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ABSTRACT

Objective Cardiac surgery may cause temporarily impaired ventricular performance and myocardial injury. We aim to characterise the response to perioperative injury for patients undergoing repair or pulmonary valve replacement (PVR) for tetralogy of Fallot (ToF).

Methods We enrolled children undergoing ToF repair or PVR from four tertiary centres in a prospective observational study. Assessment—including blood sampling and speckle tracking echocardiography—occurred before surgery (T1), at the first follow-up (T2) and 1 year after the procedures (T3). Ninety-two serum biomarkers were expressed as principal components to reduce multiple statistical testing. RNA Sequencing was performed on right ventricular (RV) outflow tract samples.

Results We included 45 patients with ToF repair aged 4.3 (3.4 – 6.5) months and 16 patients with PVR aged 10.4 (7.8 – 12.7) years. Ventricular function following ToF repair showed a fall-and-rise pattern for left ventricular global longitudinal strain (GLS) (−18±4 to −13±4 to −20±2, $p < 0.001$ for each comparison) and RV GLS (−19±5 to −14±4 to 20±4, $p < 0.002$ for each comparison). This pattern was not seen for patients undergoing PVR. Serum biomarkers were expressed as three principal components. These phenotypes are related to: (1) surgery type, (2) uncorrected ToF and (3) early postoperative status. Principal component 3 scores were increased at T2. This increase was higher for ToF repair than PVR. The transcriptomes of RV outflow tract tissue are related to patients' sex, rather than ToF-related phenotypes in a subset of the study population.

Conclusions The response to perioperative injury following ToF repair and PVR is characterised by specific functional and immunological responses. However, we did not identify factors relating to (dis)advantageous recovery from perioperative injury.

Trial registration number Netherlands Trial Register: NL5129.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Cardiac surgery with cardiopulmonary bypass can lead to (temporarily) impaired ventricular performance and myocardial injury, which may affect long-term outcomes. The mechanisms have been studied scarcely.

WHAT THIS STUDY ADDS

⇒ We characterised the functional and immunological biomarker response to perioperative injury for patients undergoing surgical repair and pulmonary valve replacement for tetralogy of Fallot. We identified a biomarker phenotype related to the response to perioperative injury.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Improved characterisation of perioperative injury and subsequent recovery may provide biomarkers to identify patients at risk for adverse events. Furthermore, it may provide novel targets for therapy, such as inhibitors of disadvantageous immune responses following cardiopulmonary bypass or perioperative myocardial protective strategies.

INTRODUCTION

Tetralogy of Fallot (ToF) is the most common type of cyanotic congenital heart disease with an incidence of 0.34 per 1000 life-born children.[1] Surgical repair can be achieved with excellent long-term survival.[1] However, lifetime morbidity of these patients remains high. Surgical repair of ToF is currently generally performed between 3 and 11 months of age.[1] Relatively earlier repair is considered to minimise the time the right ventricle (RV) is exposed to increased pressure load and cyanosis. However, earlier repair more often requires a transannular patch, which may result in worse long-term outcomes.[1] Furthermore, the neonatal repair is associated with a more complicated postoperative course.[2] Palliative procedures, such as a modified Blalock-(Thomas-)Taussig shunt (mBT), prior to repair may limit cyanosis and allow for pulmonary vascular growth.[1] However, there may be associated risks with repeat interventions.[1] There is currently no

consensus on the optimal treatment strategy or timing of repair for ToF, but treatment strategy in early life affects lifelong outcomes of ToF repair.[1 2]

Surgical procedures for ToF expose the heart to injury. Cardiopulmonary bypass (CPB) is required to gain intracardiac access. CPB exposes the heart to, among others, ischaemia and reperfusion injury.[3] Other aspects of perioperative conditioning relating to injury include oxidative stress, surgical trauma and inflammation.[3 4] Following surgery, an extensive immune response is observed and ventricular function may be impaired for several months.[5] RV function is more severely impaired compared with left ventricular (LV) function, which may relate to (1) the abnormally loaded RV in ToF, (2) the RV's impaired metabolic and antioxidant response to hypoxia,[5 6] (3) the anterior position of the RV—which may expose the RV to room temperature, limiting the protective effects of cooling[7 8] and (4) the coronary blood supply of the RV—which is more sensitive to increased afterload than that of the LV.[9] The recovery of ventricular function and the role of the immune system in the recovery from perioperative injury are poorly understood but may have important implications for long-term biventricular function.

We performed a multicentre prospective study to characterise the functional and immunological response to perioperative injury for patients undergoing surgical repair or pulmonary valve replacement (PVR) for ToF. Furthermore, we characterised the transcriptome—that is, the complete set of coding and non-coding RNA transcripts—of the right ventricular outflow tract (RVOT) from tissue samples obtained during ToF repair. Transcriptome analysis provides detailed phenotype information, which may relate to differences in patient characteristics or differences in the response to perioperative injury.

We hypothesise that functional and immunological biomarkers may identify specific phenotypes of (dis) advantageous recovery from—and vulnerability to—injury and that these patterns may differ between patients undergoing ToF repair and PVR.

