

Association between Subclavian Artery Plaques and Future Cardiovascular Events in a Hypertensive Cohort

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Abstract

Background

Atherosclerosis is one of the leading contributors to cardiovascular diseases. Subclinical atherosclerosis could occur long time before the presence of clinical symptoms and may have predictive value for future cardiovascular events. Existing carotid subclinical atherosclerotic markers have limited value.

Subjects and Methods

Patients admitted to the cardiac vascular center of Beijing Tiantan Hospital due to essential hypertension were prospectively consecutively enrolled. Those with histories of coronary artery disease (CAD), cerebrovascular diseases, and significant stenosis of main arteries were excluded. Patients diagnosed with carotid and/or subclavian artery stenosis were also excluded after admission. All participants received a series of routine blood test at admission into hospital and ultrasonic examinations of bilateral carotid arteries and right subclavian artery. All data were recorded. After discharged from hospital all participants were followed up with cardiovascular events, including death, heart failure, myocardial infarction, unstable angina pectoris and stroke.

Results

473 participants were finally involved in the study, including 284 men and 189 women.

9/473 (8.2%, 95% C.I. [5.9-11.1%]) participants suffered from endpoint events, including 19(4.0%) cases of myocardial infarction (15 undergoing PCI and 5 death), 4(0.8%) heart failure (all also involved in the myocardial infarction group), 3(0.6%) ischemic stroke (1 death), and 17(3.6%) angina pectoris (7 undergoing PCI). No death due to other reasons was recorded. After the adjustment of potential risk factors based on the results of univariate analyses, the prevalence of right subclavian plaque (RSP) (OR=2.428, 95% C.I. [1.098, 5.370], $p=0.028$) and both carotid and subclavian plaques (MPs) (OR = 3.539, 95% C.I. [1.547, 8.096], $p = 0.003$) were both significantly associated with endpoint events. Globulin and albumin had significantly correlations with future events despite the atherosclerotic marker used in the regression model.

Conclusion

The prevalence of right subclavian plaques was independently significantly associated with the incidence of future cardiovascular events in the cohort. Higher level of globulin and lower level of albumin tended to be predictors for future events. Combination of both right subclavian artery and carotid artery atherosclerotic plaques may have strongest predictive value for future cardiovascular events in the hypertensive cohort without clinical atherosclerotic diseases.

Introduction

Atherosclerosis is a fundamental contributor of cardiovascular and cerebrovascular diseases which contribute most highly to the death in most countries around the world. ^[1] Many studies have shown that subclinical atherosclerosis could occur long time before the presence of clinical symptoms associated with various atherosclerotic vascular diseases and would significantly correlated to the risk for future cardiovascular and cerebrovascular diseases. ^[2-6] Therefore, recent guidelines are attempting to incorporate subclinical

atherosclerotic markers into the screen tools or risk stratification strategy for future vascular events. [2, 7, 8] Among these markers, carotid atherosclerosis, including carotid intima-media thickness (IMT) and carotid plaques (CP), and coronary atherosclerosis indicated by coronary artery calcium scores (CACs) are most frequently studied and used. [3, 4, 9] The incorporation of these new markers enhanced the predictive value of traditional risk scores to some extent. However, these markers also have reported limitations. [10, 11] Some studies reported that the predictive value of the markers of subclinical atherosclerosis in carotid and coronary arteries should be meaningful but not yet valuable enough to be used in routine risk assessment strategies in clinical practice. [11–14]

Subclavian artery is relatively near to both the carotid artery and coronary artery and is relatively convenient to be additionally examined in routine carotid ultrasonography during clinical practice. Due to its special anatomical position, a few recent research have studied the atherosclerotic markers of subclavian artery and their potential relationship with cardiovascular and cerebrovascular events and yielded promising results. [15, 16] Data based on the progression of early subclinical atherosclerosis (PESA) study showed that the presence of subclavian atherosclerosis could be found in subjects with low traditional risk score and absence of carotid and coronary atherosclerosis. [10] Furthermore, the relationship between the prevalence of subclinical atherosclerosis in subclavian artery and that of carotid territories is controversial in recent meta-analyses and studies. [10, 17] In order to evaluate the prevalence of subclinical atherosclerosis in subclavian artery and its association between further cardiovascular events and stroke, we designed and performed this study in a hypertensive inpatient cohort.

