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Associations between blood biomarkers, cardiac function and adverse outcome in a young tetralogy of Fallot cohort

Eva van den Bosch [a,b,c], Wouter J. van Genuchten [a,c], Saskia E. Luijnenburg [a,b], Nienke Duppen [a,b], Vivian P. Kamphuis [c,d], Jolien W. Roos-Hesselink [e], Beatrijs Bartelds [a], Arno A.W. Roest [d], Johannes M.P.J. Breur [f], Nico A. Blom [d,g], Eric Boersma [e], Laurens P. Koopman [a], Willem A. Helbing [a,b,h,*]

[a] Erasmus University Medical Center, Department of Pediatrics, division of Pediatric Cardiology, Rotterdam, the Netherlands
[b] Erasmus University Medical Center, Department of Radiology, Rotterdam, the Netherlands
[c] Netherlands Heart Institute, Utrecht, the Netherlands
[d] Leiden University Medical Center, Department of Pediatrics, division of Pediatric Cardiology, Leiden, the Netherlands
[e] Erasmus University Medical Center, Department of Cardiology, Rotterdam, the Netherlands
[f] University Medical Center Utrecht, Department of Pediatric Cardiology, Utrecht, the Netherlands
[g] Academic Medical Center, Department of Pediatrics, division of Pediatric Cardiology, Amsterdam, the Netherlands
[h] Radboud University Medical Center, Department of Pediatrics, division of Pediatric Cardiology, Nijmegen, the Netherlands

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ABSTRACT

Background: To determine the potential prognostic value and clinical correlations of blood biomarkers in a cohort of patients with Tetralogy of Fallot (TOF).

Methods: In the setting of multicenter prospective research studies TOF patients underwent blood sampling, cardiopulmonary exercise testing and low-dose dobutamine stress cardiac magnetic resonance (CMR) imaging. In the blood sample NT-proBNP, GDF-15, Galectin-3, ST-2, DLK-1, FABP4, IGFBP-1, IGFBP-7, MMP-2, and vWF were assessed. During subsequent follow-up, patients were evaluated for reaching the study endpoint (cardiac death, arrhythmia-related hospitalization or cardioversion/ablation, VO_2 max ≤65% of predicted). Regression analysis was used to explore the correlation between blood biomarkers (corrected for age and gender) and other clinical parameters. The potential predictive value of blood biomarkers and events were assessed with Kaplan-Meier analysis and Cox proportional hazard analysis.

Results: We included 137 Fallot patients, median age 19.2 (interquartile range: 14.6–25.7) years, median age at TOF-repair 0.9 (0.5–1.9) years. After a median follow-up of 8.7 (6.3–10.7) years, 20 (14.6%) patients reached the composite endpoint. In a multivariable cox-regression analysis corrected for age at study baseline, elevated IGFBP-7 and MMP-2 levels were associated with the composite endpoint. We also noted a correlation between DLK-1 and relative change in right ventricular end systolic volume during dobutamine stress CMR ($\beta = -0.27$, $p = 0.010$), a correlation between FABP4 and Max VO_2 ($\beta = -0.41$, $p \leq 0.001$ and between MMP-2 and tricuspid valve E/A ratio ($\beta = -0.15$, $p = 0.037$).

Conclusions: IGFBP-7, MMP-2 and DLK-1 levels are related to cardiac function and long-term outcome in TOF patients.

1. Introduction

Tetralogy of Fallot (TOF) is the most common cyanotic congenital heart disease (ConHD) [1]. Nowadays survival after surgical repair is good with long-term survival-rates of 95% at 10-year and > 90% at 25 [1–3].

Despite good survival-rates, patients experience long-term problems, mainly related to residual pulmonary regurgitation (PR) [1]. Right ventricular dilatation, ventricular dysfunction and arrhythmias are common at long-term follow-up [2,4–7].

* Corresponding author at: Academic Centre for Congenital Heart Disease, Department of Pediatric Cardiology, Erasmus MC-Sophia Children's Hospital, PO Box 2060, 3000 CM Rotterdam, the Netherlands.
E-mail address: w.a.helbing@erasmusmc.nl (W.A. Helbing).

