Growth Differentiation Factor-15 is a Biomarker for All-cause Mortality but Less Evident for Cardiovascular Outcomes: A Prospective Study

Xue Bao
Department of Cardiology, Drum Tower Hospital, Medical School of Nanjing University, Nanjing, China
Yan Borné
Department of Cardiology, Drum Tower Hospital, Medical School of Nanjing University, Nanjing, China
BIAO XU
Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden. https://orcid.org/0000-0003-3404-8582
Marju Orho-Melander
Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden.
Jan Nilsson
Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden.
Olle Melander
Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden.
Gunnar Engström (gunnar.engstrom@med.lu.se)
Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden.

Original investigation

Keywords: growth differentiation factor-15, cardiovascular, mortality, competing risk, cohort study

Posted Date: August 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-48854/v1

Version of Record: A version of this preprint was published at American Heart Journal on April 1st, 2021.
See the published version at https://doi.org/10.1016/j.ahj.2020.12.020.

Abstract

Background

Previous studies have proposed growth differentiation factor-15 (GDF-15) as a predictor of adverse cardiovascular outcomes and mortality. The present study aimed to determine if such associations remain after accounting for markers of inflammation and cardiac dysfunction as well as death as a competing risk.

Methods

Plasma GDF-15 levels and cardiovascular risk factors were measured in individuals without cardiovascular diseases (n = 4,518, aged 57.4 ± 5.96 years, 39.2% men), who participated in Malmö Diet and Cancer-Cardiovascular Cohort during 1991–1994 and were followed up for more than 20 years. Incidence of coronary events, ischemic stroke, cardiovascular mortality and all-cause mortality was studied in relation to GDF-15 using Cox proportional hazards regression, with adjustment for potential confounders including high sensitive C-reactive protein (hsCRP) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP). Confounding from death as competing risk was carefully checked using the Fine and Gray subdistribution hazard model.

Results

During follow-up, 473 coronary events, 366 ischemic stroke, 405 cardiovascular deaths, and 1,445 all-cause deaths occurred. The associations of GDF-15 with coronary events, ischemic stroke, or cardiovascular mortality were attenuated and non-significant after adjusting for NT-proBNP or controlling for death from other causes as a competing risk. All-cause mortality remained significantly related to GDF-15. After multivariate adjustment, the hazard ratio (95% confidence interval) for all-cause mortality was 1.60 (1.34, 1.91), in the top compared with the bottom quartile of GDF-15.

Conclusions

GDF-15 concentration is a robust biomarker for all-cause mortality but less reliable for coronary event, ischemic stroke or cardiovascular mortality.

Background

Growth differentiation factor-15 (GDF-15), also known as macrophage inhibitory cytokine-1 (MIC-1), is a member of TGF-β cytokine superfamily [1]. Under healthy conditions, GDF-15 is expressed at low levels in all organs and at high level in the placenta [2]. However, its expression in many tissues is highly activated by stress and injury and is linked to inflammatory and apoptotic pathways essential for development,

differentiation and tissue repair [1]. In cultured rat and human cardiomyocytes, GDF-15 expression and secretion were strongly induced by ischemia/reperfusion and were closely associated with smaller infarct sizes and less apoptosis in the infarct border zone [3]. In contrast, in patients with cardiovascular diseases (CV diseases, CVDs), high plasma GDF-15 has consistently been associated with adverse prognosis [4]. High GDF-15 levels have also been found to predict subsequent cardiovascular event, adding incremental value to traditional cardiovascular risk factors and C-reactive protein (CRP) [5]. Pareek *et al*. demonstrated that among multiple candidate biomarkers, GDF-15 showed superior capabilities in predicting a composite of adverse CV outcomes, independent of N-terminal prohormone of brain natriuretic peptide (NT-proBNP) [6]. Due to a modest number of endpoints, only a composite endpoint was examined, despite the potentially different pathogenic mechanisms for individual CV outcomes [6]. More recently, Ho *et al.* used a proteomic platform to investigate 85 protein biomarkers of CVD risk in relation to incident atherosclerotic CVD, heart failure, CVD death and all-cause mortality in 3523 participants with a median follow-up of 14.3 years [7]. As a result, GDF15 was the only biomarker that was associated with all outcomes in multi-marker models. However, again in this study, a composite endpoint of atherosclerotic CV events was used and detailed information regarding the GDF15-CVD association was not reported [7]. In addition, previous studies examining the associations between GDF-15 and CV outcomes were based on traditional Cox proportional hazards regression. It is worth noting that the competing risk from death could substantially alter the risk estimates, particularly in studies of older adults [8, 9].

In the present study, we aim to explore whether plasma GDF15 is associated with incidence of coronary events, ischemic stroke, CVD mortality or all-cause mortality in a population-based cohort study, and whether the associations are independent of markers of inflammation (CRP) and cardiac dysfunction (NT-proBNP) as well as accounting for the competing risk of death from other causes.

Methods

Participants

Malmö Diet and Cancer-Cardiovascular Cohort (MDC-CV)

The Malmö Diet and Cancer Study (MDC study) is a prospective population-based cohort study from the city of Malmö in southern Sweden, with 230,000 inhabitants at the time of recruitment [10]. A total of 28,449 individuals (men = 11,246, born 1923–1945 and women = 17,203, born 1923–1950) underwent a baseline examination between March 1991 and September 1996. Samples of peripheral venous blood, blood pressure measurements and anthropometric measures were taken on the first visit. Participants also filled out self-administered questionnaires [10]. Between October 1991 and February 1994, a group of 6,103 individuals was randomly selected to be recruited in MDC-CV, which was designed to study the epidemiology of carotid artery disease [11]. Out of these, 5,540 participants attended the second visit when fasting plasma samples were collected. Subjects were excluded from the present study due to incomplete data on confounders (n = 415), insufficient plasma samples stored for protein biomarker

measurement (n = 380) or failing the internal quality control for the biomarker analysis (n = 118). Individuals with a history of CVDs (n = 109) at baseline were also excluded. After exclusions, at total of 4,518 individuals remained for the analysis (1,773 men and 2,745 women, aged 57.4 ± 5.96 years) (Fig. 1).

