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Growth differentiation factor 15 in heart failure with preserved vs. reduced ejection fraction

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Aim Growth differentiation factor 15 (GDF15) is a cytokine highly expressed in states of inflammatory stress. We aimed to study the clinical correlates and prognostic significance of plasma GDF15 in heart failure with preserved ejection fraction (HFpEF) vs. reduced ejection fraction(HFrEF), compared with *N*-terminal pro-brain natriuretic peptide (NT-proBNP), an indicator of haemodynamic wall stress.

Methods Plasma GDF15 and NT-proBNP were prospectively measured in 916 consecutive patients with HFrEF (EF <50%; $n = 730$) and HFpEF (EF ≥50%; $n = 186$), and measured again at 6 months in 488 patients. Patients were followed up for a composite outcome of death or first HF rehospitalization.

Results Median $GDF15_{baseline}$ values were similarly elevated in HFpEF [2862 (1812 represent the 25th percentile and 4176 represent the 75th percentile) ng/L] and HFrEF [2517 (1555, 4030) ng/L] ($P = 0.184$), whereas NT-proBNP was significantly lower in HFpEF than HFrEF (1119 ng/L vs. 2335 ng/L, $P < 0.001$). Independent correlates of $GDF15_{baseline}$ were age, systolic blood pressure, New York Heart Association (NYHA) class, diabetes, atrial fibrillation, sodium, haemoglobin, creatinine, diuretic therapy, high sensitivity troponin T (hsTnT) and NT-proBNP (all $P < 0.05$). During a median follow-up of 23 months, there were 379 events (307 HFrEF, 72 HFpEF). GDF15 remained a significant independent predictor for composite outcome even after adjusting for important clinical predictors including hsTnT and NT-proBNP (adjusted hazard ratio 1.76 per 1 Ln U, 95% confidence interval 1.39–2.21; $P < 0.001$), regardless of HF group ($P_{interaction} = 0.275$). $GDF15_{baseline}$ provided incremental prognostic value when added to clinical predictors, hsTnT and NT-proBNP (area under receiver operating characteristic curve increased from 0.720 to 0.740, $P < 0.019$), with a net reclassification improvement of 0.183 ($P = 0.004$). Patients with ≥20% $GDF15_{6months}$ increase had higher risk for composite outcome (adjusted hazard ratio 1.68, 95% confidence interval 1.15–2.45; $P = 0.007$) compared with those with $GDF15_{6months}$ within ± 20% of baseline.

Conclusions The similarly elevated levels and independent prognostic utility of GDF15 in HFrEF and HFpEF suggest that beyond haemodynamic stress (NT-proBNP), inflammatory injury (GDF15) may play an important role in both HF syndromes.

Keywords Heart failure with preserved ejection fraction • Growth differentiation factor 15 • Natriuretic peptides • Prognosis

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Introduction

Growth differentiation factor 15 (GDF15) is a member of the transforming growth factor β cytokine superfamily that is highly expressed in states of inflammatory stress. Circulating GDF15 levels are known to be elevated in heart failure (HF).[1] However, the clinical correlates and prognostic utility of GDF15 have not been directly compared between HF with reduced ejection fraction (HFrEF) vs. HF preserved ejection fraction (HFpEF) from the same community.

Given that elevated GDF15 may signify additional inflammatory stress in HF beyond haemodynamic wall stress [as indicated by *N*-terminal pro-brain natriuretic peptide (NT-proBNP) levels] we hypothesized that GDF15 may have different clinical correlates and provide incremental prognostic information from that of NT-proBNP. Furthermore, these associations may differ between HFrEF and HFpEF. We therefore aimed to determine the clinical correlates and prognostic utility of GDF15, as well as any intermediate-term (6 month) change of the same patients.

As high sensitivity troponin T (hsTnT) (a marker of myocardial injury) is increasingly being recognized as a predictor of outcomes in both stable and decompensated HF patients, we have also included hsTnT as a comparative covariate in our survival analysis.[2,3]

Methods

Study population

Patients with HF were identified from the nation-wide prospective multicentred Singapore Heart Failure Outcomes and Phenotypes (SHOP) study (the study design has been published previously).[4] In brief, consecutive patients were identified from any of the six public health-care institutions (together serving >80% of Singapore's population), who must have presented to the hospital with a primary diagnosis of HF, or to the clinic for management of a HF episode which occurred within 6 months before recruitment. All HF episodes were determined by cardiologists blinded to biomarker values, and in accordance with the European Society of Cardiology (2012) criteria defining HF, and excludes patients with HF secondary to specific aetiologies (e.g. infiltrative or congenital heart disease), with end-stage renal failure (defined as estimated glomerular filtration rate <15 mL/min.m^2) and life-limiting comorbidities resulting in <1 year life expectancy (e.g. advanced cancer). To complying with the Declaration of Helsinki, informed consent was provided by all patients participating in the protocol, which was approved by the ethics committee from each participating institution.

