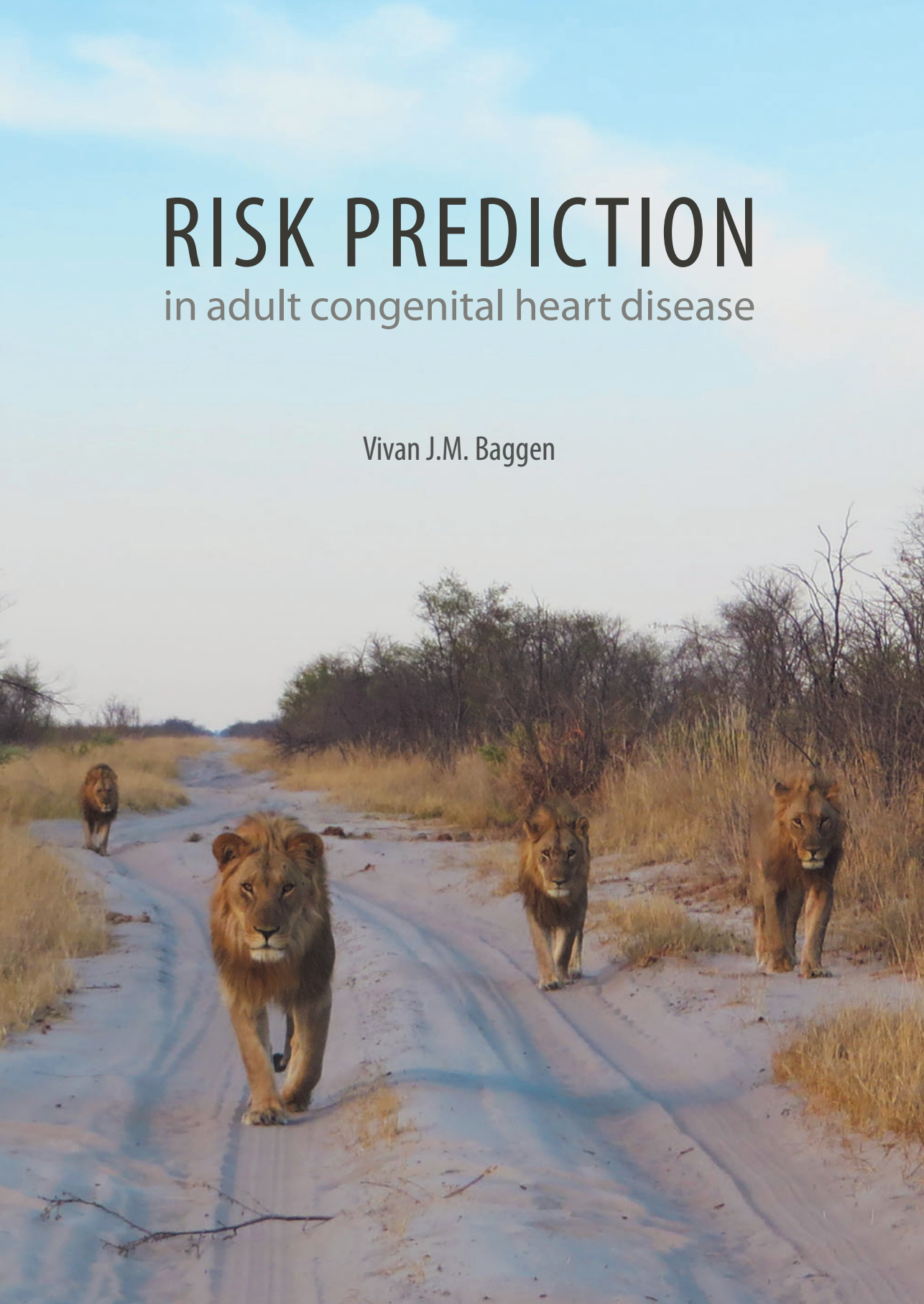
RISK PREDICTION

in adult congenital heart disease

Vivan J.M. Baggen



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Risk Prediction

In adult congenital heart disease

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Prof.dr.ir. H. Boersma

Overige leden: Prof.dr. J.J.M. Takkenberg
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GENERAL INTRODUCTION

Adult congenital heart disease

Worldwide each 2.3 seconds a child is born with a congenital heart defect, which corresponds to approximately 1.35 million newborns with congenital heart disease each year. This is based on a reported birth prevalence of congenital heart defects of ~1%, and 4.3 births every second worldwide.[1, 2] With the great advances in cardiothoracic surgery, postoperative care, and pediatric cardiology over the past decades, the survival of these patients has considerably improved and, consequently, the population of adults with congenital heart disease is steadily growing (Figure 1).[3, 4]

Adult congenital heart disease (ACHD) consist of a wide variety of diagnoses, based on the type of congenital heart defect and the type of corrective surgery that was performed. The complexity of the congenital diagnosis is a major determinant of clinical outcome.[5, 6] At the most favorable end of the spectrum, patients with an isolated repaired atrial or ventricular septal defect and no hemodynamic residuals have an excellent prognosis with a life expectancy equal or close to the general population.[5, 7-10] In contrast, patients with moderate or severe lesions such as tetralogy of Fallot, a systemic right ventricle, or a univentricular heart are at increased risk of complications such as heart failure, arrhythmia, re-interventions and early demise.[5, 11-15] These patients therefore require lifelong follow-up in specialized cardiac centers and are monitored by routine assessments including physical examination, ECG, echocardiography and exercise testing.[16]

A subset of patients with ACHD (~3%)[17] develop pulmonary arterial hypertension, which is defined as an elevated mean pulmonary artery pressure at rest (≥ 25 mmHg) with a low pulmonary capillary wedge pressure (≤ 15 mmHg) as measured by right heart catheterization.[18] Pulmonary hypertension is usually the result of large systemic-to-pulmonary shunts, such as an unrepaired atrial or ventricular septal defect. The subsequent chronic volume overload of the pulmonary vasculature causes adverse vascular remodeling and endothelial dysfunction.[19] Other types of pulmonary hypertension can develop as a result of left heart disease, primarily non-cardiac diseases including obstructive sleep apnea or chronic obstructive pulmonary disease, or chronic pulmonary thromboembolism.[20] Elevated pulmonary pressures have direct impact on the right ventricle, leading to right ventricular hypertrophy, dilatation, and dysfunction. Therefore, these patients are at increased risk of complications and most patients with pulmonary hypertension are followed-up and treated by a multidisciplinary team, including both cardiologists and pulmonologists.[18]

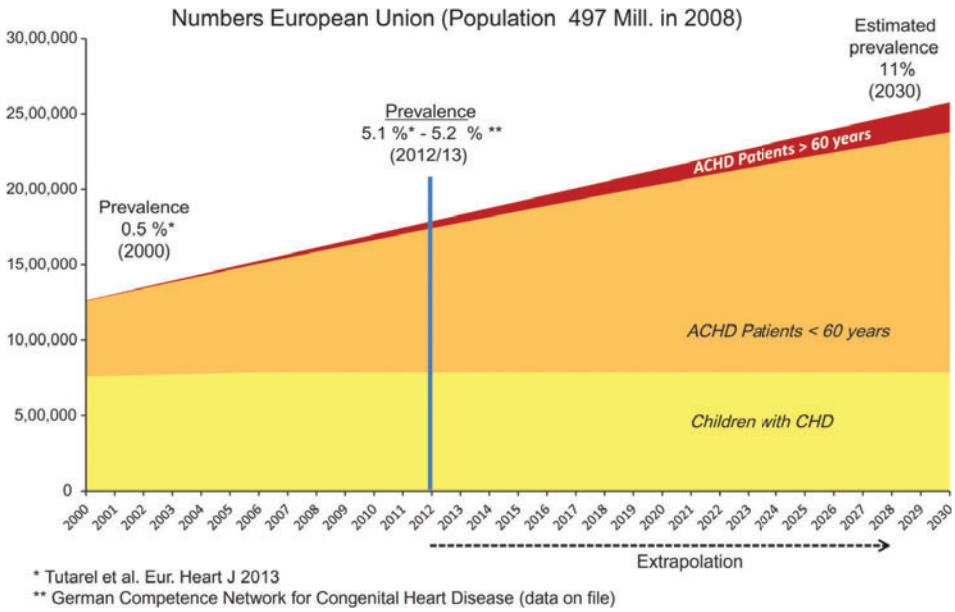

FIGURE 1 - Increasing prevalence of ACHD in the European Union. Reprinted with permission from Eur Heart J 2014;35:683-685; Baumgartner et al.

Risk prediction

Risk can be defined as "a probability of damage, injury, liability, loss, or any other negative occurrence that is caused by external or internal vulnerabilities, and that may be avoided through preemptive action".[21] This general definition directly explains the ultimate reason why physicians continuously strive to foresee a patients' life path: in cases of presumed high risk, interventions will be considered, aimed at preventing adverse events. Moreover, risk prediction is essential to provide accurate patient information, to determine adequate follow-up strategies, and to optimize cost efficiency of healthcare. Elements of the medical history, physical examination, and diagnostic tests (including ECG, echocardiography, cardiac magnetic resonance imaging, exercise testing, and blood biomarkers) may all provide information on which risk prediction can be based. Before the start of this thesis, cardiac function (measured with echocardiography or cardiac magnetic resonance imaging) and exercise capacity (measured with cardiopulmonary exercise testing or 6-minute walking test) were already known to be predictors of outcome in both patients with ACHD[22-24] and in patients with pulmonary hypertension.[25, 26] Patients with an impaired ventricular function or a decreased exercise capacity are more likely to develop heart failure, which is the leading cause of death in both patient groups.[27, 28] Other variables that correlate with cardiac function or exercise capacity may also be useful for risk stratification. To identify potential new prognostic parameters, it

can therefore be worthwhile to investigate the cross-sectional associations with these so-called 'surrogate' endpoints. Subsequently, longitudinal studies should relate these variables to the occurrence of adverse events during clinical follow-up.

A biomarker can be defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[29] Biomarkers, such as an echocardiographic measurement or a molecular substance, gene, enzyme, or hormone, can be used for the diagnosis and staging of disease, as indicators of disease prognosis, or to monitor treatment effect.[30]
Considering the established role of blood biomarkers such as N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitive Troponin-T in the general cardiology field, the potential of these biomarkers in patients with ACHD is huge. Before the start of this thesis, both biomarkers were already part of the pulmonary hypertension guidelines in 2009.[31] However, no prospective and longitudinal studies were available on the prognostic value of these biomarkers in patients with ACHD, and biomarkers did not play a role in the routine work-up of these patients. None of the guidelines that were available on patients with ACHD recommend the clinical use of any biomarker, due to the lack of substantial evidence.

This thesis

Most chapters in this thesis are based on analyses of the BioCon study: a single-center, prospective, observational follow-up study of 602 patients with ACHD, who were consecutively included during their routine visit at the outpatient clinic between 2011 and 2013. At baseline and at four subsequent annual follow-up visits, patients underwent clinical assessment, ECG, echocardiography (every two years), and venous blood sampling for biomarker assessment (Figure 2). Before the start of this thesis, all patients had been included in this cohort, and at baseline measurements had been performed of NT-proBNP,[32, 33] high-sensitive troponin-T,[34] and growth-differentiation factor 15,[35] which were found to be related to measurements of cardiac function and exercise capacity (thesis Jannet Eindhoven). During this thesis, the follow-up of all patients according to the study protocol was continued, and was completed in 2017.

Year 0 (2011-2013)	Year 1 (2012-2014)	Year 2 (2013-2015)	Year 3 (2014-2016)	Year 4 (2015-2017)
Physical examination	Physical examination	Physical examination	Physical examination	Physical examination
12-lead ECG	12-lead ECG	12-lead ECG	12-lead ECG	12-lead ECG
Blood sampling	Blood sampling	Blood sampling	Blood sampling	Blood sampling
Echocardiography		Echocardiography		Echocardiography

FIGURE 2 - Schematic overview of the BioCon study protocol for inclusion and follow-up.

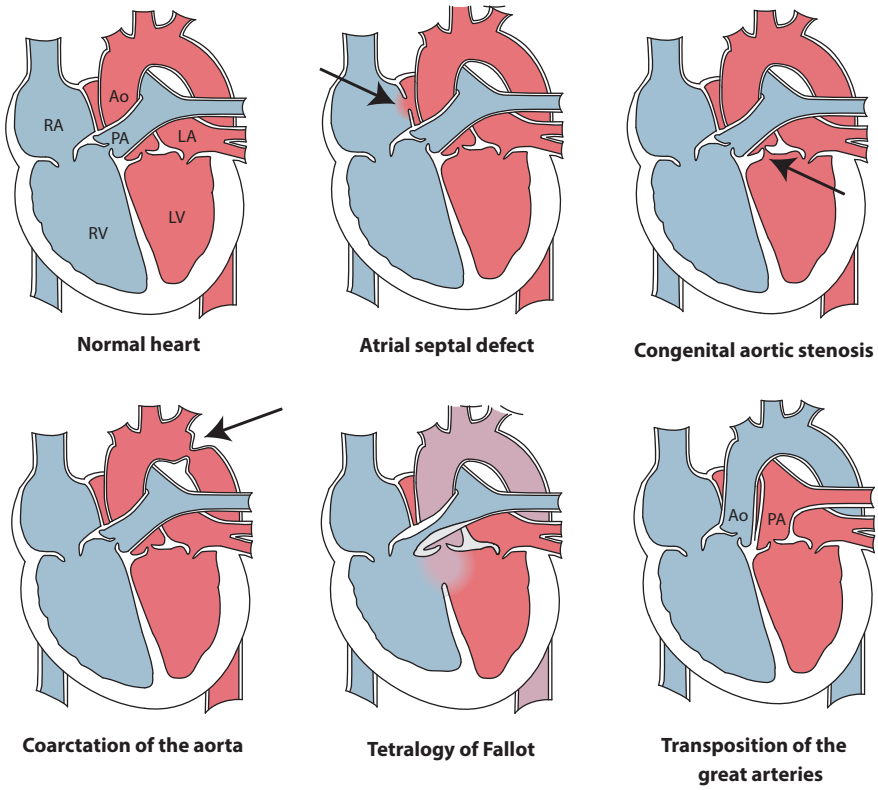

FIGURE 3 - Overview of the main congenital heart defects included in this thesis. Courtesy of dr. M.E. Menting, MD, PhD.

Atrial septal defect: closure of hemodynamically significant defects can be performed either surgically or percutaneously. The repair is sometimes performed in adults, when the atrial septal defect is diagnosed at a later age. Without repair, the left-to-right shunt causes a chronic right-sided volume overload. This may eventually result in right ventricular enlargement, dysfunction and pulmonary arterial hypertension (PAH), which is associated with a worse prognosis.

Congenital aortic stenosis: there may be an obstruction at the valvular level (mostly caused by a bicuspid aortic valve), at the supravalvular level, or at the subvalvular level. Repair can be performed surgically (e.g. resection, valve repair or replacement) or percutaneously (e.g. balloon dilatation or valve replacement).

Coarctation: aortic coarctation can be repaired surgically by an end-to-end anastomosis, subclavian flap angioplasty, bypass graft or patch or percutaneously by an aortic stent.

Tetralogy of Fallot: this cyanotic heart defect is defined by the combination of a ventricular septal defect, overriding aorta, pulmonary artery stenosis and right ventricular hypertrophy. It is repaired in childhood by an interventricular patch and resection of the pulmonary artery stenosis (and back in the days, often a transannular patch).

Transposition of the great arteries: the systemic and pulmonary circulations are completely separated. Surgical repair in the neonatal period is essential, because without any possibility to mix oxygenated with unoxygenated blood (by e.g. a septal defect), this condition is not compatible with life. From the 1960s, surgical repair was performed by an atrial switch operation (Mustard/Senning): unoxygenated blood is redirected towards the left ventricle, and oxygenated blood is redirected to the right ventricle which pumps the blood into the systemic circulation. The current treatment of choice is anatomical correction by arterial switch operation, as described for the first time in 1976.

The BioPulse study is an ongoing prospective observational cohort study of consecutive patients with pulmonary hypertension who were screened in our center from 2012 up to now. All patients underwent clinical assessment, ECG, echocardiography, computed tomography, right heart catheterization, and venous blood sampling at study inclusion and are two-yearly followed at the outpatient clinic. Before the start of this thesis, 53 patients had already been included. During this thesis, the inclusion and follow-up was continued and the analysis of multiple baseline biomarker measurements in the first 104 patients was performed. Other projects which are embedded in this thesis are based on separate cross-sectional or retrospective cohort studies. In Figure 3, an overview of the main congenital heart defects that are included in this thesis is shown.

Aim

The aim of this thesis is to establish novel prognostic tools that can be used for the risk stratification of patients with ACHD or pulmonary hypertension.

