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ORIGINAL RESEARCH

Growth Differentiation Factor-15 Predicts Death and Stroke Event in Outpatients With Cardiovascular Risk Factors: The J-HOP Study

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BACKGROUND: Growth differentiation factor-15 (GDF-15) has emerged as a novel biomarker to predict all-cause death in community-dwelling individuals and patients with cardiovascular disease. We evaluated the prognostic value of GDF-15 in outpatients with cardiovascular risk factors.

METHODS AND RESULTS: GDF-15 levels were measured in 3562 outpatients with cardiovascular risk factors in the J-HOP (Japan Morning Surge-Home Blood Pressure) study, a nationwide prospective study. Participants were stratified according to tertiles of GDF-15 and followed up for all-cause death and cardiovascular disease. During a mean follow-up period of 6.6 years, there were 155 all-cause deaths, 81 stroke events including cerebral infarction and intracranial hemorrhage, and 141 cardiac events including cardiac artery disease and heart failure. Patients with higher GDF-15 levels were associated with risks of all-cause death and stroke events (except for cardiac events) after adjustment for traditional risk factors and other prognostic biomarkers (NT-proBNP [N-terminal pro-B-type natriuretic peptide], high-sensitivity troponin T; all-cause death, hazard ratio, 2.38; 95% CI, 1.26–4.48; P=0.007; stroke events, hazard ratio, 2.93; 95% CI, 1.31–6.56, P=0.009; compared with the lowest tertile). Furthermore, incorporating GDF-15 to the predictive models for all-cause death improved discrimination and reclassification significantly. For stroke events, GDF-15 showed similar diagnostic accuracy to NT-proBNP and high-sensitivity troponin T.

CONCLUSIONS: In Japanese outpatients with cardiovascular risk factors, GDF-15 improves risk stratification for all-cause death when compared with NT-proBNP and high-sensitivity troponin T. GDF-15 was associated with increased risks of stroke events beyond conventional risk factors and other prognostic markers; however, the predictive ability for stroke events was equivalent to NT-proBNP and high-sensitivity troponin T.

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Established cardiovascular risk factors, including hypertension, diabetes, and dyslipidemia, have been used in risk assessments designed to prevent cardiovascular disease (CVD).[1,2] Practical guidelines recommend that not only cardiovascular risk factors but also biomarkers are useful to identify individuals who at risk for the development of CVD.[3,4] The representative biomarkers of high-sensitivity troponin T (hs-TnT) and NT-proBNP (N-terminal pro-B-type natriuretic peptide) are well recognized as important clinical biomarkers for diagnoses and for targeting preventive measures in patients with coronary artery disease and heart failure,

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CLINICAL PERSPECTIVE

What Is New?

- This study confirms that elevated growth differentiation factor-15 (GDF-15) levels are associated with stroke events in Asian outpatients, independently of traditional risk factors and other specific prognostic biomarkers (NT-proBNP [N-terminal pro-B-type natriuretic peptide] and high-sensitivity troponin T).
- The relationship between GDF-15 and risks of cardiac events disappear after adjusting for traditional risk factors different from previous studies in the Western population.

What Are the Clinical Implications?

- GDF-15 might be helpful for risk management in the Asian population who are likely to develop stroke.
- The risk stratification for cardiovascular disease previously reported cannot be extrapolated to the Asian population because of unique property that GDF-15 do not relate with risks of future cardiac events despite a strong association between GDF-15 and stroke risks.

Nonstandard Abbreviations and Acronyms

GDF-15	growth differentiation factor-15
hs-TnT	high-sensitivity troponin T

respectively.[5,6] These 2 biomarkers have been associated with morbidity and mortality even in general and hypertensive populations.[7,8]

A novel biomarker, growth differentiation factor-15 (GDF-15), is a member of the transforming growth factor-β superfamily.[9] GDF-15 is a stress-induced cytokine and is expressed in multiple organs. Several prospective studies have reported that GDF-15 is a stronger predictor of all-cause death in community-dwelling individuals.[10–12] Therefore, GDF-15 may be an unspecific prognostic biomarker compared with other biomarkers, such as hs-TnT and NT-proBNP.

GDF-15 is highly expressed in the central nervous system in healthy conditions[13] and predicts an unfavorable functional outcome in patients with ischemic stroke.[14] Even in community-dwelling individuals, blood GDF-15 levels were associated with subclinical brain injury and cognitive impairment.[15] Taking into consideration this evidence suggesting an association between GDF-15 and cerebrovascular disease, we speculated that GDF-15 may have prognostic power for stroke incidence rather than other CVD events in general clinical practice. No previous study has specifically assessed the association between GDF-15 and stroke events or investigated whether the addition of GDF-15 provides more predictive power for stroke events compared with other biomarkers in patients with cardiovascular risk factors.

To address this gap in knowledge, we examined the predictive power of the addition of GDF-15 to traditional cardiovascular risk factors for the prediction of distinct stroke and cardiac events, and we investigated whether GDF-15 provides prognostic power compared with hs-TnT and NT-proBNP in a large general practice population of patients with cardiovascular risk factors.

METHODS

All supporting data within the article are available upon reasonable request from any qualified investigator.

Study Design

All subjects were recruited from the J-HOP (Japan Morning Surge-Home Blood Pressure) study.[16] The J-HOP study was a nationwide prospective study conducted in Japan that included 4310 outpatients with risk factors for CVD. Details of the study design and methods are described in Data S1. The study protocol was registered on University Hospital Medical Information Network Clinical Trials Registry (registration number: UMIN000000894). All participants provided written informed consent, and the Institutional Review Board of Jichi Medical School approved the study (Institutional Review Board number: EKI 04-17; approval date: January 18, 2005).

Laboratory Testing

Blood samples were collected in the morning in a fasting state at enrollment. The blood samples were centrifuged at 3000*g* for 15 minutes at room temperature. The supernatants were stored at 4 °C, sent to a commercial laboratory (SRL Inc., Tokyo, Japan), frozen in aliquots, and stored at −80 °C in a deep freezer. All routine biochemical analyses were performed within 24 hours of sample collection at this single laboratory center. Using the stored serum samples, NT-proBNP and hs-TnT were measured as previously described.[17] The lower limits of detection of NT-proBNP and hs-TnT were 10 and 3 ng/L, respectively. The intracoefficients/intercoefficients of variation were 1.93%/3.13% for NT-proBNP and 2.02%/3.02% for hs-TnT. Serum GDF-15 levels were measured with an automated platform (Cobas e 411 analyzer, Roche Diagnostics, Indianapolis, IN). The assay has a limit of detection below 400 ng/L, a linear measuring range up to 20 000 ng/L, and an interassay imprecision of 2.3% and 1.8% at GDF-15 concentrations of 1100 and 17 200 ng/L, respectively.