All participants provided written informed consent. The study was approved by the Regional Ethical Review Board in Lund, Sweden (LU 51/90) and was carried out in accordance with the Helsinki Declaration.

Measurements And Definitions

Information on the current use of blood pressure-lowering medications, smoking habits, and alcohol consumption was gathered through self-administered questionnaires. Subjects were grouped into current smokers, former smokers and never-smokers. High alcohol consumption was defined as > 40 g alcohol per day for men and > 30 g per day for women. Waist circumference was measured midway between the lowest rib margin and iliac crest in centimetres. Blood pressure was measured using mercury column sphygmomanometer after 10 minutes of rest in the supine position. Fasting blood glucose and lipids were measured from fresh blood samples using standard procedures at the Department of Clinical Chemistry, University Hospital Malmö. Low density lipoprotein (LDL) concentrations were determined according to Friedewald's formula. The biomarkers were measured from fasting plasma samples that had been frozen at − 80 °C immediately after collection. High sensitive CRP (hsCRP) was analysed using the Tina-quant CRP latex assay (Roche Diagnostics) on an ADVIA® 1650 Chemistry System (Bayer Healthcare). GDF-15 and NT-proBNP levels were measured using Proseek® Multiplex CVD $I^{96\times96}$ reagent kit by the SciLifeLab analysis service (Uppsala, Sweden), based on a Proximity Extension Assay technology [12]. The assay procedure is described in detail elsewhere [13]. Briefly, the assay involved three core steps: incubation, extension and detection. Raw Proseek data were adjusted using a pre-processing normalization procedure and set relative to a fixed background level, which generated Normalized Protein Expression (NPX, log^2 scale) values. A high NPX value corresponds to a high protein concentration. The NPX values of GDF-15 by Proseek assay correlated closely with values obtained using an electrochemiluminescence immunoassay (Roche Diagnostics) [13].

Outcomes

All participants were followed from baseline examination until the first end point (coronary events, ischemic stroke, cardiovascular mortality or all-cause mortality), emigration from Sweden, death or December 31st, 2016, whichever came first. Separate follow-up times were calculated for each endpoint. Ascertainment of cases was carried out with the help of validated national and local registers [14, 15]. A coronary event was defined as a fatal or non-fatal myocardial infarction according to International Classification of Diseases 9th revision (ICD-9: Code 410) or 10th revision (ICD-10: Code I21), or death

attributed to ischemic heart disease (ICD-9: Codes 411–414; ICD-10: Codes I22-I25). Ischemic stroke was defined according to ICD-9 code 434 or ICD-10 code I63. Cardiovascular mortality was defined as an ICD-10 diagnosis in chapter I as underlying cause of death.

Statistical Analysis

HsCRP and fasting glucose were logarithmically transformed due to skewed distributions. Subjects were classified into quartiles with sex-specific cut points; i.e. equal number of men and women in each quartile based on the concentrations of GDF-15. Baseline characteristics are presented as mean ± standard deviation (SD) or median (interquartile range) for continuous variables and percentages for categorical variables. Cross-sectional relations of GDF-15 quartiles to CV risk factors were assessed using linear regression for continuous variables and logistic regression for dichotomous variables. *P*-values from trend tests across quartiles were used.

The associations between GDF-15 quartiles and outcomes were examined using Cox proportional hazards regression models with time-on-study as the time-scale and the lowest quartile as the reference category. HR with 95% confidence intervals (CI) were estimated in Model 1 adjusted for age and sex, and Model 2 adjusted for CV risk factors. HsCRP and NT-proBNP was additionally added into the model one after another (Model 3 and Model 4) to determine whether the observed associations were dependent of existing systematic inflammation or CV dysfunction. A Cox regression restricted cubic spline model [13] was used to check the assumption of linearity of the associations between GDF-15 and outcomes with knots placed at equal intervals of GDF-15 concentration (20%, 40%, 60% and 80%). HR per 1 SD increase of GDF-15 (1 SD = 0.56 NPX, log2 scale) was calculated for linear associations. For non-linear associations, HR was obtained by fitting piecewise linear functions (post hoc). The competing risks of death was accounted for in a sensitivity analysis by the Fine and Gray proportional subdistribution hazards models method [16]. The cumulative incidence of outcomes at different GDF-15 levels was plotted with mortality treated as a censored observation or as a competing risk, respectively, while all other covariates were fixed at their average values [16]. The interplay between GDF-15 levels and risk factors was examined by introducing an interaction term into the multivariate model one by one. A two tailed p-value < 0.05 was considered significant. Statistical Analysis System version 9.4 for Windows (SAS Institute, Cary, NC, USA) was used for the statistical analysis.

Results

Baseline Characteristics

The study population included 4,518 individuals aged 57.4 ± 5.96 years, of whom 39.2% were male. Baseline characteristics across GDF-15 quartiles are presented in Table 1. Age, waist circumference, current smoking, glucose, systolic blood pressure (SBP), BP-lowering medication, LDL, hsCRP and NT-proBNP showed positive associations with GDF-15 levels at baseline, while former smoking and never

smoking showed the opposite pattern. No statistical significance was observed between GDF-15 and alcohol consumption.