Study procedures

Once recruited, all patients will only be assessed after stabilization from the acute episode. Baseline assessment involves standardized history taking, physical examination, a resting 12-lead electrocardiogram, blood sampling and comprehensive transthoracic Doppler echocardiography using standardized equipment (Vivid ultrasound systems, General Electric, Milwaukee, WI, USA) and complying with recommendations from the American Society of Echocardiography (2009). The biplane method of disks was used to measure left ventricular (LV) EF. Patients were then stratified into HFrEF (LVEF <50%) and HFpEF (LVEF ≥50%) according to this measured baseline LVEF. The ratio of early transmitral flow to early medial–mitral annular diastolic velocity (E/e′) was recorded as an index of LV filling pressure. The simplified Modification of Diet in Renal Disease (MDRD) equation was used to provide an estimated glomerular filtration rate (eGFR).

Patients returned at 6 weeks and 6 months for full clinical review, resting 12-lead electrocardiogram and blood sampling. All participants were followed-up for clinical outcomes at 1- and 2-year time-points. The primary outcome was defined as a composite endpoint of all-cause mortality or first rehospitalization for HF. Analyses for death and hospitalization separately were undertaken as individual secondary endpoints.

Measurement of biomarkers

The GDF15 concentrations were determined by a quantitative sandwich enzyme immunoassay technique (Quantikine®; R&D Systems, Inc. Minneapolis, MN, USA). Quality control (QC) samples from R&D Systems, which had a total coefficient of variation (CV) of 6.3% at low concentrations (159 ng/L), 9.7% at medium concentrations (436 ng/L), and 15.1% at high concentrations (827 ng/L), were included in each assay and results were accepted when QCs fell within manufacturer-specified lot-specific concentration. Overall range of GDF15 detection was 308–13790 ng/L. The NT-proBNP and hsTnT concentrations were measured by electro-chemiluminescence immunoassay using the NT-proBNP II and troponin T high-sensitivity assays respectively, on a Cobas analyser (Roche Diagnostics GmbH, Mannheim, Germany). Quality controls included in both were within lot-specific target ranges. Detection of NT-proBNP ranged from 12 ng/L to 35 000 ng/L and hsTnT ranged between 3 ng/L and 1172 ng/L.

Statistical analysis

All data were analysed with STATA version 11.0 (StataCorp LP, College Station, Texas). All statistical evaluations were made assuming a two-sided test at the 5% level of significance.

Baseline characteristics were reported as follows: categorical variables as percentages (%), continuous variables as means ± standard deviation (SD) if normally distributed or medians (25th and 75th percentiles) if not normally distributed. For comparisons between HFrEF and HFpEF, the Mann–Whitney *U*-test (non-parametric), *t*-test (parametric) and chi-square test (categorical) were used as appropriate. Covariates were compared across GDF15 tertiles using chi-square test (categorical variables), one-way analysis of variance (parametric), and Kruskal–Wallis test (non-parametric). Multiple linear regression analysis with a backward selection procedure was carried out to determine covariates independently associated with baseline GDF15.

For survival analyses, incidence and Kaplan–Meier curves were constructed to estimate event rates across individual GDF15 tertiles. The strengths of unadjusted and adjusted associations of baseline GDF15 with outcomes were evaluated by univariable and multivariable Cox proportional regression models. Using backward selection, only significant variables $P < 0.05$ were kept in the final model and all factors were tested for interaction with GDF15. The incremental prognostic utility of GDF15 was assessed by comparing the areas under the curve (AUCs) of receiver operating characteristics (ROC) curves with and without GDF15 using the method of Delong *et al.*[5] In addition, the net reclassification improvement (NRI) was determined when GDF15 was added to a full model of existing putative predictors. All hazard ratios for biomarkers (GDF15, NT-proBNP, and hsTnT) are presented as per

Table 1 Baseline characteristics according to tertiles of baseline growth differentiation factor 15 (GDF15) in heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF)