Outcome Ascertainment

We divided the patient outcomes into the following 3 categories: (1) all-cause death; (2) stroke events, defined as first-ever cerebrovascular events including cerebral infarction, cerebral hemorrhage, and subarachnoid hemorrhage except for transient ischemic attack; and (3) cardiac events as the composite of coronary artery disease and hospitalization for heart failure, and coronary artery disease, defined as acute myocardial infarction and angina pectoris requiring percutaneous coronary intervention. Hospitalization for heart failure was defined as an event requiring the patient's admission to a hospital with a primary diagnosis of heart failure and the initiation or intensification of treatment for heart failure. Additional details are given in Data S1.

Statistical Analysis

Demographics and other baseline characteristics were compared across GDF-15 tertile groups. Continuous variables are presented as means and standard deviations, and the groups were compared using 1-way ANOVA. Some variables are presented as medians and interquartile ranges because of their skewed distributions, and the groups were compared using the Kruskal-Wallis test. Categorical variables are presented as counts and percentages, and groups were compared using χ^2 tests. GDF-15, NT-proBNP, and hs-TnT values were logarithmically transformed because of skewed distributions. Blood concentrations under the measuring limit of each biomarker were calculated as the half-value of the limit, that is, GDF-15 at 200 ng/L, NT-proBNP at 5 ng/L, and hs-TnT at 1 ng/L.

The relationship between the baseline GDF-15 measurements and each clinical outcome was assessed by Kaplan-Meier plots. The proportionality assumption for Cox analyses was confirmed graphically. We evaluated the association between the biomarkers and the risk of each clinical outcome using multivariable Cox proportional hazards models. Model 1 adjusted for traditional risk factors; model 2 adjusted for the variables in model 1 and other prognostic biomarkers (NT-proBNP and hs-TnT). Traditional risk factors included age, sex, body mass index, current smoking, diabetes, previous CVD, statin use, antihypertensive drug use, total cholesterol, high-density lipoprotein cholesterol, office systolic blood pressure, and estimated glomerular filtration rate.[2]

The independent variables of the multivariable analysis were continuous or categorical variables as follows: GDF-15 tertile groups and dichotomous models divided by cut points of each biomarker (ie 1200 ng/L of GDF-15,[18,19] 125 ng/L of NT-proBNP,[5,6] and 3 ng/L of hs-TnT[20]). Hazard ratios (HRs) and 95% CIs were expressed per 1 SD increase in the GDF-15 level or relative to the lowest tertile, respectively.

We analyzed the additional contribution of GDF-15 beyond traditional risk factors in predicting each outcome by using multiple metrics of biomarker performance, including discrimination (c-statistics) and reclassification (integrated discrimination index and net reclassification index). We estimated c-statistics to assess discriminatory ability of each model. For reclassification analyses, we estimated risk at 10 years; 95% CIs of each metric were estimated by using 1000 bootstrap samples. Because no established categories exist that guide clinical decisions for CVD risk in Asians with cardiovascular risk factors, we calculated a category-free net reclassification index from proportional hazards models. Additionally, we calculated measures of diagnostic accuracy (sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, diagnostic odds, and Youden index) of each model by using SAS system, version 9.4 (SAS Institute, Cary, NC). *P* values <0.05 were considered significant. All analyses except diagnostic accuracy tests were performed by using R software version 3.6.0 with the package "survival" (version 3.2.13) for c-statistics and "survIDINRI" (version 1.1.1) for integrated discrimination index and net reclassification index.

RESULTS

Baseline Characteristics

Of the 4310 patients who were enrolled in the J-HOP study, the following were excluded: 221 patients whose blood samples were not sufficient for measurement of GDF-15, 456 patients whose blood samples were not measured NT-proBNP or hs-TnT, and 71 patients whose data were incomplete. The data of the final total of 3562 patients were included in the analyses.

Table 1 provides the baseline clinical characteristics of the overall population and the patients as divided by the tertiles of GDF-15. In the overall population, the median age of the patients was 66 years, and there were more women than men. Most of the patients had hypertension and were taking antihypertensive drugs. Characteristics of the excluded population were not quite different from the included population (Table S1).

The median concentration of GDF-15 was 967.1 ng/L (interquartile range, 709.0–1347.8 ng/L). The following GDF-15 concentrations comprised the tertiles: The range of the first tertile was <788.4 ng/L; that of the second tertile was 788.6–1187.0 ng/L; and the third tertile was >1188.0 ng/L. The patients' age and the prevalence of hypertension, dyslipidemia, and diabetes were all incrementally higher in each tertile in order from the lowest tertile to the third tertile (Table 1).

Table 1. Baseline Clinical Characteristics

Variable	Overall n=3562	Tertile of GDF-15			*P* value
		First tertile n=1186	Second tertile n=1187	Third tertile n=1189	
GDF-15, ng/L	967.1 (709.0–1347.8)	619.5 (524.8–708.6)	966.9 (872.5–1067.0)	1582.0 (1347.0–2044.0)	…
Age, y	65.0±10.6	57.6±9.5	65.8±8.6	71.5±8.7	<0.001
Male, %	46.0	39.4	45.2	53.4	<0.001
Prior CVD, %	12.6	8.2	12.0	17.7	<0.001
SBP, mm Hg	141.3±16.3	139.2±15.3	141.6±15.9	143.1±17.5	<0.001
DBP, mm Hg	81.3±10.4	84.1±10	81.3±10	78.6±10.6	<0.001
Heart rate, bpm	71.3±10.8	71.7±10.2	71±10.5	71.3±11.5	0.217
BMI, kg/m^2	24.2±3.5	24.5±3.5	24.1±3.4	24.1±3.6	0.008
Waist circumference, cm	84.3±9.7	83.8±9.8	83.9±9.7	85.2±9.6	<0.001
Current smoking, %	12.1	10.0	12.1	14.2	<0.001
Daily drinking, %	27.6	27.7	27.9	27.3	0.955
Hypertension, %	91.0	88.6	91.2	93.1	<0.001
Diabetes, %	24.5	17.5	24.9	31.1	<0.001
Dyslipidemia, %	42.1	45.8	42.5	38.1	<0.001
Atrial fibrillation, %	3.8	2.0	4.1	5.4	<0.001
Chronic kidney disease, %	4.5	1.6	2.4	9.6	<0.001
Anti-hypertensive drugs, %	79.0	73.6	78.8	84.7	<0.001
Statin, %	23.8	24.6	24.3	22.5	0.444
NT-proBNP, ng/L	50.6 (25.5–97.4)	34.1 (16.8–62.2)	51.1 (27.0–92.0)	77.6 (41.2–168.5)	<0.001
hs-TnT, ng/L	3 (1–7)	1 (1–4)	3 (1–6)	6 (1–11)	<0.001
hs-CRP, mg/dL	525.0 (259.2–1130.0)	443.0 (229.0–848.5)	543.0 (265.0–1120.0)	641.0 (287.0–1430.0)	<0.001
eGFR, mL/min per 1.73 m^2	73.2±17.3	81.4±14.7	73.9±14.6	64.2±18.1	<0.001
Hemoglobin, g/dL	13.8±1.5	14.0±1.3	13.9±1.4	13.5±1.7	<0.001
Platelet, ×10^9/L	23.0±6.0	24.2±5.7	22.9±6.0	21.9±6.0	<0.001
Triglyceride, mg/dL	126.1±85.8	128.6±100.1	126.3±80.1	123.5±75.3	0.356
Total cholesterol, mg/dL	202.6±32.5	209.1±32.2	203.1±30.7	195.8±33.3	<0.001
HDL-C, mg/dL	57.7±15.1	59.9±14.9	57.9±14.5	55.4±15.6	<0.001
Non–HDL-C, mg/dL	144.9±31.9	149.1±32.4	145.2±30.7	140.4±31.9	<0.001
Fasting glucose, mg/dL	107.4±28.1	103.6±21.4	107.9±29.5	110.7±31.8	<0.001
HbA_{1c}, %	5.9±0.8	5.7±0.6	5.9±0.8	6.0±0.9	<0.001

