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Blood biomarkers to detect functional impairment in adult patients with repaired tetralogy of Fallot

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A B S T R A C T

Background: The relationship between plasma brain natriuretic peptide (NT-proBNP) and soluble suppression of tumorigenicity-2 (sST2) with structural adaptions and exercise capacity remains incompletely described in patients with repaired Tetralogy of Fallot (rTOF).

Methods: Peripheral venous blood samples were drawn for 99 patients with repaired TOF, 59 patients with severe pulmonary regurgitation (PR) and 40 patients with no or mild PR. NT-proBNP was measured using enzyme-linked immunosorbent assays (Roche Diagnostics, Indianapolis, IN). Soluble ST2 levels were assessed on Aspect-plus ST2 quantitative rapid test.

Results: The mean value of NT-proBNP was 160 ± 137 pg/ml, and sST2 was 29 ± 13, ng/ml in the entire population. 58 % had an elevated NT-proBNP, while sST2 was abnormal in 40 %. Mean NT-proBNP was significantly higher in patients with severe PR (169 ± 150 vs145 ± 118, pg/ml, $p < 0.001$), while similar sST2 levels were observed in both groups (29 ± 14 vs30 ± 12, ng/ml, $p > 0.05$). NT-proBNP and sST2 levels were higher in patients with transannular patch when compared to other RVOT intervention (174 ± 145 vs 107 ± 100, pg/ml, $p < 0.001$); (31 ± 13 vs 29 ± 15, ng/ml, $p < 0.05$). Both biomarkers were significantly associated with exercise capacity, but NT-proBNP ($r = -0.60$, $p < 0.001$) was stronger. The optimal cut-off of 90 pg/ml for NT-proBNP had a sensitivity of 74 % and specificity of 63 % for detection of impaired exercise capacity.

Conclusions: Serum levels of sST2 and NT-proBNP are elevated in patients with repaired TOF, with higher values observed in those with severe PR, but also in patients undergoing transannular patch repair. Incorporating both markers in these patients increased the ability to detect impairment in exercise capacity.

1. Introduction

Circulating biomarkers are increasingly part of contemporary patient management across a range of cardiovascular conditions. Plasma brain natriuretic peptide (NT-proBNP) secreted by ventricular myocytes in response to pressure, volume overload and increased wall stress; it is the recommended biomarker for heart failure diagnosis [1,2]. Soluble suppression of tumorigenicity-2 (sST2) is an emerging prognostic biomarker in heart failure. It is a member of the IL1 receptor family and is released by both myocytes and fibroblasts in the myocardium in response to injury. It is also linked to myocardial stress but it overcomes some of the limitations of NT-ProBNP with fewer biological confounders, and with stronger ability to predict mortality [3,4]. The two markers are synergistic in predicting adverse prognosis in heart failure.

Although NT-proBNP is performed in congenital heart disease (CHD), including patients with repaired TOF [5,6], its role and associations are less well understood. There is very little data describing sST2 in this population [7,8]. Pulmonary regurgitation with subsequent RV dilatation and dysfunction is still considered to drive of late morbidity and mortality [9–11]. NT-proBNP and sST2 may offer direct physiological markers of adverse remodelling and inform selection for interventions. This study tested the hypotheses that NT-proBNP and ST2 would be associated with pulmonary regurgitation, adverse cardiac remodelling or objective measures of exercise performance in asymptomatic rTOF patients.

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Abbreviation and acronyms

CPET	cardiopulmonary exercise test
PR	pulmonary regurgitation
VO_2 peak (ml/min)	peak absolute maximum oxygen uptake
CR	contractile reserve
NT-proBNP	Plasma brain natriuretic peptide
sST2	Soluble suppression of tumorigenicity-2

