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ORIGINAL RESEARCH

GDF-15 (Growth Differentiation Factor 15) Is Associated With Hospitalization and Mortality in Patients With a Fontan Circulation

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BACKGROUND: We investigated serial serum levels of GDF-15 (growth differentiation factor 15) in Fontan patients and their relation to outcome.

METHODS AND RESULTS: In this single-center prospective study of consecutive Fontan patients, serial serum GDF-15 measurement and clinical assessment was done at baseline (n=81) and after 2 years (n=51). The association between GDF-15 and the combined end point of all-cause mortality, heart transplant listing, and Fontan-related hospitalization was investigated. Median age at baseline was 21 years (interquartile range: 15–28 years). Median GDF-15 serum levels at baseline were 552 pg/mL (interquartile range: 453–729 pg/mL). GDF-15 serum levels correlated positively with age, age at Fontan initiation, New York Heart Association class, and serum levels of NT-proBNP (N-terminal pro-B-type natriuretic peptide) and γGT (γ-glutamyltransferase) and negatively with exercise capacity. During a median follow-up of 4.8 years (interquartile range: 3.3–5.5 years), the combined end point occurred in 30 patients (37%). Multivariate Cox regression showed that patients with the highest baseline GDF-15 (n=20, defined as the upper quartile) had a higher risk of hospitalization or death than the lowest 3 quartiles (hazard ratio [HR], 2.76; 95% CI, 1.27–6.00; *P*=0.011). After 2 years of follow-up, patients in whom serum level of GDF-15 increased to >70 pg/mL (n=13) had a higher risk of hospitalization or death than the lowest 3 quartiles (HR, 2.69; 95% CI, 1.03–6.99; *P*=0.043).

CONCLUSIONS: In Fontan patients, elevated serum levels of GDF-15 are associated with worse functional status and predict Fontan-related events. Furthermore, serial measurements showed that an increase in GDF-15 serum level was associated with increased risk for adverse outcome.

Key Words: GFD-15 (growth differentiation factor 15) ■ follow-up studies ■ Fontan procedure ■ univentricular heart

Patients with a functionally univentricular heart are currently palliated with a sequence of operations culminating in the Fontan circulation.[1] The data on long-term survival remain unsatisfactory because the nonphysiologic Fontan circulation deteriorates over time, ultimately leading to a state known as "Fontan failure." Fontan failure is a state of multiorgan failure with heterogeneous presentation; significant interindividual differences regarding timing, severity, and mode[2–4]; and limited treatment options.[5] Insufficient information is available on the exact mechanisms responsible for the failure of the Fontan circulation and the long-term risk factors. To date, no conventional heart failure biomarker has been identified that reliably

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CLINICAL PERSPECTIVE

What Is New?

- This study showed that in Fontan patients, elevated serum levels of GDF-15 (growth differentiation factor 15) are associated with worse functional status.
- GDF-15 serum levels identified a cohort of Fontan patients at high risk of hospitalization or death.
- Serial measurements of GDF-15 serum level have an additive benefit in predicting Fontan patients at high risk for adverse events.

What Are the Clinical Implications?

- These findings serve as the basis for larger scale evaluations into the role of GDF-15 in the Fontan population and provide evidence to support the routine use of single and serial GDF-15 measurements for risk stratification of patients with a Fontan circulation in clinical practice.

Nonstandard Abbreviations and Acronyms

CMR	cardiac magnetic resonance
CPET	cardiopulmonary exercise testing
EF	ejection fraction
GDF-15	growth differentiation factor-15
IQR	interquartile range
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
pVo_2	peak oxygen uptake
TGF-β	transforming growth factor β

predicts deterioration and adverse events in this heterogeneous group.[6]

GDF-15 (growth differentiation factor 15), a cytokine of the TGF-ß (transforming growth factor ß) superfamily, is involved in various stress pathways.[7,8] Under pathologic conditions, such as inflammation, tissue hypoxia, and injury, GDF-15 is upregulated in various organ systems, including the liver, the kidneys, the pulmonary vascular system, and the cardiovascular system.[8–10] Previous studies have identified that GDF-15 levels are increased in patients with hepatocellular carcinoma and cirrhosis[11,12] and in patients with chronic failure of the structurally normal heart and that these levels are of prognostic value for adverse cardiovascular events.[7,13,14] In a mixed cohort of patients with congenital heart diseases, single GDF-15 measurements correlated with functional status and predicted cardiovascular events.[15,16] The fact that GDF-15 is excreted by not only the heart but also by other organs in response to stress suggests GDF-15 serum levels to be particularly suitable for early detection and monitoring of Fontan failure, characterized by multiorgan involvement. However, only 1 relatively small cross-sectional study (n=38) has addressed the value of GDF-15 in the Fontan circulation and identified a correlation with systemic ventricular function.[17] The aim of the current study was to investigate serial serum GDF-15 measurements over time in Fontan patients and to relate these measurements to outcome. We hypothesized that high GDF-15 serum level or a rise in GDF-15 serum level predicts adverse outcomes.

METHODS

Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Patients

Consecutive patients ≥10 years old with a Fontan circulation who were followed at the Center for Congenital Heart Disease, University Medical Center Groningen, The Netherlands, between 2012 and 2014 were included. At this center, all Fontan patients undergo standardized follow-up, including cardiopulmonary exercise testing (CPET), cardiac magnetic resonance (CMR) examination, echocardiography, and venipuncture for laboratory measurements every 2 years. Patients were screened for kidney failure, defined as creatinine levels >200 µmol/L, but none were found. All patients who had at least 1 venipuncture for laboratory measurement done were included. Exclusion criteria were CMR, echocardiography, and CPET tests done >2 months from venipuncture and <1 year follow-up after baseline venipuncture.

