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# Combination of Endoglin and ASCVD Risk Assessment Improves Carotid Subclinical Atherosclerosis Recognition

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**Aim:** Our study investigated the association between soluble endoglin and carotid subclinical atherosclerosis.

**Methods:** We used endoglin as an adjunct to atherosclerotic cardiovascular disease (ASCVD) risk, in recognition of carotid clinical atherosclerosis, in order to explore a new model to refine risk assessment. Out of 3,452 participants, 978 subjects with detected soluble endoglin were enrolled in a cross-sectional investigation in Fujian Province were enrolled. Soluble endoglin concentration in serum samples was evaluated using an enzyme-linked immunosorbent assay method. Carotid ultrasonography was used to detect intima-media thickness and carotid plaque.

**Results:** The mean 10-year ASCVD risk by the new Pooled Cohort Equations accounted for 10.04% (±12.35). The mean soluble endoglin level was 15.35 ng/ml (±6.64). Multivariable regression demonstrated that age, systolic blood pressure, diastolic blood pressure, total cholesterol, high density lipoprotein cholesterol, and serum uric acid were independent determinants of soluble endoglin. Adding tests of ASCVD and endoglin together, in parallel, will increase the sensitivity and decrease specificity in recognizing carotid subclinical atherosclerosis. Evaluating the added value of endoglin to the ASCVD risk model showed significantly improved discrimination with analysis of C-statistics, continuous net reclassification index and integrated discrimination index. Both ASCVD risk and soluble endoglin showed positively linear correlation with carotid intima-media thickness (cIMT) ($\beta = 0.006$, $P < 0.001$; $\beta = 0.485$, $P < 0.001$). Even with adjustment for other factors, the relationship between log-transformed soluble endoglin with cIMT was still significant ($\beta = 0.369$, $P < 0.001$).

**Conclusions:** The combination of ASCVD risk and endoglin levels increases carotid atherosclerosis recognition.

**Key words:** Carotid subclinical atherosclerosis, Cardiovascular disease risk factors, Endoglin

## Introduction

Over the past several decades, the Chinese population has experienced increased incidence of cardiovascular disease[1, 2], especially from myocardial infarction and stroke[3]. Many previous reports have proven that carotid subclinical atherosclerosis, including elevated carotid intima-media thickness (cIMT) and carotid plaque, is an independent risk factor of myocardial infarction, coronary heart disease, and stroke[4-6]. Even in low Framingham risk subjects, initial screening by cIMT and plaque assessment is likely to improve subclinical atherosclerosis detection[7]. The American College of Cardiology/American Heart Association recommended new equations to estimate the 10-year risk for developing initial atherosclerotic

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cardiovascular disease (ASCVD) events (defined as first occurrence of nonfatal myocardial infarction or coronary heart disease death, or fatal or nonfatal stroke) in 2013 [8]. Until now, the association of 10-year ASCVD risk with carotid subclinical atherosclerosis has been well demonstrated[9]. However, whether potential novel markers could improve the utility and accuracy of ASCVD risk assessment is still under exploration.

Endoglin (CD105, TGF-$\beta$ receptor Ⅲ) is a transmembrane glycoprotein, mainly expressed on activated vascular endothelial cells[10], as a marker of tumor microvessels and is associated with solid tumor progression[11]. The soluble form of endoglin is generated by cleavage of the extracellular domain from the intact membrane by metalloprotease, may downregulate eNOS expression, and serves as a natural antagonist for TGF-$\beta$ signaling[12].

Recently, soluble endoglin was shown to have a role in endothelial injury, activation, inflammation, and senescence; it could contribute to preeclampsia, hypertension, diabetes mellitus, and hypercholesterolemia[12-17]. In addition, soluble endoglin could be a marker for predicting atherosclerosis. Blann *et al.*[14] first reported soluble endoglin levels were higher in peripheral arterial disease patients, but not in coronary artery disease (CAD) patients, than in controls. Li *et al.*[18] stated the circulating concentration of soluble endoglin increased in early stages of atherosclerosis, due to damage of endothelial cells, and then decreased in later stages. However, Cruz-Gonzalez *et al.*[19] investigated soluble endoglin levels in 183 patients with acute myocardial infarction. They found soluble endoglin levels in acute myocardial infarction patients were low and decreased further after acute myocardial infarction. A recent study showed that CAD patients had low soluble endoglin levels in circulation, especially patients with 3-vessel disease. The study also demonstrated an inverse association between soluble endoglin and the severity of coronary atherosclerosis[20]. Taking those controversial results into account, investigating the association between soluble endoglin and subclinical atherosclerosis would elucidate endoglin's effect on atherosclerosis progression.

Thus, our study aimed to investigate the association between soluble endoglin and carotid subclinical atherosclerosis. Furthermore, we used the ASCVD risk as an adjunct to endoglin in recognition of carotid subclinical atherosclerosis in order to explore a new model to refine risk assessment.

