Plasma growth differentiation factor-15 (GDF-15) independently predicts all-cause and cardiovascular mortality as well as deterioration of kidney function in type 1 diabetic patients with nephropathy

Running title: GDF-15 predicts cardiovascular disease and kidney function

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Objective- GDF-15 is involved in inflammation and apoptosis. Expression is induced in the heart in response to ischemia and in atherosclerotic plaques. The aim was to investigate GDF-15 levels in relation to all-cause mortality, cardiovascular mortality and morbidity, decline in glomerular filtration rate (GFR) and progression towards end-stage renal disease (ESRD).

Research design and methods- The study was a prospective observational follow-up study; including 451 type 1 diabetic patients with diabetic nephropathy (274 men; age 42.1±0.5 years [mean±SD], diabetes duration 28.3±8.9 years, GFR 76±33 ml/min/1.73m²) and a control group of 440 patients with longstanding type 1 diabetes and persistent normoalbuminuria (232 men; age 45.4±11.5 years, duration of diabetes 27.7±10.1 years). The patients were followed for 8.1(0.0-12.9) years (median (range)).

Results- Among normoalbuminuric patients, GDF-15 above the median predicted an adjusted (age, systolic BP and estimated GFR) increased risk of all-cause mortality (hazard ratio (HR) 3.6[1.3–10.3]; p=0.014).
Among patients with diabetic nephropathy, higher (fourth quartile) vs. lower (first quartile) GDF-15 levels predicts all-cause mortality (covariate-adjusted (sex, age, smoking, BP, HbA1c, cholesterol, GFR, NT-proBNP, antihypertensive treatment and previous cardiovascular events); HR 4.86[1.37-17.30] as well as fatal and non-fatal cardiovascular events (adj. HR 5.59[1.23-25.43] and 3.55[1.08-11.64], respectively). In addition, higher GDF-15 levels predicts faster decline in GFR (p<0.001) but not development of ESRD).

Conclusions- Higher levels of GDF-15 are a predictor of all-cause and cardiovascular mortality and morbidity in patients with diabetic nephropathy. Furthermore, higher levels of GDF-15 are associated with faster deterioration of kidney function.
Diabetes mellitus is associated with accelerated atherosclerosis and an increased risk of cardiovascular disease (CVD), which has become the major cause of morbidity and mortality among patients with diabetic nephropathy(1). Left ventricular hypertrophy, hypertension and diabetes are leading predictors for the development of heart failure and sudden death(2,3). In general, the hypertrophic growth of the myocardium is regulated by a number of pro- and anti-growth factors e.g. angiotensin-II and B-type natriuretic peptide (BNP) related to the transforming growth factor-β superfamily(4-6).

Recently, the growth-differentiation factor-15 (GDF-15) has been identified as a novel anti-hypertrophic regulatory factor(7). GDF-15 is generated as a 40-kDa propeptide from which the N-terminus is cleaved and a 30-kDa protein secreted as the active form(8).

GDF-15 is induced in the hypertrophic and dilated cardiomyopathy following hypertension/volume overload, ischemia and heart failure, possibly via pro-inflammatory cytokine and oxidative stress-dependent signalling pathways(9,10). GDF-15 is highly expressed in the infarcted myocardium in predominantly non-diabetic patients suffering an acute myocardial infarction (MI)(9) and in atherosclerotic plaques obtained from carotid artery surgery(11). In a nested case-control study, GDF-15 was shown to be associated with adverse cardiovascular outcome in women(12). Furthermore, GDF-15 has been shown to predict mortality in patients with both non-ST-elevation MI and ST-elevation MI independently of known biomarkers such as NT-proBNP(13,14).

Therefore, we investigated the predictive value of circulating GDF-15 levels on all-cause mortality, fatal and non-fatal CVD, decline in GFR as well as progression to ESRD in a well-characterized population of type 1 diabetic patients with or without diabetic nephropathy.