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Peak oxygen uptake (VO_2), QRS duration and cardiac magnetic resonance imaging (CMR) parameters have been associated with adverse outcome, often in older cohorts of TOF patients [8–11]. Nonetheless, additional parameters are needed to better identify patients at risk for adverse outcome. This search is hampered by long symptom-free intervals and therefore surrogate outcome markers are often used [12,13].

Blood biomarkers are a new potential tool in risk stratification in ConHD patients. In recent years a number of pathways and related blood biomarkers of myocardial fibrosis, remodelling and vascularization have been discovered, mostly in adult acquired heart disease patients [14–18]. However in young TOF patients relatively few biomarkers have been studied. Therefore, the aim of this study was to explore the value of several promising biomarkers in a relatively young TOF cohort.

2. Methods

2.1. Patients

We included all TOF patients with at least 5 year follow-up after TOF-repair from whom blood samples were stored in the setting of cross-sectional and prospective studies in five tertiary referral centers between 2002 and 2018 [19–21] Patients with contra-indications for CMR were excluded, as were patients with mental retardation.

The institutional review boards approved the studies. All participants, and if necessary their parents, gave written informed consent before inclusion in these studies. At the baseline study assessment all patients underwent blood sampling, CMR and cardiopulmonary exercise testing (CPET) according to a standard protocol. The patients were subsequently followed in the setting of usual care.

2.2. Blood sample analysis

Blood samples were taken from a peripheral vein and collected in EDTA tubes. Samples were stored at −80 °C. The frozen samples were shipped to Olink Proteomics AB (Uppsala, Sweden) for analysis with the Olink Cardiovascular panel III. Using proximity extension assay (PEA) technology the levels of biomarkers were measured, this PEA technique has been described extensively before [22]. All blood samples were coded, therefore the laboratory staff was blinded for the patients clinical and study data. The biomarker values are presented as normalized protein expression (NPX) units on a Log2 scale.

For the aim of the current study, we examined ten biomarkers that have been associated with ConHD, TOF, cardiac fibrosis or heart failure in general [16,23–28]. These biomarkers were: NT-proBNP, GDF-15, Galectin-3, ST2, DLK-1, FABP-4, IGFBP-1, IGFBP-7, MMP-2, and vWF. The biomarkers were selected prior to the data analysis.

2.3. CMR acquisition and analysis

All participants underwent CMR on the locally available scanners with dedicated phased-array cardiac surface coils. All images were obtained during breath-hold. CMR imaging was performed at rest and repeated during continuous infusion of low-dose (7.5 μg/kg/min) dobutamine hydrochloride (Centrafarm Services, Etten-Leur, The Netherlands). The dobutamine infusion was decreased (or stopped if necessary) when the heartrate increased >50%, if the systolic and/or diastolic blood pressure increased >50% or decreased >20%, if serious arrhythmias occurred, or if a patient did not tolerate the dobutamine effect. Technical details on our rest and dobutamine stress protocol have been published previously [19–21,29].

Analysis were performed with the software packages MASS and FLOW (Medis Medical Imaging Systems, Leiden, the Netherlands). Contours were manually drawn in end-diastole and end-systole, papillary muscle and trabeculae were excluded from the blood pool. All CMR's were analysed by one of the authors (EvdB) under supervision of one of the authors (WH) with longstanding experience in CMR. Biventricular end diastolic volume (EDV) and end systolic volume (ESV) were obtained and used to calculate the ejection fraction (EF). All ventricular volumes were indexed (i) for body surface area.

Relative changes in CMR parameters during stress were calculated as follows: relative $\text{parameter}_{\text{change}} = [(\text{parameter}_{\text{stress}} - \text{parameter}_{\text{rest}})/\text{parameter}_{\text{rest}}]*100$.

2.4. Echocardiography acquisition and analysis

All patients underwent a transthoracic echocardiographic examination following the recommendations of the AmericanSociety of Echocardiography in children. This was conducted either in clinical follow-up or study related [20,21]. All echocardiograms were made by an experienced ((pediatric) sonographer supervised by a) pediatric/congenital cardiologist. Mitral and tricuspid E and A were measured by a single observer (W.G.) Every measurement was taken during free breathing and performed and averaged over three cardiac cycles for improved reliability.