## Methods

Our study was in a framework of stroke research involving 4,932 participants. Cluster sampling was performed from May 2016 to May 2017. We randomly selected participants from Xing village in Wuyishan City, the Xiangcheng District, Zhangzhou City, the Dongjie community, Gulou District, and Qingkou Township, Minhou County, Fuzhou City, for this cross-sectional investigation. All participants were required to complete a standardized questionnaire, carotid ultrasonography, anthropometric, and biochemical measurements. Voluntary participants aged 40 years and above living in their current place of residence for at least five years were recruited in our study. Pregnant and breastfeeding women, individuals who had acute and chronic inflammatory diseases, malignancy, or hematological disorders were excluded. Ultimately, we acquired complete data on 3,452 subjects. In the final analysis, 978 subjects were selected by computer-generated random numbers to detect soluble endoglin levels. The Institutional Review Board of the Fujian Provincial Hospital approved the study, which was performed according to the Declaration of Helsinki. All participants provided signed informed consent after they received a detailed description of the potential benefits and risks associated with the study.

The age, smoking habits, alcohol consumption, medical history (including hypertension, diabetes, CAD, cardiomyopathy, congenital heart disease, peripheral vascular disease, congestive heart failure, stroke, acute and chronic inflammatory diseases, malignancy, or hematological disorder) and medications were investigated using a questionnaire. Physical examinations included height, weight, and blood pressure, which was measured with a standard vertical mercury sphygmomanometer. Blood samples were collected after overnight fasting of at least 8 h. Standard laboratory evaluations determined the plasma levels of triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), glycosylated hemoglobin ($HbA_{1c}$), serum creatinine, and serum uric acid (SUA). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared ($kg/m^2$). Pulse pressure was estimated as the difference between the mean values of the systolic and diastolic blood pressure. The estimated glomerular filtration rate (eGFR) was calculated using Modification of Diet in Renal Disease method[21]. Subjects who had a history of hypertension and were taking antihypertensive drugs were defined as hypertensive[22]. Participants diagnosed with diabetes, currently taking antidiabetic medications, or had an $HbA_{1c} \geq 6.5\%$ were defined as diabetic[23].

The soluble endoglin concentration in serum

samples was evaluated using an enzyme-linked immunosorbent assay method (Human CD105; Abcam, Cambridge, UK) according to the manufacturer's instructions. The optical density was recorded using a microplate reader (Sunrise; Tecan Trading AG, Switzerland) at 450 nm. The standard curve was drawn using computer software. The soluble endoglin concentration of the corresponding sample was checked with the optical density value curve of the standard sample.

A single-blinded carotid artery exam was conducted by a single experienced sonographer using a high-resolution B-mode scanner (M-turbo; FUJIFILM SonoSite, Bothell, USA) with the subject in the supine position according to American Society of Echocardiography Guidelines[24]. The cIMT was measured at 1 cm proximal to carotid bifurcation and along at least 1 cm of the axial length, recorded as the distance between the intimal-luminal and the medial-adventitial interfaces of the carotid artery wall represented as a double-line density on an ultrasound image. The mean cIMT values from the far walls of the bilateral common carotid arteries (mean-mean) were reported. The definition of elevated cIMT was an absolute cIMT value ≥ 0.9 mm[25]. Carotid plaque was reported when the thickness of the most thickened region was 50% greater than that of the surrounding vessel wall or where cIMT was greater than 1.5 mm.

The 10-year ASCVD risk was estimated using the new Pooled Cohort Equations for non-Hispanic whites recommended by 2013 American College of Cardiology/American Heart Association[8]. The equation was calculated from components of gender, age, treated or untreated SBP, TC, HDL-C, current smoking, and diabetes. According to the 10-year ASCVD risk, participants were stratified into two groups (<7.5%, low ASCVD risk; ≥ 7.5%, high ASCVD risk)[8, 26].

All statistical analyzes were performed using R version 3.5.3 and MedCalc® statistical software. Continuous variables were presented as mean ± standard deviation, and categorical variables were expressed as frequencies and percentages. Comparisons between continuous and categorical variables were assessed using the analysis of covariance and chi-square test, respectively. The soluble endoglin levels were log-transformed before analysis. The participants' demographic characteristics were presented in two groups according to the median of log-transformed soluble endoglin. The significant variables in covariance analysis, except for ASCVD risk and cIMT, were considered preliminary covariates of endoglin and included in multivariable regression analysis.

We computed Pearson correlations of soluble endoglin levels with 10-year ASCVD risk and cIMT. A receiver operating characteristic (ROC) curve was applied to evaluate the soluble endoglin level and the findings of carotid subclinical atherosclerosis (elevated cIMT and carotid plaque). We divided the subjects into two groups, at cutoff points determined according to the Youden index, for carotid subclinical atherosclerosis against log-transformed endoglin or 10-year ASCVD risk. Sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio for predicting atherosclerosis were reported in binary groups separately and both together in parallel or series manners.