	HFrEF (*N* = 730)				HFpEF (*N* = 186)			
	<1933 ng/L	1933 to <3451 ng/L	≥3451 ng/L	*P*-value	<1933 ng/L	1933 to <3451 ng/L	≥3451 ng/L	*P*-value
Clinical characteristics								
Age, years	55 ± 11	61 ± 11	63 ± 12	<0.001	66 ± 12	68 ± 10	71 ± 11	0.029
Male, %	85	87	82	0.211	47	45	37	0.485
Race, %: Chinese; Malay; Indian	63; 24; 12	58; 31; 10	64; 23; 11	0.378	65; 21; 11	63; 26; 11	69; 24; 6	0.690
Body mass index, kg/m^2	26 ± 5	26 ± 6	26 ± 5	0.263	29 ± 7	28 ± 5	26 ± 5	0.006
Heart rate, beats/min	77 ± 14	78 ± 13	78 ± 15	0.434	72 ± 14	70 ± 14	74 ± 14	0.229
Systolic BP, mmHg	125 ± 24	122 ± 22	120 ± 19	0.063	127 ± 20	134 ± 20	131 ± 23	0.196
Diastolic BP, mmHg	75 ± 15	71 ± 13	69 ± 12	<0.001	69 ± 11	69 ± 10	69 ± 18	0.976
NYHA class, %				<0.001				0.740
I	40	23	15		28	23	18	
II	51	60	59		61	60	63	
III	8	16	24		11	16	18	
IV	1	1	2		0	1	1	
Ischaemic aetiology of HF, %	47	70	76	<0.001	37	37	43	0.699
Coronary artery disease, %	49	65	71	<0.001	31	40	32	0.515
Hypertension, %	63	71	72	0.048	72	92	87	0.010
Diabetes mellitus, %	32	61	72	<0.001	32	65	73	<0.001
Atrial fibrillation/flutter, %	12	21	25	0.001	35	31	34	0.856
Laboratory values								
Sodium, mmol/L	139 ± 3	138 ± 3	137 ± 4	<0.001	138 ± 6	137 ± 6	137 ± 5	0.659
Haemoglobin, g/dL	14.1 ± 1.7	13.3 ± 1.8	12.5 ± 2.0	<0.001	12.7 ± 1.9	12.0 ± 1.9	10.8 ± 2.0	<0.001
Creatinine, μmol/L	94 ± 27	115 ± 42	142 ± 73	<0.001	92 ± 40	121 ± 53	141 ± 75	<0.001
eGFR, mL/min	78 (62, 96)	63 (50, 81)	52 (35, 68)	<0.001	73 (61, 89)	51 (36, 67)	47 (27, 62)	<0.001
NT-proBNP, ng/L	1287 (510, 2295)	2395 (1248, 4295)	4698 (2498, 9542)	<0.001	554 (180, 1042)	1225 (532, 2764)	2059 (862, 5594)	<0.001
hsTnT, ng/L	20 (12, 34)	31 (23, 50)	48 (28, 81)	<0.001	15 (10, 19)	26 (15, 48)	42 (24, 56)	<0.001
Medication								
Diuretic, %	89	95	96	0.002	77	90	91	0.045
ACEi/ARB, %	93	89	87	0.098	86	76	76	0.303
Beta-blocker, %	92	93	91	0.843	79	92	82	0.120
Spironolactone, %	59	62	60	0.770	12	19	9	0.213
Digoxin, %	28	34	37	0.077	14	16	12	0.791
Statin, %	81	88	91	0.007	86	87	91	0.649
Echocardiographic data								
LVEF in %	28 ± 10	28 ± 9	28 ± 10	0.810	59 ± 6	60 ± 6	60 ± 6	0.687
Mitral E/e′ ratio	16 ± 7	19 ± 7	20 ± 9	<0.001	13 ± 6	17 ± 10	16 ± 8	0.035

ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; BP, blood pressure; eGFR, estimated glomerular filtration rate; hsTnT, high sensitivity troponin T; NT-proBNP, *N*-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association Classification.

1 unit increase in Ln'Biomarker', which is equivalent to a 172% increase of absolute 'Biomarker' value.

The prognostic utility of GDF15 and NT-proBNP in HFpEF compared with HFrEF, and between other clinically relevant patient subgroups, was assessed through interaction analysis between each biomarker and covariate. A proportional sub-distribution hazard regression model adjusted for relevant clinical covariates via the Fine and Gray Method[6] has been adopted to study the association of GDF-15 with all-cause mortality and first HF re-hospitalization respectively. A comparison of the time-varying hazards ratios (HR) of GDF15, hsTnT and NT-proBNP in the subset of patients with serial measurements at 6 weeks and 6 months was done to determine the effect of time on the prognostic value of the biomarkers.

Lastly, the prognostic value of serial measurements of GDF15 was investigated in patients with GDF15 re-measured at 6 months, using the Wilcoxon matched-pair signed rank test to compare serial GDF15 measurements in the same individual. To account for baseline differences in GDF15 levels, the per cent change in GDF15 after 6 months was calculated and used to stratify patients into three categories using ±20% as a cut-off for variability (based on an estimate of the reference change value of GDF15 previously reported in stable heart failure patients):[7] 'maintained'(M) if GDF15 change was between −20% and +19%, 'decreased' (D) if GDF15 decreased by more than 20%, and 'increased' (I) if GDF15 increased by 20% or more. Similar analyses were performed for NT-proBNP using cut-offs of ±20% to allow comparison between biomarkers.

Results

Baseline characteristics

Our study consisted of 916 patients (76% men), with a mean age of 61 ± 12 years. Median $GDF15_{baseline}$ level was 2581 (25th & 75th%, 1627 & 4056) ng/L, with 85.5% above 1200 ng/L (upper limit of normal).[8] Stratification by baseline LVEF identified 79.7% of our study sample as HFrEF, and 20.3% HFpEF. Patients with HFpEF were older, more often female, had higher body mass

Table 2 Independent correlates of baseline growth differentiation factor 15 (GDF15) from multiple linear regression