Continuous variables are presented as mean±standard deviation, and categorized data are presented as number (%). Values of GDF-15, NT-proBNP, hs-TnT, and hs-CRP are median (interquartile range). Prior CVD includes preexisting angina pectoris, myocardial infarction, and stroke. BMI indicates body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; HbA_{1c}, hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and SBP, systolic blood pressure.

Association of GDF-15 With Patient Outcomes

The number and incidence of each patient outcome is shown in Table 2. During the mean follow-up of 6.6±3.9 years, there were 155 all-cause deaths (6.6 per 1000 person-years), of which 48 (2.0 per 1000 person-years) were cardiovascular deaths and 107 (4.5 per 1000 person-years) were noncardiovascular deaths. Stroke events occurred in 81 patients (3.5 per 1000 person-years) and were mainly ischemic stroke (57 ischemic strokes, 7 cerebral embolisms, 16 cerebral hemorrhages, and 1 subarachnoid hemorrhage). Cardiac events occurred in 141 patients (6 per 1000 person-years) and consisted of 70 angina pectoris, 31 acute myocardial infarctions, and 40 heart failures. Despite the fact that women outnumbered men in our study population, male patients were prone to have all-cause death and cardiac events. The incidence of stroke events is higher among men; however, it was not significant in χ^2 tests (Table S2).

The cumulative Kaplan-Meier plots of each event by tertile of GDF-15 are shown in Figure 1. Higher GDF-15 levels at baseline were significantly associated with increased event rates of all-cause death, stroke, and cardiac events.

Table 2. Number and Incident Rate of Outcomes

Outcome	Parameters	Overall n=3562	Tertile of GDF-15		
			First tertile n=1186	Second tertile n=1187	Third tertile n=1189
All-cause death	No. of events (%)	155 (4.4)	15 (1.3)	39 (3.3)	101 (8.5)
	Incident rate, 1000 person-years	6.6	1.8	4.8	13.9
Stroke event	No. of events (%)	81 (2.3)	10 (0.8)	16 (1.3)	55 (4.6)
	Incident rate, 1000 person-years	3.5	1.2	2.0	7.7
Cardiac event	No. of events (%)	141 (4.0)	26 (2.2)	44 (3.7)	71 (6.0)
	Incident rate, 1000 person-years	6.0	3.2	5.6	10.1

GDF-15 indicates growth differentiation factor-15.

In multivariable Cox proportional hazards models adjusted for traditional risk factors, patients in the third tertile of GDF-15 were at increased risk of all-cause death and stroke events compared with the patients in the first tertile (model 1 in Figure 2), and this relationship remained after adjustment for NT-proBNP and hs-TnT (model 2 in Figure 2). Higher GDF-15 levels modeled as a dichotomous and continuous variable, as well as in the tertile analyses, were associated with an increased risk for all-cause death and stroke events after adjusting for traditional risk factors (Table S3). After adjustment for NT-proBNP and hs-TnT (model 2), GDF-15 levels >1200 ng/L and 1 SD increase of the continuous model also related an increased risk for all-cause death and stroke events (dichotomous model [relative to <1200 ng/L at GDF-15], all-cause death: HR, 1.85; 95% CI, 1.24–2.74; *P*=0.002; stroke events: HR, 2.59; 95% CI, 1.48–4.51; *P*=0.001; continuous model [1 SD increase], all-cause death: HR 2.94, 95% CI, 2.12–4.07; *P*<0.001; stroke events: HR, 1.86; 95% CI, 1.10–3.16; *P*=0.021).

Among the 81 patients who experienced stroke events, 17 patients (0.7 per 1000 person-years) suffered from intracranial bleeding. However, the patients in the higher GDF-15 tertile were not associated with the risk of intracranial bleeding in Cox proportional hazard model adjusted for traditional risk factors (third tertile: HR, 1.89; 95% CI, 0.43–8.32; *P*=0.402, relative to the first tertile).

Although higher GDF-15 levels were associated with a high risk of cardiac events in the unadjusted models, the association became attenuated and no longer statistically significant after adjustment for traditional risk factors (model 1 in Figure 2). In contrast, higher NT-proBNP and hs-TnT levels were associated with an increased risk of cardiac events in the dichotomous and continuous models after adjusting for traditional risk factors (Table S3).