Prognostic comparisons during models were done using Harrell's concordance C-statistic, continuous net reclassification improvement (NRI), and integrated discrimination index (IDI) to evaluate the stratification of effects from 10-year ASCVD risk model to a complete model, including 10-year ASCVD risk, log-transformed soluble endoglin, $HbA_{1c}$, eGFR, and BMI.

We also examined the linear relationship of cIMT with ASCVD risk and soluble endoglin by a multivariate-adjusted model with stepwise selection method. All reported *P*-values were two-tailed, and $P < 0.05$ was considered statistically significant.

## Results

The study enrolled 978 subjects; 38.85% ($n=380$) were male and 61.15% ($n=598$) were female. The participants had a mean age of 57.24 years (± 9.7). The mean 10-year ASCVD risk by the new Pooled Cohort Equations accounted for 10.04% (± 12.35). The mean soluble endoglin level was 15.35 ng/ml (± 6.64), and 395 subjects were defined as having high ASCVD risk (10-year risk ≥ 7.5%). Subjects in the high endoglin group manifested higher ASCVD risk than those in the low endoglin group (15.14 ± 14.71% vs. 4.93 ± 6.07%, $P<0.001$). The individuals with high soluble endoglin were significantly older, had higher SBP, diastolic blood pressure (DBP), pulse pressure, BMI, $HbA_{1c}$, triglycerides, TC, LDL-C, eGFR, SUA, and had lower HDL-C. The cases of current smoking, alcohol consumption, hypertension, Diabetes mellitus, and antihypertensive therapy were higher in high endoglin group, and the difference was significant ($P<0.001$). The mean cIMT was significantly higher in the high ASCVD risk group than in the low risk group (0.91 ± 0.19 mm vs. 0.77 ± 0.15 mm, $P<0.001$). The high soluble endoglin group had a higher prevalence of CAD than the low group, but the difference showed no statistical significance

**Table 1.** Baseline characteristics of study subjects

| Variable | Total | log-transformed soluble endoglin | | *p* |
|---|---|---|---|---|
| | | Low group (< 1.126 ng/ml) | High group (≥ 1.126 ng/ml) | |
| N | 978 | 489 | 489 | - |
| Age, years | 57.24 ± 9.70 | 54.07 ± 8.54 | 60.41 ± 9.76 | < 0.001 |
| SBP, mmHg | 134.62 ± 19.22 | 123.54 ± 14.25 | 145.71 ± 17.04 | < 0.001 |
| DBP, mmHg | 80.67 ± 11.55 | 75.95 ± 9.70 | 85.40 ± 11.55 | < 0.001 |
| pulse pressure, mmHg | 53.95 ± 14.09 | 47.59 ± 10.92 | 60.31 ± 14.04 | < 0.001 |
| BMI, kg/m$^2$ | 23.98 ± 3.08 | 23.59 ± 3.01 | 24.37 ± 3.11 | < 0.001 |
| $HbA_{1c}$, % | 5.84 ± 1.07 | 5.76 ± 1.03 | 5.92 ± 1.11 | 0.019 |
| TG, mmol/L | 1.66 ± 1.48 | 1.45 ± 1.28 | 1.86 ± 1.63 | < 0.001 |
| TC, mmol/L | 5.12 ± 1.08 | 4.97 ± 1.02 | 5.27 ± 1.12 | < 0.001 |
| HDL-C, mmol/L | 1.38 ± 0.36 | 1.42 ± 0.36 | 1.33 ± 0.36 | < 0.001 |
| LDL-C, mmol/L | 3.25 ± 1.15 | 3.06 ± 1.09 | 3.43 ± 1.19 | < 0.001 |
| eGFR, ml/min/1.73 m$^2$ | 99.82 ± 26.96 | 102.40 ± 27.32 | 97.24 ± 26.36 | 0.003 |
| SUA, μmol/L | 329.99 ± 93.19 | 312.10 ± 87.28 | 347.87 ± 95.55 | < 0.001 |
| Soluble endoglin, ng/ml | 15.35 ± 6.64 | 10.34 ± 1.96 | 20.35 ± 5.85 | < 0.001 |
| ASCVD risk, % | 10.04 ± 12.35 | 4.93 ± 6.07 | 15.14 ± 14.71 | < 0.001 |
| cIMT, mm | 0.84 ± 0.19 | 0.77 ± 0.15 | 0.91 ± 0.19 | < 0.001 |
| Male, *n* (%) | 380 (38.85) | 151 (30.88) | 229 (46.83) | < 0.001 |
| Current smokers, *n* (%) | 191 (19.53) | 77 (15.75) | 114 (23.31) | 0.003 |
| Alcohol, *n* (%) | 202 (20.65) | 85 (17.38) | 117 (23.93) | 0.012 |
| Diabetes mellitus, *n* (%) | 147 (15.03) | 50 (10.22) | 97 (19.84) | < 0.001 |
| Hypertension, *n* (%) | 266 (27.20) | 43 (8.79) | 223 (45.60) | < 0.001 |
| Antihypertensive therapy, *n* (%) | 181 (18.51) | 25 (5.11) | 156 (31.90) | < 0.001 |
| Coronary artery disease, *n* (%) | 28 (2.86) | 9 (1.84) | 19 (3.86) | 0.055 |