OUTLINE OF THE THESIS

Part I and II focus on separate tools that are of potential importance in the risk stratification of patients with ACHD and/or pulmonary hypertension: imaging by echocardiography and cardiac magnetic resonance imaging (Part I), and blood biomarkers (Part II). The combination of multiple clinical characteristics, imaging findings and blood biomarkers in order to derive clinically useful risk predictions is described in Part III. A schematic overview of the study design, study cohorts, exposure and outcome of all chapters that are part of this thesis is provided in Table 1.

TABLE 1 - Schematic outline of the thesis.

	Study design				**Study cohort**		**Exposure** (potential prognostic tools)			**Outcome** (surrogate endpoints)			
	Cross-sectional cohort	Retrospective cohort	Prospective cohort	Review	ACHD	PAH	CMR	Echocardiography	Biomarkers	Ventricular function	Exercise test	Presence of PAH	Clinical endpoints
Part I - Imaging													
Chapter 2	●				●	●	●			●			
Chapter 3	●				●		●			●	●		
Chapter 4			●		●			●					●
Chapter 5				●	●	●		●				●	
Chapter 6		●			●	●		●				●	●
Chapter 7				●		●		●					●
Chapter 8				●		●	●						●
Part II – Blood biomarkers													
Chapter 9	●				●				●	●	●		
Chapter 10			●		●				●	●			●
Chapter 11			●		●				●	●			●
Chapter 12			●		●				●	●			●
Chapter 13			●		●				●				●
Chapter 14			●			●			●	●			●
Part III - Risk prediction													
Chapter 15				●	●	●	●	●	●				●
Chapter 16		●	●		●			●	●				●
Chapter 17				●	●	●	●	●	●	●	●	●	●

Part I – Imaging

Right ventricular function and exercise capacity are established prognostic markers in ACHD or pulmonary hypertension; however, accurate measurements can be difficult due to trabeculae.[16, 18] **Chapter 2** describes the impact of a novel method to deal with trabeculae in the measurement of right ventricular volumes and function with cardiac magnetic resonance imaging (CMR), in order to improve the accuracy and reproducibility of these measurements in patients with pressure overloaded right ventricles. The

potential prognostic value of specific imaging findings within congenital diagnostic groups is evaluated in **Chapter 3**; which focuses on the cross-sectional association between pulmonary artery size and exercise capacity in patients after arterial switch operation, and in **Chapter 4**; which focuses on the association of left atrial size and function with clinical outcome during prospective follow-up in adults with repaired tetralogy of Fallot.

Pulmonary arterial hypertension may develop in patients with an atrial septal defect and is associated with a worse prognosis. The prevalence of pulmonary hypertension before and after atrial septal defect closure at adult age is reviewed in **Chapter 5**, and is investigated using retrospective echocardiographic analysis of right ventricular pressures in **Chapter 6**. Echocardiographic findings and CMR findings that can be useful for the stratification in patients with pulmonary arterial hypertension are reviewed in **Chapter 7** and **Chapter 8**, respectively.

Part II – Blood biomarkers

The cross-sectional association of matrix metalloproteinases with cardiac function and exercise capacity in patients with ACHD is described in **Chapter 9**. The prognostic value of N-terminal pro-B-type natriuretic peptide, troponin-T, and growth-differentiation factor 15, galectin-3, and red cell distribution width in patients with ACHD is prospectively investigated and described in **Chapter 10**, **Chapter 11**, and **Chapter 12**, respectively. In **Chapter 13**, we evaluate repeated N-terminal pro-B-type natriuretic peptide measurements in patients with ACHD and relate these to the occurrence of clinical events. **Chapter 14** describes the prospectively investigated prognostic value of multiple biomarkers including N-terminal pro-B-type natriuretic peptide, troponin-T, growth-differentiation factor 15, and galectin-3 in adults with pulmonary hypertension due to different etiologies.

Part III – Risk prediction

In **Chapter 15**, an overview is provided of the wide range of factors that could be useful to predict adverse clinical outcome in the entire cohort of patients with ACHD and within specific congenital subgroups, including components of the medical history, physical examination, ECG, echocardiography, presence of pulmonary arterial hypertension, cardiac magnetic resonance imaging, exercise testing, and biomarkers. In **Chapter 16** we combined a set of clinically relevant predictors into a validated risk prediction model for ACHD. **Chapter 17** provides a summary and general discussion of all findings, and formulates implications for future research.

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PART I

IMAGING BIOMARKERS

Chapter 02

Pressure overloaded right ventricles: a multicenter study on the importance of trabeculae in RV function measured by CMR

Vivan J.M. Baggen,* Mieke M.P. Driessen,* Hendrik G. Freling, Petronella G. Pieper, Arie P.J. van Dijk, Pieter A. Doevendans, Repke J. Snijder, Marco C. Post, Folkert J. Meijboom, Gertjan Tj. Sieswerda, Tim Leiner,* Tineke P. Willems*

*Equal contributions

Int J Cardiovasc Imaging. 2014;30:599-608.

ABSTRACT

Background Cardiac magnetic resonance (CMR) imaging is the preferred method to measure right ventricular (RV) volumes and ejection fraction (RVEF). This study aimed to determine the impact of excluding trabeculae and papillary muscles on RV volumes and function in patients with RV pressure and/or volume overload and healthy controls and its reproducibility using semi-automatic software.

Methods Eighty patients (pulmonary hypertension, transposition of the great arteries after arterial switch operation and after atrial switch procedure and repaired tetralogy of Fallot) and 20 controls underwent short-axis multislice cine CMR. End-diastolic volume (EDV), end-systolic volume (ESV), RV mass and RVEF were measured using 2 methods. First, manual contour tracing of RV endo- and epicardial borders was performed. Thereafter, trabeculae were excluded from the RV blood volume using semi-automatic pixel-intensity based software. Both methods were compared using a Student *T* test and 25 datasets were reanalyzed for reproducibility.

Results Exclusion of trabeculae resulted in significantly decreased EDV, ranging from -5.7 ± 1.7 mL/m^2 in controls to -29.2 ± 6.6 mL/m^2 in patients after atrial switch procedure. RVEF significantly increased in all groups, ranging from an absolute increase of $3.4 \pm 0.8\%$ in healthy controls to $10.1 \pm 2.3\%$ in patients after atrial switch procedure. Interobserver agreement of method 2 was equal to method 1 for RVEDV, RVESV and RVEF and superior for RV mass.

Conclusions In patients with overloaded RVs exclusion of trabeculae from the blood volume results in a significant change in RV volumes, RVEF and RV mass. Exclusion of trabeculae is highly reproducible when semi-automatic pixel-intensity based software is used.

INTRODUCTION

Both in patients with pulmonary hypertension (PH) and in patients with different types of congenital heart disease (CHD), the right ventricle (RV) performs under increased pressure loading. The RV adapts by hypertrophying, however at a certain point the RV is unable to cope with the increased pressures and RV failure will ensue. Consequently, RV function is an important determinant of prognosis and of therapeutic strategy in these patients. For instance, in patients with pulmonary valvular (PV) stenosis, timing of intervention is partly dependent on RV function.[1] In patients with PH, deterioration of right ventricular ejection fraction (RVEF), increased RV end-diastolic volume (RVEDV) and stroke index are associated with poor outcome.[2,3] Furthermore, for patients with transposition of the great arteries (TGA) after an atrial switch operation, in which the RV supplies the systemic circulation (i.e. systemic RV), decline in RV function is one of the most important clinical problems. Therefore, RV volumes and function are frequently used in follow-up of these patients, making accurate and reproducible measurements highly important.

As both 2D and 3D echocardiography of the RV remain less reproducible than cardiac magnetic resonance imaging (CMR), the latter is still considered to be the reference standard for the quantification of RV volumes and EF.[4-7] Whether trabeculae and papillary muscles should be included or excluded from the blood volume is subject of debate. Throughout literature both methods are used.[2,8-10] However, many studies have not clearly described whether trabeculae and papillary muscles were included or excluded from the RV blood volume.[11-14] The impact of trabeculae is assumed to be small in healthy individuals, but Winter et al. showed that exclusion of trabeculae from the RV blood volume resulted in a substantial difference of RVEDV, RVESV and RVEF in patients with a systemic right ventricle.[15] Although theoretically more accurate, Winter et al. also showed that manual tracing of trabeculae has low reproducibility and therefore can be considered less favorable for longitudinal follow-up.[5,15]

Freling et al. recently reported that semi-automatic pixel-intensity based segmentation software is able to exclude trabeculae and papillary muscles from the RV blood volume with high reproducibility in tetralogy of Fallot (ToF) patients with predominantly volume overloaded RVs. Moreover, this resulted in a substantial difference in RV volumes and RVEF compared to the method that includes these structures in the RV blood volume.[16] In patients with increased RV pressure the trabeculae are likely to be coarser. The impact and reproducibility of excluding trabeculae and papillary muscles with this semi-automatic software in patient groups with RV pressure overload has not been investigated up to now.

The purpose of this multicenter study was to determine the impact of excluding trabeculae and papillary muscles, on RV volumes and function as assessed by CMR in

patients with pressure or combined pressure and volume overload of the RV and healthy controls. Secondly, we aimed to determine the reproducibility of this methodology when semi-automatic pixel-intensity based software is used.

METHODS

Study design and population

One hundred CMR studies were included in the analysis (median age 36.2 years, 51% male). Four groups of 20 adult patients with pressure overloaded RVs were analyzed: patients with pre-capillary PH, patients with right ventricular outflow tract obstruction (RVOTO) after arterial switch operation (ASO) for TGA, patients with repaired ToF and patients with TGA and atrial switch procedure (Mustard or Senning operation). A reference group of 20 healthy controls was also included.

PH was defined in accordance with the ESC/ESR guidelines as a mean pulmonary artery pressure of ≥ 25 mmHg and a pulmonary capillary wedge pressure of ≤ 15 mmHg.[17] Only patients with pre-capillary (i.e. with arterial vascular changes) PH were included, all were diagnosed with either chronic trombo-embolic or idiopathic PH. In all patients RV systolic pressure (RVSP) was measured using Doppler echocardiography on the day of CMR investigation. RVSP was measured using the peak velocity of tricuspid regurgitation plus estimated right atrial pressure. Patients with repaired ToF were included if a RVSP of ≥ 36 mmHg was measured by Doppler echocardiography.[18] Patients after ASO were included if RVSP measured by Doppler echocardiography was ≥ 36 mmHg or if, using Doppler echocardiography, a mild or moderate RVOTO was measured, defined as a maximum gradient of ≥ 25 mmHg. For patients with TGA and atrial switch procedure systolic blood pressure was used to determine RVSP. Basic patient characteristics for each patient group are illustrated in Table 1. Degree of pulmonary (PR) and tricuspid valve regurgitation (TR) were assessed semi-quantitatively with echocardiography, based on color-Doppler and continuous wave Doppler pattern and graded as: none or trace, mild, moderate or severe.

In this retrospective study, CMR images from two tertiary referral hospitals were analyzed. One centre contributed 59 patient CMR datasets and 20 control subjects. The second centre provided the remaining 21 patient CMR datasets. The datasets in this study were obtained between May 2008 and July 2012. Prior to analysis, all patient and control data were encoded to preserve anonymity. All CMR datasets were acquired in a routine clinical setting and anonymized for analysis. The medical ethics committees waived the need for informed consent.

TABLE 1 - Baseline characteristics of each patient group and healthy controls.

	PH n = 20	ASO n = 20	TOF n = 20	Atrial switch n = 20	Controls n = 20
Male gender	7 (35)	11 (55)	11 (55)	12 (60)	10 (50)
Age, year	55.0 ± 14.1	24.9 ± 4.0	29.1 ± 7.8	33.0 ± 6.3	36.7 ± 10.1
BSA, m^2	1.93 ± 0.18	1.88 ± 0.18	1.87 ± 0.19	1.96 ± 0.21	1.88 ± 0.21
RVSP, mmHg	54 [37–65] *n = 20*	40 [37–53] *n = 15*	45 [41–50] *n = 20*	120 [106–125] *n = 20*	-
RVOT gradient, mmHg	-	35 [29–42] *n = 8*	33 [30–40] *n = 15*	-	4 [3–8] *n = 20*
TR grade					
- No/trace	7 (35)	12 (60)	10 (50)	1 (5)	20 (100)
- Mild	9 (45)	7 (35)	7 (35)	14 (70)	-
- Moderate	4 (20)	1 (5)	3 (15)	4 (20)	-
- Severe	-	-	-	1 (5)	-
- Missing	-	-	-	-	-
PR grade*					
- No/trace	14 (70)	14 (70)	9 (45)	13 (65)	20 (100)
- Mild	6 (30)	3 (15)	4 (20)	2 (10)	-
- Moderate	-	-	-	-	-
- Severe	-	-	6 (30)	-	-
- Missing	-	2 (10)	1 (5)	5 (25)	-

Data is presented as n (%), mean ± SD or median [IQR]. *Aortic regurgitation grade in patients after atrial switch. **Abbreviations:** ASO, arterial switch operation; BSA, body surface area; PH, pulmonary hypertension; PR, pulmonary regurgitation; RVOT, right ventricular outflow tract; RVSP, right ventricular systolic pressure; TOF, tetralogy of Fallot; TR, tricuspid regurgitation.

CMR imaging protocol

Datasets were obtained using commercially available 1.5 T MR scanners (Ingenia R4.1.2; Philips Healthcare, Best, The Netherlands (n = 79); Magnetom Sonata, Siemens Healthcare; (n = 7) and Magnetom Avanto; Siemens Healthcare, Erlangen, Germany (n = 14)). For all studies dedicated chest or torso phased array parallel-imaging capable surface coils were used with 12–28 elements. CMR images were acquired during repeated end-expiratory breath holds. Cine images were acquired using a retrospectively gated balanced steady state free precession sequence with 25–30 cardiac phases per cardiac cycle. Slice thickness used were 6 mm with 4 mm gap (n = 21) and 8 mm with 0 mm gap (n = 79). Sequences included multi-slice, multi-phase cine short-axis, longitudinal four-chamber, vertical two-chamber and RV outflow views. The multi-slice cine short-axis acquisitions were planned from above the mitral valve up to and including the cardiac apex. The following ranges of other scan parameters were used: TR 2.7–3.4 ms; TE 1.1–1.7 ms; flip angle 80º–90º; matrix 171–192; voxel size: 1.25 x 1.25 x 8.0 mm and 1.7 x 1.7 x 6.0 mm. Parallel imaging factors varied between 0–3.

CMR image analysis

Image analysis was performed using Qmass MR Research edition version 7.4.14.0 (Medis, Leiden, the Netherlands).[16] Segmentation was performed on end-diastolic and end-systolic phases only. The end-diastolic and end-systolic phases were selected by visual assessment as the phases with the largest and smallest RV cavity sizes respectively, taking into account the longitudinal four-chamber, vertical two-chamber and RV outflow tract as reference views. If visual assessment was difficult, multiple frames were contoured to determine the correct end-diastolic or end-systolic phase. Using a previously described RV analysis protocol the RV epicardial and endocardial contours were manually traced from the most apical to the most basal short-axis slice.[19] Only the portion of the outflow tract below the pulmonary valve was included in the blood volume in the basal slice in which the pulmonary valve was visible. If more than 50% of the tricuspid annulus or atrium was visible in a basal slice the valve area was excluded from the blood volume. Epicardial and endocardial contours overlapped at valve borders and septum, as the septum was considered part of the left ventricle. For patients with a systemic RV, the septum was considered to be part of the RV and included in the RV myocardial volume.

Based on the methodology described above, two methods were used for determining RV volumes, function and mass. With method 1 trabeculae and papillary muscles were included in the blood volume. With method 2, trabeculae and papillary muscles were excluded from the blood volume and added to the myocardial volume (Figure 1). For both methods the volume between the endo- and epicardial contour was considered as myocardial volume. Selection of trabeculae and papillary muscles was done using semi-automatic pixel-intensity based segmentation software. The segmentation software is based on the signal intensity distribution of MR images and has been described in detail by Freling et al.[16] In brief, voxels within the epicardial contour are classified as either blood volume or myocardial volume according to their signal intensity, taking into account spatial variations in signal intensity. Based on this algorithm, trabeculae and papillary muscles were excluded from the blood volume and included in the myocardial volume. The algorithm works similar for images generated by the different scanners used in this study. It was possible to manually change the threshold for every slice, in order to select the same trabeculae in end-diastole and end-systole. Observers selected only trabeculae with a signal intensity similar to the intensity of the RV myocardium. Individual voxels could also be selected or deselected in case of artifacts due to nonlaminar flow.

For both methods, RV volumetric parameters were calculated by the sums of the traced contours multiplied by slice thickness in all short-axis slices. For method 1 the volume of trabeculae and papillary muscles was included in the RV blood volume and for method 2 this was excluded from the blood volume. Stroke volume (SV) was defined

as the difference between end-diastolic volume (EDV) and end-systolic volume (ESV). All volumetric data were indexed for body surface area (BSA), which was calculated using the Dubois-Dubois formula (0.20247 x height(m)$^{0.725}$ x weight(kg)$^{0.425}$). EF was calculated by SV / EDV * 100%. For method 1 myocardial volume was defined as epicardial minus the endocardial contour, for method 2 end-diastolic trabecular volume was added to the myocardial volume. RV mass was quantified by multiplying the specific density of myocardium (1.05 g/mL) with the end-diastolic myocardial volume.

