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Association of hemoglobin with ankle-brachial index in general population

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OBJECTIVES: Previous studies have demonstrated that both low and high hemoglobin concentrations are predictive of adverse cardiovascular outcomes in various populations. However, an association of hemoglobin with the ankle-brachial index, which is widely used as a screening test for peripheral arterial disease, has not yet been identified.

METHODS: We examined 786 subjects (236 women and 550 men) who received routine physical check-ups. The ankle-brachial index and several hematological parameters, including the hemoglobin level, hematocrit and red blood cell count and other demographic and biochemical characteristics were collected. Univariate and multivariate linear regression analyses were performed to assess the relationships between the ankle-brachial index and the independent determinants. Receiver operating characteristic curve analysis was conducted to calculate the cut-off level of hemoglobin for detecting a relatively low ankle-brachial index (less than 20% of all subjects, which was 1.02).

RESULTS: The hemoglobin level, hematocrit and red blood cell count were correlated with the ankle-brachial index in the males (r=-0.274, r=-0.224 and r=-0.273, respectively, $p<0.001$ for all), but these associations were not significant in the females. Multivariate linear regression analysis revealed that the independent determinants of the ankle-brachial index included age, total cholesterol, high-density lipoprotein cholesterol and the white blood cell count for the females and age, hypertension, total cholesterol and hemoglobin (β=-0.001, $p<0.001$) for the males after adjusting for confounding factors. Receiver operating characteristic curve analysis revealed that the cut-off level of hemoglobin for detecting a low ankle-brachial index was 156.5 g/L in the males.

CONCLUSIONS: A high hemoglobin concentration was independently correlated with a low ankle-brachial index in the healthy males, indicating that an elevation in this level may be associated with an increased atherosclerosis risk.

KEYWORDS: Hemoglobin; Ankle-brachial Index; Peripheral Arterial Disease; Atherosclerosis.

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INTRODUCTION

Previous epidemiologic studies have shown that the hemoglobin concentration is associated with unfavorable cardiovascular events in the general population and in patients with coronary arterial disease (CAD), hypertension and heart failure (1-8). Some investigations have revealed that a decreased hemoglobin concentration or anemia is predictive of adverse outcomes (1-4); however, accumulating evidence demonstrates a J- or U-shaped curve for the relationship between hemoglobin and poor prognosis in patients with acute coronary syndrome or heart failure and in the elderly general population (5-8). In addition, the clinical beneficial effects of therapeutic approaches using erythropoietin to increase the hemoglobin level are controversial, an elevation in the hematocrit to above 42% increases cardiovascular mortality (9). Therefore, a low hemoglobin level or anemia, as well as an elevated hemoglobin level, should be taken into consideration in evaluation of the total risk of cardiovascular disease (CVD).

The ankle-brachial index (ABI), the ratio of lower extremity blood pressure to upper extremity blood pressure, is a simple and convenient method used to diagnose peripheral arterial disease (PAD), which is a local manifestation of systemic atherosclerosis. An ABI of <0.9 has been shown to have a high sensitivity (90–97%) and specificity (98–100%) for detecting lower extremity stenosis (10). Therefore, it has been widely used as a screening index for PAD. PAD often coexists with CAD and cerebrovascular disease; approximately 70% of patients with PAD have atherosclerotic disease in other vascular beds (11). In addition, a high ABI of $\geqslant 1.3$ is indicative of medial arterial calcification (MAC) (12). Thus, the normal range for the ABI may be considered to be between 0.9 and 1.3. The ABI is associated with

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increased risks of CVD, cardiovascular mortality and all-cause mortality (13). Hence, it has been applied extensively as a screening index for cardiovascular risk estimation in asymptomatic subjects.

Few studies have assessed the association between the hemoglobin concentration and ABI. Moreover, the predictive ability of hemoglobin for unfavorable outcomes has been assessed mainly in subjects with a high CVD risk. Therefore, the purpose of this study was to determine the relationship between the hemoglobin level and ABI in the general population with a normal ABI, whose CVD risk is relatively low, to increase understanding of the potential mechanisms underlying the poor prognosis caused by either a low or high hemoglobin concentration.