The investigation conforms to the principles outlined in the Declaration of Helsinki. The institutional ethics committee approved the conduct of this investigation. Informed consent was obtained from all study participants and/or their parents.

Clinical and Outcomes Assessment

Patient characteristics were collected from medical records, including age, sex, body mass index, body surface area, New York Heart Association (NYHA) class, cardiac anatomy, type of initial Fontan operation, follow-up since both Fontan initiation (bidirectional cavopulmonary shunt or 1-stage Fontan) and completion and current cardiac medication use, oxygen

saturation measured by pulse oximetry, and renal function measured by serum creatinine.[18] Diffusion-weighted imaging of the liver, a magnetic resonance imaging–based technique that can be used to detect liver fibrosis and cirrhosis, was done in a subset of patients and apparent diffusion coefficient values were calculated, as reported previously.[19]

For this study, follow-up data were collected up to September 1, 2018. The combined end point was defined as Fontan-related hospitalization, heart or heart–lung transplantation, listing for these procedures, and death. Fontan-related hospitalization was defined as an overnight admission for cardiac decompensation, arrhythmias, thromboembolic events, Fontan-associated liver disease, protein-losing enteropathy, or a Fontan-related intervention.

Laboratory Measurements

Venous blood samples were drawn during routine follow-up. Liver function tests and creatinine and hemoglobin measurements were performed using standard laboratory techniques. For NT-proBNP (N-terminal pro-B-type natriuretic peptide) measurements, the specimens were collected in 4.5-mL lithium-heparin tubes, centrifuged, and directly determined by immunoassay using the Roche Modular E (Roche Diagnostics).[20] Blood samples were processed and stored within 2 hours at −80 °C. GDF-15 was determined by a quantitative sandwich enzyme immunoassay technique using the electrochemiluminescence immunoassay Elecsys GDF-15 (Roche Diagnostics) on the Cobas e 801 immunoassay analyzer. For quality control, 2 control measurements were done. The lower limit of detection for the GDF-15 assay was 400 pg/mL.

Echocardiography

Transthoracic echocardiography was performed using a commercially available General Electric ultrasound machine with a 3.5-MHz probe. A standardized protocol was used that included parasternal, apical, subcostal, and suprasternal views. For the assessment of diastolic function, the peak early (E) and late (A) inflow velocities across the dominant atrioventricular valve were measured using a pulsed Doppler sample on the apical chamber view. Pulsed wave tissue Doppler imaging was used to assess the peak early (e′) diastolic velocities of the atrioventricular annulus of the nonseptal (free) wall. The E/e′ ratio and E/A ratio were calculated.

Cardiopulmonary Exercise Testing

CPET was performed on an upright cycle ergometer in children or on a treadmill in adults, as described previously.[21] Peak oxygen uptake (pVO_2) was calculated as the mean of the last 30 seconds during exercise and was indexed for body weight (pVO_2 indexed). The pVO_2 as percentage of predicted was calculated using reference values.[21] Adequate performance of the CPET was defined as an respiratory exchange ratio of at least 1.0.[22] Patients who did not reach this threshold were excluded. The actual number of patients included for analysis was reported as appropriate.

CMR Acquisition and Analysis

CMR studies were performed on a 1.5-T system (Magnetom Avanto; Siemens) without sedation, as previously described.[23] Imaging analysis was performed using Qmass (v7.6.14.0; Medis Medical Imaging). The end-systolic and end-diastolic blood volumes were calculated from the endocardial contours; both the volumes of the systemic and hypoplastic ventricle were included. The ejection fraction (EF) was calculated from end-diastolic and end-systolic blood volumes. Cardiac output was defined as ascending aortic flow. Cardiac index was calculated using body surface area.

Because patients with a pacemaker or claustrophobia were not able to undergo CMR examination, the actual numbers included for CMR analysis were reported as appropriate.

Statistical Analysis

All continuous variables were tested for normality using the Shapiro–Wilk test and are presented as mean±SD if parametric tests were used or as median (interquartile range [IQR]) if nonparametric tests were used. Categorical data were presented as frequency (percentage of total). The distribution of GDF-15 was skewed and remained skewed after log, square root, and reciprocal transformation. Patients were classified according to quartiles of GDF-15 in concordance with previously published literature.[24–27] Continuous variables stratified by GDF-15 were analyzed using linear regression, and differences in categorical variables were evaluated using the χ^2 Mantel–Haenszel test for trend. NT-proBNP had an exponential distribution and thus was log-transformed for statistical analysis, but untransformed median and IQR are reported for ease of interpretation. Comparisons between baseline and follow-up measurements were done using the paired *t* test or Wilcoxon signed rank test. Cox regression analysis was used to perform univariate survival analysis, stratified by baseline GDF-15 quartiles 1, 2, and 3 versus quartile 4. Multivariate Cox regression was performed with baseline GDF-15 stratified by quartiles 1, 2, and 3 versus quartile 4 as the independent variable. Specified covariates were individually adjusted for, and thereafter all variables significantly ($P<0.05$) associated with outcome were included and tested using backward selection with $P>0.1$ as a removal criterion. Primary end point–free survival was defined as

the time from study inclusion to the occurrence of the first event (ie, Fontan-related hospitalization, heart or heart–lung transplant or listing for these procedures, or death). Event-free participants were censored at the most recent clinical follow-up date when event status was known. Follow-up time was truncated at 5.5 years from baseline and at 3 years from follow-up to ensure sufficient group size for adequate analysis. Because the amount of patients with CPETs was lower than the total cohort, 2 multivariate models were tested, with and without CPET variables. The interval change (Δ) between GDF-15 levels at baseline and at follow-up, and of all conventional parameters, was calculated, and patients were classified according to quartiles. Cox regression with all parameters stratified by quartiles 1, 2,and 3 versus quartile 4 was performed. All analyses were performed using IBM SPSS Statistics for Windows v26. A 2-sided $P \leq 0.05$ was considered statistically significant.