Table 1
Quartiles of GDF-15 and risk factors of participants in MDC-CV (n = 4,518).

	Q1 (n = 1,131)	Q2 (n = 1,129)	Q3 (n = 1,129)	Q4 (n = 1,129)	*p* for trend
GDF-15 (NPX)	8.08 ± 0.27	8.56 ± 0.11	8.90 ± 0.13	9.47 ± 0.38	
Men n (%)	444 (39.3)	443 (39.2)	443 (39.2)	443 (39.2)	0.99
Age (Years)	54.6 ± 5.57	56.6 ± 5.72	58.5 ± 5.56	60.1 ± 5.55	< 0.0001
Waist circumference (cm)	81.7 ± 11.6	82.2 ± 12.2	83.5 ± 12.9	85.4 ± 13.6	< 0.0001
Smoking n (%)					
Never	552 (12.2)	483 (10.7)	449 (9.94)	386 (8.54)	< 0.0001
Former	473 (10.5)	448 (9.92)	408 (9.03)	352 (7.79)	< 0.0001
Current	106 (2.35)	198 (4.38)	272 (6.02)	391 (8.65)	< 0.0001
High alcohol consumption n (%)	43 (0.95)	38 (0.84)	34 (0.75)	40 (0.89)	0.77
Glucose (mmol/L) [a]	4.81 (4.61–5.21)	4.91 (4.61–5.21)	4.90 (4.6–5.30)	5.00 (4.70–5.40)	< 0.0001 [b]
SBP (mmHg)	135.9 ± 16.7	140.1 ± 18.9	142.4 ± 19.4	144.7 ± 19.2	< 0.0001
BP-lowering medication n (%)	121 (2.68)	147 (3.25)	180 (3.98)	225 (4.98)	< 0.0001
LDL (mmol/L)	4.10 ± 0.94	4.07 ± 0.94	4.25 ± 0.99	4.25 ± 1.03	< 0.0001
hsCRP (mg/L) [a]	1.00 (0.50–1.90)	1.10 (0.60–2.20)	1.40 (0.70–2.90)	1.90 (1.00-4.20)	< 0.0001 [b]
NT-proBNP (NPX)	-0.31 ± 0.92	-0.08 ± 0.94	0.08 ± 0.97	0.22 ± 1.01	< 0.0001

GDF-15, growth differentiation factor-15; MDC-CV, Malmö Diet and Cancer-Cardiovascular Cohort; SBP, systolic blood pressure; BP, blood pressure; hsCRP, high sensitive C-reactive protein; LDL, low density lipoprotein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

[a] Glucose and hsCRP are presented as median (interquartile range in brackets) due to skewed distributions. All other continuous values are presented as means ± SD, unless otherwise stated.

	Q1 (n = 1,131)	Q2 (n = 1,129)	Q3 (n = 1,129)	Q4 (n = 1,129)	*p* for trend
[b] *p* value for log-transformed value of Glucose and hsCRP.					

Follow-up Analysis

A total of 473 individuals (273 men and 200 women) suffered a coronary event after a mean follow-up of 20.9 ± 5.55 years, 336 individuals (181 men and 185 women) developed ischemic stroke after a mean follow-up of 20.9 ± 5.53 years. In addition, during a 21.5 ± 5.01 years follow-up period, a total of 1,445 death (687 men and 758 women) occurred, of which 405 (212 men and 193 women) were attributed to CVD. The incidences per 1000 person years for coronary events, ischemic stroke, CVD mortality and all-cause mortality were 5.01, 3.88, 4.17 and 14.90, respectively.

The crude and adjusted associations between GDF-15 quartiles and outcomes can be seen in Table 2. In multivariate model (Model 2), GDF-15 was significantly associated with all the outcomes except for ischemic stroke (*p* for trend = 0.07). Adding hsCRP into the model slightly attenuated the associations (Model 3). However, after additionally adjusting for NT-proBNP (Model 4), only the associations of GDF-15 with coronary events and all-cause mortality remained significant. The HR (95% CI; *p* for trend) for coronary events and all-cause mortality in the 4th vs. 1st quartile of GDF-15 was 1.39 (1.04–1.87; *p* = 0.01) and 1.60 (1.34–1.91; *p* < 0.0001), respectively. The restricted cubic spline analysis confirmed no association between GDF-15 and ischemic stroke (*p* for effect = 0.20) while a significant and linear association (*p* for effect, *p* for non-linearity) was detected for GDF-15 with coronary events (0.049, 0.59), CVD mortality (0.04, 0.41) and all-cause mortality (< 0.0001, 0.49). The adjusted HRs (95% CI; *p*) for coronary events, CVD mortality and mortality per SD increase in GDF-15 were 1.15 (1.03–1.28; *p* = 0.01), 1.16 (1.03–1.30; *p* = 0.01) and 1.29 (1.21–1.37; *p* < 0.0001), respectively (Table 2). Table 3 presents results generated by subdistribution hazards models. In multivariate model without NT-proBNP, considering death from other causes as competing events completely eliminated the association between GDF-15 and coronary events (HR per SD increase, 1.08; 95% CI, 0.98–1.20; *p* = 0.11), and substantially moderated the association between GDF-15 and CVD mortality (HR per SD increase, 1.12; 95% CI, 1.00-1.26; *p* = 0.04). This association was no longer significant after additionally adjusting for NT-proBNP (HR per SD increase, 1.06; 95% CI, 0.94–1.19; *p* = 0.36). Correspondingly, as visualized in Additional file 1, traditional Cox analysis compared with the competing risk approach exaggerated the effect of GDF-15 on the cumulative incidence of coronary events, ischemic stroke and CVD mortality. In contrast, the cumulative incidence of all-cause mortality clearly increased with elevated GDF-15 levels. Significant interaction between GDF-15 and hsCRP was found on the risk of all-cause mortality (*p* for interaction < 0.01). Subset analyses after stratifying participants by median hsCRP (1.3 mg/L) showed that the association of GDF-15 with all-cause mortality was stronger in participants with higher baseline hsCRP concentration as compared to those with lower baseline hsCRP concentration. The adjusted HR per SD

increase in GDF-15 was 1.37 (95% CI, 1.27–1.48; $p < 0.0001$) for those with CRP above median and 1.19 (95% CI, 1.08–1.30; $p < 0.001$) for those with lower CRP.

