grade 1 <10 µmol/L; grade 2 ≥ 10 to 15 µmol/L) on BP control and HMOD using two sample *t* test with equal variances. All statistical analyses were performed in STATA 15.1 [Stata Corp].

3 | RESULTS

3.1 | Baseline characteristics

The clinical characteristics of the study population are shown in Tables 1 and 2. Overall, 145 patients were included in the analysis. The mean ± SD age of the participants was 56 ± 17 years, and there

TABLE 2 Comorbidities and medication history of the patient cohort

Clinical characteristics	
Previous MI, *n* (%)	9 (6%)
IHD, *n* (%)	6 (4%)
TIA, *n* (%)	2 (3%)
CAD, *n* (%)	32 (22%)
Stroke: Ischemic/hemorrhagic	7 (4%)/4 (2%)
Renal insufficiency (eGFR < 60 ml/min/1.73 m^2), *n* (%)	29 (20%)
Dyslipidemia, *n* (%)	24 (16%)
Diabetes Mellitus, *n* (%)	55 (38%)
Medications	
ACEi, *n* (%)	35 (25%)
ARB, *n* (%)	71 (49%)
CCB, *n* (%)	75 (52%)
Diuretics, *n* (%)	15 (10%)
β blockers, *n* (%)	49 (34%)
Statins, *n* (%)	53 (38%)

Note: History of comorbidities and medications. Data are given as *n* (%). Medications: ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; CCB, calcium channel blockers.
Abbreviations: CAD, coronary artery disease; IHD, ischemic heart disease; MI, myocardial infarction; TIA, transient ischemic attacks.

was a greater proportion of males (*n* = 89, 61%) than females (*n* = 56, 37%). The cohort had an average height of 168 ± 10.5 cm, weight 92.7 ± 23.9 kg, and BMI of 30.4 ± 10.9 kg/m^2. Around 38% of patients were on anti-diabetic medication, and 16% were on lipid-lowering therapy, predominantly statins. The patients included in this study had an average homocysteine concentration of 9.8 ± 2.4 μmol/L and were diagnosed with essential hypertension following exclusion of secondary forms via biochemistry and imaging of kidneys and renal arteries. The average 24 h ambulatory systolic BP was 135 ± 17 mmHg and diastolic BP was 78 ± 11 mmHg. Hcy concentrations positively correlated with 24 h systolic BP average (β = 0.16; p = .03) but not with 24 h diastolic average (β = −0.06; p = .43). The average values of the arterial parameters focused in this cohort include PWV of 8.6 ± 2 m/s, ACR of 8.3 ± 29 mg/mmol, and eGFR of 79 ± 14.5 ml/min/1.73 m^2, with an average 24 h urinary protein excretion of 0.2 ± 0.46 mmol/day. These patients were treated as per clinical guidelines with antihypertensive medications including ACEi (*n* = 35, 25%) or ARBs (*n* = 71, 49%), calcium channel blockers, CCB (*n* = 75, 52%) diuretics (*n* = 15, 10%) and β blockers (*n* = 49, 34%). Further baseline characteristics of the patients are described in Tables 1 and 2.

3.2 | Association of homocysteine with HMOD

The association between plasma Hcy and vascular parameters is shown in Table 3 and Figure 1. Univariate linear regression showed that plasma Hcy concentration was associated with PWV (p < .001), logACR (p = .001), 24 h urinary protein (p < .001), and inversely with

TABLE 3 Linear regression analysis of the association of Homocysteine with markers of arterial damage in hypertension