	Coefficient	95% CI	*P*-value
Age	0.007	0.004–0.010	<0.001
Systolic BP	−0.002	−0.004 to −0.001	0.004
NYHA class			0.001
II vs. I	0.166	0.078–0.253	<0.001
III vs. I	0.194	0.081–0.308	0.001
IV vs. I	0.170	−0.127–0.467	0.262
Diabetes mellitus	0.318	0.246–0.391	<0.001
Atrial fibrillation	0.093	0.008–0.179	0.033
Sodium	−0.016	−0.026 to −0.007	0.001
Haemoglobin	−0.031	−0.049 to −0.012	0.001
Creatinine	0.002	0.001–0.003	<0.001
Diuretic	0.172	0.036–0.308	0.013
LnNT-proBNP	0.101	0.069–0.134	<0.001
LnhsTnT	0.124	0.072–0.177	<0.001

BP, blood pressure; hsTnT, high sensitivity troponin T; NT-proBNP, *N*-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association Classification.
Natural logarithm of GDF15 levels is used as a dependent variable.

index (BMI) and greater prevalence of hypertension (HTN) and atrial fibrillation/flutter (AF), but a lower prevalence of coronary artery disease (CAD), compared with those with HFrEF. The prevalence of diabetes mellitus (DM) was high in both HFpEF (58%) and HFrEF (55%) in our Asian cohort. Interestingly, median $GDF15_{baseline}$ values were similarly elevated in HFpEF [2862 (1812, 4176) ng/L] and HFrEF [2517 (1555, 4030) ng/L], ($P = 0.184$), whereas NT-proBNP was significantly lower in HFpEF compared with HFrEF (1119 ng/L vs. 2335 ng/L, $P < 0.001$).

Baseline clinical correlates of growth differentiating factor 15

Baseline characteristics of patients according to $GDF15_{baseline}$ tertiles and HF group are presented in *Table 1*. In both HF groups, increasing GDF15 tertile correlated directly with age, presence of HTN, DM, AF, use of diuretic therapy, hsTnT, and NT-proBNP levels, and inversely with haemoglobin concentration and eGFR (all $P < 0.05$). For both HF groups, Increasing LV filling pressures (mitral E/e′ ratio) ($P < 0.05$), but not LVEF ($P > 0.05$), were related to increasing $GDF15_{baseline}$ tertiles. In contrast, higher NT-proBNP levels were related to lower LVEF as well as higher LV filling pressures (see the Supplementary material online, *Table 1*).

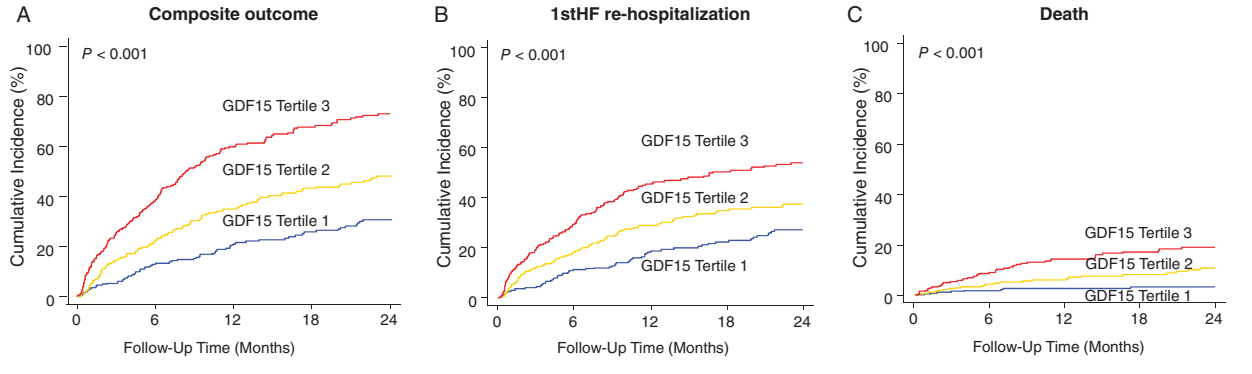
In multivariable linear regression analyses, age, systolic blood pressure, New York Heart Association (NYHA) class, DM, sodium levels, haemoglobin concentration, creatinine levels, baseline NT-proBNP levels, and diuretic treatment were independently related to $GDF15_{baseline}$ (all $P < 0.05$) (*Table 2*).

Association of growth differentiating factor 15 with outcomes

Primary outcome

During a median follow-up of 23 (25th–75th percentile, 12–24) months, the composite outcome occurred in 379 patients (81 deaths, 298 first HF rehospitalizations). A higher $GDF15_{baseline}$ was associated with a higher composite event rate, and similarly in first HF rehospitalization or all-cause mortality individually (*Figure 1*). Separate analysis for HFrEF ($n = 307$; 64 deaths, 243 HF rehospitalizations) and HFpEF ($n = 72$; 17 deaths, 55 HF rehospitalizations) yielded similar results (*Figure 2*).

