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Significance of CAVI, hs-CRP and Homocysteine in subclinical arteriosclerosis among a healthy population in China

Abstract

Purpose: The purpose of this study is to evaluate the ability of cardio-ankle vascular index (CAVI), high-sensitivity C-reactive protein (hs-CRP) levels and homocysteine (Hcy) levels to screen for subclinical arteriosclerosis (subAs) in an apparently healthy population, with the view to obtaining an optimal diagnostic marker or profile for subAs.

Methods: Subjects (152) undergoing routine health examinations were recruited and divided into two groups: carotid arteriosclerosis (CA) and non-carotid arteriosclerosis (NCA), according to carotid intima-media thickness (CMIT). CAVI was calculated based on blood pressure and pulse wave velocity. Serum hs-CRP and Hcy levels were also measured. A Receiver Operating Characteristic (ROC) curve was plotted to evaluate the efficacy of each in carotid arteriosclerosis screening. Ten parameter combinations, designated W1 to W10, were compared in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Results: The levels of all three parameters were significantly higher in the CA group, compared with the NCA group. ROC curves showed that the area under the curve (AUC) for CAVI was 0.708 (95%CI: 0.615-0.800), which is significantly larger than that of either hs-CRP (0.622) or Hcy (0.630), respectively ($P < 0.001$). Maximum sensitivity (100%) and NPV (100%) were attained with W10, while maximum specificity (86.2%) and PPV (46.7%) were obtained with W7. With W9, the maximum Youden index (0.416) was obtained, with a sensitivity of 77.8% and specificity of 63.8%.

Conclusions: CAVI is more effective than hs-CRP or Hcy for subAs screening. The optimal profile was obtained with a combination of CAVI and other parameters.

Manuscript submitted September 4, 2012
Manuscript accepted February 10, 2013

Clin Invest Med 2013; 36 (2): E81-E86.

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With the aging of the population, vascular disorders are increasingly prominent in China, including cardiovascular diseases (CVD) and cerebrovascular diseases, resulting in high mortality and morbidity. In addition, an increasing number of younger patients have been diagnosed with vascular diseases, owing to significant changes in lifestyles and food habits. Research has shown that arteriosclerosis is a major contributor to CVD, cerebrovascular disease and other vascular disorders [1]; therefore, early evaluation and prevention of arteriosclerosis is one of the most important challenges for medical scientists. For the timely prediction and prevention of vascular events, it is important to establish a simple and effective way to identify subclinical arteriosclerosis (subAs) in apparently healthy populations.

Recent studies have focused on a novel arteriosclerosis index, the cardio-ankle vascular index (CAVI), developed by measuring brachial-ankle pulse wave velocity (baPWV) and blood pressure (BP) [2]. This index is independent of blood pressure, has commendable clinical reproducibility [3], and reflects stiffness of the aorta, femoral artery and tibial artery [4]. A number of other new laboratory markers have been associated with arteriosclerosis, such as the inflammatory biomarker, high-sensitivity C-reactive protein (hs-CRP) [5]. Zou and colleagues reported that homocysteine (Hcy) promotes the proliferation of vascular smooth muscle cells (VSMCs) in the pathophysiology of the atherosclerotic process by means of reactive oxygen species [6] and that hyperhomocysteinemia is significantly associated with atherosclerotic change [7].

No reports to date have performed a comparative assessment of diagnostic efficacies among CAVI, hs-CRP and Hcy or combinations of the three parameters for subAs within a healthy population in China. In the present study, the status of carotid artery arteriosclerosis was evaluated based on carotid intima-media thickness (CIMT) as a surrogate marker for systemic subAs [8], which is an effective way to assess the severity of pathological changes of the arterial wall. Subsequently, the screening significance of parameters, including CAVI, serum hs-CRP and Hcy, assessed either alone or in combination, in subAs was evaluated in an apparently healthy population in China.

Materials and Methods

Subjects

From February 2010 to October 2010, 184 healthy individuals who consented to undergo a health medical examination in The First Affiliated Hospital of Chongqing Medical University were subjected to CAVI measurement as well as the related

instrumental examinations and laboratory tests. Excluded from the study were those subjects who had a history of coronary heart disease, heart failure, chronic atrial fibrillation, aortic disease, valvular heart disease, stroke or peripheral vascular disease. Individuals over 80 years of age ($n=1$), and those suspicious for having an infection ($\text{hs-CRP} \geq 10 \text{ mg/L}$, $n=5$) or those for whom data were missing ($n=26$) were excluded. In total, 152 subjects were enrolled in the present study (male: 71, female: 81). Informed consent was obtained from all subjects, and the study was approved by the ethics committee of the hospital.