	Model 1: Baseline model $p > \|t\|$ [β: 95% Conf. Interval]	Model 2 : age and sex adjusted	Model 3 : adjusted for systolic and diastolic BP (24 h)	Model 4: adjusted for inflammatory status (HsCRP)	Model 5: adjusted for smoking status	Model 6: adjusted for DM (fasting glucose)	Model 7: adjusted for dyslipidemia (lipid profile) adjusted for dyslipidemia (lipid profile)
PWV m/s (Macrovascular damage, arterial stiffness)	p = .000 β = 1.99 CI 0.99, 3.0	p = .041 β = 0.96 CI 0.42, 1.9	p = .001 β = 1.63 CI 0.67, 2.6	p = .026 β = 1.3 CI 0.16, 2.5	p = .003 β = 1.84 CI 0.63, 3.05	p = .008 β = 1.5 CI 0.41, 2.62	p = .002 β = 1.9 CI 0.73, 3.07
Log ACR mg/mmol (Microvascular damage)	p = .001 β = 1.14 CI 0.47, 1.8	p = .008 β = 0.96 CI 0.25, 1.7	p = .013 β = 0.88 CI 0.19, 1.6	p = .005 β = 1.15 CI 0.36, 1.9	p = .006 β = 1.08 CI 0.31, 1.8	p = .011 β = 0.99 CI 0.23, 1.7	p = .002 β = 1.2 CI 0.45, 1.95
24 h urinary protein mmol/24 h (Microvascular damage)	p = .000 β = 5.75 CI 2.8, 8.7	p = .000 β = 6.5 CI 3.3, 9.6	p = .000 β = 6.2 CI 2.9, 9.4	p = .000 β = 7.5 CI 3.4, 11.6	p = .000 β = 7.1 CI 3.4, 10.9	p = .000 β = 6.9 CI 3.6, 10.9	p = .000 β = 6.9 CI 3.3, 10.4
eGFR ml/min/1.73 m^2 (Microvascular damage)	p = .000 β = −29.4 CI −36.35, −22.4	p = .000 β = −24.4 CI −31.4, −17.4	p = .000 β = −24.4 CI −31.7, −17.4	p = .000 β = −27.3 CI −35.1, −19.5	p = .000 β = −28.3 CI −36.3, −20.2	p = .000 β = −29.8 CI −37.6, −21.9	p = .000 β = −29.76 CI −37.6, −21.6

Note: Univariate model results: ACR, albumin-creatinine ratio; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; hsCRP, high sensitivity C-reactive protein; PWV, pulse wave velocity; SBP, systolic blood pressure.

