Prevalence of Subclinical Carotid Atherosclerosis and Associated Risk Factors in Individuals Without Cardiovascular Risk Factors: Insights from General Population

Yuanmeng Tian
First Hospital of China Medical University

Suli Zhang
Central Hospital of Chao Yang City

Ru Li
First Hospital of China Medical University

Weishuang Xue
First Hospital of China Medical University

Lijing Xing
Liaoning Provincial Center for Disease Control and Prevention

Liming Zhang
First Hospital of China Medical University

Li Jing
Liaoning Provincial Center for Disease Control and Prevention

Jun Yang
First Hospital of China Medical University

Chunyan Ma
First Hospital of China Medical University

Shuang Liu (liushuang_cmu1h@163.com)
First Hospital of China Medical University

Guangxiao Li
First Hospital of China Medical University

Research Article

Keywords: atherosclerosis, carotid arteries, cardiovascular disease, risk factors, prevalence

DOI: https://doi.org/10.21203/rs.3.rs-137958/v1
Abstract

Objective: Individuals without conventional cardiovascular risks (CVRFs) still have the risk of adverse outcomes, and subclinical carotid atherosclerosis is a known predictor of cardiovascular events. We aimed to assess the prevalence and associated risk factors of subclinical carotid atherosclerosis in CVRF-free population.

Methods: The cross-sectional study was conducted in rural northeast China in 2017-2018. CVRFs freedom was defined as untreated blood pressure < 140/90 mmHg, fasting plasma glucose < 7.0 mmol/L, untreated total cholesterol < 6.22 mmol/L, low-density lipoprotein cholesterol < 4.14 mmol/L, high-density lipoprotein cholesterol (HDL-C) $\geq$ 1.04 mmol/L, and no current smoking. This subgroup population included 1449 individuals, and ultrasound was used to detect carotid atherosclerosis.

Results: The mean carotid intima-media thickness is 0.74 ± 0.14 mm. The prevalence of carotid plaque is 23.4% (95%CI: 21.2%-25.6%) among CVRFs-free population, significantly higher in men than in women (37.1% vs 20.0%, p<0.001), and rises steeply with advancing age. 1.31% have moderate-to-severe carotid stenosis. Advancing age, man, glycosylated hemoglobin (OR, 1.90; 95% CI, 1.20-1.32), HDL-C level (OR, 2.31; 95% CI, 1.75-3.04), and pulse pressure (OR, 1.03; 95% CI, 1.01-1.05) are potentially related to presence of carotid atherosclerosis. Adjusted-dose-response association shows a linear relationship between HDL-C and prevalence of carotid atherosclerosis.

Conclusions: The prevalence of subclinical carotid atherosclerosis in CVRF-free population was relatively high, indicating poorly defined factors might contribute to the early atherogenesis. Moreover, we observed a paradoxical response between subclinical carotid atherosclerosis and HDL-C levels, suggesting that treatment targeting to increase HDL-C levels might not reduce future cardiovascular risks.

Background

Asymptomatic individuals without traditional cardiovascular risk factors (CVRFs) are considered to have low risk of cardiovascular events, and preventive strategies in healthy individuals without conventional cardiovascular risk factors are usually not recommended according to current preventive guideline. However, previous studies consistently reported that nearly half individuals were reclassified as intermediate or high risk according to subclinical atherosclerosis detection. Individuals with unrecognized atherosclerosis continue to occur cardiovascular events, especially in young adults and women, because they were misclassified into low-risk population by conventional risk factors, and not receiving risk-reducing preventive therapies.

Moreover, high incidence of mortality in asymptomatic individuals free of CVRFs was confirmed previously. The CAFES-CAVE study showed that in asymptomatic individuals without CVRFs, the annual events rate was up to 3%-5%, on the contrary, the annual events rate was only 0.1% in the population without plaque. The mismatch between low conventional CVRFs, the presence of
subclinical atherosclerosis and future cardiovascular events, further warranted subclinical atherosclerosis
detection in a population absent from conventional CVRFs.\(^7\)

Carotid ultrasound is a non-invasive and effective imaging for modality of assessing subclinical
atherosclerosis.\(^8\) However, current statements recommended against screening for asymptomatic carotid
artery stenosis in general population in view to offer carotid surgery, they failed to address asymptomatic
carotid atherosclerosis as a marker of cardiovascular adverse outcomes.\(^9,10\) Early detection of the
underlying atherosclerosis before symptoms manifestation could improve risk stratification and provide a
great opportunity to reduce future cardiovascular risks, in light of many effective nonpharmacologic and
pharmacologic interventions.\(^2\) Therefore, in the present study, we aimed to determine the prevalence of
subclinical carotid atherosclerosis and potential related risk factors in the population free of major
CVRFs, and to provide reliable data for formulating effective strategies to reduce cardiovascular risks in
those individuals.

