Newer biomarker-based prediction of atherosclerosis risk

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Newer biomarker-based prediction of atherosclerosis risk

Directed by Professor Hyeon Chang Kim

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ABSTRACT

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Background: Angiopoietin-1 and its receptor, tunica interna endothelial cell kinase 2 (Tie-2), maintain endothelial integrity and homeostasis, while Angiopoietin-2 counteracts these effects. Given their biological actions, Angiopoietin-2, Angiopoietin-1, and soluble Tie-2 may have the potential to serve as predictive biomarkers for subclinical atherosclerosis. In this prospective ancillary study of the Cardiovascular and Metabolic Diseases Etiology Research Center (CMERC) and CMERC High-Risk (CMERC-HI) cohorts, we aimed to (1) construct a biomarker subcohort for the exploration of Angiopoietin-related biomarkers (i.e., Angiopoietin-2, Angiopoietin-1, and soluble Tie-2); (2) delineate the baseline characteristics of these biomarkers; (3) examine whether these biomarkers improve risk prediction for subclinical atherosclerosis beyond traditional risk factors; and (4) develop and validate subclinical atherosclerosis risk prediction models using these biomarkers.

Methods: We enrolled a total of 924 participants from the CMERC and CMERC-HI cohorts between 2021 and 2023. Data on traditional risk factors, including age, sex, current smoking, systolic blood pressure, blood pressure-lowering drug use, diabetes, total and high-density lipoprotein cholesterol levels, and lipid-lowering drug use, were collected at baseline. Serum levels of Angiopoietin-related biomarkers were measured using enzyme-linked immunosorbent assay from the blood samples collected at baseline. The coronary artery calcium (CAC) was measured by cardiac computed tomography; the CAC score was

calculated using the Agatston method. The primary measures of subclinical atherosclerosis were severe coronary atherosclerosis (defined as a CAC score >400) and coronary atherosclerosis (defined as a CAC score >0). The predictive performance of the models was evaluated using the Harrell's C-index, Hosmer-Lemeshow chi-square statistic, and calibration plot. The additive utility of Angiopoietin-related biomarkers beyond traditional risk factors was assessed using the ΔC-index, continuous net reclassification improvement (cNRI), and integrated discrimination improvement (IDI). We developed subclinical atherosclerosis risk prediction models incorporating Angiopoietin-related biomarkers (Angiopoietin-based prediction models) and performed internal and holdout validation.

Results: Of the 924 participants (median age, 62 years; 60.5% female), severe coronary atherosclerosis (CAC score >400) and coronary atherosclerosis (CAC score >0) were present in 60 (6.5%) and 390 (42.2%) participants, respectively. Angiopoietin-related biomarkers showed a poor correlation with each other and weak or no association with traditional risk factors. Angiopoietin-related biomarkers significantly improved risk discrimination and stratification for both severe coronary atherosclerosis (ΔC-index, 0.019-0.030; cNRI, 0.46-0.53; IDI, 0.037-0.061) and coronary atherosclerosis (ΔC-index, 0.007-0.014; cNRI, 0.18-0.20; IDI, 0.011-0.023) when added to traditional risk factor-based models. The Angiopoietin-based prediction models showed good discrimination in internal validation (optimism-corrected C-index, 0.793-0.849) and decent discrimination in holdout validation (C-index, 0.678-0.791). The predicted probabilities were in good agreement with the observed probabilities. Integer risk scores for subclinical atherosclerosis were also developed and showed satisfactory performance (C-index, 0.790-0.837).

Conclusion: Angiopoietin-related biomarkers significantly improved risk prediction for subclinical atherosclerosis when added to traditional risk factor-based models. Angiopoietin-based prediction models, as well as integer risk scores, showed good predictive performance. The newly developed prediction models are expected to be utilized

for identifying high-risk populations for the primary prevention of cardiovascular disease (CVD) and for surveilling individuals with a CAC score of 0. Whether Angiopoietin-related biomarkers improve risk prediction for clinical CVD events needs to be determined in future studies.

Key words: Angiopoietin; atherosclerosis; coronary artery calcium; prediction

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I. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide and the second leading cause of death in Korea.[1,2] Atherosclerotic CVD, which includes ischemic heart disease and stroke, constitutes the majority of CVD events. As the population ages, the burden of atherosclerotic CVD is rapidly increasing in Korea; between 2002 and 2018, the hospitalization rate for ischemic heart disease increased 2.6-fold (from 146 to 383 hospitalizations per 100,000 persons) and that for stroke increased 2.9-fold (from 183 to 523 hospitalizations per 100,000 persons).[3]

Preventing disease in the general population (i.e., primary prevention) is one of the core strategies for reducing the disease burden. However, the scarcity of dedicated cohorts and a lower absolute risk of atherosclerotic CVD in Korea than in Western populations present significant challenges when conducting prospective studies on atherosclerotic CVD events in Korea. In this regard, subclinical atherosclerosis, which represents an earlier, subclinical phase of overt CVD events, has been widely recognized as a surrogate for atherosclerotic CVD and used as such in various studies.[4,5] Not only is the presence of subclinical atherosclerosis strongly associated with incident atherosclerotic CVD risk,[6-8]

but its prevention and early detection through accurate risk assessment may create opportunities for interventions to deter atherosclerotic CVD events earlier in their course.[9]

The development and progression of atherosclerosis is largely affected by cardiovascular risk factors, including tobacco smoking, high blood pressure, diabetes, and high blood lipids.[10-15] These risk factors can explain a significant portion of an individual's risk of atherosclerosis and be used for its prediction. However, given the complex molecular and biological pathways underlying atherosclerosis,[16] the accuracy of atherosclerosis prediction is expected to be further improved by using the biomarkers involved in these pathways.[5,16-23]

Angiopoietins are a group of growth factors that contribute to the regulation of vascular functions and consist of Angiopoietin-1, Angiopoietin-2, and Angiopoietin-4.[24] Angiopoietin-1, together with its receptor tunica interna endothelial cell kinase 2 (Tie-2), maintains endothelial quiescence and promotes vascular integrity through its effect on vascular remodeling and inflammation and endothelial cell migration.[19] Angiopoietin-2 is a competitive inhibitor of Angiopoietin-1 and counteracts these effects, thereby disrupting vascular stability and homeostasis.[19] Given their biological actions, Angiopoietin-2, Angiopoietin-1, and soluble Tie-2 may have the potential to serve as predictive biomarkers for subclinical atherosclerosis.

It has been reported that the levels of Angiopoietin-related biomarkers (i.e., Angiopoietin-2, Angiopoietin-1, and soluble Tie-2) are altered and associated with clinical outcomes in conditions related to vascular endothelial dysfunction, such as myocardial ischemia,[25] cardiogenic shock,[26,27] kidney failure,[28,29] sepsis,[30,31] and acute lung injury.[32,33] However, the associations of Angiopoietin-related biomarkers with subclinical atherosclerosis are yet to be elucidated. The understanding of these associations may not

only facilitate the accurate prediction of subclinical atherosclerosis risk but also provide deeper mechanistic insights into the role of Angiopoietin-related biomarkers in the development and progression of atherosclerosis.

In this prospective ancillary study of the Cardiovascular and Metabolic Diseases Etiology Research Center (CMERC) and CMERC High-Risk (CMERC-HI) cohorts, we aimed to (1) construct a biomarker subcohort for the exploration of Angiopoietin-related biomarkers; (2) delineate the baseline characteristics of these biomarkers; (3) examine whether these biomarkers improve risk prediction for subclinical atherosclerosis beyond traditional risk factors; and (4) develop and validate subclinical atherosclerosis risk prediction models using these biomarkers.

II. MATERIALS AND METHODS

1. Data source

The primary data sources of the current study were the CMERC and CMERC-HI cohorts. The CMERC and CMERC-HI are prospective, multicenter, observational studies in South Korea designed to identify novel risk factors of CVD and develop evidence-based strategies for its prevention.

In the CMERC cohort, a total of 8,097 community-dwelling adults aged 30 to 64 years and free of CVD were recruited between December 2013 and March 2018. Further details of the database have been previously published.[34,35]

In the CMERC-HI cohort, a total of 3,267 adults aged 20 to 80 years and at a high risk of, but without, CVD were recruited between December 2013 and June 2018 (ClinicalTrials.gov identifier: NCT02003781). The inclusion criteria of the CMERC-HI were: (1) high-risk hypertension; (2) carotid intima-media thickness ≥0.9 mm or carotid plaque; (3) ankle-brachial index <0.9; (4) abdominal aortic aneurysm with a diameter >3 cm; (5) electrocardiographic or echocardiographic left ventricular hypertrophy; (6) atrial fibrillation with a CHA_2DS_2-VASc score ≥1; (7) asymptomatic old cerebrovascular accident or coronary artery disease; (8) family history of premature myocardial infarction at age <55 years for males and <65 years for females; (9) albuminuria with diabetes; (10) early morning spot urine albumin-creatinine ratio ≥30 mg/g within 6 months; (11) stage 1 or 2 chronic kidney disease with target organ damage; (12) stage 3 or higher chronic kidney disease; (13) end-stage kidney disease under chronic dialysis or at kidney transplantation status for >3 months; (14) retinopathy on fundoscopy; and (15) aged ≥40 years with rheumatoid arthritis under methotrexate or steroid therapy. The exclusion criteria were: (1) acute coronary syndrome; (2) symptomatic coronary artery disease; (3) symptomatic

peripheral artery disease; (4) symptomatic heart failure; (5) life expectancy <6 months due to severe, non-cardiovascular diseases; (6) pregnancy or breastfeeding status; and (7) contrast allergy.

This study complied with the Declaration of Helsinki; the study protocol was approved by the Institutional Review Board of Severance Hospital, Seoul, Korea (4-2023-1160). Written informed consent was obtained from all participants prior to the baseline examination.

2. Study population

We constructed biomarker subcohorts within the CMERC and CMERC-HI cohorts as detailed below.

A. Biomarker subcohort 1

Of the 8,097 participants in the CMERC cohort, we enrolled 760 individuals in our biomarker substudy between July 15, 2022, and April 11, 2023. These participants underwent baseline health examinations at enrollment. After excluding those with missing data on traditional risk factors (N=8) or who did not undergo cardiac computed tomography (CT) scan at baseline examination (N=2), a final analytical sample of 750 participants resulted (biomarker subcohort 1; Figure 1).

B. Biomarker subcohort 2

Of the 3,267 participants in the CMERC-HI cohort, we enrolled 228 individuals in our biomarker substudy between June 23, 2021, and January 14, 2022. These participants had undergone baseline health examinations between December 18,

2013, and June 28, 2018 (CMERC-HI baseline examination). After excluding those with missing data on traditional risk factors (N=25) or who did not undergo cardiac CT scan at baseline examination (N=29), a final analytical sample of 174 participants resulted (biomarker subcohort 2; Figure 1).

C. Primary biomarker subcohort

The primary biomarker subcohort consisted of a total of 924 participants, 750 from the biomarker subcohort 1 and 174 from the biomarker subcohort 2 (Figure 1).

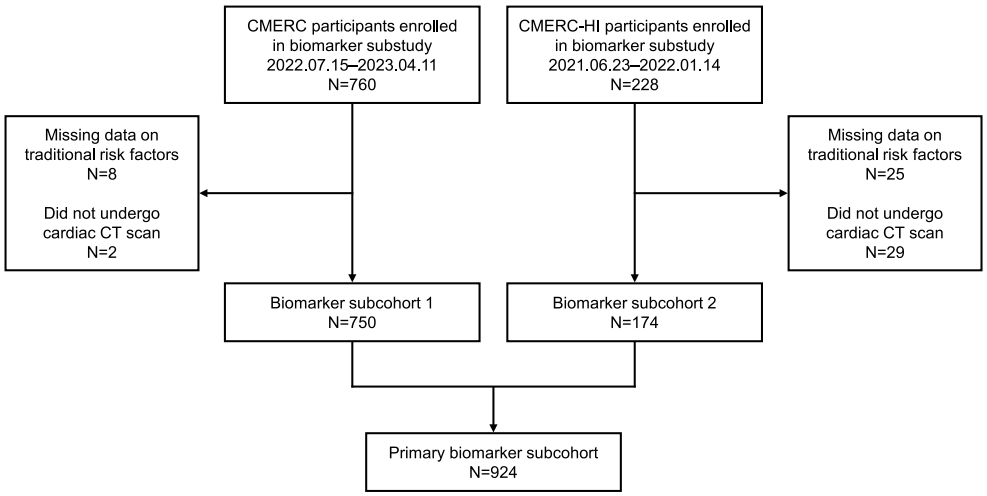

Figure 1. Study flowchart. CMERC, Cardiovascular and Metabolic Diseases Etiology Research Center; CMERC-HI, Cardiovascular and Metabolic Diseases Etiology Research Center High-Risk; CT, computed tomography.

3. Data collection

A. Traditional risk factors

Data on age, sex, smoking status (current vs. past or never), systolic blood pressure (BP), BP-lowering drug use, diabetes, total cholesterol and high-density lipoprotein cholesterol (HDL-C) levels, and lipid-lowering drug use were collected during respective baseline examinations in each subcohort.