2.5. Cardiopulmonary exercise tests

Cardiopulmonary exercise tests (CPETs) were performed on a bicycle ergometer according to protocols that were used in previous studies by our group [19–21]. From these exercise tests the VO_2 peak was assessed and expressed as percentage of predicted values. Exercise tests with a peak respiratory exchange rate (RER peak) of ≥1.0 were included in the analysis.

2.6. Composite study endpoint

After the baseline study assessment, patients received regular patient specific care. For the purpose of the current study the medical records of the latest outpatients visit were reviewed and all cardiac events during follow-up were recorded until June 2019. The survival status of the patients was checked in the Municipal Population Register.

The study endpoint was defined as a composite of cardiac death, hospitalization for arrhythmias or cardioversion/ablation for arrhythmias or reaching a CPET VO_2 max below 65% (due to cardiac reasons) during follow-up after the study CPET [11,13,30].

2.7. Statistical methods

Continuous variables with a normal distribution were summarized as mean (SD). Differences between patients with and without events, between dominant ventricles and Fontan technique were analysed using the Student's *t*-tests. Variables with a non-normal distribution were presented as median (25-75th percentile), and between-group differences were analysed by Mann-Whitney *U* tests. Categorical variables were presented as numbers and percentages, whereas between-group differences were evaluated by chi-squared tests. In regression analysis we assessed correlations between several clinical parameters and the Olink biomarkers, adjusted for gender and age.

The endpoint-free survival were estimated by Kaplan–Meier curves and differences between patients groups, defined by the level of biomarkers, were evaluated using the log-rank test. We applied Cox proportional hazard regression analyses to explore the association between the biomarkers and the endpoint-free survival. In the Cox-model we used z-scores (the standardized form) of the Olink biomarkers. We analysed the crude Cox-model (univariable analysis of the standardized Olink biomarker) and a Cox-model corrected for age.

All analyses were performed using SPSS statistical software package version 24.0 (IBM Corp. in Armonk, NY, USA), a two-sided p-value <0.05 was considered statistically significant.

3. Results

The blood samples of 138 patients were shipped for analysis. The available biomarker was successfully analysed in 137 patients, in one patient the blood sample was of insufficient quality. This patients was excluded from the study. At baseline a CMR was performed in all 137 patients and an adequate CPET was performed in 108 patients.

The median age at the baseline study assessment was 19.2 years (14.6–25.7), median 17.3 years (13.9–23.8) after the TOF-repair. Table 1 shows the patient characteristics and CMR and CPET parameters for all patients and the patients who did and did not reach the composite endpoint.

3.1. Composite endpoint and baseline characteristics

During a median follow-up of 8.7 (6.3–10.7) years, 20 (14.6%) reached the composite endpoint median 6.0 (1.8–8.1) years after the baseline study measurement (Table 2). All patients were alive at latest follow-up. A total of 13 (9.5%) patients developed an arrhythmia and 7 (5.1%) patients reached a VO_2 max ≤65% of predicted. Patients who reached the composite endpoint were significantly older at the baseline study measurement, 29.6 vs 19.2 years, p = 0.040. Patients who reached the composite endpoint had a higher left ventricular (LV) mass, a higher LVSV, a higher RVEF and a higher tricuspid valve A wave, see Table 1.

3.2. Composite endpoint and biomarkers

Table 3 shows the Cox-regression analysis. An 1-SD increase of IGFBP-7 (Hazard ratio (HR): 1.71/ 1-SD, 95% CI: 1.11–2.63) and NT-proBNP (HR: 1.82/ 1-SD, 95% CI: 1.15–2.90) were associated with the composite endpoint during follow-up. However, corrected for age at study baseline, NT-proBNP lost its predictive value for the composite endpoint. In the multivariable analysis higher levels of IGFBP-7 and MMP-2 were associated with the composite endpoint during follow-up.