METHODS

Study subjects

We reviewed the medical records of 802 participants who underwent routine physical examinations at the Health Management Center of Xiangya Hospital, Central South University, between September 2013 and December 2013. Subjects meeting any of the following criteria were excluded: an age of <18 or $\geqslant 80$ years; any missing information; an abnormal ABI (<0.9 or $\geqslant 1.3$); severe hepatic and/or renal dysfunction; a malignant tumor; infectious or systematic inflammation; or a significant hematologic disorder. A total of 786 participants (550 men and 236 women) were ultimately included in this study. The study was approved by the Xiangya Hospital ethics committee (201512536) and informed consent was obtained from each participant.

Baseline information collection

Examinations were performed by trained medical staff of the Health Management Center according to standard procedures. The participants were asked about their cigarette smoking status and past medical histories of hypertension and diabetes mellitus. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Obesity was defined as a BMI $\geqslant 28.0$ according to the criteria of the Working Group on Obesity in China (WGOC) (14). Systolic and diastolic blood pressure (SBP and DBP, respectively) was measured using a mercury sphygmomanometer in a seated position after at least 5 minutes of rest.

Fasting blood samples were collected from an antecubital vein after a 12-hour overnight fast. White blood cell (WBC), hemoglobin, hematocrit, red blood cell (RBC) and platelet counts were determined using an automated blood cell analyzer (Beckman LH750, Bera, CA, USA). Total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid, C-reactive protein (CRP) and creatinine levels were measured using an automatic biochemistry analyzer (Beckman AU5800, Koutou-ku, Tokyo, Japan). The estimated glomerular filtration rate (eGFR) was calculated using a modified Modification of Diet in Renal Disease (MDRD) equation (15).

Hypertension was defined as an SBP and/or DBP of $\geqslant 140$ and/or $\geqslant 90$ mmHg, a self-reported history of diagnosed hypertension, or current use of an anti-hypertensive drug (16). Diabetes mellitus was defined as a fasting serum glucose level of $\geqslant 7.0$ mmol/L, a self-reported history of diagnosed diabetes, or receipt of hypoglycemic therapy (17).

Measurement of the ABI

The participants rested in the supine position for at least 5 minutes, and then SBP was measured at the ankle level and in the brachial arteries on both sides of the body using an automatic waveform analyzer (model BP-203RPE, Colin, Komaki City, Japan) as previously described. Validation of this automatic device and its reproducibility have been previously demonstrated (18). The ABI was calculated as ratio of ankle pressure to brachial pressure and the lower of the bilateral ABI values for each participant was used in analysis. Participants with an ABI of <0.9 or $\geqslant 1.3$ were excluded from the study.

Statistical analysis

The participants were initially classified as female or male. The distribution of the data was assessed for normality using the one-sample Kolmogorov-Smirnov test. Continuous variables are presented as the mean and standard deviation (SD) and categorical variables are presented as counts and percentages. Comparisons between groups were performed using the two-sample t-test for continuous variables and the chi-square test for categorical variables. Correlations between the hematological parameters (WBC, hemoglobin, hematocrit, RBC and platelet counts) and ABI were examined by Pearson or Spearman correlation analysis as appropriate. Multivariate stepwise linear regression analyses were performed to assess the independent determinants of the ABI. A low ABI was defined as less than 1.02 ($<20^{th}$ percentile of all participants). Receiver operating characteristics (ROC) curves were constructed to determine the predictive values of the hemoglobin level and hematocrit and RBC counts for a low ABI. All tests were two-tailed, and a p-value of 5% or less was considered statistically significant. SPSS software package (SPSS 19.0; SPSS Inc; Chicago, IL, USA) was used for all statistical analyses.

RESULTS

A total of 786 eligible participants (236 women and 550 men) were included in the current study. Both the hemoglobin level and ABI followed normal distributions according to gender, as determined using the Kolmogorov-Smirnov test ($p>0.05$ for all). The baseline demographic and clinical characteristics and laboratory indexes of the enrolled subjects according to gender are summarized in Table 1. In brief, fewer female subjects had hypertension, diabetes mellitus or current smoking status; these subjects were unlikely to be obese and the female subjects tended to have a lower BMI, triglyceride, total cholesterol, uric acid and hemoglobin levels, WBC and RBC counts and ABI and a higher HDL-cholesterol level, eGFR and platelet count. There were no differences in age, LDL-cholesterol or CRP between the males and females.

