

Usefulness of controlled attenuation parameter and liver stiffness measurement for detecting increased arterial stiffness in asymptomatic populations in China

Xin-yan Yu, MD^a, Xiao-xiao Song, MD^b, Yu-ling Tong, MD^a, Ling-yan Wu, MD^a, Zhen-ya Song^{a,*} 

Abstract

In recent studies, vibration-controlled transient elastography (FibroScan) has been reported as an alternative noninvasive approach for measuring liver steatosis and fibrosis. The present study aimed to investigate the feasibility of FibroScan controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) in the detection of increased arterial stiffness in asymptomatic populations in China.

A retrospective cohort recruiting 4747 asymptomatic patients with no underlying causes of liver disease and having FibroScan and brachial-ankle pulse wave velocity (baPWV) during wellness check-up was covered. Nonalcoholic fatty liver disease (NAFLD) was defined as a CAP ≥ 238 dB/m. NAFLD with significant fibrosis was defined as an LSM ≥ 7.3 kPa in the presence of NAFLD. Increased arterial stiffness was determined as a BaPWV ≥ 1.4 m/second.

Among the 4747 study participants, 1596 subjects (33.6%) suffered from increased arterial stiffness. The prevalence of increased arterial stiffness progressively increased across CAP quartiles and LSM quartiles in NAFLD (23.5%, 30.8%, 38.3%, 43.7%, $P < .001$ and 33.1%, 36.8%, 40.4%, 48.2%, $P < .001$, respectively). After conventional cardiovascular risk factors were adjusted (age, sex, overweight, diabetes mellitus, hypertension, hypercholesterolemia, and current smoking habits), multivariate logistic regression analysis revealed that CAP (odds ratio [OR] = 1.005; 95% confidence interval [CI]: 1.003–1.006; $P < .001$), NAFLD (OR = 1.427; 95% CI: 1.212–1.681; $P < .001$), LSM in NAFLD (OR = 1.073; 95% CI: 1.023–1.125; $P = .003$), and significant fibrosis in NAFLD (OR = 1.480; 95% CI: 1.090–2.010; $P = .012$) were independently associated with increased arterial stiffness. Furthermore, in a multivariate logistic regression analysis, OR (95% CI) for the maximal vs. the minimal quartile of CAP was 1.602 (1.268–2.024), and that of LSM in NAFLD was 1.362 (1.034–1.792) after adjustment for the above-mentioned risk factors. Notably, NAFLD and significant fibrosis in NAFLD were significantly correlated only with increased arterial stiffness in subjects without hypertension or diabetes mellitus after adjustment for the above-mentioned risk factors.

CAP-defined NAFLD and LSM-defined significant fibrosis in NAFLD showed significant and independent relationships with increased arterial stiffness even after adjustment for conventional cardiovascular risk factors, which can be conducive to stratifying relative risk of subjects having undergone screening assessment for cardiovascular disease.

Abbreviations: ALT = alanine aminotransferase, AST = aminotransferase, BaPWV = brachial-ankle pulse wave velocity, BMI = body mass index, CAP = controlled attenuation parameter, CI = confidence interval, CIMT = carotid intima-media thickness, CVD = cardiovascular disease, FPG = fasting plasma glucose, HbA1c = glycated hemoglobin, HDL-C = high-density lipoprotein cholesterol, IQR = interquartile range, LDL-C = low-density lipoprotein cholesterol, LSM = liver stiffness measurement, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OR = odds ratio, T2DM = type 2 diabetes mellitus, TC = total cholesterol, TG = triglyceride.

Keywords: arterial stiffness, brachial-ankle pulse wave velocity, controlled attenuation parameter, fibroscan, fibrosis, liver stiffness measurement, nonalcoholic fatty liver disease

Editor: Robert Chen.

The authors have no funding and conflicts of interests to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

^a Department of General Practice and Health Management Center, ^b Department of Endocrinology, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.

* Correspondence: Zhen-ya Song, Department of General Practice and Health Management Center, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China (e-mail: songzhenya@zju.edu.cn).

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How to cite this article: Yu Xy, Song Xx, Tong Yi, Wu Ly, Song Zy. Usefulness of controlled attenuation parameter and liver stiffness measurement for detecting increased arterial stiffness in asymptomatic populations in China. *Medicine* 2020;99:48(e23360).

