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Beta-2-Microglobulin is an Independent Risk Factor for Asymptomatic Carotid Atherosclerosis in Patients with Primary Aldosteronism

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Aim: To identify the association between serum beta-2-microglobulin (B2M) or cystatin C (CysC) and asymptomatic carotid atherosclerosis in patients with primary aldosteronism (PA).

Methods: In this cross-sectional study, 265 subjects were enrolled, including 83 patients with PA, 91 with essential hypertension (EH), and 91 normotensive (NT) controls. B2M, CysC, plasma renin activity (PRA), and plasma aldosterone concentration (PAC) were measured, and the aldosterone-to-renin ratio (ARR) was calculated. Carotid intima-media thickness (cIMT), increased cIMT, and presence of carotid plaque or carotid stenosis < 50% in the carotid artery were measured *via* ultrasonography to evaluate the degree of asymptomatic carotid atherosclerosis.

Results: CIMT increased in the NT, EH, and PA groups (0.60 (0.50, 0.80) mm vs. 0.80 (0.60, 1.00) mm vs. 0.90 (0.70, 1.10) mm, $P<0.01$), so as the prevalence of increased cIMT and presence of carotid plaque (both $P<0.05$). The B2M and CysC levels exhibited the same trend (B2M: 1.60 ± 0.34 mg/L, 1.80 ± 0.41 mg/L, 1.98 ± 0.64 mg/L, $P<0.05$; CysC: 0.76 ± 0.12 mg/L, 0.88 ± 0.17 mg/L, 0.94 ± 0.23 mg/L, $P<0.05$). B2M, CysC, PAC, and ARR were all positively associated with cIMT (all $P<0.01$) in the PA group. After adjusting for potential confounders, B2M, PAC, but not CysC or ARR were independently associated with increased cIMT and presence of carotid plaque and carotid stenosis <50%, respectively. The receiver operating characteristic (ROC) curve analysis revealed that B2M and PAC demonstrated significant predictive ability for increased cIMT and presence of carotid plaque and carotid stenosis <50%.

Conclusion: B2M is an independent risk factor for asymptomatic carotid atherosclerosis in patients with PA.

Key words: Asymptomatic carotid atherosclerosis, Beta-2-microglobulin (B2M), Cystatin C (CysC), Primary aldosteronism (PA)

Introduction

The prevalence of primary aldosteronism (PA), characterized by excessive aldosterone secretion from the zona glomerulosa of the adrenal cortex, was over 5% and possibly over 10% in general hypertension[1, 2] and 17%–23% in resistant hypertension[3]. Compared with essential hypertension (EH), long-term exposure to increased aldosterone levels may lead to renal, cardiac, and vascular injury, which eventually results in the significant increase in the morbidity and mortality rates due to arteriosclerotic cardiovascular

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disease (ASCVD) in PA patients[4, 5]. However, it was demonstrated that ASCVD was still prevalent in PA patients after the correction of excessive aldosterone secretion[6, 7], which implicated other possible risk factors for ASCVD in PA patients.

Carotid atherosclerosis plays a significant role in the prediction of cardiovascular morbidity and mortality[8]; however, it often remains asymptomatic until the onset of ASCVD. According to the different progressions of carotid atherosclerosis, increased carotid intima-media thickness (cIMT) and presence of carotid plaque and carotid stenosis were generally used to assess the different severities of asymptomatic carotid atherosclerosis[8-10]. As a result, early detecting asymptomatic carotid atherosclerosis and controlling the relevant risk factors may contribute to the reduction of ASCVD.

Accumulated studies have demonstrated that creatinine was not an optimal indicator for renal function, whereas beta-2-microglobulin (B2M) and cystatin C (CysC) have emerged as more suitable indicators for renal function in the early stage of renal impairment[11, 12]. B2M is an 11.7-kD nonglycosylated polypeptide, a small subunit of the major histocompatibility class I molecule present on all nucleated cells. Conversely, CysC is a 13-kD endogenous cysteine proteinase inhibitor and a non-glycosylated basic protein and is stably produced by most nucleated cells. Numerous studies have demonstrated that B2M and CysC were both closely associated with ASCVD, including subclinical atherosclerosis, such as increased cIMT[13-15] and presence of carotid plaque and arterial stiffness[14, 16], and clinical atherosclerosis, such as coronary artery disease[17, 18] and peripheral artery disease[19]. Moreover, both B2M and CysC could independently predict cardiovascular mortality regardless of renal function[20, 21]. Taken together, it has been suggested that B2M and CysC are both potential risk factors for ASCVD, increasing as early as the subclinical stage of atherosclerosis. However, in PA patients with excessive aldosterone, whether B2M or CysC is still a risk factor independent of aldosterone for asymptomatic carotid atherosclerosis has not yet been investigated. It is of clinical importance to address the issue and try to find the simple and convenient measurement for asymptomatic carotid atherosclerosis in PA patients.

Aim

This study aimed to investigate the relationship between B2M or CysC and asymptomatic carotid atherosclerosis in PA patients in order to explore a novel risk factor for asymptomatic carotid atherosclerosis in PA.

Materials and Methods

Study Population

In the present cross-sectional study, 83 patients with PA, 91 patients with EH, and 91 NT controls were enrolled from our hospital from August 2011 to June 2016. According to the American Endocrine Society clinical practice guideline about PA[1, 2], all of the PA patients were diagnosed by plasma aldosterone concentration (PAC) to plasma renin activity (PRA) (aldosterone-to-renin ratio (ARR), ng/dL per ng/mL per hour) higher than 30 ng/dL per ng/mL per hour in the upright position, further confirmed by the failure of aldosterone suppression after the sodium infusion test and captopril challenge test[1, 2]. All EH patients were confirmed under the following conditions: systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg at different days at least three times of measurement or intake of anti-hypertension drugs prior to enrollment, with ARR < 20 ng/dL per ng/mL per hour consistent with the guideline[1, 2]. Normotensive (NT) controls were defined as subjects with SBP < 140 mmHg and DBP < 90 mmHg, without hypokalemia. All subjects did not experience transient ischemic attack, stroke, or other associated neurological symptoms in the past.

The exclusion criteria for this study were (1) other secondary hypertension (Cushing's syndrome, hyperthyroidism, pheochromocytoma, renal artery stenosis, etc.); (2) diabetes mellitus; (3) recent stress states, such as infection, surgery, and severe trauma; (4) serious cardiac insufficiency, liver insufficiency (serum transaminase or bilirubin increased), and kidney insufficiency (eGFR lower than 60 mL/min·per 1.73 m^2); (5) hematologic disease, malignancy, and rheumatologic diseases.

Data Collection

Clinical data, including gender, age, height, and weight, were obtained from all patients by a well-trained researcher. Body mass index (BMI) was calculated as body weight (kg) divided by the square of the height (m). The blood pressure levels were calculated by the mean of two consecutive blood pressure values taken 10 min apart in a sitting position. Health history and lifestyle behavior were determined through unified questionnaire, including hypertension and corresponding course, medications (hypotensive agents and lipid-lowering agents), smoking status, and alcohol consumption.

Laboratory Testing

In the morning after a 10-h overnight fast, the serum concentrations of total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c), uric acid, plasma glucose, and creatinine (Cr) were measured using the automated enzymatic method. The serum concentrations of sodium and potassium were measured using the ion electrode selection method; the serum concentration of CysC *via* particle-enhanced turbidimetric immunoassay; and the serum concentrations of B2M, apolipoprotein A-1 (Apo-A), and apolipoprotein B100 (Apo-B) via transmission turbidimetric immunoassay. All of above indexes were detected using an autonomic analyzer (Hitachi, Japan, 7600-020).