We evaluated the associations of the hematological parameters (WBC, hemoglobin, hematocrit, RBC and platelet counts) with the ABI by Spearman or Pearson correlation analysis, as appropriate, in the males and females separately (Table 2). The results revealed that the WBC count (r=-0.155, p=0.017) was negatively correlated with the ABI in the females and that the hemoglobin level (r=-0.274, $p<0.001$), hematocrit (r=-0.273, $p<0.001$) and RBC count (r=-0.224, $p<0.001$) were negatively correlated with the ABI in the males. Figure 1 shows the correlations of the hemoglobin

Table 1 - Baseline characteristics of all subjects according to gender.

Variables	Female (n=236)	Male (n=550)	p value
Age (years)	46.0 ± 10.2	46.6 ± 8.9	0.398
Hypertension (%)	38 (16.1)	140 (25.5)	0.004
Diabetes mellitus (%)	6 (2.5)	54 (9.8)	<0.001
Current smoker (%)	13 (6.0)	348 (68.2)	<0.001
Obesity (%)	15 (6.4)	102 (18.5)	<0.001
BMI (kg/m^2)	22.9 ± 3.0	25.5 ± 3.0	<0.001
Triglycerides (mmol/L)	1.40 ± 1.16	2.41 ± 2.31	<0.001
Total cholesterol (mmol/L)	4.85 ± 0.95	5.12 ± 1.01	<0.001
HDL-cholesterol (mmol/L)	1.68 ± 0.37	1.41 ± 0.36	<0.001
LDL-cholesterol (mmol/L)	2.56 ± 0.75	2.67 ± 0.84	0.069
Uric acid (μmol/L)	268.7 ± 64.9	375.8 ± 73.4	<0.001
CRP (mg/L)	2.38 ± 1.99	2.86 ± 4.16	0.096
eGFR (ml/min/1.73 m^2)	91.4 ± 14.8	82.84 ± 13.0	<0.001
WBCs (x10^9/L)	5.88 ± 1.47	6.44 ± 1.65	<0.001
Hemoglobin (g/L)	129.9 ± 10.0	152.8 ± 10.3	<0.001
Hematocrit (%)	38.6 ± 3.0	44.9 ± 3.5	<0.001
RBCs (x10^{12}/L)	4.33 ± 0.39	4.98 ± 0.42	<0.001
Platelet count (x10^9/L)	223.5 ± 62.5	209.8 ± 53.7	0.002
ABI	1.07 ± 0.06	1.08 ± 0.06	<0.001

The data are expressed as the mean ± SD or count (percentage).
BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; eGFR: estimated glomerular filtration rate; WBCs: white blood cells; RBCs: red blood cells; ABI: ankle-brachial index.
The two-sample t-test was used for continuous variables and the chi-square test was used for categorical variables.

Table 2 - Correlations between hematological parameters and ankle-brachial index according to gender.

	Female		Male	
Variable	r	*p* value	r	*p* value
WBCs	-0.155	0.017	-0.058*	0.176
Hemoglobin	-0.012	0.851	-0.274	<0.001
Hematocrit	-0.051	0.438	-0.273	<0.001
RBCs	-0.058	0.374	-0.224	<0.001
Platelet count	-0.076	0.244	-0.065	0.128

WBCs: white blood cells; RBCs: red blood cells.
*: Pearson correlation analysis was performed for all variables except for WBCs in males, as the data of WBCs did not follow a normal distribution, necessitating the use of Spearman correlation analysis.

Table 3 - Multivariate stepwise regression analysis of ankle-brachial index according to gender.

Covariate	β	95% CI	partial R^2	*p* value
Female				
Age	0.002	0.001, 0.003	0.037	<0.001
Total cholesterol	0.011	0.001, 0.020	0.024	0.029
HDL-cholesterol	-0.035	-0.059, -0.012	0.038	0.003
WBCs	-0.008	-0.014, -0.003	0.022	0.005
Male				
Age	0.002	0.001, 0.002	0.033	<0.001
Hypertension	0.019	0.007, 0.032	0.018	0.002
Total cholesterol	-0.007	-0.012, -0.002	0.001	0.011
Hemoglobin	-0.001	-0.002, -0.001	0.008	<0.001

β: nonstandard regression coefficient; CI: confidence interval; WBCs: white blood cells; HDL: high-density lipoprotein.

level and RBC and hematocrit counts with the ABI according to gender.

