



**Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases**

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Complete List of Authors:	Liu, Jinbo; Department of Endocrinology Wang, Hongyu; Peking University Shougang Hospital, Department of Vascular Medicine Wang, Qi; Peking University Shougang Hospital, Department of Vascular Medicine Zhao, Hongwei; Peking University Shougang Hospital, Department of Vascular Medicine Shi, Hongyan; Peking University Shougang Hospital, Department of Vascular Medicine Yu, Xiaolan; Peking University Shougang Hospital, Department of Vascular Medicine Fu, Xiaobao; Peking University Shougang Hospital, Department of Vascular Medicine
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4 Descriptive study of possible link between cardio-ankle vascular index  
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6 and homocysteine in vascular-related diseases  
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9 Hongyu Wang<sup>\*,1,#</sup>, Jinbo Liu<sup>\*,1</sup>, Qi Wang<sup>1</sup>, Hongwei Zhao<sup>1</sup>, Hongyan Shi<sup>1</sup>,  
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11 Xiaolan Yu<sup>1</sup>, Xiaobao Fu<sup>1</sup>  
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15 <sup>1</sup> Department of Vascular Medicine; Peking University Shougang Hospital, Beijing  
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17 100144, P. R. of China.  
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21 \* Equal contributors  
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25 #Corresponding Author: Hongyu Wang, Vascular Medicine; Peking University  
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27 Shougang Hospital; Beijing 100144, P. R. of China.  
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31 Tel (Fax): +8610-57830226; +8610-57830226  
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34 Email: [hongyuwang@188.com](mailto:hongyuwang@188.com)  
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38 *Running title:* CAVI and Homocysteine  
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## Abstract

**Objectives:** Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

**Design:** Descriptive Research.

**Participants:** 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

**Methods:** CAVI, carotid-femoral pulse wave velocity (CFPWV) and carotid-radial pulse wave velocity (CRPWV) were measured by VS-1000 and Complior apparatus.

**Results:** There was significant correlation between Hcy and CF-PWV, CR-PWV, and CAVI in the entire group ( $r=0.33$ ,  $p=0.002$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.42$ ,  $p<0.001$ ; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy  $\geq 15\mu\text{mol/L}$  group than in Hcy  $<15\mu\text{mol/L}$  group ( $13.7\pm 3.0$  vs  $10.8\pm 2.5$ ,  $p<0.001$ ;  $10.6\pm 2.1$  vs  $9.2\pm 1.6$ ,  $p=0.001$ ;  $9.30\pm 2.1$  vs  $7.79\pm 2.1$ ,  $p=0.001$ ; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI ( $\beta=0.421$ ,  $p=0.001$ ;  $\beta=-0.309$ ,  $p=0.006$ ;  $\beta=0.297$ ,  $p=0.012$ ; respectively).

**Conclusions:** CAVI was positively correlated with homocysteine in vascular-related diseases.

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*Keywords:* Cardio-ankle vascular index; Homocysteine; Vascular-related diseases

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3 Article summary  
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5 1. Article focus  
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7 To investigate the relationship between homocysteine (Hcy) and Cardio-ankle  
8 vascular index (CAVI) in vascular-related diseases.  
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11 2. Key messages

12 Homocysteine was positively correlated with CAVI in vascular-related diseases.

13 3. Strengths and limitations of this study

14  
15 Strengths of this study: our present study firstly showed the relationship between  
16 Hcy and CAVI in vascular-related diseases.  
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20 Limitations of this study: the small simple size, cases, and controls were not  
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## 1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclerosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

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4 resulted from many factors such endothelial dysfunction, smooth muscle cells  
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6 proliferation, thickening of vascular wall. Kadota et al had showed positive  
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8 correlation between Hcy and CAVI in general population [12]. However, the  
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10 relationship between CAVI and Hcy in vascular-related diseases such as hypertension,  
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12 coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still  
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14 unknown, especially in patients with one more kinds of vascular-related diseases. In  
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16 the present study, we investigated the possible link between CAVI and homocysteine  
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18 in vascular-related diseases such as hypertension, CAD and ASO.  
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## 26 **2. Materials and methods**

### 27 *2.1 Subjects*

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31 88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from  
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33 vascular medicine department of Peking University Shougang Hospital from February  
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35 2012 to April 2012 were enrolled into our study. There were 57 patients with  
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37 hypertension, 43 with CAD, and 25 patients with ASO in the whole study groups.  
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41 Hypertension was defined as known cases of hypertension or blood pressure  
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43 measurement  $\geq 140/90$ mmHg in three occasions at rest. CAD or ASO was defined as  
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45 the narrowing or blockage of coronary artery or lower extremity artery diagnosed by  
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47 angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy  $\geq$   
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49 15umol/L[11].  
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54 Enrolled patients were divided into four groups according to numbers of  
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56 suffering vascular-related diseases. Also they were divided into two groups according  
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4 to level of Hcy (Hcy <15umol/L group, N=43, and Hcy  $\geq$ 15umol/L group, N=45).

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6 All participants gave their written informed consent. This study was approved by the  
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8 ethics committee of the Health Science Center, Peking University.  
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## 10 11 12 13 14 *2.2 Pulse wave velocity measurement*

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16 Arterial stiffness was evaluated by measuring automatic PWV using the  
17  
18 Complior apparatus. The basic principle of PWV assessment is that pressure pulse  
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20 generated by ventricular ejection is propagated along the arterial system at a speed  
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22 determined by elasticity of the arterial wall. Knowing the distance and pulse transit  
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24 time, the velocity can be calculated. Patients were placed in recumbent position and,  
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26 after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV  
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28 (CFPWV) and carotid-radial PWV (CRPWV) was obtained automatically.  
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## 39 40 41 42 43 44 45 46 47 48 *2.3 The assessment of CAVI*

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50 CAVI was recorded using a VaseraVS-1000 vascular screening system  
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52 (Fukuda Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG  
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54 electrodes were placed on both wrists, a microphone for detecting heart sounds was  
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56 placed on the sternum, and cuffs were wrapped around both the arms and ankles.  
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58 After automatic measurements, obtained data were analyzed by software, and the  
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60 value of CAVI was obtained automatically [16].

## 61 62 63 64 65 66 67 68 69 70 *2.4 Laboratory measurements*



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4 Blood samples were drawn from an antecubital vein in the morning after  
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6 overnight fasting and collected into vacuum tubes containing EDTA for the  
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8 measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density  
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10 lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric  
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12 enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo,  
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14 Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting  
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16 plasma glucose, homocysteine, hs-C reactive protein were determined at the central  
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18 chemistry laboratory of the Peking University Shougang Hospital.  
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### 27 *2.5 Statistical analysis*

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29 The difference between groups were analyzed by Student' *t*-test and one-way  
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31 ANOVA. Correlation coefficient was done to find linear relation between different  
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33 variables using Spearman correlation coefficient. Multiple linear regressions were  
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35 used to estimate the coefficients of the linear equation, involving independent  
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37 variables that affected the value of the dependent variables. Values were shown as  
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39 mean  $\pm$  SD unless stand otherwise.  $p < 0.05$  (2-tailed) was considered statistically  
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41 significant.  
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## 49 **3. Results**

### 50 *3.1 Clinical characteristics of the study participants*

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52 The clinical characteristics of study participants are shown in Table 1. Among  
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54 these subjects, 33 patients had only one of these three vascular-related diseases, 34  
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3 patients had two of these three vascular-related diseases, 9 patients had all of these  
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5 three diseases, and 12 subjects with none of vascular-related diseases. Our results  
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7 showed that with the increasing numbers of suffered vascular-related diseases, the  
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9 level of Hcy was increasing. Similar results were also found in the parameters of  
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11 CF-PWV and CAVI. However, we found there was significant difference about age  
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13 between these four groups.  
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19 Next, we divided subjects into two groups according to the level of Hcy. As  
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21 shown in Table 2, the level of CAVI was significant higher in patients with  $Hcy \geq$   
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23 15umol/L than in group with  $Hcy < 15umol/L$ . The similar result was also found in  
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25 another evaluation index of arterial stiffness-PWV. However, there was significant  
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27 difference about age between these two groups.  
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### 33 3.2 Pearson correlations between PWV, CAVI and Hcy in the entire study group

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36 PWV is a golden evaluation of arterial stiffness of vascular diseases. There are  
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38 some kinds of PWV according to different arteries, such as carotid-femoral pulse  
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40 wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV). As  
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42 shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group ( $r=0.33$ ,  
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44  $p=0.002$ , Fig 1A). There was also significant positive correlation between CR-PWV  
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46 and Hcy in all patients ( $r=0.51$ ,  $p<0.001$ , Fig 1B).  
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51 CAVI, a new index of arterial stiffness independent of blood pressure, is recently  
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53 developed by measuring of PWV and blood pressure. And CAVI was not affected by  
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55 immediately blood pressure. As shown in Fig2, there was significant positive  
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4 correlation between CAVI and Hcy in all patients ( $r=0.42$ ,  $p<0.0001$ ).  
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6 As shown in Table 1 and Table 2, there was significant difference about age  
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8 between groups. So next, we investigated the possible relationship between CAVI,  
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10 PWV with Hcy after adjusting the variable of age. Our results showed that there was  
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12 still significant correlation between CAVI and Hcy after adjustment for age ( $r=0.293$ ,  
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14  $p=0.008$ ). Also a positive correlation between PWV and Hcy was found after age  
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16 adjusted (CFPWV vs Hcy,  $r=0.282$ ,  $p=0.010$ ; CRPWV vs Hcy,  $r=0.462$ ,  $p<0.001$ ;  
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18 respectively).  
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### 26 *3.3 Multiple linear regression analysis*

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28 Multiple linear regressions were used to estimate the coefficients of the linear  
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30 equation, involving independent variables that affected the value of CAVI. Our results  
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32 showed that Hcy, BMI, and age were independent influencing factors of CAVI  
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34 ( $\beta=0.421$ ,  $p=0.001$ ;  $\beta= -0.309$ ,  $p=0.006$ ;  $\beta=0.297$ ,  $p=0.012$ ; respectively).  
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#### 4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy  $\geq 15\mu\text{mol/L}$ , and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. However, PWV itself is essentially dependent on blood pressure, especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness, is derived from stiffness parameter  $\beta$ , which is detected by carotid ultrasonic measurement [16].

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4 CAVI is a new evaluation index of arterial stiffness independent of immediate  
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6 blood pressure. Recent studies have showed the role of CAVI in the prediction of  
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8 vascular events in vascular-related diseases such as metabolic syndrome (MS),  
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10 diabetes, CAD, and so on. In MS patients, there was significant positive correlation  
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12 between CAVI and waist circumference, and CAVI increased significantly with the  
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14 number of metabolic syndrome components [17]. In another MS study, they found  
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16 that CAVI was significantly decreased after 3-month period weight-reduction therapy  
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18 through diet and exercise, so the determination of arterial stiffness by CAVI may be  
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20 useful for evaluating and managing the CVD risks of MS patients [18]. In a  
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22 comparative study, researchers showed that the diagnostic accuracy of CAD was  
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24 significantly higher in the CAVI than in the brachial ankle PWV, which suggested that  
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26 CAVI had increased performance over brachial ankle PWV in predicting the coronary  
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28 artery disease [16, 17]. Namekata showed that the CAVI method was a useful tool to  
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30 screen persons with moderate to advanced levels of arteriosclerosis. CAD is one of  
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32 fatal and disabling diseases, some researchers found that CAVI was significantly  
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34 correlated with percentage plaque area in coronary arterial disease [21]. A lot of  
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36 studies have showed that CAVI was a reliable evaluation index of vascular-related  
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38 diseases. Our present study showed that with the increasing numbers of  
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40 vascular-related diseases suffering, the level of CAVI was increasing (Table 1). And  
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42 we also found significant correlation between PWV and CAVI in the entire group  
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44 (CAVI &CF-PWV:  $r=0.382$ ,  $p<0.001$ ; CAVI &CR-PWV:  $r=0.225$ ,  $p=0.039$ ).  
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56 Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases.  
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4 Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with  
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6 hypertension. The possible mechanism of this process includes endothelial cell  
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8 damage, vascular endothelial dysfunction and enhanced oxidative stress[9,10]. Our  
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10 previous study showed that chronic hyperhomocysteinemia contributed to coronary  
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12 artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So  
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14 Hcy might damage the endothelium through complex mechanisms resulting  
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16 endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle  
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18 cells through inflammation and so on. Endothelial dysfunction and proliferation of  
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20 smooth muscle cells of arterial medium could lead to the increasing of arterial  
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22 stiffness. Previous study had showed positive correlation between Hcy and CAVI in  
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24 general population [12]. However, there was little research about the relationship  
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26 between Hcy and CAVI in patients with one more kinds of vascular-related diseases.  
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28 In the present study, we found that CAVI was positively correlated with Hcy even  
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30 after adjustment of other parameters. The similar result was also found between PWV  
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32 and Hcy. Hcy increases not only in hypertension patients but also in other vascular  
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34 diseases. Hcy participates in the pathophysiological process of these diseases.  
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36 Hyperhomocysteinemia was defined as the level of Hcy  $\geq 15\mu\text{mol/L}$ . Last, we  
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38 compared the arterial stiffness between HHcy group and patients with Hcy  $< 15\mu\text{mol/L}$ .  
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40 As shown in Table 2, the levels of PWV and CAVI were significantly higher in group  
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42 with Hcy  $\geq 15\mu\text{mol/L}$  than that in group with Hcy  $< 15\mu\text{mol/L}$ . Finally, our research  
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44 showed that Hcy was an independent influencing factor of CAVI. Our study suggested  
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46 that CAVI was higher in HHcy patients, so treatment should be made to lower  
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4 homocysteine in HHcy patients in order to reduce arterial stiffness.  
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7       However, there were some limitations in the study: the small sample size, cases,  
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9 and controls were not perfectly matched. Also some patients with hypertension and  
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11 (or) CAD had oral medication such as amlodipine before coming to the hospital, this  
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13 might affect our results to a certain extent. So thorough research should be  
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15 investigated in future. However, our study suggested that CAVI was a useful  
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17 evaluation index for arterial stiffness, and there was positive correlation between  
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19 CAVI and Hcy.  
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24       In conclusion, our study showed that CAVI and Hcy are closely associated  
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26 among vascular-related diseases. More studies should be made to investigate the role  
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28 of Hcy in the development of arterial stiffness.  
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### Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