Measurement of Plasma Renin Activity and Plasma Aldosterone Concentration

Before sample collection, all patients stopped the intake of agents that interfered with the measurement of aldosterone and renin for 2–4 weeks, except for non-dihydropyridine calcium channel blocker antagonist and α-adrenergic blocker according to the guideline[1, 2]. After a whole night of sleep in the supine position, the patients were instructed to sit, stand, or walk for at least 2 h and then sit for 5 to 15 min. Subsequently, their blood samples were drawn in the midmorning. Plasmas were isolated *via* centrifugation at room temperature. PAC and PRA were detected using radioimmunoassay kits according to the manufacturer's protocol (North Institute of Biotechnology Co., Ltd., Beijing, China). The intra-assay coefficients of variation for PAC and PRA were both lower than 10%, respectively. The inter-assay coefficients of variation for PAC and PRA were both lower than 15%, respectively. The reference range of PAC was 6.5 to 29.6 ng/dL, whereas the reference range of PRA was 0.93 to 6.56 ng/mL per hour. ARR was calculated as the ratio of PAC to PRA.

Carotid Ultrasonography

The study was conducted by the same specialist in the ultrasound department using a high-resolution color Doppler ultrasound (Vivid E9, GE, Norway) equipped with a 2.4–8.0 MHz linear-array probe. The subjects were in supine position. Using the anterior or posterior margin of the sternocleidomastoid muscle as the transverse section, the position of the common carotid artery (CCA) was determined. The probe scanned the CCA laterally from the medial end of the clavicle, followed by the carotid bifurcation, internal carotid, and external carotid arteries successively as high up as possible. Then, the probe was rotated 90° to show their longitudinal section. The cIMT was measured as vertical distance from the front edge of the lumen–intima interface to the front edge of the media–adventitia interface of the CCA, and the thickest values were used for this study. Asymptomatic carotid atherosclerosis included the following parameters: increased cIMT, considered as higher than 0.9 mm[10, 22]; carotid plaque, defined as a focal region with a carotid IMT >1.5 mm that protrudes into the lumen[10, 22]; and carotid artery stenosis less than 50%, judged as a local blood filling defect with peak systolic velocity less than 125 cm/s in the presence of plaque or increased cIMT[23].

Statistical Analysis

Continuous data are expressed as either mean ± SD for normally distributed variables or median (interquartile range) for non-normally distributed variables. The normality test was confirmed by the Shapiro–Wilk test. Categorical data are expressed as numbers and percentages. Quantitative data, consistent with the normal distribution and homogeneity of variance, were analyzed *via* one-way ANOVA. Subsequently, pairwise comparison was performed using the least significant difference test (LSD-t); quantitative data inconsistent with the normal distribution were analyzed using the Kruskal–Wallis test, and then pairwise comparisons were analyzed using the Bonferroni method. The relationships between different continuous data were evaluated using Spearman's rank correlation analysis. Univariate and multivariate analyses were conducted to determine the independent risk factors of presence of increased cIMT, carotid plaque and carotid stenosis $<50\%$, expressed as odds ratios (OR) with 95% confidence intervals (CI). A receiver operating characteristic (ROC) curve analysis was designed to identify the cutoff values (thresholds) of the risk factors that best predicted asymptomatic carotid atherosclerosis. The optimal cutoff value for the ROC curve was decided by the Youden index. The differences of the area under the curve (AUC) among the variables were compared *via* pairwise comparisons of the ROC curves using MedCalc 20.0. All other statistical analyses were conducted using SPSS 24.0 (IBM, Armonk, New York, United States). Significance was defined as $P<0.05$.

Results

Clinical and Biochemical Characteristics

The characteristics of the subjects are presented in **Table 1**. There were no statistically significant differences in age, gender, BMI, lipid profile (TC, LDL, HDL, TG, Apo-A, and Apo-B), creatinine,

Table 1. Clinical and laboratory characteristics

	Primary aldosteronism ($n=83$)	Essential hypertension ($n=91$)	Normotensive controls ($n=91$)	*P* Value
Clinical characteristics				
Age (years)	47.93 ± 13.55	49.12 ± 11.51	47.09 ± 12.11	0.539
Sex [F/M (%F)]	42/41 (50.60)	33/58 (36.26)	41/50 (45.05)	0.156
BMI (kg/m^2)	24.42 ± 3.06	24.94 ± 2.94	23.28 ± 4.24	0.100
SBP (mmHg)	158.87 ± 18.06†††	156.11 ± 14.46†††	117.85 ± 13.02	< 0.001
DBP (mmHg)	95.22 ± 13.54†††	93.24 ± 11.25†††	74.82 ± 8.34	< 0.001
Duration of hypertension (years)	3.00 (0.50, 10.00)	5.00 (1.00, 8.00)	NA	0.261
Number of antihypertensive drugs (*n*)	1.47 ± 1.14	1.59 ± 1.11	NA	0.450
Administration of lipid-lowering agents [*n* (%)]	19 (22.89)††	26 (28.57)†††	5 (5.49)	< 0.001
Alcohol drink [*n* (%)]	11 (13.25)	13 (14.29)	11 (12.09)	0.908
Smoking [*n* (%)]	16 (19.28)	18 (19.78)	17 (18.68)	0.982
Laboratatory data				
TC (mmol/L)	4.77 ± 0.92	4.94 ± 1.15	4.97 ± 1.06	0.403
LDL-C (mmol/L)	3.01 ± 0.74	3.14 ± 0.98	3.13 ± 0.92	0.598
HDL-C (mmol/L)	1.15 ± 0.27	1.18 ± 0.34	1.24 ± 0.27	0.110
TG (mmol/L)	1.35 (0.84, 2.00)	1.44 (1.00,1.95)	1.10 (0.84,1.63)	0.083
Apo-A (g/L)	1.35 ± 0.26	1.37 ± 0.22	1.40 ± 0.19	0.434
Apo-B (g/L)	1.15 ± 0.41	1.18 ± 0.32	1.06 ± 0.32	0.082
FBG (mmol/L)	5.68 ± 1.17†	5.72 ± 1.34†	5.23 ± 1.19	0.042
Uric acid (μmol/L)	353 ± 108.18*†	388.53 ± 134.53	392.36 ± 95.37	0.049
Creatinine (μmol/L)	71.31 ± 15.47	73.89 ± 16.66	70.16 ± 14.54	0.257
eGFR (mL/min·per 1.73 m^2)	99.30 ± 16.36	95.75 ± 16.14††	101.94 ± 14.27	0.029
CysC (mg/L)	0.94 ± 0.23*†††	0.88 ± 0.17†††	0.76 ± 0.12	< 0.001
B2M (mg/L)	1.98 ± 0.64*†††	1.80 ± 0.41††	1.60 ± 0.34	< 0.001
Blood potassium (mmol/L)	3.28 ± 0.63***†††	3.85 ± 0.35	3.92 ± 0.25	< 0.001
Blood sodium (mmol/L)	142.46 ± 2.67*††	141.64 ± 2.13	141.43 ± 2.25	0.011
PAC (ng/dL)	20.77 (16.00, 37.04)***	11.63 (8.63, 15.68)	NA	
PRA [ng/ (mL·h)]	0.09 (0.04, 0.10)***	1.90 (1.45, 2.98)	NA	
ARR[ng/dL per ng/ (mL·h)]	344.70 (194.35, 600.00)***	5.26 (3.45, 9.43)	NA	

Variables are shown as means ± SD, medians (interquartile range) or absolute numbers and percentages. NA, not available; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; Apo-A, apolipoprotein A-1; Apo-B, apolipoprotein B100; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate; CysC, cystatin C; B2M, beta-2-microglobulin; PAC, plasma aldosterone concentration; PRA, plasma renin activity and ARR, aldosterone–renin ratio. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. essential hypertension group; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$ vs. normotensive controls.

alcohol consumption, and smoking habit among the PA, EH, and NT groups (all $P>0.05$). In addition, the PA group well matched the EH group in terms of SBP, DBP, duration of hypertension, number of antihypertensive drugs, fasting blood glucose, and eGFR (all $P>0.05$).