In patients with complete covariate data, $GDF15_{baseline}$ remained a strong independent predictor of composite outcome, even after adjusting for significant covariates identified for outcome,



Number of patients at risk (Primary Outcome in %)

Tertile 1	296	232 (13)	152 (21)	122 (26)	63 (31)
Tertile 2	295	204 (23)	130 (35)	95 (43)	61 (48)
Tertile 3	293	165 (38)	83 (60)	56 (68)	25 (73)

Number of patients at risk (Primary Outcome in %)

Tertile 1	296	232 (11)	152 (18)	122 (22)	63 (27)
Tertile 2	295	204 (18)	130 (29)	95 (35)	61 (37)
Tertile 3	293	165 (29)	83 (45)	56 (50)	25 (54)

Number of patients at risk (Primary Outcome in %)

Tertile 1	296	232 (2)	152 (3)	122 (3)	63 (3)
Tertile 2	295	204 (5)	130 (6)	95 (8)	61 (11)
Tertile 3	293	165 (9)	83 (14)	56 (17)	25 (19)

Figure 1 Cumulative incidence of composite outcome, first heart failure (1stHF) rehospitalization and death by tertiles of baseline growth differentiation factor 15 (GDF15). Increasing $GDF15_{baseline}$ tertiles was related to increasing risk of (a) the composite outcome of all-cause mortality or 1stHF rehospitalization, (b) 1stHF re-hospitalization alone, and (c) all-cause mortality alone (all Log Rank $P < 0.001$).

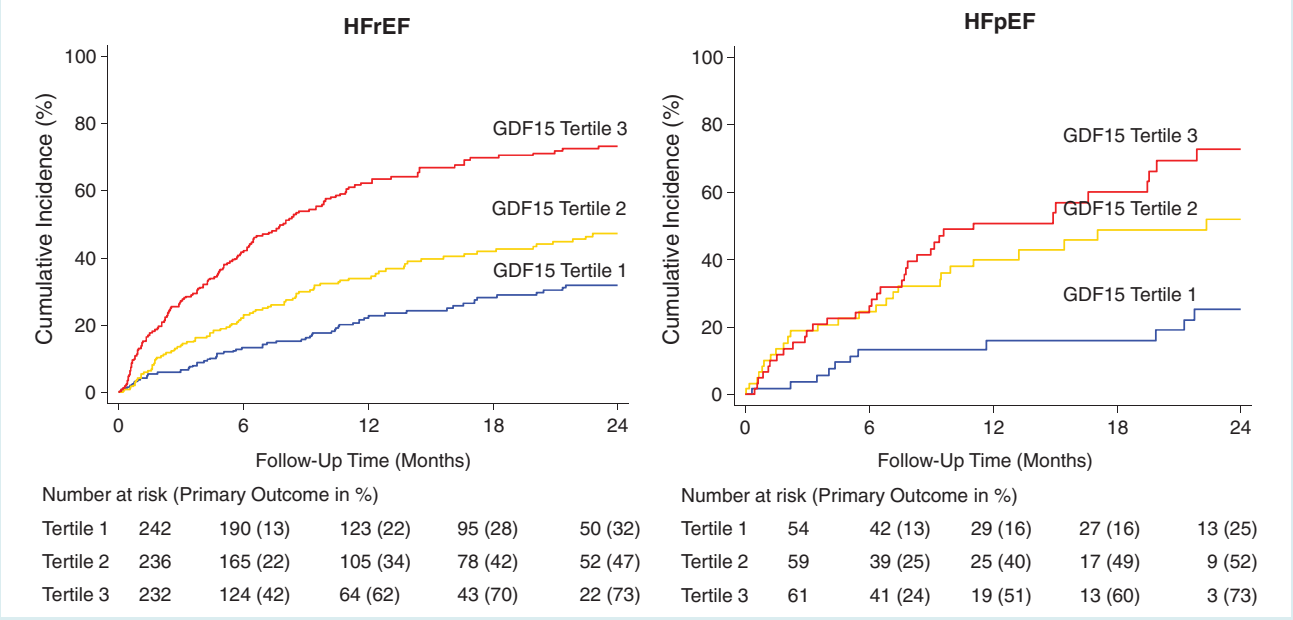


Figure 2 Cumulative survival probability of composite outcome by tertiles of baseline growth differentiation factor 15 (GDF15) in heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). In respective HF groups, Kaplan–Meier curves show increasing risk for composite outcome with increasing $GDF15_{baseline}$ tertiles. Log Rank pooled $P < 0.001$ for both HF groups.

Table 3 Multivariable Cox regression for composite outcome

	HR	95% CI	*P* Value
LnNT-proBNP	1.11	0.98–1.26	0.090
LnhsTnT	1.34	1.14–1.57	<0.001
LnGDF15	1.76	1.39–2.21	<0.001

CI, confidence interval; GDF15, growth differentiation factor 15; HR, hazard ratio per 1 unit increase in LnNT-proBNP, LnhsTnT or LnGDF15; hsTnT, high sensitivity troponin T; NT-proBNP, *N*-terminal pro-B-type natriuretic peptide. The model is adjusted for NYHA class, ischaemic aetiology of heart failure, diabetes mellitus, atrial fibrillation, sodium, creatinine, and E/e′.