Quantitative variables are presented as means±standard deviation, quanlitative variables are presented as numbers with percentages. ASCVD risk, 10-year predicted risk for atherosclerotic cardiovascular disease calculated by the new Pooled Cohort Equations; BMI, body mass index; cIMT: carotid intima-media thickness; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; SUA, serum uric acid; TC, total cholesterol; TG, triglycerides.

($P = 0.055$). **Table 1** shows more details of the comparison of characteristics. The multivariable regression showed that the independent determinants of log-transformed soluble endoglin were age, SBP, DBP, TC, HDL-C, and SUA, which were in partial accord with ASCVD parameters (**Table 2**).

Significant positive correlations between the soluble endoglin level with 10-year ASCVD risk ($r = 0.543$, $P < 0.001$) and cIMT ($r = 0.457$, $P < 0.001$) were presented by Pearson's correlation analysis (**Fig. 1**).

Among the 978 participants, nearly half (47.14%, $n = 461$) had unilateral or bilateral elevated cIMT (mean cIMT ≥ 0.9 mm), and at least 1 carotid plaque was present in 354 subjects. The cutoff point of ASCVD risk was 9.34% for the elevated cIMT performance curve, and 9.46% for carotid plaque presence, both selected by the Youden index. Adopting the similar method, the cutoff point of log-transformed endoglin was set at 1.16 ng/ml for the elevated cIMT performance curve, and 1.17 ng/ml for carotid plaque presence. After categorizing individuals into binary endoglin level groups based on cutoffs, we found higher sensitivity for identifying carotid atherosclerosis than in groups divided by ASCVD risk. Adding the tests together in parallel will increase sensitivity and decrease specificity, and vise versa, in series (**Table 3**).

For identifying elevated cIMT, the ASCVD risk model showed acceptable discrimination (C-statistics 0.698, 95%CI 0.668-0.726). As for carotid plaque, the ASCVD risk model showed fair discrimination (C-statistics 0.754, 95%CI 0.722-0.786). Evaluating the added value of endoglin to the ASCVD risk model showed significantly improved discrimination with analysis of C-statistics, continuous NRI, and IDI. The largest C-statistics were seen with the complete model,

**Table 2.** Multivariate linear regression analysis for log-transformed soluble endoglin levels

| Variable | $\beta$ | SE | standardized $\beta$ | t | $P$ |
|---|---|---|---|---|---|
| Age, years | 0.003 | <0.001 | 0.193 | 7.544 | <0.001 |
| SBP, mmHg | 0.005 | <0.001 | 0.506 | 15.044 | <0.001 |
| DBP, mmHg | 0.001 | <0.001 | 0.07 | 2.14 | 0.033 |
| BMI, kg/m$^2$ | -0.002 | 0.001 | -0.038 | -1.538 | 0.124 |
| $HbA_{1c}$, % | 0.007 | 0.004 | 0.04 | 1.683 | 0.093 |
| TG, mmol/L | 0.003 | 0.003 | 0.027 | 1.02 | 0.308 |
| TC, mmol/L | 0.022 | 0.006 | 0.135 | 3.835 | <0.001 |
| HDL-C, mmol/L | -0.05 | 0.013 | -0.105 | -3.944 | <0.001 |
| LDL-C, mmol/L | 0.002 | 0.005 | 0.014 | 0.416 | 0.677 |
| eGFR, ml/min/1.73 m$^2$ | <0.001 | <0.001 | -0.045 | -1.852 | 0.064 |
| SUA, umol/L | <0.001 | <0.001 | 0.063 | 2.481 | 0.013 |

Multivariate regression analysis excluded pulse pressure for the multicollinearity. 1 SD log-transformed soluble endoglin equals 0.175 ng/ml. SE, standard error; BMI: body mass index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; SBP: systolic blood pressure; SUA: serum uric acid; TC: total cholesterol; TG: triglycerides.