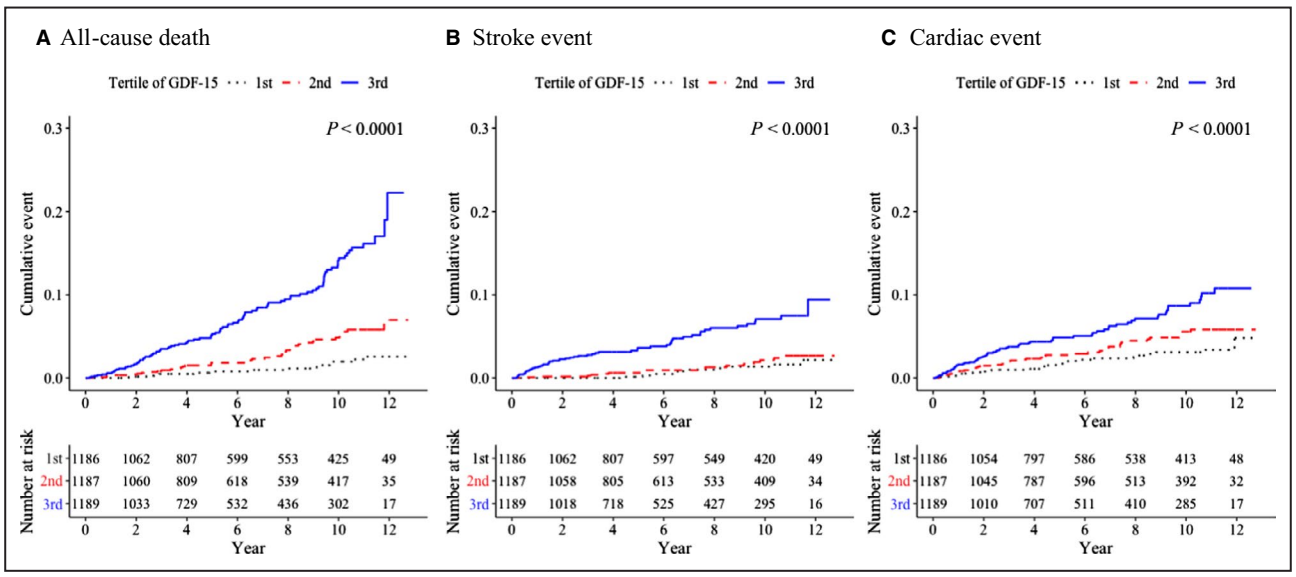

Figure 1. Cumulative incidence by outcome and GDF-15 tertile.
Kaplan-Meier curves of the cumulative incidence rates by tertile of GDF-15 (ng/L) of the following events: (**A**) all-cause death, (**B**) stroke events, and (**C**) cardiac events. Gradual increases in stroke and cardiac events were revealed among the 3 GDF-15 tertiles, but only the patients in the third tertile of GDF-15 showed a significant increase in stroke events compared with those in the first tertile. GDF-15 indicates growth differentiation factor-15.

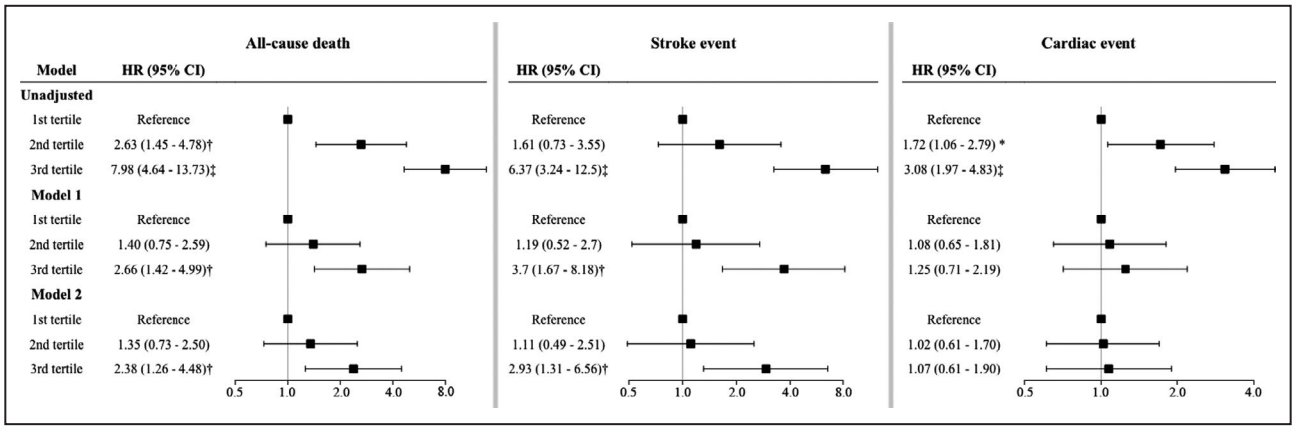

Figure 2. Unadjusted and multivariable-adjusted association between GDF-15 tertiles and outcomes.
Cox proportional hazards analysis by tertile analyses of GDF-15 and outcomes in the unadjusted model, model 1, and model 2. Model 1 was adjusted for traditional risk factors (age, sex, body mass index, current smoking, diabetes, previous cardiovascular disease, statin use, antihypertensive drug use, total cholesterol, high-density lipoprotein cholesterol, office systolic blood pressure, and estimated glomerular filtration rate). Model 2 was the model incorporating log NT-proBNP and log hs-TnT to model 1. Patients in the third tertile of GDF-15 were independently and positively related to all-cause death and stroke events, however, GDF-15 was not associated with the risk of cardiac events in model 1. Hazard ratios (HRs) with 95% CIs represent comparisons vs patients in the first tertile. GDF-15 indicates growth differentiation factor-15; hs-TnT, high-sensitivity troponin T; and NT-proBNP, N-terminal pro-B-type natriuretic peptide. **P*<0.05, †*P*<0.01, and ‡*P*<0.001.

Added Predictive Value of GDF-15

The model performance for the prediction of all-cause death was significantly improved when GDF-15 was incorporated into model 1 (Table 3) or model 2 (Table 4). NT-proBNP and hs-TnT were also associated with all-cause death, but the dichotomous model of hs-TnT did not show a significant relationship with an increased risk for all-cause death after adjusting for traditional risk factors (Table S4). Unlike GDF-15, adding NT-proBNP or hs-TnT into the predictive models did not provide the advantage of discrimination and reclassification (Table 3 and Table S4). These findings suggested that GDF-15 was a strong predictor for mortality enough to exhibit an

Table 3. Change in Risk Predictive Metrics by Incorporating Prognostic Biomarkers to the Base Model

	c-statistics (95% CI)	Category-free NRI (95% CI)	IDI (95% CI)
All-cause death			
Model 1	0.786 (0.748 to 0.824)		
Model 1+log GDF-15	0.804 (0.767 to 0.840)*	0.238 (0.123 to 0.337)‡	0.035 (0.014 to 0.063)‡
Model 1+log NT-proBNP	0.787 (0.748 to 0.826)	0.031 (–0.046 to 0.146)	0.018 (0.004 to 0.046)‡
Model 1+log hs-TnT	0.788 (0.749 to 0.827)	0.122 (0.002 to 0.223)*	0.008 (0.001 to 0.025)*
Stroke event			
Model 1	0.762 (0.712 to 0.812)		
Model 1+log GDF-15	0.787 (0.741 to 0.833)*	0.221 (0.023 to 0.320)*	0.009 (0.001 to 0.033)*
Model 1+log NT-proBNP	0.800 (0.755 to 0.844)*	0.206 (0.097 to 0.346)†	0.030 (0.012 to 0.076)†
Model 1+log hs-TnT	0.795 (0.748 to 0.842)*	0.334 (0.202 to 0.450)†	0.013 (0.003 to 0.037)*
Cardiac event			
Model 1	0.777 (0.737 to 0.816)		
Model 1+log GDF-15	0.777 (0.737 to 0.816)	0.060 (–0.120 to 0.153)	0.000 (–0.001 to 0.006)
Model 1+log NT-proBNP	0.787 (0.747 to 0.827)	0.115 (–0.019 to 0.210)	0.017 (0.003 to 0.047)†
Model 1+log hs-TnT	0.783 (0.743 to 0.822)	0.119 (–0.043 to 0.218)	0.005 (0.000 to 0.018)

