Differential use of pulse wave velocity and ankle-brachial index in elderly Chinese and novel serum bio-markers for predicting arterial stiffness: a cross-sectional study

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Research article

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Abstract

Background Arterial stiffness is characterized by the loss of vessel elasticity and arterial compliance. Brachial-ankle pulse wave velocity (PWV) and ankle-brachial Index (ABI) are widely used in clinic for evaluating arterial stiffness. This study aimed to compare the efficiency of PWV and ABI in elderly Chinese and investigate novel bio-markers for aging related arterial stiffness.

Methods PWV and ABI were evaluated in 169 elderly Chinese with mean age of 85.7 years. 86 of the participants had serum concentrations of angiotensin II, vascular cell adhesion molecule (VCAM), osteopontin and glutathione peroxidase tested in further.

Results Compared with normotensive participants, PWV levels elevated in both limbs of hypertensive patients (2219 ± 90 cm/s v.s. 1970 ± 66 cm/s, P = 0.090 and 2141 ± 52 cm/s v.s. 1932 ± 67 cm/s, P = 0.023, separately). Compared with non-diabetes mellitus participants, ABI levels declined in both limbs of diabetes mellitus (DM) patients(0.96 ± 0.02 v.s. 1.05 ± 0.02, P = 0.002 and 0.99 ± 0.03 v.s. 1.03 ± 0.02, P = 0.071, separately). VCAM was included in both regression models for predicting PWV in left limbs and PWV in right limbs (Beta= - 0.307, P= 0.048; Beta= - 0.358, P= 0.020, separately). Osteopontin was included in both regression models for predicting ABI in left limbs and ABI in right limbs (Beta= - 0.320, P= 0.039; Beta= - 0.290, P= 0.011, separately).

Conclusions: Differential use of PWV and ABI should be considered in elderly with hypertension and/or DM. Serum levels of VCAM and osteopontin might be independent bio-markers for predicting arterial stiffness.

KEYWORDS Arterial stiffness in elderly; Pulse wave velocity; Ankle-brachial Index; Vascular cell adhesion molecule; Osteopontin

1. Background

Cardiovascular disease and cerebral vascular disease are the leading causes of mortality and morbidity worldwide.1–3

Arterial stiffness has been recognized to be involved in the process of atherosclerosis.4, 5 Arterial stiffness is characterized by the loss of vessel elasticity and arterial compliance. The structural changes of the arterial wall are associated with aging, which are labeled by a decrease in the elastin content and collagen content of the arterial media.6

Angiotensin II (Ang II) plays an important role in the regulation of arterial contraction and dilation.7 Ang II has been proved to induce arterial stiffness through structural and material remodeling.

Adherence of leukocytes to the vascular endothelium and their ensuing migration through the arterial wall into its intima where they instigate the migration of vascular smooth muscle cells (VSMCs) is one of the
fundamental steps of atherosclerosis. Vascular cell adhesion molecule (VCAM) has been proved to induce leukocytes migration in the process of atherosclerosis.

Our previous study found osteopontin (OPN) regulates migration and proliferation of VSMCs in animal models.

Glutathione peroxidase (GPX) activity progressively decreased with decades of age in old people. Age-related decline of GPX may represent a mechanism for the enhanced cardiovascular risk in the elderly population.

Brachial-ankle pulse wave velocity (PWV) and ankle-brachial Index (ABI) are widely used in clinic for evaluating arterial stiffness. But there is no guide for choose of method for evaluating arterial stiffness in elderly population with different diseases, such as hypertension or diabetes mellitus (DM).

Our study aimed to compare the efficiency of PWV and ABI and investigate independent bio-markers for aging related arterial stiffness. We tested serum concentrations of Ang II, VCAM, OPN, GPX and evaluated indexes of arterial stiffness in elderly Chinese.