3.3. Association biomarkers with other clinical parameters

In Table 4 associations, corrected for age and gender, between the biomarkers and several baseline CMR and CPET parameters are shown. We observed an significant association between DLK-1 levels and the relative decrease in RVESV during dobutamine stress, DLK-1 decreased with factor β = −0.27 for every percent less decrease in RVESV during stress. Furthermore an increase in FABP4 was correlated with a decrease in max VO_2 with a β = −0.41. Lastly a significant inverse relation was observed between MMP-2 and TV E/A ratio.

4. Discussion

In this study in relatively young TOF patients we demonstrated an association between several blood biomarkers (NT-proBNP, MMP-2, FABP-4, IGFBP7 and DLK-1) and clinical condition, cardiac function and events during 8 years of follow-up.

Patients with TOF have an increased risk of arrhythmias, impaired exercise capacity, diminished ventricular function and increased mortality during follow-up [1]. Several predictors for these adverse events are known, such as peak VO_2, QRS duration and CMR parameters like RVEF, LVEF, RV mass volume ratio, RV strain and RA area [1,8–11,31,32]. However, many of these studies have been performed in older TOF patients operated at an older age than has been common practice more recently [4,9,10,33,34]. Risk-stratification is necessary to identify young TOF patients at risk for deterioration. Assessment of blood biomarker levels is a relatively simple method that may be used to monitor clinical condition.

There may be differences in pathways involved in heart failure in children with ConHD compared to adults, including adults with ConHD [17,18,35]. As such, our study explored pathways involved in the

Table 1
Baseline patient characteristics for patients with and without a composite endpoint.

	Patients (n = 137)	Composite endpoint (n = 20)	No composite endpoint (n = 117)	P-value
Male (n, %)	92 (67.2)	12 (60.0)	80 (68.4)	0.45
22q11 (n, %)	5 (3.6)	1 (5.0)	4 (3.4)	0.55
Age at study (years)	19.2 (14.6–25.7)	29.6 (14.6–52.3)	19.0 (14.6–24.2)	**0.040**
Time after TOF repair (years)	17.3 (13.9–23.8)	25.9 (14.0–41.4)	17.1 (13.7–22.4)	**0.035**
Length (cm)	168 ± 14	167 ± 14	169 ± 14	0.59
Weight (kg)	62.7 ± 17.4	62.7 ± 18.9	62.7 ± 17.3	1.00
BSA (m^2)	1.70 ± 0.30	1.69 ± 0.32	1.70 ± 0.29	0.88
QRS duration (ms)	133 ± 24	142 ± 27	132 ± 24	0.087
Palliative shunt (n, %)	31 (22.6)	8 (40.0)	23 (19.7)	0.078
Age at TOF-repair (years)	0.9 (0.5–1.9	1.9 (0.4–10.6)	0.9 (0.5–1.7)	0.097
Transannular patch (n, %)	92 (67.2)	12 (60.0)	80 (68.4)	0.45
PVR at baseline (n, %)	18 (13.1)	1 (5.0)	17 (14.5)	0.47
Maximal exercise parameters	n = 108	n = 15	n = 93	
VO_2 max (ml/min/kg)	36.5 ± 8.1	35.1 ± 7.8	36.7 ± 8.2	0.45
VO_2 max (% of predicted)	86.4 ± 18.5	92.5 ± 21.0	85.4 ± 18.0	0.17
Echo Parameters	n = 83	n = 9	n = 74	
Mitral valve E' cm/s	98.7 (77.5–110.9)	103 (78.5–110.4)	98.65 (77.4–111.0)	0.63
Mitral Valve A' cm/s	45.9 (40.6–56.7)	43 (34.8–44.9)	46.8 (40.6–57.6)	0.11
Mitral Valve E/A ratio	2.1 (1.5–2.5)	2.7 (1.9–2.9)	1.9 (1.5–2.5)	0.09
Tricuspid Valve E' cm/s	66.8 (54.6–76.7)	75.7 (66.4–92.3)	66.0 (53.8–73.9)	0.10
Tricuspid Valve A' cm/s	40.0 (32.3–49.0)	52.3 (50.25–61.0)	39.5 (31.1–44.5)	**0.002**
Tricusp[id valve E/A ratio	1.6 (1.35–2.03)	1.5 (1.3–1.5)	1.6 (1.4–2.1)	0.16
Rest CMR	n = 137	n = 20	n = 117	
LV EDV (ml/m^2)	84 ± 13	88 ± 14	83 ± 12	0.11
ESV (ml/m^2)	35 ± 8	34 ± 9	35 ± 8	0.82
SV (ml/m^2)	50 ± 8	54 ± 8	49 ± 8	**0.005**
EF (%)	59 ± 6	62 ± 7	59 ± 6	0.063
Mass (g/m^2)	53 ± 11	58 ± 13	53 ± 10	**0.031**
Mass/ EDV ratio (g/ml)	0.63 ± 0.13	0.66 ± 0.11	0.64 ± 0.12	0.47
RV EDV (ml/m^2)	129 ± 35	124 ± 38	129 ± 34	0.53
ESV (ml/m^2)	64 ± 23	57 ± 24	66 ± 22	0.11
SV (ml/m^2)	65 ± 16	67 ± 18	64 ± 16	0.44
EF (%)	51 ± 7	55 ± 7	50 ± 7	**0.002**
Mass (g/m2)	26 ± 10	27 ± 10	26 ± 10	0.84
Mass/ EDV ratio (g/ml)	0.21 ± 0.10	0.22 ± 0.07	0.21 ± 0.10	0.63
PR (%)	28 (10–41) (n = 128)	21 (1–29) (n = 20)	31 (13–43) (n = 108)	**0.027**
Relative change during stress	n = 71	n = 9	n = 62	
LV EDV (%)	-3 ± 9*	-3 ± 10	-3 ± 9*	0.93
ESV (%)	−34 ± 16*	−33 ± 14*	−34 ± 15*	0.80
SV (%)	19 ± 15*	18 ± 16*	19 ± 15*	0.81
EF (%)	22 ± 11*	21 ± 12*	22 ± 11*	0.83
RV EDV (%)	−5 ± 10*	−7 ± 12	−7 ± 12*	0.34
ESV (%)	−29 ± 16*	−20 ± 18*	−29 ± 16*	0.80
SV (%)	19 ± 18*	12 ± 13*	20 ± 18*	0.22
EF (%)	26 ± 15*	23 ± 14*	26 ± 15*	0.60