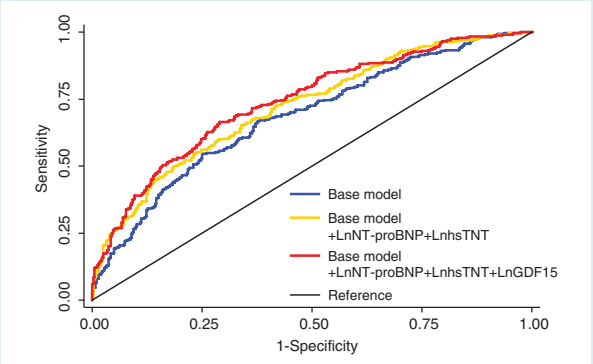


Figure 3 Comparison of areas under the receiver operating characteristics (ROC) curves for prediction of composite outcome. The ROC curves for a base model of significant covariates for outcomes (previously identified in regression analysis), model including *N*-terminal pro-brain natriuretic peptide (NT-proBNP) and high sensitivity troponin (hsTnT), and a full model including growth differentiation factor 15 (GDF15) with their respective area under the curves (AUCs). *Comparison of AUCs between the full model and the model without LnGDF15 was significant ($P = 0.019$). Net reclassification improvement calculated for addition of GDF15 to the model = 0.183, (95% confidence interval 0.062–0.266, $P = 0.004$).

hsTnT and NT-proBNP (adjusted HR 1.76 per 1 LnGDF15, 95% CI 1.39–2.21, $P < 0.001$), irrespective of HF group (interaction between LnGDF15 and HF group for primary outcome, $P = 0.275$) (*Table 3*).

To determine the incremental predictive utility of GDF15, we compared *c*-statistics before and after the addition of GDF15 to the base model with hsTnT and NT-proBNP, and found a significant increase with GDF15 ($AUC_{model\ without\ GDF15} = 0.720$ vs. $AUC_{model\ with\ GDF15} = 0.740$, $P = 0.019$) (*Figure 3*). Moreover, the NRI for addition of GDF15 to the standard fully adjusted model was significant (NRI = 0.183, 95% CI 0.062–0.266, $P = 0.004$).

In all patient subgroups explored (**see the Supplementary material online**, *Figure S1*), the unadjusted HRs for LnGDF15 and LnNT-proBNP remained statistically significant individually (all $P < 0.05$), with the exception of NYHA class IV (unadjusted HR 2.28 per LnGDF15, 95% CI 0.49–10.74, $P = 0.296$, and unadjusted HR 1.53 per LnNT-proBNP, 95% CI 0.71–3.30, $P = 0.276$). The latter finding is likely attributable to the small sample of NYHA class IV patients ($n = 5$). None of the selected variables significantly modified the relationship between GDF15 and the composite primary

Table 4 Comparison of time-varying effects of *N*-terminal pro-brain natriuretic peptide (NT-proBNP), high sensitivity troponin T (hsTnT) and growth differentiation factor 15 (GDF15) on composite outcome

	LnNT-proBNP		LnhsTnT		LnGDF15	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Baseline	1.07 (0.92–1.25)	0.368	1.30 (1.09–1.56)	0.004	1.51 (1.13–2.03)	0.006
6 weeks	1.09 (0.94–1.26)	0.267	1.32 (1.11–1.56)	0.002	1.56 (1.18–2.07)	0.002
6 months	1.13 (0.99–1.30)	0.077	1.37 (1.15–1.62)	<0.001	1.74 (1.34–2.28)	< 0.001

CI, confidence Interval; HR, hazard ratio per 1 unit increase in LnNT-proBNP, LnhsTnT, or LnGDF15.
The model is adjusted for New York Heart Association (NYHA) class, ischemic aetiology of HF, diabetes mellitus, atrial fibrillation, sodium, creatinine and E/e′.

outcome ($P_{interaction} > 0.05$). Conversely, LVEF categories significantly altered the association of NT-proBNP with composite outcome ($P_{LVEF\ interaction} = 0.037$), with the highest risk associated with increased NT-proBNP in patients with LVEF between 30 and 39.

Secondary analyses

To further delineate the prognostic utility of $GDF15_{baseline}$, a competing risks analysis of death and first HF rehospitalization was undertaken (see the Supplementary material online, *Table S2*). After adjusting for clinical predictors, respective subgroup HRs of GDF15 for death (without previous HF hospitalization) and first HF rehospitalization remained significant (SHR_{death} 2.16, $P = 0.004$; $SHR_{1st\ HF\ re\text{-}hospitalization}$ 1.44, $P = 0.006$). Notably, the subgroup HR of $GDF15_{baseline}$ was larger for the outcome of death compared with first HF rehospitalization, and conferred greater risk prediction than hsTnT and NT-proBNP. This effect was similar between HF groups for either outcome ($P_{interaction} > 0.05$ in both outcome categories).

At least one hospitalization event was experienced in 513 patients (see the Supplementary material online, *Table S3*). Higher $GDF15_{baseline}$ was associated with a greater number of hospitalizations per patient (both all-cause and HF-related hospitalizations). Interestingly, higher GDF15 levels related to more cardiovascular-related deaths, whereas higher NT-proBNP levels related to more non-cardiovascular related deaths.