B. Angiopoietin-related biomarkers

Serum levels of Angiopoietin-2, Angiopoietin-1, and soluble Tie-2 were measured with the use of enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). According to the manufacturer, the lower limit of detection is 8.29 pg/mL for Angiopoietin-2, 3.45 pg/mL for Angiopoietin-1, and 0.014 ng/mL for soluble Tie-2. These assays showed excellent reliability; the intraclass correlation coefficients (95% confidence intervals [CIs]) were 0.94 (0.88-0.97) for Angiopoietin-2, 0.98 (0.96-0.99) for Angiopoietin-1, and 0.99 (0.97-0.99) for soluble Tie-2 in our batch sample (N=36) (Table 1).

Table 1. Sensitivity and reliability of the assays for Angiopoietin-related biomarkers

Biomarker	LLD*	ICC (95% CI)†
Angiopoietin-2	8.29 pg/mL	0.94 (0.88-0.97)
Angiopoietin-1	3.45 pg/mL	0.98 (0.96-0.99)
Soluble Tie-2	0.014 ng/mL	0.99 (0.97-0.99)

*Indicated by the manufacturer. †Assessed by repeated measurements in a batch sample (N=36). CI, confidence interval; ICC, intraclass correlation coefficient; LLD, lower limit of detection; Tie-2, tunica interna endothelial cell kinase 2.

In the biomarker subcohort 1, serum levels of Angiopoietin-related biomarkers were measured from fresh blood samples collected during the baseline examination. In the biomarker subcohort 2, the levels were measured from frozen blood samples collected during the baseline examination.

C. Coronary artery calcium (CAC) score

Cardiac CT scans were performed to measure CAC during respective baseline examinations in each subcohort. CAC scores were calculated by independent radiologists at Severance Hospital, Seoul, Korea using the Agatston method.[36] In the biomarker subcohort 2, all but 1 of the participants (173 [99.4%]) underwent follow-up cardiac CT scans after a median follow-up of 5.3 [interquartile range, 4.4-6.8] years.

4. Study outcomes

The primary measures of subclinical atherosclerosis were: (1) severe coronary atherosclerosis, defined as a CAC score >400; and (2) coronary atherosclerosis, defined as a CAC score >0. The secondary measures were: (1) log-transformed CAC score, defined as log (CAC+1); and (2) coronary atherosclerosis progression, defined as any increase in CAC score during the follow-up among those with available data.

5. Statistical analysis

All statistical analyses were performed using R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

A. Baseline cohort and biomarker characteristics

Baseline characteristics of the participants were presented as median [interquartile range] or number (%) as appropriate. The distributions of continuous variables, including the biomarker levels, were visually inspected using distribution plots. Variables with right-skewed distributions were log-transformed. Age was also log-transformed despite its left-skewed distribution to maximize comparability with contemporary 10-year atherosclerotic CVD risk models.[37-39] Pairwise correlations between serum levels of Angiopoietin-related biomarkers were visualized using scatter plots and quantified by Spearman's rank correlation.

The associations between traditional risk factors and serum levels of Angiopoietin-related biomarkers were assessed by linear regression models. The associations of serum biomarker levels with primary atherosclerosis measures (i.e., severe coronary atherosclerosis and coronary atherosclerosis) were assessed using logistic regression models. To account for potential non-linearity in the associations, restricted cubic spline terms were placed on the biomarker levels with 4 knots at the 5th, 35th, 65th, and 95th percentiles. Because there existed signs of non-linearity, we defined binary cut-offs for the biomarker levels based on Youden's index.

B. Predictive utility of Angiopoietin-related biomarkers

Models for predicting primary atherosclerosis measures were developed by logistic regression analysis. The traditional risk factor-based models included age, sex, current smoking, systolic BP, BP-lowering drug use, diabetes, and total cholesterol and HDL-C levels—the same variables as those included in contemporary 10-year atherosclerotic CVD risk models[37-39]—as well as lipid-lowering drug use. The

biomarker-added models further included Angiopoietin-related biomarker levels on top of the traditional risk factor-based models.

We assessed models' discriminative capacity with Harrell's C-index and calibration with Hosmer-Lemeshow chi-square statistic and calibration plot of predicted vs. observed probabilities.[40] The additive utility of Angiopoietin-related biomarkers for predicting subclinical atherosclerosis was assessed by comparing traditional risk factor-based models with biomarker-added models—the changes in C-index (ΔC-index), continuous net reclassification improvement (cNRI), and integrated discrimination improvement (IDI) were calculated.[18,41-43]

In subgroup analyses, participants were stratified by age (<65 vs. ≥65 years), sex, hypertension, diabetes, lipid-lowering drug use, subcohort (1 vs. 2), and 10-year atherosclerotic CVD risk (<7.5 vs. 7.5-<10 vs. ≥10% for severe coronary atherosclerosis; <2.5 vs. 2.5-<5 vs. ≥5% for coronary atherosclerosis). The main analyses were also repeated for secondary atherosclerosis measures (i.e., log-transformed CAC score and coronary atherosclerosis progression). Models for log-transformed CAC score were developed by linear regression analysis, with an adjusted R^2 assessed as a performance metric for goodness of fit.

We conducted two sensitivity analyses. First, we constructed expanded traditional risk factor-based models by further including weekly minutes of moderate-to-vigorous physical activity, waist circumference, estimated glomerular filtration rate (eGFR) (calculated using the Chronic Kidney Disease Epidemiology Collaboration equation developed in 2009),[44] and log-transformed high-sensitivity C-reactive protein (hsCRP)[21,45-47] and assessed the additive utility of Angiopoietin-related biomarkers. Second, we excluded those with an eGFR <60 mL/min/1.73 m^2

and repeated the main analyses, as serum levels of Angiopoietin-related biomarkers can be affected by renal dysfunction.[28,29]

C. Angiopoietin-based subclinical atherosclerosis risk prediction models

We developed Angiopoietin-based subclinical atherosclerosis risk prediction models by logistic regression analysis. Internal validation of the models was performed by (1) bootstrapping with 1,000 iterations and (2) 5-fold cross-validation over 200 repetitions. For holdout validation, the models were derived from the biomarker subcohort 1 and validated in the biomarker subcohort 2.

To enhance the utility of prediction models and accelerate their adoption into real-world clinical practice, we additionally developed integer risk scores for subclinical atherosclerosis. For this, the biomarker-added logistic regression models were reconstructed with all the predictors dichotomized using previously defined (for Angiopoietin-related biomarkers) or clinically relevant cut-offs (for traditional risk factors). The β coefficient for each predictor was rounded to the nearest integer and then adjusted as necessary to assign integer scores to the predictors. According to the total risk score, participants were classified into very low (0-3), low (4-6), intermediate (7-9), high (10-12), and very high (≥13) risk groups. Internal validation of the risk score was performed by (1) simple method without optimism correction and (2) bootstrapping with 1,000 iterations.

D. Secondary analysis of carotid atherosclerosis

We constructed the secondary biomarker subcohort for the analysis of carotid atherosclerosis. The subcohort consisted of 772 participants, 201 from the CMERC-

HI-based biomarker subcohort 2.5 and 571 from the CMERC-based biomarker subcohort 3 (Appendix 1). Carotid atherosclerosis was measured using carotid ultrasonography and defined as the mean carotid intima-media thickness (IMT) in quartile 4 (vs. quartile 1 to 3).

III. RESULTS

1. Baseline cohort and biomarker characteristics

A total of 924 participants (median age, 62 years; 60.5% female) were enrolled in the primary biomarker subcohort—750 (81.2%) from the subcohort 1 and 174 (18.8%) from the subcohort 2 (Figure 1). In the primary biomarker subcohort, the current smoking rate was 8.3%; median systolic and diastolic BP were 121 mm Hg and 76 mm Hg, respectively; 37.8% were taking BP-lowering drugs; 18.2% had diabetes; median total cholesterol and HDL-C levels were 188 mg/dL and 58 mg/dL, respectively; 39.1% were taking lipid-lowering drugs; and median 10-year atherosclerotic CVD risk was 6.4% (Table 2). Compared with the participants in the subcohort 1, those in the subcohort 2 were younger and less likely to be female, were more likely to be currently smoking, taking BP- and lipid-lowering drugs, and having diabetes, had lower total cholesterol and HDL-C levels, and exhibited higher 10-year atherosclerotic CVD risk. Serum levels of Angiopoietin-related biomarkers were largely similar between the subcohorts; the overall median levels of Angiopoietin-2, Angiopoietin-1, and soluble Tie-2 were 1.8 ng/mL, 40.4 ng/mL, and 9.4 ng/mL, respectively. Severe coronary atherosclerosis and coronary atherosclerosis were present in 60 (6.5%) and 390 (42.2%) participants, respectively. CAC score was generally higher in the subcohort 2 than in the subcohort 1 (Table 2). The baseline characteristics did not differ appreciably between the included and excluded participants in either CMERC or CMERC-HI (Appendix 2).

Table 2. Baseline characteristics of the participants

Variable	Primary biomarker subcohort (N=924)	Biomarker subcohort 1 (N=750)	Biomarker subcohort 2 (N=174)
Age, years	62 [54-66]	62 [55-66]	59 [50-65]
Female	559 (60.5)	488 (65.1)	71 (40.8)
Current smoking	77 (8.3)	57 (7.6)	20 (11.5)
Systolic BP, mm Hg	121 [110-132]	120 [110-132]	122 [114-132]
Diastolic BP, mm Hg	76 [69-82]	76 [68-82]	75 [70-82]
BP-lowering drug use	349 (37.8)	212 (28.3)	137 (78.7)
Diabetes	168 (18.2)	98 (13.1)	70 (40.2)
Total cholesterol, mg/dL	188 [159-215]	192 [163-220]	168 [144-188]
HDL-cholesterol, mg/dL	58 [49-69]	60 [51-72]	48 [42-55]
Lipid-lowering drug use	361 (39.1)	272 (36.3)	89 (51.1)
10-year ASCVD risk, %			
<2.5	152 (16.5)	126 (16.8)	26 (14.9)
2.5-<5	203 (22.0)	165 (22.0)	38 (21.8)
5-<7.5	199 (21.5)	168 (22.4)	31 (17.8)
7.5-<10	132 (14.3)	114 (15.2)	18 (10.3)
≥10	238 (25.8)	177 (23.6)	61 (35.1)
Angiopoietin-2, ng/mL	1.8 [1.5-2.1]	1.8 [1.5-2.1]	1.8 [1.5-2.1]
Angiopoietin-1, ng/mL	40.4 [33.8-47.7]	40.3 [34.0-47.7]	40.7 [33.2-47.7]
Soluble Tie-2, ng/mL	9.4 [8.2-10.7]	9.2 [8.1-10.4]	10.6 [9.2-12.4]
CAC score			
0	534 (57.8)	459 (61.2)	75 (43.1)
1-10	79 (8.5)	63 (8.4)	16 (9.2)
11-100	172 (18.6)	129 (17.2)	43 (24.7)
101-400	79 (8.5)	57 (7.6)	22 (12.6)
>400	60 (6.5)	42 (5.6)	18 (10.3)

Values are presented as median [interquartile range] or number (%). ASCVD, atherosclerotic cardiovascular disease; BP, blood pressure; CAC, coronary artery calcium; HDL, high-density lipoprotein; Tie-2, tunica interna endothelial cell kinase 2.

Before log-transformation, the distribution of age was left-skewed, while those of systolic BP, total cholesterol level, and HDL-C level were right-skewed (Figure 2A). After log-transformation, the latter distributions became normalized (Figure 2B). The distributions of Angiopoietin-related biomarker levels were also right-skewed before and became normalized after log-transformation (Figure 3).

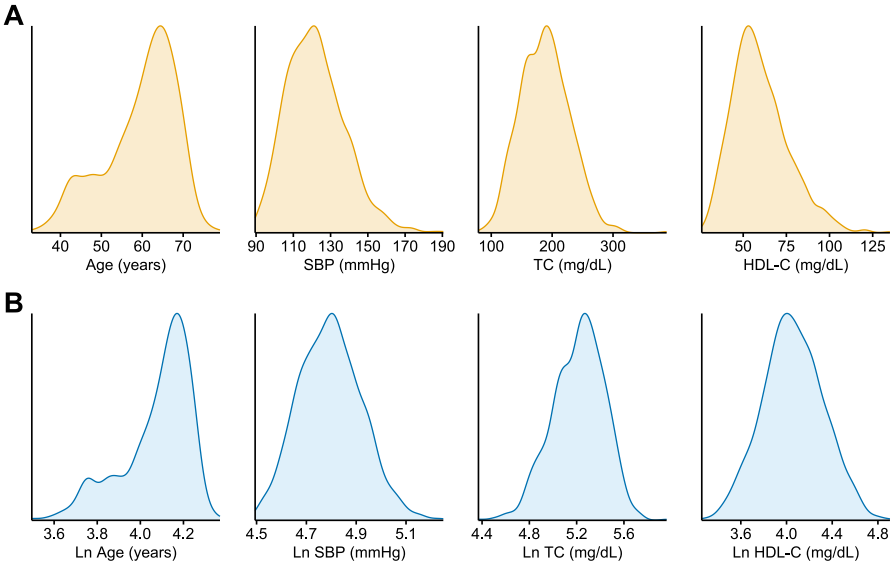

Figure 2. Distributions of continuous traditional risk factors. (A) Before log-transformation. (B) After log-transformation. HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol.

