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Biomarkers of Cardiovascular Stress and Subclinical Atherosclerosis in the Community

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BACKGROUND: Biomarkers of cardiovascular stress have been associated with incident cardiovascular outcomes. Their relations with measures of subclinical atherosclerosis, as assessed by carotid intima-media thickness, have not been well described.

METHODS: We measured plasma growth differentiation factor-15 (GDF-15), soluble ST2 (sST2), and high-sensitivity troponin I (hsTnI) in 3111 Framingham Offspring participants who also underwent carotid ultrasonography during the sixth examination (1995–1998, mean age 58 years, 54% women). Carotid measurements included maximal internal carotid artery (ICA) intima-media thickness (IMT), plaque presence (defined as ICA IMT >1.5 mm), and mean common carotid artery IMT. We carried out multivariable regressions for carotid measurements vs biomarkers using linear and logistic models; $P < 0.0056$ was deemed statistically significant.

RESULTS: Maximal ICA IMT was significantly associated with plasma GDF-15 [β-estimate 0.04 per 1-U increase in log(GDF-15), SE 0.01, $P < 0.0001$]. Similarly, the odds of having carotid plaque increased 33% [odds ratio 1.33 per 1-U increase in log(GDF-15), 95% CI 1.20–1.48, $P < 0.0001$]. In contrast, there was no significant association of maximal ICA IMT or plaque presence with sST2 or hsTnI, and none of the 3 biomarkers was significantly associated with mean CCA IMT. GDF-15 was a stronger predictor of maximal ICA thickness and plaque presence compared with BNP and CRP when these conventional biomarkers were tested together.

CONCLUSIONS: Increased GDF-15 concentrations are associated with subclinical atherosclerosis, including maximal ICA IMT and carotid plaque presence. Future studies investigating the role of GDF-15 for screening and management of patients with subclinical atherosclerosis are warranted.

Carotid ultrasonography, via carotid intima-media thickness (IMT)[11] assessment, not only has been well established as a surrogate of generalized atherosclerosis *(1, 2)* and linked with cardiovascular risk factors *(3, 4)*, but also has been shown to be a predictor for myocardial infarction, stroke, coronary heart disease, and combined cardiovascular outcomes *(5–7)*. In addition, carotid IMT has been shown to track subclinical atherosclerosis and help improve classification of cardiovascular risk in asymptomatic cohorts *(4, 8)*.

Recently, biomarkers of cardiovascular stress, including growth differentiation factor-15 (GDF-15), soluble ST2 (sST2), and high-sensitivity troponin I (hsTnI), have emerged as strong predictors of cardiovascular risk and outcomes in various community-based populations *(9–12)*. The exact mechanism by which these biomarkers are linked to cardiovascular disease, particularly in asymptomatic, population-based cohorts, are incompletely described to date.

Our group has previously demonstrated the longitudinal association of GDF-15, sST2, and hsTnI to incident cardiovascular outcomes in the community *(11)*. Given that all 3 biomarkers have been previously

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[11] Nonstandard abbreviations: IMT, intima-media thickness; GDF-15, growth differentiation factor-15; sST2, soluble ST2; hsTnI, high-sensitivity troponin I; BNP, B-type natriuretic peptide; CRP, C-reactive protein; CCA, common carotid artery; ICA, internal carotid artery; OR, odds ratio.

related to incident cardiovascular disease, we hypothesized that these biomarkers would be associated cross-sectionally with measures of subclinical atherosclerosis, as assessed via carotid IMT of the common carotid artery and internal carotid artery. Further characterizing associations with subclinical cardiovascular disease measures may offer deeper insights into the role of these biomarkers in the development of atherosclerosis.