General information

General information was obtained from each subject, including body weight and height, waist circumference and blood pressure. A questionnaire was completed under the guidance of the medical staff and past medical history of diabetes mellitus (DM) and hypertension was collected. In addition, body mass index (BMI) and pulse pressure (PP) were calculated according to the formula:

$$BMI = \frac{\text{weight}}{\text{height}^2}, PP = SBP - DBP$$

Carotid intima-media thickness

Doppler carotid ultrasonography was performed with linear-array 5-12 MHz transducers (PHILIPS HDI-5000). The patient lay in a supine position without a pillow to allow exposure of the neck, and the head was turned to the contralateral side during examination. A region approximately 1.5 cm proximal to the flow divider in the common carotid artery was identified by a professional sonographer for CINT measurement. $\text{CINT} \geq 0.9 \text{ mm}$ was defined as carotid arteriosclerosis (CA) and $< 0.9 \text{ mm}$ as non-carotid arteriosclerosis (NCA). All CINT measurements were performed by the same technician blinded to the clinical data.

CAVI scores

CAVI scores were measured using a VaSeraVS-1000 (Fukuda Denshi, Japan). In keeping with earlier studies [9], CAVI was calculated using the formula:

$$CAVI = a \times \frac{2\rho}{PP} \times \ln \frac{SBP}{DBP} \times PWV^2 + b$$

(a and b: constant; ρ : blood density; PWV: pulse wave velocity between heart and ankle)

TABLE 1. Clinical characteristics of the study subjects

Parameters	CA group (n=36)	NCA group (n=116)	<i>P</i>
Male n (%)	21 (58.3%)	50 (43.1%)	0.110
Age (years old)	58.7±9.1	50.1±8.5	0.000*
BMI (kg/m ²)	24.7±3.6	24.5±3.1	0.775
WC (cm)	85.0±11.0	81.0±8.5	0.119
PP (mmHg)	59.4±22.3	48.6±14.1	0.015*
MBP (mmHg)	105.2±16.7	103.5±15.0	0.440
Hypertension n (%)	6 (16.7%)	13 (11.2%)	0.384
DM n (%)	12 (33.3%)	9 (7.8%)	0.000*
Creatinine (umol/L)	58.36±16.09	63.75±18.47	0.092
Smoking n (%)	15 (41.7%)	27 (23.3%)	0.031*

CA, carotid arteriosclerosis; NCA, non-carotid arteriosclerosis; BMI, body mass index; WC, waist circumference; PP, pulse pressure; MBP, mean blood pressure; DM, diabetes mellitus; * $P < 0.05$.

Cuffs were applied to the four extremities and electrocardiographic electrodes attached to both wrists. A microphone was placed on the sternal angle for phonocardiography. After the subjects had rested in the supine position for 5 min, automatic measurements were performed and left and right CAVI were obtained. Average CAVI scores were calculated.

Serum hs-CRP and Hcy levels

Blood samples were collected through the peripheral venous system after fasting for at least 10 h. Serum was separated within 30 min. Serum hs-CRP was measured with an immunoturbidimetric assay using a commercial kit (Roche, Switzerland), and serum Hcy was assessed using an enzymatic cycle method with a commercial kit (Leadman, USA). All measurements were performed with an automatic biochemical analyzer (Modular DDP, Roche, Switzerland).

Statistical Analysis

Numerical variables were described as the average and standard deviation under normal distribution. In case of skewed distribution, data were measured as median and quartiles. Categorical variables were expressed as proportions. The chi-square test was applied for categorical variables, and the Student's *t*-test or Mann-Whitney U test for numerical variables between groups. The Receiver Operator Characteristic (ROC) curve was plotted to determine the area under the curve (AUC), sensitivity and specificity of CAVI, serum hs-CRP and Hcy for determining the presence of arteriosclerosis. Statistical analysis was per-

TABLE 2. CAVI, serum hs-CRP and Hcy levels in subjects with and without carotid arteriosclerosis

Parameters	CA group (n=36)	NCA group (n=116)	<i>U</i>	<i>p</i>
CAVI	8.2(7.7-9.3)	7.5(7.1-8.3)	1222.0	<0.001**
hs-CRP (mg/L)	1.19(0.39-1.85)	0.64(0.31-1.11)	1577.5	0.027*
Hcy (μmol/L)	14.2(11.4-18.4)	11.9(9.6-14.6)	1543.5	0.018*

CA, carotid arteriosclerosis; NCA, non-carotid arteriosclerosis; †Variables presented as median and quartile; ‡ Mann-Whitney U test, * $P < 0.05$; ** $P < 0.01$.

FIGURE 1. ROC curves of CAVI, serum hs-CRP and Hcy for assessing presence of carotid arteriosclerosis

formed using the Statistical Package for Social Sciences (SPSS) 17.0 software.