Compared with the EH group, PAC, ARR, and serum sodium levels were higher in the PA group.But PRA and serum potassium levels were lower in the PA group than those in the EH group (all $P<0.05$). B2M and CysC both exhibited an increasing trend in the NT, EH, and PA groups (B2M: 1.60 ± 0.34 mg/L vs. 1.80 ± 0.41 mg/L vs. 1.98 ± 0.64 mg/L, respectively, $P<0.05$; CysC: 0.76 ± 0.12 mg/L vs. 0.88 ± 0.17 mg/L vs. 0.94 ± 0.23mg/L, respectively, $P<0.05$).

CIMT and Prevalence of Increased cIMT and Presence of Carotid Plaque and Carotid Stenosis < 50%

As presented in **Table 2**, cIMT in the PA and EH groups were higher than that in the NT group (both $P<0.001$). In the two hypertensive groups, cIMT in the PA group was higher than that in the EH group (0.90 (0.70, 1.10) mm vs. 0.80 (0.60, 1.00) mm, $P<0.01$).

Similarly, the prevalence of increased cIMT and presence of carotid plaque exhibited an increasing trend in the NT, EH, and PA groups (prevalence of

Table 2. CIMT and prevalence of increased cIMT, carotid plaque and carotid stenosis <50%

	Primary aldosteronism (n=83)	Essential hypertension (n=91)	Normotensive controls (n=91)	P Value
cIMT (mm)	0.90 (0.70, 1.10)**†††	0.80 (0.60, 1.00)†††	0.60 (0.50, 0.80)	<0.001
Increased cIMT [n (%)]	32/ (38.55)*†††	19/ (20.88)††	6/ (6.59)	<0.001
Carotid plaque [n (%)]	30 (36.14)*†††	18 (19.78)†	8 (8.79)	<0.001
Carotid stenosis<50% [n (%)]	14 (16.87)†	10 (10.99)	5 (5.49)	0.056

Variables are shown as medians (interquartile range) or absolute numbers and percentages. cIMT, common carotid artery intima-media thickness. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. essential hypertension group; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$ vs. normotensive controls.

Table 3. Subgroup analysis of B2M, CysC, PAC and ARR among different groups with increased cIMT, carotid plaque and carotid stenosis <50% or not in patients with PA

	Increased cIMT		Carotid plaque		Carotid stenosis<50%	
	Yes	No	Yes	No	Yes	No
B2M (mg/L)	2.34±0.74$^{\#\#\#}$	1.76±0.46	2.36±0.75§§§	1.77±0.47	2.53±0.81$^{\infty\infty\infty\infty}$	1.87±0.55
CysC (mg/L)	1.03±0.27$^{\#\#}$	0.89±0.18	1.02±0.26§	0.90±0.20	1.01±0.29	0.93±0.21
PAC (ng/dL)	38.02 (20.70, 48.09)$^{\#\#\#}$	19.00 (15.00, 25.14)	42.13 (20.77, 49.77)§§§	19.00 (15.00, 25.23)	43.34 (25.23, 56.50)$^{\infty\infty\infty}$	20.32 (15.27, 30.99)
ARR [ng/L per ng/(mL·h)]	448.31 (198.20, 602.88)$^{\#}$	306.33 (197.18, 553.66)	494.55 (216.40, 700.87)§	300.00 (194.35, 407.94)	448.31 (180.00, 610.81)	340.00 (200.00, 596.95)

Variables are shown as means±SD or medians (interquartile range). B2M, beta-2-microglobulin; CysC, cystatin C; PAC, plasma aldosterone concentration; ARR, aldosterone–renin ratio; cIMT, common carotid artery intima-media thickness and PA, primary aldosteronism. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. PA group without increased cIMT; $^{\S}P<0.05$, $^{\S\S}P<0.01$, $^{\S\S\S}P<0.001$ vs. PA group without carotid plaque; $^{\infty}P<0.05$, $^{\infty\infty\infty}P<0.01$, $^{\infty\infty\infty\infty}P<0.001$ vs. PA group without carotid stenosis <50%.

increased cIMT: 6.59% vs. 20.88% vs. 38.55%, respectively, $P<0.05$; prevalence of carotid plaque: 8.79% vs. 19.78% vs. 36.14%, respectively, $P<0.05$). The prevalence of carotid stenosis <50% in the PA group was higher than that in the NT group (16.87% vs. 5.49%, $P<0.05$), whereas no significant difference was observed among the other groups.

Correlations between cIMT and other Variables

In the pooled data, cIMT was significantly positively correlated with age, SBP, DBP, TG, Apo-B, FBG, creatinine, CysC, and B2M and negatively correlated with eGFR (all $P<0.05$). In the PA group, cIMT was significantly positively correlated with age, duration of hypertension, TG, creatinine, CysC, B2M, PAC, and ARR and negatively correlated with eGFR (all $P<0.05$). In the EH group, cIMT was positively correlated with age, DBP, and LDL-C and negatively correlated with eGFR (all $P<0.05$) but not significantly correlated with CysC, B2M, creatinine, PAC, or ARR ($P>0.05$). The above data are presented in **Supplemental Table 1 and Supplemental Figs. 1, 2, and 3**. The results of the logistic regression analysis conducted with increased cIMT(higher than 0.9 mm) as a dependent variable and relevant risk factors as independent variables in PA patients are as follows.

Risk Factors of Increased cIMT and Presence of Carotid Plaque and Carotid Stenosis <50% in the PA Group

B2M, CysC, PAC, and ARR were analyzed after the patients with PA were divided into different subgroups as follows: patients with or without increased cIMT and carotid plaque and carotid stenosis <50%. As presented in **Table 3**, in the PA group, B2M, CysC, PAC, and ARR were all higher in patients with increased cIMT or with carotid plaque than those without (all $P<0.01$), and B2M and PAC were both higher in patients with carotid stenosis <50% than those without (all $P<0.001$). Moreover, logistic regression analysis revealed that B2M, CysC, and PAC were all significantly associated with increased cIMT (all $P<0.05$); B2M, CysC, PAC, and ARR were all significantly associated with the presence of carotid plaque (all $P<0.05$); B2M and PAC were both significantly associated with the presence of carotid stenosis <50% (both $P<0.01$) **(Table 4)**. Furthermore, multivariate logistic regression analysis was conducted, with increased cIMT or presence of carotid plaque or carotid stenosis <50% as dependent variable and B2M, CysC, eGFR, PAC divided by 10 (PAC/10), ARR divided by 10 (ARR/10), age, sex, duration of hypertension, SBP, and LDL-C as

Table 4. Univariate logistic regression analysis for B2M, CysC, PAC and ARR respectively with increased cIMT, carotid plaque and carotid stenosis <50% in PA group, with data expressed as odds ratio (95%CI)