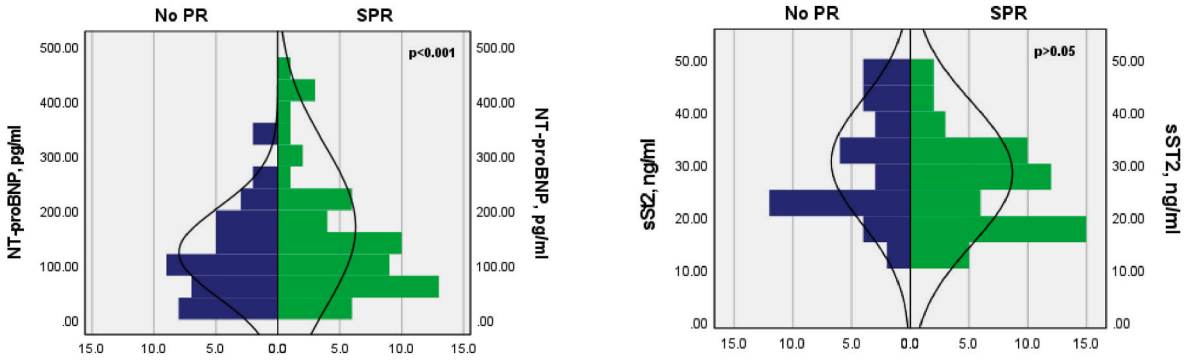

Fig. 1. Difference in mean (a) NT-proBNP and (b) sST2 levels between groups (SPR and no PR groups).

2. Methods

2.1. Study design and population

We included patients with a diagnosis of repaired TOF enrolled in a prospective study under medical follow up who had either severe PR or negligible/no PR. Inclusion criteria were 1) diagnosis of repaired TOF; 2) NYHA status III or less. Exclusion criteria included, intermediate severity PR, absence of residual pulmonary, branch PA stenosis or right ventricular outflow tract (RVOT), significant pulmonary stenosis, severe left ventricular outflow obstruction, ventricular arrhythmias, pacemaker, significant pulmonary hypertension, inability to exercise on the exercise bicycle, and poor acoustic windows.

Echocardiography was performed using a VIVID E95 (Vingmed-General Electric, Horten, Norway) ultrasound platform. Standard 2D, M-mode, colour Doppler, pulsed, continuous wave and colour tissue Doppler were performed according to British Society of Echocardiography guidelines [12]. In order to facilitate strain analyses a frame rate was selected between 35 and 70 frames per second. For 4 dimensional LV and RV analyses, a full volume single beat acquisition was stored with a frame rate not less than 30 (Hz) during end-expiratory breath--hold. For the purposes of defining normality, reference ranges were taken from a range of contemporary sources [12–14].

Exercise echocardiographic tests were undertaken following a specific image acquisition protocol (Appendix A, Fig. 1). Imaging was undertaken at baseline and at low intensity exercise (defined as 65–75 % peak age predicted heart rate). All strain and volume echo parameters were evaluated at low intensity exercise. Peak exercise acquisitions were taken after the respiratory exchange ratio (RER) was>1.0. This provided a predefined point beyond ventilatory threshold but with sufficient remaining exercise time to allow measurements to be made during high intensity exercise. Strain imaging was not undertaken at high intensity exercise because of the effect of heart rate on analysis algorithms [15].

2.2. Cardiopulmonary exercise testing (CPET)

All patients underwent a semi-recumbent tilting cycle ergometer (ERG 911 S/L, Schiller, Switzerland). $\dot{V}O_2$ was continuously measured using a calibrated breath-by-breath analyzer (Cosmed Quark CPET, Rome, Italy). A 2-min resting period was included followed by a 3-min unloaded warm up period. Exercise protocols were individually determined and based on the patient functional status. The work rate of the test was ranged between 5 and 20W, increased every minute until voluntary exhaustion. Heart rate (HR), blood pressure, and oxygen saturation were monitored throughout. All participants paddled at a self-selected rhythm between 60 and 79 revolutions per minute. A RER >1 was used to indicate adequate effort.

3. Blood biomarkers assays

3.1. Plasma brain natriuretic peptide (NT-proBNP)

Peripheral venous blood samples were drawn before the cardiopulmonary exercise testing visit (CPET), the EDTA-containing tubes were then centrifuged immediately and stored at −70 °C for subsequent analysis. Amino-terminal pro-brain natriuretic peptide (Nitro-pro BNP) was measured using enzyme-linked immunosorbent assays (Roche Diagnostics, Indianapolis, IN). One patient refused to give a blood sample. All NT-proBNP analyses were performed at the Royal London Hospital core laboratory blinded to clinical data.

3.2. Soluble suppression of tumorigenicity-2 (sST2)

Soluble ST2 levels were assessed on Aspect-plus ST2 test using a low and high level of external quality control (Critical diagnostics, Cambridge, United Kingdom). The measurement of sST2 ASPECT-Plus ST2 test was manually performed for 99 patients following the manufacturer's instruction. sST2 test cassette was warmed to room temperature for 15 min, the foil pouch was removed and 35 μl of plasma sample was pipetted into the sample well. 2 drops (i.e., ~110 μL) of test buffer were added into the test buffer well after 60s of loading the plasma sample. Finally, the ASPECT-PLUS sST2 test cassette was inserted into the ASPECT reader and the quantitative sST2 results were analysed approximately in 20 min and calculated in ng/ml. sST2 plasma concentrations were measured with a lower limit of detection of 25 ng/ml, and upper limit of detection of 200 ng/ml.