2. Methods
2.1 Subjects and clinical parameters

202 elderly individuals referred to Geriatrics department in Shanghai sixth people's hospital were evaluated. The inclusion criteria were: (1) aged above 65 years; (2) nonsmoker; (3) no alcoholic addiction. The exclusion criteria were: (1) tumor; (2) hepatic disease; (3) end-stage kidney disease (chronic kidney disease, CKD ≥ stage 4); (4) serious cardiovascular disease (acute myocardial infarction or New York Heart Association classification IV); (5) stroke within 3 months; (6) acute infection. A standard questionnaire was used to collect information, including age, sex, history of hypertension, history of DM, renal disease and use of antihypertensive or antidiabetic drugs or insulin. Hypertension was defined by a blood pressure of 140/90 mm Hg or more, or a history of taking antihypertensive medications. DM was defined as fasting plasma glucose (FPG) equal or more than 7.0 mmol/L, postprandial plasma glucose (PPG) equal or more than 11.1 mmol/L, or the use of hypoglycemic agents. Body weight and body height were measured by professionals, and body mass index (BMI) was computed.

2.2 Biological parameters

Blood samples were obtained when the participants were in a fasting state in the morning. Biological variants were tested in the clinical laboratory of Shanghai Sixth People's Hospital. Serum levels of FPG, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), highdensity lipoprotein cholesterol (HDL) and creatinine were measured using an autoanalyzer (COBAS Integra 800, Roche
Diagnostics, Basel, Switzerland). Hematocrit determination (HCT) was measured in blood samples using an automated blood analyzer (XE–5000, Sysmex, Kobe, Japan).

### 2.3 Echocardiographic examination

Echocardiography was performed at Medical Ultrasound department of Shanghai Jiaotong University affiliated Shanghai Sixth People's Hospital. Echocardiographic assessment was performed with an ACUSON Sequoia™ 512 ultrasound system (Siemens Medical Solutions, Erlangen, Germany) equipped with an ultrabroad band frequency transducer (3–8MHz). The ejection fraction (EF) was assessed using Simpson's biplane method.

### 2.4 Brachial-ankle Pulse Wave Velocity (PWV)

In this study, an Oscillometry-based device (BP–203RPE III; Colin Omron, Co, Ltd, Tokyo, Japan) was used to measure the brachial-ankle PWV. After rested for at least ten minutes, each subject was laid in the supine position with PWV cuffs wrapped around both ankles and upper arms. Bilateral PWV were recorded for analysis after the examination of both sides. All measurements were performed by trained clinician with the manufacturer's guide.

### 2.5 Ankle-brachial Index (ABI)

ABI was defined as the ratio of ankle and brachial systolic blood pressure (SBP). Both ankle and brachial SBP were automatically and simultaneously measured by the validated device (VP–1000, Omron, Japan) on each side, the values of ABI could be read directly from the device. Bilateral ABI were used for further analyses. Measurements were performed by the same staff member, who was trained with the manufacturer’s guide.

### 2.6 Carotid Ultrasonography

Carotid artery imaging was obtained by an experienced clinician using an ultrasound system (Acuson Sequoia 512, Siemens, German). The probe frequency was 7.0MHz and the axis resolution was 0.1mm. Carotid intima media thickness (IMT), as the gap between the media-adventitia interface and lumen-intima interface, was measured 1.0 cm distal to both common carotid artery (CCA) bifurcations. The measurement of IMT was carried out within a CCA region free of plaque.

### 2.7 Assays for vascular endothelial cell adhesion molecule (VCAM), angiotensin II (Ang II), osteopontin (OPN) and glutathione peroxidase (GPX)
Blood samples for plasma Ang II, VCAM–1, OPN and GPX measurements were collected in ice-cold vacutainer tubes containing ethylenediaminetetraacetic acid to inhibit metalloproteases. A previous method was optimized for recovery and stability. The concentrations of Ang II, VCAM–1, OPN and GPX was measured by enzyme-linked immunosorbent assay (Rayto RT–6100, Rayto Life and Analytical Sciences Co., Shenzhen, China).