	Increased cIMT	Carotid plaque	Carotid stenosis<50%
B2M (mg/L)	5.73 (2.18 to 15.05)***	5.64 (2.15 to 14.78)***	4.76 (1.73 to 13.12)**
CysC (mg/L)	16.52 (1.85 to 147.47)*	10.29 (1.26 to 84.25)*	4.15 (0.40 to 43.16)
PAC (ng/dL)	1.10 (1.05 to 1.14)***	1.10 (1.06 to 1.16)***	1.07 (1.03 to 1.12)**
ARR [ng/L per ng/(mL·h)]	1.001 (0.999 to 1.002)	1.002 (1.000 to 1.004)*	1.000 (0.998 to 1.002)

B2M, beta-2-microglobulin; CysC, cystatin C; PAC, plasma aldosterone concentration; ARR, aldosterone–renin ratio; cIMT, common carotid artery intima-media thickness and PA, primary aldosteronism. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

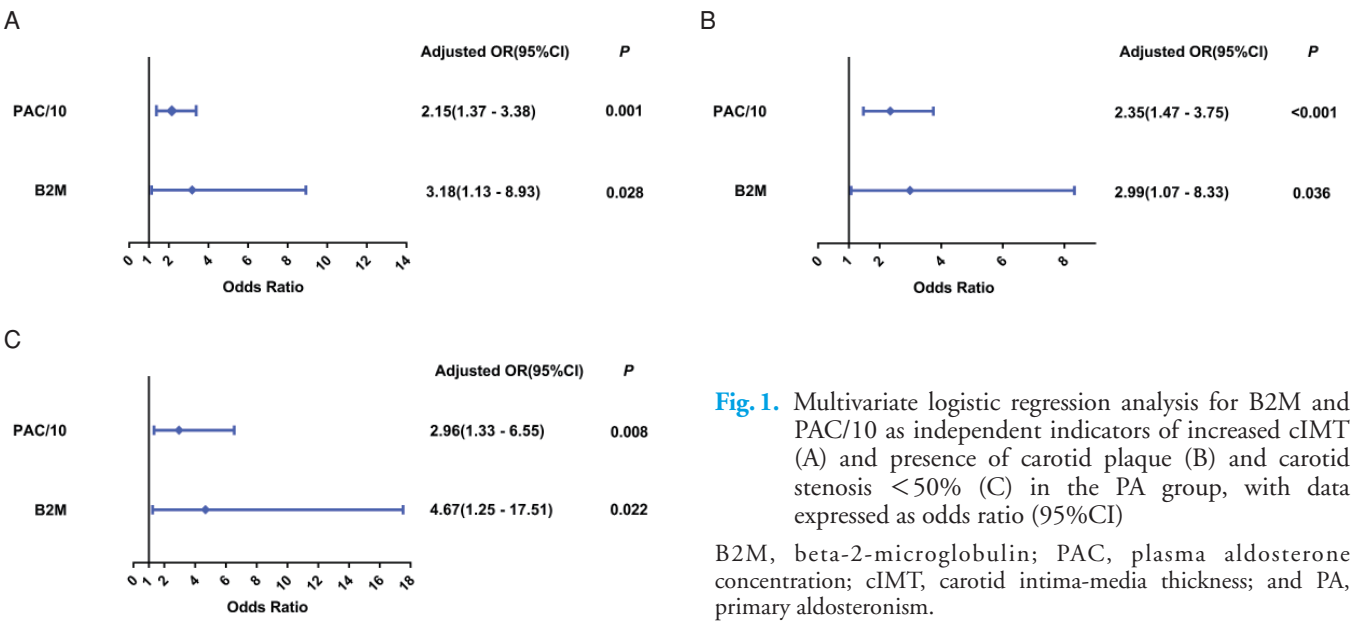

Fig. 1. Multivariate logistic regression analysis for B2M and PAC/10 as independent indicators of increased cIMT (A) and presence of carotid plaque (B) and carotid stenosis <50% (C) in the PA group, with data expressed as odds ratio (95%CI)

B2M, beta-2-microglobulin; PAC, plasma aldosterone concentration; cIMT, carotid intima-media thickness; and PA, primary aldosteronism.

independent variables. It is noteworthy that PAC and ARR were divided by 10 to make the effect estimates of PAC or ARR in the logistic regression more obvious. In this model, only B2M and PAC/10 were independent risk factors for asymptomatic carotid atherosclerosis **(Fig. 1A, 1B, 1C)**. With regard to B2M, the ORs for increased cIMT and presence of carotid plaque and carotid stenosis <50% were 3.18 (1.13 to 8.93), 2.99 (1.07 to 8.33), and 4.67 (1.25 to 17.51), respectively ($P<0.05$). For PAC/10, the ORs for increased cIMT and presence of carotid plaque and carotid stenosis <50% were 2.15 (1.37 to 3.38), 2.35 (1.47 to 3.75), and 2.96 (1.33 to 6.55), respectively ($P<0.01$).

Correlations between the Indicators of Renal Function and PAC, PRA, and ARR in the PA or EH Group

As the indicators of renal function, B2M, CysC, and eGFR except for creatinine were significantly correlated with PAC (r=0.42, r=0.32, and r=–0.32, $P<0.01$) in PA **(Table 5)**. Only B2M was significantly correlated with ARR (r=0.26, $P=0.02$), and none of the indicators of renal function was significantly correlated with PRA in PA. No correlation was observed between B2M and PAC or ARR in the EH group. Among them, B2M and CysC are the key observed indicators, therefore it's also provided the correlations between B2M or CysC and other variables, shown in **Supplemental Table 2 and 3**.

ROC Curve Analysis for Predicting Increased cIMT and Presence of Carotid Plaque and Carotid Stenosis <50%

The area under the ROC curve for PAC, B2M, CysC, and ARR in detecting increased cIMT was 0.80 (95% CI: 0.70–0.90, $P<0.001$), 0.75 (95% CI: 0.63–0.86, $P<0.001$), 0.67 (95% CI: 0.54–0.79, $P=0.011$), and 0.57 (95% CI: 0.44–0.70, $P=0.268$), respectively **(Fig. 2A)**. The area under the ROC curve for PAC, B2M, CysC, and ARR in detecting the presence of carotid plaque was 0.81 (95% CI: 0.70–0.91, $P<0.001$), 0.75 (95% CI: 0.63–0.86, $P<0.001$), 0.65 (95% CI: 0.52–0.78, $P=0.021$), and

Table 5. Correlations between indicators of renal function and PAC, PRA and ARR in PA group

	PAC(ng/dL)	PRA [ng/(mL·h)]	ARR[ng/dL per ng/(mL·h)]
Creatinine (μmol/L)	r=0.21	r=-0.04	r=0.13
eGFR (mL/min·per 1.73 m^2)	r=-0.32**	r=-0.13	r=-0.03
CysC (mg/L)	r=0.32**	r=0.05	r=0.12
B2M (mg/L)	r=0.42***	r=-0.02	r=0.26*

PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone–renin ratio; PA, primary aldosteronism; eGFR, estimated glomerular filtration rate; CysC, cystatin C and B2M, beta-2-microglobulin. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