Materials and Methods

STUDY SAMPLE

The Framingham Offspring Study cohort, initiated in 1971 and comprising offspring (and their spouses) of the original Framingham cohort, underwent examinations every 4–8 years. The present investigation included participants who attended the sixth offspring examination (1995–1998) and underwent carotid ultrasonography. From 3380 participants, 37 were excluded for missing carotid IMT data, 21 for missing covariate data, 173 for prevalent cardiovascular disease, 4 for missing sST2 data, and 34 for missing hsTnI data, leaving 3111 participants for analysis. The Institutional Review Board at Boston Medical Center approved the study protocol, and all study participants provided written informed consent.

RISK FACTOR ASSESSMENT

Each examination included a comprehensive history and physical examination. Cardiovascular risk factors pertinent to our study included systolic blood pressure, diastolic blood pressure, antihypertensive drug treatment, diabetes [defined as fasting glucose ≥126 mg/dL (≥7 mmol/L) or the use of oral hypoglycemic medications or insulin], current smoking (defined as smoking at least 1 cigarette per day over the past year), total and HDL cholesterol concentrations (measured after an overnight fast), and body mass index.

BIOMARKER MEASUREMENTS

We obtained blood samples from study participants in the morning after an overnight fast. The citrated plasma samples were centrifuged immediately and stored at −80 °C until assayed. No freeze-thaw cycles were performed before the assays described below. Reference limits and assay characteristics have been described *(11, 13, 14)*. In brief, we used a precommercial, automated electrochemiluminiscent immunoassay utilizing a Cobas e 411 analyzer (Roche Diagnostics) at a core laboratory (K.C. Wollert) to measure plasma GDF-15 concentrations. The assay had an intraassay CV of 0.8% and interassay CV of 2.3% at low concentrations (1120 ng/L), and intra- and interassay CVs of 1.1 and 1.0% at high concentrations (9031 nL/L). We measured sST2 concentrations with a high-sensitivity sandwich immunoassay (Presage™ ST2, Critical Diagnostics) at Critical Diagnostics. This assay had an interassay CV of 7.5% at low (25.6 ng/mL) and 6.0% at high (70.9 ng/mL) sST2 concentrations. We used single-molecule counting technology (Erenna hsTnI, Singulex) for measurement of hsTnI at Singulex. The assay had an interassay CV of 10.0% at low (4.7 ng/L) and 7.7% at high (19.0 n/L) hsTnI concentrations. B-type natriuretic peptide (BNP) and C-reactive protein (CRP) concentrations were measured as previously described *(15)*. In brief, BNP was measured by use of a high-sensitivity immunoradiometric assay at Shionogi with mean interassay CV of 12.2%, and CRP was measured at the Framingham Heart Study core laboratory by use of a Dade Behring BN100 nephelometer with an mean interassay CV of 2.2%.

CAROTID ULTRASONOGRAPHY

A single sonographer, certified by the Registry of Diagnostic Medical Sonographers, performed all carotid ultrasonography. Ultrasound image acquisition was obtained at the end of the R wave of an electrocardiogram to capture end-diastole. By use of a standard 45° projection from vertical, we acquired data from images obtained at 3 levels: the common carotid artery (CCA), level of the carotid artery bulb, and proximal internal carotid artery (ICA). One additional image was taken of the carotid artery bulb and proximal ICA centered on the largest plaque. Intima-media thickness values were calculated from the intima-media interface lines drawn by a certified reader on each acquired image *(4)*. Using a distance of approximately 0.5 cm below the carotid-artery bulb and measuring IMT over a 1-cm-long segment (deemed not to have any plaque presence), we determined the mean IMT of the CCA. The largest IMT in either the left or right ICA from 1 cm above the carotid sinus to the bulb determined the maximum ICA IMT. We also examined maximum ICA IMT as a dichotomous variable, defined as plaque presence if ICA IMT >1.5 mm. Replicate measurements in 37 participants was performed for assessment of reproducibility with Pearson correlation coefficients (between replicate measurements) for the mean CCA IMT and maximum ICA IMT, 0.94 and 0.76, respectively *(4)*.