**RESEARCH DESIGN AND METHODS**

**Patients.** From 1993 to 2000, adult Caucasian patients with type 1 diabetes and diabetic nephropathy attending the outpatient clinic at Steno Diabetes Center have been invited to participate in a study of genetic risk factors for the development of diabetic complications. Of these, 73% accepted. Type 1 diabetes was considered present if the age at onset of diabetes was <35 years and time to definite insulin therapy >1 year. In total, 458 patients with diabetic nephropathy defined by persistent albuminuria (>300 mg/24h) in two out of three consecutive measurements, presence of retinopathy, and absence of other kidney or urinary tract disease were enrolled as cases.

Absence of diabetic nephropathy (controls) was defined as persistent normoalbuminuria (<30 mg/24h) after more than 15 years of type 1 diabetes in patients not treated with ACE inhibitors or angiotensin-II receptor blockers. In total, 442 were included as controls.

**Baseline clinical and laboratory investigations.** All patients had blood samples and phenotypic characteristics collected as part of the EURAGEDIC project(15). Office blood pressure was measured twice using a sphygmomanometer after at least 10 min rest in the sitting position. The average value of three readings, 2 min apart was used for calculation. From venous samples, HbA1c was measured by standard high-performance liquid chromatography (normal range:4.1–6.4%) (Tosoh automated glycohemoglobin analyser; Tosoh Bioscience, Minato, Japan) and cholesterol concentrations were determined by standard methods (Hitachi 912 system; Roche Diagnostics).

Urinary albumin excretion rate was measured in 24-h urine collections by an enzyme
immunoassay. Serum creatinine concentration was determined by a modified Jaffe's method. GFR was measured annually in patients with diabetic nephropathy after a single injection of 3.7 MBq $^{51}$Cr-EDTA by determination of radioactivity in venous blood samples taken 180, 200, 220, and 240 min after injection(16). The results were standardized for 1.73 m$^2$ body surface area, using the patient's surface area at the start of the study. The mean day-to-day coefficient of variation is 4% in our laboratory. Linear regression analysis of the GFR determinations in each individual was used to estimate the rate of decline in kidney function.

In patients with normoalbuminuria, the GFR at baseline was estimated by the four variable Modification of Diet in Renal Disease (MDRD) equation(17). ESRD was defined as chronic dialysis or kidney transplantation. Diabetic retinopathy was assessed by fundus photography after pupillary dilatation and graded nil, simplex, and proliferative retinopathy. On the basis of standardized questionnaires, current smokers of one or more cigarettes/cigars/pipes per day were classified as smokers and all others as non-smokers. Major CVD events were diagnosed as stroke, myocardial infarction, coronary artery bypass graft, and/or percutaneous coronary intervention.

Measurements of GDF-15, NT-proBNP and CRP. Blood samples for determination of biomarkers were collected in EDTA tubes, centrifuged and plasma stored at –80°C until analysis. Plasma GDF-15 was measured using a one-step enzyme immunoassay based on the electrochemiluminescence (ECLIA) technology (Elecsys 2010 instrument). This method shows excellent comparibility to the IRMA-assay, previously described in details(18) and the antibodies used for both assays are identical (capture antibody: PAb $>$GDF-15$>$ goat IgG(IS); detection antibody: Mab $>$GDF-15$>$ mouse-147627-IgG (R&D Systems). The N-terminal end of pro-B-type natriuretic peptide (NT-proBNP) was determined by sandwich immunoassay (Elecsys 2010 instrument) also as previously described(19). C-reactive protein (CRP) was analyzed with a chemiluminescent enzyme-labeled immunometric assay (Hitachi instrument). All measurements of GDF-15, NT-proBNP and CRP were performed by Roche Diagnostics GmbH, Penzberg, Germany by investigators blinded to the characteristics and outcome of the patients.