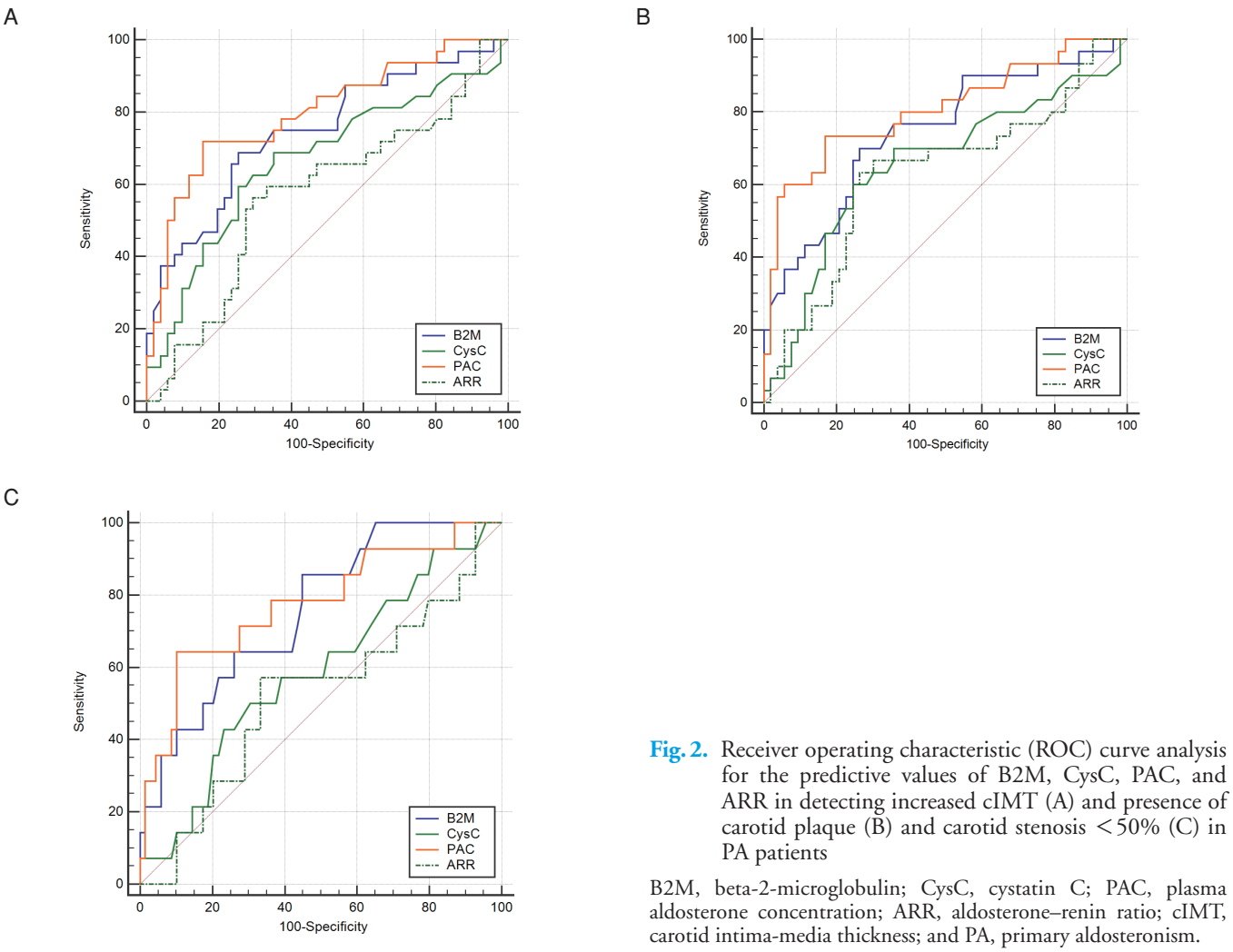

Fig. 2. Receiver operating characteristic (ROC) curve analysis for the predictive values of B2M, CysC, PAC, and ARR in detecting increased cIMT (A) and presence of carotid plaque (B) and carotid stenosis <50% (C) in PA patients

B2M, beta-2-microglobulin; CysC, cystatin C; PAC, plasma aldosterone concentration; ARR, aldosterone–renin ratio; cIMT, carotid intima-media thickness; and PA, primary aldosteronism.

0.63 (95% CI: 0.50–0.76, $P=0.049$), respectively (Fig. 2B). The area under the ROC curve for PAC, B2M, CysC, and ARR in detecting the presence of carotid stenosis <50% was 0.77 (95% CI: 0.63–0.92, $P=0.001$), 0.76 (95% CI: 0.63–0.89, $P=0.003$), 0.59 (95% CI: 0.42–0.75, $P=0.319$), and 0.52 (95% CI: 0.35–0.70, $P=0.794$), respectively (Fig. 2C). Accordingly, only PAC and B2M had good diagnostic performance for the three surrogate markers of asymptomatic carotid atherosclerosis. However, the differences in the AUC between PAC and B2M were not significant when pairwise comparisons of ROC curves were performed ($P=0.45$ for increased cIMT, $P=0.39$ for the presence of carotid plaque, and $P=0.84$ for the presence of carotid stenosis <50%). Based on the ROC curve, the optimal cutoff for PAC in detecting increased cIMT and presence of carotid plaque and carotid stenosis <50% was 28.24, 28.24,

and 42.77 ng/dL, respectively. With regard to B2M, the optimal cutoff in detecting increased cIMT and presence of carotid plaque and carotid stenosis < 50% was 2.02, 2.02, and 1.89 mg/L, respectively.

Discussion

Previous studies found that PA patients manifested less-severe abnormalities of glucose and lipid metabolism[24] but much earlier emergence of albuminuria[25] and more severe endothelial dysfunction contrary to EH patients[26]. This indicated that PA was more predisposed to prominent renal and vascular damages, especially to much earlier and more severe atherosclerosis [27, 28]. Most of the past researches usually adopted cIMT as the surrogate marker of subclinical carotid atherosclerosis[29] and found that PA patients had significantly higher cIMT compared with the EH or NT subjects [28]. As another creditable surrogate and more serious stage for carotid atherosclerosis, carotid plaque and carotid stenosis are even more powerful in predicting cardiovascular outcomes than cIMT[30, 31]. However, whether the prevalence of carotid plaque and carotid stenosis is higher in PA patients is still unknown. To the best of our knowledge, this was the first study to compare the prevalence of carotid plaque and carotid stenosis < 50% among the PA, EH, and NT groups. Significantly increasing trends of cIMT and prevalence of increased cIMT and carotid plaque were observed in the NT, EH, and PA groups. However, though the prevalence of carotid stenosis <50% in the PA group was slightly higher than that in the EH group, no significant difference was observed. As carotid stenosis was the advanced and later stage of carotid atherosclerosis compared with increased cIMT and presence of carotid plaque and the mean durations of EH and PA in the present study were both no more than 5 years, we assumed that there was no sufficient time of exposure to increased aldosterone levels in the PA group to ultimately differentiate carotid stenosis between the EH and PA groups.

A large number of studies have proved that hypertension plays a vital role in carotid atherosclerosis[32] and can partially explain why these surrogate markers for carotid atherosclerosis are higher in PA patients than in NT subjects. The German Conn's Registry reported that cardiovascular disease (CVD) was the main cause of death in the PA group, accounting for 50% of the mortality rate[33]. Aside from hypertension, CVD in PA is attributed to another important and indispensable contributor, namely, excessive aldosterone, which could induce endothelial dysfunction, fibrosis, and thickening of the arterial wall and subsequent vascular remodeling[34, 35], manifesting as increases in cIMT[36]. Consistently, we found that PAC was independently associated with the surrogate markers of asymptomatic carotid atherosclerosis, including increased cIMT and presence of carotid plaque and carotid stenosis < 50%. These findings contribute to the identification of the critical role of aldosterone excess in the occurrence and development of carotid atherosclerosis. As cIMT in PA patients could be significantly reduced after adrenalectomy and treatment with aldosterone blocker, eplerenone, or spironolactone[37-39], the findings in the present study also suggested that we should pay more attention to the early screening of PA in hypertensive patients and correct it in a timely manner.