**Statistical analysis**

The unpaired Student's t-test, analysis of variance (ANOVA) and analysis of covariance were used for analysis of continuous variables. Quantitative data were presented as the mean ± standard error (S. E. M.), while categorical data are presented as numbers. Associations between variables were conducted using Pearson correlation analysis, partial correlation analysis and multivariate linear stepwise regression analyses. Two sided p-values < 0.05 were considered significant and p-values < 0.1 were considered slightly significant. The software SPSS 19.0 for Windows (SPSS Inc., IBM, United States) was used for statistical analysis.

**3. Result**

**3.1 Clinical and laboratory characteristics**

Of the original 202 participants, 12 dropped out of the study and 21 were excluded because of various diseases, leaving 169 participants who were included in this cross-sectional study. Of the 169 participants, 119 were diagnosed with hypertension (70.4%) and 62 were diagnosed with DM (36.7%). There were 150 male participants (88.2%), the mean age was 85.7 ± 0.4 years and the mean BMI was 23.8 ± 0.3 kg/m². Laboratory parameters, PWV, ABI, and ultrasound parameters are reported in Table 1.

**3.2 Comparison of clinical characteristics between hypertension group and normotension group**

To identify better method of evaluating arterial stiffness in elderly hypertensive patients, we compared PWV and ABI levels of hypertension group with those of normotension group. As shown in table 2, we observed higher TG concentrations and lower TC concentrations in hypertension group than in normotension group. Compared with normotensive participants, PWV levels of hypertensive patients were slightly higher in left limbs (2219 ± 90 cm/s v.s. 1970 ± 66 cm/s, P = 0.090) and significantly higher in right limbs (2141 ± 52 cm/s v.s. 1932 ± 67 cm/s, P = 0.023) separately. The ABI levels of right limbs in hypertensive patients were lower than normotensive participants (1.00 ± 0.02 v.s. 1.06 ± 0.02, P = 0.049), but no significant different ABI levels were reported in left limbs. The levels of age, BMI, FPG, LDL, HDL, HCT, Creatinine, EF and IMT were not significantly different between hypertension group and normotension group.
3.3 Comparison of clinical characteristics between DM group and non-DM group

To identify better method of evaluating arterial stiffness in elderly DM patients, we compared PWV and ABI levels of DM group with those of non-DM group. FPG concentrations of DM patients were higher, while TC concentrations of DM patients were lower than those of non-DM participants. Compared with non-DM participants, ABI levels of DM patients were significantly lower in left limbs (0.96 ± 0.02 v.s. 1.05 ± 0.02, P = 0.002) and slightly lower in right limbs (0.99 ± 0.03 v.s. 1.03 ± 0.02, P = 0.071) separately. No difference was observed between PWV levels of DM patients and non-DM participants either in left limbs or in right limbs. TG concentrations were slightly higher and EF levels were slightly lower in DM patients compared to non-DM participants. The levels of age, BMI, FPG, LDL, HDL, HCT, creatinine and IMT were not significantly different between DM group and non-DM group. (Table 3)

3.4 Peripheral expressions of candidate bio-markers for arterial stiffness

For investigating potential bio-markers for arterial stiffness in elderly population, 86 candidates had the tests of serum levels of VCAM, Ang, OPN and GPX. The mean levels of VCAM, Ang, OPN and GPX were 318.8 ± 7.1 ng/ml, 116.2 ± 2.6 pg/ml, 33.8 ± 0.7 ng/ml and 615.7 ± 14.2 U/L, separately. As shown in table 4a, the VCAM concentrations of hypertensive patients were significantly lower than normotensive participants (310.3 ± 7.9 ng/ml v.s. 344.9 ± 14.1 ng/ml, P = 0.034). No significant difference of Ang, OPN or GPX was observed between hypertension group and normotension group. However, the Ang levels of DM patients were significantly lower than non-DM participants (107.5 ± 4.5 pg/ml v.s. 121.9 ± 2.8 pg/ml, P = 0.005). The levels of VCAM, OPN and GPX of DM patients were not significantly different from those of non-DM participants.