Follow-up data. In a prospective observational study design, the patients were followed until an endpoint was reached, to the last visit at Steno Diabetes Center or until the 1st of September 2006. The endpoints were all-cause mortality, cardiovascular mortality, major cardiovascular events, decline in GFR and development of ESRD. All patients were traced through the National Death Register and if a patient died before the 1st of September 2006, the date of death was recorded and information on cause of death was obtained. Two observers reviewed all death certificates independently and the primary cause of death was recorded. Additional available information from necropsy reports was included. All deaths were classified as cardiovascular deaths unless an unequivocal non-cardiovascular cause was established. Information about CVD events and ESRD was obtained from patient records or discharge letters from other hospitals.

In total, plasma GDF-15 levels were determined and follow-up data were available for 451 (98.5%) patients with diabetic nephropathy and 440 (99.5%) controls. The study was performed in accordance with the Helsinki Declaration, the local ethics committee approved the study and all patients gave their informed consent.

Statistical Analysis. Normally distributed variables are given as means±SD, whereas non-normally distributed variables were log10
transformed before analysis and are given as medians (range). Comparisons between groups were performed by an unpaired Student's t-test, analysis of variance (ANOVA) or linear regression when appropriate. The Chi²-test was used to compare non-continuous variables. A two-tailed p-value of 0.05 or less was considered statistically significant.

Multiple linear regression analysis was used to determine the influence of baseline level of GDF-15 on decline in kidney function (dGFR). All time-to-end point variables were analyzed using a log-rank test and displayed in Kaplan–Meier plots. The relations between baseline GDF-15 levels and endpoints during follow-up were assessed using Cox regression enter model (all-cause mortality, fatal and non-fatal CVD and ESRD) by univariable and multivariable analyses displayed as unadjusted and adjusted hazard ratios (HRs), respectively. HRs and 95% confidence intervals (CIs) for risk factors and significance level for χ² (likelihood ratio test) are given. Levels of NT-proBNP and GDF-15 were normalized by log10 transformation and thus the corresponding HRs refers to a 10-fold rise in the variables.

In addition, the predictive accuracy of GDF-15 were compared by generating receiver operating characteristic (ROC) curves for all-cause mortality and CVD mortality, respectively and the areas under the curves (AUCs) were calculated for patients with diabetic nephropathy. All calculations were performed using a commercially available program (SPSS, version 14.0, Chicago, USA).

**RESULTS**

**Baseline characteristics.** The study population included two groups: 451 patients with type 1 diabetes and diabetic nephropathy and 440 controls with type 1 diabetes for more than 15 years and persistent normoalbuminuria. Baseline clinical and laboratory characteristics of the 891 patients are shown in Table 1. As expected, the patients with diabetic nephropathy received more antihypertensive medication, had higher HbA1c, blood pressure, s-creatinine, total cholesterol than patients with normoalbuminuria (p<0.05). In parallel, the plasma GDF-15 concentration was higher in patients with diabetic nephropathy than in normoalbuminuric patients (median (range) 1322 (443–17735) vs. 749 (158–13933)(ng/L), respectively; p< 0.001)). On average, GFR was well preserved among the patients with diabetic nephropathy (GFR 76±33 (ml/min/1.73m²)).

Plasma concentration of GDF-15 did not differ significantly between type 1 diabetic men and women (p=0.99), but correlated positively with age (r=0.06, p<0.001) and systolic blood pressure (r=0.15, p<0.001), and inversely related to increased estimated glomerular filtration rate (r=−0.42, p<0.001). **Follow-up data.** The study was a prospective observational follow-up study with a median follow-up time until endpoint or last visit of 8.1 (0.0–12.9) years. Because of the low number of events in the normoalbuminuric group, the analyses are restricted to the median (749 ng/L) level of GDF-15 among these patients. During the follow-up period, five (2%) with GDF-15 levels below the median and 29 (13%) with GDF-15 levels above the median died. The log rank test demonstrated a significant difference between the groups (p<0.001). This corresponded to a six-fold increased risk for all-cause mortality (hazard ratio (HR) 6.3 with 95% CI [2.4–16.3]; p<0.001) in a Cox regression analysis. This relationship persisted after adjusting for age, systolic BP and eGFR; (HR) 3.6 [1.3–10.3]; p=0.014).