Methods

This present study derived from a cross-sectional survey conducted in rural areas of northeast China
from September 2017 to May 2018. A multi-stage, geologically stratified and cluster random sampling
method was employed to ensure the samples were representative. A total of 13 villages were randomly
selected from 2 counties (Chaoyang and Lingyuan) of Liaoning province. All permanent residents aged
more than 40 years old (n=6830) were recognized as eligible participants. Participants with pregnancy,
cancer or mental disorders were excluded from the study. We finally enrolled 5838 (response rate: 85.5%)
individuals into further analyses. The study protocol was approved by the central ethics committee of
China National Center for Cardiovascular Disease. Written consents were obtained from all subjects. If
the subjects were disabled, their proxies provided written informed consents for them.

The methods of data collection have been described previously.\(^11\) According to Adult Treatment Panel III
CVRF definitions,\(^12\) CVRF-free population was defined as follows: (1) no current smoking, (2) untreated
systolic blood pressure (SBP) < 140 mm Hg and diastolic blood pressure < 90 mmHg, (3) untreated
fasting plasma glucose (FPG) < 7.0 mmol/L (126 mg/dl), (4) untreated total cholesterol (TC) < 6.22
mmol/L (240 mg/dl), low-density lipoprotein cholesterol (LDL-C) < 4.14 mmol/L (160 mg/dl), and high-
density lipoprotein cholesterol (HDL-C) \(\geq\) 1.04 mmol/L (40 mg/dl).\(^7\) This subgroup population included
1449 (24.8%) individuals without CVRFs (Figure 1).

Ultrasound Protocol

All scans were performed using a high-resolution B-mode ultrasonography (Mindray M7, Shenzhen,
China) with a broadband 7L4S linear array transducer. Carotid ultrasound was performed by a certified
sonographer with >3 years of experience in vascular ultrasound imaging. All subjects were examined in
prone position with both left and right carotids scanned. A transverse scan starting at the clavicle and
moving cranially up to the mandible was performed for orientation firstly, thereby longitudinal images of carotid artery as well as plaque was obtained subsequently.

Plaque was displayed both in short-axis view and long-axis sweep, color and pulsed Doppler imaging were obtained if necessary. The highest peak systolic and end-diastolic velocity measurements from the common carotid artery and the internal carotid artery were obtained to confirm the degree of carotid stenosis. The Doppler velocity thresholds were used to determine the degree of stenosis according to the criteria that were previously described. Moderate carotid stenosis was defined as the degree of carotid artery stenosis between 50%-69%, and severe carotid stenosis was defined as the degree of carotid stenosis ≥70%. Carotid atherosclerosis was defined as mean carotid intima-media thickness (CIMT) ≥1.0mm and/or presence of carotid plaque. For each participant, CIMT was measured within a region free of plaque from a common carotid artery video acquisition according to Mannheim consensus. 3 mean CIMT measurements were taken on each artery (left/right side), and CIMT measurements from both sides were averaged to create mean CIMT. The presence of plaques was assessed in the common carotid artery, bulb, and internal carotid artery segments, defined as a focal thickness of CIMT ≥1.5mm, or focal intraluminal protrusion >50% of the surrounding CIMT according to Mannheim carotid intima-media thickness and plaque consensus.

**Statistical Analysis**

Descriptive statistics were calculated for all variables. Continuous variables with normal distribution are reported as means and standard deviations. Otherwise, continuous variables are reported as medians and interquartile ranges. Differences between groups were compared using a $\chi^2$ test for categorical variables. Univariate and stepwise multivariate logistic regression analyses were performed to evaluate the association between parameters and subclinical carotid atherosclerosis. Statistical analyses were conducted by using SPSS22.0 (SPSS Inc., Chicago, IL, USA).

We employed the restricted cubic spline function of linear model to characterize C-R relationships between potential risk factors and subclinical carotid atherosclerosis. Concentration-response relationships (C-R relationships) were analyzed using the SAS 9.4 software (SAS, Institute, Cary, NC).

CIMT was examined twice after a 1-week interval in a blinded manner. Variability was determined by calculating coefficients of variation, which was calculated as the standard deviation of differences. Paired $t$ test was employed to test the difference between the 2 measurements. $P$ values <0.05 were considered statistically significant.