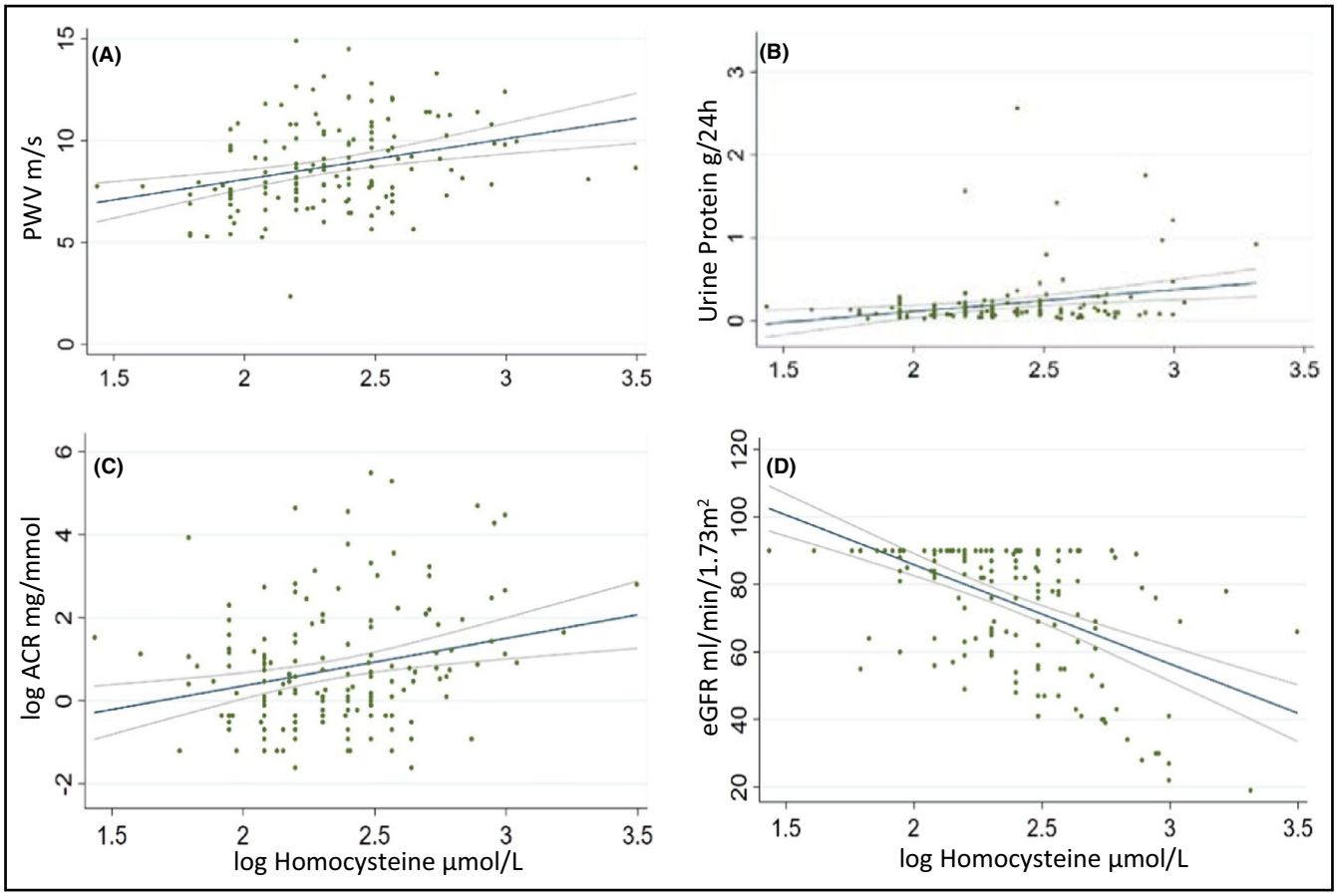


FIGURE 1 Homocysteine was associated with measures of hypertension mediated arterial damage/dysfunction. Scatterplots of measures of hypertension mediated organ damage, including the unadjusted linear regression model fits (blue line) with the mean (gray area). (A) Pulse wave velocity (PWV); (B) 24 urinary protein excretion (C) log ACR (albumin-creatinine ratio); (A) estimated glomerular filtration rate (eGFR >90 included)

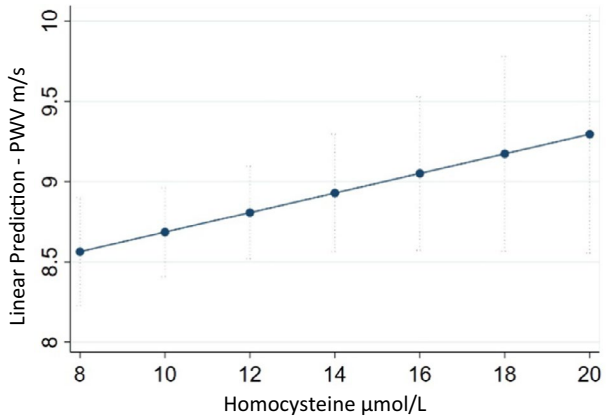
eGFR (p < .001). The linear associations between plasma Hcy and measures of HMOD remained following adjustment for age, sex, ambulatory BP measures, inflammation, smoking, DM, and lipid status (Table 3). The linear association between plasma Hcy and PWV remained significant following adjustment for renal clearance, eGFR (β = 1.19; 95% CI 0.28 to 3.47; p = .021). Predictive margin analysis indicated a positive predictive relationship between plasma Hcy levels and PWV in the age and systolic BP adjusted regression model (β = 0.61; 95% CI 0.18 to 1.03; p = .006) suggesting that a 1 µmol/L increase in Hcy corresponds to a 0.1 m/s increase in PWV in patients with hypertension (Figure 2).