Similarly, 12 normoalbuminuric patients (3%) with GDF-15 levels below the median vs. 36 (8%) above suffered a fatal or non-fatal cardiovascular event during follow-up; (HR) 3.5 [1.8–6.6]; p<0.001. However, when
adjusting for age, systolic BP and eGFR this was no longer significant (covariate-adjusted HR 1.8 [0.8–3.6], p=0.16).

The 451 patients with diabetic nephropathy were divided into quartiles according to GDF-15 levels (≤969, 970–1327, 1328–2172 and ≥2173 ng/L, respectively). Six (5%) patients with GDF-15 levels in the first quartile, 26 (23%) in the second, 47 (42%) in the third and 61 (54%) patients in the fourth quartile died during follow-up (p<0.001). A fifteen-fold increased risk of all-cause mortality was found for high (fourth quartile) vs. low (first quartile) GDF-15 levels; (HR) 15.84 [6.84–36.69], p<0.001. High GDF-15 levels remained independently predictive of all-cause mortality in the Cox regression model (covariate-adjusted (sex, age, smoking, BP, HbA1c, cholesterol, GFR, NT-proBNP, antihypertensive treatment and previous cardiovascular events) HR 4.86 [1.37–17.30], p=0.015) (Table 2).

Regarding risk of fatal cardiovascular events, four (4%) patients with GDF-15 levels in the first quartile, 17 (15%) in the second, 29 (26%) in the third, and 39 (35%) in the fourth died of cardiovascular disease during follow-up. Figure 1 show the Kaplan–Meier curve for cardiovascular mortality (p<0.001). High (fourth quartile) vs. low (first quartile) GDF-15 levels predicted an increased risk of cardiovascular mortality; HR 14.64 [5.22–41.03], p<0.001; which persisted following covariate-adjustment; HR 5.59 [1.23–25.43], p=0.026) (Table 2). A similar independent predictive value of GDF-15 was seen for non-fatal cardiovascular events (n=99). High levels (fourth quartile) vs. low (first quartile) increased the risk of non-fatal cardiovascular events when adjusting for confounding factors (HR 3.55 [1.08–11.64], p=0.036) (Table 2).

GDF-15 concentrations correlated with the cardiovascular risk factors NT-proBNP (r=0.42, p<0.001) and more weakly with CRP (r=0.01, p=0.016).

The overall prognostic value of GDF-15 levels and other risk markers for all-cause mortality was evaluated by receiver operating characteristic (ROC) curves. For GDF-15, the mean area under the curve (AUC) was 0.79 (0.74–0.84) as compared with NT-proBNP (0.75 (0.69–0.81)) and GFR (0.71 (0.64–0.77)). In comparison, the AUCs for a combined risk score model including the previous mentioned covariates with and without GDF-15 were 0.84 (0.80–0.88) and 0.83 (0.78–0.87), respectively. Regarding CVD mortality, similar AUCs for the predictive value of GDF-15 levels with and without covariates was found (data not shown). Evaluating GDF-15 as a continuous variable introduced only minor changes in the statistical outputs. Finally, when performing the survival analyses for all patients combined (n=891) adjusting for the previous mentioned covariates and nephropathy status, GDF-15 continued to be associated with all-cause and CVD mortality; HR 4.61 [1.93–11.00], p<0.001 and 5.04 [1.71–14.85], p=0.003), respectively.

**Progression of nephropathy.** The mean (SD) rate of decline in GFR was 3.0 (2.9), 3.4 (3.6), 4.8 (4.0) and 6.3 (4.8) ml/min/1.73 m²/year (p<0.001), respectively when dividing the patients according to quartiles of GDF-15. Furthermore, when including the baseline variables sex, age, HbA1c, systolic blood pressure, GFR, cholesterol and AHT as covariates in the analysis, the GDF-15 levels still predicted a faster rate of decline in GFR during follow-up (p<0.001).