Results are given as mean (standard deviation), as median (range) or as counts (percentages) *indicates a significant difference between the rest and stress measurement.
Abbreviations; BSA: body surface area, CMR: cardiovascular magnetic resonance

imaging, EDV: end diastolic volume, EF: ejection fraction, ESV: end systolic volume, LV: left ventricle, RV: right ventricle, SV: stroke volume, VO_2 max: maximum oxygen uptake.

Table 2
Clinical state at latest follow-up.

	Patients (n = 137)
Median age at latest follow-up (years)	26.8 (22.7–37.1)
Median time after study (years)	8.7 (6.3–10.7)
Composite endpoint (n, %)	20 (14.6)
Median time after baseline study (years)	6.0 (1.8–8.1)
Median time after TOF-repair (years)	32.2 (19.9–45.3)
Arrhythmias (n, %)	13 (9.5)
Atrial arrhythmia (n, %)	6 (4.4)
Ventricular arrhythmia (n, %)	5 (3.6)
AV block (n, %)	2 (1.5)
VO_2 max ≤65% of predicted (n, %)	7 (5.1)

Abbreviations; TOF: Tetralogy of Fallot, VO_2 max: maximum oxygen uptake.

Table 3
Cox-regression analyses for biomarkers and their relation to the composite endpoint.

	Crude univariable model			Model adjusted for age at study		
	HR	95% CI	P-value	HR	95% CI	P-value
Levels (per 1 SD increase)						
DLK-1	1.05	0.68–1.61	0.83	1.11	0.73–1.69	0.63
FABP4	1.20	0.80–1.81	0.37	0.84	0.53–1.32	0.44
Gal-3	1.60	0.93–2.75	0.092	1.10	0.64–1.88	0.73
GDF-15	1.12	0.80–1.57	0.52	0.70	0.34–1.43	0.32
IFGBP-1	1.25	0.80–1.96	0.33	1.43	0.95–2.17	0.088
IFGBP-7	**1.71**	**1.11–2.63**	**0.014**	**1.54**	**1.03–2.31**	**0.035**
MMP-2	1.54	0.97–2.46	0.066	**1.68**	**1.03–2.74**	**0.039**
NT-proBNP	**1.82**	**1.15–2.90**	**0.011**	1.42	0.88–2.28	0.15
ST2	0.97	0.63–1.49	0.89	0.95	0.47–1.91	0.88
vWF	1.25	0.86–1.82	0.24	1.10	0.71–1.72	0.67