STATISTICAL ANALYSES

All biomarkers and 2 carotid IMT outcomes (maximum ICA IMT and mean CCA IMT) were natural-logarithmically transformed owing to their right-skewed distributions. In primary analyses, we used multivariable linear regression to examine the associations of maximum ICA IMT and mean CCA IMT with each biomarker (GDF-15, sST2, and hsTnI). β-Estimates were expressed as relative change in IMT per 1-SD change in log-transformed biomarker, and were back-transformed for

interpretation. For the carotid outcome of plaque presence, we applied multivariable logistic regression modeling, with reported odds ratios (ORs) per 1-SD change in log-transformed biomarker. Adjustment for age, sex, systolic blood pressure, hypertension treatment, total cholesterol, HDL cholesterol, diabetes, smoking, body mass index, and baseline estimated glomerular filtration rate was used in primary models. To account for testing of multiple biomarkers (n = 3) and carotid measures (n = 3), results for primary analyses were considered significant at a Bonferroni-corrected *P*-value threshold of 0.05/9 = 0.0056.

In secondary analyses, we tested effect modification by age and sex for associations of 3 biomarkers and carotid outcomes. In addition, we further adjusted multivariable models for CRP and BNP, which have previously been shown to be associated with carotid IMT measures *(16, 17)*. A *P*-value threshold of 0.05 was deemed significant in secondary analyses. Analyses were conducted with SAS version 9.2.

Results

The study sample contained 3111 Framingham Offspring cohort participants with a mean age of 58 years, 54% of whom were female. Twenty-six percent were treated for hypertension, 15% were current smokers, and 9% had diabetes. Clinical, biomarker, and carotid measurements are shown in Table 1.

Table 1. Baseline characteristics of 3111 participants free of prevalent cardiovascular disease.[a]

Characteristic	Result
Clinical	
Age, years	58 (10)
Female sex	1687 (54)
Systolic blood pressure, mmHg	128 (19)
Treatment for high blood pressure	809 (26)
Diabetes	274 (9)
Body mass index, kg/m^2	27.9 (5.2)
Current smoker	470 (15)
Total cholesterol, mg/dL	206 (40)
HDL cholesterol, mg/dL	51 (16)
Biomarker	
GDF-15, ng/L	1028 (809, 1334)
sST2, ng/mL	20.8 (16.6, 26.0)
hsTnI, ng/L	1.3 (0.9, 2.2)
IMT	
Maximum ICA IMT, mm	1.11 (0.81, 1.78)
Plaque presence (ICA IMT >1.5 mm)	1025 (33)
Mean CCA IMT, mm	0.58 (0.51, 0.66)

[a] Data are mean (SD), n (%), or median (25th, 75th percentile). To convert cholesterol in mg/dL to mmol/L, multiply by 0.02586.

ASSOCIATIONS OF CAROTID MEASURES WITH GDF-15, sST2, AND hsTnI

In age- and sex-adjusted regression models, maximal ICA IMT was significantly associated with each biomarker. After multivariable adjustment (including age, sex, systolic blood pressure, hypertension treatment, total and HDL cholesterol, diabetes, smoking, body mass index) the association remained significant only for log(GDF-15) (β-estimate 0.04, SE 0.01, $P < 0.0001$) (Table 2). Specifically, maximal ICA IMT increased 4% per 1-SD increase in log(GDF-15). Neither sST2 nor hsTnI was associated with maximal ICA IMT after multivariable adjustment.

In age- and sex-adjusted models, plaque presence (defined as ICA IMT >1.5 mm) was significantly associated with GDF-15 and sST2. After multivariable adjustment, plaque presence remained significantly related to GDF-15 [OR 1.33 per 1-SD increase in log(GDF-15), 95% CI 1.20–1.48, $P < 0.0001$] (Table 2). Neither sST2 nor hsTnI was associated with plaque presence after multivariable adjustment.