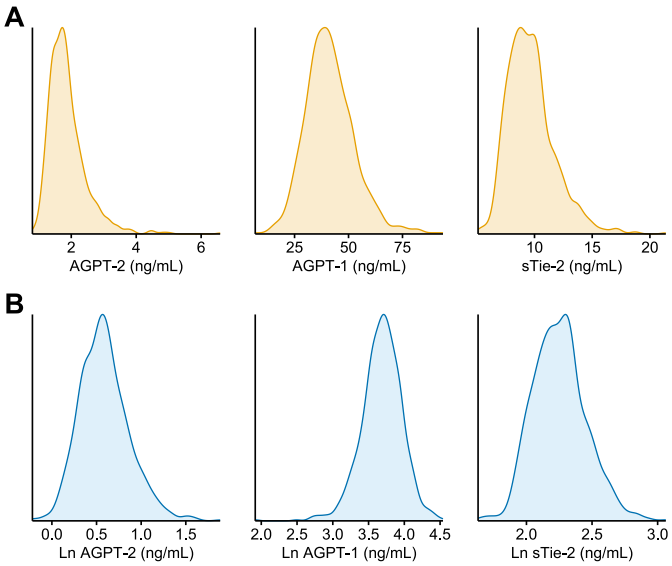

Figure 3. Distributions of serum Angiopoietin-related biomarker levels. (A) Before log-transformation. (B) After log-transformation. AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; sTie-2, soluble tunica interna endothelial cell kinase 2.

Angiopoietin-related biomarkers showed a poor correlation with each other; the Spearman's rho was 0.048 between Angiopoietin-2 and Angiopoietin-1, 0.150 between Angiopoietin-2 and soluble Tie-2, and 0.032 between Angiopoietin-1 and soluble Tie-2 (Figure 4).

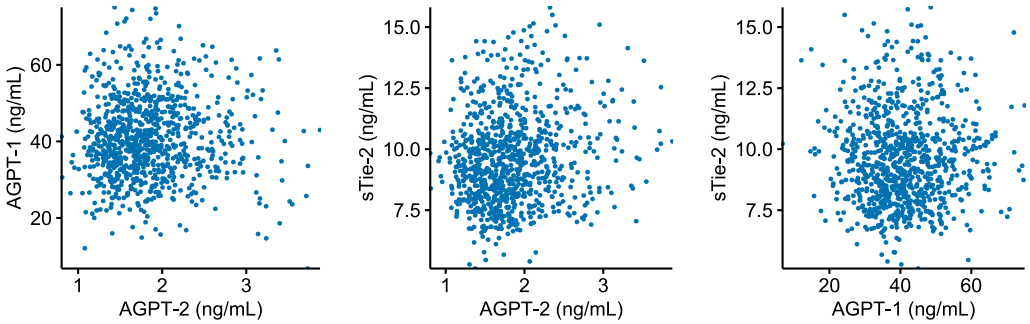

Figure 4. Pairwise correlation between Angiopoietin-related biomarkers. AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; sTie-2, soluble tunica interna endothelial cell kinase 2.

The associations of traditional risk factors with Angiopoietin-related biomarkers were generally weak and heterogeneous: sex, current smoking, and HDL-C level were significantly associated with Angiopoietin-2; age, sex, total cholesterol level, and lipid-lowering drug use were significantly associated with Angiopoietin-1; and age, systolic BP, and HDL-C level were significantly associated with soluble Tie-2 (Table 3).

Table 3. Associations of traditional risk factors with Angiopoietin-related biomarkers

Outcome Predictor	β	(95% confidence interval)
Ln Angiopoietin-2, ng/mL		
Ln Age, years	0.074	(-0.044 to 0.191)
Female sex	0.109	(0.067 to 0.151)
Current smoking	0.069	(0.001 to 0.137)
Ln systolic BP, mmHg	-0.014	(-0.164 to 0.136)
BP-lowering drug use	-0.012	(-0.053 to 0.029)
Diabetes	0.009	(-0.039 to 0.058)
Ln total cholesterol, mg/dL	-0.066	(-0.164 to 0.032)
Ln HDL-C, mg/dL	-0.117	(-0.194 to -0.041)
Lipid-lowering drug use	-0.010	(-0.053 to 0.033)
Ln Angiopoietin-1, ng/mL		
Ln Age, years	-0.212	(-0.332 to -0.093)
Female sex	-0.043	(-0.086 to -0.001)
Current smoking	0.040	(-0.029 to 0.109)
Ln systolic BP, mmHg	0.112	(-0.040 to 0.264)
BP-lowering drug use	-0.006	(-0.047 to 0.035)
Diabetes	0.012	(-0.038 to 0.061)
Ln total cholesterol, mg/dL	0.208	(0.108 to 0.307)
Ln HDL-C, mg/dL	-0.059	(-0.137 to 0.018)
Lipid-lowering drug use	0.052	(0.008 to 0.095)
Ln soluble Tie-2, ng/mL		
Ln Age, years	-0.116	(-0.201 to -0.032)
Female sex	-0.012	(-0.042 to 0.018)
Current smoking	0.002	(-0.047 to 0.050)
Ln systolic BP, mmHg	0.123	(0.015 to 0.230)
BP-lowering drug use	0.009	(-0.020 to 0.038)
Diabetes	0.028	(-0.007 to 0.063)
Ln total cholesterol, mg/dL	0.007	(-0.064 to 0.077)
Ln HDL-C, mg/dL	-0.118	(-0.173 to -0.063)
Lipid-lowering drug use	-0.014	(-0.045 to 0.017)

Multivariable linear regression models were used. BP, blood pressure; HDL-C, high-density lipoprotein cholesterol.

There existed non-linear associations between Angiopoietin-related biomarkers and subclinical atherosclerosis risk (Figure 5). We therefore determined binary cut-offs for the biomarker levels based on Youden's index (Table 4).

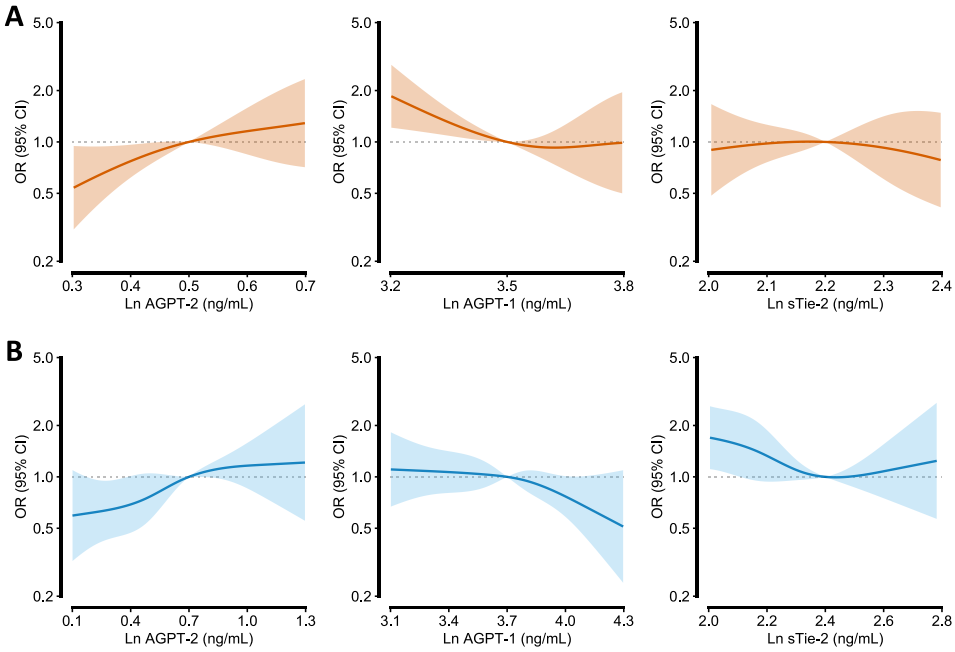

Figure 5. Associations of Angiopoietin-related biomarkers with (A) severe coronary atherosclerosis and (B) coronary atherosclerosis. Restricted cubic spline terms were used with 4 knots at the 5th, 35th, 65th, and 95th percentiles. The models were adjusted for traditional risk factors, subcohort category (1 vs. 2), and the remaining biomarkers. AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; OR, odds ratio; sTie-2, soluble tunica interna endothelial cell kinase 2.

Table 4. Binary cut-offs for Angiopoietin-related biomarkers

Biomarker	Cut-off, ng/mL	
	Severe coronary atherosclerosis	Coronary atherosclerosis
Angiopoietin-2	1.67	1.75
Angiopoietin-1	26.3	44.4
Soluble Tie-2	10.3	8.19

Tie-2, tunica interna endothelial cell kinase 2.

For severe coronary atherosclerosis, the strengths of the associations for Angiopoietin-related biomarkers were as strong as, if not stronger than, those for the traditional risk factors (Table 5). The associations became stronger when the biomarkers were dichotomized using the binary cut-offs (Table 5). The findings were similar for coronary atherosclerosis (Table 6).

Table 5. Associations of Angiopoietin-related biomarkers and traditional risk factors with severe coronary atherosclerosis

Variable	Odds ratio (95% confidence interval)	
	Linear biomarkers	Binary biomarkers
Angiopoietin-2	1.63 (1.23-2.16)	3.46 (1.72-6.98)
Angiopoietin-1	0.74 (0.56-0.97)	0.20 (0.09-0.46)
Soluble Tie-2	0.83 (0.63-1.11)	0.71 (0.38-1.33)
Ln Age, z-score	2.49 (1.57-3.96)	2.58 (1.63-4.07)
Female sex	0.18 (0.09-0.36)	0.17 (0.08-0.34)
Current smoking	0.99 (0.39-2.51)	1.02 (0.40-2.62)
Ln systolic BP, z-score	1.14 (0.84-1.55)	1.12 (0.82-1.53)
BP-lowering drug use	1.76 (0.94-3.29)	1.92 (1.01-3.63)
Diabetes	2.81 (1.51-5.21)	2.65 (1.41-5.01)
Ln total cholesterol, z-score	1.07 (0.77-1.49)	1.06 (0.77-1.48)
Ln HDL-C, z-score	0.89 (0.64-1.25)	0.89 (0.63-1.24)
Lipid-lowering drug use	1.24 (0.64-2.39)	1.32 (0.68-2.57)

Multivariable logistic regression models were used. Continuous variables, including linear biomarkers, were z-transformed to maximize comparability between the variables. BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; Tie-2, tunica interna endothelial cell kinase 2.

Table 6. Associations of Angiopoietin-related biomarkers and traditional risk factors with coronary atherosclerosis

Variable	Odds ratio (95% confidence interval)	
	Linear biomarkers	Binary biomarkers
Angiopoietin-2	1.24 (1.05-1.47)	1.64 (1.19-2.27)
Angiopoietin-1	0.86 (0.74-1.01)	0.64 (0.46-0.90)
Soluble Tie-2	0.86 (0.73-1.01)	0.50 (0.35-0.73)
Ln Age, z-score	2.04 (1.69-2.46)	2.03 (1.68-2.45)
Female sex	0.24 (0.16-0.34)	0.24 (0.16-0.34)
Current smoking	0.83 (0.46-1.50)	0.84 (0.47-1.52)
Ln systolic BP, z-score	1.28 (1.08-1.50)	1.29 (1.09-1.52)
BP-lowering drug use	1.71 (1.22-2.41)	1.77 (1.26-2.50)
Diabetes	2.68 (1.76-4.09)	2.66 (1.74-4.06)
Ln total cholesterol, z-score	1.04 (0.87-1.26)	1.06 (0.88-1.28)
Ln HDL-C, z-score	0.80 (0.67-0.96)	0.78 (0.65-0.94)
Lipid-lowering drug use	1.48 (1.03-2.15)	1.49 (1.02-2.16)

Multivariable logistic regression models were used. Continuous variables, including linear biomarkers, were z-transformed to maximize comparability between the variables. BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; Tie-2, tunica interna endothelial cell kinase 2.

2. Predictive utility of Angiopoietin-related biomarkers

Traditional risk factor-based model for severe coronary atherosclerosis showed good discrimination (C-index, 0.830 [95% CI, 0.785 to 0.876]). Angiopoietin-2, Angiopoietin-1, and soluble Tie-2, in continuous forms, did not significantly improve risk discrimination when added separately to the traditional risk factor-based model (Table 7). However, the discrimination was significantly improved when these biomarkers were added to the model at once (ΔC-index, 0.019 [95% CI, 0.001 to 0.040]). The increase in C-index was even larger when the biomarkers were dichotomized (ΔC-index, 0.030 [95% CI, 0.007 to 0.051]) (Table 7). Angiopoietin-related biomarkers also improved risk discrimination for coronary atherosclerosis, although the degree of improvement was smaller than that for severe coronary atherosclerosis (Table 8).