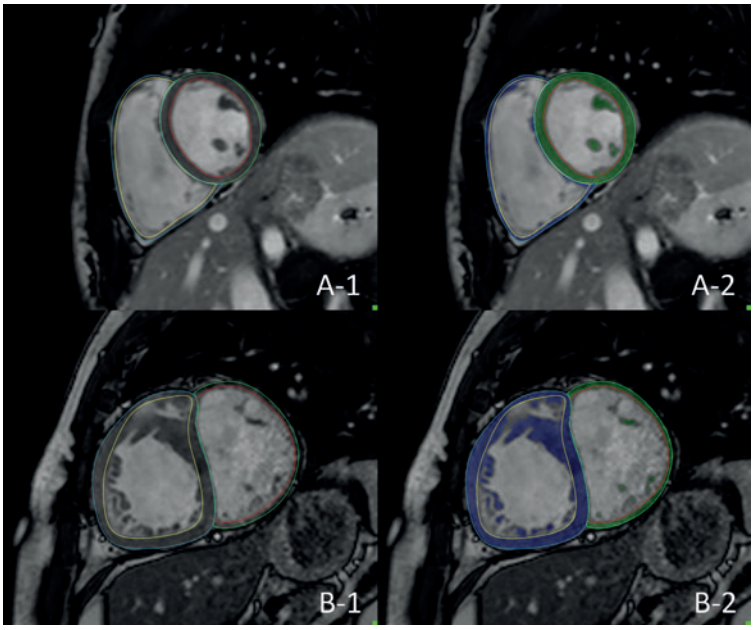

FIGURE 1 - RV contour tracing only (A-1 and B-1) and with semiautomatic selection of trabeculae (A-2 and B-2).

Two methods of measuring RV volumes in a healthy control (A) and patient after atrial switch procedure (B). Method 1: inclusion trabeculae in the blood volume (A-1 and B-1); Method 2: exclusion of trabeculae from the blood volume, using identical endocardial contours (A-2 and B-2).

Reproducibility

Intraobserver reproducibility of both methods was assessed by reanalyzing 5 randomly selected CMR datasets from every patient group, as well as the healthy control subjects by the primary observer. In total 25 datasets were reanalyzed. To determine interobserver variability a second observer reanalyzed the same 25 datasets. Observers were unaware of the results of the first analysis and there was an interval of at least two weeks between the first and second analysis. The observers had equal experience in RV volumetric analysis and received the same training for Qmass MR research edition.

Statistical analysis

Continuous data were expressed as median and interquartile range (IQR) or mean value ± standard deviation (SD) as appropriate. Mean differences ± SD between method 1 and 2 were calculated for RVEDV/m^2, RVESV/m^2, RVSV/m^2, RVEF and RV mass/m^2, using the paired Student's *T*-test. Differences in RVEDV/m^2, RVESV/m^2 and RVEF found in the patient groups were compared to the healthy control group using a one-way ANOVA with posthoc Dunnett's test. For the one-way ANOVA data underwent logarithmic transformation if necessary (i.e. if homogeneity of variances was inequal). Intra- and interobserver agreement were assessed using Bland-Altman plots and intraclass correlation coefficients (ICC). Paired Student's *T*-test was used to test for significant differences between observer 1 and 2 and between the first and second measurements of observer 1. Mean differences ± SD for all measurements were calculated. Lastly to compare reproducibility of both methods the inter- and intraobserver agreement coefficient (AC) of method 1 and 2 were calculated for each measurement. The AC was calculated using the following formula: $AC = 100 * (1 - 2 * |Obs_1 - Obs_2| / Obs_1 + Obs_2)$; in which Obs_1 and Obs_2 are the first and the second observation (or observer). The AC calculated for method 1 and 2 were compared using a paired Wilcoxon signed rank test. Using a Bonferroni correction for multiple measurements *p*-values of < 0.01 were considered statistically significant. Data analysis was performed in IBM SPSS statistics version 20.0 (IBM SPSS, Chicago, IL).

RESULTS

Exclusion of trabecular volume

RVEDV/m^2, RVESV/m^2, RVEF and RV mass/m^2 measured including (method 1) and excluding (method 2) RV trabeculae from the RV blood volume (method 2) are listed in Table 2. For all patient groups and for healthy controls, exclusion of trabeculae and papillary muscles from the blood volume resulted in a significantly decreased RVEDV/m^2 and RVESV/m^2 and a significantly increased RVEF and RV mass/m^2 (Table 2). Of note, the differences in EDV/m^2, ESV/m^2, RVEF, and RV mass between both methods were most pronounced in the patients after atrial switch procedure and least pronounced in the PH patients, with mean absolute differences in EF of 10.1 ± 2.3% and 4.7 ± 1.6%, respectively. In healthy controls an absolute increase in RVEF of 3.4 ± 0.8% was measured. Of note, the differences in EDV/m^2, EDV/m^2, RVEF and RV mass were significantly larger in all patient groups compared with the healthy controls ($p < 0.01$).

TABLE 2 - RV volumes and function.

	PH (mean ± SD)	ASO (mean ± SD)	TOF (mean ± SD)	Atrial switch (mean ± SD)	Controls (mean ± SD)
RVEDV (mL/m²)					
Method 1	117.4 ± 31.8	99.4 ± 23.3	147.0 ± 42.5	139.9 ± 33.6	96.9 ± 18.9
Method 2	105.1 ± 28.4	88.3 ± 21.2	124.8 ± 38.0	110.7 ± 28.7	91.2 ± 17.8
Difference	–12.3 ± 4.6†	–11.1 ± 3.3†	–22.2 ± 6.0†	–29.2 ± 6.6†	–5.7 ± 1.7†
RVESV (mL/m²)					
Method 1	75.4 ± 30.0	50.1 ± 13.3	85.5 ± 27.8	85.9 ± 26.2	47.5 ± 11.5
Method 2	62.7 ± 25.9	39.2 ± 11.3	63.7 ± 23.2	57.1 ± 21.7	41.6 ± 10.5
Difference	–12.7 ± 4.8†	–10.9 ± 3.3†	–21.8 ± 6.0†	–28.8 ± 6.5†	–5.9 ± 1.5†
RVSV (mL/m²)					
Method 1	42.0 ± 7.9	49.3 ± 11.7	61.5 ± 19.4	54.0 ± 14.9	49.4 ± 8.6
Method 2	42.4 ± 8.0	–0.2 ± 0.6	61.0 ± 19.6	53.6 ± 14.7	49.6 ± 8.6
Difference	–0.4 ± 0.6*	–0.2 ± 0.6	–0.4 ± 0.8*	–0.4 ± 0.9	0.2 ± 0.5
RV mass (gr/m²)					
Method 1	18.5 ± 5.5	20.1 ± 5.0	25.4 ± 7.1	43.3 ± 9.1	13.0 ± 3.0
Method 2	31.4 ± 9.8	31.1 ± 7.5	48.7 ± 12.3	73.9 ± 15.4	19.0 ± 4.2
Difference	12.9 ± 4.9†	11.0 ± 4.8†	23.3 ± 6.3†	30.6 ± 6.9†	6.0 ± 1.8†
RVEF (%)					
Method 1	37.2 ± 8.5	49.6 ± 5.0	42.1 ± 6.9	39.2 ± 7.8	51.3 ± 3.8
Method 2	41.9 ± 9.1	55.8 ± 5.1	49.4 ± 12.3	49.3 ± 9.7	54.7 ± 4.1
Difference	4.7 ± 1.6†	6.1 ± 1.7†	7.2 ± 1.7†	10.1 ± 2.3†	3.4 ± 0.8†

RV volume, mass and ejection fraction measured with inclusion (method 1) and exclusion of trabeculae from the RV blood volume (method 2). All volumetric data are indexed for BSA. **Legend:** *$p < 0.05$ using paired Student's *T*-test; †$p < 0.001$ using paired Student's *T*-test. **Abbreviations:** ASO, arterial switch operation; PH, pulmonary hypertension; RVEDV, right ventricular end-diastolic volume; RVEF; right ventricular ejection fraction; RVESV, right ventricular end-systolic volume; RVSV, right ventricular stroke volume; SD, standard deviation; TOF, tetralogy of Fallot.

Reproducibility

For both methods, inter- and intraobserver agreement was high in all measurements, as illustrated by high ICCs with small limits of agreement (Table 3; Figure 2). For both methods, RVEDV, RVESV and RV mass showed significant differences between repeated measurements. However, mean differences were small and considered not clinically relevant. In Figure 2, Bland-Altman plots show interobserver variability for RVESV, RVEDV and RVEF for both methods. For RVEDV, RVESV and RV mass the limits of agreement were narrower when trabeculae and papillary muscles were excluded from the RV blood volume (method 2). The inter- and intraobserver AC of both methods was not

statistically significantly different for RVEDV, RVESV and RVEF ($p > 0.1$). Method 2 had a significantly better interobserver AC than method 1 for RV mass measurement, with a median (IQR) interobserver AC of respectively 94.1 (92.1–97.1)% and 77.2 (72.1–82.6)%.

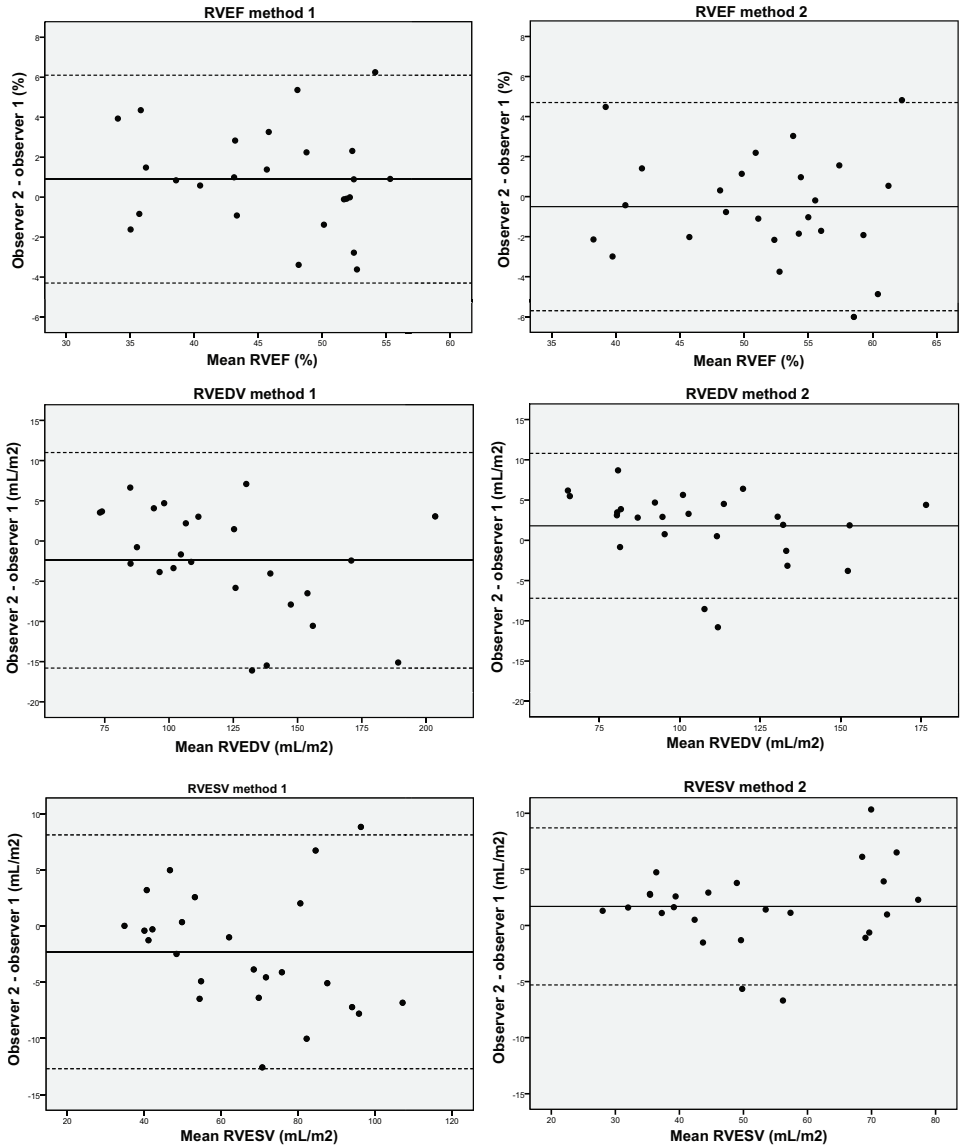

FIGURE 2 - Bland-Altman plots for method 1 and method 2.

Bland-Altman plots showing the mean value of both observers on the x-axis and absolute differences between the observers on the y-axis for each paired observation. Limits of agreement are defined as the mean difference ± 2 SD.

TABLE 3 - Inter- and intraobserver agreement.

	Interobserver (Obs_2 – Obs_1^I)			Intraobserver (Obs_1^{II} – Obs_1^I)		
	ICC	**Mean difference ± SD**	***P*-value**	**ICC**	**Mean difference ± SD**	***P*-value**
RVEDV (mL/m²)						
Method 1	0.981	–2.4 ± 6.7	0.089	0.990	2.8 ± 5.1	0.012
Method 2	0.987	1.8 ± 4.5	0.059	0.985	3.0 ± 5.0	0.006
RVESV (mL/m²)						
Method 1	0.970	–2.3 ± 5.2	0.039	0.982	1.1 ± 4.2	0.209
Method 2	0.974	1.7 ± 3.5	0.027	0.969	1.0 ± 3.8	0.194
RVEF (%)						
Method 1	0.934	0.9 ± 2.6	0.086	0.965	0.4 ± 1.8	0.241
Method 2	0.934	–0.5 ± 2.6	0.354	0.954	0.6 ± 2.2	0.189
RV mass (gr/m²)						
Method 1	0.965	5.8 ± 3.5	0.000	0.983	0.5 ± 2.3	0.283
Method 2	0.993	1.4 ± 2.6	0.012	0.990	0.3 ± 3.2	0.694

P-value obtained using paired student *T*-test. **Abbreviations:** ICC, intraclass correlation coefficient; Obs_2 = second observer; Obs_1^I = first measurement of the first observer; Obs_1^{II} = second measurement of the first observer; RVEDV, right ventricular end-diastolic volume; RVEF; right ventricular ejection fraction; RVESV, right ventricular end-systolic volume; SD, standard deviation.

DISCUSSION

Exclusion of trabeculae and papillary muscles resulted in substantial alterations of RV volumes, RVEF and RV mass in a wide range of patient populations with pressure and volume overloaded RVs. Furthermore, we found that these differences in RV parameters vary widely depending on the exact condition underlying RV overload. Although prior studies already established this fact in general terms, the major impediment to widespread adoption of this method in clinical practice was the lack of a fast and reproducible way to measure the exact amount of RV trabeculae and papillary muscles. We found that exclusion of RV trabeculae using semi-automatic pixel-intensity based software resulted in fast and highly reproducible RV measurements. This is opposed to manual tracing of trabeculae which has previously been shown to be unreliable.[15,20]

Accurate and reproducible measurement of RV volume and function is mandatory because of the prognostic and therapeutic implications in patients with PH and CHD.[1,3,21,22] The current study underscores that exclusion of trabeculae has a significant impact on RV volumes, RVEF and RV mass in both CHD and PH patients with overloaded RVs. Moreover, the impact of excluding trabeculae varied widely between patient groups, from a change in RVEDV of –12.3 ± 4.6 mL/m² in PH patients to –29.2 ± 6.6 mL/

m^2 in patients with a systemic RV. Healthy controls also exhibited significant differences in all RV measurements, but these were significantly smaller ($p < 0.01$) compared to the differences observed in patient groups. Consequently, RV volume and function in most patients will be closer to or in the normal range after exclusion of trabeculae from the RV blood volume.

Currently, there is no clear standard for RV volumetric analysis or consensus on how trabecular structures should be handled. Major obstacles to exclude trabeculae and papillary muscles from the RV blood volume have been the time investment of performing manual tracing of these structures and the low reproducibility.[15,20] Several studies in CHD patients differ on the point of in- or excluding RV trabeculae and papillary muscles from the RV blood volume[3,4,11,12,20,22,23] or are not clear about the methodology used.[13,14] In the current study we found that using semi-automatic pixel-intensity based segmentation software results in highly reproducible RV volumetric measurements. Because in- or exclusion of trabeculae has a major impact on RV parameters as measured with CMR, studies using different methodologies are incomparable. Application of the method described in this study may be a step forward to achieve uniformity of RV volumetric measurements, which is important to compare the effect of interventions aimed at preserving or improving RV function. However, there are only few reports using this new methodology and it is of great importance that new studies are undertaken to determine clinically relevant cut-off values using this semi-automated method.