3.5 Correlations between PWV and candidate bio-markers

To investigate the roles of VCAM, Ang, OPN and GPX in arterial stiffness in elderly population, we performed partial correlation analysis between PWV and candidate bio-markers. Both PWV of left limbs (L-PWV) and PWV of right limbs (R-PWV) were negatively correlated to VCAM (R = -0.316, P = 0.028; R = -0.310, P = 0.031, separately) after adjusted for age, BMI, FPG, TC and HCT. OPN was negatively correlated with R-PWV (R = -0.278, P = 0.009), but not correlated with L-PWV. Neither Ang nor GPX was found to be correlated to PWV. (Table 5)

3.6 Correlations between ABI and candidate bio-markers

Partial correlation analysis between ABI and candidate bio-markers was performed. After adjusted for age, BMI, FPG, TC and HCT, ABI of left limbs (L-ABI) was significantly correlated to Ang (R = -0.381, P =
0.010), but ABI of right limbs (R-ABI) was only slightly correlated to Ang (R = -0.234, P = 0.082). OPN was negatively correlated with R-ABI (R = -0.303, P = 0.005), but not correlated with L-ABI. Neither VCAM nor GPX was found to be correlated to ABI. (Table 6)

### 3.7 Bio-markers for predicting arterial stiffness in regression models

Since VCAM and OPN were found to be correlated to PWV, linear regression analysis of PWV with variate of age, BMI, FPG, TC, HCT, VCAM and OPN were performed. Only VCAM was included in the regression models of L-PWV and R-PWV (Beta = -0.307, P = 0.048; Beta = -0.358, P = 0.020, separately). Table 7

Linear regression analysis of ABI with variate of age, BMI, FPG, TC, HCT, Ang and OPN were performed. OPN was included both in the regression models of L-ABI and R-ABI (Beta = -0.320, P = 0.039; Beta = -0.290, P = 0.011, separately). In the second model of R-ABI, both OPN and FPG were included. However, Ang was not included in any model of ABI. Table 8

### Discussion

Population aging leads to more patients suffered from diseases related to arterial stiffness, such as cardiovascular disease, cerebral vascular disease and diabetes nephropathy. Not only high plasm glucose, lipid and high blood pressure, but also aging itself can cause arterial stiffness. Our study included subjects at an average age of 85 years old, which represented elderly Chinese population.

We found PWV levels were elevated in both limbs of hypertensive patients compared with non-hypertensive subjects. However, only ABI of right limb was slightly lower in hypertensive patients. We deduce PWV might be better method for evaluating arterial stiffness in hypertensive patients.

ABI levels of DM patients were lower in both limbs compared with non-DM participants. But no significant difference of PWV was observed between DM patients and non-DM participants. ABI might be better method for evaluating DM related vascular disease.

The mechanisms of arterial stiffness are not single. There are plenty of studies revealing the potential bio-markers for arterial stiffness, including VCAM, Ang, OPN and GPX.

Recently, the serum VCAM level was reported to be elevated in patients with peripheral artery disease and systemic sclerosis. However, we found the serum VCAM levels of hypertensive patients were lower than normotensive participants. In previous published study, serum VCAM levels were not found to be elevated in pulmonary hypertension or gestational hypertension, and low VCAM level might lead to fetal growth restriction. Our study found VCAM was negatively correlated with PWV in the total sample of elderly subjects. VCAM was the only factor included in the model for predicting PWV in our further linear regression analysis of PWV with variate of age, BMI, FPG, TC, HCT, VCAM and OPN. We deduced VCAM might be an independent biomarker for arterial stiffness caused by hypertension.
Renin-Angiotensin-Aldosterone System has been proved to play important roles in arterial remodeling of DM.\textsuperscript{18} However, we observed higher serum Ang \( \text{II} \) concentration in non-DM group than DM group. It might be associated with the advocated use of angiotensin converting enzyme inhibitors and Ang \( \text{II} \) receptor blockers as blood pressure lowering agents in DM patients.\textsuperscript{19} We also found Ang \( \text{II} \) was related to L-ABI with adjustment for age, BMI, FPG, TC & HCT, but it was not related to R-ABI. However, Ang \( \text{II} \) was not included in any model of biomarkers for ABI in further linear regression analysis.