The risk of plaque presence across increasing quartiles of the 3 biomarkers, GDF-15, sST2, and hsTnI, are displayed in Fig. 1. Increasing quartiles of GDF-15 were associated with increasing risk of plaque in multivariable analyses (*P* for trend <0.0001). Specifically, participants in the upper quartile of GDF-15 had a nearly 2-fold increased odds of carotid plaque compared with the lowest quartile (OR 1.95, 95% CI 1.44–2.64, $P < 0.0001$).

The associations of the biomarkers, GDF-15, sST2, and hsTnI, were evaluated with the outcome mean CCA IMT (Table 2). GDF-15, but not sST2 or hsTnI, was significantly associated with mean CCA IMT in age- and sex-adjusted models, and none of the 3 biomarkers was significantly associated after multivariable adjustment at the prespecified statistical threshold of $P = 0.0056$.

COMPARISON WITH ESTABLISHED BIOMARKERS

After further adjusting multivariable models for BNP and CRP, biomarkers that previously have been linked with carotid measures, GDF-15 remained a significant predictor of maximal ICA IMT, conferring a 1.04-fold increase in carotid ICA thickness per 1-SD increase of log(GDF-15) ($P = 0.0002$). In contrast, BNP was not associated with maximal ICA IMT in the multimarker model ($P = 0.48$), and CRP only nominally so ($P = 0.04$). Both GDF-15 and CRP were significant predictors of plaque presence in multivariable-adjusted models, and the association with GDF-15 appeared more significant [OR per

Table 2. Association of carotid measures with GDF-15, sST2, and hsTnI.[a]

Outcome and biomarker	Age- and sex-adjusted model	*P*[c]	Multivariable-adjusted model[b]	*P*[c]
Maximum ICA IMT (log-transformed)				
GDF-15	0.070 (0.009)	<0.0001	0.04 (0.010)	<0.0001
sST2	0.025 (0.008)	0.003	0.013 (0.008)	0.10
hsTnI	0.024 (0.008)	0.004	0.02 (0.008)	0.01
Plaque presence				
GDF-15	1.48 (1.34–1.63)	<0.0001	1.33 (1.20–1.48)	<0.0001
sST2	1.15 (1.05–1.25)	0.002	1.10 (1.00–1.20)	0.05
hsTnI	1.11 (1.02–1.21)	0.02	1.09 (1.00–1.19)	0.04
Mean CCA IMT (log-transformed)				
GDF-15	0.014 (0.004)	0.0003	0.004 (0.004)	0.30
sST2	0.009 (0.003)	0.01	0.003 (0.003)	0.31
hsTnI	0.009 (0.003)	0.007	0.007 (0.003)	0.03

[a] Data are β-estimate (SE) or OR (95% CI). β-estimate and OR reflect per 1-SD increase in log-transformed biomarker.
[b] Adjusted for age, sex, systolic blood pressure, hypertension treatment, total cholesterol, HDL cholesterol, diabetes, smoking, body mass index, and estimated glomerular filtration rate.
[c] Corrected *P*-value threshold 0.0056.

1-SD unit increase in log(GDF-15) 1.32; 95% CI, 1.18–1.47; $P < 0.0001$; OR per 1-SD unit increase in log(CRP) 1.15; 95% CI, 1.04–1.27; $P = 0.008$] (Table 3).

For a given CRP tertile, GDF-15 added further information with regard to plaque risk (Fig. 2). For example, when examining individuals in the highest tertile of CRP, those in the lowest tertile of GDF-15 had a 1.44-fold increased odds of plaque, compared with a 2.7-fold increased odds in the highest GDF-15 tertile (with those in the lowest tertile for both biomarkers serving as the reference group).