Table 2
CVD outcomes and mortality in relation to quartiles and per SD change of GDF-15.

	Q1 (n = 1131)	Q2 (n = 1129)	Q3 (n = 1129)	Q4 (n = 1129)	*p* for trend	per SD	*p*
Coronary events							
Incidence	81	95	117	180	-	- -	-
Incidence per 1000 person years	3.22	3.84	5.00	8.59	-	- -	-
Model 1 [a]	Reference	1.07 (0.79, 1.44)	1.21 (0.90, 1.62)	2.06 (1.56, 2.72)	< 0.0001	1.33 (1.21, 1.47)	< 0.0001
Model 2 [b]	Reference	0.98 (0.73, 1.32)	1.02 (0.76, 1.37)	1.55 (1.16, 2.07)	< 0.01	1.20 (1.08, 1.33)	< 0.001
Model 3 [c]	Reference	0.98 (0.72, 1.32)	1.00 (0.74, 1.34)	1.49 (1.11, 1.99)	< 0.01	1.18 (1.06, 1.31)	< 0.01
Model 4 [d]	Reference	0.95 (0.70, 1.28)	0.95 (0.70, 1.28)	1.39 (1.04, 1.87)	0.01	1.15 (1.03, 1.28)	0.01
Ischemic stroke							
Incidence	56	76	119	115	-	- -	-
Incidence per 1000 person years	2.22	3.09	5.12	5.41	-	- -	-
Model 1 [a]	Reference	1.17 (0.97, 1.40)	1.50 (1.27, 1.79)	2.25 (1.90, 2.66)	< 0.001	1.25 (1.12, 1.40)	< 0.0001
Model 2 [b]	Reference	1.10 (0.78, 1.56)	1.46 (1.05, 2.04)	1.30 (0.92, 1.84)	0.07	1.14 (1.01, 1.28)	0.03
Model 3 [c]	Reference	1.10 (0.77, 1.55)	1.45 (1.04, 2.02)	1.27 (0.90, 1.81)	0.09	1.13 (1.00, 1.28)	0.04
Model 4 [d]	Reference	1.06 (0.75, 1.50)	1.36 (0.98, 1.90)	1.17 (0.83, 1.67)	0.23	1.10 (0.97, 1.24)	0.14
CVD mortality							
Incidence	51	78	111	165	-	-	-

	Q1 (n = 1131)	Q2 (n = 1129)	Q3 (n = 1129)	Q4 (n = 1129)	*p* for trend	per SD	*p*
Incidence per 1000 person years	1.99	3.10	4.60	7.48	-	-	-
Model 1 [a]	Reference	1.20 (0.84, 1.71)	1.43 (1.02, 2.00)	2.16 (1.56, 3.00)	< 0.0001	1.44 (1.30, 1.60)	< 0.0001
Model 2 [b]	Reference	1.11 (0.78, 1.59)	1.19 (0.84, 1.67)	1.49 (1.06, 2.10)	< 0.01	1.24 (1.11, 1.39)	< 0.001
Model 3 [c]	Reference	1.11 (0.78, 1.59)	1.18 (0.83, 1.66)	1.47 (1.05, 2.07)	0.01	1.23 (1.10, 1.38)	< 0.001
Model 4 [d]	Reference	1.04 (0.73, 1.48)	1.04 (0.73, 1.47)	1.26 (0.89, 1.78)	0.13	1.16 (1.03, 1.30)	0.01
All-cause mortality							
Incidence	203	277	399	566	-	-	-
Incidence per 1000 person years	7.91	11	16.5	25.7	-	-	-
Model 1 [a]	Reference	1.17 (0.97, 1.40)	1.50 (1.27, 1.79)	2.25 (1.90, 2.66)	< 0.0001	1.46 (1.39, 1.54)	< 0.0001
Model 2 [b]	Reference	1.09 (0.91, 1.31)	1.30 (1.09, 1.55)	1.72 (1.45, 2.05)	< 0.0001	1.32 (1.25, 1.40)	< 0.0001
Model 3 [c]	Reference	1.08 (0.90, 1.30)	1.28 (1.08, 1.53)	1.67 (1.40, 1.99)	< 0.0001	1.31 (1.23, 1.39)	< 0.0001
Model 4 [d]	Reference	1.06 (0.89, 1.28)	1.24 (1.04, 1.48)	1.60 (1.34, 1.91)	< 0.0001	1.29 (1.21, 1.37)	< 0.0001

GDF-15, growth differentiation factor-15; CVD, cardiovascular disease.

[a] Adjusted for age and sex.

[b] Adjusted for age, sex, waist circumference, smoking, alcohol consumption, glucose level, systolic blood pressure, BP-lowering drug, and LDL.

[c] Adjusted for age, sex, waist circumference, smoking, alcohol consumption, glucose level, systolic blood pressure, BP-lowering drug, LDL, and high sensitive C-reactive protein level.