3.3. Ethical and statistical considerations

VO_2 peak was the primary end point for the study. We estimated a sample size of 94 would permit the detection of a medium association and permit the inclusion of an additional 5 factors into the primary prediction model.

Continuous variables were presented as mean and standard deviations (SD). Student's t tests were performed for all parameters.

Table 1
Baseline charactristics.

Baseline characteristics n = 99	Severe PR group Mean ± SD	No PR group Mean ± SD	P value
Age (yrs)			
Sex	35 ± 13	33 ± 11	**<0.05**
Male	31 (52 %)	19 (48 %)	**<0.05**
Female	29 (48 %)	21 (52 %)	**<0.05**
NYHA (I-II)	50 (II)	40 (I)	-
	10 (I)	–	
Height (cm)	166 ± 11	169 ± 8	NS
Weight (kg)	71 ± 16	73 ± 14	NS
BMI (Kg/m^2)	25 ± 5	26 ± 5	NS
ORS duration (ms)	153 ± 20	150 ± 22	NS
Type of surgery			
TAP	51 (85 %)	27 (68 %)	**<0.05**
Other RVOT intervention	9 (15 %)	13 (32 %)	**<0.05**

BMI = body mass index; TAP = transannular patch. * Bold values indicate significant level ($p < 0.05$); NS = non-significant.

Univariate correlation analyses were constructed to determine relationships between hemodynamic echocardiographic, blood biomarkers and exercise parameters at rest and at peak exercise for the right and left ventricles. For regression analysis, cases and controls were considered together and the presence of severe PR (0/1) was entered into the models as a factor. A multivariable regression analysis model was constructed with blood biomarkers and absolute VO_2 (ml/min) for the primary end point analysis. The predictive power to identify the deteriorated functional capacity was assessed by a receiver operating characteristic (ROC) curve analysis to identify the optimal cutoff point of blood biomarkers concentration for predicting low exercise capacity. A p value of < 0.05 was considered as statistically significant. All statistical analyses were performed using IBM SPSS statistics version 27 (IBMCorp, London, United Kingdom).

The study was approved by the Health Research Authority-Queen Square Research Ethics Committee (18/LO/0092). All participants provided written, informed consent.

4. Results

4.1. Baseline characteristics

99 consecutive patients, 59 with severe PR and 40 without were recruited between 2017 and 2021. Baseline characteristics are presented in Table 1. The screening population was 120, 20 patients were excluded due to severity of pulmonary stenosis, inability to exercise or right ventricular arrythmia, and one patient who refused to give a blood sample. The PR group was older than controls (35 ± 13 vs 33 ± 11, years, $p < 0.05$), and were marginally more frequently male.

There were no significant baseline differences between the two groups apart from age and sex (Table 1). The PR group also had a high frequency of transannular patch (TAP) (85 vs 68 %, in controls, $p < 0.05$) as the primary operation. 9 patients in the PR group (15 %) had reintervention with either surgical pulmonary valve replacement or other conduits, while 21 patients (53 %) in the controls had either previous surgical or percutaneous pulmonary valve intervention.

For the cardiopulmonary exercise testing a variety of load-based protocols were used averaging 15W/minute, with minimum 5W/minute and maximum 20W/minute. The average exercise time was 10 ± 2 min in the PR group and 9 ± 1 min in the control group with similar maximal workload achieved and in objective exercise performance between groups (max VO_2 1695 ± 627vs 1744 ± 521, ml/min, $p > 0.05$) (Appendix 1, Table 1). A RER of >1.01 was achieved in 97 patients, with a mean value of 1.2 ±0 .1 across the entire cohort. The termination of exercise was either due to leg fatigue, shortness of breath or general fatigue.

The mean value of NT-proBNP in the entire population was 160 ± 137 pg/ml and ranged from 35 to 703 pg/ml (Table 2). 58 % of the population had an elevated NT-proBNP, when compared to normal range (normal <100 pg/ml [16]). Mean NT-proBNP was significantly higher in the PR group (169 ± 150 vs 145 ± 118, pg/ml, $p < 0.001$) (Fig. 1a). 58 % of the PR group had an elevated level of NT-proBNP compared to 53 %, in those without PR. Higher NT-proBNP levels were observed in patients with TAP surgical approach when compared to other RVOT approaches (174 ± 145 vs 107 ± 100, pg/ml, $p < 0.001$, Fig. 2a).