Interestingly, we found OPN was negatively correlated to PWV and ABI in right limbs, but not left limbs. Moreover, OPN was included in all of the regression models of biomarkers for ABI, both in left limbs and right limbs. Although OPN was not found to be differently expressed in serum of hypertension or DM patients in our study, it might be an independent bio-marker for predicting arterial stiffness in elderly population. This finding is consistent with a former published animal study, which reported that OPN exerts stage-specific roles in arteriosclerosis by regulating vascular calcification and fibrosis.\textsuperscript{20}

### Conclusion

Differential use of PWV and ABI should be considered in elderly with hypertension and/or DM. Serum levels of VCAM and OPN might be independent bio-markers for predicting arterial stiffness.

### Abbreviations

PWV: brachial-ankle pulse wave velocity; ABI: ankle-brachial Index; VCAM: vascular cell adhesion molecule; Ang \( \text{II} \): angiotensin \( \text{II} \); VSMCs: vascular smooth muscle cells; OPN: osteopontin; GPX: glutathione peroxidase; DM: diabetes mellitus; FPG: fasting plasma glucose; PPG: postprandial plasma glucose; BMI: body mass index; TG: triglycerides; TC: total cholesterol; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; HCT: hematocrit determination; EF: ejection fraction; SBP: systolic blood pressure; IMT: carotid intima media thickness; CCA: common carotid artery; L-PWV: PWV of left limbs; R-PWV: PWV of right limbs; L-ABI: ABI of left limbs; R-ABI: ABI of right limbs.

### Declarations

#### Ethics approval and consent to participate

The protocol of the study was approved by the ethics committee of Shanghai Jiaotong University Affiliated Sixth People's Hospital (Shanghai, China). Each participate gave written informed consent to the procedure.

#### Consent for publication

Not applicable.
**Availability of data and materials**

The datasets used and/or analysed during the current study are de-identified but not available because of privacy protection.

**Competing interests**

The authors declare no conflict of interest in relation to the current study.

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**Authors’ contributions**

Guoxiang Fu and Yuan Zhong contributed equally to the work.

Guoxiang Fu provided funding and administrative support. Yuan Zhong recruited the participants. Chanchan Xu took responsibility for all aspects of the reliability and freedom from bias of the data presented. Chanchan Xu was a major contributor in writing the manuscript and took responsibility for research design. Chanchan Xu, Guoxiang Fu and Fengfeng Pan were involved in data collection. Chanchan Xu and Guoxiang Fu were involved in statistical analysis. All authors read and approved the final manuscript.

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Tables
Table 1. Clinical characteristics of total subjects

| Clinical characteristics | Mean value | SE  |
|--------------------------|------------|-----|
| FPG (mmol/L)             | 5.64       | 0.13|
| TG (mmol/L)              | 1.89       | 0.12|
| TC (mmol/L)              | 3.36       | 0.12|
| LDL (mmol/L)             | 2.16       | 0.06|
| HDL (mmol/L)             | 1.09       | 0.03|
| HCT (%)                  | 38.4       | 0.5 |
| Creatinine(umol/L)       | 89.1       | 3.0 |
| L- PWV (cm/s)            | 2145       | 67  |
| R- PWV (cm/s)            | 2079       | 42  |
| L- ABI                   | 1.01       | 0.01|
| R- ABI                   | 1.02       | 0.01|
| EF (%)                   | 63         | 0.69|
| IMT (mm)                 | 0.86       | 0.01|