Subjects And Methods

Subject Selection and Baseline Data Collection

Between July 1st 2017 and November 30st 2019, patients admitted to hospital due to essential hypertension in the cardiac vascular center of Beijing Tiantan Hospital, Capital Medical University (CCMU) were prospectively consecutively involved in this study. The exclusion criteria included histories of coronary artery diseases (CAD) (including previous coronary revascularization, myocardial infarction (MI), and/or known >50% stenosis of any coronary artery), cerebrovascular diseases (including ischemic, hemorrhagic stroke, transient ischemic attack (TIA) and etc.), significant stenosis (>50%) and/or surgeries of procedures of other main arteries, which included but were not limited to renal, carotid, and iliac arteries, congestive heart failure, congenital heart defects, and cardiomyopathy. All histories of these diseases were required to be confirmed based on medical records. Those that were diagnosed with above conditions during hospitalization would be also excluded. For each participant, relying on the standardized medical record collecting system, clinical data were recorded, including findings of physical examinations, results from a series of imaging examinations (including echocardiography, sonography on carotid artery and subclavian artery, and etc.), and results from blood biochemical tests. Two trained staff (one physician and one assistant physician) were in charge of the completeness of data collection of each subjects enrolled. A written confirmed consent was acquired for each participant. The protocol of this study was approved by the medical ethics committee institutional review board of Beijing Tiantan Hospital, CCMU.

Definitions of hypertension and other diseases

Essential hypertension was defined as either systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) is ≥ 90 mmHg according to Chinese Guidelines for hypertension.^[18] Those receiving anti-hypertensive medication without a SBP and/or DBP reach the standards were also diagnosed with hypertension. The diagnosis of essential hypertension would be verified during the hospital stay and differential diagnosis of secondary hypertension was also performed. Dyslipidemia was defined as low density lipoprotein cholesterol (LDL-C) ≥ 3.4 mmol/L, total triglycerides (TG) ≥ 1.7 mmol/L, total cholesterol (TC) ≥ 5.2 mmol/L, and/or high-density lipoprotein cholesterol (HDL-C) ≤ 1.0 mmol/L in line with China's guidelines for dyslipidemia.^[19] Diagnoses of all disorders would be carefully verified and confirmed during the hospital stay based on guidelines for clinical practice.^[20]

Ultrasonography on Carotid Artery and Subclavian Artery

Each participant routinely received ultrasonography assessment on carotid artery and subclavian artery during the first two days of hospital admission. The device used was Canon Aplio 900 (Canon Medical Systems, Japan) ultrasound system, equipped with a 7.5-MHz linear probe. Multimode ultrasonography examination (including B-mode, color Doppler, and spectral Doppler) was performed using a standardized vascular ultrasound protocol.^[13] Bilateral carotid arteries and right subclavian artery were scanned in the longitudinal and transverse planes respectively with the subjects in their supine position. All the ultrasonography were performed by two sonographers, who were experienced and well-trained at the beginning of this study. Although bilateral subclavian arteries are both anatomically close to the carotid arteries, left subclavian artery is by far more difficult to be identified precisely at the time of a routine carotid ultrasonography than the right side. Therefore, in this study, we only used the right subclavian artery as the target for the evaluation. Bilateral carotid arteries were scanned focusing on both the near and far walls of the distal 2 cm of the common carotid artery proximal to its bifurcation. Either carotid or subclavian atherosclerosis plaque was defined as a diffuse thickness greater than 1.5mm from the intima-lumen interface to the media-adventitia interface or demonstrated as a distinct area of carotid intima-media thickness (cIMT) $\geq 50\%$ more than the surrounding arterial wall or a focal structure into carotid arterial lumen of 0.5 mm or greater.^[13,21] The carotid cIMT (including the plaque) was obtained as the maximal internal carotid plaque thickness when the image showed the thickest plaque.

Cardiovascular Events and Follow-up

The endpoint cardiovascular events were defined as suffering from at least one of the following situations: 1) acute myocardial infarction (AMI), 2) unstable angina pectoris (UA), 3) hospitalization for percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), 4) stroke/TIA, 4) heart failure, 5) death. Since the discharge of the first hospitalization, all the participants were asked to be regularly followed-up at the outpatient-service of Beijing Tiantan Hospital and community health service centers. As for those who did not come to the follow-up service, we contacted them via telephone every 3 months. Cardiovascular mortality with the major underlying cause of death being CAD or stroke and all-cause mortality defined as the death from any cause were also recorded. All events recorded were verified with death certificates and medical records. Stroke/TIA was defined as focal neurological disorder lasting 24 hours or longer or until death with a clinically relevant brain lesion identified via computed tomography. The diagnosis of stroke was verified by a neurologist. Two well-trained cardiologists were in charge of the follow-up information collection.