**Results**
Characteristics of the CVRFs-free population

The characteristics of the CVRFs-free individuals are shown in Table 1. The 1449 participants include 283 men (19.5%) and 1166 women (80.5%), whose average age is 54.7 ± 9.6 years. 52.4% have a primary school education or less, 27.0% have an annual income less than 5000 yuan (approximately $ 700). The plasma HbA1c is 5.32 ± 0.33%, and the plasma HDL-C is 2.24 ± 0.57 mmol/L.
| Characteristics                                      | Male   | Female | Total  |
|------------------------------------------------------|--------|--------|--------|
| **Participant, n(%)**                                | 283(19.5) | 1166(80.5) | 1449(100.0) |
| **Mean age, years**                                  | 58.2 ± 10.3 | 53.8 ± 9.3 | 54.7 ± 9.6 |
| 40–49                                                | 23.3 | 38.8 | 35.7 |
| 50–59                                                | 29.0 | 33.4 | 32.5 |
| 60–69                                                | 33.9 | 21.8 | 24.2 |
| 70–79                                                | 11.3 | 4.9 | 6.1 |
| >=80                                                 | 2.5 | 1.2 | 1.4 |
| **Education**                                        |        |        |        |
| Primary school or lower                              | 37.5 | 56.1 | 52.4 |
| Middle school                                        | 53.4 | 39.5 | 42.2 |
| High school or above                                 | 9.2 | 4.4 | 5.3 |
| **Incoming, Yuan**                                   |        |        |        |
| < 5000                                               | 29.3 | 26.4 | 27.0 |
| 5000–9999                                            | 23.0 | 23.6 | 23.5 |
| 10000–19999                                          | 20.5 | 23.8 | 23.1 |
| >=20000                                              | 27.2 | 26.2 | 26.4 |
| **Current drinking**                                 |        |        |        |
|                                                      | 49.1 | 13.4 | 20.4 |
| **Lack of Exercise**                                 | 12.7 | 11.2 | 11.5 |
| **Body mass index, kg/m²**                           |        |        |        |
|                                                      | 23.69 ± 3.33 | 24.27 ± 3.37 | 24.16 ± 3.37 |
| **Waist, cm**                                        |        |        |        |
|                                                      | 81.50 ± 8.88 | 79.85 ± 9.24 | 80.17 ± 9.19 |
| **Hipline, cm**                                      |        |        |        |
|                                                      | 92.46 ± 6.20 | 92.94 ± 6.67 | 92.85 ± 6.58 |
| **Systolic blood pressure, mmHg**                    |        |        |        |
|                                                      | 123.66 ± 9.81 | 121.03 ± 11.12 | 121.54 ± 10.92 |
| **Diastolic blood pressure, mmHg**                   |        |        |        |
|                                                      | 78.85 ± 6.46 | 77.91 ± 6.68 | 78.09 ± 6.65 |
| **Fasting plasma glucose, mol/L**                    |        |        |        |
|                                                      | 5.51 ± 0.59 | 5.39 ± 0.56 | 5.41 ± 0.57 |
| **Glycosylated hemoglobin,%**                        |        |        |        |
|                                                      | 5.28 ± 0.34 | 5.34 ± 0.33 | 5.32 ± 0.33 |
| **Total cholesterol, mol/L**                         |        |        |        |
|                                                      | 4.54 ± 0.73 | 4.65 ± 0.75 | 4.63 ± 0.75 |
**Prevalence of subclinical carotid atherosclerosis in CVRF-free population**

Overall, among the 1449 participants without conventional CVRFs adults aged ≥ 40 years in rural northeast China, the mean cIMT is 0.74 ± 0.14 (men 0.80 ± 0.16, women 0.73 ± 0.13, respectively). The correlation coefficient between the first and second CIMT measurements is 0.88 (p < 0.05), with no significant difference. The mean difference and standard deviation is 0.02 ± 0.06 mm.

Among conventional CVRFs-free individuals, 339 have subclinical carotid atherosclerosis, including 82 have mean cIMT ≥ 1.0 mm, 316 have carotid plaques, 59 have both. The prevalence of subclinical carotid atherosclerosis is shown in Table 2. The overall prevalence of carotid plaque is 23.2% (95%CI: 21.2%-25.6%), significantly higher in men than in women (37.1% vs 20.0%, p < 0.001). It rises steeply with advancing age, ranging from 5.0% (95%CI: 3.1%-6.9%) among participants 40 to 49 years to 85.7% (95%CI: 69.4%-102.0%) among participants 80 years or older.