In the past two decades, B2M and CysC have been gaining increasing attention as risk factors of CVD[13-19], including asymptomatic carotid atherosclerosis[13-16]. There have been no reports yet exploring the relationships between B2M or CysC and asymptomatic carotid atherosclerosis in PA. In the present study, a significantly increasing trend of B2M or CysC in the NT, EH, and PA groups was observed. Within the PA group, both B2M and CysC were higher in patients with increased cIMT or with carotid plaque than those without, whereas only B2M was higher in patients with carotid stenosis <50% than those without. All of these findings suggested that both B2M and CysC might be potential risk factors of asymptomatic carotid atherosclerosis in PA. However, after adjusting for confounding factors, only B2M was found to be independently associated with asymptomatic carotid atherosclerosis.

In addition, the risk of presence of increased cIMT, carotid plaque and carotid stenosis <50% was increased by 2.18, 1.99, 3.67 times respectively per 1 mmol/L increase in B2M levels, obviously higher than PAC. This indicated that B2M could be a valued risk factor of asymptomatic carotid atherosclerosis in PA. The potential mechanisms may be as follows. First, high B2M levels may exert an atherogenic effect through amyloid deposit, which could damage the vessel walls[40, 41]. Second, atherosclerosis is presumed to be a chronic inflammatory response[42], in which B2M could take part through inflammation[43, 44] and oxidative stress[45]. B2M was found to be correlated with oxidative stress biomarkers estimated as total antioxidant capacity and superoxide dismutase in patients undergoing dialysis[46]. Some of the deposited B2M might be converted to advanced glycation end products (AGE-modified B2M), which can lead to monocyte activation, cytokine production, and further reactive oxygen species formation[47]. These collectively

suggested that inflammation and oxidative stress might be the potential ways for the atherogenic role of B2M. Third, a positive correlation between PAC and B2M was observed in the PA group, as presented in **Table 5**. Excessive aldosterone can facilitate the occurrence and development of atherosclerosis, including renal artery sclerosis[48, 49]. Meanwhile, long-term exposure to increased aldosterone levels will increase the renal perfusion pressure and damage the intrarenal vessels, subsequently leading to early damage to renal function[50, 51]. Compared with creatinine, B2M is a more sensitive indicator of renal function, which can increase when renal function begins to decline before creatinine increases[11]. As a result, B2M was even higher in the PA group than in the EH group when creatinine was still normal. In view of the mechanisms discussed above, higher B2M levels could exert more severe atherosclerogenic damage to the carotid artery in PA. This also helps explain the significant correlation between B2M and cIMT in the PA group; however, the same correlation was not observed in the EH group (result shown in **Table S1**). With the dual effects of declined renal function and excessive aldosterone, B2M may cause higher risks of asymptomatic carotid atherosclerosis than aldosterone in PA patients. Therefore, B2M could be a notable risk factor for atherosclerotic vascular complications in PA. In PA patients with elevated B2M levels, further measures to restore renal function and correct excessive aldosterone should be considered. In the ROC curve analysis, B2M demonstrated a significant predictive ability for increased cIMT and presence of carotid plaque and carotid stenosis $<50\%$, as presented in **Fig. 2A, 2B, and 2C**. These findings suggested that further measurement of asymptomatic carotid atherosclerosis *via* ultrasonography should be considered when the B2M levels are increased in PA patients. In addition, for the return visit of PA patients for checking and review of asymptomatic carotid atherosclerosis, detection of B2M levels as a much easier and cheaper method is of clinical importance.

Although many studies demonstrated that CysC is a good predictor of cardiovascular events and mortality[15, 16, 18, 21], several studies failed to find an association between serum CysC and CVD[52-54]. Eriksson *et al.* even reported that decreased CysC levels were associated with myocardial infarction[55]. The present study also failed to find that CysC was independently associated with asymptomatic carotid atherosclerosis. This discrepancy may be partly attributed to the genetic variations in CysC production[55], and partly because CysC is the most potent endogenous inhibitor of cysteine proteinases. As a matter of fact, CysC may exhibit both pro- and antiatherogenic properties in general[15, 16, 18, 55, 56]. A prospective research with a larger sample size to elucidate the role of CysC in atherosclerosis and ASCVD is required in the future.

Limitations

This study has some limitations. First, the number of PA patients enrolled was relatively small. Second, the possible causal relationship between B2M and asymptomatic carotid atherosclerosis could not be elucidated due to the cross-sectional study design. Third, details of the medications that the participants received and the lifestyle risk factors were not collected and adjusted in the data analyses, which may lead to bias to some extent. Prospectively designed studies with larger cohorts in multi-center will be required in future investigations.

Conclusions

In conclusion, cIMT was higher and increased cIMT and presence of carotid plaque were more prevalent in PA patients than in the NT group and EH group. B2M and PAC but not CysC were independent risk factors for asymptomatic carotid atherosclerosis in PA patients. Both B2M and PAC demonstrated significant predictive ability for increased cIMT and presence of carotid plaque and carotid stenosis $<50\%$. Because of the requirement of special position and time for detecting PAC, it is more recommended to use B2M as a simple and convenient replacement for the early detection of asymptomatic carotid atherosclerosis in PA patients.

Declarations

Ethics Approval and Consent to Participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University ([2020]02-020-01). Informed consent was obtained from all individual participants included in the study.

Consent for Publication

Consent to publish from the participant to report individual patient data: not applicable (no patient identifier or personalized data shown).

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Dr. Xubin Yang, Dr. Wen Xu and Dr. Bin Yao designed, organized and supervised the study. Dr. Xubin Yang provided funding support. All authors contributed to running the study, material preparation and data collection. Shangyan Liang, Xubin Yang and Qingling Li performed data analysis. The first draft of the manuscript was written by Shangyan Liang and Xubin Yang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of Data and Materials