Table 7. Improvement in risk discrimination for severe coronary atherosclerosis

Biomarker form	Index	TRF model	TRF model + AGPT-2	TRF model + AGPT-1	TRF model + sTie-2	TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	C-index (95% CI)	0.830 (0.785 to 0.876)	0.842 (0.798 to 0.887)	0.837 (0.790 to 0.884)	0.832 (0.787 to 0.877)	0.849 (0.805 to 0.894)
	ΔC-index (95% CI)		0.012 (-0.006 to 0.032)	0.007 (-0.006 to 0.018)	0.002 (-0.002 to 0.006)	0.019 (0.001 to 0.040)
Binary	C-index (95% CI)	0.830 (0.785 to 0.876)	0.851 (0.811 to 0.891)	0.839 (0.790 to 0.887)	0.830 (0.785 to 0.876)	0.860 (0.817 to 0.903)
	ΔC-index (95% CI)		0.021 (0.001 to 0.040)	0.008 (-0.006 to 0.027)	0.000 (-0.001 to 0.001)	0.030 (0.007 to 0.051)

AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Table 8. Improvement in risk discrimination for coronary atherosclerosis

Biomarker form	Index	TRF model	TRF model + AGPT-2	TRF model + AGPT-1	TRF model + sTie-2	TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	C-index (95% CI)	0.798 (0.770 to 0.827)	0.801 (0.773 to 0.829)	0.800 (0.772 to 0.829)	0.800 (0.772 to 0.829)	0.805 (0.777 to 0.833)
	ΔC-index (95% CI)		0.003 (-0.002 to 0.007)	0.002 (-0.002 to 0.006)	0.002 (-0.002 to 0.005)	0.007 (0.000 to 0.015)
Binary	C-index (95% CI)	0.798 (0.770 to 0.827)	0.801 (0.773 to 0.829)	0.803 (0.774 to 0.831)	0.805 (0.777 to 0.833)	0.812 (0.785 to 0.840)
	ΔC-index (95% CI)		0.003 (-0.002 to 0.008)	0.004 (0.000 to 0.009)	0.007 (-0.001 to 0.014)	0.014 (0.004 to 0.025)

AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Angiopoietin-related biomarkers, in continuous forms, also improved risk stratification for severe coronary atherosclerosis (cNRI, 0.46 [95% CI, 0.20 to 0.72]; IDI, 0.037 [95% CI, 0.008 to 0.066]). The improvements were even greater when the biomarkers were dichotomized (cNRI, 0.53 [95% CI, 0.29 to 0.77]; IDI, 0.061 [95% CI, 0.027 to 0.095]) (Table 9). The biomarkers improved risk stratification for coronary atherosclerosis as well, although the degree of improvement was smaller than those for severe coronary atherosclerosis (Table 10).

In subgroup analyses, improvements in risk discrimination for severe coronary atherosclerosis were generally more pronounced in higher-risk subgroups (Table 11), while the trend was less evident for coronary atherosclerosis (Table 12). Improvements in risk stratification for severe coronary atherosclerosis and coronary atherosclerosis are presented in Appendix 3 and Appendix 4, respectively.

Table 9. Improvement in risk stratification for severe coronary atherosclerosis

Form	Index	Added biomarker*			
		AGPT-2	AGPT-1	sTie-2	AGPT-2, AGPT-1, sTie-2
Cont.	cNRI (95% CI)	0.39 (0.13 to 0.65)	0.15 (-0.12 to 0.41)	0.07 (-0.19 to 0.33)	0.46 (0.20 to 0.72)
	IDI (95% CI)	0.024 (0.005 to 0.044)	0.009 (-0.005 to 0.022)	-0.001 (-0.004 to 0.002)	0.037 (0.008 to 0.066)
Bin.	cNRI (95% CI)	0.36 (0.14 to 0.59)	0.46 (0.23 to 0.70)	-0.15 (-0.41 to 0.10)	0.53 (0.29 to 0.77)
	IDI (95% CI)	0.021 (0.004 to 0.039)	0.037 (0.009 to 0.065)	0.000 (-0.001 to 0.001)	0.061 (0.027 to 0.095)

*Added to the traditional risk factor-based model. AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; cNRI, continuous net reclassification improvement; IDI, integrated discrimination improvement; sTie-2, soluble tunica interna endothelial cell kinase 2.

Table 10. Improvement in risk stratification for coronary atherosclerosis

Form	Index	Added biomarker*			
		AGPT-2	AGPT-1	sTie-2	AGPT-2, AGPT-1, sTie-2
Cont.	cNRI (95% CI)	0.18 (0.05 to 0.31)	0.09 (-0.04 to 0.22)	0.10 (-0.03 to 0.23)	0.20 (0.07 to 0.33)
	IDI (95% CI)	0.004 (0.000 to 0.009)	0.004 (0.000 to 0.007)	0.003 (-0.001 to 0.006)	0.011 (0.005 to 0.018)
Bin.	cNRI (95% CI)	0.15 (0.02 to 0.28)	0.14 (0.01 to 0.26)	0.15 (0.04 to 0.26)	0.18 (0.05 to 0.31)
	IDI (95% CI)	0.005 (0.000 to 0.009)	0.005 (0.001 to 0.010)	0.011 (0.004 to 0.018)	0.023 (0.013 to 0.033)

*Added to the traditional risk factor-based model. AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; cNRI, continuous net reclassification improvement; IDI, integrated discrimination improvement; sTie-2, soluble tunica interna endothelial cell kinase 2.

Table 11. Improvement in risk discrimination for severe coronary atherosclerosis across subgroups

Subgroup	N	ΔC-index (95% CI)*
Age <65 years	601	0.025 (-0.001 to 0.080)
Age ≥65 years	323	0.035 (0.000 to 0.076)
Female	559	0.019 (-0.036 to 0.078)
Male	365	0.062 (0.021 to 0.119)
Without hypertension	488	0.033 (0.012 to 0.079)
With hypertension	436	0.047 (0.005 to 0.087)
Without diabetes	756	0.034 (0.000 to 0.065)
With diabetes	168	0.060 (0.005 to 0.135)
Not taking lipid-lowering drug	563	0.011 (-0.013 to 0.025)
Taking lipid-lowering drug	361	0.061 (0.015 to 0.119)
Subcohort 1	750	0.018 (-0.002 to 0.040)
Subcohort 2	174	0.057 (0.008 to 0.120)
10-year ASCVD risk <7.5%	554	0.039 (0.007 to 0.099)
10-year ASCVD risk 7.5-<10%	132	0.044 (-0.022 to 0.313)
10-year ASCVD risk ≥10%	238	0.073 (0.016 to 0.140)

*Obtained after adding binary Angiopoietin-related biomarkers to the traditional risk factor-based models. ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval.

Table 12. Improvement in risk discrimination for coronary atherosclerosis across subgroups

Subgroup	N	ΔC-index (95% CI)*
Age <65 years	601	0.013 (0.002 to 0.024)
Age ≥65 years	323	0.033 (0.007 to 0.062)
Female	559	0.037 (0.015 to 0.066)
Male	365	0.007 (-0.008 to 0.021)
Without hypertension	488	0.018 (-0.001 to 0.037)
With hypertension	436	0.022 (0.002 to 0.045)
Without diabetes	756	0.019 (0.006 to 0.033)
With diabetes	168	0.015 (-0.015 to 0.046)
Not taking lipid-lowering drug	563	0.012 (0.002 to 0.025)
Taking lipid-lowering drug	361	0.023 (-0.004 to 0.050)
Subcohort 1	750	0.019 (0.006 to 0.032)
Subcohort 2	174	0.007 (-0.008 to 0.027)
10-year ASCVD risk <2.5%	152	0.016 (-0.013 to 0.044)
10-year ASCVD risk 2.5-<5%	203	0.016 (-0.022 to 0.053)
10-year ASCVD risk ≥5%	569	0.023 (0.003 to 0.045)

*Obtained after adding binary Angiopoietin-related biomarkers to the traditional risk factor-based models. ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval.

Concerning log-transformed CAC score, Angiopoietin-related biomarkers significantly improved model goodness of fit when added to the traditional risk factor-based model (ΔR^2, 0.011 [95% CI, 0.001 to 0.023]) (Appendix 5). As for coronary atherosclerosis progression, the biomarkers led to numerical improvements in risk discrimination when added to the traditional risk factor-based model, but they did not reach statistical significance (ΔC-index, 0.014 [95% CI, -0.030 to 0.057] in continuous form; 0.025 [95% CI, -0.019 to 0.069] in binary form) (Appendix 6).

In sensitivity analyses, dichotomized Angiopoietin-related biomarkers still improved risk discrimination for severe coronary atherosclerosis and coronary atherosclerosis when (1) the traditional risk factor-based models were expanded to further include weekly minutes of moderate-to-vigorous physical activity, waist circumference, eGFR, and hsCRP (Appendix 7, Appendix 8); and (2) participants with an eGFR <60 mL/min/1.73 m^2 were excluded from the analyses (Appendix 9, Appendix 10).

Angiopoietin-related biomarkers did not improve model calibration when added to the traditional risk factor-based model for severe coronary atherosclerosis—the Hosmer-Lemeshow chi-square statistic was 3.4 in traditional risk factor-based model, 4.9 in continuous biomarker-added model, and 7.8 in binary biomarker-added model (Figure 6). However, the biomarkers did improve model calibration for coronary atherosclerosis—the Hosmer-Lemeshow chi-square statistic was 13.7 in traditional risk factor-based model, 8.4 in continuous biomarker-added model, and 3.4 in binary biomarker-added model (Figure 7).

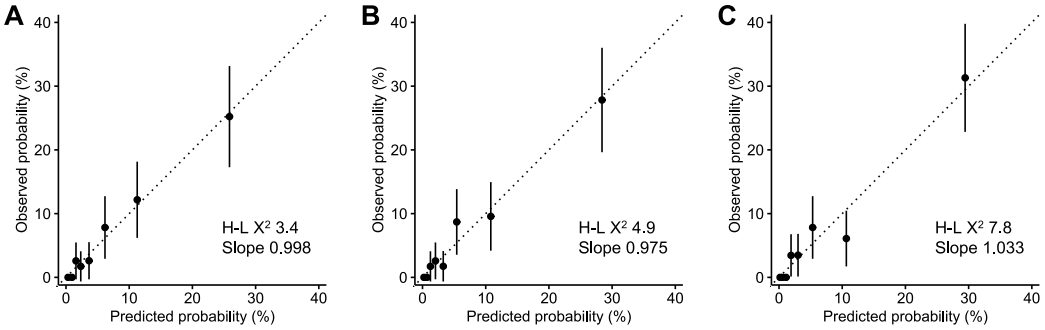

Figure 6. Calibration plots of the models for severe coronary atherosclerosis. (A) Traditional risk factor-based model. (B) Continuous biomarker-added model. (C) Binary biomarker-added model. H-L, Hosmer-Lemeshow.

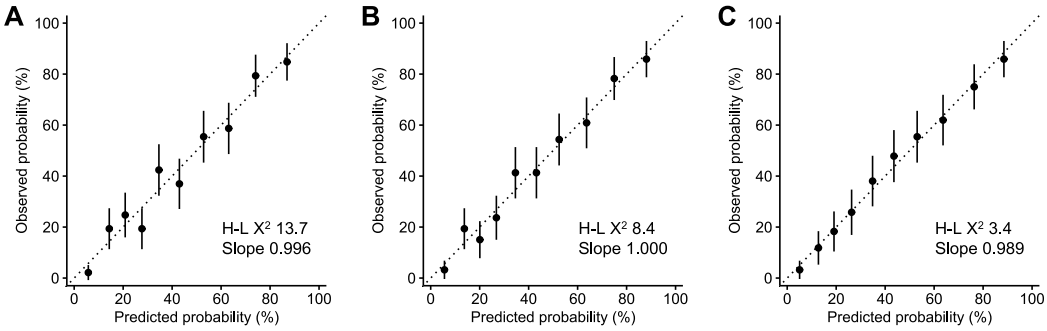

Figure 7. Calibration plots of the models for coronary atherosclerosis. (A) Traditional risk factor-based model. (B) Continuous biomarker-added model. (C) Binary biomarker-added model. H-L, Hosmer-Lemeshow.