METHODS

Study design and subjects

We performed a multicentre prospective observational study. The study protocol was published in the Netherlands Trial Register (NL5129). From December 2015 to September 2019, patients undergoing ToF repair and surgical PVR were recruited from the Erasmus MC Sophia Children's Hospital, Rotterdam; Willem Alexander Children's Hospital, Leiden; Wilhelmina Children's Hospital, Utrecht and Beatrix Children's Hospital, Groningen. Exclusion criteria were multiple congenital anomaly syndromes or pulmonary atresia. The study protocol was approved by the research ethics committees of the participating centres (protocol no MEC-2014-326/NL48188.078.14). All patients, and/or their legal guardians, provided written informed consent prior to inclusion in accordance with Dutch legislation. The public was not involved in the study design or conduct. Subjects were assessed at three time points: before surgery (T1), at the first outpatient follow-up or during the second postoperative week (T2) and at 1-year follow-up (T3). At each of the study time points, subjects underwent physical examination, echocardiography and blood sampling. Tissue samples of the RVOT were obtained during ToF repair.

Echocardiography

Transthoracic echocardiography was performed in accordance with the study echocardiography protocol in all participating centres. Studies were performed by experienced cardiac sonographers on a Vivid7 or Vivid E9 cardiac ultrasound system (General Electric Vingmed Ultrasound, Horten, Norway). No subjects were sedated for the echocardiography study. Postprocessing of images was performed using commercially available software (EchoPac V.11.2; General Electric Vingmed Ultrasound). To limit interobserver errors, all postprocessing was performed in two core centres (Erasmus Medical Center—Sophia Children's Hospital and Willem Alexander Children's Hospital—Leiden University Medical Center). All M-mode and pulsed wave tissue Doppler measurements were calculated as an average value from three consecutive heartbeats. Speckle tracking myocardial strain was performed (Echopac V.11.2; General Electric Vingmed Ultrasound). The end-diastolic phase was identified automatically by the software. Global longitudinal peak systolic strain of the LV was obtained from available segments from the apical two-chamber, three-chamber and four-chamber views. RV global longitudinal peak systolic strain was obtained from the free wall on the apical four-chamber view. Biventricular global longitudinal strain (GLS) was considered the primary measure of ventricular function, as this parameter relies on a few geometric assumptions, which may not be applicable in ToF. Ventricular dimensions were indexed according to published paediatric references.[10 11]

Blood sample analysis

At each study time point, blood samples were collected in EDTA tubes, centrifuged and plasma was stored at −80°C. Samples were analysed using a protein biomarker panel of 92 cardiovascular and immunological biomarkers (Olink Cardiovascular panel III; Olink Bioscience, Uppsala, Sweden).[12] Biomarker concentrations were assessed using a proximal extension array, which has previously been described in detail.[13] This panel was chosen as it contains many biomarkers of interest, as previously determined by a literature study.[14] Concentrations are expressed as normalised protein expression (NPX), a measure of relative concentration, rather than absolute concentrations. NPX is a logarithmic scale, where a 1 unit increase represents a doubling in concentration. If the limit of detection was not reached for a sample, the

reported NPX (under the limit of detection) was used, in consultation with Olink. Biomarkers for which the limit of detection was not reached in more than half of the subjects were excluded entirely from data analysis. N-terminal pro-brain natriuretic peptide (NT-proBNP) obtained by biomarker panel analysis was compared with NT-proBNP assessed by the participating centres' clinical laboratories.

Myocardial tissue analysis

During ToF repair, RVOT tissue was resected as part of the normal surgical treatment. Samples of the tissue were directly frozen in liquid nitrogen in the operating room and were subsequently stored at −80°C. Due to time and resource restraints, RVOT tissue samples from 20 patients had to be selected for RNA sequencing (RNA-Seq), rather than all collected tissue samples. Patients were selected based on the completeness of follow-up data and clinical characteristics (to cover a broad range of phenotypes). RNA was isolated using the ReliaPrep Kit (Z6112, Promega). RNA quality was determined using the Agilent 2100 Bioanalyzer (G2939BA, Agilent Technologies). The RNA-Seq library was prepared using the KAPA mRNA HyperPrep kit (KK8581, Roche) with an input of 500 ng RNA with an RNA integrity score >8. The library was sequenced on two HiSeq 4000 lanes single-end 50 bp reads (Illumina).

Statistical analysis

Continuous data are presented as 'mean±SD' for normal distributions and 'median (IQR)' for non-normal distributions. Categorical data are presented as 'count (percentage)'. Paired t-tests and Wilcoxon tests, depending on the distribution, were used for comparisons between study time points. Data analysis is performed in R (R Foundation for Statistical Computing, Vienna, Austria).

Serum biomarkers were analysed with principal component analysis (PCA) in R base (*prcomp* function). This is a statistical method to reduce the number of parameters to be analysed from 92 serum biomarkers to a few principal components (PCs) with minimal information loss. We used this approach to limit the risks of false-positive findings associated with multiple testing. Biomarkers that are linearly correlated with each other are summarised into uncorrelated PCs. For serum biomarker analysis, PCs were considered until less than 5% of variance (ie, information) in the dataset was explained by the PC. Information regarding the individual biomarker's contribution to PCs was abstracted from the PCA analysis. The five highest contributing biomarkers to each PC were used to determine common biological features by protein enrichment.[15] Findings related to PCs were also assessed for each of the five highest contributing biomarkers within a PC individually. For echocardiographic and biomarker analyses, no further corrections for multiple statistical testing were performed, as the number of parameters to be analysed was reduced to an amount that is conventional in clinical research. A p value <0.05 was considered statistically significant.