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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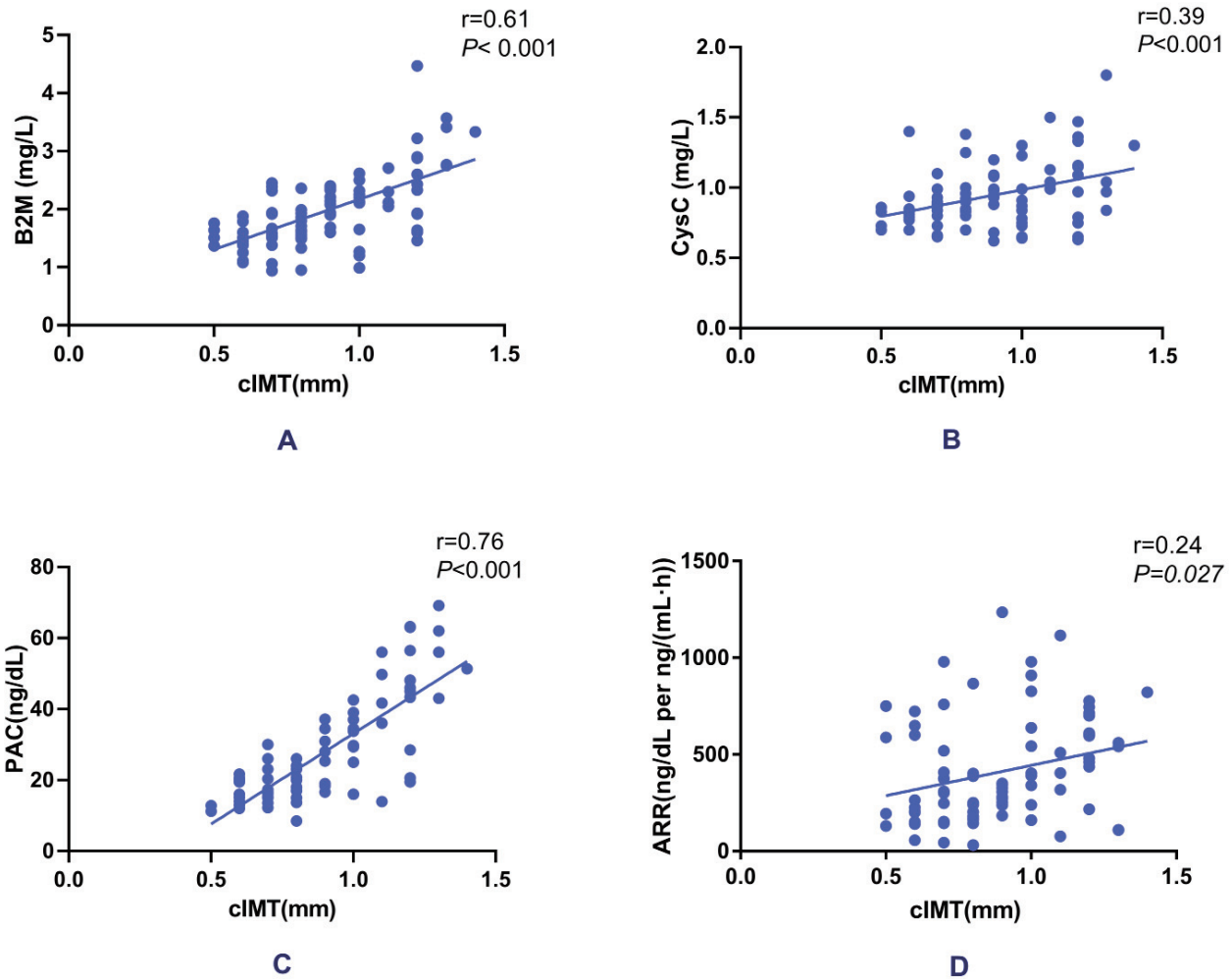
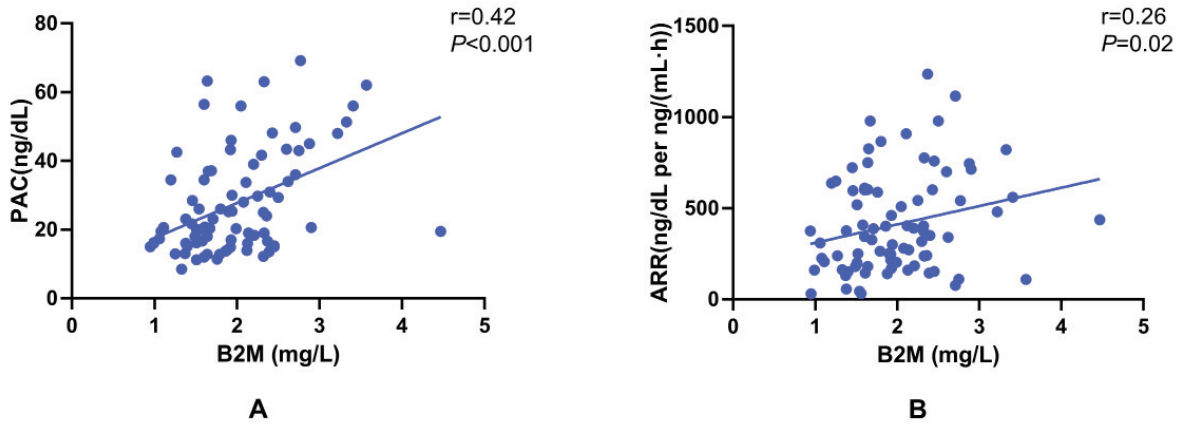


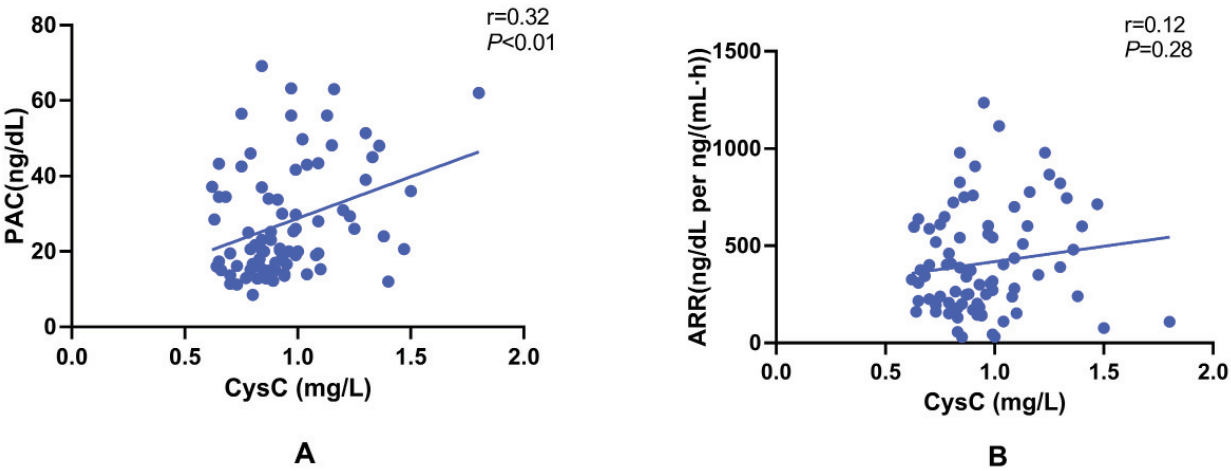
Supplemental Fig. 1. Correlations between cIMT and B2M (A), CysC (B), PAC (C), or ARR (D) in PA patients

B2M, beta-2-microglobulin; CysC, cystatin C; PAC, plasma aldosterone concentration; ARR, aldosterone–renin ratio; cIMT, carotid intima-media thickness; and PA, primary aldosteronism.



Supplemental Fig. 2. Correlations between B2M and PAC (A) or ARR (B) in PA patients

B2M, beta-2-microglobulin; PAC, plasma aldosterone concentration; ARR, aldosterone–renin ratio; and PA, primary aldosteronism.



Supplemental Fig. 3. Correlations between CysC and PAC (A) or ARR (B) in PA patients

CysC, cystatin C; PAC, plasma aldosterone concentration; ARR, aldosterone–renin ratio; and PA, primary aldosteronism.