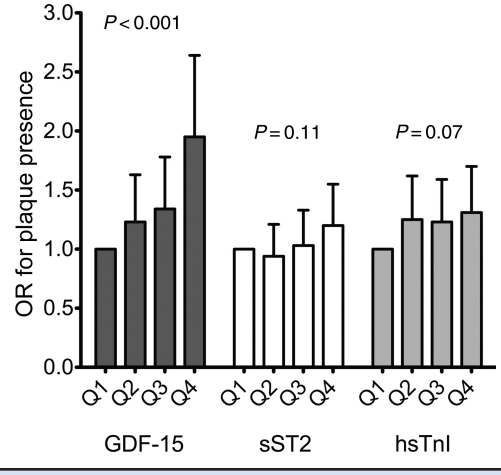

Fig. 1. Multivariable-adjusted ORs for carotid plaque presence across quartiles of GDF-15, sST2, and hsTnI.
Error bars represent 95% CI, *P* values for trend.

Discussion

GDF-15, sST2, and hsTnI have recently emerged as predictors of cardiovascular outcomes in patients with existing cardiovascular disease, as well as in the community-dwelling population. The present investigation extends these findings and supports the concept that GDF-15 is strongly associated with subclinical atherosclerosis as measured by carotid ultrasonography, even before clinical cardiovascular disease is recognized. Moreover, GDF-15 was associated with carotid plaque independent of established biomarkers of cardiovascular risk, CRP and BNP.

GDF-15 is a divergent member of the transforming growth factor β cytokine family *(18)* that is upregulated in response to stressors, including in macrophages exposed to oxidized LDL in atherosclerotic carotid arteries *(19)*. It is expressed in several cell types, including macrophages, vascular smooth muscle cells, and endothelial cells in response to oxidative, inflammatory, or metabolic stressors *(19–21)*.

Specific to atherosclerosis, GDF-15 has shown predictive abilities of coronary heart disease mortality and composite outcomes in stable and acute coronary syndromes in patients with prevalent cardiovascular risk factors *(22–25)*. In community cohorts, GDF-15 has conferred prognostic abilities for composite car-

Table 3. Association of GDF-15 and established biomarkers with carotid measures.[a]

	Maximum ICA IMT		Plaque presence		Mean CCA IMT	
Model	β-estimate (SE)	P[b]	OR (95% CI)	P	β-estimate (SE)	P[b]
Individual biomarker						
GDF-15	0.040 (0.01)	<0.0001	1.33 (1.20–1.48)	<0.0001	0.004 (0.004)	0.30
Biomarker combination						
GDF-15	0.037 (0.01)	0.0002	1.32 (1.18–1.47)	<0.0001	0.004 (0.004)	0.36
BNP	0.006 (0.009)	0.48	0.98 (0.89–1.08)	0.71	0.003 (0.004)	0.33
CRP	0.019 (0.009)	0.04	1.15 (1.04–1.27)	0.008	0.002 (0.004)	0.53

[a] All models adjusted for age, sex, systolic blood pressure, hypertension treatment, total cholesterol, high-density cholesterol, diabetes, smoking, body mass index, and estimated glomerular filtration rate. β-estimate and OR reflect per 1-SD increase in log-transformed biomarker.
[b] Corrected *P*-value threshold 0.0056.

diovascular outcomes as well as hard mortality outcomes *(9–11, 26)*. Our findings support the concept that GDF-15 is in fact associated with subclinical atherosclerosis as assessed by maximal ICA IMT as well as the presence of carotid plaque.

In contrast to the strong association of GDF-15 with maximal ICA IMT, we found no such association with mean CCA IMT. Previous studies suggest that maximum ICA IMT and mean CCA IMT may reflect different underlying processes, the former related to localized atherosclerotic plaque *(27)* and the latter representing diffuse arterial wall hypertrophy *(28)*. Interestingly, maximal ICA and plaque presence, but not mean CCA IMT, improved risk classification for incident cardiovascular disease in a previous study *(29)*. Our findings suggest that GDF-15 is associated with focal atherosclerosis, rather than diffuse inflammatory processes and arterial thickening. Similar results were found in an older community-based cohort, in which GDF-15 was associated with carotid plaque but not IMT *(10)*. Our results extend these findings to a population more than a decade younger than the previous study, without known prevalent cardiovascular disease, and directly compare GDF-15 to other emerging and established biomarkers.