When comparing the current study to prior studies investigating the impact of trabeculae and papillary muscles on RV volume and function, some important differences can be observed. Winter et al. studied 29 patients with systemic right ventricles and found an increase in RVEF of 7.4 ± 3.9 % compared to 10.1 ± 2.3 % in our report. In contrast to our results, which are based on semi-automated pixel-intensity based segmentation, manual exclusion of trabeculae was substantially less reproducible in the study of Winter et al.[15] Moreover, both our study and the study by Freling et al. even demonstrated a higher reproducibility for RVEDV and RV mass, using this semi-automatic method to exclude trabeculae compared with only endocardial contour tracing.[16] We attribute this finding to observer variation in handling of trabeculae adjacent to the endocardial border. This can result in small differences for endocardial contour tracing, which will be rectified if all trabeculae are excluded. Sievers et al. studied the effect of trabeculae on RV volumes in healthy controls and reported a difference in RVEF of only 1.72% compared with 3.4 ± 0.8% in our study, however baseline RVEDV values also differed considerably with ours, indicating that these study populations are not comparable.[24] Freling et al. investigated a different group of ToF patients with volume overloaded RVs, using the same software package as described in the current study, and found a similar increase in RVEF of 7 ± 4 % versus 7 ± 2% in our study.[16]

The current study only focused on one of the possible sources of error in RV volumetric assessment with CMR. An important source of error remains basal slice selection and delineation of the tricuspid valve. In this study a short-axis orientation for RV volumetric measurement was used, as this is standard practice in our hospitals. Axial orientation, however, might result in higher reproducibility than short-axis orientation in CHD patients with severely dilated RVs, decreasing the difficulty of valve delineation in the basal slices.[25,26] To minimize errors at the tricuspid and pulmonic valve, images were cross-linked to RV two-chamber, four-chamber and RV outflow tract views. Furthermore, only a small portion of the patients had severely dilated RVs, therefore it is unlikely that the slice orientation would have resulted in important differences for the current study. The impact and reproducibility of the semi-automatic software used in the current study will likely be similar in axial slice orientation, as the software is not restricted by geometric assumptions and uses signal intensity to select trabeculae. Finally, another source of error might be inadequate selection of the RV end-systolic frame. In daily practice both RV ESV and LV ESV are often assessed in the LV end-systolic frame. However, in patients with CHD, who often have a right bundle branch block, timing of the RV end-systolic frame can be delayed compared to the LV end-systolic frame.[27] Therefore RV end-diastolic and end-systolic phase was based solely on RV cavity size.

Study limitations

This study is unable to determine whether in- or excluding trabeculae best represents true RV volumes, as a gold standard in vivo is lacking. Because the stroke volume remains equal with both methodologies, other CMR measurements are unable to serve as a reference standard. However, theoretically exclusion of trabeculae is more accurate as these do not contribute to RV blood volumes in end-diastole or end-systole.

No invasive measurements were available to determine the true RV pressure in these patients. Therefore, estimations of RVESP and RVOT gradient based on Doppler-derived flow velocities were used, which might not always be accurate and have limitations. Nonetheless these are the best available non-invasive alternatives to assess the degree of RV pressure overload or RVOT stenosis.

CONCLUSIONS

Exclusion of trabeculae and papillary muscles has a significant impact on measured RV volumes, mass and EF. The magnitude of the differences varies between patient groups and is significantly larger in all investigated patient groups with overloaded RVs than in healthy controls. Importantly, exclusion of trabeculae with semi-automatic pixel-intensity based software is highly reproducible and superior compared to manual contour tracing for RV mass.

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Chapter 03

Main pulmonary artery area limits exercise capacity in patients long-term after arterial switch operation

Vivan J.M. Baggen,* Mieke M.P. Driessen,* Folkert J. Meijboom, Gertjan Tj. Sieswerda, Nicolaas J.G. Jansen, Sebastiaan W.H. van Wijk, Pieter A. Doevendans, Tim Leiner, Paul H. Schoof, Tim Takken, Johannes M.P.J. Breur

*Equal contributions

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ABSTRACT

Background Despite excellent survival in patients after the arterial switch operation, re-intervention is frequently required and exercise capacity is decreased in a substantial number of patients. This study relates right-sided imaging features in patients long-term after the arterial switch operation to exercise capacity and ventilatory efficiency, in order to investigate which lesions are functionally important.

Methods Patients operated in the UMC Utrecht, the Netherlands (1976–2001) and healthy controls underwent cardiac magnetic resonance imaging and cardiopulmonary exercise testing within 1 week. We measured main, left and right pulmonary artery cross-sectional areas, pulmonary blood flow distribution, peak oxygen uptake (VO_2 peak%), and minute ventilation relative to carbon dioxide elimination (VE/VCO_2 slope).

Results A total of 71 patients (median age 20 [range 12–35] years, 73% male) and 21 healthy controls (median age 26 [range 21–35] years, 48% male) were included. Main, left, and right pulmonary artery areas were decreased compared with controls (190 vs. 269 mm^2/m^2, 59 vs. 157 mm^2/m^2, 98 vs. 139 mm^2/m^2, respectively, all $p < 0.001$); however, pulmonary blood flow distribution was comparable ($p = 0.722$). VO_2 peak% and VE/VCO_2 slope were 88 ± 20% and 23.7 ± 3.8, respectively, with 42% and 1% of patients demonstrating abnormal results (≤ 84% and ≥ 34, respectively). The main pulmonary artery area significantly correlated with VO_2 peak% ($r = 0.401$, $p = 0.001$) and pulmonary blood flow distribution with VE/VCO_2 slope ($r = –0.329$, $p = 0.008$). Sub-analysis (< 18, 18–25, > 25 years) showed that the main pulmonary artery area was smaller in older age groups. In multivariable analysis, the main pulmonary artery area was independently associated with VO_2 peak% ($p = 0.032$).

Conclusion In adult patients after the arterial switch operation, narrowing of the main pulmonary artery is a common finding and is the main determinant of limitation in functional capacity, rather than pulmonary branch stenosis.

INTRODUCTION

Transposition of the great arteries is the most common cyanotic congenital heart defect, occurring in 4.7 of 10.000 newborns.[1] The current treatment of choice is neonatal arterial switch operation, as described in 1976 by Jatene and colleagues.[2]

Follow-up studies show excellent results with low perioperative mortality and high 25-year survival (> 95%). However, the cumulative risk of re-intervention increases up to 25% in adult patients.[3,4] Most of these patients have supravalvular neopulmonary artery or pulmonary branch stenosis and undergo balloon dilatation or stenting by catheter intervention or surgical pulmonary artery reconstruction.[4] Apart from re-intervention, long-term follow-up studies show that exercise capacity is decreased in a significant subset of patients.[5-7] Reduced exercise capacity has been associated with right-sided obstructive lesions.[6,7] Still, long-term follow-up data are limited, and defined management strategies for subclinical anatomic or physiologic abnormalities are lacking.[8]

This study compared patients long-term after the arterial switch operation with healthy controls, focusing on right ventricular (RV) function, pulmonary artery and branch cross-sectional areas, pulmonary branch relative area change, and pulmonary blood flow (PBF) distribution. Second, these imaging features were related to exercise capacity and ventilatory efficiency, in order to determine which lesions are functionally important and therefore potentially amenable to re-intervention.

METHODS

Study population

We performed a cross-sectional cohort study between August 2011 and February 2014. All patients who underwent an arterial switch operation in our center who were aged more than 12 years (1976-2001) were approached. Patients prospectively underwent cardiac magnetic resonance (CMR) imaging, echocardiography, and cardiopulmonary exercise testing within 1 week, without any change in clinical condition. Healthy control subjects (aged 18–35 years) underwent CMR and echocardiography with the same study protocol. Exercise testing was not performed in healthy controls because reference values are well established.[9] The institutional review committee of the University Medical Center Utrecht approved this study. Informed consent was obtained from all patients, parents if aged less than 18 years, and healthy controls. Patient characteristics were obtained from the patient chart. Patients with a homograft, signs of myocardial ischemia during the exercise test, or claustrophobia were excluded.

Cardiac magnetic resonance acquisition

Patients and controls were scanned according to a predefined imaging protocol without anesthesia or sedation. A 1.5-T system (Ingenia R4.1.2, Philips Healthcare, Best, The Netherlands) was used with a dedicated chest phased-array parallel imaging-capable surface coil with a maximum of 36 active elements.

Steady-state free precession cine images were acquired in various orientations (short axis, 4-chamber and 2-chamber long axis, right and left ventricular outflow tract views in 2 planes) during repeated end-expiratory breath holds. Multi-slice cine short-axis acquisition was planned from the apex to well above the tricuspid and mitral valve: TR / TE 3.4 / 1.69 ms, voxel size 1.25 × 1.25 × 8 mm, flip angle 90°, matrix 192 × 171 mm, 30 frames / cycle.

Gadolinium-enhanced magnetic resonance angiography (MRA) images of the pulmonary vasculature were performed with non-electrocardiography gated time-resolved 3-dimensional (3D) spoiled gradient echo sequence: TR / TE 5.2 / 1.49, voxel size 0.94 × 0.94 × 2 mm, 60 slices, field of view 300, matrix 308 × 125, keyhole percentage 25%, 3 dynamics, keyhole scan time 2.8 seconds. Gadopentetate dimeglumine (0.2 mL/kg, injection rate 1.5 mL/s) was injected intravenously followed by saline flush (25 mL).

Quantitative through-plane flow through the left pulmonary artery (LPA) and right pulmonary artery (RPA) was measured with a retrospectively electrocardiography gated, velocity encoded phase-contrast sequence: TR / TE 5.2 / 3.1 ms, voxel size 2.5 × 2.5 × 8 mm, flip angle 12°, field of view 320, matrix 128 × 100, temporal resolution 20 frames / cycle.[10] In case of aliasing, the sequence was rescanned at a higher velocity encoding range. LPA and RPA flow measurements were performed on the narrowest points identified on scout, black-blood, and MRA images.

Cardiac magnetic resonance analysis

RV volumetric analysis was performed by manual tracing of endocardial and epicardial contours, using Qmass MR Research edition (version 7.4, Medis, Leiden, The Netherlands), with a previously described RV analysis protocol.[11] Trabeculae and papillary muscles were selected and excluded from the blood volume using semiautomatic threshold-based segmentation software, which is based on the signal intensity distribution of the voxels.[12]

Diameters and areas of the main pulmonary artery (MPA), LPA, and RPA were measured on 3D MRA images in OsiriX Imaging Software for MacOS 10.7 (version 5.5.2, 32-bit, Pixmeo, Geneva, Switzerland). Narrowest vessel lumina were selected in 2 perpendicular views to obtain the smallest cross-sectional area in the third view. The full width at half maximum method was used to distinguish vessel from surrounding tissue.[13]

Velocity-encoded CMR data were analyzed using QFlow (version 5.6, Medis, Leiden, The Netherlands). Contours were manually traced for the LPA and RPA lumina in all phases. In this way, maximal and minimal areas of the pulmonary branches were obtained. Flow data were determined with a velocity analysis of each voxel in all phases. One researcher performed all CMR analyses and was blinded for the results of the cardiopulmonary exercise test.

Calculation of right-sided imaging features

All volumetric data were indexed for body surface area, calculated by the Dubois formula. Pulmonary branch relative area change was calculated by (maximal area – minimal area) / minimal area x 100%. To express the degree of unilateral pulmonary branch stenosis, relative branch area was calculated by smallest pulmonary branch area / (LPA + RPA area) x 100%. Significant unilateral pulmonary branch stenosis was defined as a relative branch area of <30%. PBF distribution was calculated by relative effective stroke volume (forward flow – backward flow per heart beat) to the left and right lung: LPA (or RPA) effective stroke volume / (LPA + RPA effective stroke volume) x 100%. Abnormal PBF distribution was defined as a greater than 15% point deviation from normal (LPA 45 : RPA 55%, thus < 30% LPA relative flow or < 40% RPA relative flow).

Echocardiography

Doppler transthoracic echocardiography was performed on a Toshiba Artida (Toshiba, Tokyo, Japan) with a 5-MHz transducer. The greatest continuous-wave velocity of the tricuspid regurgitation jet was used to express RV systolic pressure, using the Bernoulli equation ($4TR^2$). The greatest continuous-wave velocity measured across the pulmonary valve was used as an index of RV outflow tract (RVOT) obstruction.[6]

Exercise testing

Patients performed a cardiopulmonary exercise test using an electronically braked cycle ergometer (Lode Corrival, Lode BV, Groningen, The Netherlands). Before exercise, forced expiratory volume in the first second (FEV_1) was measured. After a rest period of 3 minutes and 3 minutes of unloaded cycling, the work rate was increased in a ramp-like protocol with 15, 20, or 25 Watts per minute. It was aimed to complete the test within 8 to 12 minutes.[14] Maximum effort was defined as a peak respiratory exchange ratio of greater than 1.0 for children and greater than 1.1 for adults. Indications for premature test termination are provided by the American Thoracic Society / American College of Chest Physicians.[9]

During exercise, oxygen uptake (VO_2), carbon dioxide elimination (VCO_2), and minute ventilation (VE) were recorded using a computerized breath-by-breath analyzer (ZAN 600, ZAN Meßgeräte GmbH, Accuramed BVBA, Herk-de-Stad, Belgium). Respiratory exchange ratio was calculated by VCO_2/VO_2 and breathing reserve by (1 – peak VE / [FEV_1 x 40]) x 100%. Peak oxygen uptake (VO_2 peak%) was expressed as percentage of the predicted value, based on age, sex, height, and body weight.[15] Ventilatory efficiency (minute ventilation relative to carbon dioxide elimination; VE/VCO_2 slope) was calculated using linear regression, up to the respiratory compensation point. Peak heart rate 85% or less, VO_2 peak 84% or less, and VE/VCO_2 slope 34 or greater were considered abnormal.[9] Specific reference values for children were applied in patients aged 12 to 18 years.[16]

Reproducibility

Intra- and interobserver reproducibility of RV function analysis was previously assessed and published.[11] In addition, we assessed reproducibility of 3D MRA analysis by blinded re-analysis of 10 random datasets by a second observer.

Statistical analysis

For all variables, the distribution was tested using the Shapiro-Wilk test. Continuous data were expressed as mean value ± standard deviation or median [interquartile range] as appropriate. Depending on the distribution of data, the independent-samples *T*-test or the Mann-Whitney *U* test was used to compare groups. For categorical data, the chi-square test was used. Associations were evaluated using Pearson's or Spearman's rho (*r*) coefficient as appropriate.

For the sub-analysis, we could not define clear changes in surgical strategies over time. Therefore, we chose to define three age groups (< 18, 18–25, > 25 years), based on approximately equal years and patients per subgroup. Sub-analysis for three pre-defined age groups was performed using chi-square Mantel-Haenszel tests and linear regression for dichotomous and continuous outcomes, respectively.

Associations were adjusted for age, sex and operative characteristics using multivariable linear regression. Reproducibility was tested with intraclass correlation coefficients and paired-samples *T*-test. Mean relative differences were calculated with $Obs_1 - Obs_2 / ((Obs_1 + Obs_2) / 2) \times 100\%$. All data analysis was performed in IBM SPSS statistics (version 20.0, IBM, Chicago, IL). Two-sided *P*-values of < 0.05 were considered statistically significant.

RESULTS

Study population

Eighty-six perioperative survivors were approached, of whom 74 participated. Three patients with a homograft were excluded, because this predisposes to obstruction and decreased exercise capacity. None of the patients had ST-T–segment dynamics typical of myocardial ischemia. The remaining 71 patients represented the cohort as analyzed in the present study (median age, 20.1 [14.5–26.1] years; range, 12–35, 52 male, 73%). Exercise testing was performed in 69 patients. Eight patients (11%) underwent pulmonary artery banding, and 48 patients (68%) underwent the Rashkind procedure. The median age at arterial switch operation was 7 (5–20) days (range, 0–755 days). The Lecompte maneuver was performed in 63 patients (89%).[17] Ventricular septal defect was surgically closed in 20 patients (28%), of whom 2 had Taussig-Bing anomaly; aortic coarctation was repaired in 5 patients (7%). Eleven patients (16%) underwent 1 or multiple RVOT re-interventions, of whom 7 (10%) underwent a surgical procedure. Twenty-one healthy controls were included (median age, 26.4 [23.2–29.9] years; range, 21–35, 10 men, 48%).

Patients after arterial switch operation compared with healthy controls

Doppler echocardiography and CMR measurements in patients after the arterial switch operation are compared with healthy controls in Table 1. All measured cross-sectional areas of the pulmonary tree (MPA, LPA, and RPA) were significantly and substantially reduced in patients ($p < 0.001$). Significant unilateral branch stenosis (relative branch area of < 30%) was present in 26 of 70 patients with available 3D MRA data (37%). Abnormal PBF distribution was present in only 5 of 66 patients with representative flow data (7%), of whom 4 had less than 30% relative LPA flow and 1 had less than 40% relative RPA flow. PBF distribution and smallest relative and absolute pulmonary branch flow in patients were not significantly different from those in controls ($p = 0.722$, $p = 0.444$, and $p = 0.210$, respectively).