3. Angiopoietin-based subclinical atherosclerosis risk prediction models

A. Risk model for severe coronary atherosclerosis

The risk prediction models for severe coronary atherosclerosis were developed separately using continuous and binary biomarkers. The model formulas are as follows:

$$Probability\ of\ having\ severe\ coronary\ atherosclerosis = \frac{e^{LP}}{1 + e^{LP}}$$

$$\begin{aligned} LP_{Continuous} = {} & 5.745 \times Ln\ Age - 1.721 \times Female\ sex - 0.008 \times Current\ smoking \\ & + 1.076 \times Ln\ SBP + 0.563 \times BP\text{-}lowering\ drug\ use + 1.033 \times Diabetes \\ & + 0.323 \times Ln\ TC - 0.429 \times Ln\ HDL\text{-}C + 0.215 \times Lipid\text{-}lowering\ drug\ use \\ & + 1.765 \times Ln\ AGPT\text{-}2 - 1.078 \times Ln\ AGPT\text{-}1 - 0.896 \times Ln\ sTie\text{-}2 \\ & - 24.904 \end{aligned}$$

$$\begin{aligned} LP_{Binary} = {} & 5.950 \times Ln\ Age - 1.776 \times Female\ sex + 0.018 \times Current\ smoking \\ & + 0.908 \times Ln\ SBP + 0.651 \times BP\text{-}lowering\ drug\ use + 0.976 \times Diabetes \\ & + 0.286 \times Ln\ TC - 0.459 \times Ln\ HDL\text{-}C + 0.276 \times Lipid\text{-}lowering\ drug\ use \\ & + 1.242 \times (AGPT\text{-}2 \geq 1.67) - 1.610 \times (AGPT\text{-}1 \geq 26.3) - 0.346 \times (sTie2 \geq 10.3) \\ & - 28.769 \end{aligned}$$

In internal validation, the optimism-corrected C-index of the continuous Angiopoietin-based model was 0.838 (95% CI, 0.765 to 0.900) by bootstrapping and 0.819 (95% CI, 0.711 to 0.903) by 5-fold cross-validation; that of the binary Angiopoietin-based model was 0.849 (95% CI, 0.777 to 0.907) by bootstrapping and 0.827 (95% CI, 0.730 to 0.915) by 5-fold cross-validation (Table 13). Calibration plots showed good concordance between observed and predicted probabilities, with the Hosmer-Lemeshow chi-square statistics of 10.0 and 11.4 for continuous and binary Angiopoietin-based models, respectively (Figure 8A, Figure 8B).

Table 13. C-indexes of risk prediction models for severe coronary atherosclerosis in internal and holdout validation

Validation	Method	C-index (95% confidence interval)	
		Continuous Angiopoietin-based model	Binary Angiopoietin-based model
Internal	Bootstrapping*	0.838 (0.765 to 0.900)	0.849 (0.777 to 0.907)
	5-fold cross-validation†	0.819 (0.711 to 0.903)	0.827 (0.730 to 0.915)
Holdout		0.680 (0.544 to 0.816)	0.678 (0.529 to 0.826)

*Conducted over 1,000 iterations. †Conducted over 200 repetitions.

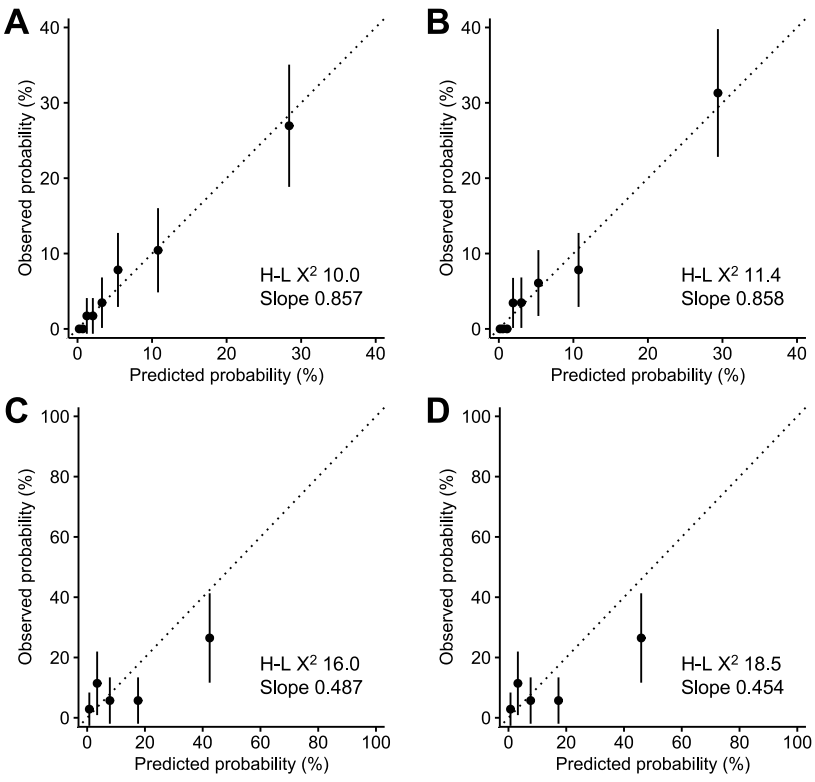

Figure 8. Calibration plots of the risk prediction models for severe coronary atherosclerosis. (A, B) Internal validation. (C, D) Holdout validation. (A, C) Continuous Angiopoietin-based model. (B, D) Binary Angiopoietin-based model. H-L, Hosmer-Lemeshow.

In holdout validation, the C-indexes of the continuous and binary Angiopoietin-based models were 0.680 (95% CI, 0.544 to 0.816) and 0.678 (95% CI, 0.529 to 0.826), respectively (Table 13). The precision of the statistics was low, evidenced by their wide confidence intervals, which was attributable to the small number of severe coronary atherosclerosis cases in the validation set (N=18). Calibration plots showed modest concordance between observed and predicted probabilities, with the Hosmer-Lemeshow chi-square statistics of 16.0 and 18.5 for continuous and binary Angiopoietin-based models, respectively (Figure 8C, Figure 8D).

B. Risk model for coronary atherosclerosis

The risk prediction models for coronary atherosclerosis were developed separately using continuous and binary biomarkers. The model formulas are as follows:

$$Probability\ of\ having\ coronary\ atherosclerosis = \frac{e^{LP}}{1 + e^{LP}}$$

$$\begin{aligned} LP_{Continuous} = {} & 4.480 \times Ln\ Age - 1.444 \times Female\ sex - 0.182 \times Current\ smoking \\ & + 1.943 \times Ln\ SBP + 0.539 \times BP\text{-}lowering\ drug\ use + 0.987 \times Diabetes \\ & + 0.201 \times Ln\ TC - 0.843 \times Ln\ HDL\text{-}C + 0.395 \times Lipid\text{-}lowering\ drug\ use \\ & + 0.783 \times Ln\ AGPT\text{-}2 - 0.517 \times Ln\ AGPT\text{-}1 - 0.772 \times Ln\ sTie\text{-}2 \\ & - 20.660 \end{aligned}$$

$$\begin{aligned} LP_{Binary} = {} & 4.454 \times Ln\ Age - 1.444 \times Female\ sex - 0.170 \times Current\ smoking \\ & + 2.000 \times Ln\ SBP + 0.572 \times BP\text{-}lowering\ drug\ use + 0.979 \times Diabetes \\ & + 0.282 \times Ln\ TC - 0.945 \times Ln\ HDL\text{-}C + 0.397 \times Lipid\text{-}lowering\ drug\ use \\ & + 0.496 \times (AGPT\text{-}2 \geq 1.75) - 0.447 \times (AGPT\text{-}1 \geq 44.4) - 0.684 \times (sTie2 \geq 8.19) \\ & - 23.617 \end{aligned}$$

In internal validation, the optimism-corrected C-index of the continuous Angiopoietin-based model was 0.799 (95% CI, 0.759 to 0.838) by bootstrapping and 0.793 (95% CI, 0.736 to 0.850) by 5-fold cross-validation; that of the binary Angiopoietin-based model was 0.807 (95% CI, 0.767 to 0.844) by bootstrapping and 0.801 (95% CI, 0.741 to 0.852) by 5-fold cross-validation (Table 14). Calibration plots showed excellent concordance between observed and predicted probabilities, with the Hosmer-Lemeshow chi-square statistics of 17.7 and 16.5 for continuous and binary Angiopoietin-based models, respectively (Figure 9A, Figure 9B).

In holdout validation, the C-indexes of the continuous and binary Angiopoietin-based models were 0.791 (95% CI, 0.724 to 0.858) and 0.785 (95% CI, 0.718 to 0.852), respectively (Table 14). Calibration plots showed good concordance between observed and predicted probabilities, with the Hosmer-Lemeshow chi-square statistics of 1.7 and 2.9 for continuous and binary Angiopoietin-based models, respectively (Figure 9C, Figure 9D).

Table 14. C-indexes of risk prediction models for coronary atherosclerosis in internal and holdout validation

Validation	Method	C-index (95% confidence interval)	
		Continuous Angiopoietin-based model	Binary Angiopoietin-based model
Internal	Bootstrapping*	0.799 (0.759 to 0.838)	0.807 (0.767 to 0.844)
	5-fold cross-validation†	0.793 (0.736 to 0.850)	0.801 (0.741 to 0.852)
Holdout		0.791 (0.724 to 0.858)	0.785 (0.718 to 0.852)

*Conducted over 1,000 iterations. †Conducted over 200 repetitions.

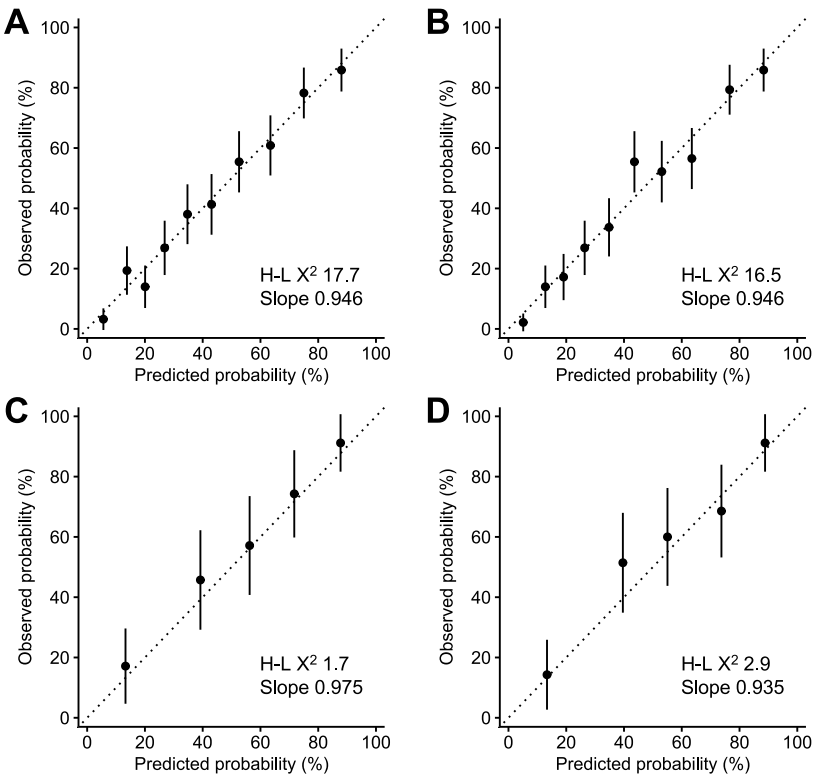

Figure 9. Calibration plots of the risk prediction models for coronary atherosclerosis. (A, B) Internal validation. (C, D) Holdout validation. (A, C) Continuous Angiopoietin-based model. (B, D) Binary Angiopoietin-based model. H-L, Hosmer-Lemeshow.

C. Integer risk score for severe coronary atherosclerosis

The integer risk score for severe coronary atherosclerosis is presented in Table 15. Given the well-established association of total cholesterol level with subclinical atherosclerosis, a total cholesterol ≥240 mg/dL was assigned a score of 1 despite the β coefficient of 0.38.

Table 15. Integer risk score for severe coronary atherosclerosis

Variable	β coefficient	Score
Age ≥65 years	1.83	2
Female sex	-3.47	-3
Current smoking	-0.40	0
Hypertension	1.59	2
Diabetes	1.86	2
Total cholesterol ≥240 mg/dL	0.38	1
HDL-cholesterol <50 mg/dL	0.58	1
Lipid-lowering drug use	0.65	1
Angiopoietin-2 ≥1.67 ng/mL	2.32	2
Angiopoietin-1 ≥26.3 ng/mL	-3.20	-3
Soluble Tie-2 ≥10.3 ng/mL	-0.61	-1
Baseline score		7

HDL, high-density lipoprotein; Tie-2, tunica interna endothelial cell kinase 2.

In internal validation, the C-index of the risk score was 0.835 (95% CI, 0.791 to 0.880) by simple method and 0.837 (95% CI, 0.770 to 0.897) by bootstrapping, suggesting that the risk score was not overfitted. The categorization of risk using the total risk score—very low (0-3), low (4-6), intermediate (7-9), high (10-12), and very high (≥13)—also demonstrated effective risk discrimination (Figure 10A).

D. Integer risk score for coronary atherosclerosis

The integer risk score for coronary atherosclerosis is presented in Table 16. Given the biological implausibility of the protective association between tobacco smoking and subclinical atherosclerosis,[10,11,48] current smoking was assigned a score of 0 despite the β coefficient of -0.72.