Received: 29 June 2020 / Received in final form: 17 September 2020 / Accepted: 24 October 2020

<http://dx.doi.org/10.1097/MD.00000000000023360>

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) has been reported as the most common cause of chronic liver disease worldwide,^[1] its spectrum includes hepatic steatosis, nonalcoholic steatohepatitis (NASH), cirrhosis, and even hepatocellular carcinoma. NAFLD is the hepatic manifestation of metabolic syndrome, and emerging data suggest NAFLD as a multisystem disease capable of predicting liver-related diseases, atherosclerotic cardiovascular disease (CVD), chronic kidney disease, and type 2 diabetes mellitus (T2DM).^[2] Although the risk of liver-related mortality is elevated in patients with NAFLD, the most common cause of death is CVD-related.^[3,4]

Arterial stiffness is considered one of the earliest detectable signs of structural and functional changes in the vessel wall of elastic arteries; it is established as a surrogate prognosticator of CVD morbidity and mortality.^[5] Brachial-ankle pulse wave velocity (baPWV) is a simple, and non-invasive measure of arterial stiffness, with high validity and reproducibility, developed with an automated system; it can be used in screening vascular damages in a large population.^[6] It is increasingly evident that baPWV is capable of predicting the risk of future cardiovascular events and total mortality.^[7-9]

Although liver biopsy is the gold standard for diagnosing, assessing activity and staging fibrosis in NAFLD, the invasiveness and possible sampling errors attributed to liver biopsy limit its clinical applications, especially in asymptomatic healthy subjects.^[10,11] Liver steatosis and fibrosis are 2 of the most commonly investigated histological variables since both variables are critical for diagnosing diseases and the degree of disease progression. Recent work has shown that controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) evaluated with vibration controlled transient elastography (FibroScan) acts as an alternative noninvasive, reliable approach for identifying and characterizing liver steatosis and fibrosis.^[12-14]

However, the associations between liver steatosis and fibrosis measured with FibroScan and arterial stiffness measured with baPWV have been rarely studied in asymptomatic subjects. In the present study, we have hypothesized that there are significant correlations between early markers of atherosclerosis and liver steatosis and fibrosis. To test this hypothesis, we examined the

association between baPWV as a measurement of arterial stiffness and CAP and LSM based on FibroScan in a large number of apparently healthy China adults. Our findings may provide clues for more effective prevention of adverse cardiovascular events in patients with NAFLD.

2. Methods

2.1. Study population

This study analyzed a retrospective cohort of apparently healthy subjects with both baPWV and liver FibroScan 502 (Echosens, Paris, France) during wellness check-up examinations at Health Management Center of the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou from April 2014 and June 2018. Among the 6419 participants, 1672 were excluded because of a history of chronic liver disease (e.g., viral hepatitis, alcoholic or drug-induced liver disease, cholestatic, autoimmune liver disease, or genetic liver disease (n=70)); alcohol consumption ≥ 210 g/week for males and ≥ 140 g/week for females (n=1327); positive serum hepatitis B surface antigen (n=224); known coronary artery disease or stroke (n=22); or insufficient laboratory and clinical data to verify relevant medical history (n=29). The study flowchart is presented in Fig. 1. This study was approved by the Institutional Review Board of the Second Affiliated Hospital, School of Medicine, Zhejiang University (2019-047).

2.2. Measurement of anthropometric and laboratory parameters

For each of the subjects in question, physicians systematically recorded any medical history of hypertension, DM, hypercholesterolemia, coronary artery disease, hepatitis and personal history of smoking and alcohol intake. Each participant underwent a standard physical examination, including measurements of height, body weight and blood pressure. Body weight and height were measured with a SK-CK ultrasonic body scale (SK-CK Corporation, Shenzhen, China); for these measurements, all subjects were required to stand barefoot and wear light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood pressure was measured with an Omron

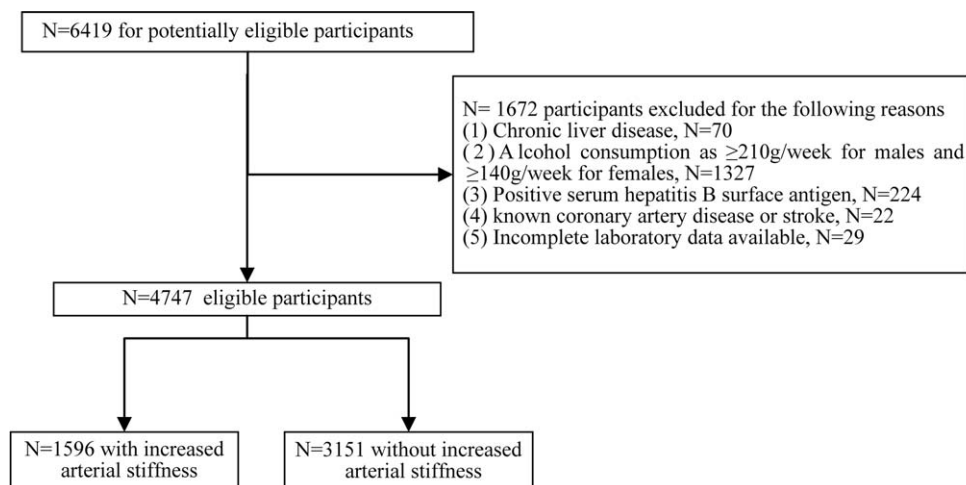


Figure 1. Study flowchart.

HBP-9020 automatic electronic sphygmomanometer (Omron Corporation, Shanghai, China) after subjects rested in a sitting for at least 10 minutes.