The mean sST2 in the population was 29 ± 13 ng/ml ranging from 15 to 80 ng/ml 40 % of the entire population had an elevated sST2, when compared to the normal range (normal <30 ng/ml [17]) (Table 2). There was no significant difference in the mean sST2 between those with and without PR (29 ± 14 vs 30 ± 12, ng/ml, $p > 0.05$, Fig. 1b). Higher sST2 levels were also observed in patients with TAP surgical approach compared to other RVOT approaches regardless of the presence of severe pulmonary regurgitation (31 ± 13 vs 29 ± 15, ng/ml, $p < 0.05$, Fig. 2b).

Both biomarkers were significantly correlated ($r = 0.67$, $p < 0.001$) (Table 3). At baseline, NT-proBNP was associated with RV size at base level ($r = 0.31$, $p < 0.001$), inversely associated with RV longitudinal function by TAPSE ($r = -0.22$, $p < 0.001$). There were no associations with LV size and function (Table 3). Conversely sST2 while not correlated with LV or RV volumes, nor conventional functional parameters, did however have a significant inverse association with both RVGLS (−0.39, $p < 0.001$), and LV global longitudinal strain (LVGLS) ($r = -0.49$, $p < 0.001$, Table 3).

NT-proBNP was associated with contractile reserve of the right ventricle. The strongest association was with RV global free wall strain (ΔRVGFWS) (Table 3). There was no association observed with contractile reserve of the LV (Table 3). Conversely sST2 was associated with both RV and LV contractile reserve parameters. For the RV both volumetric and deformation augmentation related to sST2 including RV diastolic volume ($r = -0.73$, $p < 0.05$) and RV free wall strain ($r = -0.38$, $p < 0.05$), while for the LV a correlation was observed with change in LV longitudinal strain ($r = -0.49$, $p < 0.05$) and LV diastolic volume ($r = -0.38$, $p < 0.05$, Table 3).

Both NT-ProBNP and sST2 showed inverse correlations with peak absolute (ml/min) and peak weighted (ml/min/kg) VO_2. The

Table 2
Baseline blood biomarkers in the entire population and subgroups analysis.

Blood biomarkers	NT-proBNP pg/ml (n = 99)	sST2, ng/ml (n = 99)	P value	Blood biomarkers subgroup	Severe PR group Mean ± S. D (n = 59)	No PR group Mean ± S (n = 40)	P value
All population (n = 99)	160 ± 137	29 ± 13	–	**NT-proBNP**	169 ± 150	145 ± 118	**<0.001**
				sST2, ng/ml	29 ± 14	30 ± 12	NS
Surgical type	**TAP**	**Non-TAP**		**Surgical type**			
NT-proBNP (n = 99)	174 ± 145	107 ± 100	**<0.001**	**NT-proBNP, TAP**	187 ± 157	150 ± 118	**<0.001**
sST2 (n = 99)	31 ± 13	29 ± 15	**<0.05**	**NT-proBNP, non-TAP**	69 ± 29	134 ± 124	**<0.001**
–		–	–	**sST2, TAP**	28 ± 13	30 ± 12	NS
–	–	–	–	**sST2, non-TAP**	29 ± 15	30 ± 15	NS

NT-proBNP = plasma brain natriuretic peptide; sST2 = soluble suppression of tumourigenicity-2; TAP = transannular patch; non-TAP = other type of surgery (e.g., pulmonary valvotomy) * Bold values indicate significant level ($p < 0.001$, <0.05); NS = non-significant.