Results

Characteristics of enrolled subjects

The characteristics of the enrolled subjects are shown in Table 1. Significant differences were observed in regard to age, PP, DM and smoking prevalence between the CA and NCA groups (p : 0.000, 0.015, 0.000 and 0.031).

CAVI, serum hs-CRP and Hcy levels in carotid arteriosclerosis

Statistical analyses indicate significantly higher CAVI (8.2(7.7-9.3) *vs.* 7.5(7.1-8.3), $p < 0.001$), serum hs-CRP (1.19(0.39-1.85) *vs.* 0.64(0.31-1.11), $p = 0.027$) and Hcy (14.2(11.4-18.4) *vs.* 11.9(9.6-14.6), $p = 0.018$) in the CA, compared with the NCA group, as shown in Table 2.

TABLE 3. Diagnostic ability of CAVI, serum hs-CRP and Hcy, determined by ROC, to detect carotid arteriosclerosis

	AUC (SE)	95%CI	P	cut-off	sensitivity	specificity
hs-CRP	0.622(0.054)	0.516-0.729	0.027	0.8	63.9%	67.2%
Hcy	0.630(0.052)	0.528-0.733	0.018	11.1	77.8%	46.6%
CAVI	0.708(0.047)	0.615-0.800	<0.001	7.525	83.3%	51.7%

CA, carotid arteriosclerosis; NCA, non-carotid arteriosclerosis

Diagnostic ability of CAVI, serum hs-CRP and Hcy to identify carotid arteriosclerosis

ROC curves for assessing the discriminatory abilities of CAVI, serum hs-CRP and Hcy as indicators of carotid arteriosclerosis were plotted (Figure 1). AUC for serum hs-CRP and Hcy were 0.622 and 0.630, respectively. Notably, AUC of CAVI was 0.708 with the highest Youden index of 0.351, and the corresponding sensitivity and specificity were 83.3% and 51.7% at the optimum cutoff CAVI value of 7.525 (Table 3).

Combined diagnostic ability of CAVI, serum hs-CRP and Hcy to identify carotid arteriosclerosis

Ten parameter combinations, designated W1 to W10 according to the cutoff value of each parameter (as summarized in Table 3 and Table 4) were compared. For each combination, a 2×2 crossing table was drawn, and the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) calculated (Table 5). Maximum sensitivity and NPV (100%, 100%) were obtained with the W10 combination, maximum specificity and PPV (86.2%, 46.7%) with the W7 combination, and maximum Youden index (0.416) with the W9 combination, with sensitivity and specificity of 77.8% and 63.8%, respectively.

Discussion

As a noninvasive assessment for arteriosclerosis, CIMT provides a direct image of vascular wall conditions, which is a reliable representative indicator of the system subclinical atherosclerosis. But CIMT is generally obtained via the common carotid artery, which overlooks the pathological changes in the internal carotid that occur with advanced arteriosclerosis [8]. In addition, a professional sonographer is needed for CIMT measurements, so the technique is not practical for routine health examinations. A more convenient method is needed.

Although previous research indicates that hs-CRP, Hcy and CAVI are each associated with the presence of arteriosclerosis, a number of limitations are associated with practical use of these individual markers. CAVI cannot be accurately measured in patients with heart systolic dysfunction, atrial

TABLE 4. Ten different combinations of CAVI, hs-CRP and Hcy

Combinations	Details
W1 : hs-CRP AND Hcy	+ hs-CRP $\geq$ 0.8 mg/L and Hcy $\geq$ 11.1 μ mol/L; - other situations
W2 : hs-CRP OR Hcy	- hs-CRP<0.8 mg/L and Hcy<11.1 μ mol/L; + other situations
W3 : hs-CRP AND CAVI	+ hs-CRP $\geq$ 0.8 mg/L and CAVI $\geq$ 7.525; - other situations
W4 : hs-CRP OR CAVI	- hs-CRP<0.8 mg/L and CAVI<7.525; + other situations
W5 : Hcy AND CAVI	+ Hcy $\geq$ 11.1 μ mol/L and CAVI $\geq$ 7.525; - other situations
W6 : Hcy OR CAVI	- Hcy<11.1 μ mol/L and CAVI<7.525; + other situations
W7 : W1 AND CAVI	+ W1 positive and CAVI $\geq$ 7.525; - other situations
W8 : W1 OR CAVI	- W1 negative and CAVI<7.525; + other situations
W9 : W2 AND CAVI	+ W2 positive and CAVI $\geq$ 7.525; - other situations
W10 : W2 OR CAVI	- W2 negative and CAVI<7.525; + other situations.