All blood samples were extracted from the antecubital vein between 7:00 and 9:30 A.M. after overnight fasting. An Olympus AU4500 automatic chemistry analyzer (Olympus Corporation, Tokyo, Japan) was used to measure total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), fasting plasma glucose (FPG), alanine aminotransferase (ALT) and aminotransferase (AST). Glycated hemoglobin (HbA1c) was determined with an Arkray HA8160 automatic analyzer (Arkray Corporation, Kyoto, Japan) within a reference value range of 4.4% to 6.4%.

Hypertensive patients were defined as those with a blood pressure $\geq 140/90$ mm Hg or those taking antihypertensive medications. Subjects with an FPG ≥ 126 mg/dl, HbA1c $\geq 6.5\%$, or a previous diagnosis of diabetes were defined as DM. In addition, subjects taking lipid-lowering medications or with TC ≥ 240 mg/dl or LDL-C ≥ 160 mg/dl were defined as hypercholesterolemic. Overweight was defined as a BMI of ≥ 25 kg/m² according to World Health Organization.^[15]

2.3. FibroScan CAP and LSM

CAP and LSM assessments were conducted on a FibroScan 502 (Echosens, Paris, France) with an M probe, following the manufacturers instruction, and carried out by 2 experienced operators blinded to the subjects clinical histories. All participants fasted for at least 2 hour before receiving the exam. The FibroScan test was performed on the right lobe of the liver via the intercostal spaces with the patient in the dorsal decubitus position; each examination required at least 10 valid measurements at the identical spot, and a success rate $\geq 60\%$. Both CAP and LSM results were computed in a single measurement. The final results were reported as the median of all valid measurements with the corresponding interquartile range (IQR). A reliable result was defined according to the criteria proposed previously for M probe examination: either an LSM < 7.1 kPa or IQR/median ≤ 0.3 if LSM exceeded 7.1 kPa.^[16]

As a working definition, the CAP levels used to define the presence and degree of hepatic steatosis according to a previous report by Sasso et al^[12] were as follows: 1/ < 238 dB/m, no steatosis (S0); 2/ 238–260 dB/m, mild steatosis (S1); 3/ ≥ 260 dB/m, moderate-severe steatosis (S2–3). In addition, significant fibrosis, measured as LSM, was defined using a cutoff value of 7.3 kPa derived from Ferraioli et al.^[17] A novel NASH prediction model, “CLA score”, combining 3 independent predictors (namely, CAP, LSM, and ALT) has recently been developed.^[18] Using CLA scores, patients with NAFLD were grouped into 3 categories of different risk for NASH: score of 0, low risk; 1 to 2, intermediate risk; and 3 to 4, high risk.

2.4. BaPWV Measurements

BaPWV was measured with an automatic waveform analyzer (BP-203RPE; Colin Medical Technology, Komaki, Japan), as described previously.^[19] The analyzer simultaneously recorded volume pulse form, arterial blood pressure electrocardiogram, and phonocardiogram at both the left and right brachial arteries and ankles. The baPWV was calculated by time-phase analysis between brachia and by volume waveforms at the ankle. The

distance between the brachium and ankle was assessed based on body height. The average value of baPWV from all subjects was used for final analysis. BaPWV values of ≥ 1.4 m/second were adopted to define increased arterial stiffness.

2.5. Statistical analysis

All statistical analyses were conducted with the SPSS software package (Version 21, SPSS Inc, Chicago, IL). Continuous variables are expressed as means \pm standard deviation, whereas categorical variables are presented as frequencies with percentage (%). Independent 2-sample unpaired t-tests were performed to compare continuous parameters, while Chi-Squared tests were conducted to compare categorical variables. Univariate and multivariate logistic regression analyses were conducted to analyze the associations between FibroScan findings and increased arterial stiffness. Multivariate models included adjustments for age, sex, overweight, DM, hypertension, hypercholesterolemia, and current smoking activity; these risk factors were selected according to their clinical and statistical significance. When the study population was stratified according to hypertension or DM, the covariates in the multivariable model included known risk factors excluding the stratified variable. All statistical tests were 2-sided, and $P < .05$ was considered statistically significant.

3. Results

3.1. Study population characteristics

Baseline demographics for participants with or without increased arterial stiffness are listed in Table 1. Among the 4747 participants of this study, 58.4% were male, the average age was 48 years and the prevalence of increased arterial stiffness was 33.6%. Individuals with increased arterial stiffness had higher age, SBP, DBP, and BMI; higher rates of hypertension, hypercholesterolemia and DM; and higher levels of TG, TC, LDL-C, AST, ALT, FPG,

Table 1
Characteristics of participants.