Repeated measurements of GDF15, NT-proBNP, and hsTnT were available in 604 patients at 6 weeks, and 488 patients at 6 months following recruitment. The time-varying effect of GDF15, hsTnT and NT-proBNP for prediction of the composite outcome was examined using serial measurements at 6 weeks and 6 months. It was found that GDF15 was consistently associated with higher HRs compared with both hsTnT and NT-proBNP at each time-point (*Table 4*), even after adjusting for covariates. There was no significant difference in the time-varying effect of GDF15, hsTnT, or NT-proBNP in HFrEF compared with HFpEF ($P_{interaction} > 0.05$ for all biomarkers).

Association of change in growth differentiating factor 15 with outcome

In the subset of 488 patients (81% HFrEF) with repeated measurements of plasma GDF15 and NT-proBNP at 6 months, GDF15 decreased over time (median change −137 ng/L, interquartile range −767–370 ng/L, $P < 0.001$), and the median change was similar in HFrEF and HFpEF ($P = 0.085$). When patients were grouped by %GDF15-change, event-free survival was longest in patients with decreased %GDF15-change, and shortest in patients with increased %GDF15-change (see the Supplementary material online, *Figure 2*). Interestingly, using 'maintained' group as the reference category, a >20%GDF15-increase appeared to be a stronger risk predictor (HR 1.67, 95% CI 1.19–2.34) compared with a >20%NT-proBNP-increase (HR 1.50, 95% CI, 1.03–2.18). After multivariable adjustment (see the Supplementary material online, *Table S4*), the 'increased' %GDF15-change group had higher risk for composite outcome (adjusted HR_{IvsM} 1.68, $P = 0.007$), whereas the 'decreased' group had lower risk for the composite outcome compared with the 'maintained' group (adjusted HR_{DvsM} 0.64, $P = 0.021$).

Discussion

In our study of simultaneous measurements of GDF15 and NT-proBNP in HFrEF and HFpEF from the same community, we found that GDF15, unlike NT-proBNP, was similarly elevated in both types of HF. We provide the first evidence of the incremental prognostic utility of GDF15 over NT-proBNP and hsTnT in both HFpEF and HFrEF from an Asian cohort. We further showed that serial measurements of GDF15 provide additional predictive information for outcomes. As GDF15 is a marker of systemic inflammation,[1] its additional prognostic value suggests that beyond haemodynamic wall stress (NT-proBNP) and myocardial necrosis (hsTnT), inflammatory stress (GDF15) may play an important role in HF (regardless of EF) and its progression.[9]

The relatively young mean age of our Asian cohort, compared with similar cohorts from Western populations, is consistent with other large registries.[10] Unlike the reported ~50% prevalence of HFpEF in Western epidemiological HF studies, the percentage of HFpEF in our cohort is 20.3%. This lower proportion of HFpEF is consistent with reports from the Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD) (25% HFpEF) and in the population of Qatar (13.3% HFpEF). Whether this reflects true ethnic differences or the younger age of onset of HF in Asian cohorts deserves further study.[11,12]

Median baseline GDF15 levels from our population were also notably higher than the DIAST-CHF cohort—the only

other study comparing baseline GDF15, but not its association with outcomes—in both HF groups (HFpEF $GDF15_{median}$ 2862 vs. 1660 ng/L; HFrEF $GDF15_{median}$ 2517 versus 1810 ng/L).[13] Notably, this was observed despite a younger cohort in SHOP vs. DIAST-CHF (HFrEF 59 years old) vs. 71 years old); HFpEF 59 years old) vs. 73 years old) and the direct correlation of GDF15 with age. Similar findings of higher median GDF15 in our HFrEF cohort compared with other Western studies were noted.[14,15] While this may be caused by assay differences, it may also indicate true population or ethnic differences. We postulate that ours was a cohort of sicker HF patients because all patients were required to have a validated diagnosis of HF and confirmation of a recent (within 6 months) episode of HF decompensation to enter our study. The higher median baseline of NT-proBNP levels in our cohort compared with DIAST-CHF ($HFrEF_{SHOPvsDIAST\text{-}CHF}$ 1119 ng/L vs. 422 ng/L; $HFpEF_{SHOPvsDIAST\text{-}CHF}$ 2335 ng/L vs. 326 ng/L), as well as the higher event rates in our HFrEF population (42.0% reached composite outcome after a median follow-up of 23 months) compared with other studies (34% reached first morbid event after 23 months in the study of Anand *et al.*),[14] are also consistent with a more severely ill cohort. Furthermore, the high prevalence of DM in our cohort may have contributed to higher GDF15 levels. Nonetheless, similar levels of circulating GDF15 found between HFrEF and HFpEF groups are consistent with findings from the DIAST-CHF cohort.[13] Despite population differences, independent associations with GDF15 identified from our study (i.e. age, renal function, presence of diabetes, NYHA class, and NT-proBNP levels) are also consistent with previous findings.[14,15]