RESULTS

Baseline Analysis

Eighty-one patients with a median age of 21 years (IQR: 14.5–27.5 years) were included at baseline, of whom 32 (40%) were aged <18 years. Median GDF-15 serum level at baseline was 552.2 pg/mL (IQR: 452.5–728.7 pg/mL). These values are higher than reported GDF-15 serum levels from healthy controls of similar age (median: 429 pg/mL)[28] and slightly lower than GDF-15 serum levels of patients with various congenital heart diseases (median: 618 pg/mL) with a median age of 33 years.[15] The control measurements of the GDF-15 analysis showed an intermediate precision of 2.69% (acceptable limit: <4.6%) and 2.94% (acceptable limit: <4.0%).

Patients in the upper GDF-15 quartile were significantly older and were more likely than those in lower quartiles to have an atriopulmonary connection, a higher NYHA class, more β-blocker use, a pacemaker, a lower cardiac index, a lower exercise capacity, and higher NT-proBNP and liver function parameters (γGT, alanine aminotransferase, alanine aminotransferase/aspartate aminotransferase ratio, apparent diffusion coefficient values). For a complete overview of the baseline characteristics, see Table 1.

Survival status and follow-up data were available for all 81 patients included at baseline. The median follow-up duration from baseline was 4.8 years (IQR: 3.3–5.5 years). The composite end point occurred in 30 patients (37%): there were 2 deaths, 1 listing for heart transplant, and 27 Fontan-related hospitalizations due to cardiac decompensation (n=3), arrhythmia (n=17, 15 atrial and 2 ventricular arrhythmias), Fontan-associated liver disease (ie, hepatocellular carcinoma; n=1), severe protein-losing enteropathy (n=2), and cardiovascular interventions (n=4). Median time to event was 3.5 years (IQR: 2.6–4.6 years).

Fontan patients with a GDF-15 serum level in the upper quartile (n=20) were at increased risk for the combined outcome ($P<0.001$; Table 2, Figure 1). Although adjustment for age and sex slightly affected the predictive value, GDF-15 serum level in the upper quartile remained significantly associated with outcome (hazard ratio [HR], 3.10; 95% CI, 1.33–7.27; $P=0.009$). After full adjustment for all variables significantly associated with outcome in the univariate analysis, upper GDF-15 quartile remained a significant predictor of outcome ($P=0.011$; Table 2). Similarly, when GDF-15 serum levels were stratified at the median value, univariate Cox regression analysis revealed a GDF-15 level above the median value to also be predictive for adverse outcome (HR: 2.11; 95% CI, 1.01–4.41; $P=0.047$). In addition, when analyzed as a continuous value with univariate Cox regression, GDF-15 baseline serum level correlated significantly with outcome (per Δ10, HR: 1.40; 95% CI, 1.07–1.82; $P=0.014$). Finally, subanalysis excluding all patients with an atriopulmonary connection Fontan did not attenuate the predictive quality of GDF-15 at univariate Cox regression (Q4 versus Q1, Q2, and Q3, HR: 3.15; 95% CI, 1.22–8.12; $P=0.018$), although in this specific subgroup at multivariate analysis, GDF-15 did not remain significantly associated with outcomes. For a full overview of the univariate and multivariate regression models, see Table S1.

Longitudinal Analysis

GDF-15 measurements from the second outpatient visit were available for 51 patients. The median time between the first and second visit was 2.1 years (IQR: 1.9–2.25 years). Median GDF-15 level of the group did not increase significantly over the course of 2 years (baseline [570 pg/mL; IQR: 451–74) pg/mL] versus follow-up [596 pg/mL; IQR: 483–873 pg/mL]; $P=0.251$). Of the 51 patients, 30 (59%) remained in the same GDF-15 quartile, 8 (16%) changed to a lower quartile, and 13 (25%) changed to a higher quartile. CMR parameters (EF, cardiac index), echocardiography parameters, and functional parameters (NYHA, CPET) did not worsen significantly. Of all conventional parameters (NYHA class, EF, cardiac index, pVO_2 index, pVO_2 predicted percentage, NT-proBNP, γGT), only interval change in NT-proBNP correlated significantly with interval change in GDF-15 (Spearman $\rho=0.35$, $P=0.014$). The composite end point occurred in 17 patients (33%): there was 1 death and 16 Fontan-related hospitalizations due to cardiac decompensation (n=1), arrhythmia (n=10, 8 atrial and 2 ventricular arrhythmias), severe protein-losing enteropathy (n=2), and cardiovascular interventions (n=3). Median time from