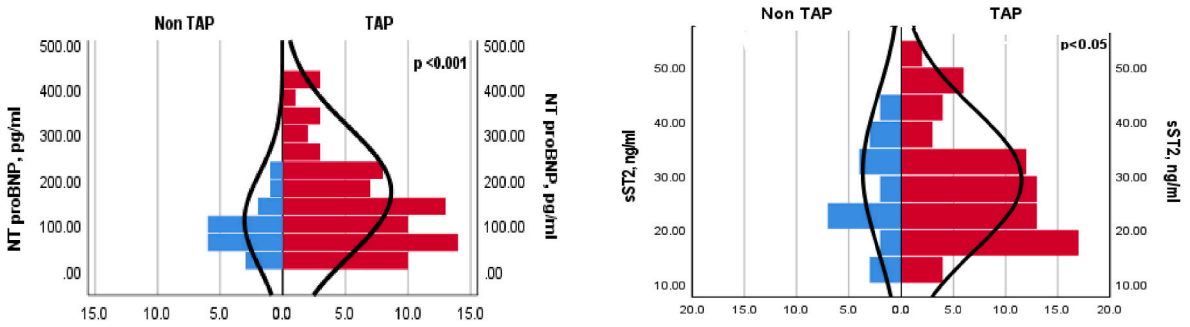

Fig. 2. (a) NT-proBNP levels comparison between TAP and non-TAP in the entire population (n = 99), and (b) sST2 levels comparison between TAP and non-TAP in the entire population (n = 99).

Table 3
Univariate correlation analyses (r values presented) between blood biomarker, contractile reserve, and exercise parameters.

Baseline echocardiographic Parameters (n = 99) (univariate)	NT-proBNP (pg/ml)	sST2 (ng/ml)	Contractile Reserve	NT-proBNP (pg/ml)	sST2 (ng/ml)
Right ventricle			Right ventricle		
RV mid (mm)	.11	.02	**Δ RVS' (%)**	**−.33***	−.11
RV base (mm)	**.31***	.11	Δ FAC (%)	−.10	−.20
RVEDVI (ml/m^2)	.18	.19	**Δ TAPSE (%)**	**−.22***	−.10
RVS' (cm/s)	−.09	−.05	**Δ RVEDVI (%)**	**−.34***	**−.73***
TAPSE (mm)	**−.22***	−.11	**Δ RVGLS**	**−.44***	**−.23***
FAC (%)	−.10	−.05	**Δ RVGFWS**	**−.69***	**−.38***
RVGLS (%)	**−.17***	**−.39***	**Left Ventricle**		
RVGFWS (%)	−.20	−.13	**Δ LVS'**	.03	**−.48***
Pulmonary regurgitation			**Δ LVEDVI**	.11	**−.38***
PG (mmHg)	−.01	−.04	**Δ LVGLS (%)**	−.06	**−.49***
PHT (ms)	−.12	−.06			
Left ventricle			**Function Capacity**		
LVEDVI (ml/m^2)	.03	.14	VO_2 (ml/min)	−.50*	−.39*
LVS' (cm/s)	−.19	−.09	VO_2 (ml/kg/min)	−.38*	−.20
LVGLS (%)	−.30	**−.49***			
Blood biomarkers	**sST2 (ng/ml)**	-			
NT-proBNP	**.67***	-			

RV mid = right ventricular mid-size; RV base = right ventricular basal-size; RVEDVI = indexed right ventricular end diastolic volume; RVS' = right ventricular systolic velocity; TAPSE = tricuspid annular plane systolic excursion; FAC = fractional area change; RVGLS = right ventricular global longitudinal strain; RVGFWS = right ventricular global free wall stain; PG = pulmonary regurgitation pressure gradient; PHT = pulmonary regurgitation pressure half time; LVEDVI = indexed left ventricular end diastolic volume; LVS' = left ventricular systolic velocity; LVGLS = left ventricular global longitudinal strain.* Bold values indicate significant level (p < 0.00.001). ΔRVS' = contractile reserve of right ventricular systolic velocity; ΔFAC = contractile reserve of fractional area change; ΔTAPSE = contractile reserve of tricuspid annular plane systolic excursion; ΔRVEDVI = contractile reserve of indexed right ventricular end diastolic volume; ΔRVGLS = contractile reserve of right ventricular global longitudinal strain; ΔRVGFWS = contractile reserve of right ventricular global free wall stain; ΔLVS' = contractile reserve of left ventricular systolic velocity; ΔLVEDVI = contractile reserve of indexed left ventricular end diastolic volume; ΔLVGLS = contractile reserve of left ventricular global longitudinal strain.* Bold values indicate significant level (p < 0.00.05). ** All values in table represent r values. NS = non-significant.

Table 4
Multivariate linear regression of blood biomarkers predictors of exercise capacity in the entire population.

Multivariable linear regression Outcome variable: VO_2 (ml/min) R^2 = −.60, p.<0.001 TAP (0/1)	B-coefficient	R	p value
Surgical approach (TAP/non-TAP)	−.20	.40	**<0.001**
NT-proBNP (pg/ml)	−9.3	**−.60**	**<0.001**
sST2 (ng/ml)	−7.5	**−.45**	**<0.001**

NT-proBNP = plasma brain natriuretic peptide; sST2 = soluble suppression of tumourigenicity-2. * Bold values indicate significant level (p.<0.0.001).

relationship with sST2 was less strong (r = −0.39, p < 0.001, Table 3). In a multivariate regression model, incorporating surgery type as a factor, the relationship with peak oxygen uptake was improved, and both blood biomarkers were independently associated, with an overall model fit was R^2 = −0.60, p < 0.001 (Table 4, Fig. 3).