| Characteristics | Increased arterial stiffness (n = 1596) | Controls (n = 3151) | P value |
|-----------------------------|---|---------------------|---------|
| Age, years | 54.44 \pm 9.87 | 45.09 \pm 8.72 | <.001 |
| Male, n (%) | 1001 (62.7) | 1773 (56.3) | <.001 |
| SBP, mm Hg | 133.65 \pm 16.82 | 115.45 \pm 13.76 | <.001 |
| DBP, mm Hg | 79.37 \pm 11.24 | 69.59 \pm 10.14 | <.001 |
| BMI, kg/m ² | 24.70 \pm 3.08 | 23.79 \pm 3.13 | <.001 |
| Total cholesterol, mmol/L | 5.23 \pm 1.07 | 5.04 \pm 0.94 | <.001 |
| Triglyceride, mmol/L | 1.98 \pm 1.51 | 1.65 \pm 1.33 | <.001 |
| HDL cholesterol, mmol/L | 1.27 \pm 0.31 | 1.31 \pm 0.32 | <.001 |
| LDL cholesterol, mmol/L | 2.90 \pm 0.79 | 2.79 \pm 0.71 | <.001 |
| FPG, mmol/L | 5.71 \pm 1.70 | 5.07 \pm 0.76 | <.001 |
| HbA1c, % | 6.20 \pm 1.05 | 5.75 \pm 0.54 | <.001 |
| ALT, U/L | 28.58 \pm 20.68 | 24.88 \pm 18.67 | <.001 |
| AST, U/L | 26.39 \pm 10.90 | 24.06 \pm 11.49 | <.001 |
| Overweight, n (%) | 698 (43.7) | 1011 (32.1) | <.001 |
| Hypertension, n (%) | 829 (51.9) | 366 (11.6) | <.001 |
| Diabetes mellitus, n (%) | 380 (23.8) | 199 (6.3) | <.001 |
| Hypercholesterolemia, n (%) | 262 (16.4) | 361 (11.5) | <.001 |
| Current smoking, n (%) | 333 (20.9) | 655 (20.8) | .950 |

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, DBP = diastolic blood pressure, FPG = fasting plasma glucose, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein, LDL = low-density lipoprotein, SBP = systolic blood pressure.

and HbA1c, and lower HDL-C than controls (all $P < .001$). Only current smoking showed no significance difference between groups.

3.2. FibroScan findings

FibroScan findings according to the presence of increased arterial stiffness are listed in Table 2. Individuals with increased arterial stiffness showed a higher CAP compared to that of normal individuals (258.42 ± 49.64 dB/m vs 240.41 ± 50.17 dB/m, $P < .001$). Among the 4747 study participants, 2557 (53.9%) were diagnosed as NAFLD, with a prevalence of NAFLD higher in the increased arterial stiffness group than in the control group (64.7% vs 48.4%, $P < .001$). Individuals with increased arterial stiffness also showed a higher prevalence of moderate-to-severe NAFLD than those without increased arterial stiffness (46.4% vs 33.0%, $P < .001$). NAFLD patients with increased arterial stiffness also showed a higher LSM compared to NAFLD patients without increased arterial stiffness (5.62 ± 2.61 kPa vs 5.03 ± 1.72 kPa, $P < .001$). Two hundred sixty five (10.4%) NAFLD patients were categorized as significant fibrosis, a condition whose incidence was also significantly higher in the increased arterial stiffness group than in the control group (14.5% vs 7.5%, $P < .001$). The proportions of increased arterial stiffness in low, intermediate and high risk groups for NASH in NAFLD were 14.2%, 78.8%, and 7.0%, respectively, 2 of which were higher than those in the control group ($P = .049$). The prevalence of increased arterial stiffness progressively increased across CAP quartiles and LSM quartiles in NAFLD (23.5%, 30.8%, 38.3%, 43.7%, $P < .001$ and 33.1%, 36.8%, 40.4%, 48.2%, $P < .001$, respectively, as shown in Fig. 2).

3.3. Association between NAFLD, significant fibrosis, NASH risk, and increased arterial stiffness

The associations between NAFLD, significant fibrosis, NASH risk, and increased arterial stiffness are presented in Table 3. After conventional cardiovascular risk factors were adjusted (namely, age, sex, overweight, DM, hypertension, hypercholesterolemia, and current smoking), the results of multivariate logistic regression analysis revealed that CAP (odd ratio [OR]=1.005; 95% confidence interval [CI]: 1.003–1.006; $P < .001$), NAFLD (OR=1.427; 95% CI: 1.212–1.681; $P < .001$), LSM in NAFLD (OR=1.073; 95% CI: 1.023–1.125; $P = .003$), and significant fibrosis in

Table 2

FibroScan findings according to the presence of increased arterial stiffness.

| Characteristics | Increased arterial stiffness (n=1596) | Controls (n=3151) | P value |
|--------------------------|---------------------------------------|-------------------|---------|
| CAP, dB/m | 258.42 ± 49.64 | 240.41 ± 50.17 | <.001 |
| CAP ≥ 238, n (%) | 1032 (64.7) | 1525 (48.4) | <.001 |
| CAP ≥ 260, n (%) | 741 (46.4) | 1039 (33.0) | <.001 |
| In NAFLD | | | |
| LSM, kPa | 5.62 ± 2.61 | 5.03 ± 1.72 | <.001 |
| LSM ≥ 7.3, n (%) | 150 (14.5) | 115 (7.5) | <.001 |
| CLA score | | | .049 |
| Low risk, n (%) | 147 (14.2) | 273 (17.9) | |
| Intermediate risk, n (%) | 813 (78.8) | 1147 (75.2) | |
| High risk, n (%) | 72 (7.0) | 105 (6.9) | |

CAP = controlled attenuation parameter, LSM = liver stiffness measurement, NAFLD = nonalcoholic fatty liver disease.