Statistical Analysis

Statistical analyses were performed mostly using the SPSS for Windows statistical software package version 23.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were calculated using mean and standard deviation, while categorical variables using proportions. CP and RSP were both categorized as present or absent. Based on the incidence of future cardiovascular events (endpoint events), participants were finally divided into two groups (presence of events or no events). Differences in continuous variables between the two groups were tested using Student t's test, including age, years of hypertension history, heart rate, blood pressures at admission, ultrasonic parameters, biochemical indicators and other blood test results (homogeneity of variance was considered). Difference between categorized variables were tested via person χ^2 test, where those with one or more respected frequency(s) < 5 were evaluated based on Fisher's exact test. The exact confidence intervals (C.I.) of the prevalence of categorized variables were calculated using StatPages.net (<http://statpages.org/confint.html>) on the basis of binomial distribution.

Multiple logistic regression models were used to explore the independent association between the potential risk factors and future endpoint events. Variables with $p < 0.100$ were chosen as independents to construct the models for multivariate analyses (backward stepwise logistic regression models). Due to the potentially high correlation between the three studied ultrasonic markers of subclinical atherosclerosis, which are CP, RSP, and MPs, they were considered as independent variables separately in logistic regression models where the future cardiovascular endpoint events (yes/no) were considered as dependent variable. All p values reported are two-tailed and $p < 0.05$ was considered to be statistically significant.

Subject Selection and Baseline Data Collection

Between July 1st 2017 and November 30st 2019, patients admitted to hospital due to essential hypertension in the cardiac vascular center of Beijing Tiantan Hospital, Capital Medical University (CCMU) were prospectively consecutively involved in this study. The exclusion criteria included histories of coronary artery diseases (CAD) (including previous coronary revascularization, myocardial infarction (MI), and/or known $>50\%$ stenosis of any coronary artery), cerebrovascular diseases (including ischemic, hemorrhagic stroke, transient ischemic attack (TIA) and etc.), significant stenosis ($>50\%$) and/or surgeries of procedures of other main arteries, which included but were not limited to renal, carotid, and iliac arteries, congestive heart failure, congenital heart infects, and cardiomyopathy. All histories of these diseases were required to be confirmed based on medical records. Those that were diagnosed with above conditions during hospitalization would be also excluded. For each participant, relying on the standardized medical record collecting system, clinical data were recorded, including findings of physical examinations, results from a series of imaging examinations (including echocardiography, sonography on carotid artery and subclavian artery, and etc.), and results from blood biochemical tests. Two trained staff (one physician and one assistant physician) were in charge of the completeness of data collection of each subjects enrolled. A written confirmed consent was acquired for each participant. The protocol of this study was approved by the medical ethics committee institutional review board of Beijing Tiantan Hospital, CCMU.

Results

A total of 542 subjects originally participated in this study. After receiving ultrasonic examination for the carotid and right subclavian arteries, 37 subjects (25 men and 12 women) were excluded due to the diagnosis of carotid and/or subclavian stenosis. Up to the date when the analysis was performed, two patients refused to be continuously followed up and seven lost contacts with the investigation team. Therefore, 473 participants were

finally involved in the study, including 284 (60.0%) men and 189 (40.0%) women. The average age of all the 473 participants at admission was 63.57 ± 10.82 (range from 23 to 81) years, where women were a few years older than men (66.93 ± 9.37 vs. 61.34 ± 11.15 , $p < 0.001$). The average follow-up period were 25.3 ± 9.6 months with a range from 10.6 to 35.8 months. The prevalence of RSP and CP at baseline examination was 244/473 (51.6%, 95% confidence interval (C.I.) [47.0%, 56.2%]) and 289/473 (61.1%, 95% C.I. [56.5%, 65.5%]) respectively. Up to the day when the paper preparation work began, 39/473 (8.2%, 95% C.I. [5.9-11.1%]) participants suffered from endpoint events, including 19(4.0%) cases of MI (15 undergoing PCI and 5 death), 4(0.8%) heart failure (all also involved in the myocardial infarction group), 3(0.6%) ischemic stroke (1 death), and 17(3.6%) UA (7 undergoing PCI). No death due to other reasons was recorded.