Furthermore, analyses were performed for eachbiomarker. P values for these analyses were subsequently adjusted for multiple testing using the Benjamini-Hochberg procedure.[16] An adjusted p value <0.05 was considered statistically significant.

For RNA-Sq analysis of the RVOT, adapter and polyA sequences and low-quality nucleotides were removed using BBDuk. Trimmed reads were mapped against the human genome using STAR, and htseq-count was used to determine read counts.[17] Differential expression analysis was performed with the DESeq2 package.[18] PCA was performed with the DESeq2 package, using default parameters. For transcriptome analyses, p-values were corrected for multiple testing by using the false discovery rate of Benjamini-Hochberg procedure (p<0.05).[16]

RESULTS

Study population

We included 45 patients who underwent ToF repair and 16 who underwent PVR. All surgical procedures were successful. Patient characteristics are shown in table 1. Patients undergoing ToF repair and PVR were comparable with regard to extracardiac defects and prior palliative procedures. Indications for PVR were the following: severe pulmonary regurgitation (PR) (n=10), pulmonary stenosis (PS) (n=3) and combined PR/PS (n=3). Parameters of cardiovascular MRI studies were available for 10 patients with PVR and are shown in table 1.

Assessment at T1 took place 1 (1–6) day before surgery, T2 at 7 (5–13) days after surgery and T3 at 372 (310–444) days after surgery. No patients died during follow-up. In total, 25 patients suffered any complication during hospital stay, which are specified in table 2. One patient was lost to study follow-up at T2, and an additional five patients at T3.

Echocardiography

Echocardiography studies were obtained for 56 patients at T1, 56 at T2 and 52 at T3. Echocardiography measurements are shown in table 3. For patients who underwent ToF repair, biventricular GLS was decreased at T2 compared with other time points (p≤0.002 for all comparisons). For patients who underwent PVR, LV GLS did not differ across study time points. However, RV GLS was reduced at T2 (−20.8±2.4 vs −15.7±4.6, p=0.014 for T1 vs T2). Other parameters of the biventricular function (tissue Doppler imaging (TDI) mitral valve lateral S′, TDI tricuspid valve lateral S′ (TV S′), and tricuspid annular systolic excursion) followed patterns similar to GLS for both patient groups. Biventricular GLS did not correlate with total intervention duration, perfusion time, aortic cross-clamp time, total hospital stay or intensive care unit (ICU) stay for any study group or the combined study population.

Table 1 Patient characteristics

	Correction (N=45)	PVR (N=16)	P value
Age	4.3 (3.4–6.5) months	10.4 (7.8–12.7) years	**<0.001**
Male sex	26 (58%)	8 (50%)	0.591
BMI (kg/m^2)	15.6±1.5	17.3±4.5	0.143
BSA (m^2)	0.34±0.05	1.21±0.39	**<0.001**
O_2 saturation	92±8	99±1	**<0.001**
Hb	8.5±1.2	8.1±0.64	0.092
Ht	0.40±0.06	0.39±0.03	0.214
Prior interventions*	8 (18%)	4 (25%)	0.624
mBT	6 (13%)	2 (13%)	
Central AP shunt	1 (2%)	1 (6%)	
RVOT stent	1 (2%)	–	
Balloon dilatation	1 (2%)	–	
Stent LPA	–	1 (6%)	
Prostin	3 (7%)	2 (13%)	0.465
Extracardiac defects	1 (2%)	2 (13%)	0.102
	Bilateral clubfoot	*Familial hypercholesterolaemia*	
		Prematurity (29+1)	
CMR parameters			
LV EDVi (mL/m^2)		76 (66–81)	
LV ESVi (mL/m^2)		30 (28–40)	
LV SVi (mL/m^2)		40 (36–45)	
LV EF (%)		56 (50–61)	
RV EDVi (mL/m^2)		153 (113–164)	
RV ESVi (mL/m^2)		74 (70–93)	
RV SVi (mL/m^2)		69 (54–79)	
RV EF (%)		42 (40–53)	
PR fraction (%)		43 (35–52)	
Intervention			
TAP	33 (73%)	–	–
Total surgical duration	310 (276–354)	261 (239–336)	**0.030**
Perfusion duration	128 (111–148)	83 (61–101)	**<0.001**
Cross-clamp duration	93 (74–106)	0 (0–26)	**<0.001**

Bold denotes a statistically significant p value.
*All patients with PVR underwent prior ToF repair, and these procedures were not considered a prior intervention for comparisons with the group undergoing ToF repair. One patient with ToF repair previously underwent both (unsuccessful) mBT and central AP shunt.
AP, aortopulmonary; BMI, body mass index; BSA, body surface area; CMR, cardiac magnetic resonance; EDVi, end-diastolic volume index; EF, ejection fraction; ESVi, end-systolic volume index; Hb, haematocrit; Hb, haemoglobin; LPA, left pulmonary artery; LV, left ventricular; mBT, modified Blalock-(Thomas-)Taussig shunt; PR, pulmonary regurgitation; PVR, pulmonary valve replacement; RV, right ventricular; RVOT, right ventricular outflow tract; SVi, stroke volume index; TAP, transannular patch; ToF, tetralogy of Fallot .