**Ethics approval:** From the ethics committee of the Health Science Center, Peking University, China.

### Contributorship

Dr. Jinbo Liu, Qi Wang, Dr. Hongwei Zhao, Dr. Hongyan Shi, Dr. Xiaolan Yu, Dr. Xiaobao Fu had contribution to the collection of information of patients. Pro. Wang designed the present study, and Pro. Wang and Dr. Liu were Equal contributors to the this paper.

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### Data Sharing

No additional data available.

### Competing Interests

None



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19 **Figure legends**

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21 Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig  
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23 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse  
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25 wave velocity. CR-PWV: carotid-radial pulse wave velocity.

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31 Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy:  
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33 homocysteine. CAVI: cardio-ankle vascular index.

Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	<i>p</i> values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
BMI, kg/m <sup>2</sup>	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL, mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL, mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heartrate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density cholesterol. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA.

Table 2 Clinical characteristics in patients with Hcy<15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	<i>p</i> values
Age, y	61.9±13.0	71.9±11.5	<0.01
BMI, kg/m <sup>2</sup>	23.9±4.2	23.3±3.8	0.48
Hypertension, No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL, mmol/L	1.72±0.4	1.84±0.6	0.31
HDL, mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001

CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean  $\pm$ SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density cholesterol. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test.

Fig 1

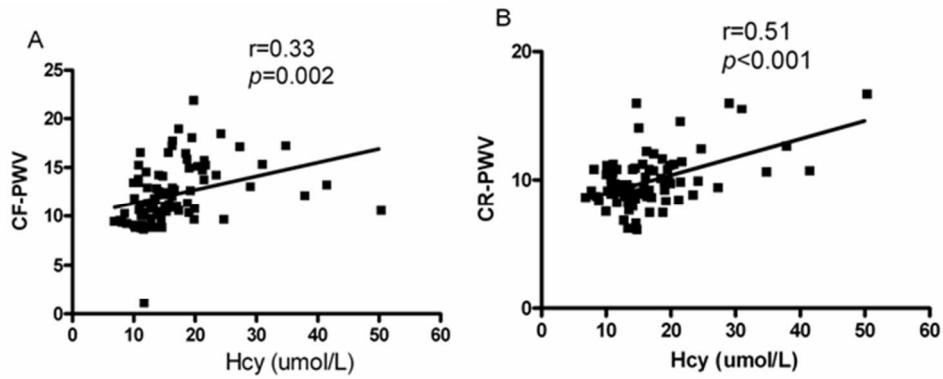
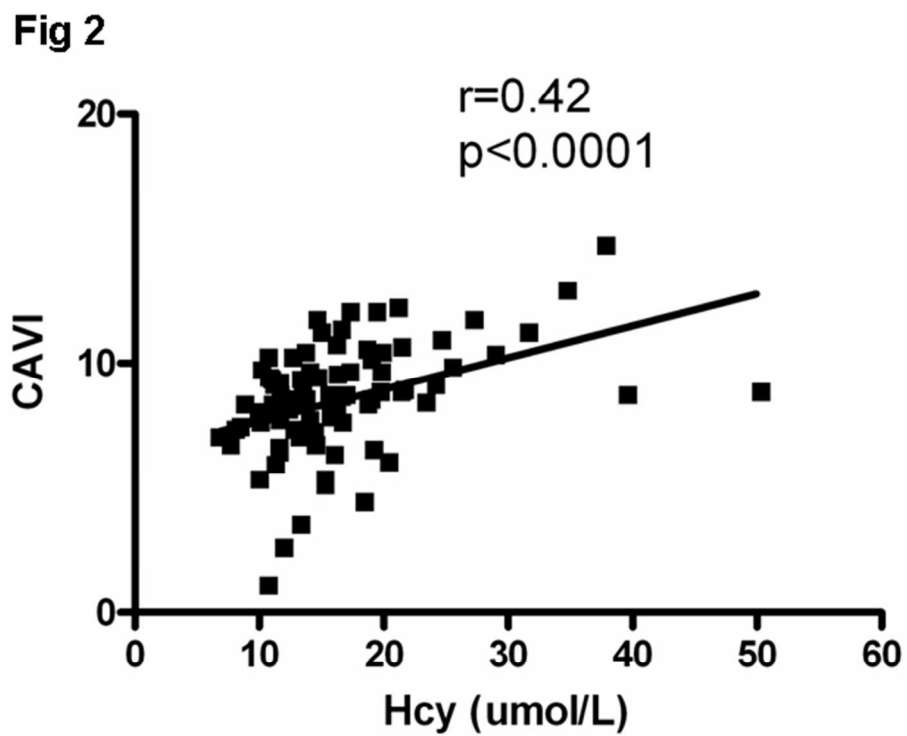


Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

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Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.  
57x43mm (300 x 300 DPI)



**Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases**

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4 Descriptive study of possible link between cardio-ankle vascular index  
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6 and homocysteine in vascular-related diseases  
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9 Hongyu Wang<sup>\*,1,#</sup>, Jinbo Liu<sup>\*,1</sup>, Qi Wang<sup>1</sup>, Hongwei Zhao<sup>1</sup>, Hongyan Shi<sup>1</sup>,  
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11 Xiaolan Yu<sup>1</sup>, Xiaobao Fu<sup>1</sup>  
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14  
15 <sup>1</sup> Department of Vascular Medicine; Peking University Shougang Hospital, Beijing  
16  
17 100144, P. R. of China.  
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21 \* Equal contributors  
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24  
25 #Corresponding Author: Hongyu Wang, Vascular Medicine; Peking University  
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27 Shougang Hospital; Beijing 100144, P. R. of China.  
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31 Tel (Fax): +8610-57830226; +8610-57830226  
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34 Email: [hongyuwang@188.com](mailto:hongyuwang@188.com)  
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38 *Running title:* CAVI and Homocysteine  
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## Abstract

**Objectives:** Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

**Design:** Descriptive Research.

**Participants:** 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

**Methods:** CAVI, carotid-femoral pulse wave velocity (CFPWV) and carotid-radial pulse wave velocity (CRPWV) were measured by VS-1000 and Complior apparatus.

**Results:** There was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the entire group ( $r=0.33$ ,  $p=0.002$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.42$ ,  $p<0.001$ ; respectively). And there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the vascular-related disease group ( $r=0.23$ ,  $p=0.048$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.392$ ,  $p=0.001$ ; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy  $\geq 15\mu\text{mol/L}$  group than in Hcy  $<15\mu\text{mol/L}$  group ( $13.7\pm 3.0$  vs  $10.8\pm 2.5$ ,  $p<0.001$ ;  $10.6\pm 2.1$  vs  $9.2\pm 1.6$ ,  $p=0.001$ ;  $9.30\pm 2.1$  vs  $7.79\pm 2.1$ ,  $p=0.001$ ; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI in the entire study group ( $\beta=0.421$ ,  $p=0.001$ ;

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4  $\beta = -0.309, p=0.006; \beta=0.297, p=0.012;$  respectively). And Hcy, BMI, and age were  
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6 independent influencing factors of CAVI in vascular-related disease group ( $\beta=0.434,$   
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8  $p=0.001; \beta = -0.331, p=0.009; \beta=0.288, p=0.022;$  respectively).

10  
11 **Conclusions:** CAVI was positively correlated with homocysteine in vascular-related  
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13 diseases.

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16 *Keywords:* Cardio-ankle vascular index; Homocysteine; Vascular-related diseases  
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3 Article summary  
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5 1. Article focus  
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7 To investigate the relationship between homocysteine (Hcy) and Cardio-ankle  
8 vascular index (CAVI) in vascular-related diseases.  
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11 2. Key messages

12 Homocysteine was positively correlated with CAVI in vascular-related diseases.

13 3. Strengths and limitations of this study

14  
15 Strengths of this study: our present study firstly showed the relationship between  
16 Hcy and CAVI in vascular-related diseases.  
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20 Limitations of this study: the small sample size, cases, and controls were not  
21 perfectly matched.  
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## 1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclerosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

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4 resulted from many factors such as endothelial dysfunction, smooth muscle cells  
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6 proliferation, thickening of vascular wall. Kadota et al had showed positive  
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8 correlation between Hcy and CAVI in general population [12]. However, the  
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10 relationship between CAVI and Hcy in vascular-related diseases such as hypertension,  
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12 coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still  
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14 unknown, especially in patients with one more kinds of vascular-related diseases. In  
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16 the present study, we investigated the possible link between CAVI and homocysteine  
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18 in vascular-related diseases such as hypertension, CAD and ASO.  
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## 27 **2. Materials and methods**

### 28 *2.1 Subjects*

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31 88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from  
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33 vascular medicine department of Peking University Shougang Hospital from February  
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35 2012 to April 2012 were enrolled into our study. There were 57 patients with  
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37 hypertension, 43 with CAD, and 25 patients with ASO in the whole study group. And  
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39 there were 12 patients without hypertension, CAD and ASO but suffering one of these  
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41 two diseases, acute upper respiratory tract infection or acute gastritis.  
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47 Hypertension was defined as known cases of hypertension or blood pressure  
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49 measurement  $\geq 140/90$ mmHg in three occasions at rest. CAD or ASO was defined as  
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51 the narrowing or blockage of coronary artery or lower extremity artery diagnosed by  
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53 angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy  $\geq$   
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55 15umol/L[11].  
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Enrolled patients were divided into four groups according to numbers of suffering vascular-related diseases. Also they were divided into two groups according to level of Hcy (Hcy <15umol/L group, N=43, and Hcy ≥15umol/L group, N=45). All participants gave their written informed consent. This study was approved by the ethics committee of the Health Science Center, Peking University.

### 2.2 *Pulse wave velocity measurement*

Arterial stiffness was evaluated by measuring automatic PWV using the Complior apparatus. The basic principle of PWV assessment is that pressure pulse generated by ventricular ejection is propagated along the arterial system at a speed determined by elasticity of the arterial wall. Knowing the distance and pulse transit time, the velocity can be calculated. Patients were placed in recumbent position and, after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV (CFPWV) and carotid-radial PWV (CRPWV) was obtained automatically. CFPWV and CRPWV are both reliable index for arterial stiffness of vascular diseases [2,26]. And we chose the right PWV for analysis.

### 2.3 *The assessment of CAVI*

CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG electrodes were placed on both wrists, a microphone for detecting heart sounds was placed on the sternum, and cuffs were wrapped around both the arms and ankles.