FPG: fasting plasma glucose; TG: triglycerides, TC: total cholesterol; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; HCT: red blood cell specific volume; PWV: pulse wave velocity; L- PWV: PWV in left limbs; R- PWV: PWV in right limbs; ABI: ankle brachial index; L- ABI: ABI in left limbs; R- ABI: ABI in right limbs; EF: ejection fraction; IMT: intima media thickness
Table 2. Clinical characteristics between hypertensive patients and normotensive participants

| Clinical parameters | Hypertension | Normotension | P-value |
|---------------------|--------------|--------------|---------|
| n                   | 119          | 50           | /       |
| Age (years)         | 86.1 ± 0.4   | 84.8 ± 0.9   | 0.156   |
| BMI                 | 23.7 ± 0.3   | 24.0 ± 0.5   | 0.670   |
| FPG (mmol/L)        | 5.76 ± 0.18  | 5.40 ± 0.05  | 0.215   |
| TG (mmol/L)         | 2.09 ± 0.16 *| 1.48 ± 0.17 *| 0.018   |
| TC (mmol/L)         | 3.09 ± 0.16 *| 3.92 ± 0.18 *| 0.002   |
| LDL (mmol/L)        | 2.11 ± 0.08  | 2.30 ± 0.11  | 0.194   |
| HDL (mmol/L)        | 1.07 ± 0.05  | 1.10 ± 0.06  | 0.760   |
| HCT (%)             | 38.0 ± 0.6   | 39.3 ± 0.7   | 0.607   |
| Creatinine (µmol/L) | 92.3 ± 3.8   | 83.1 ± 4.5   | 0.141   |
| L-PWV (cm/s)        | 2219 ± 90 *  | 1970 ± 66 *  | 0.090   |
| R-PWV (cm/s)        | 2141 ± 52 *  | 1932 ± 67 *  | 0.023   |
| L-ABI               | 1.00 ± 0.02  | 1.03 ± 0.02  | 0.313   |
| R-ABI               | 1.00 ± 0.02 *| 1.06 ± 0.02 *| 0.049   |
| EF (%)              | 63.4 ± 0.6   | 62.3 ± 1.9   | 0.534   |
| IMT (mm)            | 0.85 ± 0.01  | 0.86 ± 0.03  | 0.734   |

* P < 0.05, *P < 0.1

BMI: body mass index; FPG: fasting plasma glucose; TG: triglycerides, TC: total cholesterol; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; HCT: red blood cell specific volume; PWV: pulse wave velocity; L-PWV: PWV in left limbs; R-PWV: PWV in right limbs; ABI: ankle brachial index; L-ABI: ABI in left limbs; R-ABI: ABI in right limbs; EF: ejection fraction; IMT: intima media thickness
Table 3. Clinical characteristics between DM patients and non-DM participants

| Clinical parameters | Diabetes Mellitus | Non- Diabetes Mellitus | P-value |
|---------------------|-------------------|------------------------|---------|
| n                   | 62                | 107                    | /       |
| Age (years)         | 85.9 ± 0.6        | 85.5 ± 0.5             | 0.638   |
| BMI                 | 23.7 ± 0.5        | 23.8 ± 0.3             | 0.818   |
| FPG (mmol/L)        | 6.69 ± 0.34 *     | 5.18 ± 0.05 *          | 0.000   |
| TG (mmol/L)         | 2.21 ± 0.23 *     | 1.73 ± 0.13 *          | 0.062   |
| TC (mmol/L)         | 2.90 ± 0.22 *     | 3.59 ± 0.15 *          | 0.008   |
| LDL (mmol/L)        | 2.13 ± 0.12       | 2.20 ± 0.08            | 0.624   |
| HDL (mmol/L)        | 1.04 ± 0.07       | 1.11 ± 0.04            | 0.377   |
| HCT                 | 37.0 ± 0.8        | 39.1 ± 0.5             | 0.607   |
| Creatinine (μmol/L)| 94.6 ± 5.6        | 86.2 ± 3.4             | 0.180   |
| L- PWV              | 2141 ± 90         | 2148 ± 92              | 0.964   |
| R- PWV              | 2126 ± 71         | 2053 ± 52              | 0.405   |
| L- ABI              | 0.96 ± 0.02 *     | 1.05 ± 0.02 *          | 0.002   |
| R- ABI              | 0.99 ± 0.03 *     | 1.03 ± 0.02 *          | 0.076   |
| EF (%)              | 61.7 ± 1.4 *      | 64.2 ± 0.6 *           | 0.071   |
| IMT (mm)            | 0.87 ± 0.02       | 0.85 ± 0.01            | 0.359   |