NAFLD (OR=1.480; 95% CI: 1.090–2.010; $P = .012$) were independently associated with increased arterial stiffness. Compared with low risk of NASH, high risk of NASH showed strong correlations with increased arterial stiffness in NAFLD (OR=1.735; 95% CI: 1.122–2.684; $P = .013$) after adjustment for the above-mentioned risk factors. Furthermore, in multivariate logistic regression analysis, a graded independent correlation was identified between the prevalence of risk for increased arterial stiffness and higher levels of CAP and LSM, respectively. OR (95% CI) for the maximal vs. the minimal quartile of CAP was 1.602 (1.268–2.024), and that of LSM in NAFLD was 1.362 (1.034–1.792) after adjustment for the mentioned risk factors (Fig. 3).

3.4. Significance of FibroScan stratified as hypertension or DM

Since hypertension and DM are generally known to be significant risk factors for CVD, the study population was stratified according to these 2 risk factors, and the relationship between NAFLD, significant fibrosis in NAFLD and increased arterial stiffness were re-evaluated (Fig. 4). Note that NAFLD showed a significant association with increased arterial stiffness in subjects without hypertension (OR=1.601, 95% CI: 1.320–1.941, $P < .001$) or DM (OR=1.464, 95% CI: 1.229–1.744, $P < .001$), but not in subjects with each of these diseases after adjustment for cardiovascular risk factors. Moreover, significant fibrosis in

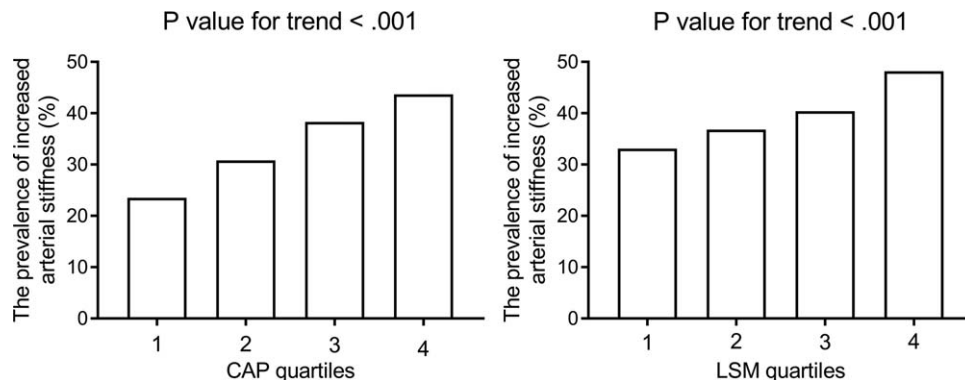


Figure 2. The prevalence of increased arterial stiffness according to quartiles of CAP and quartiles of LSM in NAFLD. CAP = controlled attenuation parameter, LSM = liver stiffness measurement, NAFLD = nonalcoholic fatty liver disease.

Table 3
Association between FibroScan findings and increased arterial stiffness.

| Variables | Univariate | | Multivariate | |
|-------------------|---------------------|---------|---------------------|---------|
| | Odds ratio (95% CI) | P value | Odds ratio (95% CI) | P value |
| CAP | 1.007 (1.006–1.008) | <.001 | 1.005 (1.003–1.006) | <.001 |
| CAP < 238 | 1 (reference) | | 1 (reference) | |
| CAP ≥ 238 | 1.951 (1.723–2.209) | <.001 | 1.427 (1.212–1.681) | <.001 |
| In NAFLD | | | | |
| LSM | 1.147 (1.101–1.196) | <.001 | 1.073 (1.023–1.125) | .003 |
| LSM < 7.3 | 1 (reference) | | 1 (reference) | |
| LSM ≥ 7.3 | 2.085 (1.612–2.696) | <.001 | 1.480 (1.090–2.010) | .012 |
| CLA score | | | | |
| Low risk | 1 (reference) | | 1 (reference) | |
| Intermediate risk | 1.316 (1.057–1.640) | .014 | 1.206 (0.935–1.556) | .149 |
| High risk | 1.273 (0.888–1.827) | .189 | 1.735 (1.122–2.684) | .013 |

CAP = controlled attenuation parameter, CI = confidence interval, LSM = liver stiffness measurement, NAFLD = nonalcoholic fatty liver disease.