Abbreviations; CI: confidence interval, DLK-1: protein delta homolog 1, FABP4: Fatty acid-binding protein 4, Gal 3: galectin 3, GDF-15: growth differentiation factor 15, HR: hazard ratio, IGFBP1: insulin-like growth factor-binding-protein 1, IGFBP-7: insulin-like growth factor-binding protein 7, MMP-2: matrix metalloproteinase-2, NT-proBNP: N-terminal pro-brain natriuretic peptide, ST2: suppression of tumorigenicity 2, vWF: von Willebrand factor.

maintenance of cardiac function and long-term outcome of relatively young TOF patients. The processes leading to myocardial changes to adapt to chronic abnormal loading conditions in ConHD have been incompletely understood [35,36]. Pathways most likely involved in the development of heart failure in ConHD are related to hypertrophy, fibrosis, remodelling, vascularization, inflammation, cardiac metabolism and repair [35]. In this study we examined ten biomarkers that have been associated with ConHD, TOF, cardiac fibrosis or heart failure in general [16,23–28]. We will subsequently discuss the biological role of the 5 biomarkers that showed a statistically significant relation / associations with clinical outcomes in our study.

4.1. NT-proBNP

In response to increased myocardial stress and ventricular volume and pressure overload, NT-proBNP is secreted [37]. It is a well-known biomarker in acquired heart failure and adult ConHD patients; elevated NT-proBNP levels are associated with mortality and adverse events [17,37–39].

In TOF patients, Westhoff-Bleck et al. [9] observed that higher NT-proBNP levels were associated with the severity of PR and adverse outcomes. In our study, NT-proBNP levels were associated with the composite endpoint in univariable analysis. However, when corrected for age at baseline NT-proBNP lost its predictive value. A possible explanation for the different relation between NT-pro BNP levels and outcome is the relative young age at baseline (19.2 years vs 26.3 years) and at TOF-repair in our cohort (0.9 vs 2.5 years) compared to the cohort of Westhoff-Bleck et al. [9]

4.2. IGFBP-7

IGF binding proteins (IGFBPs) are a family of proteins that regulate and modulate IGF activity and have indirect effects on growth hormone [40]. IGFBP-7 is highly expressed in endothelial cells and has been linked to collagen deposition [41–43]. Interestingly, IGFBP-7 has been linked to post infarction myocardial repair [44]. IGFBP-7 has been identified as a potential biomarker for adverse outcome in acquired heart failure patients [26], and is associated with diastolic dysfunction and lower VO_2 max [42]. In ConHD patients, the role of IGFPBs in cardiac function or prognosis is largely unexplored, but has been linked to general growth, failure to thrive, nutritional status and subclinical kidney injury [45–47].

In young Fontan patients we also observed an association between IGFPB-7 levels (corrected for age and gender) and cardiac function and VO_2 max [48]. In our current study we found that higher IGFBP-7 levels were associated with higher RV mass volume ratio and with adverse outcomes. These observations indicate a possible role of IGFBP-7 in the follow-up of TOF patients and perhaps other types of ConHD.

4.3. MMP-2

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases and are controlled through tissue inhibitors of metalloproteinases (TIMPs) [49]. MMPs can degrade components of the extra cellular matrix (ECM) including collagens and play and important role in ECM remodelling [50–52]. In rat, canine and ovine models of acute pulmonary embolism pre-treatment with doxycycline -a non-selective MMP inhibitor- reduced RV dilation [52].

In patients with congestive heart failure, higher MMP-2 levels have been associated with higher NYHA class, older age and with hospitalization for heart failure during subsequent follow-up [51]. In adult patients with hypertrophic cardiomyopathy higher levels of MMP-2 have been associated with LV systolic dysfunction [53]. In a large ConHD cohort Baggen et al. [24] have observed an association between MMP-2 levels and exercise capacity.