In addition, progression to chronic dialysis or transplantation (ESRD) during the follow-up period was seen in three patients in the first quartile (3%), four patients in the second quartile (4%), 21 patients in the third quartile (19%) and 45 patients in the fourth quartile (47%) (p<0.001). Cox regression analysis revealed a 40 times increased risk of ESRD in patients with the highest GDF-15 levels (fourth quartile) compared to the lowest (first
CONCLUSIONS
In the prospective observational analysis including 8 years of follow-up, GDF-15 levels were independently predictive of all-cause mortality among the type 1 diabetic patients with and without diabetic nephropathy. In addition, among the 451 patients with overt diabetic nephropathy, elevated levels of GDF-15 independently predicted CVD mortality and morbidity as well as deterioration of renal function (dGFR) after adjustment for conventional CVD and renal risk factors, including GFR and NT-proBNP in multivariable models. The elevated levels of GDF-15 may reflect several underlying conditions including acute/or chronic ischemia associated with adverse cardiovascular outcomes or merely indicate reduced renal excretion of GDF-15 due to renal dysfunction. In particular, renal dysfunction appears to mediate part of the prognostic impact of GDF-15 given the strong inverse relationship between baseline levels of GDF-15 and eGFR in our study. Thus, among the several confounding factors, impaired renal function may be particularly important since a reduced GFR represents a cardiovascular risk factor by itself and raises the possibility of residual confounding by decreased renal clearance. Therefore, in our study we adjusted for several conventional CVD covariates including GFR at baseline. The remaining strong independent predictive value of GDF-15 suggests mechanisms other than renal dysfunction to account for the correlation between CVD risk and GDF-15 levels. The possibility remains that elevated levels of GDF-15 may reflect a compensatory production in individuals suffering more severe cardiovascular disease. Circulating GDF-15 levels have previously been shown to be an independent risk factor for adverse cardiovascular events, including stroke and AMI among women(12). At the cellular level, GDF-15 mRNA and propeptide expression is strongly induced by both permanent and transient ischemia in mice and in patients who died after an MI(9). In addition, the gdf-15 gene targeted mice have virtually normal hearts under non-stressed conditions. In contrast, they develop greater infarct sizes and greater extent of cardiomyocyte cell death following the ischemia/reperfusion injury which could be prevented by treatment with recombinant GDF-15(9). Thus, GDF-15 may function as a necessary protective factor to the heart in association with immediate and long-term alterations in the hypertrophic response antagonizing the onset or severity of heart failure, analogous to BNP. Secretion of BNP and NT-proBNP is induced from the heart following acute and chronic stimulation associated with cardiac injury and long-standing disease, signalling a protective and anti-hypertrophic response(6,20). Previously, circulating levels of NT-proBNP levels have been shown to be associated with increased mortality due to chronic heart failure and acute coronary syndromes in various disease states such as hypertension(21), ESRD(22) and of excess overall and cardiovascular mortality in both type 1(19) and type 2(23) diabetic patients with diabetic nephropathy. Thus, we also included NT-proBNP levels in the multivariate analysis. Still, elevated GDF-15 levels remained independently associated with CVD in an analogous manner to circulating NT-proBNP levels and as such, may offer future therapeutic targets for the treatment of hypertrophic and dilated cardiomyopathy. However, studies showing that GDF-15 levels are modified by intervention are needed.
Recent data suggest that patients with non-ST-elevation acute coronary syndrome having elevated GDF-15 levels benefit from early revascularization whereas patients with lower levels of GDF-15 do not(24). Thus, future studies should elucidate whether GDF-15 could be informative as part of a multimarker strategy identifying high-risk patients. The ROC curve analysis further illustrated GDF-15 as a strong marker of mortality risk with an AUC of 0.79 as compared with NT-proBNP (0.75 (0.69-0.81)) and GFR (0.71 (0.64-0.77)). However, in a combined risk score model including the previous mentioned covariates, GDF-15 only slightly improved the predictive accuracy of the model. This could be due to the fact, that the combined model without GDF-15 has an AUC of 0.83 and hence the predictive accuracy is difficult to increase. Still, combining markers of renal dysfunction, inflammation and natriuretic peptides enhance risk stratification of type I diabetic patients with and without diabetic nephropathy and may provide additional insight into pathophysiological mechanisms. Although the cellular sources, upstream regulators and functional effects of GDF-15 in the cardiovascular system remain to be fully elucidated, several details are emerging. Basal GDF-15 expression has so far only been detected in liver and placenta tissue. However, studies of cultured human kidney cells showed a rapid rise in secretion of mature GDF-15 following injury, hypoxia or cytokine/growth factor stimulation due to a dramatic induction of expression of GDF-15 not release of stored GDF-15(8,11).