LPA and RPA relative area change did not significantly differ from controls; however, the range of data distribution was larger. The maximal flow velocities across the pulmonary valve (echocardiography) and pulmonary branches (CMR phase-contrast) were increased compared with controls ($p < 0.001$).

Cardiopulmonary exercise testing

Exercise testing results are presented in Table 2. Patients achieved a median peak heart rate of 98% of predicted (interquartile range, 94–102), 4 out of 69 patients (6%) had an

inadequate increase of heart rate during exercise (range, 71 to 83% of predicted). Mean VO_2 peak% was 88 ± 20%, and 29 patients (42%) demonstrated abnormal results (69 ± 11%). Mean VE/VCO_2 slope was 23.7 ± 3.8, and 1 patient (1%) had an abnormal VE/VCO_2 slope (35).

TABLE 1 - Patients after the arterial switch operation compared with healthy controls.

	Patients (n = 71)	**Controls (n = 21)**	***P*-value**
Age, years	20.1 [14.5–26.1]	26.4 [23.2–29.9]	< 0.001
Sex, male n (%)	52 (73)	10 (48)	0.028
RV systolic pressure, mmHg	29 ± 10	-	-
Maximal velocity across pulmonary valve, cm/s	207 ± 68	107 ± 15	< 0.001
RV function			
RV end-diastolic volume, mL/m²	95 [85–104]	104 [91–116]	0.014
RV end-systolic volume, mL/m²	41 [35–48]	50 [45–58]	0.003
RV stroke volume, mL/m²	53 ± 9	56 ± 9	0.160
RV ejection fraction, %	55 ± 5	53 ± 4	0.048
RV mass, gr/m²	30 [27–35]	23 [21–26]	< 0.001
Pulmonary artery cross-sectional areas			
MPA area, mm²/m²	190 [137–238]	269 [242–301]	< 0.001
LPA area, mm²/m²	59 [41–86]	157 [141–186]	< 0.001
RPA area, mm²/m²	98 [63–147]	139 [123–175]	< 0.001
LPA + RPA area, mm²/m²	175 [109–228]	300 [272–343]	< 0.001
Smallest branch area, mm²/m²	51 [37–77]	139 [123–169]	< 0.001
Qflow measurements			
LPA relative area change, %	43 [31–61]	42 [35–53]	0.754
RPA relative area change, %	56 [40–66]	51 [45–65]	0.966
LPA : RPA flow, %	45 : 55 ± 8	45 : 55 ± 4	0.722
Smallest branch flow, %	44 [39–47]	46 [41–47]	0.444
Smallest branch flow, mL/m²	21 [19–25]	23 [21–26]	0.210
LPA peak flow velocity, cm/s	168 [139–204]	73 [66–82]	< 0.001
RPA peak flow velocity, cm/s	166 [140–207]	88 [77–97]	< 0.001
LPA regurgitation fraction, %	2.7 [0.6–10.0]	3.0 [2.2–7.0]	0.565
RPA regurgitation fraction, %	5.8 [1.6–8.5]	1.7 [0.6–2.7]	0.001

Median [interquartile range] and Mann-Whitney *U* test; mean ± SD and independent-samples *T*-test; or n (%) and chi-square test. **Abbreviations:** RV, right ventricular; MPA, main pulmonary artery; LPA, left pulmonary artery; RPA, right pulmonary artery.

TABLE 2 - Exercise testing results of patients after the arterial switch operation (n = 69).

Variable	Mean ± SD or median [IQR]
Weight, kg	64.2 ± 17.7
Body surface area, m^2	1.75 ± 0.29
Body mass index, kg/m^2	21.4 ± 4.0
Peak minute ventilation, L/min	83.4 ± 24.1
Forced expiratory volume in first second, L	3.44 ± 0.91
Breathing reserve, %	37 [27–47]
Peak heart rate	
Beats/min	184 [177–192]
% predicted	98 [94–102]
Peak systolic blood pressure, mmHg	183 ± 27
Peak workload	
Watt	208 ± 55
% predicted	96 ± 19
Peak oxygen uptake	
mL/kg/min	40.1 ± 10.8
% predicted	88 ± 20
Abnormal (≤ 84%), n (%)	29 (42)
mL/peak heart rate	13.6 ± 3.9
% predicted	90 ± 18
Minute ventilation/carbon dioxide elimination	
Slope	23.7 ± 3.8
Abnormal slope (≥ 34), n (%)	1 (1)
Peak respiratory exchange ratio	1.17 ± 0.10

Abbreviations: IQR, interquartile range; SD, standard deviation.

Age, sex and imaging features related to functional outcome

Univariable correlations of age, sex and imaging features with exercise capacity and ventilatory efficiency are presented in Table 3. Age negatively correlated with VO_2 peak% ($r = –0.315$, $p = 0.008$) and male patients generally had a better exercise capacity ($r = 0.318$, $p = 0.008$). RV end-diastolic volume and stroke volume were both significantly associated with exercise capacity and ventilatory efficiency. MPA area significantly correlated with VO_2 peak% ($r = 0.401$, $p = 0.001$), however the sum of LPA and RPA area, and smallest pulmonary branch area were not related to VO_2 peak%. Smallest pulmonary branch area (mm^2/m^2) and smallest relative branch flow (%) were significantly associated with VE/VCO_2 slope ($r = –0.301$, $p = 0.013$ and $r = –0.307$, $p = 0.014$ respectively).

The MPA area was also significantly related to RV stroke volume ($r = 0.273$, $p = 0.022$) and RV systolic pressure ($r = -0.493$, $p = 0.001$). We repeated all analyses with exclusion of patients after pulmonary artery banding, because this is usually not an accepted practice in the contemporary era. This did not yield different conclusions.

TABLE 3 - Univariable correlations of age, sex and imaging features with exercise capacity and ventilatory efficiency in patients after the arterial switch operation.

	VO_2 peak% (*r*)	**VE/VCO_2 slope (*r*)**
Age, years†	–0.315**	0.073
Sex, male	0.318**	–0.279*
RV systolic pressure, mmHg	–0.112	–0.076
Maximal velocity across pulmonary valve, cm/s	–0.304*	0.012
RV function		
RV end-diastolic volume, mL/m²†	0.305*	–0.306*
RV end-systolic volume, mL/m²†	0.159	–0.200
RV stroke volume, mL/m²	0.400**	–0.347**
RV ejection fraction, %	0.142	0.025
RV mass, gr/m²†	0.208	–0.235
Pulmonary artery cross-sectional areas		
MPA area, mm²/m²	0.401**	–0.043
LPA area, mm²/m²†	0.044	–0.249*
RPA area, mm²/m²†	0.115	–0.182
LPA + RPA area, mm²/m²†	0.093	–0.233
Smallest branch area, mm²/m²†	0.109	–0.301*
Qflow measurements		
LPA relative area change, %†	–0.032	–0.178
RPA relative area change, %†	–0.051	0.152
LPA : RPA flow, %	0.141 : –0.141	–0.329** : 0.329**
Smallest branch flow, %†	0.105	–0.307*
Smallest branch flow, mL/m²†	0.260*	–0.264*
LPA peak flow velocity, cm/s	–0.070	0.154
RPA peak flow velocity, cm/s†	–0.249*	0.288*
LPA regurgitation fraction, %†	–0.076	–0.156
RPA regurgitation fraction, %†	0.002	0.245

*$p < 0.05$; **$p < 0.01$; †Spearman's correlation; other Pearson's correlation. **Abbreviations:** VE/VCO_2 slope, minute ventilation relative to carbon dioxide elimination; VO_2 peak%, percentage of predicted peak oxygen uptake; other as defined in Table 1.

Sub-analysis in three age groups

Results of the sub-analysis in 3 pre-defined age groups (< 18, 18–25 and > 25 years) are presented in Figure 1 and Table 4. As presented in Figure 1, MPA area was not associated with exercise capacity in patients aged less than 18 years (β = –0.034, p = 0.635), who had the largest MPA areas, whereas it was significantly related to exercise capacity both in patients aged 18 to 25 years and in patients aged more than 25 years (β = 0.134, p = 0.008 and β = 0.219, p = 0.045, respectively).

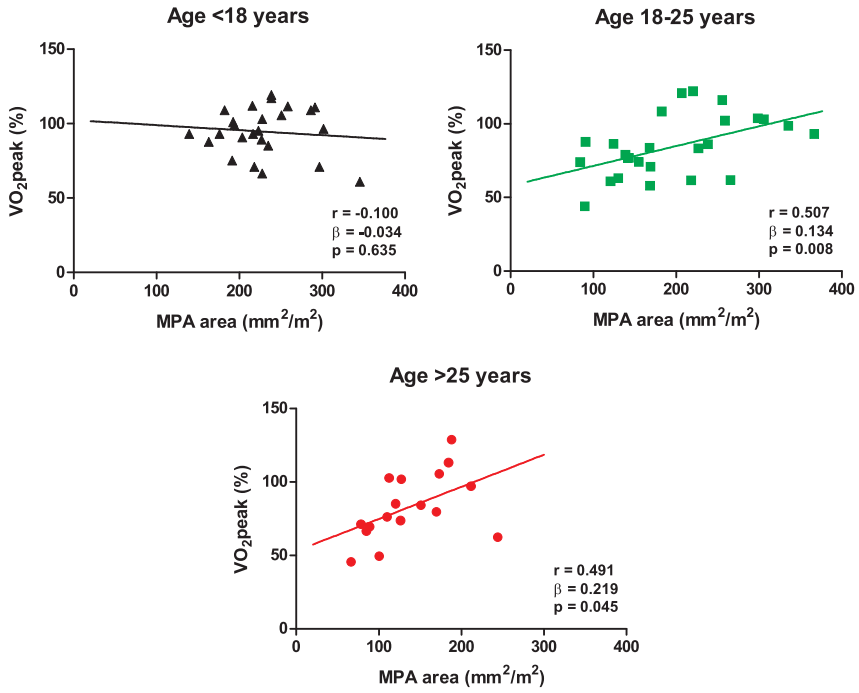

FIGURE 1 - Association between MPA area and VO_2 peak% in different age groups.

MPA area is not associated with exercise capacity in patients < 18 years, who have largest MPA areas (β = –0.034, p = 0.635), while it is significantly related to exercise capacity in patients 18–25 and > 25 years (β = 0.134, p = 0.008 and β = 0.219, p = 0.045, respectively). **Abbreviations:** MPA, main pulmonary artery; VO_2 peak%, exercise capacity reflected by percentage of predicted peak oxygen uptake.

Table 4 shows the operative characteristics and imaging features per age group. Pulmonary artery banding was almost exclusively performed in the oldest age group (p < 0.001). Patients from the earliest surgical era underwent repair at an older age (p < 0.001), and for the reconstruction of the MPA mostly separate patches were used (vs. pantaloon-type patch in younger patients) using pericardium treated with

glutaraldehyde (vs. untreated pericardium in younger patients). Oldest patients also had smallest MPA areas (229.6 ± 47.7 mm^2/m^2, 196.3 ± 77.7 mm^2/m^2, and 133.6 ± 51.2 mm^2/m^2, $p < 0.001$), highest maximal velocity across the pulmonary valve ($p < 0.001$), highest RV mass ($p = 0.035$), highest RV systolic pressures ($p < 0.001$), and a trend towards a lower VO_2 peak% ($p = 0.051$). Pulmonary branch areas, flow, and VE/VCO_2 slope were not significantly different among the 3 age groups.

TABLE 4 - Sub-analysis for operative characteristics, imaging features and functional outcome among three age groups.

Variable	< 18 years (n = 25)	18–25 years (n = 26)	> 25 years (n = 20)	*P*-value
Sex, male n (%)	18 (72)	20 (77)	14 (70)	0.910
Operative characteristics				
Rashkind procedure, n (%)	22 (88)	14 (54)	12 (60)	0.089
Pulmonary artery banding, n (%)	0 (0)	1 (4)	7 (35)	< 0.001
Age at surgical repair, days	9.2 ± 8.8	15.5 ± 26.9	125.8 ± 201.2	0.001
Pantaloon-type patch, n (%)	23 (92)	10 (40)*	1 (8)†	< 0.001
Untreated pericardium, n (%)	25 (100)	14 (56)*	0 (0)†	< 0.001
Lecompte maneuver, n (%)	23 (92)	23 (89)	17 (85)	0.463
Ventricular septal defect repair, n (%)	5 (20)	8 (31)	7 (35)	0.260
Cardiopulmonary bypass time, min	153 ± 59	207 ± 53	187 ± 45	0.104
Cross clamp time, min	92 ± 18	125 ± 34	106 ± 27	0.171
RV outflow tract re-intervention, n (%)	0 (0)	2 (8)	9 (45)	< 0.001
Imaging features				
RV systolic pressure, mmHg	22.7 ± 9.6	29.2 ± 6.8	36.5 ± 9.9	< 0.001
Maximal velocity across pulmonary valve, cm/s	183.7 ± 58.0	194.5 ± 72.4	254.6 ± 52.7	0.001
RV end-diastolic volume, mL/m^2	94.1 ± 12.0	96.9 ± 12.4	94.5 ± 23.7	0.909
RV stroke volume, mL/m^2	51.7 ± 8.3	53.3 ± 7.0	52.6 ± 10.5	0.690
RV mass, gr/m^2	29.6 ± 4.7	30.6 ± 6.0	34.1 ± 9.5	0.035
MPA area, mm^2/m^2	229.6 ± 47.7	196.3 ± 77.7	133.6 ± 51.2	< 0.001
LPA + RPA area, mm^2/m^2	171.0 ± 55.5	176.2 ± 81.4	184.5 ± 102.1	0.581
Smallest pulmonary branch area, mm^2/m^2	57.7 ± 28.9	58.3 ± 35.0	67.6 ± 44.7	0.388
Smallest pulmonary branch flow, mL/m^2	22.0 ± 4.5	21.0 ± 4.0	21.2 ± 4.3	0.494
Smallest pulmonary branch flow, %	43.7 ± 6.0	41.7 ± 5.9	42.5 ± 5.0	0.435
Functional outcome				
VO_2 peak, %	94.7 ± 16.1	84.4 ± 20.6	83.4 ± 21.9	0.051
VE/VCO_2 slope	23.0 ± 2.9	24.3 ± 3.6	24.0 ± 5.1	0.379

Mean ± SD and univariable linear regression; or n (%) and chi-square Mantel-Haenszel test. *Data available in 25 of 26 patients; †data available in 12 of 20 patients. **Abbreviations:** as defined in Table 1 and 3.

Lecompte Maneuver

Pulmonary branch areas were smaller in patients who underwent the Lecompte maneuver compared with patients who did not (LPA 52 [39–75] mm^2/m^2 vs. 128 [91–149] mm^2/m^2, $p < 0.001$ and RPA 89 [58–139] mm^2/m^2 vs. 145 [109–187] mm^2/m^2, $p = 0.053$); however, this did not translate into an abnormal PBF distribution (mean LPA : RPA relative flow 46 : 54 vs. 47 : 53%, $p = 0.181$). MPA area, VO_2 peak%, and VE/VCO_2 slope were comparable in both groups ($p = 0.971$, $p = 0.216$, and $p = 0.555$, respectively).

Multivariable analysis

MPA area remained significantly associated with exercise capacity ($\beta = 0.102$, $p = 0.032$) after adjustment for age, sex and operative characteristics (age at repair, pulmonary artery banding, patch type and treatment, RVOT re-intervention). In this multivariable model, MPA area was the only significant predictor of exercise capacity.

Reproducibility

Inter- and intraobserver variability for the measurement of the MPA area on MRA were reflected by intraclass correlation coefficients of 0.993 and 0.997 and mean differences of 2.1 ± 10.7 mm^2/m^2 ($p = 0.564$) and 7.3 ± 8.0 mm^2/m^2 ($p = 0.019$), respectively. Mean relative differences for inter- and intraobserver variability were 0.7 ± 5.3% and –3.7 ± 3.2%.

DISCUSSION

In this cross-sectional cohort study of patients long-term after arterial switch operation, both MPA and pulmonary branch areas were significantly decreased compared with healthy controls, and abnormal exercise capacity was common (42%). Cross-sectional MPA area was independently associated with exercise capacity in patients aged more than 18 years and correlated with stroke volume at rest and RV systolic pressure.

Significant unilateral pulmonary branch stenosis was present in 37% of patients; however, this resulted in abnormal blood flow distribution in only 7% of patients. Other factors that could inhibit exercise capacity in this group of patients, such as RV dysfunction or chronotropic incompetence, were uncommon in our cohort. These results indicate that narrowing of the MPA is a common finding in adult patients after the arterial switch operation and that it is the main right-sided lesion limiting functional capacity, rather than RV dysfunction or unilateral pulmonary branch stenosis.

Although abnormal PBF distribution was uncommon in our cohort, smallest relative pulmonary branch flow was associated with ventilatory efficiency. This was also reported

by Giardini and colleagues, who identified abnormal PBF distribution as an independent predictor of both decreased exercise capacity and ventilatory efficiency.[7] In contrast to our results, abnormal PBF distribution was more common in their study (18%) and MPA area was not an independent predictor of exercise capacity. This is probably explained by the younger age of the patient group in their study (13.3 ± 3.4 years), because MPA area was not related to exercise capacity in the youngest patients of our cohort.