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4 After automatic measurements, obtained data were analyzed by software, and the  
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6 value of CAVI was obtained automatically [16]. And we chose the right CAVI for  
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8 analysis.  
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#### 10 11 12 13 14 *2.4 Laboratory measurements*

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16 Blood samples were drawn from an antecubital vein in the morning after  
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18 overnight fasting and collected into vacuum tubes containing EDTA for the  
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20 measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density  
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22 lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric  
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24 enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo,  
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26 Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting  
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28 plasma glucose, homocysteine, hs-C reactive protein were also determined by  
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30 colorimetric methods of related metabolic products using the same autoanalyzer at the  
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32 central chemistry laboratory of the Peking University Shougang Hospital.  
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#### 41 42 *2.5 Statistical analysis*

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44 SPSS 13.0 was used as statistical software in the present study. The differences  
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46 between groups were analyzed by Student' *t*-test and one-way ANOVA. Proportions  
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48 were analyzed by  $\chi^2$ -test. Correlation coefficient was done to find linear relation  
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50 between different variables using Spearman correlation coefficient. Multiple linear  
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52 regressions were used to estimate the coefficients of the linear equation, involving  
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54 independent variables that affected the value of the dependent variables. Values were  
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4 shown as mean  $\pm$  SD unless stand otherwise.  $p < 0.05$  (2-tailed) was considered  
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6 statistically significant.  
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### 10 11 **3. Results**

#### 12 13 *3.1 Clinical characteristics of the study participants*

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16 The clinical characteristics of study participants are shown in Table 1. Among  
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18 these subjects, 33 patients had only one of these three vascular-related diseases, 34  
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20 patients had two of these three vascular-related diseases, 9 patients had all of these  
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22 three diseases, and 12 subjects with none of vascular-related diseases. Our results  
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24 showed that with the increasing numbers of suffered vascular-related diseases, the  
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26 level of Hcy was increasing. Similar results were also found in the parameters of  
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28 CF-PWV and CAVI. However, we found there was significant difference about age  
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30 between these four groups.  
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37 Next, we divided subjects into two groups according to the level of Hcy. As  
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39 shown in Table 2, the level of CAVI was significant higher in patients with Hcy  $\geq$   
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41 15umol/L than in group with Hcy < 15umol/L. The similar result was also found in  
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43 another evaluation index of arterial stiffness-PWV. However, there was significant  
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45 difference about age and sex between these two groups.  
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#### 51 52 *3.2 Pearson correlations between PWV, CAVI and Hcy in the entire study group*

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54 PWV is a golden evaluation of arterial stiffness of vascular diseases. There are  
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56 some kinds of PWV according to different arteries, such as carotid-femoral pulse  
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4 wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV). CFPWV  
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6 and CRPWV are both reliable index for arterial stiffness of vascular diseases [2,26].  
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9 As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group  
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11 ( $r=0.33$ ,  $p=0.002$ , Fig 1A). There was also significant positive correlation between  
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13 CR-PWV and Hcy in all patients ( $r=0.51$ ,  $p<0.001$ , Fig 1B). In addition, our results  
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15 showed that there was significant correlation between Hcy and CF-PWV, CR-PWV in  
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17 the vascular-related disease group ( $r=0.23$ ,  $p=0.048$ ;  $r=0.51$ ,  $p<0.001$ , respectively).  
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21 CAVI, a new index of arterial stiffness independent of blood pressure, is recently  
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23 developed by measuring of PWV and blood pressure. And CAVI was not affected by  
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25 immediate blood pressure. As shown in Fig2, there was significant positive correlation  
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27 between CAVI and Hcy in all patients ( $r=0.42$ ,  $p<0.0001$ ). Also we found there was  
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29 significant correlation between Hcy and CAVI in the vascular-related disease group  
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31 ( $r=0.392$ ,  $p=0.001$ ). However, there was no significant correlation between Hcy and  
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33 CF-PWV, CR-PWV, CAVI in patients without vascular-related diseases in the present  
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35 study ( $r=0.14$ ,  $p=0.661$ ;  $r=0.152$ ,  $p=0.620$ ;  $r=0.056$ ,  $p=0.855$ ; respectively).  
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41 As shown in Table 1 and Table 2, there was significant difference about age or  
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43 sex between groups. So next, we investigated the possible relationship between CAVI,  
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45 PWV and Hcy after adjusting the variable of age or sex. Our results showed that there  
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47 was still significant correlation between CAVI and Hcy after adjustment for age in the  
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49 entire study group ( $r=0.293$ ,  $p=0.008$ ). Also a positive correlation between PWV and  
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51 Hcy was found after age adjusted in the entire study group (CFPWV vs Hcy,  $r=0.282$ ,  
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53  $p=0.010$ ; CRPWV vs Hcy,  $r=0.462$ ,  $p<0.001$ ; respectively). In addition, there was  
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4 significant correlation between Hcy and CF-PWV, CR-PWV, CAVI after age and sex  
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6 adjusted in the entire study group ( $r=0.26$ ,  $p=0.022$ ;  $r=0.38$ ,  $p=0.001$ ;  $r=0.27$ ,  $p=0.014$ ;  
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8 respectively).

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11 There were 12 patients without vascular-related diseases in the entire study group,  
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13 so in next step, we analyzed relationship between PWV, CAVI and Hcy in patients  
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15 with vascular-related diseases. Our results showed that there was significant  
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17 correlation between Hcy and CR-PWV, CAVI after adjustment for age in the  
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19 vascular-related disease group ( $r=0.48$ ,  $p<0.001$ ;  $r=0.321$ ,  $p=0.007$ ; respectively),  
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21 without significant correlation between Hcy and CFPWV ( $r=0.21$ ,  $p=0.079$ ). After  
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23 adjustment for age and sex, significant correlation between Hcy and CR-PWV, CAVI  
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25 was found in the vascular-related disease group ( $r=0.40$ ,  $p=0.001$ ;  $r=0.298$ ,  $p=0.013$ ;  
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27 respectively). However, there was no significant correlation between Hcy and  
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29 CFPWV after age and sex adjusted ( $r=0.193$ ,  $p=0.115$ ).  
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### 39 *3.3 Multiple linear regression analysis*

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41 Multiple linear regressions were used to estimate the coefficients of the linear  
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43 equation, involving independent variables that affected the value of CAVI. Our results  
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45 showed that Hcy, BMI, and age were independent influencing factors of CAVI in the  
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47 entire study group ( $\beta=0.421$ ,  $p=0.001$ ;  $\beta= -0.309$ ,  $p=0.006$ ;  $\beta=0.297$ ,  $p=0.012$ ;  
48  
49 respectively). And Hcy, BMI, and age were independent influencing factors of CAVI  
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51 in vascular-related disease group ( $\beta=0.434$ ,  $p=0.001$ ;  $\beta= -0.331$ ,  $p=0.009$ ;  $\beta=0.288$ ,  
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53  $p=0.022$ ; respectively).  
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#### 4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy  $\geq 15\mu\text{mol/L}$ , and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. Recent study showed that CRPWV was a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation and CRPWV may predict the severity of the CAD [25,26]. Our present study showed that CFPWV and

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4 CRPWV were higher in patients with vascular-related diseases than in subjects  
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6 without vascular-related diseases ( $12.80 \pm 2.9$  vs  $9.17 \pm 2.6$ ,  $p < 0.001$ ;  $10.00 \pm 2.2$  vs  
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8  $9.35 \pm 0.9$ ,  $p = 0.08$ ). However, PWV itself is essentially dependent on blood pressure,  
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10 especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new  
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12 index of arterial stiffness, is derived from stiffness parameter  $\beta$ , which is detected by  
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14 carotid ultrasonic measurement [16].  
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19 CAVI is a new evaluation index of arterial stiffness independent of immediate  
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21 blood pressure. Recent studies have showed the role of CAVI in the prediction of  
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23 vascular events in vascular-related diseases such as metabolic syndrome (MS),  
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25 diabetes, CAD, and so on. In MS patients, there was significant positive correlation  
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27 between CAVI and waist circumference, and CAVI increased significantly with the  
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29 number of metabolic syndrome components [17]. In another MS study, they found  
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31 that CAVI was significantly decreased after 3-month period weight-reduction therapy  
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33 through diet and exercise, so the determination of arterial stiffness by CAVI may be  
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35 useful for evaluating and managing the cardiovascular diseases risks of MS patients  
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37 [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD  
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39 was significantly higher in the CAVI than in the brachial ankle PWV, which suggested  
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41 that CAVI had increased performance over brachial ankle PWV in predicting the  
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43 coronary artery disease [16, 17]. Namekata showed that the CAVI method was a  
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45 useful tool to screen persons with moderate to advanced levels of arteriosclerosis.  
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47 CAD is one of fatal and disabling diseases, some researchers found that CAVI was  
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49 significantly correlated with percentage plaque area in coronary arterial disease [21].  
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4 A lot of studies have showed that CAVI was a reliable evaluation index of  
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6 vascular-related diseases. Our present study showed that with the increasing numbers  
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8 of vascular-related diseases suffering, the level of CAVI was increasing (Table 1).  
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10 CAVI was significantly higher in patients with vascular-related diseases than in  
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12 control subjects ( $8.73 \pm 2.3$  vs  $7.51 \pm 0.9$ ,  $p=0.002$ ). And we also found significant  
13  
14 correlation between PWV and CAVI in the entire group (CAVI & CF-PWV:  $r=0.382$ ,  
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16  $p<0.001$ ; CAVI & CR-PWV:  $r=0.225$ ,  $p=0.039$ , respectively).  
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21 Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases.  
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23 Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with  
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25 hypertension. The possible mechanism of this process includes endothelial cell  
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27 damage, vascular endothelial dysfunction and enhanced oxidative stress [9,10]. Our  
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29 previous study showed that chronic hyperhomocysteinemia contributed to coronary  
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31 artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So  
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33 Hcy might damage the endothelium through complex mechanisms resulting  
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35 endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle  
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37 cells through inflammation and so on. Endothelial dysfunction and proliferation of  
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39 smooth muscle cells of arterial medium could lead to the increasing of arterial  
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41 stiffness. Previous study had showed positive correlation between Hcy and CAVI in  
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43 general population [12]. However, there was little research about the relationship  
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45 between Hcy and CAVI in patients with one more kinds of vascular-related diseases.  
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47 In the present study, we found that CAVI was positively correlated with Hcy even  
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49 after adjustment of other parameters, such as age and sex. The similar result was also  
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4 found between PWV and Hcy. Hcy increases not only in hypertension patients but  
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6 also in other vascular diseases. Hcy participates in the pathophysiological process of  
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8 these diseases. Hyperhomocysteinemia was defined as the level of Hcy  $\geq 15\mu\text{mol/L}$ .  
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10 Next, we compared the arterial stiffness between HHcy group and patients with Hcy  
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12  $< 15\mu\text{mol/L}$ . As shown in Table 2, the levels of PWV and CAVI were significantly  
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14 higher in group with Hcy  $\geq 15\mu\text{mol/L}$  than in group with Hcy  $< 15\mu\text{mol/L}$ . Finally,  
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16 our research showed that Hcy was an independent influencing factor of CAVI in  
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18 vascular-related diseases. Folate administration has been consistently shown to reduce  
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20 plasma Hcy even in healthy individuals without elevated Hcy levels [22]. Lange et al  
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22 found that folic acid treatment could reduce frequency of restenosis after angioplasty  
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24 in patients with markedly elevated homocysteine levels [23]. Another study showed  
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26 that low-dose folic acid treatment improves vascular function in CAD patients [24].  
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28 Our study suggested that CAVI was higher in HHcy patients, so treatment should be  
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30 made to lower homocysteine in HHcy patients in order to reduce arterial stiffness.  
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32 And thorough clinical research should be investigated in future.  
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42 However, there were some limitations in the study: the small sample size, cases,  
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44 and controls were not perfectly matched. Also some patients with hypertension and  
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46 (or) CAD had oral medication such as amlodipine before coming to the hospital, this  
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48 might affect our results to a certain extent. So thorough research should be  
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50 investigated in future. However, our study suggested that CAVI was a useful  
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52 evaluation index for arterial stiffness, and there was positively correlation between  
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54 CAVI and Hcy.  
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4 In conclusion, our study showed that CAVI and Hcy are closely associated  
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6 among vascular-related diseases. More studies should be made to investigate the role  
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8 of Hcy in the development of arterial stiffness.  
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No conflicts of interest, financial or otherwise, are declared by the authors.

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**Figure legends**

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.



Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	<i>p</i> values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
Sex(Male/Female)	4/8	20/13	16/18	6/3	0.283
BMI, kg/m <sup>2</sup>	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL, mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL, mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heart rate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA. Proportions were analyzed by  $\chi^2$ -test.

Table 2 Clinical characteristics in patients with Hcy&lt;15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	<i>p</i> values
Age, y	61.9±13.0	71.9±11.5	<0.01
Sex(Male/Female)	14/29	32/13	<0.001
BMI, kg/m <sup>2</sup>	23.9±4.2	23.3±3.8	0.48
Hypertension, No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL, mmol/L	1.72±0.4	1.84±0.6	0.31
HDL, mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001
CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test. Proportions were analyzed by  $\chi^2$ -test.

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4 Descriptive study of possible link between cardio-ankle vascular index  
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9 Hongyu Wang<sup>\*,1,#</sup>, Jinbo Liu<sup>\*,1</sup>, Qi Wang<sup>1</sup>, Hongwei Zhao<sup>1</sup>, Hongyan Shi<sup>1</sup>,  
10  
11 Xiaolan Yu<sup>1</sup>, Xiaobao Fu<sup>1</sup>  
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15 <sup>1</sup> Department of Vascular Medicine; Peking University Shougang Hospital, Beijing  
16  
17 100144, P. R. of China.  
18

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20  
21 \* Equal contributors  
22

23  
24 #Corresponding Author: Hongyu Wang, Vascular Medicine; Peking University  
25  
26 Shougang Hospital; Beijing 100144, P. R. of China.  
27

28  
29  
30 Tel (Fax): +8610-57830226; +8610-57830226  
31

32  
33 Email: [hongyuwang@188.com](mailto:hongyuwang@188.com)  
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37 *Running title:* CAVI and Homocysteine  
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## Abstract

**Objectives:** Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

**Design:** Descriptive Research.

**Participants:** 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

**Methods:** CAVI, carotid-femoral pulse wave velocity (CFPWV) and carotid-radial pulse wave velocity (CRPWV) were measured by VS-1000 and Complior apparatus.