In TOF patients MMP-9 is thought to have a modulating effect on aortic stiffness and aortic root dilatation [54]. We observed that elevated MMP-2 levels correlated with changes in right ventricular diastolic function and corrected for age at study, were associated with an increased risk for the composite endpoint.

4.4. DLK-1

DLK-1 is part of the epidermal growth factor-like family and plays a role in muscular differentiation, angiogenesis, and fibrosis [16,55]. DLK-1 knock-out mice display an increased collagen deposition, LV dilatation and reduced myocardial contractility [16]. In human ischemic myocardial tissue DLK-1 mRNA expression was down-regulated compared to healthy tissue [16].

Recently we observed that young Fontan patients with higher DLK-1 levels have a better event-free survival [48] and a higher functional reserve (EF_{stress}– EF_{rest}) during dobutamine stress CMR, which is a predictor for outcome [13]. In the current study in TOF patients we also observed an association between DLK-1 levels and the response to dobutamine stress CMR. Higher DLK-1 levels were associated with a larger relative decrease in RVESV during stress. Recently an impaired relative decrease in RVESV during stress has been linked to adverse outcome [13].

Table 4
Association between study parameters and biomarker levels, corrected for age and gender.

Dependent variable		Max VO_2 max (per 1 ml/min/kg)	RVEF (per 1%)	Relative decrease in RVESV during stress (per %)	TV E/A ratio
DLK-1	β			−0.269	
	95% CI			−0.022 to −0.003	
	p-value	NS	NS	0.010	NS
FABP4	β	−0.413			
	95% CI	−0.052 to −0.020			
	p-value	<0.001	NS	NS	NS
Gal-3	β				
	95% CI				
	p-value	NS	NS	NS	NS
GDF-15	β				
	95% CI				
	p-value	NS	NS	NS	NS
IGFBP-1	β				
	95% CI				
	p-value	NS	NS	NS	NS
IGFBP-7	β				
	95% CI				
	p-value	NS	NS	NS	NS
MMP-2	β				−0.149
	95% CI				−0.290 to −0.009
	p-value	NS	NS	NS	0.037
NT-proBNP	β				
	95% CI				
	p-value	NS	NS	NS	NS
ST2	β				
	95% CI				
	p-value	NS	NS	NS	NS
vWF	β				
	95% CI				
	p-value	NS	NS	NS	NS

Interpretation: for every increase %, the biomarker increases or decreases with factor β.
Abbreviations; CMR: cardiovascular magnetic resonance imaging, EDV: indexed end diastolic volume, EF: ejection fraction, DLK-1: protein delta homolog 1, FABP4: Fatty acid-binding protein 4, Gal 3: galectin 3, GDF-15: growth differentiation factor 15, IGFBP1: insulin-like growth factor-binding protein 1, IGFBP-7: insulin-like growth factor-binding protein 7, MMP-2: matrix metalloproteinase-2, NS; not significant, NT-proBNP: N-terminal pro-brain natriuretic peptide, ST2: suppression of tumorigenicity 2, VO_2 peak: maximum oxygen uptake, vWF: von Willebrand factor.

4.5. FABP-4

FABP-4 is highly expressed in adipocytes and elevated levels of FABP-4 are associated with adiposity, female gender, diabetes and hypertension [26,56,57]. FABP-4 displays some expression in macrophages and it is thought that FABP-4 increases foam cell formation and induces an inflammatory response [56,57].

FABP-4 levels have been associated with LV hypertrophy and systolic and diastolic dysfunction [56]. In patients with chronic heart failure, higher FABP4-levels were independently associated with adverse events during follow-up [26].

Patients with TOF have increased levels of FABP-4 RNA expression in RV tissue compared to patients with a VSD [58]. Recently we observed in a young Fontan cohort a negative association between FABP-4 levels and age at Fontan and peak VO_2 [48]. Remarkably, we also observed a negative association between FABP-4 levels and peak VO_2 in the current TOF cohort. Therefore more research on the role of FABP4 levels in ConHD is required.