Supplemental Table 1. Correlations of cIMT(mm) with clinical and biochemical parameters

Variables	Pooled group (n=265)	PA group (n=83)	EH group (n=91)	NT group (n=91)
Age (years)	r=0.39***	r=0.40***	r=0.36***	r=0.45***
BMI (kg/m^2)	r=0.12	r=0.14	r=-0.02	r=0.30
SBP (mmHg)	r=0.34***	r=0.03	r=-0.02	r=0.28**
DBP (mmHg)	r=0.19**	r=-0.05	r=-0.25*	r=0.10
Duration of hypertension (years)	NA	r=0.31**	r=0.20	NA
Number of antihypertensive drugs (n)	NA	r=0.20	r=-0.13	NA
TC (mmol/L)	r=0.06	r=0.04	r=0.18	r=0.13
LDL-C (mmol/L)	r=0.04	r=-0.19	r=0.24*	r=0.13
HDL-C (mmol/L)	r=-0.11	r=-0.05	r=0.01	r=-0.16
TG (mmol/L)	r=0.22***	r=0.25*	r=0.13	r=0.25*
Apo-A (g/L)	r=-0.07	r=0.08	r=-0.02	r=-0.13
Apo-B (g/L)	r=0.18**	r=0.13	r=0.19	r=0.20
FBG (mmol/L)	r=0.18**	r=0.09	r=0.06	r=0.25*
Uric acid (μmol/L)	r=0.01	r=0.02	r=0.07	r=0.13
Creatinine (μmol/L)	r=0.12*	r=0.30**	r=-0.03	r=0.13
eGFR (mL/min·per 1.73 m^2)	r=-0.38***	r=-0.47***	r=-0.34**	r=-0.35**
CysC (mg/L)	r=0.35***	r=0.39***	r=0.18	r=0.19
B2M (mg/L)	r=0.39***	r=0.61***	r=0.19	r=0.17
Blood potassium (mmol/L)	r=-0.11	r=-0.03	r=0.14	r=0.08
Blood sodium (mmol/L)	r=0.04	r=-0.02	r=-0.17	r=0.12
PAC(ng/dL)	NA	r=0.76***	r=0.07	NA
PRA [ng/(mL·h)]	NA	r=-0.18	r=-0.03	NA
ARR [ng/dL per ng/(mL·h)]	NA	r=0.24*	r=0.05	NA

cIMT, common carotid artery intima-media thickness; PA, primary aldosteronism; EH, essential hypertension; NT, normotension; NA, not available; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; Apo-A, apolipoprotein A-1; Apo-B, apolipoprotein B100; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate; CysC, cystatin C; B2M, beta-2-microglobulin; PAC, plasma aldosterone concentration; PRA, plasma renin activity and ARR, aldosterone–renin ratio. *P<0.05, **P<0.01, ***P<0.001.

Supplemental Table 2. Correlations of B2M (mg/L) with clinical and biochemical parameters

Variables	Pooled group (n=265)	PA group (n=83)	EH group (n=91)	NT group (n=91)
Age (years)	r=0.33***	r=0.51***	r=0.26*	r=0.23*
BMI (kg/m^2)	r=-0.12	r=-0.18	r=-0.14	r=0.01
SBP (mmHg)	r=0.26***	r=0.16	r=0.05	r=-0.04
DBP (mmHg)	r=0.06	r=-0.08	r=-0.25*	r=-0.09
Duration of hypertension (years)	NA	r=0.29**	r=0.17	NA
TC (mmol/L)	r=-0.01	r=0.01	r=0.12	r=-0.05
LDL-C (mmol/L)	r=0.04	r=-0.08	r=0.23*	r=0.03
HDL-C (mmol/L)	r=-0.06	r=0.05	r=-0.04	r=-0.06
TG (mmol/L)	r=-0.01	r=0.09	r=-0.09	r=-0.06
Apo-A (g/L)	r=-0.03	r=0.02	r=0.08	r=-0.12
Apo-B (g/L)	r=0.03	r=-0.01	r=0.12	r=0.00
FBG (mmol/L)	r=-0.04	r=-0.03	r=-0.19	r=-0.01
Uric acid (μmol/L)	r=0.05	r=-0.06	r=0.06	r=0.26*
Creatinine (μmol/L)	r=0.25***	r=0.33**	r=0.13	r=0.26*
eGFR (mL/min·per 1.73 m^2)	r=-0.42***	r=-0.55***	r=-0.40***	r=-0.28**
CysC (mg/L)	r=0.62***	r=0.69***	r=0.61***	r=0.45***
Blood potassium (mmol/L)	r=-0.15*	r=-0.18	r=-0.06	r=0.10
Blood sodium (mmol/L)	r=0.16*	r=0.19	r=-0.02	r=0.19
PAC (ng/dL)	NA	r=0.42***	r=-0.05	NA
PRA [ng/(mL·h)]	NA	r=-0.02	r=0.01	NA
ARR [ng/dL per ng/(mL·h)]	NA	r=0.26*	r=0.01	NA

B2M, beta-2-microglobulin; PA, primary aldosteronism; EH, essential hypertension; NT, normotension; NA, not available; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; Apo-A, apolipoprotein A-1; Apo-B, apolipoprotein B100; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate; CysC, cystatin C; PAC, plasma aldosterone concentration; PRA, plasma renin activity and ARR, aldosterone–renin ratio. *P<0.05, **P<0.01, ***P<0.001.

Supplemental Table 3. Correlations of CysC (mg/L) with clinical and biochemical parameters

Variables	Pooled group (n=265)	PA group (n=83)	EH group (n=91)	NT group (n=91)
Age (years)	r=0.24***	r=0.39***	r=0.25*	r=0.11
BMI (kg/m^2)	r=0.06	r=-0.03	r=0.14	r=-0.03
SBP (mmHg)	r=0.37***	r=0.19	r=0.09	r=-0.02
DBP (mmHg)	r=0.17**	r=-0.10	r=-0.18	r=-0.13
Duration of hypertension (years)	NA	r=0.26*	r=0.34**	NA
TC (mmol/L)	r=-0.11	r=-0.06	r=-0.05	r=-0.04
LDL-C (mmol/L)	r=-0.05	r=-0.14	r=0.14	r=-0.02
HDL-C (mmol/L)	r=-0.21**	r=-0.06	r=-0.20	r=-0.18
TG (mmol/L)	r=0.09	r=0.24*	r=-0.12	r=0.06
Apo-A (g/L)	r=-0.17**	r=-0.01	r=-0.16	r=-0.17
Apo-B (g/L)	r=0.04	r=0.06	r=-0.07	r=0.06
FBG (mmol/L)	r=-0.02	r=0.06	r=-0.17	r=-0.15
Uric acid (μmol/L)	r=0.10	r=0.03	r=0.07	r=0.43***
Creatinine (μmol/L)	r=0.41***	r=0.45***	r=0.33**	r=0.51***
eGFR (mL/min·per 1.73 m^2)	r=-0.44***	r=-0.52***	r=-0.45**	r=-0.31**
B2M (mg/L)	r=0.62***	r=0.69***	r=0.61***	r=0.45***
Blood potassium (mmol/L)	r=-0.21***	r=-0.31**	r=-0.08	r=0.09
Blood sodium (mmol/L)	r=0.17***	r=0.23*	r=0.07	r=0.14
PAC (ng/dL)	NA	r=0.32**	r=-0.01	NA
PRA [ng/(mL·h)]	NA	r=0.05	r=-0.06	NA
ARR [ng/dL per ng/(mL·h)]	NA	r=0.12	r=0.08	NA

CysC, cystatin C; PA, primary aldosteronism; EH, essential hypertension; NT, normotension; NA, not available; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; Apo-A, apolipoprotein A-1; Apo-B, apolipoprotein B100; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate; B2M, beta-2-microglobulin; PAC, plasma aldosterone concentration; PRA, plasma renin activity and ARR, aldosterone–renin ratio. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.