	Q1 (n = 1131)	Q2 (n = 1129)	Q3 (n = 1129)	Q4 (n = 1129)	*p* for trend	per SD	*p*

[d] Adjusted for age, sex, waist circumference, smoking, alcohol consumption, glucose level, systolic blood pressure, BP-lowering drug, LDL, high sensitive C-reactive protein level, and NT-proBNP.

Table 3
Subdistribution Hazards Models for Associations of GDF-15 with CVD outcomes and mortality (competing risk analysis).

	Q1 (n = 1131)	Q2 (n = 1129)	Q3 (n = 1129)	Q4 (n = 1129)	*p* for trend	per SD	*p*
Coronary events							
Multivariate model [a]	Reference	0.99 (0.73, 1.33)	0.99 (0.74, 1.35)	1.35 (1.00, 1.82)	0.04	1.08 (0.98, 1.20)	0.11
Multivariate model + NT-proBNP	Reference	0.97 (0.72, 1.31)	0.96 (0.71, 1.30)	1.28 (0.94, 1.73)	0.09	1.06 (0.96, 1.17)	0.27
Ischemic Stroke							
Multivariate model [a]	Reference	1.11 (0.78, 1.57)	1.47 (1.06, 2.05)	1.17 (0.83, 1.65)	0.24	1.05 (0.94, 1.17)	0.38
Multivariate model + NT-proBNP	Reference	1.08 (0.76, 1.53)	1.40 (1.00, 1.95)	1.09 (0.77, 1.55)	0.47	1.02 (0.91, 1.14)	0.75
CVD mortality							
Multivariate model [a]	Reference	1.12 (0.79, 1.60)	1.16 (0.82, 1.64)	1.32 (0.94, 1.86)	0.09	1.12 (1.00, 1.26)	0.04
Multivariate model + NT-proBNP	Reference	1.06 (0.74, 1.51)	1.03 (0.73, 1.47)	1.14 (0.81, 1.61)	0.45	1.06 (0.94, 1.19)	0.36
All-cause mortality [b]							
Multivariate model [a]	Reference	1.08 (0.90, 1.30)	1.28 (1.08, 1.53)	1.67 (1.40, 1.99)	< 0.0001	1.31 (1.23, 1.39)	< 0.0001
Multivariate model + NT-proBNP	Reference	1.06 (0.89, 1.28)	1.24 (1.04, 1.48)	1.60 (1.34, 1.91)	< 0.0001	1.29 (1.21, 1.37)	< 0.0001

GDF-15, growth differentiation factor-15; CVD, cardiovascular disease; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

[a] Adjusted for age, sex, waist circumference, smoking, alcohol consumption, glucose level, systolic blood pressure, BP-lowering drug, LDL, and high sensitive C-reactive protein level.

[b] Estimated based on traditional Cox proportional hazards

Discussion

This large, population-based cohort study showed a robust association between plasma GDF-15 levels and all-cause mortality. GDF-15 was also observed to be associated with coronary events, ischemic stroke and CVD mortality, but at least in part, the associations were likely to be confounded by pre-existing CV dysfunction or by the competing risk of death.

In line with previous studies [13, 17–19], the present study observed positive correlations between GDF-15 and traditional CV risk factors, including age, waist circumference, smoking, glucose, SBP, LDL and hsCRP. Since GDF-15 is an anti-inflammatory cytokine by nature [20] and all of these CV risk factors are related to systemic inflammation, the correlations could at least be partly explained by a compensatory mechanism of GDF-15 in response to inflammation. Of note, GDF-15 correlated strongly with NT-proBNP [17, 19], a commonly used marker of left ventricular dysfunction in clinical practice. Under pressure or volume overload, NT-proBNP is produced on cardiomyocytes to cope with LV hypertrophy and fibrosis due to myocardial stretch [21]. Similar to NT-proBNP, the production of GDF-15 by cardiomyocytes is stimulated in response to CV injury [3, 22, 23]. Thus, individuals with simultaneously elevated GDF-15 and NT-proBNP in this study represented a group with suboptimal CV health even in the absence of overt cardiac pathology.

In longitudinal analyses, GDF-15 was found to be associated with incidence of coronary events, ischemic stroke, CVD mortality and all-cause mortality before correction for the confounding from NT-proBNP. This observation was in agreement with previous studies, supporting an increased risk of a wide range of CVDs in community-dwelling individuals with elevated GDF-15 levels [6, 7, 17, 19]. Our data, along with evidence from another Swedish cohort [24], suggested that the association between GDF-15 and ischemic stroke was confounded by NT-proBNP. Whereas additional adjustment for NT-proBNP greatly affected all associations with cardiovascular outcomes, its impact on the association between GDF-15 and all-cause mortality was relatively small. Previous cohort studies have consistently shown that GDF-15 and NT-proBNP are prognostic markers for adverse CV outcomes and mortality among asymptomatic subjects [6, 7, 19]. When comparisons were made between these two biomarkers, Pareek *et al*. [6] demonstrated that, as compared to NT-proBNP, GDF-15 showed stronger association with composite outcomes including all-cause mortality, but weaker association with incident heart failure or CVD mortality (sample size 1,324, aged 66 years, followed up for 8.6 years). Ho *et al*. [7] showed that GDF-15 is a better predictor of atherosclerotic CVD and all-cause mortality but a weaker predictor of CVD death than NT-proBNP (sample size 3,523, aged 62 years, followed up for 14.3 years). Similarly, Daniels *et al*. [19] reported that GDF-15 was a stronger predictor of all-cause mortality while NT-proBNP was a stronger predictor of CVD mortality (sample size 1,391, aged 70 years, followed up for 11 years). In addition, GDF-15 but not NT-proBNP was independently associated with non-CV death and cancer death [19]. Therefore, it seems that whereas GDF-15 might not be a better marker than NT-proBNP in a cardiovascular context, it is a powerful marker for all-cause mortality.