RV basal and diameter Z scores were decreased following both ToF repair and PVR. RV end-diastolic dimension Z scores at T3 were higher for patients with ToF repair with a TAP compared with those without ToF repair (1.0±0.7 vs −0.4±0.2, p=0.006).

Serum biomarkers

Two samples from one patient with PVR did not pass the quality assessment and were excluded from the analysis. Panel biomarker analysis was performed for 31 patients at T1, 13 at T2 and 25 at T3. Subjects with available blood samples did not significantly differ from those without. A table of patient characteristics denoting the differences between the aforementioned groups at T2 is included in the online supplemental file. Expression of one biomarker (SPON1) was below the limit of detection in all samples and was excluded from the analysis.

Table 2 Complications during postoperative hospital stay

Complication (n)	Patient group	Days since surgery
Postoperative resuscitation (1)	ToF repair	0
Second pump run for TAP placement (1)	ToF repair	0
Delirium and convulsion (1)	ToF repair	1
Cardiac tamponade (1)	ToF repair	2
Chylothorax (3)	ToF repair	5, 6, 10
Infection or fever (9)	7 ToF repair/2 PVR	2 (2–5)
Arrhythmia (9)	8 ToF repair/1 PVR	0 (0–1)

One patient required a second pump run to create a TAP because of a narrow right ventricular outflow tract following the initial repair. The most common arrhythmia was junctional ectopic tachycardia. PVR, pulmonary valve replacement; TAP, transannular patch; ToF, tetralogy of Fallot.

Biomarkers were expressed as three PCs, which together accounted for 59% of the total variance in the dataset. An overview of these PCs is presented in table 4 and figure 1. PC1 scores primarily differentiated between patients undergoing ToF repair and PVR (2.3±5.4 vs −4.3±5.7, p≤0.001, combined for all study time points). As these patient groups differ importantly in age, we investigated the relationship between age and PC1 scores within these patient groups. PC1 scores did not correlate with age for patients undergoing ToF repair (r=0.08, p=0.720) or PVR (r=−0.12, p=0.752).

PC2 scores primarily differentiated between patients before and after ToF repair (−2.7±2.1 for T1 of ToF repair vs 1.2±2.7 for other time points including PVR, p≤0.001). PC2 scores did not relate to hospital or ICU stay at any time point. Furthermore, PC2 scores at T1 correlated with preoperative haematocrit for patients undergoing ToF repair (r=0.56, p=0.006). No relation between O_2 saturation and PC2 scores could be established at this study time point.

PC3 scores were increased at the early postoperative time point (T2) for ToF repair (4.9±1.1 vs −0.8±1.8, p≤0.001). For patients with PVR, PC3 followed a similar pattern, but differences between time points were not statistically significant. PC3 scores at T2 did not relate to total intervention duration, perfusion time, aortic cross-clamp time, hospital stay or ICU stay.

Analyses for individual biomarkers are supplied in the online supplemental file. Biomarkers highly contributing to a certain PC generally behave in a similar fashion to the PC itself. PCs did not distinguish patients with regard to sex, biventricular GLS, staged versus primary ToF repair, RV dimensions or the occurrence of complications. NT-proBNP assessed by the clinical laboratories (after log transformation) correlated well with panel biomarker–derived measurements of NT-proBNP (r=0.84, p≤0.001). For the subset of patients with complete blood samples at each study time point (n=10), two biomarkers differed across study time points as per the repeated measurements analysis of variance: suppression of tumorigenicity-2 (ST2) (5.4±1.2 to 6.8±0.9 to 5.4±0.5, adjusted p=0.004) and NT-proBNP (3.3±0.9 to 5.4±2.1 to 3.1±0.5, adjusted p=0.011).

Characterisation of right ventricular outflow tract transcriptomes

We performed whole-tissue RNA-Seq of RVOT samples of 20 patients who underwent ToF repair. Patient characteristics are provided in the online supplemental file. Unsupervised PC analysis shows clustering of the samples according to the sex of the patients, rather than any clinical characteristic (figure 2A). Patient 20 does not cluster with the other patient samples. This sample had higher expression of transcripts associated with fibroblasts, rather than cardiomyocytes. Significantly differential expressed genes between female and male patients were located on the Y-chromosomes such as *TTTY14*, *TMSB4Y*, or are involved in the inactivation of the X-chromosomes such as *XIST* and *TSIX*, consistent with the sex of the patients (figure 2B). Five genes—among which *HECW2*, *PIGN* and *ADAMTS9*—were differentially expressed between patients above and below the median haematocrit, a surrogate for cyanosis. No genes were differentially expressed between patients with and without a previous palliative procedure. No transcriptome phenotypes were related to the parameters of clinical outcome following ToF repair. Expression levels of *NPPB*, encoding for the biomarker NT-proBNP, in RVOT transcriptomes correlated with the measured serum concentrations at T1 (r=0.52, p=0.045).