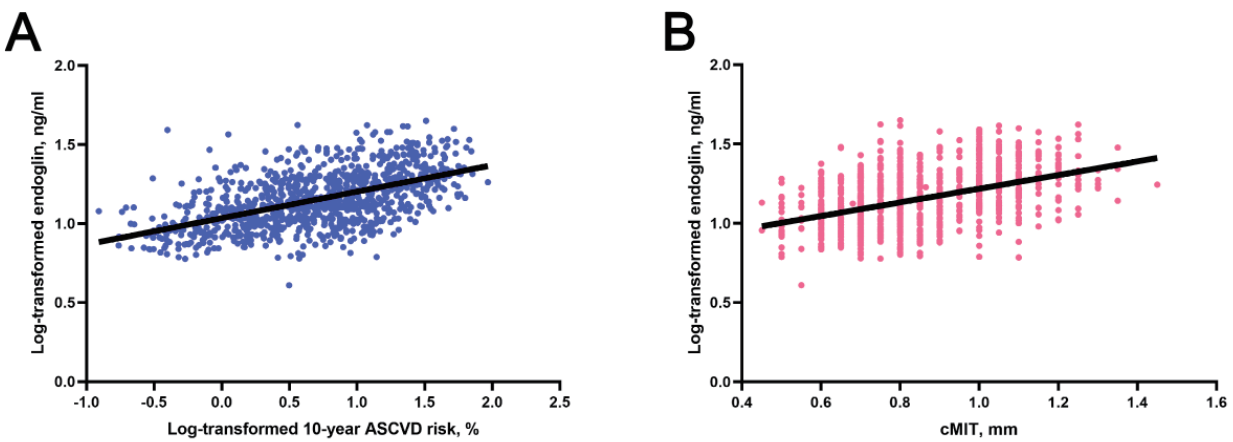


**Fig. 1.** Linear relationship between soluble endoglin levels and: (A) 10-year ASCVD risk (r=0.543, $P$<0.001), and (B) cIMT (r=0.457, $P$<0.001)

10-year ASCVD risk: 10-year predicted risk for atherosclerotic cardiovascular disease calculated by the new Pooled Cohort Equations; cIMT, carotid intima-media thickness.

including 10-year ASCVD risk, log-transformed soluble endoglin, $HbA_{1c}$, eGFR, and BMI (**Table 4, Fig. 2**).

In addition, both ASCVD risk and soluble endoglin showed positively linear correlation with cIMT ($\beta$ =0.006, $P$<0.001; $\beta$ =0.485, $P$<0.001). Even with adjustment for BMI, $HbA_{1c}$, and ASCVD risk, the relationship between log-transformed soluble endoglin with cIMT was still significant ($\beta$ =0.369, $P$<0.001; **Table 5**).

## Discussion

In the present study, we assessed the association between soluble endoglin, ASCVD risk, and carotid subclinical atherosclerosis in a Chinese community population. Our results showed that soluble endoglin was significantly associated with a range of cardiovascular risk factors and remained positively correlated with carotid atherosclerosis after adjusting for confounding factors. We, for the first time, combined both soluble endoglin and ASCVD risk to recognize elevated cIMT and carotid plaque, which revealed a better sensitivity of 76.14% and 83.33% in a parallel manner, respectively.

To the best of our knowledge, this study, for the first time, reported serum soluble endoglin in a Chinese community population. Our data showed that soluble endoglin was significantly related to a series of risk factors for atherosclerosis, which were age, SBP, DBP, pulse pressure, BMI, $HbA_{1c}$, triglycerides, TC, HDL-C, LDL-C, eGFR, and SUA. The findings were

**Table 3.** Diagnostic performance of ASCVD risk and endoglin for predicting carotid subclinical atherosclerosis separately and together

| | sensitivity(%) | specitivity(%) | +LR | -LR |
|---|---|---|---|---|
| elevated cIMT | | | | |
| ASCVD risk | 54.66 | 72.34 | 1.98 | 0.63 |
| log-sEng | 66.38 | 64.60 | 1.88 | 0.52 |
| parallel connection | 76.14 | 53.58 | 1.64 | 0.45 |
| series connection | 44.90 | 83.37 | 2.70 | 0.66 |
| carotid plaque | | | | |
| ASCVD risk | 59.04 | 80.93 | 3.10 | 0.51 |
| log-sEng | 75.42 | 75.48 | 3.08 | 0.33 |
| parallel connection | 83.33 | 65.71 | 2.43 | 0.25 |
| series connection | 51.13 | 90.71 | 5.50 | 0.54 |

Elevated cIMT, carotid intima-media thickness values ≥ 0.9 mm. ASCVD risk: 10-year predicted risk for atherosclerotic cardiovascular disease calculated by the new Pooled Cohort Equations; cIMT, carotid intima-media thickness; log-sEng, log-transformed soluble endoglin; +LR, positive likelihood ratio; -LR, negative likelihood ratio. Cutoff points in elevated cIMT: 9.34% for ASCVD risk, 1.16 for log-sEng; Cutoff points in carotid plaque: 9.46% for ASCVD risk, 1.17 for log-sEng. parallel connection: ASCVD risk higher than cutoff point or log-sEng higher than cutoff point. series connection: ASCVD risk higher than cutoff point together with log- sEng higher than cutoff point.