Noteworthy, although almost all relevant studies were conducted on an elderly population and most of them were with a long-term follow-up, hardly any study has carefully evaluated the death as a competing risk. Emphasis has been made that the competing risk of death is an important consideration in studies evaluating the long-term association among older adults, ignoring of which might lead to substantially overestimated associations [8, 9]. Kaplan-Meier estimates and traditional Cox proportional hazards regression models assume that subjects are censored from causes unrelated to the primary outcome. Therefore, those who died from other causes are still considered to be at risk of the primary outcomes at the point of censoring and are thought to have the same distribution of time-to-event as incident cases. However, such subjects cannot possibly develop the primary outcomes, thus ignoring that the competing risk of mortality could exaggerate the HRs for the primary outcomes, particularly for subjects with high mortality, e.g. older adults with long-term follow-up [8, 9]. Our study added important information on the competing risk of death and indicates that the associations of GDF-15 with coronary events and CVD mortality may be markedly overestimated if this competing risk is ignored, especially given that high GDF-15 contributed largely to mortality. As a result, only all-cause mortality remained significantly related to GDF-15 in this study. The association between GDF-15 and all-cause mortality was strengthened in subjects with higher baseline hsCRP, which may be due to an enhanced secretion and function of GDF-15 in pro-inflammatory conditions.

Of note, accumulating evidence has indicated that GDF-15 has a role beyond inflammation [1, 17]. It has multiple roles in, for example, oxidative stress, tumorigenesis, strain and ischemia, and its elevation is associated with a wide range of diseases involving different systems [1, 17]. Therefore, it is speculated that GDF-15 concentration reflects overall health status and thus correlates with all-cause mortality. Elevated GDF-15 levels mirror cellular stress and senescence, which might also explain the association between GDF-15 and life span. As a downstream target of p53, GDF15 gene is expressed via a p53-dependent pathway in cellular response to a broad range of stress [25, 26]. During this process, p53 mediate cellular senescence and apoptosis by functioning as a transcription factor [26], and thus plays a role in regulating longevity [26]. At structural levels, p53 directly influences mitochondrial respiration and impairs clearance of defective mitochondria [27, 28], leading to mitochondrial dysfunction, a well-known driver of biologic ageing [29]. Consistently, emerging studies have proposed GDF-15 as a specific biomarker of mitochondrial disorder [30, 31]. In further support of GDF-15 as a marker of ageing, GDF-15 has recently been identified as a soluble senescence-associated secretory protein and a key of cellular senescence [32]. Nevertheless, it remains largely obscure whether and exactly how GDF-15 itself promotes ageing.

The study is based on a large number of subjects from the general population, with a long-term follow-up and sufficient number of cases. Less than 1% was loss to follow-up [10]. CV endpoints were retrieved from validated national and local registers [14, 15]. However, there are some limitations that might have affected our study. High-risk individuals are less likely to participate in population-based studies. Thus, the proportion of individuals with high risk could be underestimated. Information on biomarkers was available for a sub-cohort, MDC-CV, of the whole MDC cohort. Nevertheless, the sub-cohort was randomly selected and should be regarded as representative of the whole MDC cohort in terms of age, sex,

biological, lifestyle and socio-economic characteristics [33]. Furthermore, the risk factors used for adjustments in the study are well-known CV risk factors but still we cannot exclude the possibility of residual confounding. Information about incident coronary events and ischemic stroke was retrieved from local and national hospital registers. The validity of this information has been confirmed in many previous studies [14, 15].

Conclusions

Our results suggest that high GDF-15 levels are associated with increased incidence of all-cause mortality while its association with coronary events, ischemic stroke and CVD mortality might be dependent of pre-existing cardiovascular dysfunction or confounded by death.

List Of Abbreviations

CI: confidence interval; CRP: C-reactive protein; CV: cardiovascular; CVD: cardiovascular disease; GDF-15: growth differentiation factor-15; hsCRP: high sensitive C-reactive protein; LDL: low density lipoprotein; MDC: Malmö Diet and Cancer; MDC-CV: Malmö Diet and Cancer-Cardiovascular Cohort; MIC-1: macrophage inhibitory cytokine-1; NPX: normalized protein expression; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; SBP: systolic blood pressure.

Declarations

Ethics approval and consent to participate: All participants provided written informed consent. The study was approved by the Regional Ethical Review Board in Lund, Sweden (LU 51/90) and was carried out in accordance with the Helsinki Declaration.ed from all individual participants included in the study.

Consent for publication: Not applicable.

Availability of data and material: The data that support the findings of this study are available from Lund University, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Lund University.

Competing interests: The authors declare that they have no competing interests.

Funding: This work was supported by the Swedish Heart–Lung Foundation (grant numbers 2016-0315, 2017-0626); andthe Natural Science Foundation of Jiangsu Province (grant numberSBK2020043796). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author's Contributions: XB, BX and GE contributed to the conception or design of the work. XB and YB contributed to the acquisition, analysis, or interpretation of data for the work. XB drafted the manuscript.

YB, BX, MOM, JN, OM and GE critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Acknowledgments: We thank all the participants in this study and the staff of the department of Clinical Chemistry at University Hospital, Malmö. We also would like to acknowledge the information provided by national and local registers and support of the Clinical biomarker facility at SciLifeLab Sweden for providing assistance in protein analyses.