DISCUSSION

In this multicentre prospective observational study, we confirmed temporarily impaired biventricular function for patients with ToF undergoing ToF repair. However, for patients undergoing PVR, only RV function was temporarily impaired. Ninety-one cardiovascular and immunological biomarkers were summarised as three PCs, which related to specific biological and clinical phenotypes. These phenotypes were related to patients undergoing ToF repair and PVR, patients before and after repair, and early postoperative status, respectively. No PC was related to either ventricular function or complications following procedures. RNA-Seq of the transcriptome of whole tissue samples of patients undergoing ToF repair differentiated patients only with regard to sex, rather than clinical features, suggesting that the RVOT—in contrast to other anatomic structures of the RV—may be relatively unaffected by clinical features such as right ventricular pressure overload.

Compared with published paediatric reference values, RV GLS of our study cohort at T1 (−19.3±4.9 for ToF repair and −20.8±2.4 for PVR) was lower than normal (although within the normal range).[19] This may relate to

Table 3 Parameters at study time points

	ToF repair						PVR					
	T1	T2	T3	P value (T1 vs T2)	P value (T2 vs T3)	P value (T1 vs T3)	T1	T2	T3	P value (T1 vs T2)	P value (T2 vs T3)	P value (T1 vs T3)
LV GLS	−18.0±3.7	−13.2±3.8*	−19.8±1.8	**<0.001**	**<0.001**	**0.024**	−17.5±3.1	−16.8±4.1*	−18.6±2.5	0.748	0.103	0.103
TDI MV S' (cm/s)	5.0±1.3***	4.8±1.7***	7.0±1.8	0.861	**<0.001**	**<0.001**	7.9±1.8***	6.5±1.1***	7.0±1.5	**0.025**	0.259	0.259
RV GLS	−19.3±4.9	−14.1±3.8	−20.4±4.0	**0.002**	**<0.001**	0.888	−20.8±2.4	−15.7±4.6	−19.6±5.3	**0.014**	0.119	0.119
TDI TV S' (cm/s)	8.3±2.1	4.3±1.8	7.6±1.8	**<0.001**	**<0.001**	0.217	8.5±1.6	5.4±1.8	8.2±1.6	**0.004**	**0.021**	**0.021**
TAPSE (mm)	12.3±2.9*	6.4±1.8***	12.1±2.0	**<0.001**	**<0.001**	0.444	14.8±4.0*	10.2±2.3***	14.6±3.9	**0.018**	0.066	0.066
RV end-diastolic diameter (Z)	0.1±1.0	−0.3±1.0	0.7±0.8*	0.643	0.096	0.872	1.1±1.0	0.6±0.7	−0.4±0.3*	0.332	0.058†	0.063†
RV basal diameter (Z)	1.9±1.0	1.2±1.6	0.9±1.8	**0.015**	0.542	**0.045**	1.5±1.8	0.5±1.5	0.1±2.2	**0.013**	0.474	0.474
RV mid diameter (Z)	1.0±1.1*	0.9±1.9	0.3±1.2	0.589	0.738	0.312	2.0±1.2*	0.6±1.3	1.2±1.4	**0.004**	0.764	0.764
Proximale RVOT diameter (Z)	−2.6±1.7*	−1.7±2.0	−1.6±2.3	0.495	0.692	0.684	−0.6±1.8*	−1.6±1.8	−1.4±2.7	0.435	0.925	0.925
PV max flow (m/s)	4.4±0.9***	2.5±0.7*	2.2±0.6	**<0.001**	**0.012**	**<0.001**	2.5±1.1***	1.7±0.8*	2.3±0.9	0.061	0.148	0.148
Biomarker PCs												
PC1	2.9±5.5**	3.8±7.4	0.8±4.4*	0.768	0.567	0.567	−5.8±5.9**	−1.6±6.0	−5.0±5.2*	0.753	0.350	0.350
PC2	−2.7±2.1***	−1.2±2.8*	0.5±1.9**	0.122	0.260	0.260	1.2±1.8***	3.1±4.1*	2.7±1.5**	0.725	0.478	0.478
PC3	−0.8±2.0	4.9±1.1*	−0.9±1.5	**0.002**	**<0.001**	**<0.001**	−0.2±1.8	1.7±2.8*	−0.8±1.3	0.401	0.179	0.179

Bold denotes a statistically significant p value.
Statistically significant difference between patients with ToF repair and PVR: *p<0.05; **p<0.01; ***p<0.001.
†Unpaired t-test shown due to insufficient pairs.
GLS, global longitudinal strain; LV, left ventricular; MV S', mitral valve lateral S′; PC, principal component; PV, pulmonary valve; PVR, pulmonary valve replacement; RV, right ventricular; RVOT, right ventricular outflow tract; TAPSE, tricuspid annular systolic excursion; TDI, tissue Doppler imaging; ToF, tetralogy of Fallot; TV, tricuspid valve lateral S'.