GDF-15 indicates growth differentiation factor-15; hs-TnT, high sensitive troponin T; IDI, integrated discrimination improvement; NRI, net reclassification improvement; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Model 1 was adjusted for traditional risk factors (age, sex, body mass index, current smoking, diabetes, previous cardiovascular disease, statin use, antihypertensive drug use, total cholesterol, high-density lipoprotein cholesterol, office systolic blood pressure, and estimated glomerular filtration rate).

**P*<0.05.

†*P*<0.01.

‡*P*<0.001.

Table 4. Change in Risk Predictive Metrics by Incorporating GDF-15 to the Model Including NT-proBNP and hs-TnT

	c-statistics (95% CI)	Category-free NRI (95% CI)	IDI (95% CI)
All-cause death			
Model 2	0.787 (0.748–0.827)		
Model 2+log GDF-15	0.802 (0.764–0.840)*	0.180 (0.069–0.276)†	0.028 (0.010–0.055)‡
Stroke event			
Model 2	0.811 (0.766–0.857)		
Model 2+log GDF-15	0.817 (0.773–0.862)*	0.134 (–0.080–0.256)	0.006 (0.000–0.027)*
Cardiac event			
Model 2	0.789 (0.749–0.829)		
Model 2+log GDF-15	0.789 (0.749–0.829)*	–0.005 (0.068–0.114)	0.000 (–0.001–0.005)

Model 2 was the model incorporating log NT-proBNP and log hs-TnT to model 1, which is described in Table 3. GDF-15, growth differentiation factor-15; hs-TnT, high sensitive troponin T; IDI, integrated discrimination improvement; NRI, net reclassification improvement; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

*$P<0.05$.

†$P<0.01$.

‡$P<0.001$.

incremental benefit for the predictive models that contain the traditional risk factors and prognostic biomarkers.

Incorporating GDF-15 into model 1 significantly improved the model performance for the prediction of stroke events. However, the improvement of the model by adding GDF-15 was smaller than the improvement obtained by adding other prognostic biomarkers in logarithmic analyses (Table 3), and then GDF-15 had only marginal effects on the predictive model including NT-proBNP and hs-TnT (Table 4). Furthermore, we calculated measures of diagnostic accuracy (sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, diagnostic odds, and Youden index) when each of these 3 markers added into model 1 for each outcome (Tables S5 through S7). As a result, GDF-15 showed equal predictive ability for stroke events to NT-proBNP and hs-TnT.

Adding GDF-15, NT-proBNP, or hs-TnT into model 1 did not improve the model performance for the prediction of cardiac events (Table 3 and Table S4). Unexpectedly, higher GDF-15 levels were not associated with the risk of cardiac events, even though NT-proBNP and hs-TnT each showed a significant and independent relationship to the incidence events.

DISCUSSION

In a large general practice population of patients with cardiovascular risk factors, our division of the tertiles of GDF-15 revealed that the third tertile (>1188.0 ng/L) was significantly and independently associated with all-cause death and stroke events (except for cardiac events) after adjustment for conventional risk factors and representative prognostic biomarkers, that is, hs-TnT and NT-proBNP. The addition of GDF-15 improved the predictive model that contained traditional risk factors for all-cause death and stroke events. However, the model improvement was different between death and stroke events compared with NT-proBNP and hs-TnT. In the models for all-cause death, the addition of GDF-15 to the reference model increased all parameters of c-statistics, net reclassification index, and integrated discrimination index and enhanced the performance of the model that contained NT-proBNP and hs-TnT. In contrast, all 3 markers improved the predictive model for stroke events, and the incremental effect of adding GDF-15 was relatively smaller compared with those of adding NT-proBNP and hs-TnT. When we incorporated GDF-15 into the models that contained NT-proBNP and hs-TnT, GDF-15 produced only a marginal effect. These findings suggested that GDF-15 is a strong predictor for all-cause death, in accord with previous investigations. Our study provides the first report of the unique property that GDF-15 has prognostic ability for stroke events (except for cardiac events) in Asian patients with cardiovascular risk factors, and the information implicates that the risk stratification for CVD previously reported cannot be extrapolated to the Asian population and that it is important for clinical application of GDF-15.

Predictive Ability of GDF-15 for Stroke Event

We observed the prognostic value of GDF-15 for stroke events in patients with cardiovascular risk factors. Only a few studies have focused on the relationship between GDF-15 and stroke, despite the high expression of GDF-15 in the central nervous system.[13] Higher GDF-15 levels were reported to be associated with incident stroke events in patients with atrial fibrillation[21,22] and ischemic heart disease.[23,24] Wang et al[25] reported that higher GDF-15 levels were associated with incident

ischemic stroke in Chinese patients with hypertension, but that study's population was small. In contrast, some population-based studies of White individuals showed no association of blood GDF-15 levels with incident stroke.[15,26] The prognostic impact of GDF-15 for stroke incidence may thus have racial differences. Cerebral small-vessel disease has been suggested to be more common in Asians compared with White individuals.[27,28] The present study is the first to reveal that GDF-15 was associated with stroke events and improved the prognostic capacity of an established risk prediction model in a large Asian population with cardiovascular risk factors.

Notably, our findings demonstrated that GDF-15 was associated with stroke events after adjustment for representative prognostic biomarkers (hs-TnT and NT-proBNP) and that the effects of GDF-15 to discrimination and risk reclassification of the predictive model consisting traditional risk factors were equivalent to NT-proBNP and hs-TnT. It was reported that NT-proBNP has predictive ability for stroke events in community-dwelling individuals[29] and that hs-TnT predicts ischemic stroke in patient with atrial fibrillation.[30] GDF-15 might be a predictive marker for stroke events as with NT-proBNP and hs-TnT.