NAFLD was also noticeably correlated with increased arterial stiffness with no hypertension (OR=1.700, 95% CI: 1.133–2.550, P=.010) or DM (OR=1.533, 95% CI: 1.072–2.193, P=.019); in the presence of each of 2 diseases, however, significant fibrosis in NAFLD showed no association with increased arterial stiffness after adjustment for cardiovascular risk factors.

4. Discussion

In this study, we evaluated the relationships between increased CAP and LSM value (assessed by FibroScan) and increased arterial stiffness (as measured by BaPWV) in apparently healthy

China adults. NAFLD, defined as a CAP ≥238 dB/m, and significant fibrosis in NAFLD, defined as an LSM ≥7.3 kPa, showed significant and independent correlation with increased arterial stiffness, even after adjustment for conventional risk factors of CVD. The feasibility of CAP and LSM was reflected primarily in subjects without hypertension or DM, while in patients with hypertension or DM, CAP, and LSM did not show an obvious association with increased arterial stiffness.

Liver biopsy is the gold standard for diagnosing and quantifying liver steatosis and fibrosis, but its invasiveness limits its value as a routine screening tool, especially in the asymptomatic general population. In recent years, serum diagnostic models have been used to diagnose and evaluate NAFLD or NASH because of the

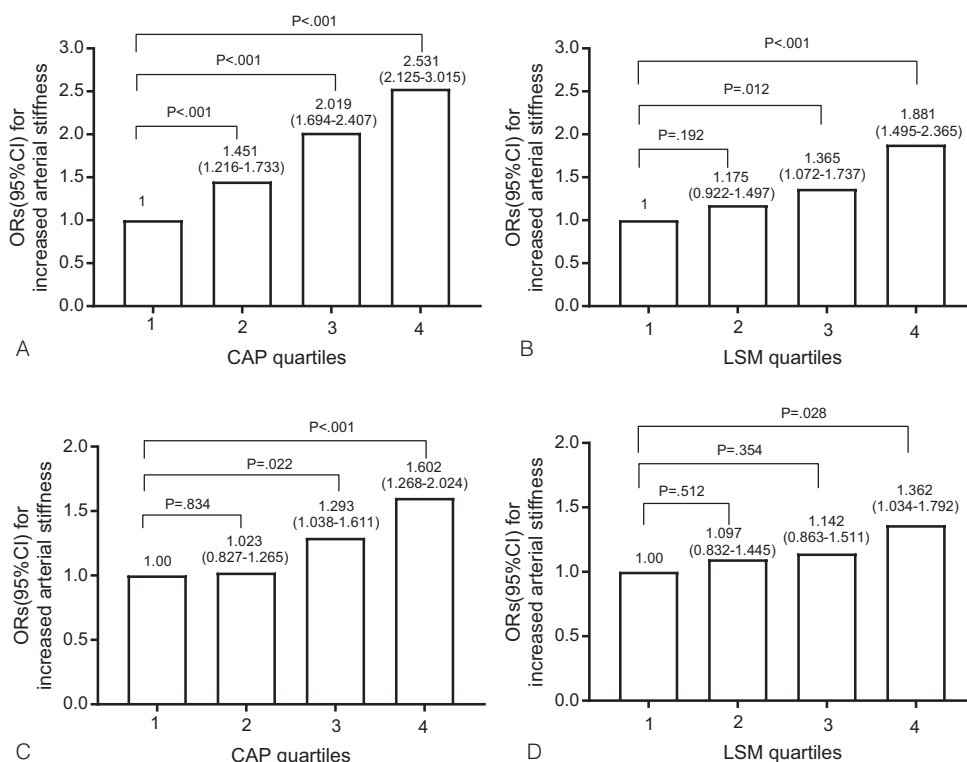


Figure 3. ORs and 95% CI for increased arterial stiffness in CAP quartiles and LSM quartiles in NAFLD using univariate analysis (A and B) and multivariate logistic regression analysis (C and D). CAP = controlled attenuation parameter, CI = confidence interval, LSM = liver stiffness measurement, NAFLD = nonalcoholic fatty liver disease, ORs = odds ratio.

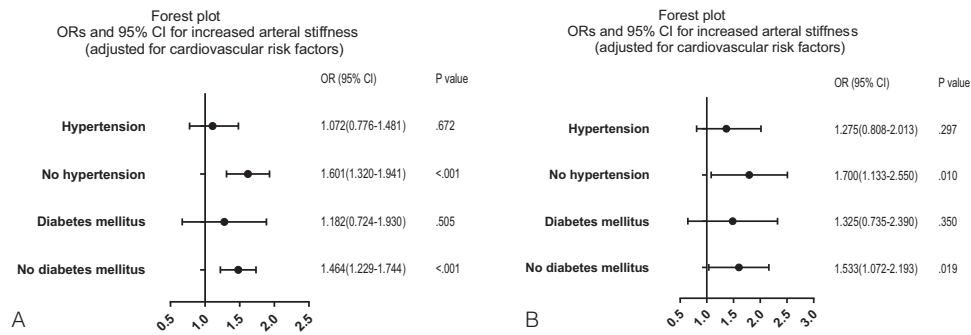


Figure 4. Forest plot of ORs with 95% CI for association of increased arterial stiffness with NAFLD (A) and significant fibrosis in NAFLD (B), stratified by diabetes mellitus or hypertension. CI = confidence interval, NAFLD = nonalcoholic fatty liver disease, ORs = odds ratios.