Receiver operator characteristics (ROC) showed that an elevated plasma BNP serum and sST2 levels were able to predict reduced functional capacity. The area under the curve for high BNP was 0.69 (p = 0.03, 95 % confidence interval 0.53–0.77, Fig. 4), while AUC for sST2 was 0.53 (p = 0.04, 95 % CI 0.38-0.68, Fig. 4). The optimal cut-off value for the plasma BNP level to detect reduced exercise capacity in this cohort was 90 pg/ml (sensitivity, 74 %; specificity, 63 %, Table 5), and 37 ng/ml for sST2 (sensitivity 65 %; specificity 70 %) (Table 6).

5. Discussion

We have demonstrated that in asymptomatic patients with repaired tetralogy of Fallot NT-proBNP is abnormal in 58 % of patients while sST2 was abnormal in 40 %. The presence of severe PR resulted in an elevated NT-proBNP but not an elevated sST2, while the use of a transannular patch as the surgical technique was associated with elevation of both biomarkers irrespective of the presence of PR. NT-proBNP was associated with RV size and function, while sST2 was influenced by both RV and LV size and function. This pattern was also demonstrated when parameters were assessed during exercise where NT-proBNP was associated with RV contractile reserve, but sST2 was associated with both LV and RV contractile reserve, suggesting that sST2 may be more sensitive to signals from the LV in this group of patients. NT-proBNP showed a better association and detection ability of low VO_2 max, confirming that the observed findings are clinically relevant, tracking directly to functional capacity.

NT-proBNP is known as a diagnostic marker which is the inactive portion of the prohormone that released to the blood stream from cardiomyocytes in response to myocardial stretch resulted from volume overload [18], while sST2 is a prognostic marker and a receptor that comes in two isoforms: transmembrane or cellular (ST2L) and soluble or circulating (sST2). It is a member of the IL-1 receptor family which is secreted by most cells in response to any cellular damage including

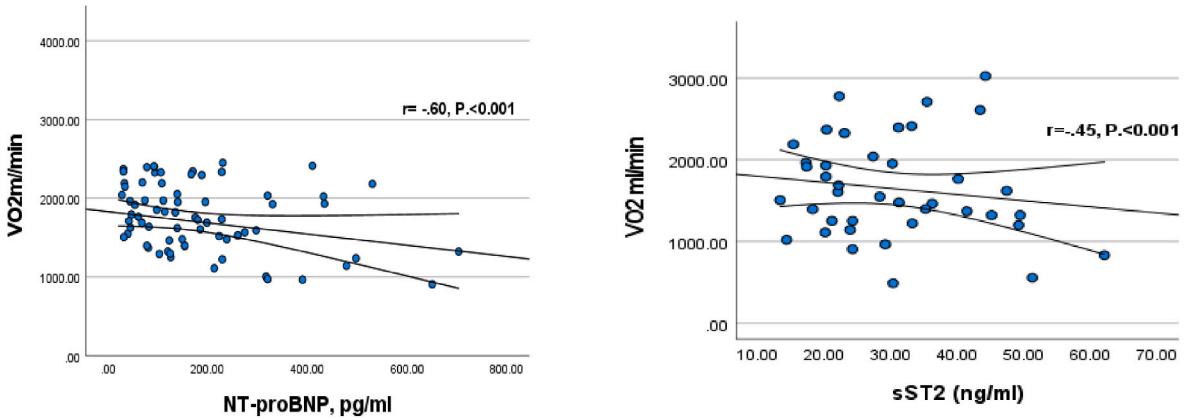

Fig. 3. Scatter plots of blood biomarkers as significant predictors of exercise capacity (overall model fit $R^2 = -0.60$, $p < 0.001$).