Table 1. Baseline Patient Characteristics

	All	GDF-15 Quartiles				*P* Value
		Quartile 1 (<453 pg/mL)	Quartile 2 (453–552 pg/mL)	Quartile 3 (553–729 pg/mL)	Quartile 4 (≥729 pg/mL)	
n (%)	81 (100)	20 (25)	21 (25)	20 (25)	20 (25)	
Age, y	21 (15–28)	15 (12–17)	21 (16–27)	20 (13–24)	28 (24–35)	<0.001
Female, n (%)	41 (51)	8 (40)	10 (48)	9 (45)	14 (70)	0.08
Age at Fontan initiation, y	1.3 (1.1–2.1)	1.1 (0.9–2.0)	1.3 (1.0–2.1)	1.4 (1.2–2.4)	2.5 (1.4–3.5)	0.68
Age at Fontan completion, y	4.5 (3.4–6.4)	4.7 (3.5–6.6)	4.1 (3.2–5.1)	4.5 (3.1–5.6)	4.8 (3.8–7.7)	0.32
Left ventricular morphology, n (%)	68 (84)	18 (90)	19 (91)	14 (70)	17 (85)	0.33
Fontan type, n (%)						<0.001
Atriopulmonary connection/Bjork	16 (20)	0 (0)	2 (10)	3 (15)	11 (55)	
Lateral tunnel	44 (54)	9 (45)	16 (76)	10 (50)	9 (45)	
Extracardiac conduit	21 (26)	11 (55)	3 (14)	7 (35)	0 (0)	
Pacemaker, n (%)	15 (19)	1 (5)	2 (10)	3 (15)	9 (45)	0.001
β-Blocker use, n (%)	20 (25)	1 (5)	6 (29)	3 (15)	10 (50)	0.005
History of arrhythmia, n (%)	22 (27)	1 (5)	3 (14)	5 (25)	13 (65)	<0.001
NYHA class, n (%)						0.002
I	35 (43)	12 (60)	11 (52)	9 (45)	3 (15)	
II	36 (44)	7 (35)	10 (48)	9 (45)	10 (50)	
III	10 (12)	1 (5)	0 (0)	2 (10)	7 (35)	
Ejection fraction, % (n=56)	58 (50–62)	61 (49–62)	56 (49–61)	58 (53–63)	57 (50–64)	0.84
Cardiac index, L/min/m^2 (n=56)	2.7 (2.1–3.3)	3.0 (2.5–3.8)	2.4 (2.0–2.8)	2.9 (2.1–3.3)	2.1 (1.7–2.7)	0.01
pVo_2 indexed, mL/min/kg (n=65)	24 (20–33)	29 (23–33)	27 (21–34)	25 (21–33)	18 (15–21)	<0.001
pVo_2 predicted, % (n=65)	58 (45–66)	57 (43–66)	64 (56–74)	63 (46–65)	49 (45–60)	0.04
O_2 saturation at rest, %	94 (91–96)	95 (91–97)	93 (90–96)	95 (93–95)	93 (89–95)	0.05
Ea, cm/s (n=55)	66 (55–78)	66 (60–76)	65 (56–79)	75 (64–83)	50 (43–57)	0.31
e′, cm/s (n=58)	10.5 (8.7–12.9)	12.6 (8.8–13.2)	9.9 (7.2–12.8)	10.4 (9.1–12.6)	10.0 (8.9–12.4)	0.30
E/A (n=54)	1.4 (1.1–1.8)	1.5 (1.1–1.9)	1.4 (1.2–1.7)	1.7 (1.2–1.9)	1.1 (0.8–1.6)	0.08
E/e′ (n=48)	6.1 (4.7–7.9)	5.6 (4.7–7.6)	6.2 (5.4–9.0)	6.4 (5.6–7.4)	4.8 (2.8–6.9)	0.79
Deceleration time, ms (n=51)	166 (133–194)	150 (130–171)	185 (119–223)	175 (142–191)	173 (120–202)	0.45
NT-proBNP ng/L*	113 (62–269)	75 (51–109)	116 (64–317)	124 (51–148)	306 (100–479)	0.005
Creatinine μmol/L*	63 (55–77)	53 (40–64)	61 (55–71)	62 (57–74)	77 (67–85)	<0.001
γGT, μ/L	59 (39–103)	54 (33–71)	50 (42–106)	52 (34–76)	94 (66–171)	0.01
AST, μ/L	30 (25–35)	32 (27–37)	27 (23–31)	30 (27–44)	30 (25–33)	0.62
ALT, μ/L	26 (21–32)	26 (19–29)	25 (21–32)	29 (19–35)	29 (23–35)	0.03
AST/ALT ratio	1.1 (0.9–1.4)	1.3 (1.2–1.5)	1.1 (0.9–1.4)	1.1 (0.9–1.4)	1.0 (0.8–1.0)	0.002
ADC value, ×10^{-3} mm^2/s (n=52)	0.83 (0.73–0.89)	0.89 (0.83–0.93)	0.79 (0.71–0.88)	0.83 (0.78–0.88)	0.76 (0.63–0.82)	0.01
End point occurrence, n (%)	30 (37)	7 (35)	4 (19)	5 (25)	14 (70)	0.001
Death	2 (6)	…	…	…	2 (14)	
Transplant listing	1 (3)	…	1 (25)	…	…	
Cardiac decompensation	3 (10)	…	…	…	3 (21)	
Arrhythmia	17 (57)	3 (43)	3 (75)	3 (60)	8 (57)	
Protein-losing enteropathy	2 (6)	1 (14)	…	1 (20)	…	
Hepatocellular carcinoma	1 (3)	…	…	…	1 (7)	
Fontan-related procedure	4 (13)	3 (43)	…	1 (20)	…	

Values are reported as median (interquartile range) or mean (SD), except as noted. Differences across quartiles were analyzed using linear regression for continuous variables and the χ^2 Mantel–Haenszel test for trend for categorical variables. Not all patients underwent cardiovascular magnetic resonance imaging and cardiopulmonary exercise testing; therefore, the exact number of included patients is indicated at the appropriate variable. ADC indicates apparent diffusion coefficient; ALT, alanine aminotransferase; AST, aspartate aminotransferase; E/A, ratio of peak early diastolic flow over peak late diastolic flow; E/e′, ratio of peak early diastolic flow over rate of acceleration of blood across the mitral valve in early diastole; e′, rate of acceleration of blood across the mitral valve in early diastole; Ea (cm/sec), peak early mitral annular tissue velocity recorded by Doppler tissue imaging; γGT, γ-glutamyltransferase; GDF-15, growth differentiation factor 15; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; and pVo_2, peak oxygen uptake.