According to the incidence of endpoints events, the participants were then divided into two groups (events $n = 39$ and no events $n = 434$). The demographic data, physical findings, ultrasonic results and blood test results were listed and compared in Table 1 and Table 2 for continuous variables and categorized variables respectively. The continuous variables included age (years), history of hypertension (years), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), heart rate (beats per minute), body mass index (BMI) (m/kg^2), levels of aspartate amino transferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), glomerular filtration rate (GFR), alkaline phosphatase, cholinesterase, albumin, globulin, fibrinogen, alpha hydroxybutyrate dehydrogenase (α -HBDH), total protein, total bilirubin, direct bilirubin, gamma-glutamyl transpeptidase (GGT), blood urea nitrogen (BUN), uric acid, glucose, glycosylated hemoglobin (HbA1c) (%), total Cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (APOA1), apolipoprotein B (APOB), electrolytes, hemoglobin (Hb), total triiodothyronine (TT3), total tetraiodothyronine (TT4), thyroid stimulating hormone (TSH), left ventricular ejection fraction (LVEF), and left ventricular end-diastolic dimension (LVEDD). The categorized variables included sex(male), histories of diabetes (yes/no), dyslipidemia (yes/no), atrial fibrillation (AF)(yes/no), smoking(current smoker, ex-smoker, and never), ultrasonic findings of CP (yes/no), RSP(yes/no) and multisite plaques (MPs) including plaques detected in both carotid and right subclavian arteries (yes/no), and baseline medications of angiotensin converting enzyme inhibitors (ACEIs)(yes/no), β -blockers (yes/no), calcium channel blockers (CCBs)(yes/no), diuretics (yes/no), aspirin (yes/no).

Table 1
Baseline characteristics (continuous variables) of the participants grouped by endpoint events (n = 473)

<i>Variables*</i>	<i>Endpoint events</i>		<i>P</i>
	<i>Yes (n=39)</i>	<i>No (n=434)</i>	
Age (years)	65.59±11.03	63.39±10.79	0.224
History of hypertension (years)	12.61±12.36	13.34±11.16	0.741
Systolic blood pressure (mmHg)	129.12±13.91	130.45±18.97	0.690
Diastolic blood pressure (mmHg)	77.62±8.07	77.14±12.31	0.823
Heart rate (beats per minute)	73.67±10.91	73.18±11.47	0.799
Levels of			
BMI (m/kg ²)	25.41±3.42	26.78±3.62	0.027
AST (U/L)	23.16±20.18	21.12±11.93	0.352
ALT (U/L)	21.09±11.18	24.86±19.04	0.231
Lactate dehydrogenase (LDH) (U/L)	200.19±60.16	182.36±55.12	0.059
Creatine kinase (CK) (U/L)	118.43±100.05	91.58±77.62	0.050
GFR (ml/min)	91.24±28.43	100.33±19.06	0.064
A Lkaline Phosphatase (U/L)	62.12±14.50	65.30±20.32	0.353
Cholinesterase(U/L)	8010.05±2046.74	8374.75±1833.52	0.252
Albumin (g/L)	39.72±3.77	40.70±2.91	0.046
Globulin (g/L)	25.42±4.25	24.09±3.87	0.056
Fibrinogen (g/L)	3.08±0.79	2.85±0.61	0.035
α-HBDH (U/L)	163.20±55.66	143.50±51.23	0.027
Total protein	65.14±6.51	64.73±4.49	0.714
Total Bilirubin (µmol/L)	12.59±6.24	12.88±5.85	0.778
Direct Bilirubin (µmmol/L)	3.88±1.91	4.14±2.13	0.487
GGT (U/L)	30.75±27.42	35.22±38.39	0.490
BUN (mmol/L)	7.18±5.20	6.10±3.43	0.223

* Values are presented as mean ± standard deviation

BMI = body mass index; AST = aspartate amino transferase; ALT = alanine transaminase; GFR = glomerular filtration rate; α-HBDH = Alpha hydroxybutyrate dehydrogenase; GGT = gamma-glutamyl transpeptidase; BUN = Blood Urea Nitrogen; HbA1c = glycosylated hemoglobin; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; APOA1 = apolipoprotein A1; APOB = apolipoprotein B; TT3 = total triiodothyronine; TT4 = total Tetraiodothyronine; TSH = Thyroid Stimulating Hormone; LVEF = left ventricular ejection fraction; LVEDD = left ventricular end-diastolic dimension.