* p < 0.05, *p < 0.1

DM: Diabetes Mellitus; BMI: body mass index; FPG: fasting plasma glucose; TG: triglycerides, TC: total cholesterol; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; HCT: red blood cell specific volume; PWV: pulse wave velocity; L- PWV: PWV in left limbs; R- PWV: PWV in right limbs; ABI: ankle brachial index; L- ABI: ABI in left limbs; R- ABI: ABI in right limbs; EF: ejection fraction; IMT: intima media thickness
Table 4a. Serum levels of VCAM, Ang II, OPN and GPX in hypertension group and normotension group

| Laboratory Variable | Hypertension   | Normotension   | P-value |
|---------------------|----------------|----------------|---------|
| VCAM (ng/ml)        | 310.3 ± 7.9 *  | 344.9 ± 14.1 * | 0.034   |
| Ang II (pg/ml)      | 115.4 ± 3.2    | 118.8 ± 3.9    | 0.575   |
| OPN (ng/ml)         | 33.29 ± 0.73   | 35.33 ± 1.85   | 0.220   |
| GPX (U/L)           | 620.5 ± 16.1   | 600.7 ± 30.3   | 0.552   |

* P < 0.05.
VCAM: vascular endothelial cell adhesion molecule; Ang II: angiotensin II; OPN: osteopontin; GPX: glutathione peroxidase

Table 4b. Serum levels of VCAM, Ang II, OPN and GPX in DM group and non-DM group

| Laboratory Variable | Diabetes Mellitus | Non- Diabetes Mellitus | P-value |
|---------------------|-------------------|------------------------|---------|
| VCAM (ng/ml)        | 311.3 ± 12.3      | 323.6 ± 8.5            | 0.396   |
| Ang II (pg/ml)      | 107.5 ± 4.5 *     | 121.9 ± 2.8 *          | 0.005   |
| OPN (ng/ml)         | 33.92 ± 0.92      | 33.70 ± 1.03           | 0.881   |
| GPX (U/L)           | 627.7 ± 23.6      | 607.8 ± 17.8           | 0.495   |

* P < 0.05.
DM: Diabetes Mellitus; VCAM: vascular endothelial cell adhesion molecule; Ang II: angiotensin II; OPN: osteopontin; GPX: glutathione peroxidase
Table 5. Pearson correlation analysis between PWV and candidate bio-markers with adjustment for age, BMI, FPG, TC and HCT.

| Variable | L- PWV | | | R- PWV | | |
|----------|--------|-----------------|-----------------|-----------------|-----------------|
|          |        | R   | P-value | R   | P-value |
| VCAM     | -0.316* | 0.028 |   | -0.310* | 0.031           |
| Ang II   | -0.141  | 0.203 |   | -0.004  | 0.489           |
| OPN      | -0.084  | 0.240 |   | -0.278* | 0.009           |
| GPX      | -0.083  | 0.314 |   | -0.094  | 0.290           |

* P < 0.05.

R: correlation coefficient.

PWV: pulse wave velocity; L- PWV: PWV in left limbs; R- PWV: PWV in right limbs; VCAM: vascular endothelial cell adhesion molecule; Ang II: angiotensin II; OPN: osteopontin; GPX: glutathione peroxidase; FPG: fasting plasma glucose; TC: cholesterol; BMI: body mass index; HCT: red blood cell specific volume.

Table 6. Pearson correlation analysis between laboratory variable and ABI with adjustment for age, BMI, FPG, TC & HCT.

| Variable | L- ABI | | | R- ABI | | |
|----------|--------|-----------------|-----------------|-----------------|-----------------|
|          |        | R   | P-value | R   | P-value |
| VCAM     | -0.171  | 0.155 |   | 0.063  | 0.356           |
| Ang II   | -0.381* | 0.010 |   | -0.234* | 0.082           |
| OPN      | -0.145  | 0.112 |   | -0.303* | 0.005           |
| GSH      | 0.148   | 0.191 |   | -0.007 | 0.483           |

* P < 0.05, * P < 0.1.