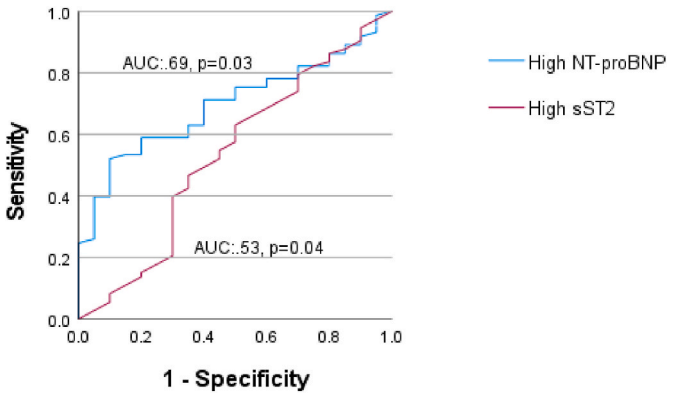

Fig. 4. ROC curve assessing ability of high NT-proBNP and sST2 serum levels to predict functional deterioration. AUC: area under the curve; BNP: B-type natriuretic peptide; ROC: receiver-operating characteristic curve.

myocardial stress [3]. It has stronger ability than BNP in prediction prognosis of heart failure patients and improving risk assessment [4]. The natriuretic peptide has been used to monitor patients with heart failure and coronary artery disease [19], left ventricular hypertrophy and valvular heart disease [20], however, sST2 has more important value in heart failure and other cardiovascular diseases when it comes to prognosis [21]. Combining both biomarkers is recommended for prevention, to guide treatment strategies and for better risk stratification in symptomatic patients suspecting heart failure [22]. These biomarkers are still under investigation in congenital heart disease including patients with repaired TOF. Developing new markers that are sensitive enough to detect ongoing hemodynamic adverse changes, and could help in streamlining care, prognostication and potentially defining treatment strategies is clinically impactful.

NT-proBNP is known to be high in this population [5,6,23], but the underlying cause for this remains unclear. We found that NT-proBNP levels were elevated in asymptomatic patients with repaired TOF with severe PR and in patients where *trans*-annular patch was used as a surgical approach rather than other RVOT intervention such as pulmonary valvotomy or infundibulectomy. This has also been highlighted in our

Table 6
Receiver operating curve for NT-proBNP and sST2 for predicting low exercise capacity.

Parameters	AUC (95 % CI)	P value	Cut-off pg/ml	Sensitivity, %	Specificity, %
High NT-proBNP, (pg/ml)	**.69 (.59–.75)**	**.03**	**90**	**.74**	**.63**
High sST2, (ng/ml)	**.53 (.52–.63)**	**.04**	**37**	**.65**	**.70**

NT-proBNP = plasma brain natriuretic peptide; sST2 = soluble suppression of tumourigenicity-2; AUC: area under the curve.

Table 5
Multivariate linear regression of potential echo predictors of increased levels of biomarkers in the entire population.

Multivariate prediction model 1 (NT-proBNP, pg/ml) $R^2 = .60$. $P < 0.001$, PR (0/1)	B-coefficient	R	P value	Multivariate prediction model 2 (sST2, ng/ml) $R^2 = .40$. $P < 0.001$, PR (0/1)	B-coefficient	R	P value
Pulmonary regurgitation	NS	NS	NS	**Pulmonary regurgitation**	NS	NS	NS
Resting Echo Parameters				**Resting Echo Parameters**			
RV base (mm)	NS	NS	NS	RVGLS (%)	NS	NS	NS
TAPSE (mm)	−.37	**−.47**	**<0.001**	**LVGLS (%)**	**−25**	**−.27**	**<0.001**
RVGLS (%)	NS	NS	NS	–	–	–	–
Exercise echo parameters				**Exercise Echo parameters**			
Δ TAPSE (%)	NS	NS	NS	ΔRVEDVI (%)	NS	NS	NS
Δ RVS' (%)	NS	NS	NS	Δ RVGLS (%)	NS	NS	NS
ΔRVEDVI (%)	**−.58**	**−.60**	**<0.001**	Δ RVGFWS (%)	NS	NS	NS
ΔRVGLS (%)	NS	NS	NS	ΔLVS' (%)	NS	NS	NS
ΔRVGFWS (%)	**−.30**	**−.52**	**<0.001**	**ΔLVEDVI (%)**	**−.12**	**−.40**	**<0.001**
–	–	–	-	**ΔLVGLS (%)**	**−.43**	**−.55**	**<0.001**