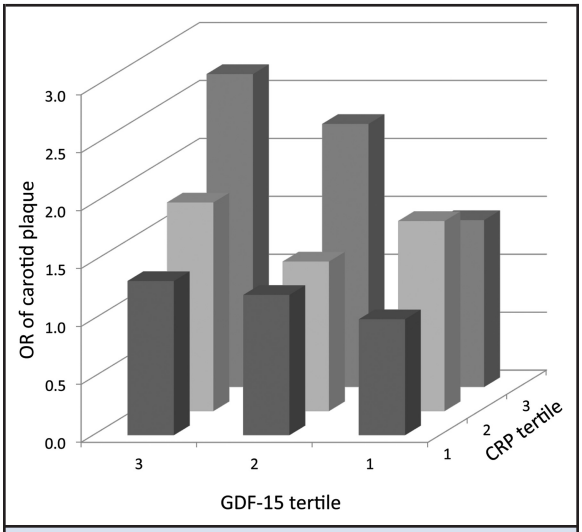

Fig. 2. Multivariable-adjusted ORs for carotid plaque presence across increasing tertiles of GDF and CRP.
Reference groups for OR were GDF-15 tertile 1 and CRP tertile 1.

We found that the association of GDF-15 and carotid plaque is independent of CRP, a marker of inflammation. Notably, in multimarker models with CRP and BNP, the association of GDF-15 with maximum ICA IMT and carotid plaque was more robust than that of either BNP or CRP. This suggests that GDF-15 may reflect an orthogonal pathway associated with cardiovascular disease, the mechanism of which remains unclear. Experimental studies suggest antithrombotic and antiplatelet effects *(30)*. However, in vascular tissue studies, there is growing evidence that GDF-15 may promote increased inflammation and atherosclerosis progression, a process that may involve p53 pathway activation *(31, 32)*. Whether GDF-15 is a mediator of cardiovascular disease or upregulated in response to cardiovascular injury remains unclear.

In comparison to GDF-15, sST2 and hsTnI exhibited no significant associations with carotid measures in our study. Soluble ST2, a member of the interleukin-1 cytokine family, has been shown to serve as a decoy receptor, binding IL-33, a cytokine shown to have protective effects in the cardiovascular system *(33, 34)*. In atherosclerosis, IL-33 administration in murine models of atherosclerosis led to significantly smaller atherosclerotic plaques, whereas sST2 administration led to larger aortic sinus atherosclerotic plaques *(35)*. Although sST2 has shown associations

with cardiovascular outcomes in acute coronary syndrome *(36, 37)*, more recent studies have not shown sST2 to be predictive of atherosclerotic plaque in patients with carotid atherosclerotic disease *(38)* or patients free of vascular disease *(39)*.

There are several limitations to the present study that merit discussion. Ours is an observational cross-sectional study, and causal inferences cannot be drawn. It is also important to note that carotid IMT may incompletely capture the complex process of atherosclerosis in multiple vascular beds *(40)*, and interval carotid IMT measurements may confer more specificity in this process and relation with atherosclerotic outcomes. The use of our Bonferroni-corrected *P*-value threshold may have been too conservative, given that 2 of the carotid measures (ICA IMT and plaque presence) are not independent of one another. However, even when using a less conservative *P*-value threshold, the main results did not differ materially. Last, our population was predominantly white, limiting generalizability to other ethnic groups.

In summary, we found that GDF-15 was associated with subclinical atherosclerosis, including higher maximum ICA IMT and the presence of carotid plaque in an ostensibly healthy community-based population without prevalent cardiovascular disease. This association was independent of other established biomarkers, including CRP and BNP. Future studies are warranted to elucidate the potential use of GDF-15 for screening and management of patients with subclinical atherosclerosis.