advantages of non-invasive, simple operation, low cost, and strong repeatability. The non-invasive diagnostic models of NAFLD mainly include fatty liver index, liver fat index, ZJU index and SteatoTest; but these models cannot quantify steatosis, nor can they effectively distinguish mild steatosis from moderate-severe steatosis.^[20–23] The NASH non-invasive diagnostic models main include NASH Test, NASH ClinLiMet score, and cytokeratin 18, but the diagnostic value of these tests needs to be further verified, and are not yet ready for clinical application.^[24–26] Recently, CAP and LSM, implemented on FibroScan have been introduced for noninvasive, quantitative evaluation of both steatosis and fibrosis, and have been widely applied in routine clinical practice. CAP is reported to be highly sensitive in detecting low grade steatosis as fat deposition >10%, which may identify either low-grade and moderate-to-severe steatosis; but this assay has limitations in distinguishing between moderate and severe fatty liver.^[12,27] Notably, LSM with transient elastography is considered and accurate assessment of the severity of hepatic fibrosis in patients with NAFLD.^[13] CLA score, a novel NASH prediction model, allows risk stratification of NASH with an acceptable performance (area under the receiver operating characteristic curve of 0.812).^[18] Thus, CAP and LSM based on transient elastography combined with ALT were used to assess the 3 stages of NAFLD (namely, steatosis, steatohepatitis, and fibrosis) and to comprehensively investigate the correlation of NAFLD with increased arterial stiffness.

Regarded as a reliable marker of arterial structural and functional alterations, arterial stiffness has been associated with numerous diseases after in numerous epidemiological studies. In CVDs, for instance, arterial stiffness has been classified as an emerging risk factor for the development of coronary artery disease and clinical hypertension.^[28,29] Arterial stiffness has also been demonstrated as a risk factor for development of fatal and nonfatal cardiovascular events.^[28] In terms of metabolic diseases, arterial stiffness has been shown to be associated with diabetes.^[30–32] Recently, a prospective cohort study including 2450 subjects with a follow-up of 4.43 years showed that increased arterial stiffness was an early marker for risk of developing diabetes.^[33] Our previous research found that arterial stiffness as measured by baPWV is associated with NAFLD in non-obese, non-hypertensive, and non-diabetic young and middle-aged population.^[19] Additionally, a systematic review has shown that arterial stiffness is associated with decreased cognitive function and cerebral small vessel disease.^[34] Therefore, early identification of risk factors for increased arterial stiffness is crucial in clinical work.

In the present study, CAP and NAFLD were found to be independent risk factors for increased arterial stiffness after adjustment for cardiovascular risk factors. Our findings are comparable to those of recent studies evaluating the relationships between liver steatosis and arterial stiffness. In a cross-sectional study, for example, Park et al. reported that CAP ≥ 222 dB/m shows an independent correlation with increased arterial stiffness measured, as measured by cardio-ankle vascular index.^[35] Harada et al. suggested a relationship between NAFLD and aortic stiffness, as measured with carotid-femoral pulse wave velocity, independent of abdominal obesity.^[36] The study of Zheng et al revealed that NAFLD is strongly correlated with subclinical atherosclerosis, as measured by carotid intima-media thickness (CIMT) and baPWV.^[37] In a prospective study, Li et al found that NAFLD is an independent predictor of faster progression of baPWV even after adjustment for other cardiovascular risk factors.^[38] Meta-analysis further suggested that NAFLD is tightly correlated with subclinical atherosclerosis that measured by 4 diverse indices.^[39]

In this study, we also found that LSM and significant fibrosis in NAFLD were independent risk factors for increased arterial stiffness after adjustment for cardiovascular risk factors. The results of this study were consistent with those of other recent studies. A community-based, cross-sectional study has shown that advanced fibrosis indicated by NAFLD fibrosis score is positively and independently associated with arterial stiffness measured with baPWV in the NAFLD patients.^[40] As revealed from a case-control study with 125 patients with biopsy-proven NAFLD, the severity of arterial and liver stiffness increases in parallel in NAFLD patients.^[41] A prospective study with a mean follow-up of 26.4 years showed that fibrosis stage is the strongest predictor for CVD mortality in NAFLD.^[3] In contrast with our study findings, a retrospective cohort study of 515 asymptomatic patients in South Korea showed that LSM values are not critical determinants of increased arterial stiffness.^[35] However, a large proportion of subjects were recruited in our study. This difference in patient number may account for the different results.