It is notable that higher levels of both GDF15 and NT-proBNP were related to increased LV filling pressures (higher mitral E/e′ ratio), whereas only NT-proBNP was associated with LVEF. Higher intracardiac pressures (higher mitral E/e′ ratio) with larger cardiac dimensions (lower LVEF) imply greater LV wall tension by Laplace's law—a known trigger for increased NT-proBNP production. In contrast, higher intracardiac pressures (higher mitral E/e′ ratio) independent of cardiac dimensions imply increased LV chamber stiffness in association with increased GDF15 levels. Thus GDF15, by reflecting increased wall stiffness from inflammatory injury, may provide complementary pathophysiological information to that of NT-proBNP, which reflects haemodynamic wall tension or stress. Our findings are further supported by demonstrating independent prognostic significance even adjusting for hsTnT, a marker of myocardial cell necrosis, suggesting that, in addition to myocardial inflammation from myocyte cell injury, ongoing systemic inflammatory damage indicated by further increases in GDF15 during follow-up may explain the additional risk associated with rising values compared with stable values.

The strong independent prognostic value of GDF15 in our HFrEF subpopulation is consistent with previous studies. Specifically in HFrEF alone, our adjusted HR for death in competing risks analysis is comparable to that reported by Kempf *et al.*[15] (HR 1.86, 95% CI 1.06–3.24 vs. HR_{Kempf} 2.26, 95% CI 1.52–3.37, per 1 LnGDF15; $P < 0.05$ for both studies). Importantly, our study provides novel findings of significant prognostic power of GDF15 in HFpEF. In fact, inspection of *Figure 2* suggests a higher gradient of risk in HFpEF vs. HFrEF with increasing GDF15 tertiles (threefold increase for HR_{HFpEF} vs. twofold increase for HR_{HFrEF}). The lack of statistical significance in our test of interaction by HF group may reflect our relatively small sample size for patients with HFpEF. Nonetheless, the prognostic significance of GDF15 in HFpEF was robust even after multivariable adjustment. HFpEF is a syndrome increasingly recognized as an inflammatory condition,[16,17] and associated with multiple comorbidities, many of which can interfere with the performance characteristics of NT-proBNP (age, sex, obesity, renal dysfunction and AF).[18,19] Our subgroup analysis revealed fairly uniform performance of GDF15 as a prognostic marker in the presence of various comorbidities.

The availability of serial measurements in our study allowed for the examination of changes in GDF15 with subsequent outcomes. Anand *et al.*[14] re-measured GDF15 at 12 months in HFrEF and reported an overall increase in GDF15. In contrast, our re-measurements at 6 months found overall reductions in GDF15, with no differences between HF groups. Differences in time of sampling, patient characteristics or disease severity are potential explanations for these divergent observations. In our cohort, compared with patients without repeated measurements, those with re-measurements at 6 months were slightly younger than those without re-measurements (60 ± 12 vs. 62 ± 13 years), but otherwise had similar baseline characteristics (heart rate, blood pressure, NYHA status, and comorbidities; data not shown). In these patients we found that the strength of the association between circulating GDF15 and risk of adverse outcomes increased over time. Furthermore, patients who displayed a greater than 20% increase in $GDF15_{6months}$ from baseline were at particularly high risk of events, with the opposite trend holding true in patients with a greater than 20% decrease in $GDF15_{6months}$. In fact, the prognostic effect of a >20% increase in GDF-15 appeared to be stronger than a similar percentage increase in NT-proBNP. It remains unknown if intervention with medical therapy or other therapeutic strategies upon raised GDF15 can lower GDF15 in HF. As such, the applicability of GDF15 in monitoring disease progression or guiding disease management in HFpEF and HFrEF are areas deserving of further study.

Our study is limited by relatively small numbers of events, particularly in our smaller HFpEF group and within subgroups with repeated measurements. Future studies are needed to validate our findings in HFpEF and determine their generalizability to other populations and ethnicities.

In conclusion, GDF15 (a marker of inflammatory stress) is similarly elevated in HFrEF and HFrEF, whereas NT-proBNP (a marker of haemodynamic wall stress) is higher in HFrEF than in HFpEF. Elevated circulating GDF15, and further elevations on follow-up, identify patients at increased risk of death or HF rehospitalization in both HFrEF and HFpEF, providing incremental prognostic information beyond that offered by standard clinical risk factors, hsTnT, and NT-proBNP. These data support an independent role of inflammatory cytokine release (GDF15) in the pathophysiology of HF regardless of EF.

Supplementary Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Prognostic value of baseline GDF15 and baseline NT-proBNP for composite outcome in different patient sub-groups.

Figure S2. Cumulative survival probability of composite outcome according to percentage change of GDF15 from baseline to 6 months

Table S1. Baseline characteristics associated with baseline GDF15, NT-proBNP or hsTnT from univariate linear regression.

Table S2. Competing risks analysis for death and first HF rehospitalization

Table S3. Baseline GDF15 and NT-proBNP by outcome.

Table S4. Association between change in GDF15 (baseline 6 months) and composite outcome.

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