4.6. Composite endpoint

In this study, as in another recent study from our group, we used a composite endpoint of cardiac death, arrhythmias and a peak $VO_2 \leq$ 65% of predicted [13]. Exercise capacity is an established prognostic marker in TOF patients [11,30,32]. Clinical guidelines recommend CPET during routine follow-up of TOF patients [12,59]. Diller et al. [30] and Giardini et al. [60] observed an association between a lower peak VO_2 and an increased risk of hospitalization and death during follow-up. Likewise in a recent study in a young (25.5 years) TOF cohort, a peak $VO_2 \leq$ 65% of predicted was associated with an increased risk for death, sustained ventricular tachycardia and cardiac related hospitalizations [11]. Based on the relations between exercise performance, particularly VO_2 max and subsequent outcome, peak $VO_2 \leq$ 65% was included in the composite endpoint used in our study.

4.7. Relation between clinical factors and endpoint

Patients that reached the endpoint were older, had their TOF-repair at an older age and had at baseline study assessment less PR, higher LVSV and mass, higher RVEF and more signs of RV diastolic impairment (higher TV A wave). This clinical profile has been associated with altered ventricular diastolic function in TOF [1]. In patients with or without an event a significant difference for MMP-2 and IGFBP-7 was noted [50,51,52]. These factors are known to increase fibrosis, which increases the risk for arrhythmias and decreased peak VO2 in TOF [61]. Ventricular fibrosis is a well-known problem in patients after repair of TOF [1]. When fibrosis occurs and how it changes over time during follow-up is less well studied but may have high clinical relevance [62]. On the basis of our current data we speculate that differences in cardiac fibrosis could be reflected in MMP-2 and IGFBP-7 expression and influence adverse events via this mechanism. A combined fibrosis imaging and biomarker approach might be important to elucidate this aspect of improved risk profiling of TOF patients.

4.8. Limitations

In our relatively young cohort of TOF patients, the number of hard endpoints was limited. This restriction is a known limitation in research in ConHD patients [12,13]. We therefore used a composite endpoint of cardiac death, arrhythmias and a diminished exercise tolerance to assess the possible association between biomarkers and cardiac outcome. However, due to the limited number of endpoints it is possible that we

have missed associations between biomarkers and endpoints in this study. Detecting biomarker cut-off values for clinical use was not part of the current study and further research is necessary to determine the potential role of the observed biomarkers in clinical practice.

Although the median age of TOF patients in our cohort was quite young, some older patients were part of the analysis. This could have influenced our results.

Late gadolinium enhancement or T1 mapping, useful in detecting local or generalized fibrosis in the myocardium, was not performed in our imaging protocol due to time constraints [63]. Therefore we could not investigate associations between myocardial fibrosis with and potential fibrosis blood biomarkers.

5. Conclusion

In this study we performed an exploratory analysis of blood biomarkers and their relation to cardiac function and subsequent outcome in a relatively young and contemporary TOF population. We observed that in addition to NT-proBNP, biomarkers such as IGFPB-7, DLK-1, MMP-2 and FABP-4 relate to cardiac function and long-term outcome. These biomarkers may have a role in the clinical follow-up and risk stratification of TOF patients.

Disclosures

The authors have nothing to disclose.

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CRediT authorship contribution statement

Eva van den Bosch: Investigation, Formal analysis, Writing – original draft. **Wouter J. van Genuchten:** Investigation, Writing – review & editing. **Saskia E. Luijnenburg:** Investigation, Writing – review & editing. **Nienke Duppen:** Investigation, Writing – review & editing. **Vivian P. Kamphuis:** Investigation, Writing – review & editing. **Jolien W. Roos-Hesselink:** Investigation, Writing – review & editing. **Beatrijs Bartelds:** Investigation, Writing – review & editing. **Arno A.W. Roest:** Investigation, Writing – review & editing. **Johannes M.P.J. Breur:** Investigation, Writing – review & editing. **Nico A. Blom:** Investigation, Writing – review & editing. **Eric Boersma:** Formal analysis, Writing – review & editing. **Laurens P. Koopman:** Supervision, Investigation, Writing – review & editing. **Willem A. Helbing:** Supervision, Investigation, Writing – original draft.

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