*NT-proBNP normal values: 42.5–106.4 ng/L (men) and 111.0–215.9 ng/L (women)[20]; creatinine reference values: 53–106 μmol/L (men) and 44–97 μmol/L (women).[18]

Table 2. Baseline GDF-15 Univariate and Multivariate Outcome Analysis

	HR	95% CI	*P* Value
Baseline GDF-15 quartile 4 vs 1, 2,and 3 (n=81)			
Univariate	4.16	2.04–8.43	<0.001
Multivariate model			
GDF-15 quartile 4 vs 1,2,3	2.76	1.27–6.00	0.01
Age, y	0.96	0.89–1.04	0.31
History of arrhythmia	1.09	0.39–3.08	0.87
NYHA class			
I			
II	1.61	0.60–3.87	0.32
III	1.47	0.26–3.84	0.54
NT-proBNP ng/L (log-transformed)	3.06	1.23–7.61	0.02

For multivariate analysis, all variables (including GDF-15) were tested using univariate Cox regression; if they were significantly associated with the outcome ($P<0.05$), they were included in the multivariate model and removed in backward fashion (removal criterion $P>0.1$). See Table S1 for a complete overview of the Cox regression analysis. GDF-15 indicates growth differentiation factor 15; HR, hazard ratio; NYHA, New York Heart Association; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

the second measurement time point to event was 1.0 year (IQR: 0.7–1.9 years). Similar to baseline analysis, at follow-up, Fontan patients in the highest GDF-15 quartile remained at higher risk for the composite outcome than patients in the lower 3 quartiles (HR: 3.19; 95% CI, 1.23–8.28; $P=0.017$). Fontan patients with an increase in GDF-15 serum levels of >70 pg/mL (n=13; ie, the upper quartile of GDF-15 interval change between baseline and follow-up visit) had a higher risk of hospitalization or death at univariate analysis (HR: 2.69; 95% CI, 1.03–6.99; $P=0.043$; Figure 2, Table 3). This association was negated after adjustment for age and sex. Ten patients in the upper baseline GDF-15 quartile did not have an increase in GDF-15 over time. Of these, 5 (50%) had an end point occurrence. All end points were atrial arrhythmias. Of the 34 patients who did not experience the primary outcome, 5 (15%) had an increase of GDF-15 of >70 pg/mL, as opposed to 8 of the 17 (47%) who did experience the primary outcome.

DISCUSSION

This study is the first to evaluate serial measurements of the circulating biomarker GDF-15 and its prognostic value in children and adults with a Fontan circulation. GDF-15 serum levels were associated with functional status and measures of liver disease. Furthermore, GDF-15 serum levels independently predicted adverse outcomes. This study is the first showing that serial

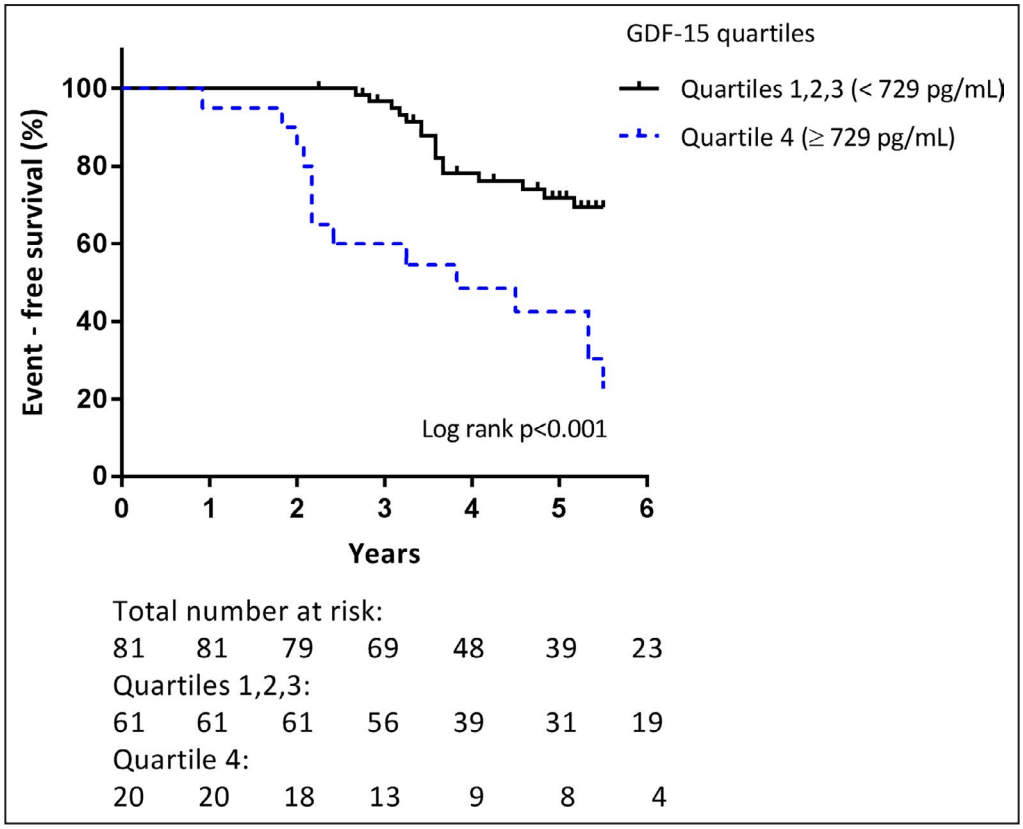

Figure 1. Event-free survival stratified by GDF-15 (growth differentiation factor 15) quartiles 1, 2, and 3 (n=61) vs quartile 4 (n=20).