R: correlation coefficient.

ABI: ankle brachial index; VCAM: vascular endothelial cell adhesion molecule; Ang II: angiotensin II; OPN: osteopontin; GPX: glutathione peroxidase; FPG: fasting plasma glucose; TC: cholesterol; BMI: body mass index; HCT: red blood cell specific volume.
Table 7.

Linear regression analysis of L- PWV with variate of age, BMI, FPG, TC, HCT, VCAM and OPN

| Variate | Beta    | P-value | State   |
|---------|---------|---------|---------|
| VCAM    | -0.307 * | 0.048   | included |
| age     | -0.109  | 0.486   | excluded |
| BMI     | -0.050  | 0.751   | excluded |
| FPG     | 0.129   | 0.400   | excluded |
| TC      | 0.034   | 0.834   | excluded |
| HCT     | -0.097  | 0.529   | excluded |
| OPN     | 0.009   | 0.952   | excluded |

Linear regression analysis of R- PWV with variate of age, BMI, FPG, TC, HCT, VCAM and OPN

| Variate | Beta    | P-value | State   |
|---------|---------|---------|---------|
| VCAM    | -0.358 * | 0.020   | included |
| age     | 0.108   | 0.481   | excluded |
| BMI     | -0.043  | 0.779   | excluded |
| FPG     | 0.154   | 0.303   | excluded |
| TC      | -0.029  | 0.855   | excluded |
| HCT     | 0.036   | 0.813   | excluded |
| OPN     | -0.227  | 0.13    | excluded |

* P < 0.05. Beta: standardized coefficients for multivariable regression model.
PWV: pulse wave velocity; L- PWV: PWV in left limbs; R- PWV: PWV in right limbs; FPG: fasting plasma glucose; TC: cholesterol; BMI: body mass index; HCT: red blood cell specific volume; VCAM: vascular endothelial cell adhesion molecule; OPN: osteopontin
Table 8.

Linear regression analysis of L-ABI with variate of age, BMI, FPG, TC, HCT, Ang II and OPN

| Variate | Beta   | P-value | State    |
|---------|--------|---------|----------|
| OPN     | -0.320*| 0.039   | included |
| age     | 0.043  | 0.779   | excluded |
| BMI     | 0.041  | 0.787   | excluded |
| FPG     | -0.201 | 0.183   | excluded |
| TC      | 0.091  | 0.549   | excluded |
| HCT     | -0.007 | 0.963   | excluded |
| Ang II  | -0.198 | 0.203   | excluded |

Linear regression analysis of R-ABI with variate of age, BMI, FPG, TC, HCT, VCAM and OPN

| Variate | Model 1 | Model 2 |
|---------|---------|---------|
|         | Beta   | P-value | State    | Beta   | P-value | State    |
| OPN     | -0.290*| 0.011   | included | -0.274*| 0.013   | included |
| age     | -0.195 | 0.082   | excluded | -0.169 | 0.124   | excluded |
| BMI     | -0.039 | 0.831   | excluded | -0.034 | 0.745   | excluded |
| FPG     | -0.257 | 0.020   | excluded | -0.257*| 0.020   | included |
| TC      | -0.036 | 0.756   | excluded | -0.004 | 0.972   | excluded |
| HCT     | -0.122 | 0.278   | excluded | -0.127 | 0.243   | excluded |
| Ang II  | 0.000  | 0.998   | excluded | -0.008 | 0.941   | excluded |

* P < 0.05. Beta: standardized coefficients for multivariable regression model.

ABI: ankle brachial index; L-ABI: ABI in left limbs; R-ABI: ABI in right limbs;
FPG: fasting plasma glucose; TC: cholesterol; BMI: body mass index; HCT: red blood cell specific volume; Ang II: angiotensin II; OPN: osteopontin;