**Results:** There was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the entire group ( $r=0.33$ ,  $p=0.002$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.42$ ,  $p<0.001$ ; respectively). And there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the vascular-related disease group ( $r=0.23$ ,  $p=0.048$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.392$ ,  $p=0.001$ ; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy  $\geq 15\mu\text{mol/L}$  group than in Hcy  $<15\mu\text{mol/L}$  group ( $13.7\pm 3.0$  vs  $10.8\pm 2.5$ ,  $p<0.001$ ;  $10.6\pm 2.1$  vs  $9.2\pm 1.6$ ,  $p=0.001$ ;  $9.30\pm 2.1$  vs  $7.79\pm 2.1$ ,  $p=0.001$ ; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI in the entire study group ( $\beta=0.421$ ,  $p=0.001$ ;

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4  $\beta = -0.309, p=0.006; \beta=0.297, p=0.012;$  respectively). And Hcy, BMI, and age were  
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6 independent influencing factors of CAVI in vascular-related disease group ( $\beta=0.434,$   
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8  $p=0.001; \beta = -0.331, p=0.009; \beta=0.288, p=0.022;$  respectively).

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11 **Conclusions:** CAVI was positively correlated with homocysteine in vascular-related  
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13 diseases.

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16 **Keywords:** Cardio-ankle vascular index; Homocysteine; Vascular-related diseases  
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3 Article summary  
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5 1. Article focus  
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7 To investigate the relationship between homocysteine (Hcy) and Cardio-ankle  
8 vascular index (CAVI) in vascular-related diseases.  
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11 2. Key messages

12 Homocysteine was positively correlated with CAVI in vascular-related diseases.

13 3. Strengths and limitations of this study

14  
15 Strengths of this study: our present study firstly showed the relationship between  
16 Hcy and CAVI in vascular-related diseases.  
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19 Limitations of this study: the small **sample** size, cases, and controls were not  
20 perfectly matched.  
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## 1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclerosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

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4 resulted from many factors such as endothelial dysfunction, smooth muscle cells  
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6 proliferation, thickening of vascular wall. Kadota et al had showed positive  
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8 correlation between Hcy and CAVI in general population [12]. However, the  
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10 relationship between CAVI and Hcy in vascular-related diseases such as hypertension,  
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12 coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still  
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14 unknown, especially in patients with one more kinds of vascular-related diseases. In  
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16 the present study, we investigated the possible link between CAVI and homocysteine  
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18 in vascular-related diseases such as hypertension, CAD and ASO.  
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## 26 **2. Materials and methods**

### 27 *2.1 Subjects*

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31 88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from  
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33 vascular medicine department of Peking University Shougang Hospital from February  
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35 2012 to April 2012 were enrolled into our study. There were 57 patients with  
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37 hypertension, 43 with CAD, and 25 patients with ASO in the whole study group. And  
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39 there were 12 patients without hypertension, CAD and ASO but suffering one of these  
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41 two diseases, acute upper respiratory tract infection or acute gastritis.  
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47 Hypertension was defined as known cases of hypertension or blood pressure  
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49 measurement  $\geq 140/90$ mmHg in three occasions at rest. CAD or ASO was defined as  
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51 the narrowing or blockage of coronary artery or lower extremity artery diagnosed by  
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53 angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy  $\geq$   
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55 15umol/L[11].  
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4           Enrolled patients were divided into four groups according to numbers of  
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6 suffering vascular-related diseases. Also they were divided into two groups according  
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8 to level of Hcy (Hcy <15umol/L group, N=43, and Hcy  $\geq$ 15umol/L group, N=45).  
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10 All participants gave their written informed consent. This study was approved by the  
11  
12 ethics committee of the Health Science Center, Peking University.  
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### 15 16 17 18 19 *2.2 Pulse wave velocity measurement*

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21           Arterial stiffness was evaluated by measuring automatic PWV using the  
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23 Complior apparatus. The basic principle of PWV assessment is that pressure pulse  
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25 generated by ventricular ejection is propagated along the arterial system at a speed  
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27 determined by elasticity of the arterial wall. Knowing the distance and pulse transit  
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29 time, the velocity can be calculated. Patients were placed in recumbent position and,  
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31 after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV  
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33 (CFPWV) and carotid-radial PWV (CRPWV) was obtained automatically. CFPWV  
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35 and CRPWV are both reliable index for arterial stiffness of vascular diseases  
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37 [2,26]. And we chose the right PWV for analysis.  
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### 46 47 *2.3 The assessment of CAVI*

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49           CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda  
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51 Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG  
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53 electrodes were placed on both wrists, a microphone for detecting heart sounds was  
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55 placed on the sternum, and cuffs were wrapped around both the arms and ankles.  
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4 After automatic measurements, obtained data were analyzed by software, and the  
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6 value of CAVI was obtained automatically [16]. And we chose the right CAVI for  
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8 analysis.  
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#### 10 11 12 13 14 *2.4 Laboratory measurements*

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16 Blood samples were drawn from an antecubital vein in the morning after  
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18 overnight fasting and collected into vacuum tubes containing EDTA for the  
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20 measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density  
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22 lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric  
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24 enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo,  
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26 Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting  
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28 plasma glucose, homocysteine, hs-C reactive protein were also determined by  
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30 colorimetric methods of related metabolic products using the same autoanalyzer at the  
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32 central chemistry laboratory of the Peking University Shougang Hospital.  
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#### 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 *2.5 Statistical analysis*

SPSS 13.0 was used as statistical software in the present study. The differences  
between groups were analyzed by Student' *t*-test and one-way ANOVA. Proportions  
were analyzed by  $\chi^2$ -test. Correlation coefficient was done to find linear relation  
between different variables using Spearman correlation coefficient. Multiple linear  
regressions were used to estimate the coefficients of the linear equation, involving  
independent variables that affected the value of the dependent variables. Values were

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4 shown as mean  $\pm$  SD unless stand otherwise.  $p < 0.05$  (2-tailed) was considered  
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6 statistically significant.  
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### 10 11 **3. Results**

#### 12 13 *3.1 Clinical characteristics of the study participants*

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15 The clinical characteristics of study participants are shown in Table 1. Among  
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17 these subjects, 33 patients had only one of these three vascular-related diseases, 34  
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19 patients had two of these three vascular-related diseases, 9 patients had all of these  
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21 three diseases, and 12 subjects with none of vascular-related diseases. Our results  
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23 showed that with the increasing numbers of suffered vascular-related diseases, the  
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25 level of Hcy was increasing. Similar results were also found in the parameters of  
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27 CF-PWV and CAVI. However, we found there was significant difference about age  
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29 between these four groups.  
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37 Next, we divided subjects into two groups according to the level of Hcy. As  
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39 shown in Table 2, the level of CAVI was significant higher in patients with Hcy  $\geq$   
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41 15umol/L than in group with Hcy < 15umol/L. The similar result was also found in  
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43 another evaluation index of arterial stiffness-PWV. However, there was significant  
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45 difference about age and sex between these two groups.  
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#### 51 52 *3.2 Pearson correlations between PWV, CAVI and Hcy in the entire study group*

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54 PWV is a golden evaluation of arterial stiffness of vascular diseases. There are  
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56 some kinds of PWV according to different arteries, such as carotid-femoral pulse  
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4 wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV). CFPWV  
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6 and CRPWV are both reliable index for arterial stiffness of vascular diseases [2,26].  
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9 As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group  
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11 (r=0.33, p=0.002, Fig 1A). There was also significant positive correlation between  
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13 CR-PWV and Hcy in all patients (r=0.51, p<0.001, Fig 1B). In addition, our results  
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15 showed that there was significant correlation between Hcy and CF-PWV, CR-PWV in  
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17 the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001, respectively).  
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21 CAVI, a new index of arterial stiffness independent of blood pressure, is recently  
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23 developed by measuring of PWV and blood pressure. And CAVI was not affected by  
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25 immediate blood pressure. As shown in Fig2, there was significant positive correlation  
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27 between CAVI and Hcy in all patients (r=0.42, p<0.0001). Also we found there was  
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29 significant correlation between Hcy and CAVI in the vascular-related disease group  
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31 (r=0.392, p=0.001). However, there was no significant correlation between Hcy and  
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33 CF-PWV, CR-PWV, CAVI in patients without vascular-related diseases in the present  
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35 study (r=0.14, p=0.661; r=0.152, p=0.620; r=0.056, p=0.855; respectively).  
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41 As shown in Table 1 and Table 2, there was significant difference about age or  
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43 sex between groups. So next, we investigated the possible relationship between CAVI,  
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45 PWV and Hcy after adjusting the variable of age or sex. Our results showed that there  
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47 was still significant correlation between CAVI and Hcy after adjustment for age in the  
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49 entire study group (r=0.293, p=0.008). Also a positive correlation between PWV and  
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51 Hcy was found after age adjusted in the entire study group (CFPWV vs Hcy, r=0.282,  
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53 p=0.010; CRPWV vs Hcy, r=0.462, p<0.001; respectively). In addition, there was  
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4 significant correlation between Hcy and CF-PWV, CR-PWV, CAVI after age and sex  
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6 adjusted in the entire study group ( $r=0.26$ ,  $p=0.022$ ;  $r=0.38$ ,  $p=0.001$ ;  $r=0.27$ ,  $p=0.014$ ;  
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8 respectively).

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11 There were 12 patients without vascular-related diseases in the entire study group,  
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13 so in next step, we analyzed relationship between PWV, CAVI and Hcy in patients  
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15 with vascular-related diseases. Our results showed that there was significant  
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17 correlation between Hcy and CR-PWV, CAVI after adjustment for age in the  
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19 vascular-related disease group ( $r=0.48$ ,  $p<0.001$ ;  $r=0.321$ ,  $p=0.007$ ; respectively),  
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21 without significant correlation between Hcy and CFPWV ( $r=0.21$ ,  $p=0.079$ ). After  
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23 adjustment for age and sex, significant correlation between Hcy and CR-PWV, CAVI  
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25 was found in the vascular-related disease group ( $r=0.40$ ,  $p=0.001$ ;  $r=0.298$ ,  $p=0.013$ ;  
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27 respectively). However, there was no significant correlation between Hcy and  
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29 CFPWV after age and sex adjusted ( $r=0.193$ ,  $p=0.115$ ).

### 30 31 32 33 34 35 36 37 38 39 3.3 Multiple linear regression analysis

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41 Multiple linear regressions were used to estimate the coefficients of the linear  
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43 equation, involving independent variables that affected the value of CAVI. Our results  
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45 showed that Hcy, BMI, and age were independent influencing factors of CAVI in the  
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47 entire study group ( $\beta=0.421$ ,  $p=0.001$ ;  $\beta= -0.309$ ,  $p=0.006$ ;  $\beta=0.297$ ,  $p=0.012$ ;  
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49 respectively). And Hcy, BMI, and age were independent influencing factors of CAVI  
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51 in vascular-related disease group ( $\beta=0.434$ ,  $p=0.001$ ;  $\beta= -0.331$ ,  $p=0.009$ ;  $\beta=0.288$ ,  
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53  $p=0.022$ ; respectively).