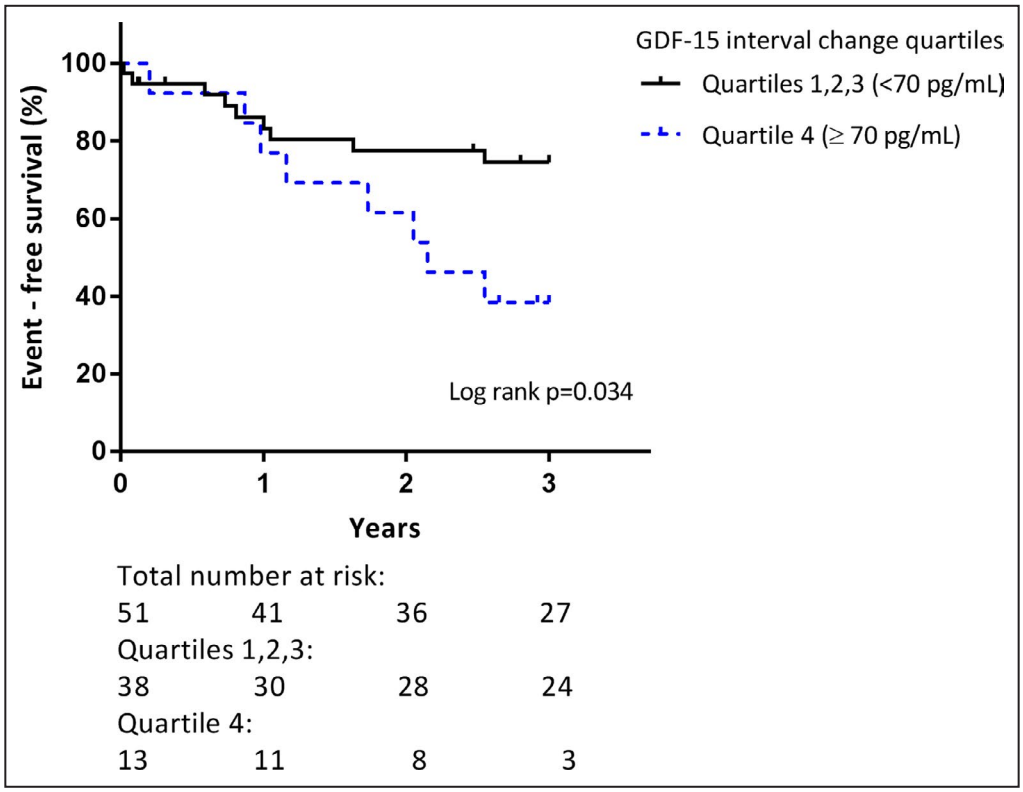

Figure 2. Event-free survival stratified by GDF-15 (growth differentiation factor 15) interval change quartiles 1, 2, and 3 (n=38) vs quartile 4 (n=13).

biomarker measurement is of additional value in predicting outcome in the Fontan population.

The attrition of the nonphysiologic Fontan circulation over time is well recognized.[5,21] However, the time course of developing Fontan failure and its heterogeneous presentation involving various organ systems is still unpredictable and poorly understood. Early identification of patients at increased risk of deterioration is key to prevent or slow progression and yet has proven to be difficult. To date, studies focusing on identifying circulating biomarkers for Fontan failure have been done cross-sectionally, accepting the limitations this approach inevitably brings.[6] In the current prospective study with serial measurements, median GDF-15 serum levels in Fontan patients were higher than GDF-15 serum levels reported in healthy controls of similar age.[16,28] Furthermore, GDF-15 serum levels in this study were slightly lower than median levels previously reported in patients with a variety of different congenital heart diseases.[15,24] Because GDF-15 serum levels increase with age independent of cardiac issues, the latter might be explained by the fact that median age in the current study was lower than in the referred studies.

We found that in Fontan patients, GDF-15 serum levels were associated with functional status. GDF-15 correlated positively with NYHA class, as has been reported previously in studies in patients with various congenital heart diseases. However, these studies failed to differentiate between various subtypes of congenital heart disease.[15,29] In the current study, lower peak oxygen uptake was associated with increased GDF-15 serum levels. This is in contrast to Eindhoven et al,[15] who, in a cohort of patients with various types of congenital heart diseases, were able to detect a significant association between workload and GDF-15 but not with peak oxygen uptake. This might have been due to the smaller sample size (n=40). The significant increase of CPET values over the course of 2 years is remarkable but can be explained by the young age of the current cohort, representing a natural increase in muscle mass and thus oxygen utilization potential up to the age of approximately 20 years, in line with previously published reference values.[30]

In this study, GDF-15 did not correlate with ventricular EF, as opposed to the findings of Raedle-Hurst et al,[17] who showed GDF-15 levels significantly higher in patients with an EF <50% compared with those with an EF >50%. The patient cohort in the current study consisted of relatively young Fontan patients with a homogenously well-preserved ventricular function, which might explain these seemingly contradictory findings. Nevertheless, patients in the upper GDF-15 quartile had a significantly worse overall functional status. This

Table 3. Interval Change Outcome Analysis (n=51)

	HR	95% CI	*P* Value
Univariate Cox regression			
GDF-15 interval change Q4 vs Q1,Q2, and Q3	2.69	1.03–6.99	0.04
NYHA class worsening, yes	1.90	0.45–8.55	0.37
Ejection fraction interval change (n=26) Q1 vs Q2, Q3, and Q4	2.95	0.79–11.04	0.11
Cardiac index interval change (n=26) Q1 vs Q2, Q3, and Q4	4.34	0.87–21.67	0.07
pVo_2 indexed interval change (n=36) Q1 vs Q2, Q3, and Q4	1.26	0.35–4.58	0.73
pVo_2 predicted interval change (n=36) Q1 vs Q2, Q3, and Q4	1.91	0.62–5.84	0.26
NT-proBNP interval change Q4 vs Q1, Q2, and Q3	3.48	1.34–9.05	0.01
γGT interval change Q4 vs Q1, Q2, and Q3	2.43	0.92–6.39	0.07
Multivariate Cox regression			
GDF-15 interval change Q4 vs Q1, Q2, and Q3 Adjusted for age and sex	2.47	0.91–6.69	0.08