Multivariate stepwise linear regression was conducted to identify independent determinants of the ABI according to gender. Age, hypertension, diabetes mellitus, current smoking status, BMI, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, uric acid, CRP, the eGFR and the WBC, hemoglobin, hematocrit, RBC and platelet counts were included in the regression model. The results showed that in the females, the independent determinants of the ABI included age (β=0.002, $p<0.001$), total cholesterol (β=0.011, $p=0.029$), HDL-cholesterol (β=-0.035, $p=0.003$) and the WBC count (β=-0.008, $p=0.005$). Alternatively, in the males, the independent determinants included age (β=0.002, $p<0.001$), hypertension (β=0.019, $p=0.002$) and total cholesterol (β=-0.007, $p=0.011$) and hemoglobin (β=-0.001, $p<0.001$).

The area under the ROC curve (AUC) for hemoglobin (AUC=0.663, 95% CI 0.605-0.721, $p<0.001$) showed strong discriminatory power for detecting a low ABI in the males (Figure 2). The optimal cut-off level was 156.5 g/L (sensitivity of 57.1% and specificity of 69.1%). The predictive values of the RBC and hematocrit counts are also indicated in Figure 2. The ability of the hemoglobin level to predict a low ABI in the males was slightly superior to the abilities of the RBC (AUC=0.621, 95% CI 0.560-0.682, $p<0.001$) and hematocrit counts (AUC=0.656, 95% CI 0.597-0.716, $p<0.001$).

DISCUSSION

To the best of our knowledge, this is the first study evaluating the relationship between the hemoglobin concentration and ABI in the general population. In this cross-sectional study, we found strong negative associations of hemoglobin, RBC and hematocrit with the ABI in healthy male subjects. After adjusting for potential risk and confounding factors, only hemoglobin remained significant in the regression model. A relatively low ABI was defined as 1.02 (20% cut-off point of the entire population) in our study, suggesting that subjects with a low ABI may have a higher risk of PAD. The ROC curve indicated that the cut-off hemoglobin level for detecting a low ABI was 156.5 g/L; notably, a correlation between hemoglobin and the ABI was only observed in the male subjects; this finding may be explained by the lower range of hemoglobin in females compared with males. Thus, the results of the present study suggest that hemoglobin has important healthcare implications in the general population, particularly in males.

Hemoglobin, which is the main functional constituent of RBCs, carries oxygen from the lungs to tissues of the body and carries carbon dioxide from the tissues to the lungs. A decreased hemoglobin concentration or anemia usually indicates a relatively poor health status and may be a consequence of or accompanied by certain types of chronic comorbidities. Thus, the clinical importance of a decrease in the hemoglobin concentration has been recognized by physicians and some investigations have demonstrated that this decrease is predictive of adverse outcomes in patients with CVD and in the general population. However, some other studies have demonstrated a J- or U-shaped curve for the relationship between hemoglobin and poor prognosis. In patients with acute coronary syndrome, both low and high baseline hemoglobin levels increase the risks of cardiovascular mortality, myocardial infarction and recurrent ischemia (5). Patients with chronic heart failure