#### 4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy  $\geq 15\mu\text{mol/L}$ , and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. Recent study showed that CRPWV was a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation and CRPWV may predict the severity of the CAD [25,26]. Our present study showed that CFPWV and

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4 CRPWV were higher in patients with vascular-related diseases than in subjects  
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6 without vascular-related diseases ( $12.80 \pm 2.9$  vs  $9.17 \pm 2.6$ ,  $p < 0.001$ ;  $10.00 \pm 2.2$  vs  
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8  $9.35 \pm 0.9$ ,  $p = 0.08$ ). However, PWV itself is essentially dependent on blood pressure,  
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10 especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new  
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12 index of arterial stiffness, is derived from stiffness parameter  $\beta$ , which is detected by  
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14 carotid ultrasonic measurement [16].  
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19 CAVI is a new evaluation index of arterial stiffness independent of immediate  
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21 blood pressure. Recent studies have showed the role of CAVI in the prediction of  
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23 vascular events in vascular-related diseases such as metabolic syndrome (MS),  
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25 diabetes, CAD, and so on. In MS patients, there was significant positive correlation  
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27 between CAVI and waist circumference, and CAVI increased significantly with the  
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29 number of metabolic syndrome components [17]. In another MS study, they found  
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31 that CAVI was significantly decreased after 3-month period weight-reduction therapy  
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33 through diet and exercise, so the determination of arterial stiffness by CAVI may be  
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35 useful for evaluating and managing the cardiovascular diseases risks of MS patients  
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37 [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD  
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39 was significantly higher in the CAVI than in the brachial ankle PWV, which suggested  
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41 that CAVI had increased performance over brachial ankle PWV in predicting the  
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43 coronary artery disease [16, 17]. Namekata showed that the CAVI method was a  
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45 useful tool to screen persons with moderate to advanced levels of arteriosclerosis.  
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47 CAD is one of fatal and disabling diseases, some researchers found that CAVI was  
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49 significantly correlated with percentage plaque area in coronary arterial disease [21].  
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4 A lot of studies have showed that CAVI was a reliable evaluation index of  
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6 vascular-related diseases. Our present study showed that with the increasing numbers  
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8 of vascular-related diseases suffering, the level of CAVI was increasing (Table 1).  
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11 CAVI was significantly higher in patients with vascular-related diseases than in  
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13 control subjects ( $8.73 \pm 2.3$  vs  $7.51 \pm 0.9$ ,  $p=0.002$ ). And we also found significant  
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15 correlation between PWV and CAVI in the entire group (CAVI & CF-PWV:  $r=0.382$ ,  
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17  $p<0.001$ ; CAVI & CR-PWV:  $r=0.225$ ,  $p=0.039$ , respectively).  
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21 Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases.  
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23 Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with  
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25 hypertension. The possible mechanism of this process includes endothelial cell  
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27 damage, vascular endothelial dysfunction and enhanced oxidative stress [9,10]. Our  
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29 previous study showed that chronic hyperhomocysteinemia contributed to coronary  
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31 artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So  
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33 Hcy might damage the endothelium through complex mechanisms resulting  
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35 endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle  
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37 cells through inflammation and so on. Endothelial dysfunction and proliferation of  
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39 smooth muscle cells of arterial medium could lead to the increasing of arterial  
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41 stiffness. Previous study had showed positive correlation between Hcy and CAVI in  
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43 general population [12]. However, there was little research about the relationship  
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45 between Hcy and CAVI in patients with one more kinds of vascular-related diseases.  
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47 In the present study, we found that CAVI was positively correlated with Hcy even  
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49 after adjustment of other parameters, such as age and sex. The similar result was also  
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4 found between PWV and Hcy. Hcy increases not only in hypertension patients but  
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6 also in other vascular diseases. Hcy participates in the pathophysiological process of  
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8 these diseases. Hyperhomocysteinemia was defined as the level of Hcy  $\geq 15\mu\text{mol/L}$ .

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11 **Next**, we compared the arterial stiffness between HHcy group and patients with Hcy  
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13  $< 15\mu\text{mol/L}$ . As shown in Table 2, the levels of PWV and CAVI were significantly  
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15 higher in group with Hcy  $\geq 15\mu\text{mol/L}$  than in group with Hcy  $< 15\mu\text{mol/L}$ . Finally,  
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17 our research showed that Hcy was an independent influencing factor of CAVI in  
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19 vascular-related diseases. Folate administration has been consistently shown to reduce  
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21 plasma Hcy even in healthy individuals without elevated Hcy levels [22]. Lange et al  
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23 found that folic acid treatment could reduce frequency of restenosis after angioplasty  
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25 in patients with markedly elevated homocysteine levels [23]. Another study showed  
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27 that low-dose folic acid treatment improves vascular function in CAD patients [24].  
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29 Our study suggested that CAVI was higher in HHcy patients, so treatment should be  
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31 made to lower homocysteine in HHcy patients in order to reduce arterial stiffness.  
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33 **And thorough clinical research should be investigated in future.**

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36 However, there were some limitations in the study: the small sample size, cases,  
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38 and controls were not perfectly matched. Also some patients with hypertension and  
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40 (or) CAD had oral medication such as amlodipine before coming to the hospital, this  
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42 might affect our results to a certain extent. So thorough research should be  
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44 investigated in future. However, our study suggested that CAVI was a useful  
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46 evaluation index for arterial stiffness, and there was positively correlation between  
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48 CAVI and Hcy.  
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4 In conclusion, our study showed that CAVI and Hcy are closely associated  
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6 among vascular-related diseases. More studies should be made to investigate the role  
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8 of Hcy in the development of arterial stiffness.  
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For peer review only

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### Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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**Figure legends**

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.



Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	p values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
Sex(Male/Female)	4/8	20/13	16/18	6/3	0.283
BMI, kg/m <sup>2</sup>	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL, mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL, mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heart rate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA. Proportions were analyzed by  $\chi^2$ -test.

Table 2 Clinical characteristics in patients with Hcy&lt;15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	p values
Age, y	61.9±13.0	71.9±11.5	<0.01
Sex(Male/Female)	14/29	32/13	<0.001
BMI, kg/m <sup>2</sup>	23.9±4.2	23.3±3.8	0.48
Hypertension, No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL , mmol/L	1.72±0.4	1.84±0.6	0.31
HDL , mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001
CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test. Proportions were analyzed by  $\chi^2$ -test.

Fig 1

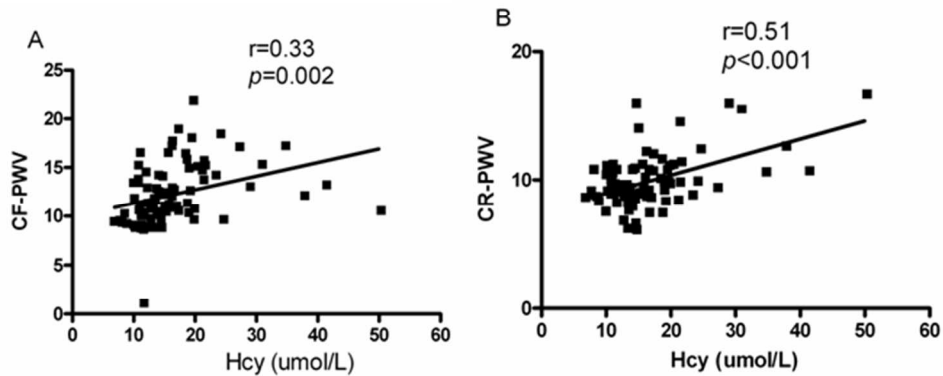
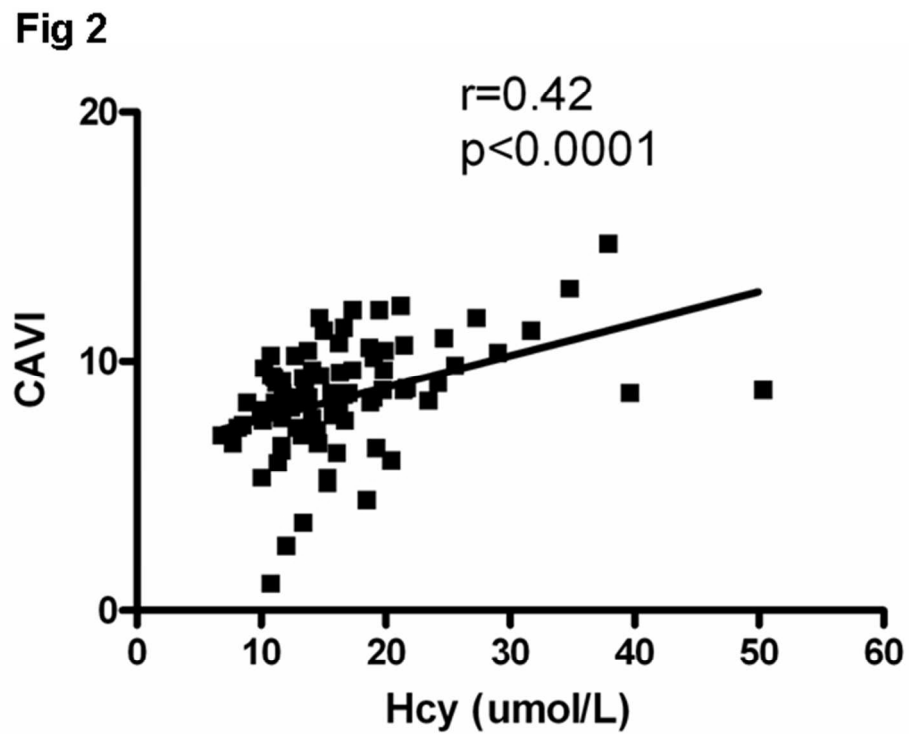


Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

58x27mm (300 x 300 DPI)



32 Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-  
33 ankle vascular index.  
34 57x43mm (300 x 300 DPI)

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**Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases**

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4 Descriptive study of possible link between cardio-ankle vascular index  
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6 and homocysteine in vascular-related diseases  
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9 Hongyu Wang<sup>\*,1,#</sup>, Jinbo Liu<sup>\*,1</sup>, Qi Wang<sup>1</sup>, Hongwei Zhao<sup>1</sup>, Hongyan Shi<sup>1</sup>,  
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11 Xiaolan Yu<sup>1</sup>, Xiaobao Fu<sup>1</sup>  
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14  
15 <sup>1</sup> Department of Vascular Medicine; Peking University Shougang Hospital, Beijing  
16  
17 100144, P. R. of China.  
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21 \* Equal contributors  
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24  
25 #Corresponding Author: Hongyu Wang, Vascular Medicine; Peking University  
26  
27 Shougang Hospital; Beijing 100144, P. R. of China.  
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31 Tel (Fax): +8610-57830226; +8610-57830226  
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34 Email: [hongyuwang@188.com](mailto:hongyuwang@188.com)  
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38 *Running title:* CAVI and Homocysteine  
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## Abstract

**Objectives:** Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

**Design:** Descriptive Research.

**Participants:** 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

**Methods:** CAVI, carotid-femoral pulse wave velocity (CF-PWV) and carotid-radial pulse wave velocity (CR-PWV) were measured by VS-1000 and Complior apparatus.

**Results:** There was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the entire group ( $r=0.33$ ,  $p=0.002$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.42$ ,  $p<0.001$ ; respectively). And there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the vascular-related disease group ( $r=0.23$ ,  $p=0.048$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.392$ ,  $p=0.001$ ; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy  $\geq 15\mu\text{mol/L}$  group than in Hcy  $<15\mu\text{mol/L}$  group ( $13.7\pm 3.0$  vs  $10.8\pm 2.5$ ,  $p<0.001$ ;  $10.6\pm 2.1$  vs  $9.2\pm 1.6$ ,  $p=0.001$ ;  $9.30\pm 2.1$  vs  $7.79\pm 2.1$ ,  $p=0.001$ ; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI in the entire study group ( $\beta=0.421$ ,  $p=0.001$ ;

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4  $\beta = -0.309, p=0.006; \beta=0.297, p=0.012;$  respectively). And Hcy, BMI, and age were  
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6 independent influencing factors of CAVI in vascular-related disease group ( $\beta=0.434,$   
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8  $p=0.001; \beta = -0.331, p=0.009; \beta=0.288, p=0.022;$  respectively).  
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11 **Conclusions:** CAVI was positively correlated with homocysteine in vascular-related  
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13 diseases.  
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16 **Keywords:** Cardio-ankle vascular index; Homocysteine; Vascular-related diseases  
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3 Article summary  
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5 1. Article focus  
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7 To investigate the relationship between homocysteine (Hcy) and Cardio-ankle  
8 vascular index (CAVI) in vascular-related diseases.  
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11 2. Key messages

12 Homocysteine was positively correlated with CAVI in vascular-related diseases.

13 3. Strengths and limitations of this study

14  
15 Strengths of this study: our present study firstly showed the relationship between  
16 Hcy and CAVI in vascular-related diseases.  
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20 Limitations of this study: the small **sample** size, cases, and controls were not  
21 perfectly matched.  
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## 1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclerosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

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4 resulted from many factors such as endothelial dysfunction, smooth muscle cells  
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6 proliferation, thickening of vascular wall. Kadota et al had showed positive  
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8 correlation between Hcy and CAVI in general population [12]. However, the  
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10 relationship between CAVI and Hcy in vascular-related diseases such as hypertension,  
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12 coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still  
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14 unknown, especially in patients with one more kinds of vascular-related diseases. In  
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16 the present study, we investigated the possible link between CAVI and homocysteine  
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18 in vascular-related diseases such as hypertension, CAD and ASO.  
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## 26 **2. Materials and methods**