γGT indicates γ-glutamyltransferase; GDF-15, growth differentiation factor 15; HR, hazard ratio; NYHA, New York Heart Association; NT-proBNP, N-terminal pro-B-type natriuretic peptide; pVo_2, peak oxygen uptake; and Q, quartile.

finding again highlights that Fontan failure and ventricular failure are not synonymous.[4]

NT-proBNP showed a significant positive correlation with GDF-15, which is in accordance with previous studies in both congenital and other heart diseases.[13,15,29] Furthermore, GDF-15 serum levels correlated positively with measures suggestive for Fontan-associated liver disease (γGT, alanine aminotransferase, alanine aminotransferase/aspartate aminotransferase ratio, apparent diffusion coefficient values), suggesting that GDF-15 may to some degree reflect the functional status of the liver in Fontan patients.[3,12,19,31] Whereas NT-proBNP and γGT are organ-specific biomarkers, GDF-15 is secreted by various organs, including both the heart and the liver, in response to stress.[8] Because the Fontan circulation has a detrimental effect on various organs, a biomarker reflecting this state of multiorgan failure, such as GDF-15, might prove invaluable.

This study is the first showing that high GDF-15 levels were predictive for death and Fontan-related adverse events, independent of age, sex, surgical history, functional parameters, imaging parameters, and other laboratory markers. The number of hospitalizations due to Fontan-related morbidities in this study was substantial, highlighting that even relatively young Fontan patients with apparently well-preserved functional status are at risk for adverse events. Event-free survival in Fontan patients has been described to be as low as 29% at 25 years,[32] and Fontan patients with an associated morbidity have been reported to have a 36-fold increase in the risk of subsequent Fontan takedown, heart transplantation, or death.[33] With this study, GDF-15 has been shown to be an important parameter that is able to predict the occurrence of these morbidities. GDF-15 serum levels might be a useful tool for timely detection of patients at risk for morbidities, allowing early adaptation of treatment strategies, including potential interventions, aiming at prevention or delaying decline into Fontan failure.

In the longitudinal analysis, patients in whom GDF-15 levels increased >70 pg/mL during the 2-year follow-up had an increased risk of adverse events. This risk could not be predicted by a change in traditional follow-up parameters in Fontan patients, such as pVo_2 and CMR parameters. When excluding patients with an atriopulmonary Fontan type, GDF-15 remained significantly associated with outcomes at univariate analysis. This observation indicates that the correlation is not solely driven by the atriopulmonary connection type of Fontan circulation, which may be not representative for contemporary Fontan patients who are predominantly treated with lateral tunnel or extracardiac type of Fontan circulation.

The patient cohort investigated in the current study, although larger than in previous studies, is still relatively small, limiting the power of extensive multivariable analyses, and includes the risk of overfit of the multivariate models, which consequently should be interpreted with care. Nevertheless, the prospective, standardized nature of this study makes it unique and ensures adequate and unbiased representation of Fontan patients in the current era. The number of hard end points (ie, all-cause mortality or heart or heart–lung transplants and listings) in this study was low, which might be explained by the young median age and the overall preserved functional status of the studied cohort. To optimize outcome analysis, we chose a combined end point of mortality, heart–lung transplant and listing, and Fontan-related hospitalization, in line with comparable studies.[24,34]

The findings of the current study should be confirmed in another independent and larger cohort of Fontan patients before GDF-15 serum levels can be used in clinical decision-making in Fontan patients.

In conclusion, in patients with a Fontan circulation, elevated serum levels of GDF-15 are associated with worse functional status and are predictive for adverse outcomes. Furthermore, an increase in GDF-15 over time is associated with increased risk of adverse outcomes; however, the additive value of serial measurements requires validation in a larger and independent cohort.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Material

Table S1

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SUPPLEMENTAL MATERIAL

Table S1. Univariate and multivariate Cox regression models, anatomy description, and GDF-15 distribution per quartile.

Univariate cox regression – baseline (n=81)			
	HR	95% CI	*P*-value
Baseline GDF-15 Q4 compared to Q1,2,3	4.16	2.04-8.43	<0.001
Age, years	1.06	1.02-1.10	0.001
Female sex	1.67	0.81-3.45	0.164
Left Ventricular morphology	0.74	0.31-1.65	0.501
Age at Fontan completion, years	1.07	0.98-1.17	0.126
Age at Fontan initiation, years	0.96	0.79-1.25	0.964
Type Fontan Atriopulmonary connection/Bjork Lateral tunnel (compared to APC) Extracardiac (compared to APC)	 2.43 0.40 0.98	 1.14-5.18 0.15-1.06 0.39-2.43	0.069 0.021 0.068 0.966
History of arrhythmia	2.79	1.37-5.67	0.005
NYHA class I II (compared to I) III (compared to I)	 0.35 2.33 5.00	 0.15-0.82 0.96-5.66 1.81-13.82	0.008 0.015 0.062 0.002
Ejection fraction % (n=56)	1.00	0.94-1.01	0.886
Cardiac index L/min/m^2 (n=56)	0.91	0.54-1.98	0.914
pVO_2 indexed (n=65)	0.93	0.88-0.99	0.014
pVO_2 predicted, % (n=65)	0.96	0.93-0.99	0.026
NT-proBNP ng/L (log-transformed)	4.86	2.07-11.37	<0.001
ɣGT, U/L	1.01	1.00-1.01	0.058
AST U/L	0.98	0.93-1.00	0.268
ALT U/L	1.00	0.96-1.05	0.858
AST/ALT ratio	0.48	0.15-1.56	0.224
ADC value (x10^{-3} mm^2/s) (n=52)	2.16	0.01-3.26	0.763
Ea (cm/s) (n=55)	0.99	0.97-1.0	0.526

e' (cm/s) (n=58)	0.97	0.86-1.10	0.663
E/A ratio (n=54)	0.41	0.15-1.14	0.087
E/e' ratio (n=48)	0.96	0.83-1.2	0.995
Deceleration time (ms) (n=51)	1.01	0.99-1.01	0.241
Cardiac diagnosis			
Tricuspid atresia	0.86	0.42-1.76	0.681
Double inlet left ventricle	1.23	0.53-2.86	0.631
Hypoplastic left heart syndrome	1.29	0.18-9.48	0.803
Atrioventricular septal defect	0.52	0.16-1.71	0.283
PA IVS	1.63	0.63-4.25	0.318
Heterogenous anomalies	1.30	0.31-5.43	0.723
Heterotaxy	1.17	0.45-3.04	0.753