3.3 | Association of homocysteine with serum aldosterone, the main mineralocorticoid of the RAAS pathway

Hcy correlated with serum aldosterone concentration (β = 0.26, p < .001) and systemic salt retention characterized by reduced 24 h renal excretion of sodium and potassium (24 h urinary sodium: β = −0.1902, p = .046; potassium, β = −0.2454, p = .003; sodium/potassium ratio, β = 0.2401, p = .012), (Table 4, Figure 3). To explore whether the association of Hcy with HMOD is potentially mediated by RAAS pathway, additional sub-group analysis (n = 90) was performed on those patients for whom PWV was available and were taking antihypertensive regimen targeting the RAAS, namely ACEi (n = 32) and ARBs (n = 58). These patients were stratified based on Hcy concentration within the physiological range (group 1: Hcy <10 µmol/L; group 2: 10 ≥ Hcy ≤ 15 µmol/L). In the ACEi treated patients, both average 24 h systolic BP and PWV were higher in group 2 (G2) compared to group 1 (G1) (G1:126 ± 11 mmHg, 7.5 ± 1.4 m/s; G2: 134 ± 17 mmHg, 9.1 ± 2.1 m/s; Δ SBP 8 mmHg, p = .048; Δ PWV 1.6 m/s p = .019) (Figure 4A,B, panel A, Table 5). In contrast, such an intergroup difference was not observed in patients on ARB treatment (G1:135 ± 19 mmHg, 8.1 ± 2.4 m/s; G2: 138 ± 12 mmHg, 8.9 ± 1.9 m/s; Δ SBP 3 mmHg, p = .174; Δ PWV 0.8 m/s p = .079) (Figure 4A,B, panel B, Table 5). None of the groups showed any significant association with 24 h diastolic BP.

4 | DISCUSSION

Our findings indicate that Hcy levels are clearly associated with well-established markers of HMOD: macrovascular damage exemplified by



Hcy µmol/L	Margin – PWV m/s	P>t	95% CI
8	8.478073 ≈ 8.5	0.000	8.14, 8.81
10	8.695146 ≈ 8.7	0.000	8.42, 8.97
12	8.912218 ≈ 8.9	0.000	8.61, 9.20
14	9.129291 ≈ 9.1	0.000	8.74, 9.52
16	9.346363 ≈ 9.3	0.000	8.83, 9.87
18	9.563435 ≈ 9.6	0.000	8.90, 10.23
20	9.780508 ≈ 9.8	0.000	8.96, 10.60

FIGURE 2 Predictive margin analysis to determine the association of homocysteine with arterial stiffening, measured by pulse wave velocity (PWV) in hypertension. A positive predictive relationship was observed between plasma homocysteine levels and PWV (homocysteine 1 µmol/L ~ 0.1 m/s increase in PWV) in the age and systolic BP adjusted regression model

TABLE 4 Homocysteine is associated with enhanced RAAS signaling

RAAS parameters	Homocysteine correlation	Mechanisms involved
Renin	β = −0.1227, p = .1131	Negative feedback inhibition
Aldosterone	β = 0.2565, p = .0008	Enhanced RAAS signaling
24 h sodium excretion in urine	β = −0.1902, p = .0456	Increased sodium retention
24 h potassium excretion in urine	β = −0.2454, p = .0029	Increased potassium retention
24 h urinary sodium/potassium ratio	β = 0.2401, p = .0123	Increased 24 h Na/K ratio suggestive of increased aldosterone activity

Note: Correlation analysis of homocysteine against the measures of Renin-Angiotensin-Aldosterone System (RAAS): Renin; Aldosterone; 24 urinary sodium excretion; 24 urinary potassium excretion; 24 urinary sodium/ potassium ratio.