§Cut-off value obtained according to Table 3.

fibrillation/flutter, aortic disease, valvular heart disease or peripheral vascular disease [10]. Elevated expression of a type of acute phase-reactive protein, hs-CRP, has been observed in several other conditions, including infection, cancer and trauma. Moreover, a number of studies have reported no independent association between hs-CRP levels and subclinical atherosclerosis in healthy adults [11]. In addition, no significant correlation between Hcy and arteriosclerosis has been established to date [12]. In view of the above findings, it is necessary to re-evaluate the ability of these parameters to detect subAs in an apparently healthy population and to construct an optimal profile for effective arteriosclerosis screening.

Data from the present study showed that CAVI, serum hs-CRP and Hcy in the CA group were higher than those in the

TABLE 5. Diagnostic ability of the ten parameter combinations

		CA group	NCA group	sensitivity	specificity	Youden index	PPV	NPV
W1	+	17	25	47.2%	78.4%	0.256	40.5%	82.7%
	-	19	91					
W2	+	34	75	94.4%	35.3%	0.297	31.2%	95.3%
	-	2	41					
W3	+	18	21	50.0%	81.9%	0.319	46.2%	84.1%
	-	18	95					
W4	+	35	73	97.2%	37.1%	0.343	32.4%	97.7%
	-	1	43					
W5	+	24	37	66.7%	68.1%	0.348	39.3%	86.8%
	-	12	79					
W6	+	34	81	94.4%	30.2%	0.246	29.6%	94.6%
	-	2	25					
W7	+	14	16	38.9%	86.2%	0.251	46.7%	82.0%
	-	22	100					
W8	+	33	65	91.7%	44.0%	0.357	33.7%	94.4%
	-	3	51					
W9	+	28	42	77.8%	63.8%	0.416	40.0%	90.2%
	-	8	74					
W10	+	36	89	100%	23.3%	0.233	28.8%	100%
	-	0	27					

§Abbreviations: CA, carotid arteriosclerosis; NCA, non-carotid arteriosclerosis; PPV, positive predictive value; NPV, negative predictive value

NCA group. CAVI was originally developed by Japanese researchers in 2004 as a novel arteriosclerotic index to evaluate the stiffness of the aorta, femoral artery and tibial artery in a non-invasive manner. Our ROC curves revealed the strength of CAVI in distinguishing carotid arteriosclerosis (Figure 1), consistent with earlier reports [9,13]. So, does CAVI alone reliably reflect the presence of arteriosclerosis? The greatest advantage of CAVI is that the value does not vary with changes in blood pressure. Kubozono *et al.* [14] demonstrated that CAVI is not influenced by changes in SBP of more than 10 mmHg. Shirai and colleagues arrived at the same conclusion based on an agent intervention experiment of β 1-adrenergic receptor blocker administration, and further suggested that CAVI reflects the systolic and diastolic function of the artery [15].

There are still some factors to influence CAVI. Diabetes mellitus, hypertension and metabolic syndrome have been shown to contribute to increased CAVI [13]. Smoking is another important factor that affects CAVI. Earlier studies showed that CAVI increased significantly after smoking just one cigarette, and, conversely, improved after complete smok-

ing cessation [16-18]. Therefore, the application of CAVI as a single parameter to assess subAs is inadequate, and a combination of parameters for assessing subAs is recommended for greater reliability.

To enhance the accuracy of screening, various parameter combinations were examined, which improved sensitivity (W10, 100%) and specificity (W7, 85.2%) significantly for detection of carotid arteriosclerosis in an apparently healthy population. A profile with maximum Youden index (0.416) and good sensitivity (77.8%) and specificity (63.8%) was identified. Based on our data, the W10 combination is recommended for arteriosclerosis screening, owing to good NPV (100%), and W9 generating the maximum Youden index for clinical usage.

A possible limitations of our study is the small size of the sample. In addition, more consideration to job occupation, ethnic compositions, eating habits and level of exercise should be followed for the study population. The clinical utility remains to be validated by a multi-center prospective study.

Conclusion

Data from the present study collectively showed that CAVI, serum hs-CRP and Hcy are higher in the CA than the NCA group. Overall, CAVI was considered more appropriate than hs-CRP and Hcy for clinical use due to its effectiveness in detecting carotid arteriosclerosis. Since CAVI is influenced by various factors, different parameter combinations were compared, and the optimal profiles were determined for health screening and clinical usage, respectively.

Acknowledgments

The authors are grateful to the members of the Health Examination Center, the First Affiliated Hospital of Chongqing Medical University. We are greatly indebted to them for their assistance with data. This study was supported by a grant from The First Affiliated Hospital of Chongqing Medical University (No. YXJJ2009-14), and a grant from Chongqing Municipal Health Bureau (2012-2-016).

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