Limitation of stroke volume increase during exercise

In normal subjects, the RV stroke volume increases during exercise with only mild elevation of RV pressures. This is a consequence of low pulmonary vascular resistance, mediated by recruitment of closed vessels and distension of already opened vessels.[18,19] We found that MPA area significantly correlated with RV stroke volume at rest and RV systolic pressure. This may imply that in patients with pulmonary artery narrowing, RV systolic pressure increases disproportionately during exercise, limiting the increase in RV stroke volume during exercise, subsequently limiting exercise capacity.

Relative area change

Decreased distensibility of the MPA or branches has been proposed as a possible mechanism for reduced exercise capacity in patients after the arterial switch operation. Grotenhuis and colleagues[20] and Voges and colleagues[21] demonstrated a reduced distensibility of the proximal aorta in these patients. This was potentially mediated by increased aortic wall stress of the dilated root, fibrous tissue, and intrinsic wall abnormalities near the suture line and pulmonary artery branches embracing the aorta after the Lecompte maneuver.[17] Most of these features could theoretically also affect the distensibility of the MPA and branches, contributing to a functional stenosis on exercise.[7,22] This study approximated distensibility of the pulmonary branches by relative area change, which is uncorrected for pulse pressure, because our study protocol did not include invasive measurements. To our knowledge, these measurements have not been performed in patients after the arterial switch operation. Our data do not provide evidence for a decreased relative area change of the LPA and RPA. However, relative area change of the MPA was not assessed in this study and could be altered because of different elastic properties of the pericardial patch.

Smaller main pulmonary artery areas in older age groups

Patients aged more than 18 years had smaller MPA areas compared with younger patients. Because this is a cross-sectional study, it is uncertain whether this diminution of size is a reflection of learning curve and changing surgical techniques over time or

the result of rapid somatic growth with inadequate growth at the anastomotic site, as suggested by Prifti and colleagues.[23] The operative characteristics of patients aged more than 25 years showed clear differences with both other age categories. Pulmonary artery banding was almost exclusively performed in this age group, patients were markedly older at time of surgical repair, and for the reconstruction of the MPA, mostly separate patches of pericardium treated with glutaraldehyde were used (vs. untreated pantaloon-type patch). Single pantaloon-type patch has been reported to result in less residual RVOT obstruction, and, in contrast to treated pericardium, untreated pericardial tissue potentially retains the ability to grow and adapt.[23] Therefore, pulmonary artery banding and type of MPA reconstruction (separate patches, treated pericardium with inadequate growth) are probably important causes of MPA narrowing.

Prolonged cyanosis and low PBF in patients who underwent the arterial switch operation at an older age also may adversely affect the microscopic pulmonary vasculature, myocardium, and systemic circulation, leading to a reduced VO_2 peak%.

By using multivariable linear regression, we adjusted for the effects of age, sex, and operative characteristics (age at repair, pulmonary artery banding, patch type and treatment, RVOT re-intervention). In multivariable analysis, MPA area was the only significant predictor of exercise capacity.

Clinical implications

In this study, the importance of MPA narrowing as a determinant for exercise capacity is highlighted. This could imply that during the arterial switch operation, optimization of MPA size is important for functional outcomes. Moreover, this suggests that the long-term follow-up of patients after the arterial switch operation should focus on presence or progression of MPA stenosis. Pulmonary branch re-interventions in patients with unilateral pulmonary branch stenosis might only improve functional capacity in the small subgroup of patients with an abnormal PBF distribution. This hypothesis requires further investigation in longitudinal studies.

Study limitations

Limitations of this study are inherent to its design, because this is a single-center, cross-sectional study. Therefore, variance in operation procedures and surgical experience among surgical centers could result in different outcomes in other patient cohorts. Second, we need longitudinal follow-up studies to assess the actual impact of RVOT re-interventions on functional outcome in adult patients after the arterial switch operation. Individual follow-up data should elucidate whether the observation of decreased MPA areas in older age groups can be partially explained by rapid somatic growth. Finally,

patients and controls were not perfectly matched, because patients were younger than healthy controls and included more male patients. To account for this, all measurements were indexed for body surface area. Moreover, because patients aged less than 18 years exhibited the smallest differences compared with healthy controls and male patients generally had a better exercise capacity, age and sex matching would have only strengthened the differences found in this study.

CONCLUSIONS

In this cross-sectional study of patients long-term after the arterial switch operation, the MPA area was increasingly smaller in older age groups. The MPA area was significantly related to exercise capacity, independently of age, sex and operative characteristics. Although branch pulmonary artery areas were smaller than in healthy controls, PBF at rest and ventilatory efficiency were generally not compromised. Therefore, our data suggest that MPA narrowing is an important and thus far underappreciated determinant in the limitation of functional capacity in adults after arterial switch operation.

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Chapter 04

Prognostic value of left atrial size and function in adults with tetralogy of Fallot

Vivan J.M. Baggen, Anne-Rose W. Schut, Judith A.A.E. Cuypers, Maarten Witsenburg, Eric Boersma, Annemien E. van den Bosch, Jolien W. Roos-Hesselink

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ABSTRACT

Background Left atrial (LA) size predicts cardiovascular outcome in chronic heart failure. Its prognostic value in adults with repaired tetralogy of Fallot (ToF) is unknown. This study therefore investigated the association of LA size and function with cardiovascular events in adults with ToF.

Methods Clinically stable adults with ToF who visited the outpatient clinic between 2011 and 2013 underwent echocardiography and were prospectively followed for the occurrence of death, heart failure, hospitalizations, arrhythmia, thromboembolic events, and re-interventions. LA maximal, minimal and pre-A wave volume, area and length were measured on the apical four-chamber view. Total, passive and active emptying fractions were calculated.

Results In total, 134 patients were included (median age 35 [IQR 29–45] years, 65% male, 91% NYHA I). Median follow-up was 40 [IQR 32–47] months. Patients with a dilated LA (≥ 34 mL/m^2, 43%) were at higher risk of cardiovascular events (n = 33, adjusted HR 2.48; 95% CI 1.09–5.62, p = 0.030). Analysis of LA volumes as continuous variables yielded similar conclusions. In addition, LA length (adjusted HR 2.49; 95% CI 1.51–4.09, $p < 0.001$), total emptying fraction (adjusted HR 0.96; 95% CI 0.93–0.99, $p = 0.008$), and active emptying fraction (adjusted HR 0.92; 95% CI 0.87–0.96, $p = 0.001$) were significantly associated with cardiovascular events. Standardized HRs indicated that LA length was the strongest prognostic marker. In addition, none of the patients with a normally sized LA died or developed heart failure.

Conclusions LA size and function can provide relevant prognostic information in clinically stable adults with repaired ToF. Especially LA length may be a valuable additional tool in the risk stratification of these patients.

INTRODUCTION

Tetralogy of Fallot (ToF) is the most common cyanotic heart defect.[1,2] Thanks to successful repair at young age, the survival of patients with ToF has tremendously improved with current survival rates over 90% up to 30 years after surgical repair.[3] Nevertheless, residual lesions are common. Especially older patients are at risk of complications such as heart failure, arrhythmia and early demise. Therefore, life-long follow-up is warranted. Identification of patients with a high risk of adverse cardiovascular events is essential, in order to optimize patient information, follow-up and therapeutic strategies.[3,4]

Echocardiography is the cornerstone in the evaluation of cardiac function in patients with ToF. Right and left ventricular function are known as important prognostic markers in these patients.[5] Atrial function has been largely neglected so far. The left atrium (LA) has multiple functions: it modulates as a reservoir, a conduit and as a pump.[6] LA size can be reliably measured with two-dimensional echocardiography and reflects the average effect of left ventricle (LV) filling pressures over time. It is known as a useful marker for both chronicity and severity of LV diastolic dysfunction.[7-9] Larger LA volumes have been associated with an increased risk of adverse cardiovascular events in patients with chronic heart failure.[10] To the best of our knowledge, the prognostic value of LA size or function in patients with ToF has not been evaluated. Therefore, the aim of this study was to investigate the association of LA size and function with a composite endpoint of cardiovascular events in clinically stable adults with repaired ToF.

METHODS

Study design and population

For the purpose of this study, all patients with repaired ToF were selected from a large prospective cohort of clinically stable adults with congenital heart disease. This cohort consisted of consecutive patients who *routinely* visited our adult congenital cardiology outpatient clinic and underwent echocardiography between April 2011 and April 2013. Patients with pulmonary valve atresia and ventricular septal defect and patients with isolated pulmonary stenosis were excluded. Other exclusion criteria were: age < 18 years, pregnancy, incapability of understanding and signing informed consent, kidney failure (creatinine > 200 µmol/L), or insufficient echocardiographic image quality. Image quality was defined as insufficient when the LA borders were not clearly visible and therefore could not be adequately traced in the apical four-chamber (A4C) view.

At the time of study inclusion, all patients underwent physical examination by a cardiologist, 12-lead ECG, venous blood sampling, and echocardiography. Patient demographics, medical history, medication use, symptoms and signs of heart failure

(NYHA classification), results of physical examination, associated lesions, surgical characteristics, re-interventions, electrocardiography, echocardiography, laboratory results and events were collected using an electronic CRF-based online system (© 2004–2012 OpenClinica, LLC and collaborators). Details have been reported previously.[11] The institutional review board of the Erasmus MC approved the study protocol and all participants provided written informed consent.

Echocardiography

Two-dimensional transthoracic echocardiographic images were acquired using a commercially available ultrasound system iE33 (Philips Medical Systems, Best, the Netherlands) with a 1.5-MHz transducer. Echocardiographic measurements were performed in agreement with the current recommendations of the American Society of Echocardiography and European Association of Cardiovascular Imaging.[12] LV end-diastolic and end-systolic volumes were calculated using the biplane method of disks summation (modified Simpson's rule). LV systolic function was quantified with LV ejection fraction, and visually graded as normal or impaired. LV diastolic function was assessed using pulsed waved Doppler of the mitral valve inflow (E/A-ratio and deceleration time) and septal tissue Doppler imaging (E' wave and E/E'-ratio). RV systolic function was quantified with RV fractional area change and tricuspid annular plane systolic excursion.

Left atrial size and function

LA volumetric measurements were performed on the A4C view in three different phases during one cardiac cycle. LA maximal and minimal volume were measured on the frames with respectively the largest and smallest atrial cavities on visual assessment. LA pre-A-wave volume was measured one frame before the atrial contraction started (also based on the movement of the mitral valve leaflets, and the onset of the P-wave on the ECG). Contours were manually traced, the atrial appendage and pulmonary veins were excluded, and at the mitral valve level the contour was closed with a straight line. For the calculation of volumes, a single plane summation of disks method was used. In addition, left atrial maximal size was quantified using the LA anteroposterior dimension on the parasternal long axis view, the LA major axis on the A4C view (LA length), and the LA maximal area on the A4C view (Supplemental File 1). All LA volumes were indexed by body surface area.

We separately considered LA reservoir, conduit and contractile function.[6,7,13] Reservoir function was quantified with LA total emptying volume (TEV), LA total emptying fraction (TEF) and the expansion index. TEV was calculated by LA maximal volume – LA minimal

volume. TEF was calculated by (TEV / LA maximal volume) *100%. Expansion index was defined as (TEV / LA minimal volume) *100%. Conduit function was quantified with LA passive emptying volume (PEV) and LA passive emptying fraction (PEF). PEV was calculated by LA maximal volume – LA pre-A-wave volume. PEF was calculated by (PEV / LA maximal volume) *100%. Contractile or active function was estimated with LA active emptying volume (AEV) and LA active emptying fraction (AEF). AEV was calculated by LA pre-A-wave volume – LA minimal volume. AEF was calculated by (AEV / LA pre-A-wave volume) *100%.

One investigator (A.S.), who was blinded for clinical events and other patient data, performed all LA measurements. Reproducibility was assessed by blinded repeated analysis of 20 randomly selected patients by the first and a second observer (V.B.).

Laboratory testing

Peripheral venous blood samples were obtained from all patients after at least 30 minutes of rest. Serum NT-proBNP was measured for research purposes only in our clinical chemistry laboratory using a commercial electrochemiluminescence immunoassay (Roche Diagnostics, Rotkreuz, Switzerland). The limit of detection was 0.6 pmol/L. The upper limit of normal for NT-proBNP was 14 pmol/L (approximately 125 pg/mL), based on the recommended low cut-off for the diagnosis of heart failure in patients presenting with non-acute symptoms.[14]

Definition and assessment of events

The primary endpoint was defined prior to the collection of data as a composite of all adverse cardiovascular events: all-cause mortality, heart failure (requiring initiation or change in heart failure medication, or requiring hospital admission), hospitalization for cardiac reasons, arrhythmia (symptomatic and recorded, or requiring treatment), thromboembolic events (ischemic cerebrovascular accident, pulmonary embolism or myocardial infarction), or cardiac re-interventions (surgical or percutaneous). A secondary composite endpoint of all-cause mortality or heart failure was defined. All patients were prospectively followed for fatal and non-fatal events by a yearly clinical evaluation at our institution. During the follow-up, patients were treated according to the physician's discretion and the study protocol did allow for adjustment of cardiac medication. Where necessary, we retrieved information from electronic patient charts and correspondence with referring hospitals. Survival status of all patients was also checked in the Municipal Population Register. Events were adjudicated by two experienced investigators (V.B. and J.R.) without knowledge of LA measurements. The investigators had direct access to the patient clinical records and the events were

independently reviewed by each investigator. For patients with multiple events, event-free survival was defined as the time from enrollment to the occurrence of the first event. Patients without any cardiovascular event were censored at the end of the follow-up duration (August 1, 2015).

Statistical analysis

Continuous normally distributed data were presented as mean ± standard deviation (SD) and skewed data as median [IQ_1–IQ_3]. Categorical data were presented as frequencies and percentages. Following the guidelines, patients were stratified according to LA volume index < 34 mL/m^2 (normal LA) and ≥ 34 mL/m^2 (dilated LA).[12] Continuous variables were compared between these two groups using the Student's *T*-test or the Mann-Whitney *U* test, depending on the distribution of data. Categorical variables were compared using the chi-squared test or Fisher's exact test when applicable. Cumulative endpoint free survival estimates and survival curves were derived by the Kaplan-Meier method; the log-rank test was used to compare groups. Cox proportional hazards regression was used to investigate the associations between LA measurements and endpoints. LA measurements were analyzed as continuous and as standardized variables (created by calculating z-scores). The association of LA measurements with the primary endpoint was adjusted for age and NYHA functional class, using a minimum of ten endpoints per degree of freedom to prevent overfitting. Inter- and intraobserver agreement of LA maximal volume, area and length was presented by Bland-Altman plots. Limits of agreement were defined as the mean difference of the two repeated measurements ± 1.96 SD. The coefficient of variation (COV) was defined as the SD of the differences of two measurements divided by the mean of two measurements *100%. Mean relative differences were calculated by dividing the difference of two measurements by the mean of two measurements *100%. All data analysis was performed using IBM SPSS Statistics Version 21.0.0.1 (IBM Corp., Armonk, NY, USA). Two-sided *p*-values of < 0.05 were considered statistically significant.

RESULTS

Baseline measurements

Of the 179 patients that were included in the initial cohort, 31 patients with pulmonary valve atresia and 1 patient with isolated pulmonary stenosis were excluded. In addition, 13 patients were excluded because of insufficient image quality. These patients did not have significant or clinically relevant differences in age, NYHA classification, body mass index, use of cardiac medication or number of re-interventions compared to the 134 adult patients with repaired ToF who were included in this study. Median age at

inclusion was 35 [29–45] years and 87 patients (65%) were male. Median age at initial repair was 3.3 [1.0–6.7] years and in 87 patients (67%) a transannular patch was used. Baseline characteristics are further detailed in Table 1.

The LA was dilated (≥ 34 mL/m^2) in 58 patients (43%). Clinical characteristics and results of electrocardiography and echocardiography are compared between patients with a normal and a dilated LA in Tables 1 and 2. Patients with a dilated LA were significantly older and were in a higher NYHA functional class. In addition, 22 patients (38%) with a dilated LA used cardiac medication compared with 9 patients (12%) with a normal LA ($p < 0.001$). The majority of patients were in sinus rhythm (n = 111, 83%) and had a prolonged QRS interval (n = 91, 75%). None of the patients fulfilled criteria of heart failure at baseline. Patients with a dilated LA had significantly larger LV volumes, impaired diastolic function (expressed by a lower E' wave and a higher E/E'-ratio), a larger RV end-diastolic annulus dimension and a higher NT-proBNP level. No significant differences in rhythm or valvular function were found between both subgroups.

TABLE 1 - Baseline characteristics of the study cohort.