**Table 4.** Added value of endoglin to the ASCVD risk model in carotid subclinical atherosclerosis

| | C-statistics (95%CI) | *P* | continuous NRI (95%CI) | *P* | IDI (95%CI) | *P* |
|---|---|---|---|---|---|---|
| elevated cIMT | | | | | | |
| Model 1 | 0.698 (0.665,0.731) | - | - | - | - | - |
| Model 2 | 0.736 (0.704,0.767) | 0.002 | 0.412 (0.289, 0.534) | <0.001 | 0.058 (0.043, 0.072) | <0.001 |
| Model 3 | 0.748 (0.717,0.778) | <0.001 | 0.563 (0.442, 0.683) | <0.001 | 0.072 (0.056, 0.088) | <0.001 |
| carotid plaque | | | | | | |
| Model 1 | 0.754 (0.722,0.786) | - | - | - | - | - |
| Model 2 | 0.842 (0.815,0.868) | <0.001 | 0.753 (0.631, 0.874) | <0.001 | 0.174 (0.148, 0.200) | <0.001 |
| Model 3 | 0.844 (0.818,0.871) | <0.001 | 0.838 (0.719, 0.957) | <0.001 | 0.179 (0.152, 0.205) | <0.001 |

*P* value, compared to Model 1. Model 1 included 10-year ASCVD risk. Model 2 included 10-year ASCVD risk and log-transformed soluble endoglin. Model 3 included 10-year ASCVD risk, log-transformed soluble endoglin, $HbA_{1c}$, eGFR and BMI. Elevated cIMT, carotid intima-media thickness values ≥ 0.9 mm.10-year ASCVD risk, 10-year predicted risk for atherosclerotic cardiovascular disease calculated by the new Pooled Cohort Equations; cIMT, carotid intima- media thickness; eGFR, estimated glomerular filtration rate; IDI, integrated discrimination index; NRI, net reclassification index.

partially consistent with previous studies in subjects with medical histories of hypertension, diabetes mellitus, or dyslipidemia[15, 16, 27]. Blazquez-Medela *et al.*[15] reported soluble endoglin levels were higher in those with high probability of 10-year cardiovascular risk by using the scale of the ESH-ESC guide 2007 [28]. Not surprising, by applying the new Pooled Cohort Equations recommended by ACC/AHA, we demonstrate a significant positive correlation between the soluble endoglin level with 10-year ASCVD risk.

As a crucial result of our study, subjects with high endoglin levels were more likely to have elevated cIMT and carotid plaque. With adjusting for 10-year ASCVD, BMI and $HbA_{1c}$, the relationship between soluble endoglin and cIMT was still significant. These results clarified that, in subjects with carotid subclinical atherosclerosis, soluble endoglin appeared to be high. Our result was similar to previous reports about elevated soluble endoglin levels in ischemic heart disease, peripheral vascular disease, and coronary disease[14, 17]. However, Cruz-Gonzalez[19] and Saita E *et al.*[20] revealed soluble endoglin levels in acute myocardial infarction patients and coronary heart disease were low, even negatively associated with the severity of coronary heart disease. Those controversial results may have occurred because soluble endoglin increased in the early stage of atherosclerosis, due to endothelial damage, and decreased in a later stage, due to enhanced atherogenesis induced by more endoglin/TGF-$\beta$ complexes[18]. Thus, the high level of serum

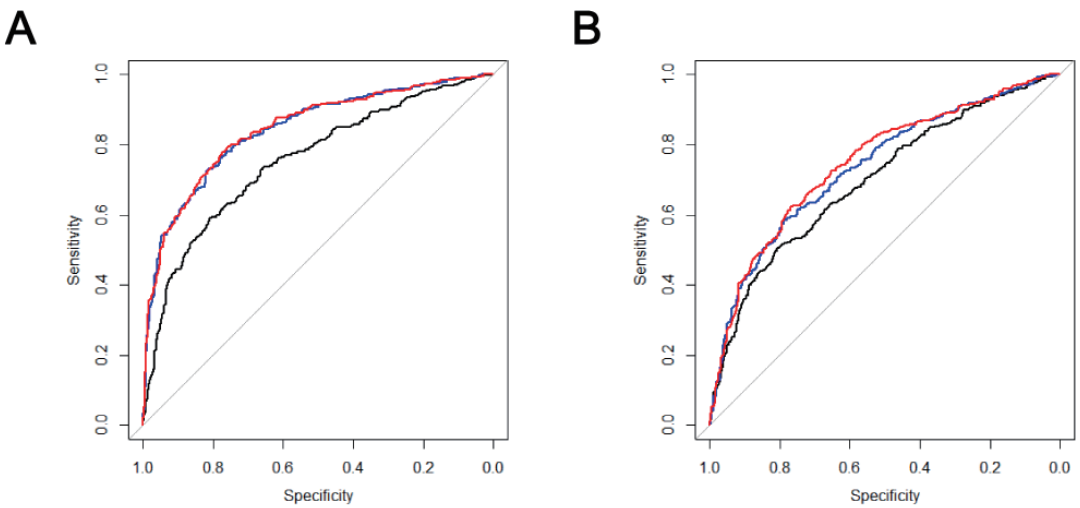


**Fig. 2.** Receiver operating characteristic curve in discriminating carotid atherosclerosis (A, elevated cIMT; B, carotid plaque)