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References

1. Bots ML, Hofman A, Grobbee DE. Common carotid intima-media thickness and lower extremity arterial atherosclerosis. The Rotterdam Study. Arterioscler Thromb 1994;14:1885–91.
2. Bots ML, Witteman JC, Grobbee DE. Carotid intima-media wall thickness in elderly women with and without atherosclerosis of the abdominal aorta. Atherosclerosis 1993;102:99–105.
3. Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C. Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. Am J Epidemiol 1991;134:250–6.
4. Polak JF, Pencina MJ, Meisner A, Pencina KM, Brown LS, Wolf PA, D'Agostino RB Sr. Associations of carotid artery intima-media thickness (IMT) with risk factors and prevalent cardiovascular disease: comparison of mean common carotid artery IMT with maximum internal carotid artery IMT. J Ultrasound Med 2010;29:1759–68.
5. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997; 96:1432–7.
6. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, Azen SP. The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med 1998;128:262–9.
7. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22.
8. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) study, 1987–1993. Am J Epidemiol 1997;146:483–94.
9. Daniels LB, Clopton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the Rancho Bernardo Study. Circulation 2011;123:2101–10.
10. Lind L, Wallentin L, Kempf T, Tapken H, Quint A, Lindahl B, et al. Growth-differentiation factor-15 is an independent marker of cardiovascular dysfunction and disease in the elderly: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Eur Heart J 2009;30:2346–53.
11. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, et al. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. Circulation 2012;126: 1596–604.
12. deFilippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, Seliger SL. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. JAMA 2010;304:2494–502.
13. Ho JE, Hwang SJ, Wollert KC, Larson MG, Cheng S, Kempf T, et al. Biomarkers of cardiovascular stress and incident chronic kidney disease. Clin Chem 2013;59:1613–20.