			LA volume index, mL/m^2		
	Valid cases, n (%)	**All patients**	**< 34 (normal, n = 76)**	**≥ 34 (dilated, n = 58)**	**P-value**
Clinical Characteristics					
Age, years	134 (100)	35 [29–45]	31 [26–39]	42 [34–49]	**< 0.001**
Sex, male n (%)	134 (100)	87 (65)	49 (65)	38 (66)	0.900
NYHA class II-III, n (%)	134 (100)	12 (9)	3 (4)	9 (16)	**0.020**
Body mass index, kg/m^2	134 (100)	24 ± 4	24 ± 4	25 ± 4	**0.046**
Body surface area, m^2	134 (100)	1.89 ± 0.20	1.89 ± 0.22	1.89 ± 0.18	0.918
Systolic blood pressure, mmHg	131 (98)	126 ± 17	124 ± 15	127 ± 19	0.355
O_2 saturation < 90%, n (%)	126 (94)	1 (1)	1 (1)	0 (0)	-
Cardiac medication, n (%)	134 (100)	31 (23)*	9 (12)	22 (38)	**< 0.001**
ACE-inhibitor	134 (100)	10 (8)	4 (5)	6 (10)	-
Beta blocker	134 (100)	17 (13)	3 (4)	14 (24)	-
Anti-arrhythmic	134 (100)	10 (8)	2 (3)	8 (14)	-
Aspirin	134 (100)	10 (8)	2 (3)	8 (14)	-
Anticoagulants	134 (100)	16 (12)	1 (1)	15 (26)	-
Diuretics	134 (100)	7 (5)	0 (0)	7 (12)	-
Associated lesions, n (%)	134 (100)	31 (23)	15 (20)	16 (28)	0.286
Atrial septal defect	134 (100)	16 (12)	7 (9)	9 (16)	-
Patent ductus arteriosus	133 (99)	11 (8)	7 (9)	4 (7)	-
Aortic valve stenosis	134 (100)	8 (6)	4 (5)	5 (7)	-

TABLE 1 - Continued.

			LA volume index, mL/m²		
	Valid cases, n (%)	**All patients**	**< 34 (normal, n = 76)**	**≥ 34 (dilated, n = 58)**	**P-value**
Surgical Characteristics					
Age at repair, years	134 (100)	3.3 [1.0–6.7]	1.6 [0.8–4.1]	5.8 [2.2–9.7]	**< 0.001**
Prior aortopulmonary shunt, n (%)	134 (100)	39 (29)	14 (18)	25 (43)	**0.002**
Blalock shunt	134 (100)	20 (15)	5 (7)	15 (26)	-
Waterston shunt	134 (100)	16 (12)	7 (9)	9 (16)	-
Other shunt	134 (100)	3 (2)	2 (2)	1 (2)	-
Transannular patch used, n (%)	129 (96)	87 (67)	52 (71)	35 (63)	0.294
Re-intervention, n (%)	134 (100)	86 (64)	48 (63)	38 (66)	0.778
More than two re-interventions	134 (100)	14 (10)	4 (5)	10 (17)	**0.025**
Surgical, n (%)	134 (100)	75 (56)	46 (61)	29 (50)	0.224
Pulmonary valve replacement	134 (100)	67 (50)	42 (55)	25 (43)	-
Residual VSD repair	134 (100)	19 (14)	8 (11)	11 (19)	-
Catheter-based, n (%)	134 (100)	19 (14)	11 (15)	8 (14)	0.911
Pulmonary valve balloon dilatation	134 (100)	11 (8)	7 (9)	4 (7)	-
Pulmonary valve replacement	134 (100)	4 (3)	2 (3)	2 (3)	-
Ablation	134 (100)	4 (3)	2 (3)	2 (3)	-
Other, n (%)	134 (100)	22 (16)	9 (12)	13 (22)	0.102

Continuous variables are reported as mean ± SD or as median [IQ_1–IQ_3], categorical variables are reported as n (%). *P*-values are given for the comparison between patients with a normal (< 34 mL/m²) and dilated (≥ 34 mL/m²) left atrium. Significant *p*-values are printed in bold. Comparisons were not made between subgroups, in order to avoid multiplicity of comparisons. *In 13 patients, the cardiac medication was changed during the follow-up. **Abbreviations:** ACE, angiotensin converting enzyme; LA, left atrial; NYHA, New York Heart Association; VSD, ventricular septal defect.

Follow-up

At the end of the follow-up (August 1, 2015), survival status and detailed follow-up data were complete in 134 patients (100%). During a median follow-up period of 40.4 [31.9–46.7] months, the primary endpoint occurred in 33 patients (25%) and the secondary endpoint occurred in 8 patients (6%). None of the patients with a normally sized LA died or developed heart failure. Kaplan-Meier estimates of event-free survival (the primary endpoint) were 88% at year one, 83% at year two and 77% at year three. Heart-failure-free survival (the secondary endpoint) was 96% at year one, 96% at year two and 94% at year three. All components of the primary and secondary endpoint are separately displayed in Supplemental File 2 for exploratory purposes.

TABLE 2 - Baseline electrocardiography, echocardiography and laboratory results of the study cohort.

			LA volume index, mL/m²		
	Valid cases, n (%)	**All patients**	**< 34 (normal, n = 76)**	**≥ 34 (dilated, n = 58)**	***P*-value**
Electrocardiography					
Heart rate, beats/minute	134 (100)	75 ± 13	76 ± 14	73 ± 13	0.298
Rhythm, n (%)					
Sinus rhythm	134 (100)	111 (83)	67 (88)	44 (76)	0.061
Pacemaker rhythm	134 (100)	12 (9)	4 (5)	8 (14)	0.087
Other*	134 (100)	11 (8)	5 (7)	6 (10)	0.531
QRS duration, ms†	122 (91)	144 [120–169]	139 [119–164]	158 [122–170]	0.286
QRS duration >120 ms, n (%)†	122 (91)	91 (75)	52 (72)	39 (78)	0.471
RBBB	91 (100)	82 (90)	50 (96)	32 (82)	-
LBBB	91 (100)	1 (1)	0 (0)	1 (3)	-
Other	91 (100)	8 (9)	2 (4)	6 (15)	-
PR interval, if sinus rhythm, ms	111 (83)	165 [146–180]	163 [146–173]	173 [148–187]	0.053
Echocardiography					
LV end diastolic volume, mL/m²	122 (91)	56 [49–66]	54 [45–64]	59 [51–71]	**0.027**
LV end systolic volume, mL/m²	121 (90)	25 [20–32]	24 [21–30]	28 [21–34]	**0.048**
LV ejection fraction, %	122 (91)	53 ± 8	54 ± 5	53 ± 10	0.446
Normal LV function, n (%)	134 (100)	58 (43)	33 (43)	25 (43)	0.971
E/A-ratio	128 (95)	1.5 [1.1–2.1]	1.5 [1.1–2.1]	1.4 [1.1–2.0]	0.338
E' wave, cm/s	129 (96)	7.7 [6.2–9.2]	8.1 [6.6–9.6]	7.0 [5.8–8.8]	**0.023**
E/E'-ratio	127 (95)	10.0 [8.1–13.6]	8.9 [7.7–11.9]	11.3 [8.4–15.8]	**0.005**
Deceleration time, ms	124 (93)	192 [158–235]	192 [158–237]	192 [159–235]	0.835
RV end-diastolic annulus, mm	119 (89)	45.7 ± 7.9	43.7 ± 7.7	48.4 ± 7.4	**0.001**
TAPSE, mm	124 (93)	17.4 ± 4.5	17.1 ± 4.4	17.8 ± 4.8	0.432
RV fractional area change, %	105 (78)	41 ± 9	41 ± 9	42 ± 9	0.443
Valvular function					
AV peak velocity, m/s	117 (87)	1.07 [0.94–1.21]	1.05 [0.91–1.16]	1.12 [0.96–1.24]	0.078
AR ≥ moderate, n (%)	89 (66)	3 (3)	0 (0)	3 (7)	0.101
PV peak velocity, m/s	131 (98)	2.20 ± 0.75	2.24 ± 0.73	2.15 ± 0.78	0.519
PR ≥ moderate, n (%)	124 (93)	47 (38)	25 (37)	22 (39)	0.773
TR ≥ moderate, n (%)	124 (93)	11 (9)	5 (7)	6 (11)	0.536
TR peak velocity, m/s	107 (80)	2.8 ± 0.5	2.8 ± 0.5	2.9 ± 0.6	0.276
MR ≥ moderate, n (%)	82 (61)	1 (1)	0 (0)	1 (2)	1.000

TABLE 2 - Continued.

		LA volume index, mL/m²			
	Valid cases, n (%)	All patients	< 34 (normal, n = 76)	≥ 34 (dilated, n = 58)	*P*-value
Laboratory results					
Hemoglobin, mmol/L	93 (69)	9.2 ± 0.9	9.3 ± 0.8	9.0 ± 1.0	0.181
Creatinine, µmol/L	134 (100)	78.4 ± 19.5	76.7 ± 13.7	80.7 ± 25.2	0.237
NT-proBNP, pmol/L	133 (99)	14.5 [6.4–27.4]	9.3 [4.9–17.0]	23.4 [14.6–42.4]	**< 0.001**

As defined in Table 1. *Three patients were in atrial fibrillation (one with LA volume index < 34 mL/m² and two with LA volume index ≥ 34 mL/m²). †Patients with a pacemaker rhythm were excluded. **Abbreviations:** AR, aortic regurgitation; AV, aortic valve; LA, left atrial; LBBB, left bundle branch block; LV, left ventricle; MR, mitral regurgitation; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; PR, pulmonary regurgitation; PV, pulmonary valve; RBBB, right bundle branch block; RV, right ventricle; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation.

Prognostic value of left atrial measurements

In Figure 1, Kaplan-Meier curves show the proportion of patients free of the primary and secondary endpoint for patients with a normal and a dilated LA. In patients with a normally sized LA, the cumulative proportion of death and heart failure was 0%. In Table 3, the association between all LA measurements and the primary endpoint is shown. Except for the anteroposterior dimension on the parasternal long-axis view, all other LA size measurements (length, area, and volume) were significantly associated with the primary endpoint, also when adjusted for age and NYHA functional class. The standardized adjusted hazard ratios (HRs) show that among the various LA size indices, LA length was most strongly associated with the primary endpoint (HR 2.05; 95% CI 1.38–3.02, $p < 0.001$).

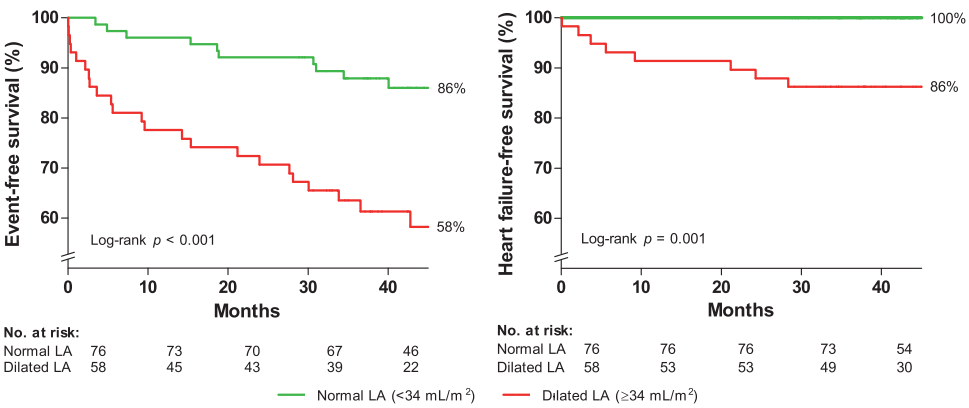

FIGURE 1 - Cardiovascular event-free survival and heart failure-free survival, stratified according to LA maximal volume index (< 34 mL/m² and ≥ 34 mL/m²).

TABLE 3 - The association of left atrial size and function with the primary composite endpoint.

							Standardized values			
	Valid cases, n (%)	Mean ± SD	Crude HR (95% CI)	*P*-value	Adjusted HR (95% CI)*	*P*-value	Crude HR (95% CI)	*P*-value	Adjusted HR (95% CI)*	*P*-value
LA size										
AP dimension, cm†	124 (93)	3.9 ± 0.8	2.59 (1.80–3.73)	**<0.001**	2.00 (1.25–3.21)	**0.004**	2.16 (1.61–2.90)	**<0.001**	1.75 (1.20–2.57)	**0.004**
AP dimension, cm/m²†	124 (93)	2.1 ± 0.4	3.85 (1.97–7.53)	**<0.001**	2.10 (0.86–5.12)	0.102	1.82 (1.35–2.45)	**<0.001**	1.39 (0.94–2.07)	0.102
Length, cm†	134 (100)	5.5 ± 0.8	3.38 (2.10–5.42)	**<0.001**	2.49 (1.51–4.09)	**<0.001**	2.60 (1.79–3.77)	**<0.001**	2.05 (1.38–3.02)	**<0.001**
Length, cm/m²†	134 (100)	2.9 ± 0.5	4.47 (2.06–9.72)	**<0.001**	2.87 (1.26–6.54)	**0.012**	2.00 (1.39–2.86)	**<0.001**	1.63 (1.11–2.38)	**0.012**
Area, cm²/m²	134 (100)	11.5 ± 2.6	1.35 (1.21–1.50)	**<0.001**	1.24 (1.07–1.43)	**0.004**	2.16 (1.65–2.84)	**<0.001**	1.73 (1.20–2.51)	**0.004**
Maximal volume ≥34 mL/m²	134 (100)	-	3.71 (1.76–7.80)	**0.001**	2.48 (1.09–5.62)	**0.030**	-	-	-	-
Maximal volume, mL/m²	134 (100)	36.0 ± 12.6	1.06 (1.04–1.08)	**<0.001**	1.04 (1.01–1.07)	**0.007**	2.06 (1.61–2.64)	**<0.001**	1.63 (1.14–2.33)	**0.007**
Minimal volume, mL/m²‡	130 (97)	20.1 ± 10.0	1.06 (1.04–1.09)	**<0.001**	1.04 (1.01–1.07)	**0.006**	1.85 (1.51–2.26)	**<0.001**	1.51 (1.13–2.03)	**0.006**
Pre-A-wave volume, mL/m²‡	129 (96)	25.6 ± 9.8	1.07 (1.04–1.10)	**<0.001**	1.06 (1.02–1.10)	**0.003**	1.93 (1.52–2.45)	**<0.001**	1.75 (1.21–2.51)	**0.003**
LA function										
Reservoir function										
TEV, mL/m²	130 (97)	16.1 ± 5.2	1.06 (1.00–1.13)	0.070	1.02 (0.95–1.09)	0.603	1.36 (0.98–1.89)	0.070	1.10 (0.78–1.54)	0.603
TEF, %	130 (97)	45.5 ± 9.2	0.94 (0.91–0.97)	**<0.001**	0.96 (0.93–0.99)	**0.008**	0.57 (0.43–0.74)	**<0.001**	0.67 (0.50–0.90)	**0.008**
Expansion index, %	130 (97)	88.4 ± 31.1	0.98 (0.96–0.99)	**0.000**	0.98 (0.97–1.00)	**0.007**	0.47 (0.32–0.70)	**<0.001**	0.59 (0.39–0.86)	**0.007**
Conduit function										
PEV, mL/m²	129 (96)	9.7 ± 4.0	1.07 (0.98–1.16)	0.127	1.05 (0.97–1.14)	0.234	1.30 (0.93–1.82)	0.127	1.22 (0.88–1.71)	0.234
PEF, %	129 (96)	27.9 ± 9.0	0.97 (0.93–1.01)	0.087	0.99 (0.95–1.03)	0.482	0.74 (0.53–1.05)	0.087	0.88 (0.61–1.26)	0.482
Contractile function										
AEV, mL/m²	127 (95)	6.4 ± 2.8	1.11 (0.99–1.25)	0.071	0.99 (0.86–1.14)	0.883	1.35 (0.97–1.88)	0.071	0.97 (0.65–1.44)	0.883
AEF, %	127 (95)	25.1 ± 7.6	0.94 (0.90–0.98)	**0.008**	0.92 (0.87–0.96)	**0.001**	0.62 (0.44–0.88)	**0.008**	0.51 (0.34–0.76)	**0.001**

*Adjusted for age and NYHA functional class. †LA anteroposterior dimension was measured on the parasternal long axis view, and LA length was measured on the apical four chamber view. ‡Minimal volume was not measured in patients with inadequate echocardiographic image quality (n = 4); pre-A-wave volume was not measured in patients with atrial fibrillation (n = 3) or inadequate echocardiographic image quality (n = 2). Significant *p*-values are printed in bold. Standardized HRs are expressed per one SD increase in the corresponding LA measurement. **Abbreviations:** AEF, active emptying fraction; AEV, active emptying volume; AP, anteroposterior; CI, confidence interval; HR, hazard ratio; LA, left atrial; PEF, passive emptying fraction; PEV, passive emptying volume; TEF, total emptying fraction; TEV, total emptying volume.