### Table 2

| Age group(years) | Male | Female | Total |
|------------------|------|--------|-------|
|                  | No   | Rate   | 95%CI | No   | Rate   | 95%CI | No   | Rate   | 95%CI | p value |
| 40–49            | 8    | 12.1   | 4.0–20.2 | 18   | 4.0   | 2.2–5.8 | 26   | 5.0   | 3.1–6.9 | 0.011 |
| 50–59            | 17   | 20.7   | 11.8–29.7 | 63   | 16.2  | 12.5–19.9 | 80   | 17.0  | 13.6–20.4 | 0.320 |
| 60–69            | 52   | 54.2   | 44.0–64.3 | 103  | 40.6  | 34.5–46.6 | 155  | 44.3  | 39.1–49.5 | 0.022 |
| 70–79            | 21   | 65.6   | 48.2–83.0 | 39   | 68.4  | 56.0–80.9 | 60   | 67.4  | 57.5–77.3 | 0.787 |
| >=80             | 7    | 100.0  | -       | 11   | 78.6  | 54.0–103.2 | 18   | 85.7  | 69.4–102.0 | 0.521 |
| **Total**        | 105  | 37.1   | 31.4–42.8 | 234  | 20.1  | 17.8–22.4 | 339  | 23.4  | 21.2–25.6 | < 0.001 |
Among participants without conventional CVRFs, 19 (1.31%) had more than moderate-to-severe carotid stenosis, including 13 (0.90%) who were diagnosed with moderate carotid stenosis (50–69%), and 6 (0.41%) who have severe carotid stenosis (≥ 70%).

**Related risk factors for prevalence of subclinical carotid atherosclerosis among CVRFs-free population**

According to the results of the multivariate regression model, our results identify that advancing age, man (OR, 1.87; 95% CI, 1.26–2.77), glycosylated hemoglobin (HbA1c) (OR, 1.90; 95% CI, 1.20–1.32), HDL-C level (OR, 2.31; 95% CI, 1.75–3.04), and pulse pressure (OR, 1.03; 95% CI, 1.01–1.05) are risk factors for presence of subclinical carotid atherosclerosis. However, ever smoking does not significantly correlated to the presence of subclinical atherosclerosis.

What is noteworthy is that participants with higher HDL-C and HbA1c levels were likely to have a high prevalence of carotid atherosclerosis in individuals without CVRFs (Table 3). We further chose HDL-C and HbA1c levels that were encoded using restricted cubic spline function with 3 knots, located at the fifth, 50th, 95th percentiles, after adjusted age, sex, current drink, triglyceride, TG, LDL-C, BMI, waist, hipline, lack of exercise, education, and pulse pressure, we found linear relationships between HDL-C and subclinical carotid atherosclerosis. In individuals without conventional CVRFs, the prevalence of subclinical carotid atherosclerosis increased significantly with high levels of HDL-C after adjusting other risk factors (p < 0.05) (Fig. 2).
Table 3
Association of risk factors with subclinical carotid atherosclerosis

|                      | OR    | 95% CI       | P value |
|----------------------|-------|--------------|---------|
| Sex                  |       |              |         |
| Female               | 1.00  | -            |         |
| Male                 | 1.87  | 1.26–2.77    | 0.002   |
| Age Group, Years     |       |              |         |
| 40-                  | 1.00  | -            |         |
| 50-                  | 2.63  | 1.62–4.27    | < 0.001 |
| 60-                  | 7.62  | 4.64–12.52   | < 0.001 |
| 70-                  | 17.29 | 8.80–33.99   | < 0.001 |
| 80-                  | 53.99 | 14.44–201.83 | < 0.001 |
| Incoming, Yuan       |       |              |         |
| < 5000               | 1.00  | -            |         |
| 5000–9999            | 0.64  | 0.43–0.94    | 0.021   |
| 10000–19999          | 0.53  | 0.35–0.80    | 0.003   |
| >=20000              | 0.47  | 0.30–0.74    | 0.001   |
| Waist, cm            | 1.02  | 1.00–1.03    | 0.051   |
| HDL-c, mmol/L        | 2.31  | 1.75–3.04    | < 0.001 |
| HbA1c, mmol/L        | 1.90  | 1.20–3.02    | 0.007   |
| Ever smoking         | 1.56  | 0.95–2.56    | 0.077   |
| Pulse pressure       | 1.03  | 1.01–1.05    | 0.003   |

Adjusted factors: sex, incoming, age, current drinking, ever smoking, education, overweight or obesity, lack of exercise, fasting plasma glucose, waist, hipline, neck circumference, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), pulse pressure, glycosylated hemoglobin (HbA1c).