Blood GDF-15 levels were increased in patients with both atherosclerotic plaques and small-vessel disease, which contributed to cerebral infarction.[15,31] In human atherosclerotic carotid arteries, GDF-15 was exclusively localized in activated macrophages and was associated with the development and progression of atherosclerotic plaques through the regulation of apoptosis and inflammatory processes of activated macrophages.[31] Although GDF-15 exerts a cardioprotective effect through the activation of anaplastic lymphoma kinase receptors and the phosphorylation of the Smad signaling pathway,[32] studies of a GDF-15–deficient model were suggested that GDF-15 plays a pathogenic role in atherosclerotic plaques that contribute to the development of cerebral infarction through regulating inflammatory responses to vascular injury.[33,34] For the small-vessel disease that is the main cause of the development and progression of cerebral infarction in Asians, there has been a paucity of information about the pathological mechanism of GDF-15. New therapeutic targets may emerge based on a better understanding of the pathogenic mechanism of GDF-15 reflected by the atherosclerotic plaques and small-vessel disease.

A cut point of the third tertile of GDF-15 was 1188 ng/L in this study, and this value is close to a cut point of 1200 ng/L proposed as the upper limit of the reference interval of GDF-15 in previous studies.[18,19] We thus also performed analyses of the model using the dichotomous variable of GDF-15 with a cut point of 1200 ng/L when comparing the improvement of the model performance by adding other biomarkers. The result of adding this dichotomous model was similar to that of the stratification of tertiles. These results suggested that the stratification by a cut point of 1200 ng/L was useful for the risk management of stroke in patients with cardiovascular risk factors.

GDF-15 Did Not Predict Cardiac Event in Outpatients With Cardiovascular Risk Factors

Although it had been widely reported that higher GDF-15 levels were also associated with coronary artery disease and heart failure in community-dwelling individuals[10–12] and patients with CVD,[23,24,35] we did not observe a relationship between GDF15 level and the incidence of cardiac events in the present population. The reason for the inconsistency of the predictive ability for cardiac events might be unique and complicated pathophysiology of GDF-15. GDF-15 is highly expressed in various organs through different mechanisms by diseases. For example, cardiomyocytes in the infarct border zone mainly provide GDF-15 in patients with ischemic heart disease.[36] In patients with nonischemic heart failure, GDF-15 appears to be produced mainly in peripheral tissues.[37] In common cancers, GDF-15 is produced in tumor tissues and is cleaved from a propeptide by furin-like proteases before its secretion, but this intracellular cleavage from a propeptide does not process efficiently in tumor tissue.[38] A half-life of GDF-15 is prolonged in the circulation, and serum levels of GDF-15 increase markedly in advanced cancer.[39] The physiological roles of GDF-15 are also different from organs. As previously mentioned, GDF-15 has a cardioprotective effect[32] but plays a pathogenic role in carotid plaques.[33,34] Emerging evidence indicates that GDF-15 regulates body weight through an effect on the appestat.[40–43] GDF-15 forms a coreceptor complex with glial cell–derived neurotrophic factor receptor alpha-like and rearranged during transfection and induces an anorexia effect via the appestat.[40–43] Weight loss in patients with CVD or cancer is clearly associated with poor prognosis as disease-related anorexia-cachexia. Because of cardioprotective effects of GDF-15, highly expressed GDF-15 in various diseases might attenuate the relationship between serum GDF-15 levels and cardiac events in patients with high risks of CVD. Given that GDF-15 might develop carotid plaque and decrease body weight, it is acceptable that high GDF-15 levels are strongly related to all-cause death and stroke events in outpatients.

We indicate that NT-proBNP and hs-TnT were generally associated with the risk of cardiac events and that NT-proBNP marginally improved the predictive model for cardiac events. These findings correspond to those

of a prospective study of a community-dwelling population.[44] Additionally, the distribution of blood concentrations of GDF-15 and the incidence of each event in the present study are not greatly different from those of other studies.

Limitations

There were some limitations of this study. First, we measured blood GDF-15 levels only 1 time at the baseline, and we thus could not assess the interaction and fluctuation of GDF-15 levels over time during the progression of each adverse event. Second, the patients in this study were all Japanese, and our findings thus may not be generalizable to other racial or ethnic groups. Third, the patients in this study were being treated mainly for hypertension. Patients being treated for primary prevention are rare subjects for clinical research regarding GDF-15. This point might have caused the unique result of cardiac events. Fourth, the small number of stroke events prevented subgroup analyses of cerebral hemorrhage, although higher GDF-15 levels are related to major bleeding.[21–23] Finally, this study was the post hoc analysis of the JHOP study, which evaluated the relationship between home blood pressure and CVD risks; therefore, we could not calculate the sample size to assess the prognostic value of GDF-15 for adverse outcomes in patients with CVD risk factors.

Future Directions

Information about GDF-15 might be helpful for risk management of stroke events and all-cause death in outpatients with cardiovascular risk factors. However, it is difficult to make available GDF-15 to clinical practice individually because of its unique and complicated pathophysiology. Recently, it was attempted to combine GDF-15 with other biomarkers for the risk management of CVD, which was incorporated in a new scoring system of bleeding risks for patients with atrial fibrillation.[45,46] Thus, GDF-15 might be useful for clinical application of a multimarker strategy for stroke events in patients with cardiovascular risk factors.

CONCLUSIONS

In a large Japanese population with cardiovascular risk factors, blood GDF-15 levels were associated with increased risks of all-cause death and stroke events beyond conventional risk factors and other prognostic markers. Predictive ability of GDF-15 for stroke events was equivalent to NT-proBNP and hs-TnT. However, GDF-15 had no prognostic value for cardiac events.

ARTICLE INFORMATION

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Supplementary Material

Data S1
Tables S1–S7

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Study design

The Japan Morning Surge Home Blood Pressure (JHOP) study was a nationwide prospective study conducted in Japan that included 4310 outpatients with any of the following risk factors for cardiovascular disease: hypertension, impaired glucose tolerance or diabetes mellitus, dyslipidemia, current smoking (and/or current chronic obstructive pulmonary disease), chronic kidney disease (CKD), atrial fibrillation, metabolic syndrome, and sleep apnea syndrome. The exclusion criteria for the J HOP Study were a recent history of cardiovascular disease events (within 6 months), current hemodialysis treatment, chronic inflammatory disease, or malignancy. Diagnostic criteria of the cardiovascular risk factors were hypertension, defined as a clinic systolic BP (SBP) of >140 mmHg and/or a diastolic BP (DBP) of >90 mmHg, or current use of antihypertensive medication; impaired fasting glucose, defined as a fasting glucose level of >110 mg/dl; impaired glucose tolerance, defined as a glucose level of >140 mg/dl at 2 hours after a 75 g oral glucose tolerance test; diabetes, defined as a fasting glucose level of >126 mg/dl and/or a casual glucose level of >200 mg/dl or treated diabetes; hyperlipidemia, defined as a total cholesterol level of >240 mg/dl or treated hyperlipidemia; CKD, defined as the presence of proteinuria or a value of <60ml/min/1.73m^2 for the estimated glomerular filtration rate; metabolic syndrome, defined according to the guidelines of the Examination Committee of the Criteria for Metabolic Syndrome in Japan published in April 2005; or sleep apnea syndrome, defined as an apnea-hypopnea index of >15 events/hour by overnight sleep polysomnography.