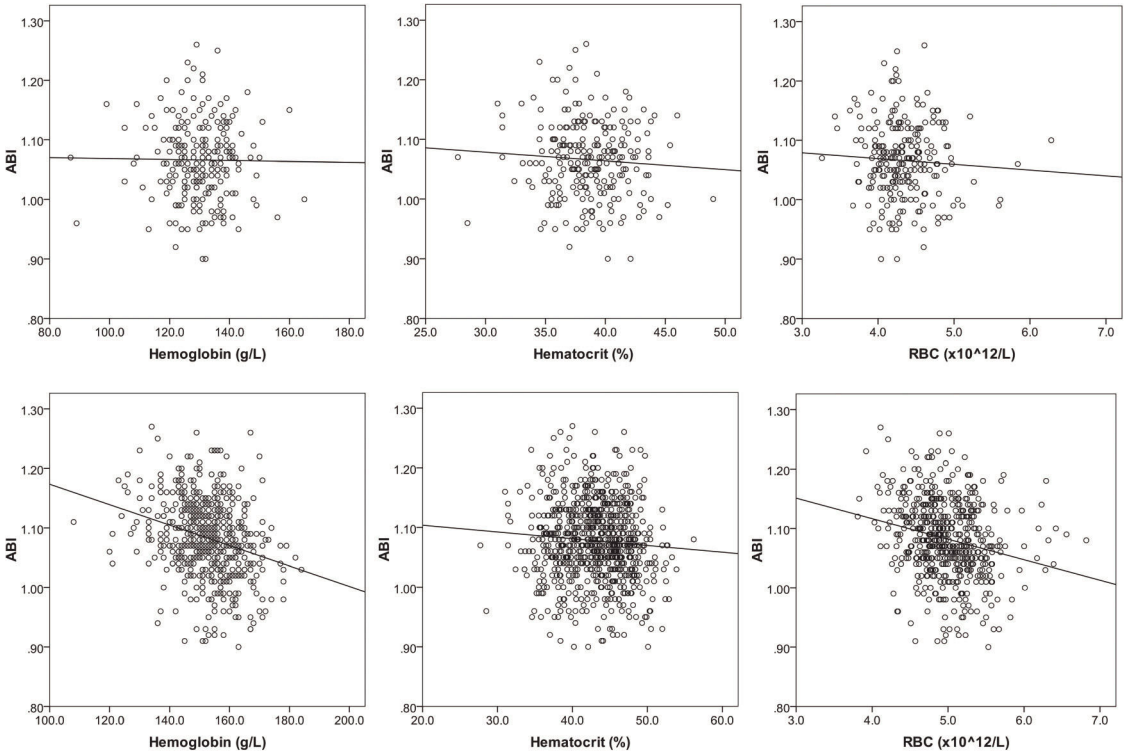

Figure 1 - Correlations of the ankle-brachial index with hemoglobin, RBC and hematocrit according to gender (the top row is for females, and the bottom row is for males). ABI: ankle-brachial index; RBC: red blood cell.

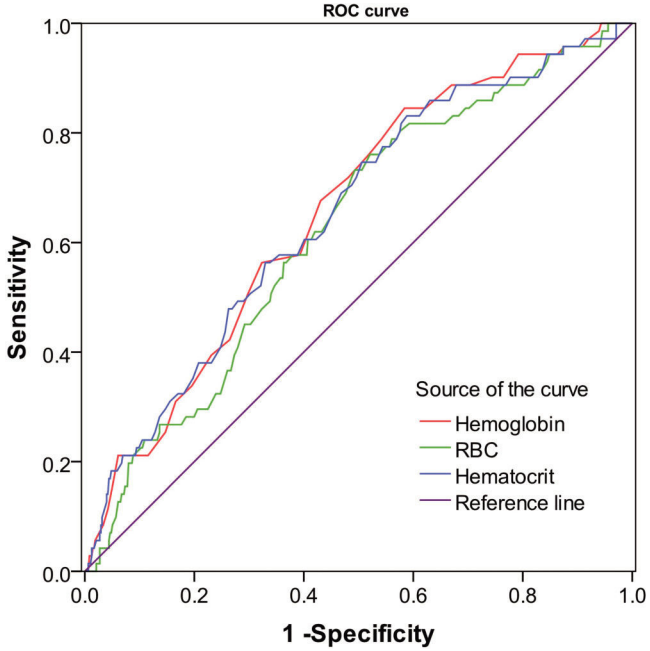

Figure 2 - Receiver operating characteristic curves for various blood parameters in detecting a low ankle-brachial index (less than 20% of all subjects, which was 1.02).

who are severely anemic or polycythemic have the worst survival rates (6). Another study of patients with chronic heart failure has reported similar results, showing that a very high ($\geq$17 g/dL) or reduced ($<$13 g/dL) hemoglobin level is independently predictive of substantially increased risks of death and hospitalization, regardless of the level of systolic function (7). In addition, in an older community-dwelling population with a normal CVD risk, low and high

hemoglobin concentrations have been demonstrated to be independently associated with increased mortality (8). Thus, the adjusted risk of poor outcome associated with a high hemoglobin level is similar to and not lower than that associated with a low hemoglobin level.