Table 4 Principal components of serum biomarkers

	PC1	PC2	PC3
Variance explained	43%	10%	6%
Contributing biomarkers	EPHB4	TIMP4	NT-proBNP
	TR-AP	IL-1RT2	ST2
	TNF-R2	COL1A1	PRTN3
	TNF-R1	GP6	MB
	U-PAR	SELP	PON3
Subcellular localisation	–	Integrin αIIb–β3 complex	Troponin complex Striated muscle thin filament Myofilament Extracellular region
Biological process	Negative regulation of extracellular matrix constituent secretion Positive regulation of apoptotic process involved in morphogenesis Pulmonary valve development Negative regulation of cardiac muscle hypertrophy Aortic valve development	–	–
Clinical associations	PC1 differentiates between patients undergoing ToF repair versus PVR (2.3±5.4 vs −4.3±5.7, p≤0.001)	PC2 differentiates between patients before versus after ToF repair (−2.7±2.1 vs 1.2±2.7, p<0.001) PC2 correlates with haematocrit for patients before ToF repair (r=0.56, p=0.006)	PC3 is increased at the early postoperative time point, compared with other time points (3.2±2.7 vs −0.7±1.7, p<0.001)

COL1A1, collagen alpha-1(I) chain; EPHB4, ephrin type-B receptor 4; GP6, platelet glycoprotein VI; IL-1RT2, interleukin-1 receptor type 2; MB, myoglobin; NT-proBNP, N-terminal pro-brain natriuretic peptide; PC, principal component; PON3, paraoxonase-3; PRTN3, myeloblastin; PVR, pulmonary valve replacement; SELP, P-selectin; ST2, suppression of tumorigenicity-2; TIMP4, metalloproteinase inhibitor 4; TNF-R, tumour necrosis factor receptor; ToF, tetralogy of Fallot; TR-AP, tartrate-resistant acid phosphatase type 5; U-PAR, urokinase receptor.

differences in loading conditions, rather than reflecting intrinsically decreased RV contractility.[20] Biventricular function was impaired at T2 for patients undergoing ToF repair and recovered at T3. These findings are in agreement with previous studies describing temporarily decreased ventricular performance following ToF repair.[21 22] Biventricular GLS following PVR did not differ statistically significantly across study time points. TDI TV S′—another parameter of RV function—did follow a pattern of temporarily impaired ventricular function. It should be noted RV GLS and TDI TV S′ followed the same fall-and-rise pattern, although differences between time points were not statistically significant. Temporarily decreased RV GLS may be confounded in this population by RV unloading, which should increase RV GLS. A previous retrospective study found temporarily impaired ventricular function following surgical—but not following transcatheter—PVR.[23] In our present study, perfusion duration in the PVR group was relatively short, aortic cross-clamping was less commonly used and—if employed—aortic cross-clamp duration was relatively short. This may also explain the limited effect on biventricular function observed in our study.

PC1, accounting for 43% of the variance in the dataset, primarily differentiated between patients undergoing ToF repair and PVR. PC1 was mostly influenced by serum levels of ephrin type-B receptor 4 (EPHB4), tartrate-resistant acid phosphatase type 5 (TR-AP), tumour necrosis factor receptors 1 and 2 (TNF-R1/TNF-R2) and urokinase receptor (U-PAR). Age differs importantly between patients undergoing ToF repair (4.3 (3.4–6.5 months) and PVR (10.4 (7.8–12.7) years). Although age did not relate to PC1 scores within these patient groups, we cannot ignore this potentially important confounder. We did not find any relation with other clinical parameters. What differences in patient characteristics cause the difference in biomarker expression is currently unclear. TNF-R1 and TNF-R2 are associated with, among others, pulmonary and aortic valve development.[24] Other biomarkers related to PC1 have been related to outcomes: TR-AP related to outcomes following acute coronary syndrome and cardiac hospitalisation among patients with chronic kidney disease.[25–27] EPHB4 marginally related to outcomes following acute coronary syndrome.[26] U-PAR has been related to outcomes in patients with congestive heart failure and coronary disease.[28 29] However, the role of these biomarkers in the perioperative setting is largely unknown.

PC2 differentiated between patients before and after ToF repair. Furthermore—across patients before ToF