The black curve represents a model that included the 10-year ASCVD; the blue curve, a model that included a 10-year ASCVD and log-transformed soluble endoglin; the red curve, a model that included a 10-year ASCVD risk, log-transformed soluble endoglin, $HbA_{1c}$, eGFR, and BMI; elevated cIMT, carotid intima-media thickness values ≥ 0.9 mm.

**Table 5.** Linear regression analysis showing association of soluble endoglin level and ASCVD risk with cIMT

| | Unadjusted | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Model 1 | | | Model 2 | | |
| Variable | *β* | t | *P* | *β* | t | *P* | *β* | t | *P* |
| ASCVD risk | 0.006 | 14.030 | <0.001 | 0.004 | 7.966 | <0.001 | 0.003 | 6.972 | <0.001 |
| log-sEng | 0.485 | 16.044 | <0.001 | 0.360 | 10.821 | <0.001 | 0.369 | 11.108 | <0.001 |

Model 1 included 10-year ASCVD risk (or log-transformed endoglin). Model 2 included $HbA_{1c}$, BMI, 10-year ASCVD risk and log-transformed endoglin. 10-year ASCVD risk: 10- year predicted risk for atherosclerotic cardiovascular disease calculated by the new Pooled Cohort Equations; cIMT, carotid intima-media thickness; log-sEng, log-transformed soluble endoglin.

endoglin in subclinical atherosclerosis measured as cIMT may support its synergistic effect in atherosclerotic pathological development. The literature most similar to our study was carried out in 2016 by Çelik, N. *et al.*[29], who measured the cIMT and brachial artery flow-mediated dilatation in children aged 10-18 years. They found no statistical difference in the endoglin level between the obese and control groups in spite of independent associations between subclinical atherosclerosis with BMI and waist circumference. Their population was small (95); moreover, the research objects had totally different ages, backgrounds, and ethnicity from ours. Hence, our result seems more convincing. Through *in vitro* research, high endoglin was expressed in atherosclerotic lesions in carotid, but not in coronary and cerebral, arteries[30]. Therefore, another assumption would be that endoglin levels might be varied in separate vascular beds. Further studies on different vascular subclinical atherosclerosis need to be assessed in new investigations.

Previous studies indicated high carotid IMT or carotid plaque to be a strong predictor of cardiovascular disease[4-6]. Since the new ASCVD risk calculator was developed, two recent analysis of large Asian subjects have demonstrated that carotid IMT correlated with the new 10-year ASCVD risk score[9, 31]. In agreement with those studies, in the present study 10-year ASCVD risk was positively corelated with cIMT and carotid atherosclerosis. In addition, a prospective study with a small sample showed that chronic kidney disease, left ventricular ejection fraction, and serum endoglin were predictors of major adverse cardiovascular events (congestive heart failure, acute myocardial infarction, stroke, and sudden cardiac death)[32]. Based on above findings, we found that a model incorporating endoglin and 10-year ASCVD would contribute to recognizing carotid subclinical atherosclerosis. Not surprisingly, we demonstrate an increased carotid ath-

erosclerosis recognition rate via taking high ASCVD risk and high endoglin levels into consideration together than that achieved with either factor alone. To elucidate the effect of endoglin in atherosclerosis progression, we established models, including soluble endoglin, ASCVD risk, BMI, $HbA_{1c}$, and eGFR for elevated cIMT and carotid plaque. In the models, we acquired higher C-statistics of 0.748 for elevated cIMT and 0.844 for carotid plaque. As cIMT increased with advancing age, elevated cIMT may be an adaptive response to changes in flow, wall tension, or lumen diameter[33]. Studies had reported that cIMT provided morphological evidence of atherosclerosis, and was strongly influenced by genetic determinants, but carotid plaque appeared to be determined by common CAD risk factors, such as age, sex, hypertension, diabetes mellitus, hypercholesterolemia, and amount of nicotine consumed other than genetic inheritance[34]. Carotid plaque also correlated with other measures of atherosclerotic vascular disease, such as aortic stiffness, whereas no such association was found for cIMT[35]. Those mechanisms may explain the different discrimination for elevated cIMT and carotid plaque by the same model. The complete model obtained by adding $HbA_{1c}$, eGFR, and BMI into the basic model (composed by soluble endoglin and ASCVD risk), showed little increase of C-statistics in predicting carotid plaque. It may indicate soluble endoglin, rather than those factors, plays a more important role in relation to carotid atherosclerosis. Another explanation may be that the adoption of soluble endoglin influenced the relative correlation between those factors and carotid plaque.