<i>Variables*</i>	<i>Endpoint events</i>		<i>P</i>
	<i>Yes (n=39)</i>	<i>No (n=434)</i>	
Uric Acid (µmmol/L)	376.75±108.31	358.07±87.82	0.225
Glucose (mmol/L)	6.96±2.94	6.27±2.57	0.116
HbA1c (%)	6.59±1.11	6.51±1.22	0.690
Total Cholesterol (mmol/L)	3.98±0.96	3.86±0.92	0.426
Triglyceride (mmol/L)	1.57±1.40	1.67±1.08	0.587
HDL-C (mmol/L)	1.15±0.38	1.07±0.27	0.094
LDL-C (mmol/L)	2.35±0.83	2.27±0.80	0.584
APOA1 (g/L)	1.29±0.30	1.28±0.24	0.641
APOB (g/L)	0.90±0.21	0.89±0.23	0.850
Ca (mmol/L)	2.30±0.10	2.31±0.09	0.665
Na (mmol/L)	139.82±3.38	140.62±3.15	0.132
K (mmol/L)	4.06±0.46	3.98±0.37	0.193
P (mmol/L)	1.12±0.21	1.17±0.17	0.145
Cl (mmol/L)	104.09±3.03	104.55±3.27	0.399
Hemoglobin (Hb) (g/L)	133.74±19.07	136.52±16.16	0.317
TT3 (pmol/L)	1.23±0.25	1.36±0.22	0.001
TT4 (pmol/L)	94.22±20.29	90.50±18.19	0.240
TSH (pmol/L)	2.13±0.96	2.32±1.48	0.777
LVEF (%)	63.37±8.52	64.46±6.59	0.343
LVEDD (mm)	45.42±6.94	45.32±4.27	0.900

* Values are presented as mean ± standard deviation

BMI = body mass index; AST = aspartate amino transferase; ALT = alanine transaminase; GFR = glomerular filtration rate; α-HBDH = Alpha hydroxybutyrate dehydrogenase; GGT = gamma-glutamyl transpeptidase; BUN = Blood Urea Nitrogen; HbA1c = glycosylated hemoglobin; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; APOA1 = apolipoprotein A1; APOB = apolipoprotein B; TT3 = total triiodothyronine; TT4 = total Tetraiodothyronine; TSH = Thyroid Stimulating Hormone; LVEF = left ventricular ejection fraction; LVEDD = left ventricular end-diastolic dimension.

Table 2

Baseline characteristics (proportion of categorized variables) of the participants grouped by endpoint events (n = 473)

	<i>Events</i> (n = 39)	<i>%</i>	<i>95% C.I.</i>	<i>No events</i> (n = 434)	<i>%</i>	<i>95% C.I.</i>	<i>P</i>
Sex (male)	23	59.0	42.1-74.4	261	60.1	55.4-64.8	0.887
Histories of							
Diabetes (yes)	17	43.6	27.8-60.1	176	40.6	35.9-45.3	0.712
Dyslipidemia (yes)	20	51.3	34.8-67.6	219	50.5	45.7-55.3	0.461
Atrial fibrillation (yes)	3	7.7	1.6-20.9	38	8.8	6.3-11.8	1.000*
Smoking							
Current smoker	8	20.5	9.3-36.5	73	16.8	13.4-20.7	0.814*
Ex-smoker	0	0.0	0.0-9.0	7	1.6	0.7-3.3	
Never	31	79.5	63.5-90.7	354	81.6	77.6-85.1	
Ultrasonic findings							
CP	32	82.1	66.5-92.5	257	59.2	54.4-63.9	0.005
RSP	29	74.4	57.9-87.0	215	49.5	44.7-54.3	0.003
MPs	30	76.9	60.7-88.9	192	44.2	39.5-49.1	<0.001
Medications							
ACEIs	25	69.4	66.5-92.5	287	69.3	61.5-70.6	0.988
β-blockers	27	75.0	52.4-83.0	275	67.4	58.6-67.9	0.349
CCBs	26	72.2	49.8-80.9	244	59.7	51.4-60.9	0.139
Diuretics	12	33.3	17.0-47.6	80	19.6	14.9-22.4	0.051
Aspirin	0	0.0	0.0-9.0	12	2.9	1.4-4.8	0.611*
* Based on Fisher's exact test							
CP = carotid artery plaques; RSP = right subclavian artery plaques; MPs = multisite plaques including plaques detected in both carotid and right subclavian arteries; ACEI = angiotensin converting enzyme inhibitors; CCB = calcium channel blockers; 95% C.I. = 95% Confidence intervals							