The exclusion criteria for the J-HOP Study were a recent history of cardiovascular disease events (within the most recent 6 months), current hemodialysis treatment, chronic inflammatory disease, and malignancy. Patients were recruited for the J-HOP study between 2005 and 2012 and followed up through March 2015 by 75 doctors at 71 institutions (45 primary practices, 22 hospital-based outpatient clinics, and four specialized university hospitals).

In Japan, there are 47 administrative divisions (prefectures). In 25 of the prefectures (Tochigi, Aichi, Yamaguchi, Nagano, Miyazaki, Ibaraki, Hiroshima, Kumamoto, Hyogo, Tottori, Chiba, Saitama, Niigata, Fukushima, Oosaka, Shiga, Gunma, Kanagawa, Tokyo, Toyama, Mie, Yamagata, Gifu, Saga, Nara), 75 doctors at 71 institutions (45 primary practices, 22 hospital-based outpatient clinics, and 4 specialized university hospitals) agreed with the aims of this study and collected prospective data from individuals who agreed to participate in this project.

Outcome ascertainment

We divided the patient outcomes into the following three categories: (1) all-cause death including cardiovascular death and non-cardiovascular death. (2) Stroke events including cerebral infarction, cerebral hemorrhage, and subarachnoid hemorrhage based on the findings of brain computed tomography or magnetic resonance imaging. Stroke events defined as a sudden onset of a neurological deficit persisting for ≥24 hours in the absence of any other disease that could account for the symptoms. Transient ischemic attack was not included. (3) Cardiac events as the composite of CAD and hospitalization for heart failure; fatal and nonfatal CAD, defined as acute myocardial infarction and angina pectoris requiring percutaneous coronary intervention. The criteria for myocardial infarction included definite electrocardiographic findings (i.e., ST elevation), typical or atypical symptoms together with electrocardiographic findings and abnormal enzymes, or typical symptoms and abnormal cardiac enzymes with or without electrocardiographic findings. Hospitalization for heart failure was defined as an event requiring the patient's admission to a hospital with a primary diagnosis of heart failure and the initiation or intensification of treatment for heart failure. If events occurred on ≥2 occasions, the first occurrence was included in the analysis. Evidence on the above CV outcomes was ascertained by ongoing reports from a general physician at each institute. The incident stroke and cardiac events were also ascertained by means of annual or more frequent reviews of patients' medical records. When patients failed to come to the hospital, they or their family members were interviewed by telephone. The end point committee adjudicated all events by reviewing the patients' files and source documents and by requesting more detailed written information from investigators when necessary.

Table S1. Baseline clinical characteristics between the analysis population and the excluded population.

Variables	Analysis population n = 3562	Excluded population n = 748	p value
Age, y	66 (58–73)	65 (57–74)	0.518
Male, %	46.0	54.8	0.003
Prior CVD, %	12.6	14.2	0.826
Office SBP, mmHg	140.2 (130.0–151.2)	139.8 (130.1–151.2)	0.742
Office DBP, mmHg	81.0 (74.5–87.8)	80.4 (73.0–87.7)	0.135
BMI, kg/m^2	23.9 (22.0–26.1)	24.3 (22.2–26.5)	0.010
Current Smoking, %	12.1	13.0	0.565
Hypertension, %	91.0	93.4	0.033
Diabetes mellitus, %	24.5	18.2	0.296
Dyslipidemia, %	42.1	33.8	<0.001
Anti-hypertensive drugs, %	79.0	79.5	0.790
Statin, %	23.8	22.6	0.507
eGFR, $mL/min/1.73m^2$	73.0 (62.3–84.0)	74.9 (63.9–85.6)	0.017
Triglyceride, mg/dl	104 (76–150)	105 (77–144)	0.722
Total cholesterol, mg/dl	202 (181–224)	200 (178–221)	0.127
HDL-C, mg/dl	56 (47–66)	54 (46–65)	0.012
Fasting glucose, mg/dl	100 (92–112)	101 (94–114)	0.013
HbA1c, %	5.7 (5.4–6.0)	5.7 (5.4–6.1)	0.536

Continuous variables are presented as median (interquartile range) and categorized data are presented as number (percentage, %). Prior CVD includes pre-existing angina pectoris, myocardial infarction, and stroke. CVD: cardiovascular disease, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, eGFR: estimated glomerular filtration rate, HDL-C: high-density lipoprotein cholesterol, HbA1c: Hemoglobin A1c.

Table S2. Outcomes by gender cross-tabulations.

	All-cause death		Stroke event		Cardiac event	
	absence	presence	absence	presence	absence	presence
Male	1530	108	1596	42	1547	91
Female	1877	47	1885	39	1874	50
χ^2 tests	$p < 0.001$		$p = 0.338$		$p < 0.001$	

Table S3. Comparison of increased risk for outcomes in the dichotomous and continuous models adjusted for traditional risk factors.

Event	Biomarker	Dichotomous model Relative to reference HR (95%CI)	Continuous model 1 SD increase HR (95%CI)
All-cause death	GDF-15	2.03 (1.37–2.99)‡	3.21 (2.35–4.38)‡
	NT-proBNP	1.65 (1.16–2.36)†	1.37 (1.17–1.60)‡
	hs-TnT	1.15 (0.79–1.68)	1.26 (1.07–1.49)†
Stroke event	GDF-15	3.13 (1.82–5.39)‡	2.38 (1.46–3.88)‡
	NT-proBNP	2.72 (1.67–4.43)‡	1.65 (1.33–2.04)‡
	hs-TnT	3.24 (1.75–6.00)‡	1.65 (1.31–2.08)‡
Cardiac event	GDF-15	1.10 (0.74–1.65)	1.12 (0.74–1.68)
	NT-proBNP	2.35 (1.61–3.42)‡	1.38 (1.18–1.61)‡
	hs-TnT	1.29 (0.87–1.92)	1.22 (1.03–1.45)*

Dichotomous models of the prognostic biomarkers were stratified according to following cut-points: 1200 ng/L of GDF-15, 125 ng/L of NT-proBNP, and 3 ng/L of hs-TnT. The Cox proportional hazards analysis was adjusted for traditional risk factors (Model 1: age, sex, body mass index, current smoking, diabetes mellitus, previous cardiovascular disease, statin use, anti-hypertensive drug use, total cholesterol, high-density lipoprotein cholesterol, office systolic blood pressure, and estimated glomerular filtration rate). Hazard ratios (HRs) with 95% confidence intervals (CIs) represent comparisons versus patients under the cut-off point. *$p<0.05$, †$p<0.01$, and ‡$p<0.001$.