14. Coglianese EE, Larson MG, Vasan RS, Ho JE, Ghorbani A, McCabe EL, et al. Distribution and clinical correlates of the interleukin receptor family member soluble ST2 in the Framingham heart study. Clin Chem 2012;58:1673–81.
15. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med 2006;355:2631–9.
16. Thakore AH, Guo CY, Larson MG, Corey D, Wang TJ, Vasan RS, et al. Association of multiple inflammatory markers with carotid intimal medial thickness and stenosis (from the Framingham Heart Study). Am J Cardiol 2007;99:1598–602.
17. Zhou W, Ni Z, Yu Z, Shi B, Wang Q. Brain natriuretic peptide is related to carotid plaques and predicts atherosclerosis in pre-dialysis patients with chronic kidney disease. Eur J Intern Med 2012;23:539–44.
18. Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proc Natl Acad Sci U S A 1997;94:11514–9.
19. Schlittenhardt D, Schober A, Strelau J, Bonaterra GA, Schmiedt W, Unsicker K, et al. Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxLDL-induced apoptosis of human macrophages in vitro and in arteriosclerotic lesions. Cell Tissue Res 2004;318:325–33.
20. Bermúdez B, López S, Pacheco YM, Villar J, Muriana FJG, Hoheisel JD, et al. Influence of postprandial triglyceride-rich lipoproteins on lipid-mediated gene expression in smooth muscle cells of the human coronary artery. Cardiovasc Res 2008;79:294–303.
21. Secchiero P, Corallini F, Gonelli A, Dell'Eva R, Vitale M, Capitani S, et al. Antiangiogenic activity of the MDM2 antagonist nutlin-3. Circ Res 2007; 100:61–9.
22. Bonaca MP, Morrow DA, Braunwald E, Cannon CP, Jiang S, Breher S, et al. Growth differentiation factor-15 and risk of recurrent events in patients stabilized after acute coronary syndrome: observations from PROVE IT-TIMI 22. Arterioscler Thromb Vasc Biol 2011;31:203–10.
23. Eggers KM, Kempf T, Lagerqvist B, Lindahl B, Olofsson S, Jantzen F, et al. Growth-differentiation factor-15 for long-term risk prediction in patients stabilized after an episode of non-ST-segment-elevation acute coronary syndrome. Circ Cardiovasc Genet 2010;3:88–96.
24. Kempf T, Sinning J-M, Quint A, Bickel C, Sinning C, Wild PS, et al. Growth-differentiation factor-15 for risk stratification in patients with stable and unstable coronary heart disease: results from the Atherogene Study. Circ Cardiovasc Genet 2009; 2:286–92.
25. Wollert KC, Kempf T, Lagerqvist B, Lindahl B, Olofsson S, Allhoff T, et al. Growth differentiation factor 15 for risk stratification and selection of an invasive treatment strategy in non ST-elevation acute coronary syndrome. Circulation 2007;116: 1540–8.
26. Rohatgi A, Patel P, Das SR, Ayers CR, Khera A, Martinez-Rumayor A, et al. Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study. Clin Chem 2012;58:172–82.
27. Dalager S, Paaske WP, Kristensen IB, Laurberg JM, Falk E. Artery-related differences in atherosclerosis expression: implications for atherogenesis and dynamics in intima-media thickness. Stroke 2007;38:2698–705.
28. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 1986;74:1399–406.
29. Polak JF, Pencina MJ, Pencina KM, O'Donnell CJ, Wolf PA, D'Agostino RB Sr. Carotid-wall intima-media thickness and cardiovascular events. N Engl J Med 2011;365:213–21.
30. Rossaint J, Vestweber D, Zarbock A. Gdf-15 prevents platelet integrin activation and thrombus formation. J Thromb Haemost 2013;11:335–44.
31. Bonaterra GA, Zugel S, Thogersen J, Walter SA, Haberkorn U, Strelau J, Kinscherf R. Growth differentiation factor-15 deficiency inhibits atherosclerosis progression by regulating interleukin-6-dependent inflammatory response to vascular injury. J Am Heart Assoc 1:e002550, 2012.
32. Yang H, Filipovic Z, Brown D, Breit SN, Vassilev LT. Macrophage inhibitory cytokine-1: a novel biomarker for p53 pathway activation. Mol Cancer Ther 2003;2:1023–9.
33. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. J Clin Invest 2007;117: 1538–49.
34. Seki K, Sanada S, Kudinova AY, Steinhauser ML, Handa V, Gannon J, Lee RT. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. Circ Heart Fail 2009;2:684–91.
35. Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N, et al. IL-33 reduces the development of atherosclerosis. J Exp Med 2008;205:339–46.
36. Sabatine MS, Morrow DA, Higgins LJ, MacGillivray C, Guo W, Bode C, et al. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction. Circulation 2008;117:1936–44.
37. Shimpo M, Morrow DA, Weinberg EO, Sabatine MS, Murphy SA, Antman EM, Lee RT. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. Circulation 2004; 109:2186–90.
38. Willems S, Quax PH, de Borst GJ, de Vries JP, Moll FL, de Kleijn DP, et al. Soluble ST2 levels are not associated with secondary cardiovascular events and vulnerable plaque phenotype in patients with carotid artery stenosis. Atherosclerosis 2013;231: 48–53.
39. Miller AM, Purves D, McConnachie A, Asquith DL, Batty GD, Burns H, et al. Soluble ST2 associates with diabetes but not established cardiovascular risk factors: a new inflammatory pathway of relevance to diabetes? PLoS One 2012;7:e47830.
40. Bots ML, Baldassarre D, Simon A, de Groot E, O'Leary DH, Riley W, et al. Carotid intima-media thickness and coronary atherosclerosis: weak or strong relations? Eur Heart J 2007;28:398–406.