increased PWV, and microvascular damage as indicated by increased 24 h urinary protein excretion, ACR, and reduced eGFR. Importantly, these associations were independent of age, sex, ambulatory BP measures, inflammation, smoking, DM, and dyslipidemia. In addition, our study demonstrated that Hcy levels were associated with arterial stiffness and circulating aldosterone concentration. Interestingly, when patients were stratified according to the level of Hcy (group 1: Hcy < 10 µmol/L; group 2: 10 ≥ Hcy ≤ 15 µmol/L) within the physiologic range, the average systolic BP and PWV differed significantly in those patients on ACEi, whereas no such difference was evident in patients on ARBs. This may indicate that in hypertensive patients with Hcy above 10 µmol/L, ACE inhibitors may be less effective in reducing BP and preventing vascular damage, whereas ARBs appear to be effective independent of Hcy concentrations. In line with these observations, it is postulated that the AT1R plays a crucial role in Hcy mediated organ damage.[19,20]

Current guidelines have not classified Hcy as cardiovascular disease risk stratification tool. However, Hcy has been prospectively validated and it has been shown that incremental increase in Hcy levels predicted adverse cardiovascular disease events beyond the Framingham Risk Score and was proposed to fulfill the criteria of a "novel" risk marker.[22] From a pathophysiological point of view, different mechanisms have been discussed to account for the vascular dysfunction related to Hcy. Possible mechanisms include the direct action of Hcy or may be due to Hcy mediated RAAS potentiation in hypertension.

Elevated Hcy levels have been shown to inhibit endothelium-dependent vasorelaxation in conduit arteries through endothelial nitric oxide inactivation.[23,24] In addition, Hcy deactivates Ca^{2+}-activated potassium channels in resistance arteries and is associated with impaired endothelium-derived hyperpolarizing factor-mediated endothelial relaxation.[25] Moreover, Hcy activates metalloproteinases, induces collagen synthesis, and causes an imbalance in the elastin/collagen ratio affecting vascular elastance,[26] which is an important determinant of vascular adaptation in high BP. Moreover, higher Hcy concentrations have been shown to attenuate bradykinin-induced changes in blood flow, vascular resistance, and BP.[27] Positive associations have been reported between Hcy and atherosclerosis in type 2 diabetics[28] and aortic stiffness in hypertensive[29,30] and chronic kidney disease[21] patients. In our study, we found that Hcy, at physiologic concentrations, was significantly associated with macrovascular damage (PWV) and microvascular