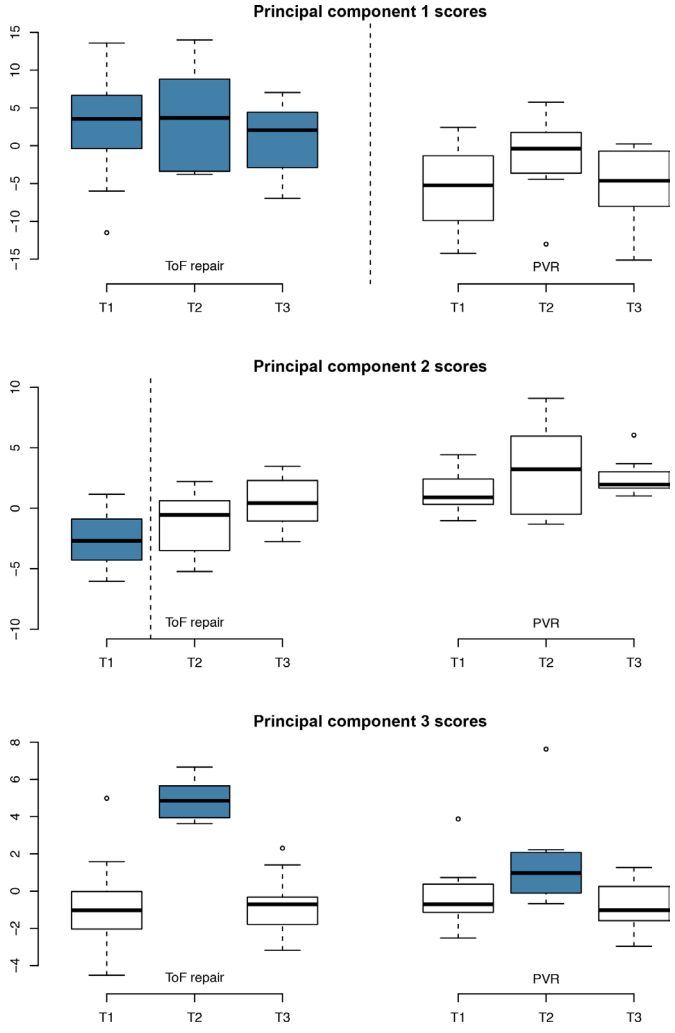

Figure 1 Principal component (PC) scores of panel biomarkers. PC1 scores were higher in the tetralogy of Fallot (ToF) repair group compared with pulmonary valve replacement (PVR). PC2 scores were lower for patients before ToF repair, compared with other time points. PC3 scores were increased at the postoperative time point (T2).

repair—PC2 correlated with haematocrit levels, which may reflect preoperative cyanosis. PC2 was mostly influenced by serum levels of metalloproteinase inhibitor 4 (TIMP4), interleukin-1 receptor type 2 (IL-1RT2), collagen alpha-1(I) chain (COL1A1), platelet glycoprotein VI (GP6) and P-selectin (SELP). GP6 and SELP regulate the activity of the integrin αIIb–β3 complex.[30] This complex is found in platelets and regulates platelet aggregation.[30] Previous research found that preoperative cyanosis may affect platelet function and increase platelet aggregation.[31] Furthermore, other biomarkers of collagen and other matrix metalloproteinase subtypes have been related to outcomes in congenital heart disease.[32–34] However, their role in the perioperative setting has scarcely been studied. Whether PC2 also relates to features of unrepaired ToF other than cyanosis—such as right ventricular pressure load, right ventricular hypertrophy or diminished flow in the pulmonary circulation—could not be established. In mice models, collagen and matrix metalloproteinases have been related to right ventricular hypertrophy resulting from pulmonary hypertension.[35] Biomarkers in PC2 have previously been related to outcomes in acute coronary syndrome (TIMP4, IL-1RT2, COL1A1, SELP),[26 36] congestive heart failure (TIMP)[37] and atherosclerosis development (TIMP4).[38]

PC3 is clinically related to early postoperative status (T2). PC3 was influenced by, among others, serum levels of NT-proBNP, ST2, and myoglobin (MB). These biomarkers are expressed in striated (ie, skeletal and cardiac) muscle tissue. NT-proBNP and ST2 are released by the myocardium in response to increased wall stress.[39 40] MB can be released in response to injury to cardiac or skeletal muscle myocytes.[41] Temporarily impaired ventricular function at this time point may lead to the expression of NT-proBNP and ST2.[39 40] Serum MB levels may be reduced at T2 due to muscle wasting related to hospitalisation or due to periprocedural early losses.[41] Patients with PVR had lower PC3 scores at T2 compared with those with ToF repair. This may relate to the limited perfusion and aortic cross-clamp time in this group, as well as the limited impairment of ventricular function at this time point. NT-proBNP and ST2 have received much attention as biomarkers for long-term outcome in cardiovascular and congenital heart disease.[14 40] Perioperative levels of NT-proBNP may predict outcome during 6 months of follow-up.[42] ST2 had not been studied in the perioperative setting for congenital heart disease.

With regard to the RVOT transcriptome at the time of operation, we found most differences related to patients' sex, rather than clinical characteristics, indicating that gene expression in the RVOT is not influenced by clinical characteristics in the relatively homogeneous study population. It should be noted that the RVOT may not be representative of the global RV myocardium, and other anatomic structures may be less preserved. A previous study by Zhao *et al*, in eight patients with ToF aged 6 (3–10) months, reported various upregulation of *HIF1A*-regulated hypoxia response genes in RVOT samples obtained from patients with cyanosis when compared with patients without cyanosis.[43] In our present study, these genes were not differentially expressed between patients with and without cyanosis. It should be noted that our present study included both patients with staged and primary ToF repair, whereas Zhao *et al* only included patients with primary repair. Although the age of repair was similar between the two studies, patient characteristics related to disease severity such as Hb, Ht, RVOT dimensions or transpulmonary valve flow velocities were not reported in the study by Zhao *et al*. Disease severity may account for the different findings across these studies.