References

1. Unsicker K, Spittau B, Krieglstein K. The multiple facets of the TGF-beta family cytokine growth/differentiation factor-15/macrophage inhibitory cytokine-1. Cytokine Growth Factor Rev. 2013;24(4):373–84.
2. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci USA. 2004;101(16):6062–7.
3. Kempf T, Eden M, Strelau J, Naguib M, Willenbockel C, Tongers J, Heineke J, Kotlarz D, Xu J, Molkentin JD, et al. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. Circulation research. 2006;98(3):351–60.
4. Eggers KM, Lindahl B. Prognostic Biomarkers in Acute Coronary Syndromes: Risk Stratification Beyond Cardiac Troponins. Curr Cardiol Rep. 2017;19(4):29.
5. Brown DA, Breit SN, Buring J, Fairlie WD, Bauskin AR, Liu T, Ridker PM. Concentration in plasma of macrophage inhibitory cytokine-1 and risk of cardiovascular events in women: a nested case control study. The Lancet. 2002;359:2159–63.
6. Pareek M, Bhatt DL, Vaduganathan M, Biering-Sorensen T, Qamar A, Diederichsen AC, Moller JE, Hindersson P, Leosdottir M, Magnusson M, et al. Single and multiple cardiovascular biomarkers in subjects without a previous cardiovascular event. European journal of preventive cardiology. 2017;24(15):1648–59.
7. Ho JE, Lyass A, Courchesne P, Chen G, Liu C, Yin X, Hwang SJ, Massaro JM, Larson MG, Levy D. Protein Biomarkers of Cardiovascular Disease and Mortality in the Community. Journal of the American Heart Association. 2018; 7(14).
8. Berry SD, Ngo L, Samelson EJ, Kiel DP. Competing risk of death: an important consideration in studies of older adults. J Am Geriatr Soc. 2010;58(4):783–7.
9. Ensrud KE, Harrison SL, Cauley JA, Langsetmo L, Schousboe JT, Kado DM, Gourlay ML, Lyons JG, Fredman L, Napoli N, et al. Impact of Competing Risk of Mortality on Association of Weight Loss With Risk of Central Body Fractures in Older Men: A Prospective Cohort Study. Journal of bone mineral research: the official journal of the American Society for Bone Mineral Research. 2017;32(3):624–32.

10. Berglund G, Elmstahl S, Janzon L, Larsson S. Malmö Diet and Cancer Study: Design and Feasibility. J Intern Med. 1993;233:45–51.
11. Hedblad B, Nilsson P, Janzont L, Berglund G. Relation between Insulin Resistance and Carotid Intima-media Thickness and Stenosis in Non-Diabetic Subjects: Results from a Cross-Sectional Study in Malmö, Sweden. British Diabetic Association Diabetic Medicine. 2000;17:299–307.
12. Lundberg M, Eriksson A, Tran B, Assarsson E, Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low abundant proteins in human blood. Nucleic Acid Res. 2011;39(15):e102.
13. Bao X, Borne Y, Muhammad IF, Nilsson J, Lind L, Melander O, Niu K, Orho-Melander M, Engstrom G. Growth differentiation factor 15 is positively associated with incidence of diabetes mellitus: the Malmo Diet and Cancer-Cardiovascular Cohort. Diabetologia. 2019;62(1):78–86.
14. Hammar N, Alfredsson L, Rosen M, Spetz C-L, Kahan T, Ysberg A-S. A national record linkage to study acute myocardial infarction incidence and case fatality in Sweden. Int J Epidemiol. 2001;30(Suppl 1):30-S4.
15. Khan FA, Zia E, Janzon L, Engstrom G. Incidence of stroke and stroke subtypes in Malmo, Sweden, 1990–2000: marked differences between groups defined by birth country. Stroke. 2004;35(9):2054–8.
16. Kohl M, Plischke M, Leffondre K, Heinze G. PSHREG: a SAS macro for proportional and nonproportional subdistribution hazards regression. Comput Methods Programs Biomed. 2015;118(2):218–33.
17. Wollert KC, Kempf T, Wallentin L. Growth Differentiation Factor 15 as a Biomarker in Cardiovascular Disease. Clinical chemistry. 2017;63(1):140–51.
18. Brown DA, Breit SN, Buring J, Fairlie WD, Bauskin AR, Liu T, Ridker PM. Concentration in plasma of macrophage inhibitory cytokine-1 and risk of cardiovascular events in women: a nested case-control study. Lancet. 2002;359(9324):2159–63.
19. 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(19):2101–10.
20. Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, Zhang HP, Donnellan M, Mahler S, Pryor K, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proc Natl Acad Sci USA. 1997;94(21):11514–9.
21. Daniels LB, Maisel AS. Natriuretic peptides. J Am Coll Cardiol. 2007;50(25):2357–68.
22. Kempf T, Zarbock A, Widera C. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. Nat Med. 2011;17:581–8.
23. Xu J, Kimball TR, Lorenz JN, Brown DA, Bauskin AR, Klevitsky R, Hewett TE, Breit SN, Molkentin JD. GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. Circulation research. 2006;98(3):342–50.