Table S4. Change in risk predictive metrics by incorporating dichotomous model of prognostic biomarkers to Model 1.

	c-statistics (95%CI)	Difference of c-statistics (95%CI)	Category-free NRI (95%CI)	IDI (95%CI)
All-cause death				
Model 1	0.786 (0.748–0.824)			
Model 1 + GDF-15	0.792 (0.755–0.829)§	0.006 (0.000–0.020)	0.228 (0.091–0.324)†	0.009 (0.001–0.024)*
Model 1 + NT-proBNP	0.787 (0.749–0.826)	0.002 (-0.003–0.011)	0.096 (-0.081–0.191)	0.005 (-0.001–0.020)
Model 1 + hs-TnT	0.786 (0.748–0.824)	0.000 (-0.002–0.004)	0.187 (-0.183–0.273)	0.001 (-0.001–0.008)
Stroke event				
Model 1	0.761 (0.711–0.812)			
Model 1 + GDF-15	0.797 (0.753–0.842)§	0.035 (0.008–0.057)	0.309 (0.139–0.433)†	0.014 (0.005–0.037)†
Model 1 + NT-proBNP	0.786 (0.739–0.833)§	0.025 (0.003–0.055)	0.207 (0.047–0.346)*	0.015 (0.004–0.048)†
Model 1 + hs-TnT	0.793 (0.746–0.840)§	0.032 (0.008–0.053)	0.355 (0.206–0.448)*	0.009 (0.002–0.028)*
Cardiac event				
Model 1	0.777 (0.737–0.816)			
Model 1 + GDF-15	0.777 (0.737–0.817)	0.000 (-0.001–0.006)	0.073 (-0.115–0.173)	0.000 (-0.001–0.006)

Model 1 + NT-proBNP	0.788 (0.748–0.828)§	0.011 (0.000–0.026)	0.181 (0.030–0.280)*	0.020 (0.005–0.043)†
Model 1 + hs-TnT	0.780 (0.740–0.819)	0.003 (-0.001–0.013)	0.143 (-0.120–0.231)	0.001 (-0.001–0.008)

NRI: net reclassification improvement, IDI: integrated discrimination improvement. Model 1 and the cut-off points of each dichotomous model were described in Table S2. The confidence intervals (CIs) of each metric were estimated by using 1000 bootstrap samples. *$p<0.05$, †$p<0.01$, and ‡$p<0.001$. §Significant improvement of c-statistics, as the 95%CIs were not <0.

Table S5. The diagnostic testing accuracy of the prognostic biomarkers for all-cause death.

Model	SE	SP	PPV	NPV	PLR	NLR	DOR	Youden index	Cutoff
Model 1	0.658	0.719	0.096	0.979	2.345	0.475	4.934	0.377	0.086
Model 1 + GDF-15	0.742	0.666	0.092	0.983	2.219	0.388	5.725	0.408	0.066
Model 1 + NT-proBNP	0.652	0.701	0.090	0.978	2.179	0.497	4.383	0.353	0.078
Model 1 + hs-TnT	0.632	0.761	0.107	0.978	2.643	0.483	5.468	0.393	0.098
Model 2	0.645	0.737	0.100	0.979	2.453	0.481	5.095	0.382	0.090
Model 2 + GDF-15	0.684	0.723	0.101	0.98	2.468	0.437	5.644	0.407	0.078

SE: sensitivity. SP: specificity. PPV: positive predictive values. NPV: positive predictive values. PLR: positive likelihood ratios. NLR: negative likelihood ratios. DOR: diagnostic odds. Model 1 were described in Table S2. Model 2 was the model incorporating log NT-proBNP and log hs-TnT to Model 1.

Table S6. The diagnostic testing accuracy of the prognostic biomarkers for stroke events.

Model	SE	SP	PPV	NPV	PLR	NLR	DOR	Youden index	Cutoff
Model 1	0.753	0.588	0.041	0.99	1.828	0.42	4.354	0.341	0.031
Model 1 + GDF-15	0.753	0.620	0.044	0.991	1.983	0.398	4.981	0.373	0.033
Model 1 + NT-proBNP	0.765	0.664	0.050	0.992	2.277	0.353	6.445	0.429	0.034
Model 1 + hs-TnT	0.864	0.495	0.038	0.994	1.712	0.274	6.244	0.359	0.021
Model 2	0.679	0.749	0.059	0.99	2.711	0.428	6.329	0.429	0.045
Model 2 + GDF-15	0.654	0.752	0.058	0.989	2.633	0.460	5.725	0.406	0.044

SE: sensitivity. SP: specificity. PPV: positive predictive values. NPV: positive predictive values. PLR: positive likelihood ratios. NLR: negative likelihood ratios. DOR: diagnostic odds. Model 1 were described in Table S2. Model 2 was the model incorporating log NT-proBNP and log hs-TnT to Model 1.

Table S7. The diagnostic testing accuracy of the prognostic biomarkers for cardiac events.

Model	SE	SP	PPV	NPV	PLR	NLR	DOR	Youden index	Cutoff
Model 1	0.716	0.681	0.085	0.983	2.242	0.417	5.378	0.397	0.052
Model 1 + GDF-15	0.745	0.656	0.082	0.984	2.163	0.389	5.554	0.4	0.049
Model 1 + NT-proBNP	0.674	0.744	0.098	0.982	2.628	0.439	5.991	0.417	0.061
Model 1 + hs-TnT	0.766	0.632	0.079	0.985	2.083	0.37	5.627	0.398	0.046
Model 2	0.823	0.566	0.073	0.987	1.897	0.313	6.056	0.389	0.038
Model 2 + GDF-15	0.823	0.571	0.073	0.987	1.919	0.31	6.18	0.394	0.038

SE: sensitivity. SP: specificity. PPV: positive predictive values. NPV: positive predictive values. PLR: positive likelihood ratios. NLR: negative likelihood ratios. DOR: diagnostic odds. Model 1 were described in Table S2. Model 2 was the model incorporating log NT-proBNP and log hs-TnT to Model 1.