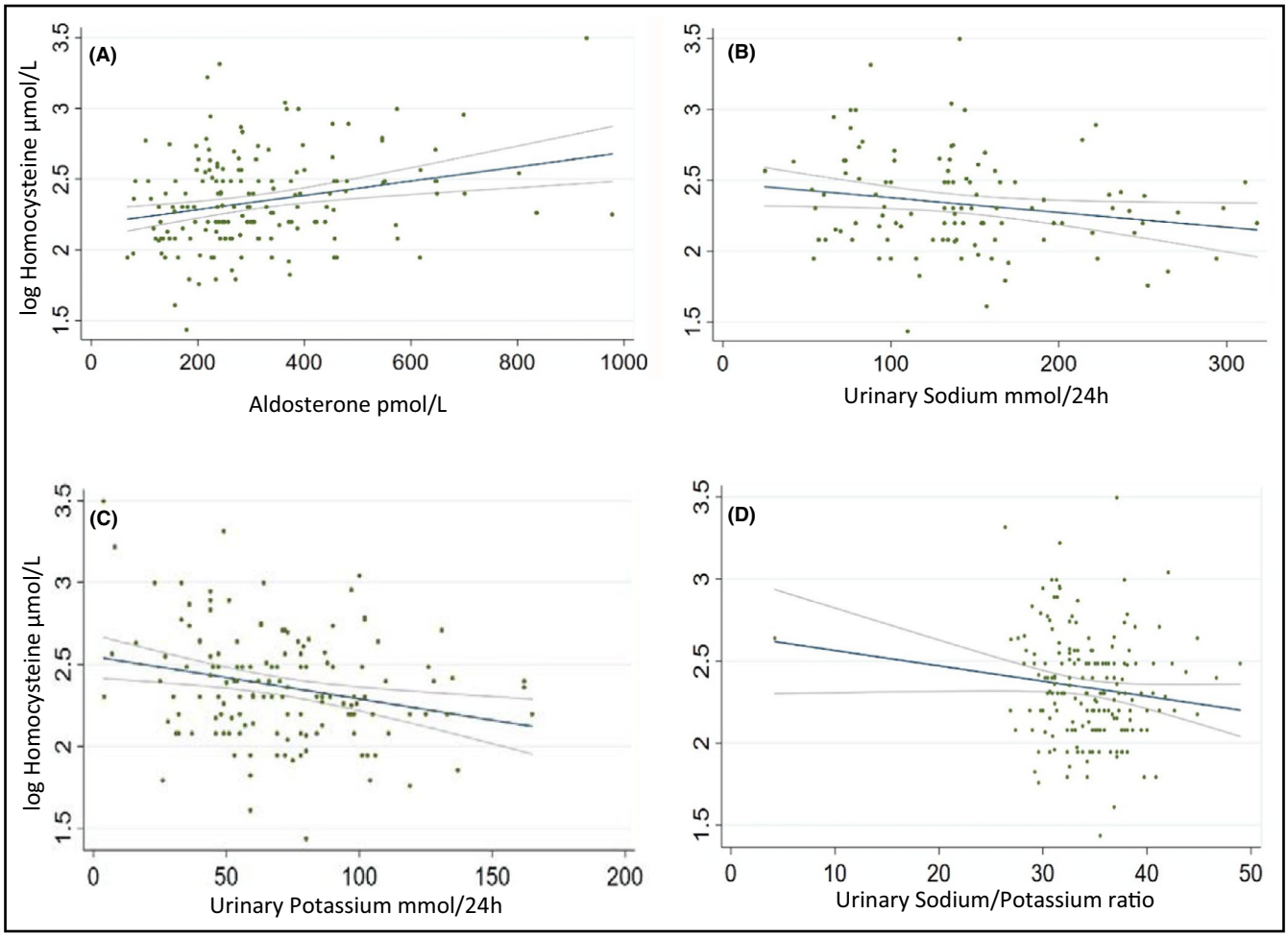


FIGURE 3 Homocysteine is associated with increased RAAS signaling, as assessed by serum aldosterone concentration and systemic salt retention. Scatterplots of log homocysteine against the measures of renin-angiotensin-aldosterone signaling (RAAS) including the unadjusted linear regression model fits (blue line) with the mean (gray line). (A) Aldosterone; (B) 24 urinary sodium excretion; (C) 24 urinary potassium excretion; (D) 24 urinary sodium/ potassium ratio