Table 16. Integer risk score for coronary atherosclerosis

Variable	β coefficient	Score
Age ≥65 years	2.03	2
Female sex	-2.91	-3
Current smoking	-0.72	0
Hypertension	1.56	2
Diabetes	2.11	2
Total cholesterol ≥240 mg/dL	0.54	1
HDL-cholesterol <50 mg/dL	0.59	1
Lipid-lowering drug use	0.94	1
Angiopoietin-2 ≥1.75 ng/mL	0.99	1
Angiopoietin-1 ≥44.4 ng/mL	-1.12	-1
Soluble Tie-2 ≥8.19 ng/mL	-1.45	-1
Baseline score		5

HDL, high-density lipoprotein; Tie-2, tunica interna endothelial cell kinase 2.

In internal validation, the C-index of the risk score was 0.791 (95% CI, 0.762 to 0.819) by simple method and 0.790 (95% CI, 0.748 to 0.831) by bootstrapping, suggesting that the risk score was not overfitted. The categorization of risk using the integer risk score—very low (0-3), low (4-6), intermediate (7-9), high (10-12), and very high (≥13)—also demonstrated effective risk discrimination (Figure 10B).

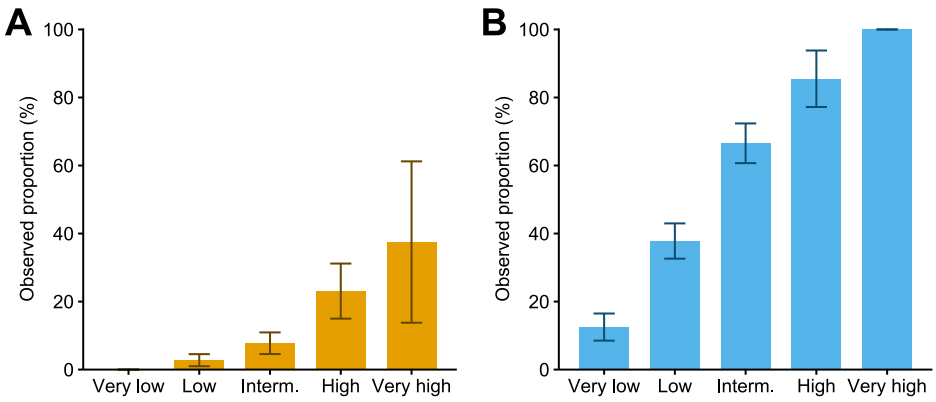

Figure 10. Categorization of subclinical atherosclerosis risk using the integer risk score. (A) Risk categorization for severe coronary atherosclerosis. (B) Risk categorization for coronary atherosclerosis. According to the total risk score, participants were classified into very low (0-3), low (4-6), intermediate (7-9), high (10-12), and very high (≥13) risk groups. Error bars denote 95% confidence intervals.

4. Secondary analysis of carotid atherosclerosis

A total of 772 participants (median age, 56 years; 68% female) were enrolled in the secondary biomarker subcohort—201 (26.0%) from the subcohort 2.5 and 571 (74.0%) from the subcohort 3 (Appendix 1). In the secondary biomarker subcohort, the current smoking rate was 9.2%, 15.7% had diabetes, and the mean carotid IMT was 0.67 mm. Compared with the participants in the subcohort 3, those in the subcohort 2.5 were older, were more likely to be male and currently smoking, exhibited worse cardiometabolic risk profiles, and had higher 10-year atherosclerotic CVD risk and mean carotid IMT (Appendix 11).

Angiopoietin-2, Angiopoietin-1, and soluble Tie-2, in continuous forms, did not significantly improve risk discrimination for carotid atherosclerosis when added separately to the traditional risk factor-based model (Appendix 12). However, the discrimination was

significantly improved when these biomarkers were added to the model at once (ΔC-index, 0.011 [95% CI, 0.001 to 0.021]). The increase in C-index was even larger when the biomarkers were dichotomized (ΔC-index, 0.021 [95% CI, 0.005 to 0.035]) (Appendix 12).

Angiopoietin-related biomarkers, in continuous forms, also improved risk stratification for carotid atherosclerosis (cNRI, 0.15 [95% CI, 0.00 to 0.30]; IDI, 0.010 [95% CI, 0.002 to 0.017]). The improvements were even greater when the biomarkers were dichotomized (cNRI, 0.44 [95% CI, 0.29 to 0.58]; IDI, 0.027 [95% CI, 0.014 to 0.040]) (Appendix 13).

IV. DISCUSSION

1. Principal findings of the study

In this prospective ancillary study of the CMERC and CMERC-HI cohorts, we constructed a biomarker subcohort for exploring Angiopoietin-related biomarkers. The subcohort, which consisted of a total of 924 participants, was broadly representative of the primary prevention population—i.e., those without diagnosed CVD—in Korea. The Angiopoietin-related biomarkers demonstrated a poor correlation with each other, exhibited weak or no association with traditional risk factors, and had a strong, non-linear relationship with the risk of subclinical atherosclerosis. The biomarkers significantly improved risk discrimination and stratification for subclinical atherosclerosis when added to traditional risk factor-based models; the improvements were more pronounced with the biomarkers in binary forms than in their linear forms. The Angiopoietin-based subclinical atherosclerosis risk prediction models were developed and demonstrated good performance, with the C-index ranging from 0.793 to 0.849 and the Hosmer-Lemeshow chi-square statistic ranging from 10.0 to 17.7 in internal validation. The integer risk scores for subclinical atherosclerosis were also developed for enhanced clinical utility and exhibited satisfactory performance, with the C-index ranging between 0.790 and 0.837.

2. Clinical implications of the findings

The newly developed Angiopoietin-based risk prediction models for severe coronary atherosclerosis (i.e., CAC score >400) are expected to help identify high-risk individuals for the primary prevention of CVD.[9] Based on our findings, we estimate that 16 individuals need to be tested for Angiopoietin-related biomarker levels to accurately identify 1 additional case or non-case of severe coronary atherosclerosis.

In a recent study from the multinational CONFIRM (Coronary CT Angiography Evaluation for Clinical Outcomes: An International Multicenter) registry, persons with no history of atherosclerotic CVD and a CAC score >300 exhibited a risk of major adverse cardiovascular events (i.e., all-cause mortality, non-fatal myocardial infarction, or hospitalization for unstable angina) comparable to that of patients with previous atherosclerotic CVD.[49] In the context of CVD prevention, this finding underscores the importance of accurately identifying individuals with a high CAC score (e.g., CAC score >300, >400, or >1000) given their notably high risk of CVD.[6,8,50] However, universal assessment of CAC score in the primary prevention population may not be feasible, cost-effective, or safe.[51] In this sense, our new risk prediction models for severe coronary atherosclerosis may facilitate appropriate candidate selection for CAC score assessment by identifying those with a high pre-test probability of having a CAC score >400.

The 2018 American Heart Association (AHA)/American College of Cardiology (ACC) guideline on the management of blood cholesterol and 2019 ACC/AHA guideline on the primary prevention of CVD recommend deferral of statin therapy for individuals with an intermediate risk of atherosclerotic CVD (i.e., 10-year risk 7.5-<20%) who have a CAC score of 0.[52,53] These recommendations are based on multiple pieces of evidence showing that the risk of CVD is considerably low in persons with a CAC score of 0, for whom the benefits of statin therapy may be limited.[54-56] Although currently not endorsed by the guidelines, a recent study from the MESA (Multi-Ethnic Study of Atherosclerosis) suggested that the evidence-based guidance for those with a CAC score of 0 would be to consider reassessing CAC score in 3 to 7 years, depending on individual demographics and risk profiles.[57] Although such “warranty period of zero” provides some reassurance for deferring statin therapy,[57,58] this does not necessarily imply that individuals with a CAC

score of 0 require no risk surveillance until the reassessment of CAC score. Our Angiopoietin-based risk prediction models for coronary atherosclerosis (i.e., CAC score >0) may help fill this "surveillance gap" and could be employed to monitor individuals' risk of having a CAC score >0 during the period. The optimal methods and interval for monitoring, including the risk cut-off to guide CAC score reassessment, should be determined by further investigations.

3. Future perspectives

The findings of our subgroup analyses hinted at an increased predictive utility of Angiopoietin-related biomarkers for severe coronary atherosclerosis in higher-risk subgroups, including older adults (≥65 years), males, individuals with hypertension or diabetes, those on lipid-lowering drugs, and those with high 10-year atherosclerotic CVD risk. Although hypothesis-generating, these findings are biologically plausible given the roles of Angiopoietin-related biomarkers in maintaining vascular integrity and homeostasis.[19,24] Indeed, previous studies have reported that the levels of Angiopoietin-related biomarkers are frequently altered in cases of vascular endothelial dysfunction, such as myocardial ischemia,[25] cardiogenic shock,[26,27] kidney failure,[28,29] sepsis,[30,31] and acute lung injury.[32,33] Future studies should determine sociodemographic, lifestyle, clinical, and genetic factors associated with the efficacy of Angiopoietin-related biomarkers to maximize their clinical utility.

4. Study strengths and limitations

This study has several distinguishing features. First, to the best of our knowledge, this is the first study to evaluate the utility of Angiopoietin-related biomarkers for predicting the

risk of subclinical atherosclerosis. Second, we focused not only on the presence but also on the severity of subclinical atherosclerosis by investigating both coronary atherosclerosis (defined as CAC score >0) and severe coronary atherosclerosis (defined as CAC score >400). Third, the consistency of the results across secondary measures of subclinical atherosclerosis, including log-transformed CAC score, coronary atherosclerosis progression, and carotid atherosclerosis, further added robustness to our findings. Fourth, our integer risk scores for subclinical atherosclerosis are expected to accelerate the adoption of the study findings into real-world clinical practice.

Our findings should be interpreted in light of certain limitations. First, the sample size was relatively small, reducing the precision of the models' coefficients, performance metrics, and other relevant statistics. This was particularly notable in the holdout validation of the Angiopoietin-based risk prediction models for severe coronary atherosclerosis, where the number of cases was only 18 in the validation set. Second, we could not perform external validation of our risk prediction models. Due to the limited availability of assays for Angiopoietin-related biomarkers, validating these models in external cohorts is expected to be challenging. Third, our analyses were limited to coronary and carotid atherosclerosis. Future studies should investigate whether our findings also pertain to atherosclerosis in other regions, such as aorta or femoral artery.[59] Fourth, the CAC score only represents calcified coronary plaques and does not reflect the presence or extent of non-calcified plaques.[60,61] However, CAC is a well-established surrogate of coronary heart disease and has been reported to have a strong and significant association with the risk of clinical CVD events.[6-8] Fifth, we lacked data on clinical outcomes, including incident atherosclerotic CVD events. Continued follow-up of our biomarker subcohort may enable future exploration of the predictive utility of Angiopoietin-related biomarkers for clinical

CVD events. Sixth, the well-known adverse effects of tobacco smoking on subclinical atherosclerosis were not adequately represented in our risk prediction models,[10,11,48] as indicated by a negative coefficient for current smoking. These findings are presumed to be data-driven; only 77 (8.3%) out of 924 participants were current smokers in our cohort, among whom the number of cases with severe coronary atherosclerosis and coronary atherosclerosis were 7 and 37, respectively. It should be noted that even among the total CMERC and CMERC-HI participants with available data, the smoking status (i.e., current, past, or never smoking) did not show a significant association with either severe coronary atherosclerosis or coronary atherosclerosis (Appendix 14, Appendix 15). Future studies should properly account for the effects of tobacco smoking in their investigation of the predictive utility of Angiopoietin-related biomarkers for subclinical atherosclerosis. Last, since the study cohort consisted entirely of Koreans, our findings may not be directly applicable to other ethnic and/or racial populations.

V. CONCLUSION

For the purpose of exploring Angiopoietin-related biomarkers, we constructed a biomarker subcohort within the CMERC and CMERC-HI cohorts. The Angiopoietin-related biomarkers showed a poor correlation with each other, had weak or no association with traditional risk factors, and demonstrated a strong, non-linear relationship with the risk of subclinical atherosclerosis. The biomarkers significantly improved risk discrimination and stratification for subclinical atherosclerosis when added to traditional risk factor-based models. The Angiopoietin-based subclinical atherosclerosis risk prediction models were developed and yielded promising performance in both internal and holdout validation. The integer risk scores for subclinical atherosclerosis were also developed and demonstrated satisfactory performance. The newly developed prediction models are expected to be utilized for identifying high-risk populations for the primary prevention of CVD and for surveilling individuals with a CAC score of 0. Whether Angiopoietin-related biomarkers can improve risk prediction for clinical CVD events needs to be determined in future studies.