Discussion

The major findings of the present study are: 1) the prevalence of subclinical carotid atherosclerosis in CVRFs-free individuals is 23.2%, which rises steeply with advanced age; 2) the prevalence of subclinical
carotid atherosclerosis in men is higher than in women; 3) the HDL-C and HbA1c levels are potentially related to high rate of subclinical carotid atherosclerosis. These findings indicate that additional poorly defined factors are likely to contribute to the process of atherogenesis in individuals without conventional CVRFs. Moreover, a significant number of individuals without conventional CVRFs have subclinical carotid atherosclerosis, which indicates that the assessment of subclinical atherosclerosis in this population might provide substantial yield. In addition, the association between HDL-C and the presence of subclinical carotid atherosclerosis is not expected, further suggesting that using pharmacologic inhibitor to increase HDL-C levels might not reduce the rate of cardiovascular events in individuals without conventional CVRFs, and a fundamental assessment of the clinical importance of HDL-C should be warranted.

Atherosclerotic cardiovascular diseases remain to be the major cause of premature death globally. Conventional risk factors for atherosclerosis including smoking, dyslipidemia, hypertension and diabetes are used within the risk factor-based approach to identify high-risk groups, such as Framingham risk scores and pooled cohort equation risk scores. However, only nearly 40% of cardiovascular events occur in those high-risk group, and approximately 40–60% adverse outcomes occur in the low-risk individuals, accounting for 1/3 of the population. Therefore, the validity of conventional risk factors assessment in identifying risk factors in the next 10 years remains controversial. In our study cohort, up to 23.2% of individuals had carotid atherosclerosis, including 1.31% of participants who have moderate-to-severe carotid stenosis, indicating the clinical importance of carotid atherosclerosis screening.

Screening for asymptomatic carotid stenosis was based on the concept that it was possible to prevent stroke by stenting or operating moderate to severe carotid stenosis. However, recent studies reported that carotid operation such as carotid endarterectomy or carotid stent might cause more stroke than it can prevent, which indicated the recommendation against screening for asymptomatic carotid stenosis in view of surgery is now outmoded. In addition, previous study reported that the presence of plaque, no matter the size, was a marker of increased risk of cardiovascular events. Therefore, subclinical atherosclerosis in regard of selecting optimal medical therapy to reduce cardiovascular risks should be recommended. Previous study suggested that carotid ultrasound is a more readily available and reliable imaging modality for detecting early atherosclerosis when compared to CAC scan. Therefore, in the present study, we used ultrasound to evaluate the subclinical atherosclerosis in CVRFs-free population.

LDL-C and HDL-C are crucially involved in the development of atherosclerosis. In contrast to LDL-C promotes the vascular atherosclerotic process, HDL-C was thought to act as a protective agent by preventing endothelial dysfunction. Previous studies reported that low concentration of HDL-C was inversely associated with the risk of adverse cardiovascular outcomes in general population. However, there are growing evidences suggesting that HDL-C might lose its protective properties in certain conditions. Elevated HDL-C levels failed to decrease cardiovascular events and high levels of HDL-C was not correlated with reduced CIMT. Our findings indicated that the inverse relationship between HDL-C and atherosclerosis may not apply to the individuals without CVRFs, further suggested that using
pharmacologic inhibitor of the cholesterol-ester transfer protein to increase HDL-C levels might not reduce
the rate of cardiovascular events in individuals without conventional CVRFs. In addition, PESA study
indicated that in CVRF-free middle-aged individuals, LDL-C was independently correlated with the
presence of atherosclerosis, even in currently considered normal levels.\textsuperscript{7} However, in our population, we
did not find the correlation, possibly because of the different population selection and definition of
atherosclerosis.

Previous study reported that HbA1c was linked to increased CAC and cIMT in 2340 nondiabetic
participants.\textsuperscript{26} In addition, other study reported that HbA1c concentration was independently associated
with cIMT progression and adverse cardiovascular outcomes in nondiabetic prospective series.\textsuperscript{27} In the
present study, we found HbA1c level was independently associated with the presence of subclinical
carotid atherosclerosis, which consistent with previous studies.\textsuperscript{26,27} This finding might be partially
explained by the association between prediabetes and increased cardiovascular risk.

Smoking is an important modifiable risk factor for atherosclerosis, and current smoking and cumulative
exposure are significantly related to cardiovascular risks.\textsuperscript{28} Therefore, smoking cessation is crucially
important in reducing cardiovascular risks. In the present study, we did not find any significant
association between subclinical atherosclerosis and ever smoking, coincident with previous studies,\textsuperscript{28}
which indicated smoking-related damage to the artery is irreversible, and smoking cessation should be
emphasized.