RV mid = right ventricular mid-size; TAPSE = tricuspid annular plane systolic excursion; RVGLS = right ventricular global longitudinal strain; ΔTAPSE = contractile reserve of tricuspid annular plane systolic excursion; ΔRVS' = contractile reserve of right ventricular systolic velocity; ΔRVEDVI = contractile reserve of indexed right ventricular end diastolic volume; ΔRVGLS = contractile reserve of right ventricular global longitudinal strain; ΔRVGFWS = contractile reserve of right ventricular global free wall stain; LVGLS = left ventricular global longitudinal strain; ΔLVS' = contractile reserve of left ventricular systolic velocity; ΔLVEDVI = contractile reserve of indexed left ventricular end diastolic volume; ΔLVGLS = contractile reserve of left ventricular global longitudinal strain.* Bold values indicate significant level (p.<0.001)); NS = non-significant.

recent meta-analysis on the available retrospective data which showed that in 1427 rTOF patients, elevated serum levels of NT-proBNP were related to the chosen surgical techniques and ongoing adverse hemodynamic response in this population [24].

Assessment of sST2 assays is a novel approach in cardiovascular medicine, and to date, there is no data that has been specifically evaluated the levels of sST2 in patients with repaired TOF [7,8]. Our result showed that mean sST2 was 29 ± 1 ng/ml for the population with 40 % of subjects were abnormal, with no significant difference between patients with severe pulmonary regurgitation and patients with no loading conditions. Interestingly we did observe higher values of sST2 in patients with TAP surgical approach than in patients with other surgical approaches (31 ± 13 vs 29 ± 15, ng/ml, $p < 0.05$). When compared to the only two published reports in ACHD patients, our sST2 levels were higher (29 vs 26, ng/ml), although these populations were more clinically deteriorated than ours. This suggests that even in asymptomatic patients with repaired TOF who have elevated levels of NT-proBNP are most likely to have increased levels of sST2.

There is ongoing debate about the echocardiographic hemodynamic causes behind the raised levels of NT-proBNP and sST2 in patients with repaired TOF. It might be because the excessive load imposed by severe pulmonary regurgitation, or RV structure and RV volume [25]. The importance of RV volume and size to explain raised levels of NT-proBNP is demonstrated in few investigations [5,23,26,27]. LV dysfunction has also been proposed as a cause for high heart failure biomarkers in TOF patients but the evidence supporting this is currently lacking [25,28]. BNP could be abnormal either because of the structural changes [25], choice of surgical technique [24] or ventricular dependency [27]. Our results showed that resting volume and contractile reserve of RV were associated with abnormal NT-proBNP levels while both RV and LV volume and functional parameters were associated with sST2 increased levels. This suggests that NT-proBNP elevation in this population is more explained by the change in RV volume and function, while sST2 is more reflective of subtle LV changes. The association was more powerful during exercise.

High concentrations of plasma natriuretic peptide are known to be associated with reduced exercise capacity in ACHD [29], with few reports in patients with repaired TOF [30–32]. This study, exclusively in adult patients with repaired TOF showed that the majority of patients who had reduced exercise capacity also had elevated levels of NT-proBNP. This confirms the findings of our recent meta-analysis [24]. Our results also showed such association with sST2, but relatively less than NT-proBNP. The combination was however stronger with exercise ability, considering together with clinical characteristics explaining 60 % of the variability in VO_2. We found that the optimal cut-off values for optimal sensitivity and specificity to predict exercise intolerance were 90 pg/ml for NT-proBNP and 37 ng/ml for sST2. This provides a useful parameter to use in clinical care especially in those unable to undertake formal exercise testing.

5.1. Clinical perspective

This study describes how both BNP and ST2 are elevated in a significant proportion of patients with rTOF. The relationship between increased levels of serum blood biomarkers and the presence of abnormal right and left ventricular remodelling as well as the association with impaired functional capacity suggest an important role in the follow up of these patients. These findings suggest that further investigation into the role of these biomarkers to predict prognosis and drive clinical therapies should be investigated.

5.2. Study limitations

This study is limited by the surgical history of our patients that might be not representative of the whole TOF population. We excluded patients with intermediate severity of pulmonary regurgitation. We specifically did not address the issue of long-term RV remodelling, fibrosis and arrhythmic potential which represent an alternative reason to undertake intervention to reduce PR. But our findings represent the first analysis of the blood biomarkers in asymptomatic patients with repaired TOF investigating their increased levels with the ongoing adverse hemodynamic changes.

6. Conclusions

NT-proBNP and sST2 levels are frequently elevated in asymptomatic patients with repaired TOF. NT-proBNP reflects changes in the RV while sST2 reflects changes in the LV and RV both at rest and on exercise. Incorporating both markers increased the ability to detect functional impairment in this population. Further work on individual, combined biomarkers and how they predict outcome is required.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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