Among these variables, the baseline prevalence of CP, RSP, MPs were significantly different between those with and without cardiovascular events (p = 0.005, 0.003 and < 0.001 respectively) (Table 2). To put it another way, the incidence of endpoint events was significantly higher in participants with plaques than in those without (see Table 3 for detail). Also, Table 2 shows that BMI, levels of albumin, fibrinogen, α-HBDH, TT3 were significantly different between the two groups (p = 0.027, 0.046, 0.035, 0.027 respectively). Then these variables and others with p < 0.100 based on univariate analyses, including levels of LDH, CK, GFR, globulin, and usage of diuretics, were entered into multivariate logistic regression models, except for CP, RSP, and MPs were entered as an

independent variable separately. A backward stepwise method was used to perform the multiple logistic regression with entry standard as $p < 0.050$ and removal standard as $p > 0.100$. Base on clinical practice and previous studies, [22] BMI was transformed to a trinomial categorized variable as 0 for normal weight ($BMI < 24\text{kg/m}^2$), 1 for overweight ($24 \text{ kg/m}^2 \leq BMI < 28 \text{ kg/m}^2$), and 2 for obese ($BMI > 28 \text{ kg/m}^2$), where group 0 were considered as reference using indicator method in SPSS logistic regression module; while the level of TT3 was categorized as a quadrinomial variable using its quartiles as cut-off points, where the first quartile was considered as reference category. The detailed results are listed in Table 4.

Table 3
Incidence of endpoint events groups by CP, RSP, and MPs.

		Endpoint events		<i>P</i>
		n	%	
CP	Yes (n=289)	32	11	0.005
	No (n=184)	7	3.8	
RSP	Yes (n=244)	29	11.9	0.003
	No (n=229)	10	4.4	
MPs	Yes (n=222)	30	13.5	< 0.001
	No (n=251)	9	3.6	

CP = carotid artery plaque, RSP = right subclavian artery plaque, MPs = multisite plaques detected in both CP and RSP

Table 4
Associations between CP/RSP and the incidence of future cardiovascular events based on multiple logistic regression (n = 473)

Variable**	Model A*			Model B*			Model C*				
	OR	95% C.I.	P	OR	95% C.I.	P	OR	95% C.I.	P		
CP	2.229	0.924, 5.375	0.074	RSP	2.428	1.098, 5.370	0.028	MPs	3.539	1.547, 8.096	0.003
Glb	1.128	1.022, 1.245	0.016		1.140	1.032, 1.259	0.010		1.150	1.038, 1.273	0.007
Alb	0.871	0.771, 0.984	0.027		0.867	0.768, 0.979	0.021		0.881	0.777, 0.997	0.046
HDL-C	2.984	0.955, 9.325	0.060		3.172	1.024, 9.828	0.045		2.742	0.875, 8.588	0.083

* CP, RSP, and MPs were entered into the multiple logistic regression model A, B, and C respectively.

** Variables entered in the first step of the logistic regression model included levels of lactate dehydrogenase (LDH), creatine kinase (CK), Glb, Alb, fibrinogen, Alpha hydroxybutyrate dehydrogenase, HDL-C, usage of diuretics, total triiodothyronine categorized by quartile, Body Mass Index categorized by obesity standards.

CP = carotid artery plaque, RSP = right subclavian artery plaque, MPs = multisite plaques detected in both CP and RSP, Glb = level of globulin, Alb = level of albumin, HDL-C = level of high-density lipoprotein cholesterol, 95% C.I. = 95% Confidence intervals.

As Table 4 presents, after adjusted for other potentially significant risk factors, the association between prevalence of CP at baseline and incidence of future endpoint events became weaker and even insignificant (p = 0.074), as seen in Model A, while that between RSP and events remained significant (OR = 2.428, 95% C.I. [1.098, 5.370], p=0.028), as seen in Model B, despite the fact that p value was lower indicating that the association also became weaker than in univariate analysis. However, the prevalence of positive detection of atherosclerosis plaque in both carotid artery and subclavian artery, when incorporated as one potential predictor, was still highly significantly correlated to the incidence of future cardiovascular events (OR = 3.539, 95% C.I. [1.547, 8.096], p = 0.003), as seen in Model C of Table 4.

Interestingly, the three models in Table 4 constantly indicates that levels of albumin and globulin at admission also tended to significantly be associated with the incidence of future events.

Discussion

Atherosclerosis is by far a fundamental promotor and contributor to many deadly cardiovascular diseases, but recent evidence is increasingly indicating that atherosclerosis could present long before clinical diseases appear. Therefore, since several years ago, researchers began to pay their attention not only to clinically significant evidence of atherosclerosis but also to some minor signs of subclinical atherosclerosis, the importance of which had long been underestimated before. Carotid atherosclerosis is one of the first targets to be noted by many medical researchers. The most frequently used markers include increased IMT and carotid plaques. With a large amount of definite evidence, it has been suggested that routine examination of carotid artery for the detection of