The strengths of the study include its population-based design in Chinese population and a
comprehensive estimation of subclinical carotid atherosclerosis by ultrasound. However, this present
study also had several limitations. Firstly, we can only provide implications for the association between
risk factors and subclinical carotid atherosclerosis, but the causality of this association still needs more
longitudinal studies to validate. Secondly, we use carotid ultrasound as a surrogate of subclinical carotid
atherosclerosis, which may have led to underestimation of atherosclerosis prevalence. However,
atherosclerosis is a systemic disorder, and previous study suggested that screening atherosclerosis by
carotid ultrasound is likely to provide the highest yield to detect atherosclerosis.\textsuperscript{2} Thirdly, our population
originated from the rural areas of northeast China, whether our results are suitable to the population in
different geographic and economic conditions also needs further studies to confirm. Lastly, women
comprised 80.5% of the population, and selection bias was inevitable as women usually had better
access to prevention strategies, and were more likely to obey the health education.\textsuperscript{29}

**Conclusions**

The present study provides insight into the presence of subclinical carotid atherosclerosis and related risk
factors in a representative population in rural northeast China, observing 23.2% had subclinical carotid
atherosclerosis, including 1.31% had moderate-to-severe carotid stenosis, indicating poorly-defined
factors play a role in the early process of atherosclerosis, and early detection of subclinical
atherosclerosis in sight of reducing cardiovascular risks should be recommended. Moreover, the HDL-C
levels are potentially related to a high rate of subclinical carotid atherosclerosis, further indicated that increase HDL-C levels might not reduce the rate of cardiovascular events in individuals without conventional CVRFs.

Abbreviations

CIMT: carotid intima-media thickness; FPG: fasting plasma glucose; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; HbA1c: glycosylated hemoglobin

Declarations

Acknowledgments

We thank neurologists and staffs from central hospital and CDC of Chaoyang, Liaoyang, Dandong and Donggang city in Liaoning province who work hard to ensure the reliability and accuracy of data.

Author’ Contributions

SL was responsible for the concept and design of the study. YT and SZ were responsible for the study coordination and conduct. YT, SZ, and RL contributed to the drafting of the manuscript. WX, LX, GL, LZ, and LJ collected and analyzed the data. JY and CM interpreted the data. All authors read and approved the final manuscript.

Funding

This study was supported by the Department of Science and Technology of Liaoning Province (2018225065) and the National Natural Science Foundation of China (81701699). The funders of this work had no role in the design of the study, data collection, data analysis, or data interpretation. The funders had no role in writing the manuscript.

Availability of data and materials

The datasets generated for and analyzed in the study are not publicly available but are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate
The study was granted approval by the Central Ethics Committee at the China National Center for Cardiovascular Disease (Clinical Research No.[2015]024. Beijing, China.). All methods were performed in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all participants.

**Consent for publication**

Not applicable.

**Competing interests**

None.

**Author details**

1 Department of Cardiology, The First Hospital of China Medical University, Shenyang 110001, People's Republic of China. 2 Department of Chronic Disease, Liaoning Provincial Center for Disease Control and Prevention, Shenyang 110005, People's Republic of China. 3 Department of Cardiovascular Ultrasound, Central Hospital of Chaoyang City, Chaoyang 122000, People's Republic of China. 4 Department of Cardiovascular Ultrasound, The First Hospital of China Medical University, Shenyang 110001, People's Republic of China. 5 Department of Neurology, The First Hospital of China Medical University, Shenyang 110001, People's Republic of China. 6 Department of Medical Record Management Center, The First Hospital of China Medical University, Shenyang 110001, People's Republic of China.

**References**

1. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, Himmelfarb CD, Khera A, Lloyd-Jones D, McEvoy JW, Michos ED, Miedema MD, Munoz D, Smith SC, Jr., Virani SS, Williams KA, Sr., Yeboah J, Ziaeian B. 2019 acc/aha guideline on the primary prevention of cardiovascular disease: A report of the american college of cardiology/american heart association task force on clinical practice guidelines. *Circulation*. 2019;140:e596-e646

2. Naqvi TZ, Mendoza F, Rafii F, Gransar H, Guerra M, Lepor N, Berman DS, Shah PK. High prevalence of ultrasound detected carotid atherosclerosis in subjects with low framingham risk score: Potential implications for screening for subclinical atherosclerosis. *J Am Soc Echocardiogr*. 2010;23:809-815

3. Peters SA, den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: A systematic review. *Heart*. 2012;98:177-184
4. Baber U, Mehran R, Sartori S, Schoos MM, Sillesen H, Muntendam P, Garcia MJ, Gregson J, Pocock S, Falk E, Fuster V. Prevalence, impact, and predictive value of detecting subclinical coronary and carotid atherosclerosis in asymptomatic adults: The bioimage study. *J Am Coll Cardiol.* 2015;65:1065-1074