### 27 *2.1 Subjects*

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31 88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from  
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33 vascular medicine department of Peking University Shougang Hospital from February  
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35 2012 to April 2012 were enrolled into our study. There were 57 patients with  
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37 hypertension, 43 with CAD, and 25 patients with ASO in the whole study group. And  
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39 there were 12 patients without hypertension, CAD and ASO but suffering one of these  
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41 two diseases, acute upper respiratory tract infection or acute gastritis.  
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47 Hypertension was defined as blood pressure measurement  $\geq 140/90$ mmHg in  
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49 three occasions at rest or subjects with known cases of diagnosed hypertension before  
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51 and taking antihypertensive drugs at present. CAD or ASO was defined as the  
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53 narrowing or blockage of coronary artery or lower extremity artery diagnosed by  
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55 angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy  $\geq$   
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4 15umol/L[11].  
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6 Enrolled patients were divided into four groups according to numbers of  
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8 suffering vascular-related diseases. Also they were divided into two groups according  
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10 to level of Hcy (Hcy <15umol/L group, N=43, and Hcy  $\geq$ 15umol/L group, N=45).  
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12 All participants gave their written informed consent. This study was approved by the  
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14 ethics committee of the Health Science Center, Peking University.  
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### 21 *2.2 Pulse wave velocity measurement*

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23 Arterial stiffness was evaluated by measuring automatic PWV using the  
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25 Complior apparatus. The basic principle of PWV assessment is that pressure pulse  
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27 generated by ventricular ejection is propagated along the arterial system at a speed  
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29 determined by elasticity of the arterial wall. Knowing the distance and pulse transit  
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31 time, the velocity can be calculated. Patients were placed in recumbent position and,  
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33 after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV  
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35 (CF-PWV) and carotid-radial PWV (CR-PWV) was obtained automatically. CF-PWV  
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37 and CR-PWV are both reliable index for arterial stiffness of vascular diseases  
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39 [2,26].And we chose the right PWV for analysis.  
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### 49 *2.3 The assessment of CAVI*

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51 CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda  
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53 Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG  
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55 electrodes were placed on both wrists, a microphone for detecting heart sounds was  
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4 placed on the sternum, and cuffs were wrapped around both the arms and ankles.  
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6 After automatic measurements, obtained data were analyzed by software, and the  
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8 value of CAVI was obtained automatically [16]. And we chose the right CAVI for  
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10 analysis.  
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#### 13 14 15 16 2.4 Laboratory measurements 17

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19 Blood samples were drawn from an antecubital vein in the morning after  
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21 overnight fasting and collected into vacuum tubes containing EDTA for the  
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23 measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density  
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25 lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric  
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27 enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo,  
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29 Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting  
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31 plasma glucose, homocysteine, hs-C reactive protein were also determined by  
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33 colorimetric methods of related metabolic products using the same autoanalyzer at the  
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35 central chemistry laboratory of the Peking University Shougang Hospital.  
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#### 44 2.5 Statistical analysis 45

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47 SPSS 13.0 was used as statistical software in the present study. The differences  
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49 between groups were analyzed by Student' *t*-test and one-way ANOVA. Proportions  
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51 were analyzed by  $\chi^2$ -test. Correlation coefficient was done to find linear relation  
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53 between different variables using Spearman correlation coefficient. Multiple linear  
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55 regressions were used to estimate the coefficients of the linear equation, involving  
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3 independent variables that affected the value of the dependent variables. Values were  
4 shown as mean  $\pm$  SD unless stand otherwise.  $p < 0.05$  (2-tailed) was considered  
5 statistically significant.  
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### 10 11 12 13 **3. Results**

#### 14 15 *3.1 Clinical characteristics of the study participants*

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17 The clinical characteristics of study participants are shown in Table 1. Among  
18 these subjects, 33 patients had only one of these three vascular-related diseases, 34  
19 patients had two of these three vascular-related diseases, 9 patients had all of these  
20 three diseases, and 12 subjects with none of vascular-related diseases. Our results  
21 showed that with the increasing numbers of suffered vascular-related diseases, the  
22 level of Hcy was increasing. Similar results were also found in the parameters of  
23 CF-PWV and CAVI. However, we found there was significant difference about age  
24 between these four groups.  
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39 Next, we divided subjects into two groups according to the level of Hcy. As  
40 shown in Table 2, the level of CAVI was significant higher in patients with Hcy  $\geq$   
41 15umol/L than in group with Hcy < 15umol/L. The similar result was also found in  
42 another evaluation index of arterial stiffness-PWV. However, there was significant  
43 difference about age and sex between these two groups.  
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#### 54 *3.2 Pearson correlations between PWV, CAVI and Hcy*

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56 PWV is a golden evaluation of arterial stiffness of vascular diseases. There are  
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4 some kinds of PWV according to different arteries, such as carotid-femoral pulse  
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6 wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV).  
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8 CF-PWV and CR-PWV are both reliable index for arterial stiffness of vascular  
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10 diseases [2,26]. In the present study, patients with ASO had bilateral vascular lesions.  
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12 And there was no significant difference between right side ankle-brachial index (ABI)  
13  
14 and left side ABI in the entire study group ( $1.08 \pm 0.13$  vs  $1.07 \pm 0.15$ ,  $p=0.612$ ). In  
15  
16 addition, there was no significant difference between right side ABI and left side ABI  
17  
18 in subjects with ASO ( $1.01 \pm 0.18$  vs  $1.05 \pm 0.14$ ,  $p=0.376$ ). And we chose the right  
19  
20 PWV and CAVI for analysis.  
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26 As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group  
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28 ( $r=0.33$ ,  $p=0.002$ , Fig 1A). There was also significant positive correlation between  
29  
30 CR-PWV and Hcy in all patients ( $r=0.51$ ,  $p<0.001$ , Fig 1B). In addition, our results  
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32 showed that there was significant correlation between Hcy and CF-PWV, CR-PWV in  
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34 the vascular-related disease group ( $r=0.23$ ,  $p=0.048$ ;  $r=0.51$ ,  $p<0.001$ , respectively).  
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39 CAVI, a new index of arterial stiffness independent of blood pressure, is recently  
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41 developed by measuring of PWV and blood pressure. And CAVI was not affected by  
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43 immediate blood pressure. As shown in Fig2, there was significant positive correlation  
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45 between CAVI and Hcy in all patients ( $r=0.42$ ,  $p<0.0001$ ). Also we found there was  
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47 significant correlation between Hcy and CAVI in the vascular-related disease group  
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49 ( $r=0.392$ ,  $p=0.001$ ). However, there was no significant correlation between Hcy and  
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51 CF-PWV, CR-PWV, CAVI in patients without vascular-related diseases in the present  
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53 study ( $r=0.14$ ,  $p=0.661$ ;  $r=0.152$ ,  $p=0.620$ ;  $r=0.056$ ,  $p=0.855$ ; respectively).  
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4 As shown in Table 1 and Table 2, there was significant difference about age or  
5  
6 sex between groups. So next, we investigated the possible relationship between CAVI,  
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8 PWV and Hcy after adjusting the variable of age or sex. Our results showed that there  
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10 was still significant correlation between CAVI and Hcy after adjustment for age in the  
11  
12 entire study group ( $r=0.293$ ,  $p=0.008$ ). Also a positive correlation between PWV and  
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14 Hcy was found after age adjusted in the entire study group (CF-PWV vs Hcy,  $r=0.282$ ,  
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16  $p=0.010$ ; CR-PWV vs Hcy,  $r=0.462$ ,  $p<0.001$ ; respectively). In addition, there was  
17  
18 significant correlation between Hcy and CF-PWV, CR-PWV, CAVI after age and sex  
19  
20 adjusted in the entire study group ( $r=0.26$ ,  $p=0.022$ ;  $r=0.38$ ,  $p=0.001$ ;  $r=0.27$ ,  $p=0.014$ ;  
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22 respectively).

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29 There were 12 patients without vascular-related diseases in the entire study group,  
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31 so in next step, we analyzed relationship between PWV, CAVI and Hcy in patients  
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33 with vascular-related diseases. Our results showed that there was significant  
34  
35 correlation between Hcy and CR-PWV, CAVI after adjustment for age in the  
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37 vascular-related disease group ( $r=0.48$ ,  $p<0.001$ ;  $r=0.321$ ,  $p=0.007$ ; respectively),  
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39 without significant correlation between Hcy and CF-PWV ( $r=0.21$ ,  $p=0.079$ ). After  
40  
41 adjustment for age and sex, significant correlation between Hcy and CR-PWV, CAVI  
42  
43 was found in the vascular-related disease group ( $r=0.40$ ,  $p=0.001$ ;  $r=0.298$ ,  $p=0.013$ ;  
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45 respectively). However, there was no significant correlation between Hcy and  
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47 CF-PWV after age and sex adjusted ( $r=0.193$ ,  $p=0.115$ ).

### 56 3.3 Multiple linear regression analysis

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4 Multiple linear regressions were used to estimate the coefficients of the linear  
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6 equation, involving independent variables that affected the value of CAVI. Our results  
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8 showed that Hcy, BMI, and age were independent influencing factors of CAVI in the  
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10 entire study group ( $\beta=0.421$ ,  $p=0.001$ ;  $\beta= -0.309$ ,  $p=0.006$ ;  $\beta=0.297$ ,  $p=0.012$ ;  
11  
12 respectively). And Hcy, BMI, and age were independent influencing factors of CAVI  
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14 in vascular-related disease group ( $\beta=0.434$ ,  $p=0.001$ ;  $\beta= -0.331$ ,  $p=0.009$ ;  $\beta=0.288$ ,  
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16  $p=0.022$ ; respectively).  
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#### 4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy  $\geq 15\mu\text{mol/L}$ , and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. Recent study showed that CR-PWV was a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation and CR-PWV may predict the severity of the CAD [25,26]. Our present study showed that CF-PWV and

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4 CR-PWV were higher in patients with vascular-related diseases than in subjects  
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6 without vascular-related diseases ( $12.80 \pm 2.9$  vs  $9.17 \pm 2.6$ ,  $p < 0.001$ ;  $10.00 \pm 2.2$  vs  
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8  $9.34 \pm 0.92$ ,  $p = 0.08$ , respectively). However, PWV itself is essentially dependent on  
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10 blood pressure, especially immediate blood pressure. Cardio-ankle vascular index  
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12 (CAVI), a new index of arterial stiffness, is derived from stiffness parameter  $\beta$ , which  
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14 is detected by carotid ultrasonic measurement [16].  
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19 CAVI is a new evaluation index of arterial stiffness independent of immediate  
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21 blood pressure. Recent studies have showed the role of CAVI in the prediction of  
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23 vascular events in vascular-related diseases such as metabolic syndrome (MS),  
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25 diabetes, CAD, and so on. In MS patients, there was significant positive correlation  
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27 between CAVI and waist circumference, and CAVI increased significantly with the  
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29 number of metabolic syndrome components [17]. In another MS study, they found  
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31 that CAVI was significantly decreased after 3-month period weight-reduction therapy  
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33 through diet and exercise, so the determination of arterial stiffness by CAVI may be  
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35 useful for evaluating and managing the cardiovascular diseases risks of MS patients  
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37 [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD  
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39 was significantly higher in the CAVI than in the brachial ankle PWV, which suggested  
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41 that CAVI had increased performance over brachial ankle PWV in predicting the  
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43 coronary artery disease [16, 17]. Namekata showed that the CAVI method was a  
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45 useful tool to screen persons with moderate to advanced levels of arteriosclerosis.  
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47 CAD is one of fatal and disabling diseases, some researchers found that CAVI was  
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49 significantly correlated with percentage plaque area in coronary arterial disease [21].  
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4 A lot of studies have showed that CAVI was a reliable evaluation index of  
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6 vascular-related diseases. Our present study showed that with the increasing numbers  
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8 of vascular-related diseases suffering, the level of CAVI was increasing (Table 1).  
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10 CAVI was significantly higher in patients with vascular-related diseases than in  
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12 control subjects ( $8.73 \pm 2.3$  vs  $7.51 \pm 0.9$ ,  $p=0.002$ ). And we also found significant  
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14 correlation between PWV and CAVI in the entire group (CAVI & CF-PWV:  $r=0.382$ ,  
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16  $p<0.001$ ; CAVI & CR-PWV:  $r=0.225$ ,  $p=0.039$ , respectively).  
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21 Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases.  
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23 Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with  
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25 hypertension. The possible mechanism of this process includes endothelial cell  
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27 damage, vascular endothelial dysfunction and enhanced oxidative stress [9,10]. Our  
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29 previous study showed that chronic hyperhomocysteinemia contributed to coronary  
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31 artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So  
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33 Hcy might damage the endothelium through complex mechanisms resulting  
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35 endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle  
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37 cells through inflammation and so on. Endothelial dysfunction and proliferation of  
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39 smooth muscle cells of arterial medium could lead to the increasing of arterial  
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41 stiffness. Previous study had showed positive correlation between Hcy and CAVI in  
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43 general population [12]. However, there was little research about the relationship  
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45 between Hcy and CAVI in patients with one more kinds of vascular-related diseases.  
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47 In the present study, we found that CAVI was positively correlated with Hcy even  
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49 after adjustment of other parameters, such as age and sex. The similar result was also  
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4 found between PWV and Hcy. Hcy increases not only in hypertension patients but  
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6 also in other vascular diseases. Hcy participates in the pathophysiological process of  
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8 these diseases. Hyperhomocysteinemia was defined as the level of Hcy  $\geq 15\mu\text{mol/L}$ .