GDF-15 has been suggested to provide endogenous protection against cardiomyocyte apoptosis possibly via a PI3K-Akt dependent pathway(9). In addition, GDF-15 treatment also transiently activated Akt and ERK1/2 signalling, both of which are thought to be cardioprotective pathways(10). Other studies indicate that bone marrow derived GDF-15 protects against macrophage accumulation within atherosclerotic lesions and promotes lesion stabilisation possibly due to inhibition of adhesion molecules(25). Finally, the potential heterogeneity in the mechanism of GDF-15 action in different tissues or cell types may be attributable to differential expression of the type-I and type-II transforming growth factor-β activin receptors with affinity for GDF-15. Thus, GDF-15 might not only be a marker of ongoing tissue damage but actively influence multiple downstream signalling effectors as part of a cardioprotective mechanism. However, a suggestive role for GDF-15 therapy in improving treatment and prevention of complications among diabetic patients remains to be investigated further in both experimental and clinical settings.

There are some limitations that should be mentioned. Firstly, the endpoint fatal CVD is based on death certificates in contrast to non-fatal CVD events which are based on information obtained from the patients medical records. However, on a positive note the number of events may be underestimated and hence the predictive value of GDF-15 could be regarded as a conservative estimate. Secondly, the stability of GDF-15 after 15+ years of storage is not known. However, all samples of this study were treated and stored at – 80ºC under the same conditions. Furthermore, GDF-15 has been shown to be stable at room temperature for 48h and resistant to at least 4 freeze-thaw cycles and equally important, the choice of anticoagulant matrix had no influence on the measurements(18).

In conclusion, higher levels of GDF-15 independently predicted all-cause and cardiovascular mortality and morbidity in patients with diabetic nephropathy beyond conventional cardiovascular risk factors including NT-proBNP and GFR. Furthermore, higher levels of GDF-15 were predictive of deterioration of kidney function. Therefore, GDF-15 may be considered as an
additional marker of inflammation and atherosclerosis involved in the pathogenesis of ischemic heart disease.

ACKNOWLEDGMENTS
The authors wish to acknowledge the technical assistance by Tina R. Juhl, Anne G Lundgaard, Berit R. Jensen and Ulla M. Smidt.

In addition, the authors wish to thank Georg Hess and Dietmar Zdunek from Roche Diagnostics for supplying the GDF-15, NT-proBNP and CRP measurements.

Sources of Funding. The EURAGEDIC study was supported by the European Commission (contract QLG2-CT-2001-01669).