5. Belcaro G, Nicolaides AN, Ramaswami G, Cesaroni MR, De Sanctis M, Incandela L, Ferrari P, Geroulakos G, Barsotti A, Griffin M, Dhanjil S, Sabetai M, Bucci M, Martines G. Carotid and femoral ultrasound morphology screening and cardiovascular events in low risk subjects: A 10-year follow-up study (the cafes-cave study(1)). *Atherosclerosis.* 2001;156:379-387

6. Postley JE, Luo Y, Wong ND, Gardin JM. Identification by ultrasound evaluation of the carotid and femoral arteries of high-risk subjects missed by three validated cardiovascular disease risk algorithms. *Am J Cardiol.* 2015;116:1617-1623

7. Fernandez-Friera L, Fuster V, Lopez-Melgar B, Oliva B, Garcia-Ruiz JM, Mendiguren J, Bueno H, Pocock S, Ibanez B, Fernandez-Ortiz A, Sanz J. Normal ldl-cholesterol levels are associated with subclinical atherosclerosis in the absence of risk factors. *J Am Coll Cardiol.* 2017;70:2979-2991

8. Johri AM, Nambi V, Naqvi TZ, Feinstein SB, Kim ESH, Park MM, Becher H, Sillesen H. Recommendations for the assessment of carotid arterial plaque by ultrasound for the characterization of atherosclerosis and evaluation of cardiovascular risk: From the american society of echocardiography. *J Am Soc Echocardiogr.* 2020;33:917-933

9. Giannoukas AD, Chabok M, Spanos K, Nicolaides A. Screening for asymptomatic carotid plaques with ultrasound. *Eur J Vasc Endovasc Surg.* 2016;52:309-312

10. LeFevre ML, Force USPST. Screening for asymptomatic carotid artery stenosis: U.S. Preventive services task force recommendation statement. *Ann Intern Med.* 2014;161:356-362

11. Xing L, Jing L, Tian Y, Liu S, Lin M, Du Z, Ren G, Sun Q, Shi L, Dai D, Liu S. High prevalence of stroke and uncontrolled associated risk factors are major public health challenges in rural northeast china: A population-based study. *Int J Stroke.* 2019;1747493019851280

12. Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Executive summary of the third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii). *JAMA.* 2001;285:2486-2497

13. Hong L, Xing L, Li R, Zhang L, Ma C, An J, Zhao L, Yang J, Liu S. Subclinical left ventricular dysfunction assessed by two-dimensional speckle tracking echocardiography in asymptomatic patients with carotid stenosis. *Int J Cardiovasc Imaging.* 2019;35:2205-2212

14. Hua Y, Jia L, Xing Y, Hui P, Meng X, Yu D, Pan X, Fang Y, Song B, Wu C, Zhang C, Sui X, Jin Y, Zhang J, Li J, Wang L, Mu Y, Zhong J, Zhu Y, Zhang H, Cai X. Distribution pattern of atherosclerotic stenosis in chinese patients with stroke: A multicenter registry study. *Aging Dis.* 2019;10:62-70

15. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Hernandez Hernandez R, Jaff M, Kownator S, Naqvi T, Prati P, Rundek T, Sitzer M, Schminke U, Tardif JC, Taylor A, Vicaut E, Woo KS. Mannheim carotid intima-media thickness and
plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis. 2012;34:290-296

16. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. Circ Res. 2016;118:535-546

17. Topel ML, Shen J, Morris AA, Al Mheid I, Sher S, Dunbar SB, Vaccarino V, Sperling LS, Gibbons GH, Martin GS, Quyyumi AA. Comparisons of the Framingham and pooled cohort equation risk scores for detecting subclinical vascular disease in blacks versus whites. Am J Cardiol. 2018;121:564-569

18. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Greenlund KJ, Hailpern SM, Heit JA, Ho PM, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, McDermott MM, Meigs JB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Rosamond WD, Sorlie PD, Stafford RS, Turan TN, Turner MB, Wong ND, Wylie-Rosett J, American Heart Association Statistics C, Stroke Statistics S. Heart disease and stroke statistics–2011 update: A report from the American Heart Association. Circulation. 2011;123:e18-e209

19. Spence JD, Pelz D, Veith FJ. Asymptomatic carotid stenosis: Identifying patients at high enough risk to warrant endarterectomy or stenting. Stroke. 2014;45:655-657