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11 Next, we compared the arterial stiffness between HHcy group and patients with Hcy  
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13  $< 15\mu\text{mol/L}$ . As shown in Table 2, the levels of PWV and CAVI were significantly  
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15 higher in group with Hcy  $\geq 15\mu\text{mol/L}$  than in group with Hcy  $< 15\mu\text{mol/L}$ . Finally,  
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17 our research showed that Hcy was an independent influencing factor of CAVI in  
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19 vascular-related diseases. Folate administration has been consistently shown to reduce  
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21 plasma Hcy even in healthy individuals without elevated Hcy levels [22]. Lange et al  
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23 found that folic acid treatment could reduce frequency of restenosis after angioplasty  
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25 in patients with markedly elevated homocysteine levels [23]. Another study showed  
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27 that low-dose folic acid treatment improves vascular function in CAD patients [24].  
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29 Our study suggested that CAVI was higher in HHcy patients, so treatment should be  
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31 made to lower homocysteine in HHcy patients in order to reduce arterial stiffness.  
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33 And thorough clinical research should be investigated in future.

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36 However, there were some limitations in the study: the small sample size, cases,  
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38 and controls were not perfectly matched. Also some patients with hypertension and  
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40 (or) CAD had oral medication such as amlodipine before coming to the hospital, this  
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42 might affect our results to a certain extent. So thorough research should be  
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44 investigated in future. However, our study suggested that CAVI was a useful  
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46 evaluation index for arterial stiffness, and there was positively correlation between  
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48 CAVI and Hcy.  
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In conclusion, our study showed that CAVI and Hcy are closely associated among vascular-related diseases. More studies should be made to investigate the role of Hcy in the development of arterial stiffness.

For peer review only

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## Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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**Figure legends**

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.

Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	p values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
Sex(Male/Female)	4/8	20/13	16/18	6/3	0.283
BMI, kg/m <sup>2</sup>	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL, mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL, mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heart rate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA. Proportions were analyzed by  $\chi^2$ -test.

Table 2 Clinical characteristics in patients with Hcy&lt;15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	p values
Age, y	61.9±13.0	71.9±11.5	<0.01
Sex(Male/Female)	14/29	32/13	<0.001
BMI, kg/m <sup>2</sup>	23.9±4.2	23.3±3.8	0.48
Hypertension, No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL, mmol/L	1.72±0.4	1.84±0.6	0.31
HDL, mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001
CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test. Proportions were analyzed by  $\chi^2$ -test.

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4 Descriptive study of possible link between cardio-ankle vascular index  
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6 and homocysteine in vascular-related diseases  
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9 Hongyu Wang<sup>\*,1,#</sup>, Jinbo Liu<sup>\*,1</sup>, Qi Wang<sup>1</sup>, Hongwei Zhao<sup>1</sup>, Hongyan Shi<sup>1</sup>,  
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11 Xiaolan Yu<sup>1</sup>, Xiaobao Fu<sup>1</sup>  
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15 <sup>1</sup> Department of Vascular Medicine; Peking University Shougang Hospital, Beijing  
16  
17 100144, P. R. of China.  
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21 \* Equal contributors  
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25 #Corresponding Author: Hongyu Wang, Vascular Medicine; Peking University  
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27 Shougang Hospital; Beijing 100144, P. R. of China.  
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31 Tel (Fax): +8610-57830226; +8610-57830226  
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34 Email: [hongyuwang@188.com](mailto:hongyuwang@188.com)  
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38 *Running title:* CAVI and Homocysteine  
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## Abstract

**Objectives:** Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

**Design:** Descriptive Research.

**Participants:** 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

**Methods:** CAVI, carotid-femoral pulse wave velocity (CF-PWV) and carotid-radial pulse wave velocity (CR-PWV) were measured by VS-1000 and Complior apparatus.

**Results:** There was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the entire group ( $r=0.33$ ,  $p=0.002$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.42$ ,  $p<0.001$ ; respectively). And there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the vascular-related disease group ( $r=0.23$ ,  $p=0.048$ ;  $r=0.51$ ,  $p<0.001$ ;  $r=0.392$ ,  $p=0.001$ ; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy  $\geq 15\mu\text{mol/L}$  group than in Hcy  $<15\mu\text{mol/L}$  group ( $13.7\pm 3.0$  vs  $10.8\pm 2.5$ ,  $p<0.001$ ;  $10.6\pm 2.1$  vs  $9.2\pm 1.6$ ,  $p=0.001$ ;  $9.30\pm 2.1$  vs  $7.79\pm 2.1$ ,  $p=0.001$ ; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI in the entire study group ( $\beta=0.421$ ,  $p=0.001$ ;



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4  $\beta = -0.309, p=0.006; \beta=0.297, p=0.012;$  respectively). And Hcy, BMI, and age were  
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6 independent influencing factors of CAVI in vascular-related disease group ( $\beta=0.434,$   
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8  $p=0.001; \beta = -0.331, p=0.009; \beta=0.288, p=0.022;$  respectively).

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11 **Conclusions:** CAVI was positively correlated with homocysteine in vascular-related  
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13 diseases.

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16 *Keywords:* Cardio-ankle vascular index; Homocysteine; Vascular-related diseases  
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3 Article summary  
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5 1. Article focus  
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7 To investigate the relationship between homocysteine (Hcy) and Cardio-ankle  
8 vascular index (CAVI) in vascular-related diseases.  
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11 2. Key messages

12 Homocysteine was positively correlated with CAVI in vascular-related diseases.

13 3. Strengths and limitations of this study

14  
15 Strengths of this study: our present study firstly showed the relationship between  
16 Hcy and CAVI in vascular-related diseases.  
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20 Limitations of this study: the small sample size, cases, and controls were not  
21 perfectly matched.  
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## 1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclerosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

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4 resulted from many factors such as endothelial dysfunction, smooth muscle cells  
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6 proliferation, thickening of vascular wall. Kadota et al had showed positive  
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8 correlation between Hcy and CAVI in general population [12]. However, the  
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10 relationship between CAVI and Hcy in vascular-related diseases such as hypertension,  
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12 coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still  
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14 unknown, especially in patients with one more kinds of vascular-related diseases. In  
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16 the present study, we investigated the possible link between CAVI and homocysteine  
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18 in vascular-related diseases such as hypertension, CAD and ASO.  
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## 26 **2. Materials and methods**

### 27 *2.1 Subjects*

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31 88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from  
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33 vascular medicine department of Peking University Shougang Hospital from February  
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35 2012 to April 2012 were enrolled into our study. There were 57 patients with  
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37 hypertension, 43 with CAD, and 25 patients with ASO in the whole study group. And  
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39 there were 12 patients without hypertension, CAD and ASO but suffering one of these  
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41 two diseases, acute upper respiratory tract infection or acute gastritis.  
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47 Hypertension was defined as blood pressure measurement  $\geq 140/90$ mmHg in  
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49 three occasions at rest or subjects with known cases of diagnosed hypertension before  
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51 and taking antihypertensive drugs at present. CAD or ASO was defined as the  
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53 narrowing or blockage of coronary artery or lower extremity artery diagnosed by  
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55 angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy  $\geq$   
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4 15umol/L[11].

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6 Enrolled patients were divided into four groups according to numbers of  
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8 suffering vascular-related diseases. Also they were divided into two groups according  
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10 to level of Hcy (Hcy <15umol/L group, N=43, and Hcy  $\geq$ 15umol/L group, N=45).  
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12 All participants gave their written informed consent. This study was approved by the  
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14 ethics committee of the Health Science Center, Peking University.  
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## 20 21 *2.2 Pulse wave velocity measurement*

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23 Arterial stiffness was evaluated by measuring automatic PWV using the  
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25 Complior apparatus. The basic principle of PWV assessment is that pressure pulse  
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27 generated by ventricular ejection is propagated along the arterial system at a speed  
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29 determined by elasticity of the arterial wall. Knowing the distance and pulse transit  
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31 time, the velocity can be calculated. Patients were placed in recumbent position and,  
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33 after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV  
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35 (CF-PWV) and carotid-radial PWV (CR-PWV) was obtained automatically. CF-PWV  
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37 and CR-PWV are both reliable index for arterial stiffness of vascular diseases  
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39 [2,26].And we chose the right PWV for analysis.  
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## 49 *2.3 The assessment of CAVI*

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51 CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda  
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53 Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG  
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55 electrodes were placed on both wrists, a microphone for detecting heart sounds was  
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3 placed on the sternum, and cuffs were wrapped around both the arms and ankles.  
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6 After automatic measurements, obtained data were analyzed by software, and the  
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8 value of CAVI was obtained automatically [16]. And we chose the right CAVI for  
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10 analysis.  
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#### 13 14 15 16 *2.4 Laboratory measurements* 17

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19 Blood samples were drawn from an antecubital vein in the morning after  
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21 overnight fasting and collected into vacuum tubes containing EDTA for the  
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23 measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density  
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25 lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric  
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27 enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo,  
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29 Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting  
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31 plasma glucose, homocysteine, hs-C reactive protein were also determined by  
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33 colorimetric methods of related metabolic products using the same autoanalyzer at the  
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35 central chemistry laboratory of the Peking University Shougang Hospital.  
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#### 44 *2.5 Statistical analysis* 45

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47 SPSS 13.0 was used as statistical software in the present study. The differences  
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49 between groups were analyzed by Student' *t*-test and one-way ANOVA. Proportions  
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51 were analyzed by  $\chi^2$ -test. Correlation coefficient was done to find linear relation  
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53 between different variables using Spearman correlation coefficient. Multiple linear  
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55 regressions were used to estimate the coefficients of the linear equation, involving  
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4 independent variables that affected the value of the dependent variables. Values were  
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6 shown as mean  $\pm$  SD unless stand otherwise.  $p < 0.05$  (2-tailed) was considered  
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8 statistically significant.  
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### 10 11 12 13 **3. Results**

#### 14 15 16 *3.1 Clinical characteristics of the study participants*

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18 The clinical characteristics of study participants are shown in Table 1. Among  
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20 these subjects, 33 patients had only one of these three vascular-related diseases, 34  
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22 patients had two of these three vascular-related diseases, 9 patients had all of these  
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24 three diseases, and 12 subjects with none of vascular-related diseases. Our results  
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26 showed that with the increasing numbers of suffered vascular-related diseases, the  
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28 level of Hcy was increasing. Similar results were also found in the parameters of  
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30 CF-PWV and CAVI. However, we found there was significant difference about age  
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32 between these four groups.  
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40 Next, we divided subjects into two groups according to the level of Hcy. As  
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42 shown in Table 2, the level of CAVI was significant higher in patients with  $Hcy \geq$   
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44  $15\mu\text{mol/L}$  than in group with  $Hcy < 15\mu\text{mol/L}$ . The similar result was also found in  
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46 another evaluation index of arterial stiffness-PWV. However, there was significant  
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48 difference about age and sex between these two groups.  
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#### 51 52 53 *3.2 Pearson correlations between PWV, CAVI and Hcy*

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56 PWV is a golden evaluation of arterial stiffness of vascular diseases. There are  
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4 some kinds of PWV according to different arteries, such as carotid-femoral pulse  
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6 wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV).  
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8 CF-PWV and CR-PWV are both reliable index for arterial stiffness of vascular  
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10 diseases [2,26]. In the present study, patients with ASO had bilateral vascular lesions.  
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12 And there was no significant difference between right side ankle-brachial index (ABI)  
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14 and left side ABI in the entire study group ( $1.08 \pm 0.13$  vs  $1.07 \pm 0.15$ ,  $p=0.612$ ). In  
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16 addition, there was no significant difference between right side ABI and left side ABI  
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18 in subjects with ASO ( $1.01 \pm 0.18$  vs  $1.05 \pm 0.14$ ,  $p=0.376$ ). And we chose the right  
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20 PWV and CAVI for analysis.  
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26 As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group  
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28 ( $r=0.33$ ,  $p=0.002$ , Fig 1A). There was also significant positive correlation between  
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30 CR-PWV and Hcy in all patients ( $r=0.51$ ,  $p<0.001$ , Fig 1B). In addition, our results  
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32 showed that there was significant correlation between Hcy and CF-PWV, CR-PWV in  
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34 the vascular-related disease group ( $r=0.23$ ,  $p=0.048$ ;  $r=0.51$ ,  $p<0.001$ , respectively).  
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39 CAVI, a new index of arterial stiffness independent of blood pressure, is recently  
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41 developed by measuring of PWV and blood pressure. And CAVI was not affected by  
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43 immediate blood pressure. As shown in Fig2, there was significant positive correlation  
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45 between CAVI and Hcy in all patients ( $r=0.42$ ,  $p<0.0001$ ). Also we found there was  
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47 significant correlation between Hcy and CAVI in the vascular-related disease group  
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49 ( $r=0.392$ ,  $p=0.001$ ). However, there was no significant correlation between Hcy and  
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51 CF-PWV, CR-PWV, CAVI in patients without vascular-related diseases in the present  
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53 study ( $r=0.14$ ,  $p=0.661$ ;  $r=0.152$ ,  $p=0.620$ ;  $r=0.056$ ,  $p=0.855$ ; respectively).  
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4 As shown in Table 1 and Table 2, there was significant difference about age or  
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6 sex between groups. So next, we investigated the possible relationship between CAVI,  
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8 PWV and Hcy after adjusting the variable of age or sex. Our results showed that there  
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10 was still significant correlation between CAVI and Hcy after adjustment for age in the  
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12 entire study group ( $r=0.293$ ,  $p=0.008$ ). Also a positive correlation between PWV and  
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14 Hcy was found after age adjusted in the entire study group (CF-PWV vs Hcy,  $r=0.282$ ,  
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16  $p=0.010$ ; CR-PWV vs Hcy,  $r=0.462$ ,  $p<0.001$ ; respectively). In addition, there was  
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18 significant correlation between Hcy and CF-PWV, CR-PWV, CAVI after age and sex  
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20 adjusted in the entire study group ( $r=0.26$ ,  $p=0.022$ ;  $r=0.38$ ,  $p=0.001$ ;  $r=0.27$ ,  $p=0.014$ ;  
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22 respectively).