Atherosclerosis is usually considered a chronic inflammatory disease as inflammation is critical to all stages of the atherosclerotic process.^[42,43] NAFLD has also been considered a chronic low-grade systemic inflammation disease. NASH refers to the progressive inflammatory state of NAFLD, which significantly stimulates the development of liver fibrosis in NAFLD and largely determines risk stratification. This study found that the risk of increased arterial stiffness is elevated stepwise with a rise in NASH

risk up to an OR of 1.632(1.127–2.362) ($P=.009$) after adjustment for cardiovascular risk factors, suggesting that NASH may have a significant independent association with increased arterial stiffness. We speculate that inflammation may be the common pathogenic mechanism for both of these diseases.

It is notable that the relationships between NAFLD and significant fibrosis in NAFLD with increased arterial stiffness exist only in subjects without hypertension or DM, findings that are consistent with those of previous studies. A retrospective study of 4860 subjects, excluding known diabetes patients, found that an association between NAFLD and arterial stiffness is apparent only in subjects with normal glucose tolerance.^[44] In a cohort enriched for T2DM, McKimmie et al reported no significant associations between hepatic steatosis measured by computed tomography and CIMT.^[45] An observational, cross-sectional study with 68 patients newly diagnosed with essential hypertension found that NAFLD is not accompanied by increased arterial stiffness in essential hypertensive patients.^[46] Though there has been no clear mechanism to explain our results, we speculate that our result might be due to the significant effect of these 2 diseases on arterial stiffness. The fact that most subjects with these 2 diseases have some degree of NAFLD and arterial stiffness, reduces the statistical power of tests to evaluate the association between NAFLD and increased arterial stiffness.^[47]

In this study, we found that CAP-defined NAFLD, LSM-defined significant fibrosis and CLA score-defined NASH risk show significant correlations with increased arterial stiffness, which may more effectively stratify the relative risk of patients having undergone screening assessment for CVD. In clinical practice, if asymptomatic wellness check-up subjects without hypertension or DM, meet the diagnostic criteria of NAFLD, they are considered to be at increased risk for CVD. Lifestyle intervention should be recommended for these patients. Furthermore, diagnosis of significant fibrosis or high NASH risk in NAFLD patients suggests higher risks for CVD. Lifestyle intervention and further examination, such as artery ultrasound, might be indicated for these patients.

This study has several limitations. First, though CAP and LSM are capable of differentiating liver steatosis and fibrosis, there are no ideal CAP and LSM cut-off values to define liver steatosis and fibrosis and the definition of NAFLD based on CAP has not yet been clearly established in asymptomatic, healthy population. However, this study was focused on early detection of NAFLD with CAP and identification of significant fibrosis with LSM, rather than determining whether the CAP values and LSM values in potentially at-risk patients with increased arterial stiffness are accurate. Second, liver biopsy data were not obtained from our study cohort, although it remains the only definitive method for diagnosis of NASH. For this reason, the risk stratification of NASH using the CLA score in our study cohort is only exploratory. We could not confirm the diagnosis of NASH histologically, because our study population only included subjects with no liver-associated symptoms; but this cohort is worth following longitudinally to evaluate development of NASH-related complications. Third, because this study was a cross-sectional observational study, a causal relationship between arterial stiffness and the presence of NAFLD and significant fibrosis in NAFLD could not be definitively determined. Future longitudinal studies are required to review this association to ascertain causality. Lastly, the study subjects, all of whom were visitors to a check-up center in a single hospital, are

not necessarily representative of the overall general population, and our results should be rigorously interpreted, or confirmed with additional studies, before being extrapolated to that general population.

5. Conclusion

CAP and LSM values based on FibroScan are feasible methods for determination of increased arterial stiffness. The presence of CAP-defined NAFLD and LSM-defined significant fibrosis in NAFLD shows an apparent correlation with increased arterial stiffness independent of known cardiovascular risk factors. The above associations are especially robust in subjects without hypertension or DM. For this reason, early detection of NAFLD and rigorous monitoring by FibroScan in high-risk individuals should be highlighted in efforts to reduce morbidity and mortality related to NAFLD. Furthermore, in those subjects with NAFLD without hypertension or DM, the development and progression of CVD and related comorbidities require active screening and management.

Acknowledgment

The authors thank Kun Chen for his advice on statistics throughout the study.

Author contributions

Conceptualization: Zhen ya Song.

Data curation: Yu ling Tong, Ling yan Wu.

Formal analysis: Xin yan Yu, Xiao xiao Song.

Investigation: Xin yan Yu, Yu ling Tong, Ling yan Wu.

Methodology: Xin yan Yu, Xiao xiao Song.

Project administration: Zhen ya Song.

Resources: Xin yan Yu, Zhen ya Song.

Software: Xiao xiao Song.

Supervision: Zhen ya Song.

Validation: Yu ling Tong, Ling yan Wu.

Writing – original draft: Xin yan Yu.

Writing – review & editing: Zhen ya Song.

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