clinical and subclinical atherosclerosis should be added to recent guidelines for cardiovascular and cerebrovascular diseases. [8] However, many authors believed subclinical carotid atherosclerosis only has limited additional value over existing cardiovascular risk prediction models. [11, 14] And hence the most current guidelines have not incorporated it into routine risk score system. [13] Consequently, markers for subclinical atherosclerosis in vessels other than carotid arteries arose interests. Subclinical artery is rather promising because of the convenience to reach and its position anatomically close to carotid artery and other great arteries. A few of preliminary studies have showed its value in terms of its association with cardiovascular diseases but it still needs more evidence. That is why we carried out this study and try to explore the importance of RSP in predicting future cardiovascular events. A previous observational study on multi-site vessel atherosclerosis, not including subclavian arteries, indicated that the CP should be most prevalent in a group of Chinese patients with clinical atherosclerotic cardiovascular diseases. [17] In the present hypertensive cohort, CP was also more prevalent than RSP at the baseline but not significantly associated with endpoint events. However, RSP showed a higher and significant correlation to the incidence of future cardiovascular events in a hypertensive cohort after the adjustment of other potential risk factors (OR = 2.428, 95% C.I. [1.098, 5.370] p = 0.028). Considering the CP's p value closed to 0.05, a larger sample size might be needed. A more important and also reasonable finding was that carotid and subclavian atherosclerosis markers as a whole had a stronger prediction value for the endpoints events than RSP and CP used alone, though this result should be further tested in other largescale studies.

We also involved other traditional risk factors for cardiovascular events in this study, but most of them did not show a significant effect on the incidence of future events in either univariate analyses or multivariate analyses. For instance, age is usually a strong predictor to most of cardiovascular diseases and events but not the case in the present study. The reason may be the characteristics of this cohort. All the participants were inpatients admitted to one cardiovascular center due to essential hypertension. Hypertension itself is a very strong risk factor for cardiovascular events. Therefore, the contribution of age as an independent variable to the incidence of endpoint events might be attenuated. Furthermore, most of the participants were relatively old with a mean age of 63.57 ± 10.82 years. A larger scaled sample size of each age group and a longer follow-up time period may contribute to its significance in terms of the prediction of future events. BMI is also a common risk factor of cardiovascular diseases and showed insignificant association with events in our study. Besides the strong interference of hypertension, obesity tends to contribute to a higher incidence of cardiovascular events in a longer run. Diabetes and dyslipidemia are both usually considered as risk factors of cardiovascular diseases and were also not of significance regarding future endpoint events. Besides the reasons mentioned above with age and BMI, patients in this cohorts all received a formal and regular therapy for their glycemic control and lipid management. As a result, the difference, in terms of events, between patients with diabetes and/or dyslipidemia and those without would not be significant in only two or three years.

With the advantage of all subjects being inpatients of this cohort, we were able to get a series of results from blood tests at baseline investigation, and in turn able to explore the difference of these potential blood markers between the two groups divided by with or without events. Based on the multivariate regression analyses, both higher level of serum globulin and lower level of serum albumin showed a significant prediction value for cardiovascular events in this study, despite the subclinical atherosclerosis marker used in the multiple logistic regression model. This may be an interesting finding and need to be further investigated both in other cohorts and in the further follow-up of the present cohort. High level of globulin may be associated with inflammation, which are reported to be associated with cardiovascular diseases in previous studies. [23] Albumin is the principal

protein that determines the oncotic pressure of plasma and play an important role in fluid exchange between body components. [24] Low blood albumin level is also associated with CAD, heart failure, stroke, and AF attributed to antioxidant, anti-inflammatory, and anti-aggregating effects. GGT participates in the regulation of glutathione catabolism, which is the most important antioxidant, [25] and it also contributes to the atherosclerotic process. [26] Salih Toal and et al. reported recently that GGT to albumin ratio (GAR) can predict severity of CAD detected by coronary computed tomography angiography (CTA). [27] In the present hypertensive cohort, however, only higher albumin showed a potential protective effect on the incidence of future endpoint events, while neither GGT nor GAR tended to have significant predictive value. Very recently Stroke (REGARDS) study found that HbA1c was associated with an increasing risk of cardiovascular disease among patients with diabetes. [28] Another recent study from the REWIND trial also reported that changes of HbA1c contribute to lower incidence of major adverse cardiovascular events. [29] However in this study, we did not see the significant effect with HbA1c either. Low T3 syndrome and subclinical hypothyroidism are commonly associated with an increased risk of cardiovascular diseases and mortality. [30, 31] T3 maintains cardiac transverse-tubule structure and function. [32] Low Serum T3 Levels contribute to all-cause and cardiovascular mortality even in peritoneal dialysis patients. [33] In this study low TT3 showed a significant association with endpoint events in univariate analysis ($p < 0.001$) but did not after the adjustment of other variables, either entering the multivariate model as continuous or as categorized variables. Associations between these blood biochemical markers and the incidence of cardiovascular events might exist and were not strong enough to be detected in such a follow-up period of the present study. Longer follow-up period of this cohort may contribute to their significance.