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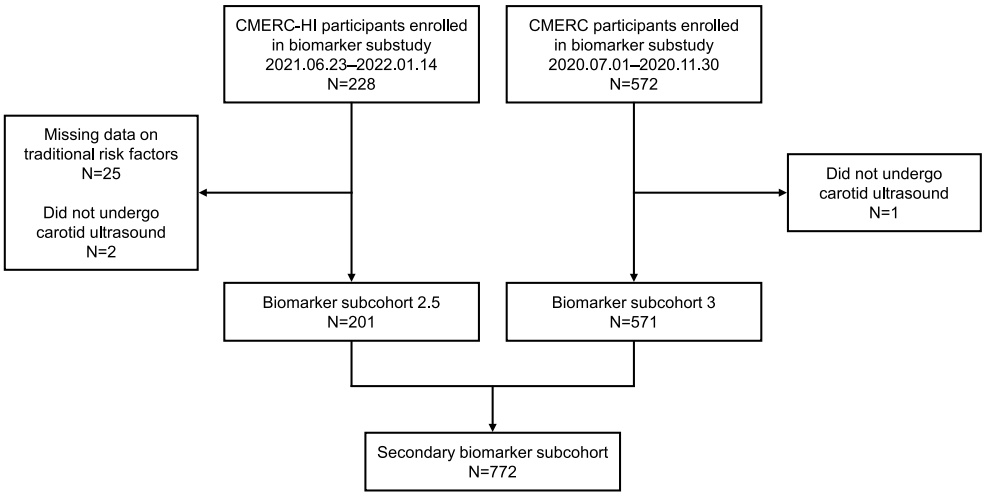
APPENDICES



Appendix 1. Study flowchart for the secondary analysis. CMERC, Cardiovascular and Metabolic Diseases Etiology Research Center; CMERC-HI, Cardiovascular and Metabolic Diseases Etiology Research Center High-Risk.

Appendix 2. Baseline characteristics of the included and excluded participants

Variable	CMERC		CMERC-HI	
	Included (N= 750)	Excluded (N=7,347)	Included (N=174)	Excluded (N=3,093)
Age, years	55 [48-58]	53 [45-58]	59 [50-65]	61 [52-69]
Female	488 (65.1)	4,801 (65.3)	71 (40.8)	1,402 (45.3)
Current smoking	77 (10.3)	983 (13.4)	20 (11.5)	419 (13.6)
Systolic BP, mm Hg	116 [108-126]	118 [108-128]	122 [114-132]	126 [116-136]
Diastolic BP, mm Hg	74 [69-81]	75 [69-82]	75 [70-82]	76 [68-82]
BP-lowering drug use	124 (16.5)	1,200 (16.3)	137 (78.7)	2,336 (75.5)
Diabetes	40 (5.3)	586 (8.0)	70 (40.2)	1,325 (42.8)
Total cholesterol, mg/dL	198 [171-221]	193 [171-217]	168 [144-188]	169 [146-195]
HDL-cholesterol, mg/dL	55 [46-66]	54 [46-64]	48 [42-55]	47 [40-57]
Lipid-lowering drug use	101 (13.5)	811 (11.0)	89 (51.1)	1,249 (40.4)
10-year ASCVD risk, %				
<2.5	266 (35.5)	2,836 (38.6)	26 (14.9)	421 (13.6)
2.5-<5	281 (37.5)	2,511 (34.2)	38 (21.8)	502 (16.2)
5-<7.5	136 (18.1)	1,262 (17.2)	31 (17.8)	410 (13.3)
7.5-<10	53 (7.1)	461 (6.3)	18 (10.3)	357 (11.5)
≥10	14 (1.9)	277 (3.8)	61 (35.1)	1,404 (45.4)

Values are presented as median [interquartile range] or number (%). ASCVD, atherosclerotic cardiovascular disease; BP, blood pressure; CMERC, Cardiovascular and Metabolic Diseases Etiology Research Center; CMERC-HI, Cardiovascular and Metabolic Diseases Etiology Research Center High-Risk; HDL, high-density lipoprotein.

Appendix 3. Improvement in risk stratification for severe coronary atherosclerosis across subgroups

Subgroup	N	cNRI (95% CI)*	IDI (95% CI)*
Age <65 years	601	0.61 (0.23 to 0.99)	0.101 (0.029 to 0.173)
Age ≥65 years	323	0.53 (0.21 to 0.85)	0.044 (0.009 to 0.080)
Female	559	0.61 (0.15 to 1.06)	0.038 (-0.001 to 0.077)
Male	365	0.58 (0.29 to 0.88)	0.089 (0.036 to 0.142)
Without hypertension	488	0.76 (0.33 to 1.19)	0.083 (0.003 to 0.164)
With hypertension	436	0.45 (0.14 to 0.75)	0.064 (0.021 to 0.106)
Without diabetes	756	0.56 (0.30 to 0.81)	0.054 (0.020 to 0.088)
With diabetes	168	0.35 (-0.05 to 0.76)	0.100 (0.024 to 0.175)
Not taking lipid-lowering drug	563	0.48 (0.16 to 0.79)	0.046 (0.008 to 0.084)
Taking lipid-lowering drug	361	0.61 (0.25 to 0.97)	0.076 (0.019 to 0.134)
Subcohort 1	750	0.71 (0.43 to 1.00)	0.064 (0.016 to 0.112)
Subcohort 2	174	0.72 (0.26 to 1.18)	0.068 (0.002 to 0.133)
10-year ASCVD risk <7.5%	554	1.20 (0.77 to 1.64)	0.091 (0.002 to 0.179)
10-year ASCVD risk 7.5-<10%	132	0.42 (-0.22 to 1.06)	0.010 (-0.048 to 0.068)
10-year ASCVD risk ≥10%	238	0.56 (0.23 to 0.88)	0.068 (0.021 to 0.115)

*Obtained after adding binary Angiopoietin-related biomarkers to the traditional risk factor-based models. ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval; cNRI, continuous net reclassification improvement; IDI, integrated discrimination improvement.

Appendix 4. Improvement in risk stratification for coronary atherosclerosis across subgroups

Subgroup	N	cNRI (95% CI)*	IDI (95% CI)*
Age <65 years	601	0.25 (0.08 to 0.42)	0.016 (0.005 to 0.026)
Age ≥65 years	323	0.30 (0.08 to 0.52)	0.051 (0.027 to 0.076)
Female	559	0.31 (0.13 to 0.49)	0.046 (0.027 to 0.065)
Male	365	0.23 (0.02 to 0.44)	0.010 (-0.001 to 0.022)
Without hypertension	488	0.35 (0.16 to 0.54)	0.025 (0.009 to 0.042)
With hypertension	436	0.29 (0.10 to 0.48)	0.036 (0.018 to 0.053)
Without diabetes	756	0.23 (0.09 to 0.38)	0.027 (0.015 to 0.039)
With diabetes	168	0.13 (-0.19 to 0.46)	0.021 (0.000 to 0.043)
Not taking lipid-lowering drug	563	0.39 (0.23 to 0.56)	0.023 (0.010 to 0.036)
Taking lipid-lowering drug	361	0.25 (0.05 to 0.46)	0.033 (0.015 to 0.052)
Subcohort 1	750	0.21 (0.07 to 0.36)	0.027 (0.015 to 0.040)
Subcohort 2	174	0.10 (-0.20 to 0.40)	0.018 (-0.001 to 0.036)
10-year ASCVD risk <2.5%	152	0.41 (-0.03 to 0.86)	0.061 (-0.005 to 0.127)
10-year ASCVD risk 2.5-<5%	203	0.39 (0.07 to 0.70)	0.026 (0.003 to 0.049)
10-year ASCVD risk ≥5%	569	0.22 (0.06 to 0.38)	0.037 (0.021 to 0.053)

*Obtained after adding binary Angiopoietin-related biomarkers to the traditional risk factor-based models. ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval; cNRI, continuous net reclassification improvement; IDI, integrated discrimination improvement.

Appendix 5. Improvement in model goodness of fit for log-transformed coronary artery calcium score

Biomarker form	Index	TRF model	TRF model + AGPT-2	TRF model + AGPT-1	TRF model + sTie-2	TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	R^2 (95% CI)	0.274	0.281	0.277	0.275	0.285
	ΔR^2 (95% CI)		0.007 (0.000 to 0.020)	0.003 (-0.001 to 0.013)	0.000 (-0.001 to 0.007)	0.011 (0.001 to 0.023)

AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Appendix 6. Improvement in risk discrimination for coronary atherosclerosis progression

Biomarker form	Index	TRF model	TRF model + AGPT-2	TRF model + AGPT-1	TRF model + sTie-2	TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	C-index (95% CI)	0.719 (0.640 to 0.797)	0.718 (0.640 to 0.796)	0.713 (0.635 to 0.791)	0.726 (0.648 to 0.804)	0.732 (0.656 to 0.808)
	ΔC-index (95% CI)		-0.001 (-0.014 to 0.010)	-0.006 (-0.038 to 0.021)	0.007 (-0.024 to 0.035)	0.014 (-0.030 to 0.057)
Binary	C-index (95% CI)	0.719 (0.640 to 0.797)	0.733 (0.656 to 0.810)	0.716 (0.638 to 0.794)	0.736 (0.660 to 0.812)	0.744 (0.669 to 0.819)
	ΔC-index (95% CI)		0.014 (-0.014 to 0.041)	-0.003 (-0.028 to 0.023)	0.017 (-0.014 to 0.053)	0.025 (-0.019 to 0.069)

Only participants with available data on follow-up coronary artery calcium score (N=173) were included. AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Appendix 7. Improvement in risk discrimination for severe coronary atherosclerosis using expanded definition of TRF

Biomarker form	Index	Exp. TRF model	Exp. TRF model + AGPT-2	Exp. TRF model + AGPT-1	Exp. TRF model + sTie-2	Exp. TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	C-index (95% CI)	0.845 (0.798 to 0.892)	0.851 (0.806 to 0.896)	0.848 (0.799 to 0.897)	0.845 (0.798 to 0.892)	0.856 (0.809 to 0.902)
	ΔC-index (95% CI)		0.006 (-0.007 to 0.017)	0.003 (-0.008 to 0.014)	0.000 (-0.002 to 0.002)	0.011 (-0.007 to 0.026)
Binary	C-index (95% CI)	0.806 (0.777 to 0.834)	0.860 (0.819 to 0.902)	0.850 (0.801 to 0.899)	0.844 (0.797 to 0.892)	0.867 (0.823 to 0.910)
	ΔC-index (95% CI)		0.016 (-0.001 to 0.033)	0.005 (-0.007 to 0.021)	0.000 (-0.001 to 0.000)	0.022 (0.002 to 0.041)

Expanded TRF model additionally included weekly minutes of moderate-to-vigorous physical activity, waist circumference, estimated glomerular filtration rate, and log-transformed high-sensitivity C-reactive protein. AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Appendix 8. Improvement in risk discrimination for coronary atherosclerosis using expanded definition of TRF

Biomarker form	Index	Exp. TRF model	Exp. TRF model + AGPT-2	Exp. TRF model + AGPT-1	Exp. TRF model + sTie-2	Exp. TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	C-index (95% CI)	0.806 (0.777 to 0.834)	0.807 (0.778 to 0.835)	0.808 (0.780 to 0.837)	0.807 (0.779 to 0.835)	0.811 (0.783 to 0.839)
	ΔC-index (95% CI)		0.001 (-0.003 to 0.005)	0.003 (-0.001 to 0.006)	0.001 (-0.002 to 0.004)	0.005 (-0.001 to 0.012)
Binary	C-index (95% CI)	0.806 (0.777 to 0.834)	0.807 (0.779 to 0.835)	0.809 (0.781 to 0.838)	0.811 (0.783 to 0.839)	0.817 (0.790 to 0.845)
	ΔC-index (95% CI)		0.001 (-0.003 to 0.005)	0.004 (-0.001 to 0.008)	0.005 (-0.001 to 0.012)	0.011 (0.002 to 0.022)

Expanded TRF model additionally included weekly minutes of moderate-to-vigorous physical activity, waist circumference, estimated glomerular filtration rate, and log-transformed high-sensitivity C-reactive protein. AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Appendix 9. Improvement in risk discrimination for severe coronary atherosclerosis among participants with eGFR ≥60

Biomarker form	Index	TRF model	TRF model + AGPT-2	TRF model + AGPT-1	TRF model + sTie-2	TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	C-index (95% CI)	0.821 (0.770 to 0.872)	0.830 (0.782 to 0.879)	0.827 (0.776 to 0.877)	0.821 (0.770 to 0.872)	0.837 (0.788 to 0.885)
	ΔC-index (95% CI)		0.010 (-0.008 to 0.030)	0.006 (-0.003 to 0.016)	0.000 (-0.003 to 0.002)	0.016 (-0.004 to 0.040)
Binary	C-index (95% CI)	0.821 (0.770 to 0.872)	0.844 (0.801 to 0.888)	0.827 (0.773 to 0.880)	0.822 (0.771 to 0.873)	0.849 (0.803 to 0.895)
	ΔC-index (95% CI)		0.024 (-0.001 to 0.047)	0.006 (-0.006 to 0.026)	0.002 (-0.005 to 0.008)	0.028 (0.003 to 0.057)

AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; eGFR, estimated glomerular filtration rate; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Appendix 10. Improvement in risk discrimination for coronary atherosclerosis among participants with eGFR ≥60