Disclosures. None.
REFERENCES


Table 1. Baseline clinical and laboratory characteristics of 891 type 1 diabetic patients followed for 8.1 (0.0–12.9) years according to nephropathy status.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nephropathy ((n=451))</th>
<th>Normoalbuminuria ((n=440))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (men/women)</td>
<td>274/177</td>
<td>232/208</td>
<td>0.018</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.1 ± 10.5</td>
<td>45.4 ± 11.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>28.3 ± 8.9</td>
<td>27.7 ± 10.1</td>
<td>0.37</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.3 ± 3.3</td>
<td>24.2 ± 3.1</td>
<td>0.77</td>
</tr>
<tr>
<td>HbA(_{1c}) (%)</td>
<td>9.4 ± 1.5</td>
<td>8.4 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensive treatment (%)(^a)</td>
<td>77.0</td>
<td>16.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>144 ± 22</td>
<td>134 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82 ± 12</td>
<td>76 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary albumin excretion rate mg/24 h</td>
<td>593 (3–14545)(^b)</td>
<td>9 (1–30)</td>
<td>–</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>102 (52–706)</td>
<td>80 (53–134)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR (ml min(^{-1}) 1.73 m(^{-2}))</td>
<td>76±33</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.6 ± 1.2</td>
<td>4.8 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>46.3</td>
<td>39.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Retinopathy (0/SR/PR)</td>
<td>6/139/306</td>
<td>159/162/119</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of MI(%)(^c)</td>
<td>4.2</td>
<td>2.1</td>
<td>0.05</td>
</tr>
<tr>
<td>History of stroke (%)(^c)</td>
<td>6.9</td>
<td>1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NT-proBNP (ng/L)</td>
<td>89.1 (5–19394)</td>
<td>42.8 (5–1552)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GDF-15 (ng/L)</td>
<td>1322 (443–17735)</td>
<td>749 (158–13933)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are \(n\), mean ± SD or median (range). \(^a\)In 2002 the recommendations at SDC were extended to include statins and low-dose aspirin for all patients with diabetic nephropathy. \(^b\)Some patients with previously persistent macroalbuminuria receiving antihypertensive treatment had values <300 mg/24 h at the time of investigation. SR, simplex retinopathy; PR, proliferative retinopathy. \(^c\)Presence of previous CVD is defined as either myocardial infarction (MI) or stroke.
Table 2. Hazard ratio (HR) of long-term cumulative mortality, cardiovascular mortality and morbidity after adjustment for confounding factors among the 451 patients with diabetic nephropathy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All-cause mortality (n=140)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Second vs. first quartile</td>
<td>1.90</td>
<td>0.60–6.03</td>
<td>0.28</td>
</tr>
<tr>
<td>Third vs. first quartile</td>
<td>3.75</td>
<td>1.22–11.57</td>
<td>0.022</td>
</tr>
<tr>
<td>Fourth vs. first quartile</td>
<td>4.86</td>
<td>1.37–17.30</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>Cardiovascular mortality (n=89)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second vs. first quartile</td>
<td>1.76</td>
<td>0.44–6.97</td>
<td>0.42</td>
</tr>
<tr>
<td>Third vs. first quartile</td>
<td>3.99</td>
<td>1.06–14.99</td>
<td>0.040</td>
</tr>
<tr>
<td>Fourth vs. first quartile</td>
<td>5.59</td>
<td>1.23–25.43</td>
<td>0.026</td>
</tr>
<tr>
<td><strong>Non-fatal cardiovascular event (n=99)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second vs. first quartile</td>
<td>2.36</td>
<td>0.91–6.13</td>
<td>0.079</td>
</tr>
<tr>
<td>Third vs. first quartile</td>
<td>2.04</td>
<td>0.75–5.56</td>
<td>0.16</td>
</tr>
<tr>
<td>Fourth vs. first quartile</td>
<td>3.55</td>
<td>1.08–11.64</td>
<td>0.036</td>
</tr>
</tbody>
</table>

All primary endpoints are adjusted for the following confounding factors: sex, age, smoking, HbA1c, systolic BP, cholesterol, GFR, NT-proBNP, antihypertensive treatment and a history of cardiovascular events at baseline.

Legend to Figure 1.
Figure 1. Unadjusted Kaplan–Meier curves for CVD mortality among the 451 patients with diabetic nephropathy according to quartiles of GDF-15 levels (≤969, 970–1327, 1328–2172 and ≥2173ng/L, respectively. Light gray line, 1st Quartile; Light gray dotted line, 2nd quartile; Dark gray dotted line, 3rd Quartile and black line, 4th Quartile. LogRank test resulted in p<0.001).
GDF-15 predicts cardiovascular disease and kidney function