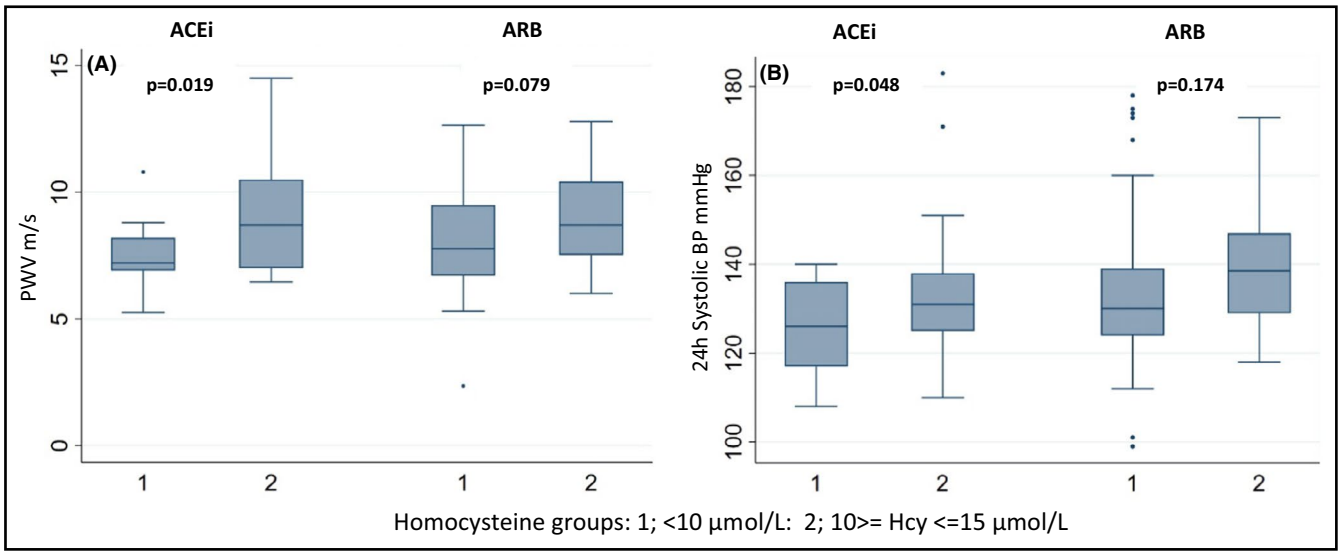


FIGURE 4 Homocysteine is associated with attenuation of ACEi mediated systolic BP lowering and regression of HMOD. Boxplot comparing the influence of antihypertensive therapy. (A) Pulse wave velocity (PWV); (B) Systolic blood pressure (SBP) in homocysteine groups

TABLE 5 Analysis of RAAS blockade in Homocysteine associated HMOD

Medications	ACEi			ARB		
	Group 1	Group 2	Two sample *T* test with equal variance	Group 1	Group 2	Two sample *T* test with equal variance
Homocysteine (Hcy) μmol/L	*n* = 13	*n* = 19		*n* = 34	*n* = 24	
PWV m/s	7.5 ± 1.4	9.1 ± 2.3	*p* = .019	8.1 ± 2.4	8.9 ± 1.9	*p* = .079
Systolic BP mmHg (24 h)	126 ± 11	134 ± 17	*p* = .048	135 ± 19	138 ± 12	*p* = .174
Diastolic BP mmHg (24 h)	73 ± 10	78 ± 10	*p* = .084	80 ± 13	77 ± 12	*p* = .854

Note: Results of *t* test with equal variances: Analyzing the results of antihypertensive therapy ACEi and ARB on PWV and systolic BP between Hcy groups (Group 1: <10 μmol/L; Group 2: 10 ≥ Hcy ≤ 15 μmol/L).

Abbreviations: ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; BP, blood pressure; HMOD, hypertension mediated organ damage; PWV, pulse wave velocity; RAAS, renin-angiotensin-aldosterone system.

damage (ACR, 24 h urinary protein excretion and eGFR) (Figure 1, Table 3).

Hcy is an important player in vascular metabolism, evident by the endothelial dependence on methionine, necessary for the generation of S-adenosyl methionine (SAM), carbon metabolism and for the methylation of DNA and proteins.[31] Hcy is metabolized by cystathionine γ-lyase to hydrogen sulfide (H_2S), a potent vasorelaxant and an endogenous inhibitor of endothelial ACE activity.[26,32] In contrast to Hcy, cysteine is the substrate of the H_2S generating enzymes: cystathionine β-synthase and cystathionine γ-lyase, all of which are inhibitors of endothelial ACE activity.[32] Elevated Hcy levels in hyperhomocysteinemia result in damage and precipitation of modified cystathionine-γ lyase attenuating H_2S generation,[26] which may explain the diminished antihypertensive response with ACE inhibitors in elevated Hcy concentrations.[33] Distinct conformational changes of AT1R were demonstrated upon binding to Ang II and Hcy. AT1R is both orthosterically (hHcy states) and allosterically regulated by Hcy and it has been suggested that AT1R blockade could mitigate hHcy-associated aneurysmal vascular injuries in mouse models using molecular dynamics and site directed mutagenesis.[19] In addition, pathological ventricular hypertrophy observed in hHcy rats was attenuated when treated with AT1R blocker (ARB), valsartan.[20] Therefore, we speculated that Hcy levels might possibly influence RAAS signaling and may influence the action of the antihypertensive drugs, especially RAAS blockers.