20. Marquardt L, Geraghty OC, Mehta Z, Rothwell PM. Low risk of ipsilateral stroke in patients with asymptomatic carotid stenosis on best medical treatment: A prospective, population-based study. Stroke. 2010;41:e11-17

21. Marz W, Kleber ME, Scharnagl H, Speer T, Zewinger S, Ritsch A, Parhofer KG, von Eckardstein A, Landmesser U, Laufs U. HDL cholesterol: Reappraisal of its clinical relevance. Clin Res Cardiol. 2017;106:663-675

22. Mineo C, Shaul PW. Novel biological functions of high-density lipoprotein cholesterol. Circ Res. 2012;111:1079-1090

23. Kobayashi D, Noto H, Shimbo T, Ino T, Osugi Y, Takahashi O, Asai K. Repeated measures of extremely high levels of high-density lipoprotein cholesterol and subsequent all-cause mortality and cardiovascular events: A longitudinal study. Atherosclerosis. 2019;288:17-25

24. Khera AV, Demler OV, Adelman SJ, Collins HL, Glynn RJ, Ridker PM, Rader DJ, Mora S. Cholesterol efflux capacity, high-density lipoprotein particle number, and incident cardiovascular events: An analysis from the JUPITER trial (justification for the use of statins in prevention: An intervention trial evaluating rosvuvastatin). Circulation. 2017;135:2494-2504

25. Lamprea-Montalegre JA, Astor BC, McClelland RL, de Boer IH, Burke GL, Sibley CT, O’Leary D, Sharrett AR, Szklo M. Ckd, plasma lipids, and common carotid intima-media thickness: Results from the multi-ethnic study of atherosclerosis. Clin J Am Soc Nephrol. 2012;7:1777-1785

26. Xing FY, Neeland IJ, Gore MO, Ayers CR, Paixao AR, Turer AT, Berry JD, Khera A, de Lemos JA, McGuire DK. Association of prediabetes by fasting glucose and/or haemoglobin a1c levels with subclinical
atherosclerosis and impaired renal function: Observations from the dallas heart study. *Diab Vasc Dis Res.* 2014;11:11-18

27. Sander D, Schulze-Horn C, Bickel H, Gnahn H, Bartels E, Conrad B. Combined effects of hemoglobin a1c and c-reactive protein on the progression of subclinical carotid atherosclerosis: The invade study. *Stroke.* 2006;37:351-357

28. Moreno-Franco B, Perez-Esteban A, Civeira F, Guallar-Castillon P, Casasnovas JA, Mateo-Gallego R, Jarauta E, Malo S, Laclaustra M. Association between alcohol consumption and subclinical femoral atherosclerosis in smoking and non-smoking men: The awhs study. *Addiction.* 2020;115:1754-1761

29. Barker-Collo S, Bennett DA, Krishnamurthi RV, Parmar P, Feigin VL, Naghavi M, Forouzanfar MH, Johnson CO, Nguyen G, Mensah GA, Vos T, Murray CJ, Roth GA, Group GBDW, Group GBDSPE. Sex differences in stroke incidence, prevalence, mortality and disability-adjusted life years: Results from the global burden of disease study 2013. *Neuroepidemiology.* 2015;45:203-214

**Figures**
Two countries were randomly selected from 14 countries in Liaoning Province

13 villages were randomly selected

10 villages in Chaoyang  3 villages in Lingyuan

Permanent residents ≥ 40 years were invited (n=6830)

992 subjects failed to complete the study

5838 completed the study (response rate 85.5%)

Subjects without conventional cardiovascular risk factors (n=1449)

Subjects without subclinical carotid atherosclerosis (n=1110)  Subjects with subclinical carotid atherosclerosis (n=339)

Moderate carotid stenosis (n=13)  Severe carotid stenosis (n=6)

**Figure 1**

Flow chart of population selection.
Association between HDL-C and risk of subclinical carotid atherosclerosis among CVRF-free population with 3 knots (5th, 50th, and 95th)

Test for overall association: df=2, x2=6.69, p=0.035  
Test for non-linear association: df=1, x2=0.020 p=0.964  
AIC = 567.27

Figure 2

C-R association between HDL-C and presence of carotid atherosclerosis in individuals without conventional CVRFs. HDL-C levels were encoded using a restrictive cubic spline (RCS) function of linear model with 3 knots located at the 5th, 50th, and 95th percentiles of distribution. Y-axis values show the change of subclinical atherosclerosis between the indicated HDL-C (Fig.2B) values and the reference value. The dashed lines are the 95% confidence intervals. HDL-C, high-density lipoprotein cholesterol; CVRFs, cardiovascular risk factors.