Limitations

Despite our study's strengths, some limitations should be considered. Patients undergoing PVR were significantly older and perioperative conditioning often varied compared with patients undergoing repair. This confounds comparisons between these

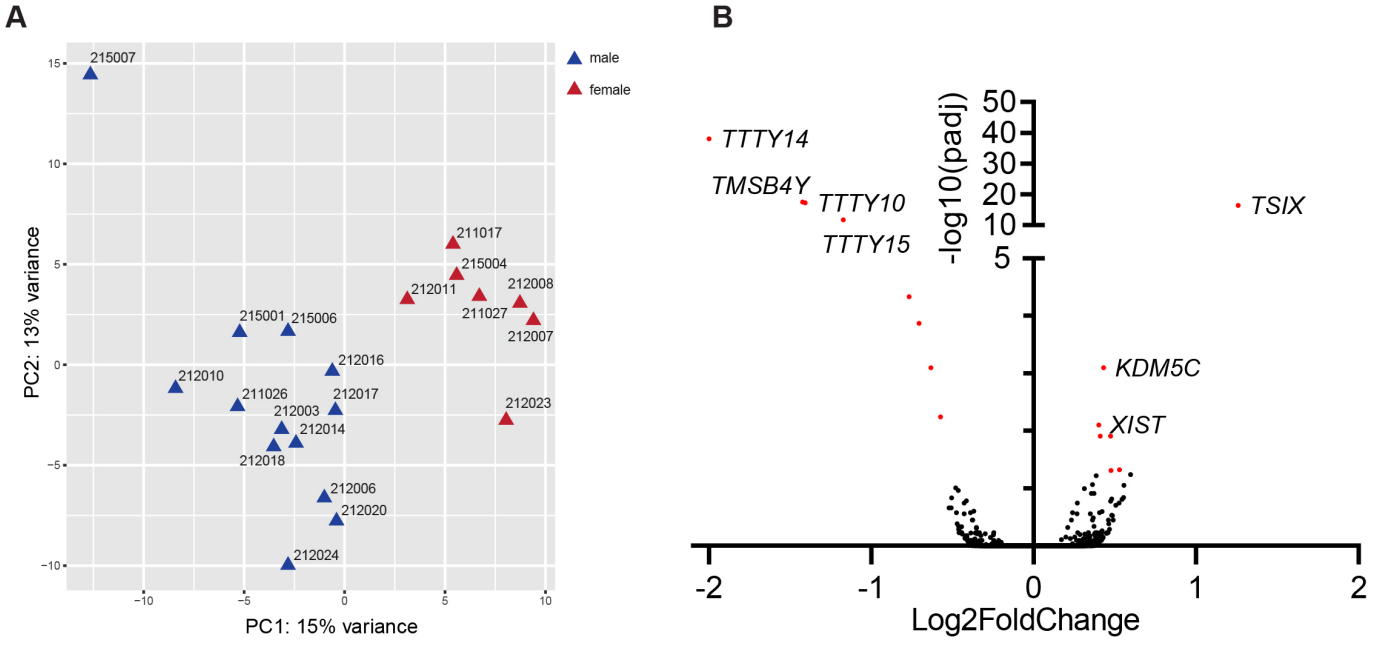

Figure 2 Right ventricular outflow tract RNA sequencing data. (A) Unsupervised principal component (PC) analysis shows patients cluster with regard to sex. (B) Significantly differential expressed genes between female and male patients are located on the X and Y chromosome, respectively.

groups. Strategies to account for confounders such as propensity score matching or weighting are not feasible considering the large inherent age difference between these groups. To minimise age differences, we excluded adult patients undergoing PVR. It should be noted that patients with ToF generally undergo PVR beyond childhood.[1]

We used haematocrit as a surrogate marker for preoperative cyanosis. Haematocrit values, in contrast to O_2 saturation values, may better reflect the long-term burden of cyanosis, rather than a single saturation measurement, which may be subject to large fluctuations. Furthermore, many patients had O_2 saturations of ≥99%, which complicates statistical analysis. It should be noted preoperative PC2 scores correlated with haematocrit, but no relation to O_2 saturation at this time point could be established. Factors other than cyanosis such as hydration status and other blood count values also may have influenced haematocrit values.

Our biomarker panel analysis results in relative expressions, which cannot be compared across study populations or with published references. Most of these biomarkers are relatively novel and do not have age-related references. Some of the variance between time points may be explained by normal somatic development (eg, normal NT-proBNP concentration rapidly declines during the first years of life).[44] The analysis of myocardial transcriptome was limited to the RVOT, as this tissue is readily obtained during ToF repair. The transcriptome may differ between the RVOT and other segments of the RV.

Despite these limitations, we provide a comprehensive analysis, including RVOT transcriptome, functional echocardiographic parameters and serum biomarkers, of patients undergoing ToF repair and PVR.

CONCLUSIONS

We provide extensive observations on the functional and immunological response to periprocedural injury for patients with ToF. We identified biomarker phenotypes that clinically relate to (1) ToF repair and PVR, (2) uncorrected versus corrected ToF and (3) early postoperative status. The identified biomarker response at the early postoperative status may relate to recovery from perioperative injury. However, we did not identify any specific functional or immunological response that related to (un)favourable recovery from surgery.

These findings add to our current understanding of periprocedural injury and subsequent recovery. Improved characterisation of perioperative injury and subsequent recovery may provide biomarkers to identify patients at risk for adverse events. Furthermore, elucidating the mechanisms of perioperative injury may identify targets for therapy, such as inhibitors of disadvantageous immune responses following CPB or perioperative myocardial protective strategies.

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