24. Wallentin L, Zethelius B, Berglund L, Eggers KM, Lind L, Lindahl B, Wollert KC, Siegbahn A. GDF-15 for prognostication of cardiovascular and cancer morbidity and mortality in men. PloS one. 2013;8(12):e78797.
25. Kelly JA, Lucia MS, Lambert JR. p53 controls prostate-derived factor/macrophage inhibitory cytokine/NSAID-activated gene expression in response to cell density, DNA damage and hypoxia through diverse mechanisms. Cancer letters. 2009;277(1):38–47.
26. Vousden KH, Lane DP. p53 in health and disease. Nature reviews Molecular cell biology. 2007;8(4):275–83.
27. Hoshino A, Mita Y, Okawa Y, Ariyoshi M, Iwai-Kanai E, Ueyama T, Ikeda K, Ogata T, Matoba S. Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. Nature communications. 2013;4:2308.
28. Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, Hwang PM. p53 regulates mitochondrial respiration. Science. 2006;312(5780):1650–3.
29. Harman D. The biologic clock: the mitochondria? J Am Geriatr Soc. 1972;20(4):145–7.
30. Poulsen NS, Madsen KL, Hornsyld TM, Eisum AV, Fornander F, Buch AE, Stemmerik MG, Ruiz-Ruiz C, Krag TO, Vissing J. Growth and differentiation factor 15 as a biomarker for mitochondrial myopathy. Mitochondrion. 2020;50:35–41.
31. Yatsuga S, Fujita Y, Ishii A, Fukumoto Y, Arahata H, Kakuma T, Kojima T, Ito M, Tanaka M, Saiki R, et al. Growth differentiation factor 15 as a useful biomarker for mitochondrial disorders. Ann Neurol. 2015;78(5):814–23.
32. Basisty N, Kale A, Jeon OH, Kuehnemann C, Payne T, Rao C, Holtz A, Shah S, Sharma V, Ferrucci L, et al. A proteomic atlas of senescence-associated secretomes for aging biomarker development. PLoS biology. 2020;18(1):e3000599.
33. Melander O, Newton-Cheh C, Almgren P, Hedblad B, Berglund G, Engstrom G, Persson M, Smith J, Magnusson M, Christensson A, et al. Novel and Conventional Biomarkers for Prediction of Incident Cardiovascular Events in the Community. JAMA. 2009;302:49–57.

Figures

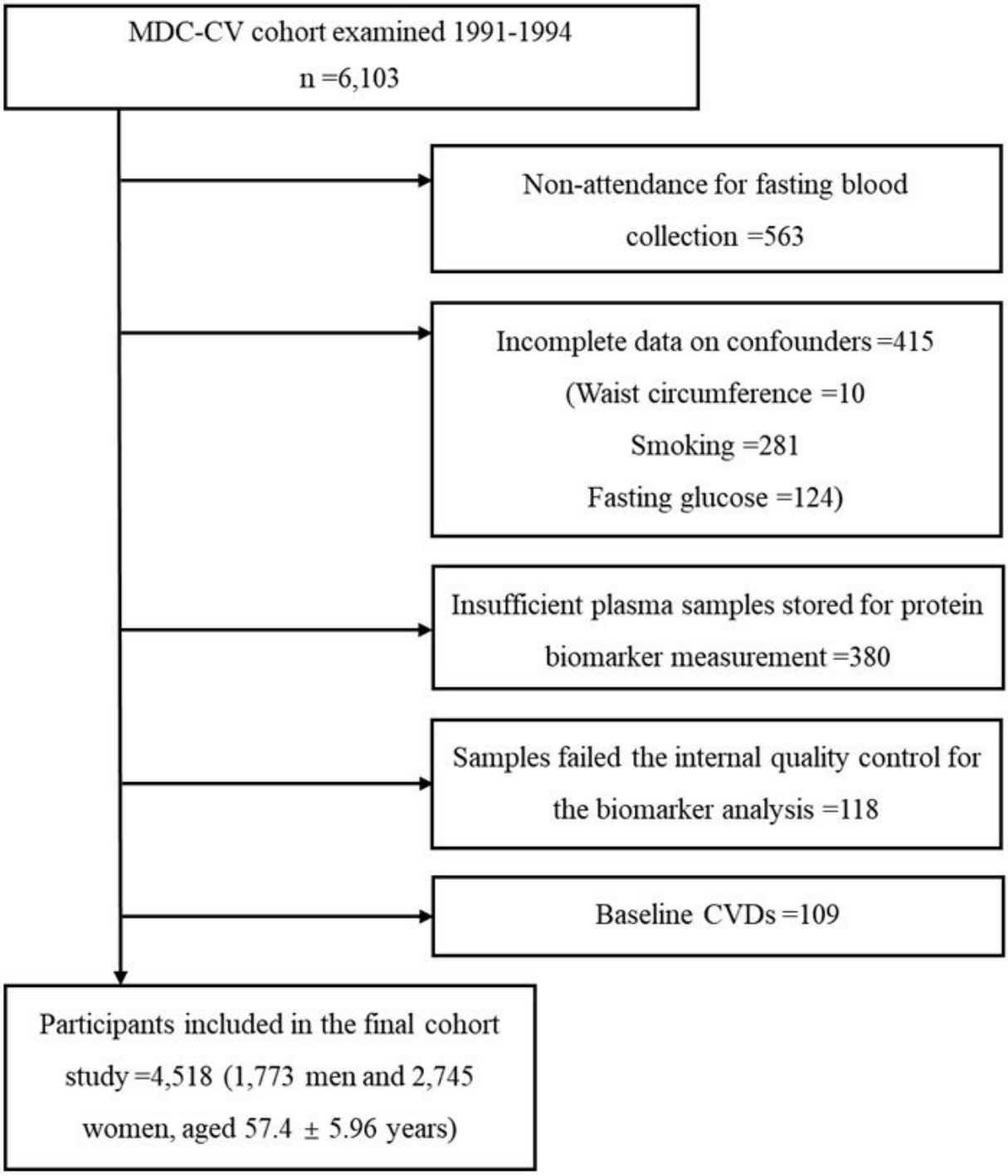

Figure 1

Study population flow.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigure1.png