Multivariate cox regression – baseline, including pVO_2 index and pVO_2 predicted (n=65) (backwards, removal >0.1)			
	HR	95% CI	*P*-value
Baseline GDF-15 Q4 compared to Q1,2,3	3.25	1.37-7.68	0.007
Age, years	1.00	0.94-1.07	0.956
History of arrhythmia	1.02	0.31-3.34	0.980
NYHA class I II III	 1.71 1.58	 0.55-5.39 0.30-8.35	 0.348 0.591
pVO_2 indexed	1.02	0.93-1.12	0.562
pVO_2 predicted, % (n=65)	0.99	0.96-1.03	0.329
NT-proBNP ng/L (log-transformed)	2.54	0.98-6.56	0.054

Multivariate cox regression - baseline, excluding pVO_2 index (n=81) (backwards, removal >0.1)			
	HR	95% CI	*P*-value
Baseline GDF-15 Q4 compared to Q1,2,3	2.76	1.27-6.00	0.011
Age, years	0.96	0.89-1.04	0.314
History of arrhythmia	1.09	0.39-3.08	0.868
NYHA class I II	 1.61	 0.60-3.87	 0.320

III	1.47	0.26-3.84	0.539
NT-proBNP ng/L (log-transformed)	3.06	1.23-7.61	0.016

Not all patients underwent cardiovascular magnetic resonance imaging and cardiopulmonary exercise testing. Therefore the exact number of included patients is indicated at the appropriate variable. ALT= alanine aminotransferase, AST= aspartate aminotransferase, ADC=apparent diffusion coefficient, GDF-15- Growth differentiation factor-15, γGT= gamma-glutamyltransferase, NYHA= New York Heart Association, NT-proBNP= N-terminal pro-B-type natriuretic peptide, pVO_2= peak oxygen uptake, for multivariate analysis, all variables (including GDF-15) were tested usings univariate cox regression, and only if significantly associated with the outcome ($P<0.05$) were included in the multivariate model and removed in backwards fashion (removal criterion $P>0.1$)

Cox regression - baseline, excluding atriopulmonary connection Fontan (n=65) (backwards, removal >0.1)			
	HR	95% CI	*P*-value
Univariate			
Baseline GDF-15 Q4 compared to Q1,2,3	3.15	1.22-8.12	0.018
Multivariate			
Baseline GDF-15 Q4 compared to Q1,2,3	2.33	0.69-7.86	0.173
Age, years	0.96	0.89-1.04	0.504
History of arrhythmia	3.73	1.41-9.84	0.008
NYHA class I II III	 1.53 1.00	 0.60-3.87 0.26-3.84	 0.373 0.990
NT-proBNP ng/L (log-transformed)	2.59	0.93-7.18	0.069

Cardiac anatomy descriptives

	All	GDF-15 quartiles				
		Quartile 1 (<453 pg/mL)	**Quartile 2** (453-552 pg/mL)	**Quartile 3** (553-729 pg/mL)	**Quartile 4** (≥ 729 pg/mL)	***P*-value**
N (%)	81 (100%)	20 (25)	21 (25)	20 (25)	20 (25)	
Diagnosis, n (%)						0.384
Tricuspid atresia	35 (43)	9 (45)	11 (52)	4 (20)	11 (55)	
Double inlet left ventricle	18 (22)	3 (15)	5 (24)	7 (35)	3 (15)	
Hypoplastic left heart syndrome	2 (3)	/	1 (5)	1 (5)	/	
Atrioventricular septal defect	12 (15)	4 (20)	1 (5)	5 (25)	2 (10)	
PA IVS	10 (12)	2 (10)	2 (10)	2 (10)	4 (20)	
Heterogenous anomalies	4 (5)	2 (10)	1 (5)	1 (5)	/	
Heterotaxy, n (%)	12 (15)	3 (15)	1 (5)	5 (25)	3 (15)	0.355

GDF-15 level distribution per quartile

	Quartile 1 N=20	Quartile 2 N=21	Quartile 3 N=20	Quartile 4 N=20
Median + IQR	408 (400-439)	514 (475-537)	618 (589-675)	1109 (841-1483)
Mean ± SD	418 ± 19	509 ± 33	626 ± 50	1435 ± 1160
Individual GDF-15 values	< 400,0 < 400,0 < 400,0 < 400,0 < 400,0 < 400,0 < 400,0 403,3 403,6 405,2 410,3 417,1 429,7 430,0 433,4 441,2 441,7 442,7 443,7 450,6	454,30 462,40 463,40 469,20 470,40 479,80 481,40 500,80 507,60 510,90 514,40 516,30 522,60 531,10 531,20 534,70 539,30 541,30 550,90 552,00 552,20	552,40 561,70 570,40 582,10 588,30 592,40 595,30 598,00 599,50 608,20 626,80 628,70 629,90 647,00 670,50 676,50 680,60 692,10 706,30 717,30	740,00 744,30 764,30 788,20 827,70 879,60 884,30 890,50 1027,00 1071,00 1146,00 1221,00 1234,00 1257,00 1276,00 1552,00 1602,00 1927,00 3069,00 5795,00