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29 There were 12 patients without vascular-related diseases in the entire study group,  
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31 so in next step, we analyzed relationship between PWV, CAVI and Hcy in patients  
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33 with vascular-related diseases. Our results showed that there was significant  
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35 correlation between Hcy and CR-PWV, CAVI after adjustment for age in the  
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37 vascular-related disease group ( $r=0.48$ ,  $p<0.001$ ;  $r=0.321$ ,  $p=0.007$ ; respectively),  
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39 without significant correlation between Hcy and CF-PWV ( $r=0.21$ ,  $p=0.079$ ). After  
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41 adjustment for age and sex, significant correlation between Hcy and CR-PWV, CAVI  
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43 was found in the vascular-related disease group ( $r=0.40$ ,  $p=0.001$ ;  $r=0.298$ ,  $p=0.013$ ;  
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45 respectively). However, there was no significant correlation between Hcy and  
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47 CF-PWV after age and sex adjusted ( $r=0.193$ ,  $p=0.115$ ).

### 56 3.3 Multiple linear regression analysis

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4 Multiple linear regressions were used to estimate the coefficients of the linear  
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6 equation, involving independent variables that affected the value of CAVI. Our results  
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8 showed that Hcy, BMI, and age were independent influencing factors of CAVI in the  
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10 entire study group ( $\beta=0.421$ ,  $p=0.001$ ;  $\beta= -0.309$ ,  $p=0.006$ ;  $\beta=0.297$ ,  $p=0.012$ ;  
11  
12 respectively). And Hcy, BMI, and age were independent influencing factors of CAVI  
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14 in vascular-related disease group ( $\beta=0.434$ ,  $p=0.001$ ;  $\beta= -0.331$ ,  $p=0.009$ ;  $\beta=0.288$ ,  
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16  $p=0.022$ ; respectively).  
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#### 4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy  $\geq 15\mu\text{mol/L}$ , and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. Recent study showed that CR-PWV was a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation and CR-PWV may predict the severity of the CAD [25,26]. Our present study showed that CF-PWV and

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4 CR-PWV were higher in patients with vascular-related diseases than in subjects  
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6 without vascular-related diseases ( $12.80 \pm 2.9$  vs  $9.17 \pm 2.6$ ,  $p < 0.001$ ;  $10.00 \pm 2.2$  vs  
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8  $9.34 \pm 0.92$ ,  $p = 0.08$ , respectively). However, PWV itself is essentially dependent on  
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10 blood pressure, especially immediate blood pressure. Cardio-ankle vascular index  
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12 (CAVI), a new index of arterial stiffness, is derived from stiffness parameter  $\beta$ , which  
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14 is detected by carotid ultrasonic measurement [16].  
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19 CAVI is a new evaluation index of arterial stiffness independent of immediate  
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21 blood pressure. Recent studies have showed the role of CAVI in the prediction of  
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23 vascular events in vascular-related diseases such as metabolic syndrome (MS),  
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25 diabetes, CAD, and so on. In MS patients, there was significant positive correlation  
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27 between CAVI and waist circumference, and CAVI increased significantly with the  
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29 number of metabolic syndrome components [17]. In another MS study, they found  
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31 that CAVI was significantly decreased after 3-month period weight-reduction therapy  
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33 through diet and exercise, so the determination of arterial stiffness by CAVI may be  
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35 useful for evaluating and managing the cardiovascular diseases risks of MS patients  
36  
37 [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD  
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39 was significantly higher in the CAVI than in the brachial ankle PWV, which suggested  
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41 that CAVI had increased performance over brachial ankle PWV in predicting the  
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43 coronary artery disease [16, 17]. Namekata showed that the CAVI method was a  
44  
45 useful tool to screen persons with moderate to advanced levels of arteriosclerosis.  
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47 CAD is one of fatal and disabling diseases, some researchers found that CAVI was  
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49 significantly correlated with percentage plaque area in coronary arterial disease [21].  
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4 A lot of studies have showed that CAVI was a reliable evaluation index of  
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6 vascular-related diseases. Our present study showed that with the increasing numbers  
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8 of vascular-related diseases suffering, the level of CAVI was increasing (Table 1).  
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10 CAVI was significantly higher in patients with vascular-related diseases than in  
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12 control subjects ( $8.73 \pm 2.3$  vs  $7.51 \pm 0.9$ ,  $p=0.002$ ). And we also found significant  
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14 correlation between PWV and CAVI in the entire group (CAVI & CF-PWV:  $r=0.382$ ,  
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16  $p<0.001$ ; CAVI & CR-PWV:  $r=0.225$ ,  $p=0.039$ , respectively).  
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21 Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases.  
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23 Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with  
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25 hypertension. The possible mechanism of this process includes endothelial cell  
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27 damage, vascular endothelial dysfunction and enhanced oxidative stress [9,10]. Our  
28  
29 previous study showed that chronic hyperhomocysteinemia contributed to coronary  
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31 artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So  
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33 Hcy might damage the endothelium through complex mechanisms resulting  
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35 endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle  
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37 cells through inflammation and so on. Endothelial dysfunction and proliferation of  
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39 smooth muscle cells of arterial medium could lead to the increasing of arterial  
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41 stiffness. Previous study had showed positive correlation between Hcy and CAVI in  
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43 general population [12]. However, there was little research about the relationship  
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45 between Hcy and CAVI in patients with one more kinds of vascular-related diseases.  
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47 In the present study, we found that CAVI was positively correlated with Hcy even  
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49 after adjustment of other parameters, such as age and sex. The similar result was also  
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4 found between PWV and Hcy. Hcy increases not only in hypertension patients but  
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6 also in other vascular diseases. Hcy participates in the pathophysiological process of  
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8 these diseases. Hyperhomocysteinemia was defined as the level of Hcy  $\geq 15\mu\text{mol/L}$ .  
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10 Next, we compared the arterial stiffness between HHcy group and patients with Hcy  
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12  $< 15\mu\text{mol/L}$ . As shown in Table 2, the levels of PWV and CAVI were significantly  
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14 higher in group with Hcy  $\geq 15\mu\text{mol/L}$  than in group with Hcy  $< 15\mu\text{mol/L}$ . Finally,  
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16 our research showed that Hcy was an independent influencing factor of CAVI in  
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18 vascular-related diseases. Folate administration has been consistently shown to reduce  
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20 plasma Hcy even in healthy individuals without elevated Hcy levels [22]. Lange et al  
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22 found that folic acid treatment could reduce frequency of restenosis after angioplasty  
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24 in patients with markedly elevated homocysteine levels [23]. Another study showed  
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26 that low-dose folic acid treatment improves vascular function in CAD patients [24].  
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28 Our study suggested that CAVI was higher in HHcy patients, so treatment should be  
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30 made to lower homocysteine in HHcy patients in order to reduce arterial stiffness.  
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32 And thorough clinical research should be investigated in future.  
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42 However, there were some limitations in the study: the small sample size, cases,  
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44 and controls were not perfectly matched. Also some patients with hypertension and  
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46 (or) CAD had oral medication such as amlodipine before coming to the hospital, this  
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48 might affect our results to a certain extent. So thorough research should be  
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50 investigated in future. However, our study suggested that CAVI was a useful  
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52 evaluation index for arterial stiffness, and there was positively correlation between  
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54 CAVI and Hcy.  
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4 In conclusion, our study showed that CAVI and Hcy are closely associated  
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6 among vascular-related diseases. More studies should be made to investigate the role  
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8 of Hcy in the development of arterial stiffness.  
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### Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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**Figure legends**

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.

Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	p values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
Sex(Male/Female)	4/8	20/13	16/18	6/3	0.283
BMI, kg/m <sup>2</sup>	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL, mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL, mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heart rate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA. Proportions were analyzed by  $\chi^2$ -test.

Table 2 Clinical characteristics in patients with Hcy&lt;15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	<i>p</i> values
Age, y	61.9±13.0	71.9±11.5	<0.01
Sex(Male/Female)	14/29	32/13	<0.001
BMI, kg/m <sup>2</sup>	23.9±4.2	23.3±3.8	0.48
Hypertension, No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL, mmol/L	1.72±0.4	1.84±0.6	0.31
HDL, mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001
CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test. Proportions were analyzed by  $\chi^2$ -test.

Fig 1

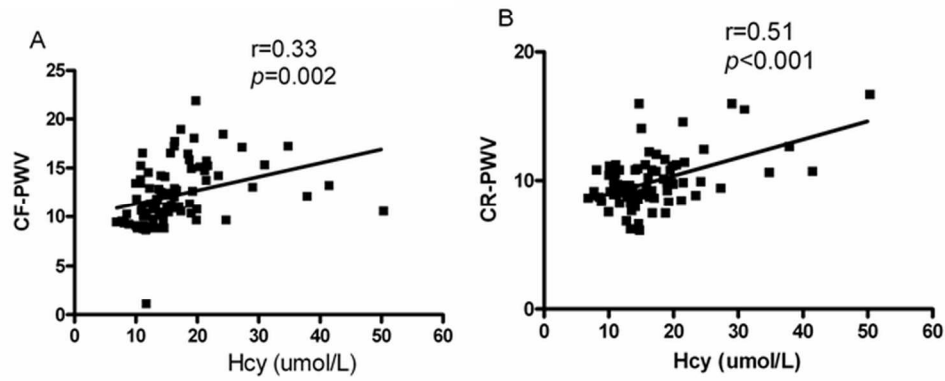


Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

189x90mm (300 x 300 DPI)



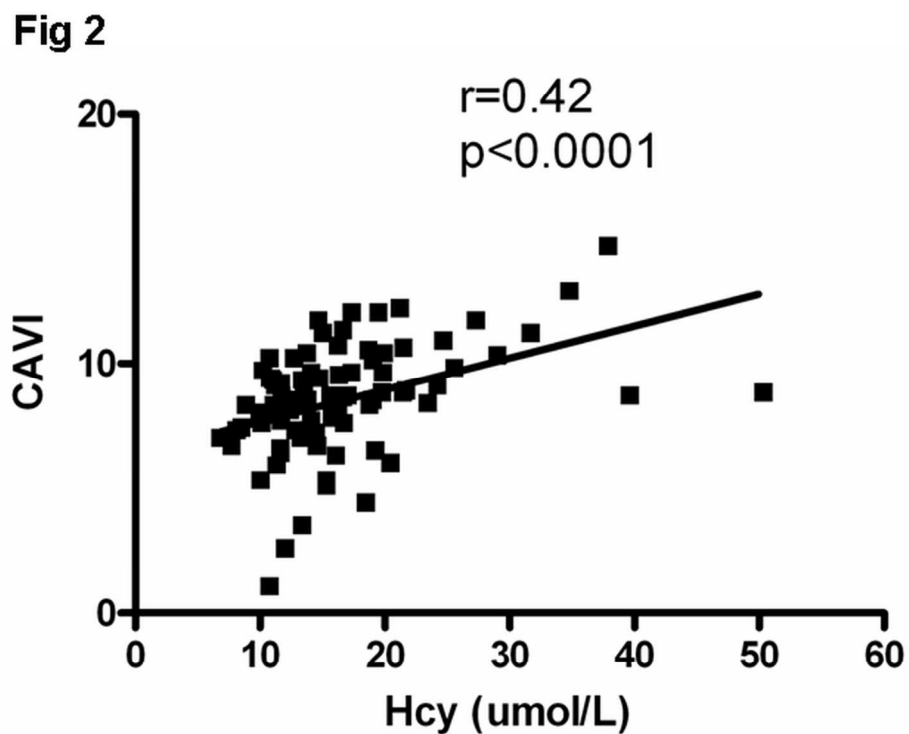


Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.  
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