A few of large longitudinal studies with a longtime follow-up, including Bioimage, [2] Multi-Ethnic Study of Atherosclerosis (MESA) [1, 6] and PESA [10], have investigated multi-territorial extent of subclinical atherosclerosis in some specific age cohorts. However, none of them reported assessment of the subclavian arteries. Data from PESA showed that the iliofemoral arteries was most frequently suffered in the early stage of atherosclerosis, followed by carotid arteries, abdominal aorta, and coronary arteries. [10] The position of subclavian atherosclerotic plaques in this sequence still needs to be determined. However, though the iliofemoral arteries tend to be most sensitive among above studied territories in the detection of early plaques, ultrasonic examination of it is less convenient to perform during routine clinical practice and in some cases magnetic resonance angiography is recommended to accurately determine multi-territory atherosclerotic plaques. [17] An additional procedure and medicare costs, as well as training for sonographers would be required. On the other hand, carotid ultrasound examination has been widely carried out, the ultrasonic examination for subclavian artery would be more easily to perform without training ultrasonographers due to the anatomical advantage. Carotid arteries and right subclavian artery can be scanned conveniently at one procedure and would be easily accepted by subjects. Last but not least, in the present study, combination of both CP and RSP showed the most predictive value for the incidence of future cardiovascular events. Besides, compared to angiography and CTA, ultrasound examinations are more inexpensive. Therefore, adding subclavian atherosclerotic markers to current risk screening method for future events is promising, especially when combination with carotid atherosclerotic markers.

The present study had limitations. Firstly, we did not routinely perform coronary CTA and coronary angiography because all participants involved at baseline were only diagnosed with essential hypertension and the two examinations are expensive and potentially harmful due to radiation and hence contraindicated for the purpose

of screening according to local medicare policies. Therefore, we could not evaluate the CAC, which is also a frequently used atherosclerotic marker.^[1] However, these data would not affect the present results of independently significant correlation between CP/MPs and endpoint events. Secondly, the present cohort was a hypertensive cohort that would be different from general population, for hypertension itself would affect the outcome of patients to an extent. Considering the lower incidence of endpoint events in general asymptomatic population, a much larger sample size and longer follow-up period are needed. Thirdly, data of detailed status of anti-hypertensive control after discharge, which might affect the results, were not available at the time of analysis. Furthermore, the period of follow-up was short. Some markers like TT3 and BMI would probably present significant correlations with endpoint events at five- or ten-years follow-up. Finally, we had not yet performed another ultrasonic scan on carotid and subclavian arteries for all the participants. The change of the cIMT and plaques might also play a role in the incidence of endpoint events.^[34] The next follow-up study in this cohort performed years later would give rise to more meaningful results.

Conclusion

The prevalence of right subclavian plaques was independently significantly associated with the incidence of future cardiovascular events in the hypertensive inpatient cohort. Carotid plaques were not proved to be independent predictor with regard to the events. Higher level of globulin and lower level of albumin tended to be predictors for the incidence of future cardiovascular events. Combination of both right subclavian artery and carotid artery atherosclerotic plaques may have strongest predictive value regarding future cardiovascular events in the hypertensive cohort. As a simple, inexpensive, and invasive technique, evaluation of subclavian artery atherosclerosis is a promising way, especially combined with assessment of carotid atherosclerosis, to provide additional information for the prediction of future cardiovascular events.

Declarations

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Authors' contributions

LM conceived the study design, collected and analyzed the data of the study and did primary paper preparation work, and serves as guarantor for the contents of this paper. LM, ZJ and CG participated in the design of the study. LD and LM gathered and reviewed the ultrasound data. HJ served as laboratory test reviewers and reviewed the laboratory content of the paper. LC served as data manager of the database. All authors participated in the interpretation of the data. All authors read and approved the final manuscript.

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Availability of data and material

All data are available by request to the corresponding author.

Declarations

Ethics approval and consent to participate

This study was conducted according to the recommendations of the Declaration of Helsinki and was approved by the institutional review boards of medical ethics committee institutional review board of Beijing Tiantan Hospital, Capital Medical University, Beijing, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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