The mean soluble endoglin of 15.35ng/ml seemed higher than prior measurements[15, 20, 27], but close to a report on Iranian women[36]. We attributed the disparity to a varied immunoassay kit of enzyme-linked immunosorbent assay and ethnic difference. The weak, but significant, association between soluble endoglin and SUA seemed to be first demonstrated. An animal experiment has revealed that elevated cardiac endoglin was accompanied by increased SUA, which might potentially explain the connection[37]. Doghish AS *et al.* found that plasma soluble endoglin increased with the development of diabetic nephropathy with microalbuminuria[27]. However, Charytan DM analyzed soluble endoglin concentrations in a large cohort of individuals, with and without chronic kidney disease, and found no association between soluble endoglin levels and eGFR or urinary albumin excretion[38].Our study showed soluble endoglin was inversely correlated with eGFR, but the association was not significant after further adjustment. The result indicates the former association may be produced by mixed effects from other risk factors.

The strength of our study is the new atherosclerosis marker endoglin and efficient 10-year ASCVD risk. By investigating a range of cardiovascular risk factors in a Chinese community population, we thoroughly explored the determinants of soluble endoglin and pioneered models, including serum endoglin and 10-year ASCVD risk, to improve recognition for carotid atherosclerosis. Our study also has a few limitations. First, the Pooled Cohort Equations was originally developed in African Americans and non-Hispanic Whites, which limited their applicability to other populations[26]. We did not apply the China-PAR to assess ASCVD risk due to insufficient information. Second, the data presented here are cross-sectional and would not clarify the causal relationship. Therefore, a larger cohort would be required. Third, carotid subclinical atherosclerosis as a single indicator of atherosclerosis or subclinical atherosclerosis would generate certain degrees of bias. Hence, the relationship between atherosclerosis and soluble endoglin levels should be detected in more vascular beds.

## Conclusion

Soluble endoglin is independently related with age, SBP, DBP, TC, HDL-C, SUA, and 10-year ASCVD risk, remained positively correlative with carotid subclinical atherosclerosis after adjusting for confounding factors. The combination of ASCVD risk and endoglin levels detects carotid atherosclerosis more often.

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## COI

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**Supplementary Table 1.** Baseline characteristics of study subjects

| Variable | selected | not selectd | *P*-value |
|---|---|---|---|
| N | 978 | 2,474 | - |
| Age, years | 57.24 ± 9.70 | 56.70 ± 9.90 | 0.141 |
| SBP, mmHg | 134.62 ± 19.22 | 134.50 ± 18.96 | 0.869 |
| DBP, mmHg | 80.67 ± 11.55 | 80.59 ± 11.67 | 0.845 |
| pulse pressure, mmHg | 53.95 ± 14.09 | 53.92 ± 13.97 | 0.949 |
| BMI, kg/m$^2$ | 23.98 ± 3.08 | 24.05 ± 3.21 | 0.546 |
| $HbA_{1c}$, % | 5.84 ± 1.07 | 5.83 ± 1.06 | 0.851 |
| TG, mmol/L | 1.66 ± 1.48 | 1.67 ± 1.42 | 0.77 |
| TC, mmol/L | 5.12 ± 1.08 | 5.10 ± 1.07 | 0.716 |
| HDL-C, mmol/L | 1.38 ± 0.36 | 1.39 ± 0.37 | 0.205 |
| LDL-C, mmol/L | 3.25 ± 1.15 | 3.26 ± 1.09 | 0.655 |
| eGFR, ml/min/1.73 m$^2$ | 99.82 ± 26.96 | 98.61 ± 21.20 | 0.209 |
| SUA, umol/L | 329.99 ± 93.19 | 331.27 ± 89.08 | 0.712 |
| 10-year ASCVD risk, % | 10.04 ± 12.35 | 9.86 ± 11.93 | 0.704 |
| Male, *n* (%) | 380 (38.85) | 1,008 (40.74) | 0.314 |
| Current smokers, *n* (%) | 191 (19.53) | 520 (21.02) | 0.33 |
| Alcohol, *n* (%) | 202 (20.65) | 517 (20.90) | 0.729 |
| Diabetes mellitus, *n* (%) | 147 (15.03) | 374 (15.12) | 0.949 |
| Hypertension, *n* (%) | 266 (27.20) | 681 (27.53) | 0.846 |
| Antihypertensive therapy, *n* (%) | 181 (18.51) | 488 (19.73) | 0.415 |

Quantitative variables are presented as means±standard deviation, quanlitative variables are presented as numbers with percentages. 10-year ASCVD risk: 10-year predicted risk for atherosclerotic cardiovascular disease calculated by the new Pooled Cohort Equations; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; TG: triglycerides; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; eGFR: estimated glomerular filtration rate; SUA: serum uric acid.