Biomarker form	Index	TRF model	TRF model + AGPT-2	TRF model + AGPT-1	TRF model + sTie-2	TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	C-index (95% CI)	0.784 (0.754 to 0.815)	0.786 (0.756 to 0.817)	0.786 (0.756 to 0.817)	0.786 (0.756 to 0.817)	0.792 (0.762 to 0.822)
	ΔC-index (95% CI)		0.002 (-0.003 to 0.007)	0.002 (-0.002 to 0.007)	0.002 (-0.002 to 0.007)	0.008 (0.000 to 0.016)
Binary	C-index (95% CI)	0.784 (0.754 to 0.815)	0.787 (0.757 to 0.817)	0.789 (0.758 to 0.819)	0.791 (0.761 to 0.821)	0.799 (0.770 to 0.829)
	ΔC-index (95% CI)		0.003 (-0.003 to 0.008)	0.004 (-0.001 to 0.010)	0.007 (-0.001 to 0.015)	0.015 (0.004 to 0.027)

AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; eGFR, estimated glomerular filtration rate; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Appendix 11. Baseline characteristics of the participants in secondary biomarker subcohort

Variable	Secondary biomarker subcohort (N=772)	Biomarker subcohort 2.5 (N=201)	Biomarker subcohort 3 (N=571)
Age, years	56 [46-60]	58 [50-65]	55 [46-59]
Female	523 (67.7)	75 (37.3)	448 (78.5)
Current smoking	71 (9.2)	24 (11.9)	47 (8.2)
Systolic BP, mm Hg	118 [108-129]	123 [115-134]	115 [106-127]
Diastolic BP, mm Hg	73 [68-80]	75 [70-82]	72 [68-79]
BP-lowering drug use	253 (32.8)	158 (78.6)	95 (16.6)
Diabetes	121 (15.7)	77 (38.3)	44 (7.7)
Total cholesterol, mg/dL	190 [165-216]	167 [144-188]	199 [175-222]
HDL-cholesterol, mg/dL	55 [46-66]	48 [41-55]	58 [48-68]
Lipid-lowering drug use	175 (22.7)	96 (47.8)	79 (13.8)
10-year ASCVD risk, %			
<2.5	253 (32.8)	31 (15.4)	222 (38.9)
2.5-<5	233 (30.2)	45 (22.4)	188 (32.9)
5-<7.5	141 (18.3)	36 (17.9)	105 (18.4)
7.5-<10	55 (7.1)	22 (10.9)	33 (5.8)
≥10	90 (11.7)	67 (33.3)	23 (4.0)
Angiopoietin-2, ng/mL	1.8 [1.4-2.2]	1.8 [1.5-2.1]	1.8 [1.4-2.2]
Angiopoietin-1, ng/mL	45.3 [37.1-53.0]	39.0 [32.6-47.2]	47.1 [39.4-54.8]
Soluble Tie-2, ng/mL	11.9 [10.3-14.0]	10.6 [9.1-12.5]	12.3 [10.7-14.5]
Maximum cIMT, mm	0.78 [0.70-0.88]	0.92 [0.76-1.00]	0.76 [0.69-0.85]
Mean cIMT, mm	0.67 [0.60-0.76]	0.74 [0.62-0.93]	0.66 [0.59-0.74]

Values are presented as median [interquartile range] or number (%). ASCVD, atherosclerotic cardiovascular disease; BP, blood pressure; cIMT, carotid intima-media thickness; HDL, high-density lipoprotein; Tie-2, tunica interna endothelial cell kinase 2.

Appendix 12. Improvement in risk discrimination for carotid atherosclerosis

Biomarker form	Index	TRF model	TRF model + AGPT-2	TRF model + AGPT-1	TRF model + sTie-2	TRF model + AGPT-2 + AGPT-1 + sTie-2
Continuous	C-index (95% CI)	0.765 (0.732 to 0.799)	0.768 (0.735 to 0.801)	0.770 (0.737 to 0.803)	0.767 (0.734 to 0.800)	0.776 (0.743 to 0.809)
	ΔC-index (95% CI)		0.003 (-0.002 to 0.007)	0.005 (-0.002 to 0.011)	0.002 (-0.003 to 0.007)	0.011 (0.001 to 0.021)
Binary	C-index (95% CI)	0.765 (0.732 to 0.799)	0.769 (0.736 to 0.802)	0.772 (0.739 to 0.804)	0.773 (0.741 to 0.806)	0.786 (0.754 to 0.818)
	ΔC-index (95% CI)		0.004 (-0.003 to 0.010)	0.006 (-0.004 to 0.017)	0.008 (-0.001 to 0.018)	0.021 (0.005 to 0.035)

AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; sTie-2, soluble tunica interna endothelial cell kinase 2; TRF, traditional risk factor.

Appendix 13. Improvement in risk stratification for carotid atherosclerosis

Form	Index	Added biomarker*			
		AGPT-2	AGPT-1	sTie-2	AGPT-2, AGPT-1, sTie-2
Cont.	cNRI (95% CI)	0.02 (-0.13 to 0.17)	0.16 (0.01 to 0.31)	0.08 (-0.07 to 0.23)	0.15 (0.00 to 0.30)
	IDI (95% CI)	0.002 (0.000 to 0.005)	0.004 (-0.001 to 0.008)	0.002 (-0.002 to 0.005)	0.010 (0.002 to 0.017)
Bin.	cNRI (95% CI)	0.21 (0.08 to 0.33)	0.38 (0.23 to 0.53)	0.14 (0.00 to 0.28)	0.44 (0.29 to 0.58)
	IDI (95% CI)	0.005 (0.000 to 0.009)	0.010 (0.002 to 0.019)	0.009 (0.001 to 0.016)	0.027 (0.014 to 0.040)

*Added to the traditional risk factor-based model.

AGPT-1, Angiopoietin-1; AGPT-2, Angiopoietin-2; CI, confidence interval; cNRI, continuous net reclassification improvement; IDI, integrated discrimination improvement; sTie-2, soluble tunica interna endothelial cell kinase 2.

Appendix 14. Association of smoking status with severe coronary atherosclerosis in primary biomarker subcohort and in total cohort

Variable	Odds ratio (95% confidence interval)	
	Biomarker subcohort (N=924)	Total cohort* (N=3,360)
Smoking status		
Never	1.00 (reference)	1.00 (reference)
Past	0.84 (0.40-1.76)	1.01 (0.73-1.38)
Current	1.01 (0.36-2.80)	1.28 (0.87-1.88)
Ln Age, z-score	2.46 (1.59-3.81)	1.76 (1.53-2.03)
Female sex	0.20 (0.09-0.46)	0.44 (0.32-0.60)
Ln SBP, z-score	1.16 (0.86-1.57)	1.22 (1.09-1.35)
BP-lowering drug use	1.68 (0.91-3.10)	1.63 (1.26-2.10)
Diabetes	2.76 (1.51-5.05)	2.17 (1.74-2.72)
Ln TC, z-score	1.03 (0.75-1.41)	0.81 (0.72-0.92)
Ln HDL-C, z-score	0.87 (0.63-1.20)	1.01 (0.90-1.14)
Lipid-lowering drug use	1.23 (0.65-2.33)	1.04 (0.83-1.30)

Multivariable logistic regression models were used. *Total participants in CMERC and CMERC-HI with available data. BP, blood pressure; CMERC, Cardiovascular and Metabolic Diseases Etiology Research Center; CMERC-HI, Cardiovascular and Metabolic Diseases Etiology Research Center High-Risk; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol.

Appendix 15. Association of smoking status with coronary atherosclerosis in primary biomarker subcohort and in total cohort

Variable	Odds ratio (95% confidence interval)	
	Biomarker subcohort (N=924)	Total cohort* (N=3,360)
Smoking status		
Never	1.00 (reference)	1.00 (reference)
Past	1.31 (0.78-2.19)	1.16 (0.90-1.48)
Current	1.01 (0.53-1.94)	1.34 (0.99-1.82)
Ln Age, z-score	2.14 (1.78-2.58)	2.09 (1.90-2.29)
Female sex	0.32 (0.20-0.51)	0.50 (0.39-0.63)
Ln SBP, z-score	1.25 (1.06-1.47)	1.30 (1.20-1.41)
BP-lowering drug use	1.67 (1.19-2.33)	1.59 (1.34-1.88)
Diabetes	2.54 (1.68-3.85)	2.42 (2.03-2.87)
Ln TC, z-score	1.01 (0.84-1.21)	0.94 (0.86-1.03)
Ln HDL-C, z-score	0.80 (0.67-0.96)	0.91 (0.83-0.99)
Lipid-lowering drug use	1.44 (1.00-2.08)	1.26 (1.07-1.49)

Multivariable logistic regression models were used. *Total participants in CMERC and CMERC-HI with available data. BP, blood pressure; CMERC, Cardiovascular and Metabolic Diseases Etiology Research Center; CMERC-HI, Cardiovascular and Metabolic Diseases Etiology Research Center High-Risk; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol.

ABSTRACT (IN KOREAN)

새로운 바이오마커를 이용한 죽상경화증의 예측

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이 혁 희

서론: 안지오포이에틴-1은 혈관내피세포의 Tie-2 수용체와 결합하여 혈관의 안정성과 항상성을 증대하며, 안지오포이에틴-2는 위 작용을 억제한다. 이러한 분자생물학적 기전 상 안지오포이에틴-2, 안지오포이에틴-1 및 수용성 Tie-2는 죽상경화증 예측 바이오마커로 활용될 잠재력이 있다. 우리는 본 연구에서 (1) 안지오포이에틴 연관 바이오마커(안지오포이에틴-2, 안지오포이에틴-1 및 수용성 Tie-2) 연구를 위한 코호트를 구축하고, (2) 해당 바이오마커들의 기본 특성을 파악하며, (3) 해당 바이오마커들이 죽상경화증 위험도 예측에 도움이 될 수 있는지 확인하고, (4) 해당 바이오마커들을 활용한 죽상경화증 위험도 예측모형을 개발하고자 하였다.

연구방법: 2021년부터 2023년 사이 CMERC 및 CMERC-HI 코호트로부터 총 924명의 대상자가 모집되었으며, 검진을 통해 대상자들의 전통적 심혈관 위험인자(나이, 성별, 현재흡연, 수축기혈압, 혈압강하제 복용, 당뇨병, 총콜레스테롤, 고밀도지단백콜레스테롤 및 지질강하제 복용), 혈중 안지오포이에틴 연관 바이오마커 농도 및 관상동맥 석회화지수 등의 정보를 수집하였다. 죽상경화증은 중증 관상동맥 죽상경화증(관상동맥 석회화지수 >400)과 관상동맥 죽상경화증(관상동맥 석회화지수 >0)의 두 가지 지표로 정의되었다. 죽상경화증 예측 로지스틱 회귀모형의 성능은 C-지수, 호스머-레메쇼 카이제곱 통계치 및 일치도 그림 등의 방법으로 평가하였으며, 안지오포이에틴 연관 바이오마커에 의한 예측능 향상은 ΔC-지수, 연속형 순재분류향상 및 통합식별향상 등으로 평가하였다. 최종 개발된 죽상경화증 위험도 예측모형에 대해서는 내적 검증 및 홀드아웃 검증을 시행하였다.

연구결과: 총 924명의 대상자(나이 중위수, 62세; 60.5% 여성) 중 중증 관상동맥 죽상경화증은 60명(6.5%), 관상동맥 죽상경화증은 390명(42.2%)에서 존재하였다. 안지오포이에틴 연관 바이오마커들은 서로 미약한 상관관계를 보였으며, 전통적 심혈관 위험인자와는 유의한 연관성을 보이지 않았다. 해당 바이오마커들은 전통적 심혈관 위험인자 기반 회귀모형의 중증 관상동맥 죽상경화증 위험도 예측력(ΔC-지수, 0.019-0.030; 연속형 순재분류향상, 0.46-0.53; 통합식별향상, 0.037-0.061)과 관상동맥 죽상경화증 위험도 예측력(ΔC-지수, 0.007-0.014; 연속형 순재분류향상, 0.18-0.20; 통합식별향상, 0.011-0.023)을 모두 향상시켰다. 최종 개발된 안지오포이에틴 기반 죽상경화증 위험도 예측모형은 내적 검증에서 우수한 식별력을 보였으며(C-지수 0.793-0.849), 홀드아웃 검증에서도 준수한 식별력을 보였다(C-지수 0.678-0.791). 일치도 그림 상 모형의 예측 위험도는 실제 위험도와 전반적으로 잘 일치하였다. 간편한 임상 적용을 위해 추가로 개발한 죽상경화증 위험도 점수 역시 좋은 식별력을 보였다(C-지수 0.790-0.837).

결론: 안지오포이에틴 연관 바이오마커들은 전통적 심혈관 위험인자 기반 회귀모형의 죽상경화증 위험도 예측력을 유의하게 향상시켰으며, 최종 개발된 안지오포이에틴 기반 죽상경화증 위험도 예측모형은 검증 결과 우수한 성능을 보였다. 본 연구에서 개발한 예측모형은 심혈관질환 일차예방을 위한 고위험 인구 선별 및 관상동맥 석회화지수 0인 대상자의 위험도 감시에 이용될 수 있을 것으로 기대된다. 안지오포이에틴 연관 바이오마커들이 임상 심혈관질환 사건 예측에도 활용될 수 있을지에 대해서는 추가적인 연구가 필요하다.

핵심되는 말: 안지오포이에틴; 죽상경화증; 관상동맥 석회화; 예측

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