The hemoglobin concentration in blood varies minimally with hematocrit, which is the proportion of the blood that consists of packed RBCs. A previous study has indicated that hematocrit is strongly correlated with the hemoglobin level (6); thus, the hematocrit is usually used as a surrogate marker for hemoglobin. A high hematocrit has been shown to be predictive of subsequent mortality in patients with ischemic stroke (19). A health survey study has demonstrated that a slightly elevated hematocrit is associated with an increase in coronary heart disease mortality after 28 years of follow-up (20). In addition, a study involving hematocrit measurement has demonstrated a U-shaped pattern for cardiovascular or non-cardiovascular mortality in hypertensive adults (21). In the current study, we recorded both the baseline hemoglobin concentration and hematocrit, and univariate regression analysis revealed that both of these parameters were correlated with the ABI and had semblable discriminating abilities for detecting a low ABI. However, multivariate stepwise regression analysis, in which hemoglobin, hematocrit and other potential covariates were simultaneously considered, revealed that after adjustments, only hemoglobin, and not hematocrit, remained as an independent determinant of the ABI. There is a lack of studies examining which parameter is superior to the others for detecting a low ABI and thus the risk of CVD therefore, further study is required.

The independent association of hemoglobin with the ABI detected in the present study was restricted to the male subjects. The main reason for this discrepancy is the differing distributions of hemoglobin levels between the males and females, as the females had a lower range of hemoglobin than the males, in agreement with previous studies (3,5-7). Further, ROC curve analysis revealed that the cut-off level for detecting a low ABI in the males was 156.5 g/L; only 0.8% of the females' hemoglobin levels were above this cut-off level (compared with 35.3% of the males). Notably, other covariates affect the relationship of hemoglobin with the ABI, but they were not adjusted for in our study. Although some studies have shown that the relationship between hemoglobin and adverse outcomes is independent after adjusting for covariates, including gender (5-7), it is better to divide participants according to gender when determining optimal hemoglobin ranges (3).

The mechanism underlying the association between hemoglobin and arterial stiffness or atherosclerosis has not yet been elucidated. The main mechanism may involve an increase in blood viscosity due to a high hemoglobin concentration. Notably, blood viscosity is closely associated with carotid and coronary atherosclerosis (22,23), cerebral atherogenesis (24), common carotid artery elasticity (25) and increased vascular resistance, resulting in decreased coronary blood flow (26) and reduced cerebral reperfusion after acute stroke Another mechanism may involve an increase in blood pressure due to an elevated hemoglobin concentration (1,8), resulting in an increased risk of thrombosis (28). Moreover, hemoglobin participate in atherogenesis. Erythrocytes are prone to lyse in advanced atheromatous lesions, releasing hemoglobin. Hemoglobin is oxidized to ferri- and ferrylhemoglobin and released heme and iron promote further oxidation of lipids, amplifying the endothelial cell cytotoxicity of plaque components (29).

The major limitations of this study are its cross-sectional and observational nature and limited sample size; in particular, the sample size of the female participants was relatively smaller than that of the males. However, potential confounding factors were adjusted for in our study, suggesting that the relationship observed between hemoglobin and the ABI in males is a valid finding. In addition, our study recruited general subjects undergoing routine physical examinations, so our findings should be interpreted with caution. Moreover, we excluded those participants with an ABI of <0.9 or $\geqslant 1.3$ before analysis, as the proportion of participants with an ABI of <0.9 was extremely low (0.8%). Therefore, our study failed to determine the predictive ability of hemoglobin for PAD and future study is warranted. Finally, the findings regarding the impact of the hemoglobin concentration on dynamic changes in the ABI over time may be more convincing and further study is necessary.

In conclusion, in healthy males, the hemoglobin concentration was negatively and independently associated with the ABI. Accordingly, subjects with an elevated hemoglobin concentration may be at an increased risk of PAD. Further investigation is necessary to confirm these findings.

AUTHOR CONTRIBUTIONS

Chenglong Z and Jing L participated in data collection and manuscript writing, Xia K contributed in data analysis and manuscript writing and Yang T designed the study plan and revised the paper.

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