In this study, Hcy significantly correlated with aldosterone concentration and reduced salt excretion possibly suggestive of enhanced RAAS activation (Table 4, Figure 3). We therefore sought to further explore whether the association of Hcy with HMOD was influenced by RAAS by performing a sub-group analysis depending on treatment with either ARB or ACEi at lower and higher physiologic concentrations of Hcy (Table 5, Figure 4). ARBs suppress aldosterone production through inhibition of expression and activation of AT1R, whereas ACEis decrease the formation of the vasoconstrictor Ang II and increase levels of the vasodilator bradykinin.[33] The overall BP lowering effect of both drug classes has been shown to be similar.[34]

Whether Hcy may influence the potency of ACEis or ARBs has to our knowledge not yet been investigated in a real-world hypertension cohort. While we did not assess this prospectively, we observed a significant difference in both BP and PWV in patients treated with ACEi depending on Hcy levels (Table 5, Figure 4) and found that those with Hcy levels above 10 μmol/L had higher BP and PWV than those with lower levels. This attenuation of BP response was not evident in patients treated with ARBs, suggesting that Hcy levels should be taken into account when a therapeutic decision to block the RAAS is considered. This may be due to involvement of the RAAS in Hcy-mediated vascular damage and the inadequate ACEi response may be due to uninhibited ACE activity at higher concentrations of Hcy, albeit still in the physiological range, as shown in previous studies.[33] The influence of Hcy is possibly attenuated by ARBs due to the offsetting of RAAS at the AT1R.

From a clinical perspective, Hcy levels could therefore not only represent a useful marker of arterial HMOD but also potentially serve to guide the choice of the most appropriate first-line RAAS blocking antihypertensive regimen, that is, ACEi or ARB, the latter being the preferred choice in the context of elevated Hcy levels above 10 μmol/L. Clearly, future studies are needed to investigate whether indeed these theoretical considerations hold true in prospective longitudinal studies and whether Hcy levels may influence the potency of antihypertensive therapy with ACEi vs ARBs in regard to lowering BP and preventing HMOD.

4.1 | Limitations

This is a cross-sectional study that included a cohort of patients referred from general practitioners to an outpatient hypertension clinic in a tertiary hospital with multiple associated comorbidities treated with various medications. While we have attempted to control for many variables, it is possible that there are residual confounders that influence the results. Moreover, we speculate that the interaction between Hcy and the RAAS is mainly based on aldosterone levels and indirect measures such as the sodium/potassium ratio. Furthermore, the relatively low numbers in certain subgroups may lead to an underestimation of effects. However, this study reflects a real-world approach with gold standard assessments providing robust clinical data which may have advantages in terms of its general applicability in clinical settings.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Revathy CARNAGARIN involved in conceptualization, data curation, formal analysis, investigation, visualization, writing—original draft, and writing—review and editing. Janis M. NOLDE, Natalie WARD, Sandi ROBINSON, Ancy JOSE, and Márcio Galindo KIUCHI involved in conceptualization and writing—review and editing. Leslie Marisol LUGO-GAVIDIA, Justine CHAN, and Anu JOYSON involved in investigation, data curation, and writing—review and editing. Omar AZZAM involved in conceptualization, investigation, and writing—review and editing. Bibombe P MWIPATAYI involved in conceptualization, and writing—review, editing, and supervision. Markus P. SCHLAICH involved in conceptualization, formal analysis, data curation, funding acquisition, project administration, supervision, writing—original draft, and writing—review, editing, and supervision.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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