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In addition, a lower reservoir function (TEF) and a lower contractile function (AEF) were significantly associated with the primary endpoint, whereas a lower conduit function (PEF) was not.

Reproducibility of left atrial measurements

Bland-Altman plots of the intra- and interobserver agreement of LA maximal volume, area and length are presented in Supplemental File 3. Mean differences of the intraobserver measurements were –0.3 ± 0.9 mL/m^2 (COV = 2.5), 0.0 ± 0.4 cm^2/m^2 (COV = 3.4) and 0.0 ± 0.2 cm/m^2 (COV = 5.7), and mean relative differences were –0.7 ± 2.8%, –0.2 ± 3.5% and –0.4 ± 5.7% for LA maximal volume, area, and length, respectively. Mean differences of the interobserver measurements were –0.3 ± 5.2 mL/m^2 (COV = 14.2), 0.0 ± 1.0 cm^2/m^2 (COV = 8.4) and 0.0 ± 0.2 cm/m^2 (COV = 5.8), and mean relative differences were –0.5 ± 15.1%, –0.2 ± 8.9% and –0.8 ± 5.6% for LA maximal volume, area, and length, respectively.

DISCUSSION

To the best of our knowledge, this is the first study that evaluates the prognostic value of LA size and function in ToF patients. In our cohort of clinically stable adults with repaired ToF, the LA was enlarged in 43% of patients. Larger LA size, lower LA reservoir function, and lower LA contractile function were significantly associated with the primary endpoint of any cardiovascular event. None of the patients with a normally sized LA died or developed heart failure. Among the different indices of LA size, LA length was most strongly associated with adverse cardiovascular events, and had the best reproducibility.

Diastolic dysfunction

A potential association of LA size and clinical outcomes in patients with repaired ToF has been suggested earlier, but this association has not further been described.[15] However from other cardiac conditions, as well as in the general population, it is known that LA size is a marker of LV diastolic dysfunction and that it is associated with adverse clinical outcome.[7-10,16,17] In our cohort, patients with a dilated LA were significantly older, had larger LV volumes, and indeed had an impaired diastolic function. Of note, the accuracy of LV diastolic parameters such as the E' wave is questioned in patients with ToF because of the presence of the interventricular septal patch. Evaluation of LA size may therefore be a more reliable alternative, or valuable addition to the set of LV diastolic function parameters.

Cardiac magnetic resonance studies have evaluated the extent of ventricular fibrosis in patients with ToF using late gadolinium enhancement.[18,19] In this relatively young patient group, more prominent LV regional and diffuse myocardial fibrosis was observed, which was related to both abnormal systolic and diastolic function. Increased myocardial fibrosis may explain why LV diastolic dysfunction and accompanied LA enlargement already develops at a relatively young age in patients with ToF.[18,19] However, the normal aging process is also likely to play a role in the development of diastolic dysfunction, which was reflected in our cohort by increased LA volumes in older patients.

In this study, a decreased LA active emptying fraction was associated with cardiovascular events, whereas the passive emptying fraction was not. This association has also been described in the general population, in chronic hypertension, and in ischemic heart disease.[13,20] When LV diastolic dysfunction progresses, adequate diastolic filling of the LV becomes more dependent on the contractile function of the LA.[20] This might explain why the contribution of the LA active emptying fraction seems to be of clinical importance in patients with ToF, in contrast to the passive emptying fraction. This is in line with a previous study in asymptomatic young patients after ToF repair, in which earlier and increased LA active contraction was suggested to indicate adaptive compensatory mechanisms to overcome latent and asymptomatic altered LV performance.[21]

Left atrial enlargement and clinical worsening

RV dilatation and dysfunction have always been the major concern in patients with repaired ToF.[1] More recently, LV systolic dysfunction has also shown to predict adverse outcomes in ToF patients.[5,22,23] Several studies showed an association between RV and LV systolic dysfunction in ToF patients, suggesting an unfavorable ventricular interaction.[5,24,25] For instance, RV dilatation – secondary to RV volume overload – may shift the interventricular septum to the left, changing LV geometry, mechanics and filling.[26] More specific, speckle-tracking echocardiography studies have suggested that RV dilatation impedes LV apical rotation in patients with repaired ToF. The subsequent reduced LV twist resulted in global LV systolic dysfunction.[24,25,27] Interestingly, the abnormal systolic LV twist was also related to LV diastolic dysfunction in these patients.[24] Diastolic dysfunction can lead to an increase in LA pressure, reflected by LA enlargement. The resulting increase in pulmonary artery pressure may augment pulmonary regurgitation, which is a common problem in ToF patients.[1] The subsequent RV dilatation may create one of the vicious circles that can tilt a stable ToF patient towards progressive clinical deterioration.

Atrial fibrillation and mitral regurgitation are also known to influence the LA size.[28] In patients with ToF, atrial fibrillation is an important cause of morbidity and poor clinical

outcome.[15,28,29] LA dilatation in patients with mitral regurgitation has been described as a compensatory mechanism and predictor of cardiovascular events.[28,29] Both conditions could in theory explain the association of LA enlargement and clinical deterioration. However, in our cohort they do not seem to play a major role, since we found no significant differences in rhythm and heart rate between patients with a normal and a dilated LA, only three patients were in atrial fibrillation, and only one patient had moderate mitral regurgitation.

Comparison of left atrial size measurements

According to the guidelines, the most reliable and accurate method to estimate LA size and function is provided by LA volume or by multiple linear dimensions. A single linear measurement of the LA may underestimate the LA size, considering the asymmetrical shape and the possibility of foreshortening.[8,12,16,30] In order to compare the prognostic value of the different measurements of LA size (volume, area, length, and anteroposterior dimension), we evaluated each measurement as a standardized variable, which allows comparison of the HRs across all variables. Interestingly, a single measurement of LA length appeared to be more predictive than LA volume, reflected by a higher standardized HR. This could be explained by the observation that LA dilatation mainly develops in the craniocaudal direction. Anatomic factors such as a dilated right ventricle, the sternum and the spine may limit LA dilatation in the anteroposterior direction.[31] As a result, LA length possibly provides a better reflection of LA enlargement. In addition, LA length showed to be the most reproducible measurement, reflected by a smaller interobserver variation than the LA area or volume measurement.

Clinical implications

In patients with ToF, accurate risk prediction is of paramount importance in order to optimize individual follow-up strategies, pharmacological treatment and timing of interventions.[1] This study shows that one-dimensional LA length is a simple and reliable measurement, which is associated with cardiovascular events in clinically stable adults with repaired ToF. This measurement comes at no extra costs, as it is quick and can be performed on the A4C view of routinely acquired echocardiographic images. Although other imaging techniques such as CT, CMR or 3D echocardiography could provide a more accurate estimation of LA size, this simple linear dimension can be easily implemented in day-to-day clinical practice. Patients with LA dilatation may require more vigilant follow-up, initiation or expansion of medical therapy, or earlier timing of a re-intervention. Importantly, LA size seems to have a very high negative predictive value for death and heart failure, which makes it particularly valuable as a screening

tool. Patients with a normally sized LA may be reassured, and may need less frequent evaluation at the outpatient clinic.

Study limitations

The most important limitation of this study is that LA volumes were measured using a single plane approach of the A4C only, because the two-chamber views were of insufficient quality in the majority of patients. Because of this limitation, the LA volumes measured in our study could be slightly underestimated.[31] Second, due to the limited number of events, we could only adjust for the variables age and NYHA functional class, which may have resulted in residual confounding. Age at initial repair and prior palliative shunts were associated with LA dilatation, and may also play a crucial role. Larger studies are warranted to investigate the prognostic value of LA size and function in addition to multiple clinical, echocardiographic and biochemical markers.

CONCLUSIONS

In this cohort of clinically stable adults with repaired ToF, the LA was enlarged in a substantial number of patients. LA size and function, measured on routine echocardiographic examination, were significantly associated with cardiovascular events. A normally sized LA could accurately rule out the risk of death and heart failure with a high negative predictive value. LA length was most strongly associated with cardiovascular events, and had the best reproducibility. This free, quick and simple one-dimensional echocardiographic measurement may therefore be a valuable additional tool in the management and risk stratification of adults with repaired ToF.

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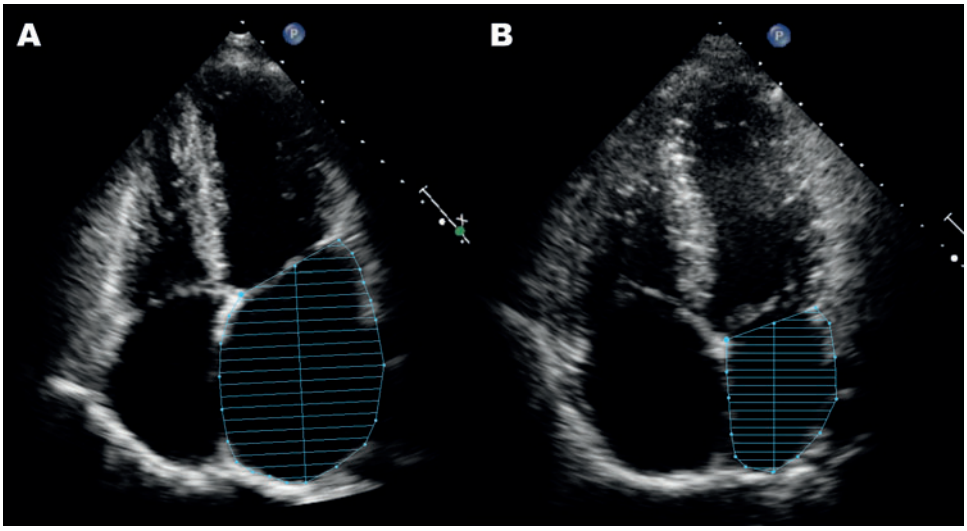
SUPPLEMENTAL MATERIAL

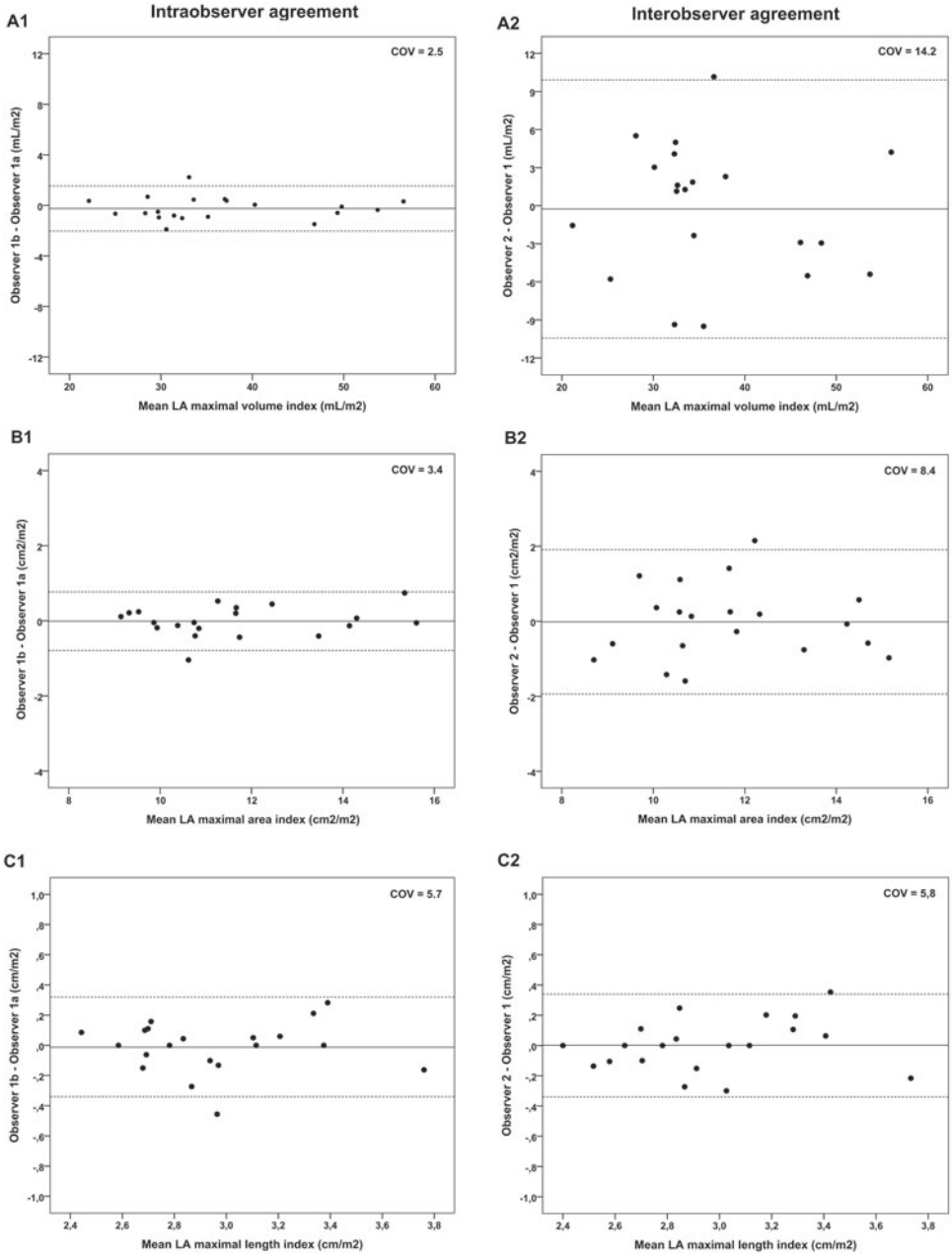


SUPPLEMENTAL FILE 1 - Left atrial length, area and volume measurement on the apical four chamber view
A, tetralogy of Fallot patient with a dilated left atrium (84 mL/m^2). B, tetralogy of Fallot patient with a normal left atrium (26 mL/m^2).

SUPPLEMENTAL FILE 2 - Patients with the primary and secondary composite endpoint.

		LA volume index, mL/m^2		
	All patients (n = 134)	**< 34 (normal, n = 76)**	**≥ 34 (dilated, n = 58)**	***P*-value**
Cardiovascular event	33 (25)	10 (13)	23 (40)	< 0.001
Death or heart failure	8 (6)	0 (0)	8 (14)	0.001
Death	4 (3)	0 (0)	4 (7)	
Heart failure*	6 (5)	0 (0)	6 (10)	
Hospitalization	26 (19)	9 (12)	17 (29)	
Arrhythmia	19 (14)	4 (5)	15 (26)	
Thromboembolic event	5 (4)	1 (1)	4 (7)	
Cardiac re-intervention	18 (13)	8 (11)	10 (17)	

For patients with multiple events, event-free and heart failure-free survival was defined as the time from enrollment to the occurrence of the first event. All components of the primary and secondary endpoint are separately displayed in this table for exploratory purposes (in which patients are not censored at the time of another endpoint than the endpoint of interest). *P*-value is calculated using the chi-squared test. Comparisons were not made between subgroups, in order to avoid multiplicity of comparisons. *Due to RV dysfunction (n = 3), LV dysfunction (n = 2) or both RV and LV dysfunction (n = 1). **Abbreviations:** LA, left atrial.



SUPPLEMENTAL FILE 3 - Bland-Altman plots demonstrating intraobserver (left) and interobserver agreement (right) for LA maximal volume (A), area (B) and length (C).

The solid lines represent the mean difference of two measurements. The dotted lines represent the limits of agreement (mean ± 1.96 SD). **Abbreviations:** COV, coefficient of variation; LA, left atrial.

Chapter 05

The prevalence of pulmonary arterial hypertension before and after atrial septal defect closure at adult age: a systematic review

Roxanne D. Zwijnenburg, Vivan J.M. Baggen, Laurie W. Geenen, Kelly R. Voigt, Jolien W. Roos-Hesselink, Annemien E. van den Bosch

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ABSTRACT

Background The development or persistence of pulmonary arterial hypertension (PAH) after atrial septal defect (ASD) closure at adult age is associated with a poor prognosis. The objective of this review was to investigate the prevalence of PAH before and after ASD closure and to identify factors that are associated with PAH.

Methods EMBASE and MEDLINE databases were searched for publications until March 2017. All studies reporting the prevalence of PAH or data on pulmonary artery pressures both before and after surgical or percutaneous ASD closure in an adult population (≥ 16 years of age) were included. Papers were methodologically checked and data was visualized in tables, bar charts and plots.

Results A total of 30 papers were included. The prevalence of PAH ranged from 29 to 73% before ASD closure and from 5 to 50% after closure; being highest in older studies, small study cohorts, and studies with high rates of loss to follow-up. The pooled systolic pulmonary artery pressure (PAP) was 43 ± 13 before ASD closure and 32 ± 10 after closure. The overall mean PAP was 34 ± 10 before closure and 28 ± 8 after closure. Studies with a higher mean PAP before closure and a higher mean age of the study cohort reported greater PAP reductions.

Conclusions The prevalence of PAH and mean pulmonary pressures decreased in all studies, regardless of the mean age or pulmonary pressures of the cohort. The reported prevalence of PAH after ASD closure is substantial, although widely varying (5–50%), which is likely affected by selection of the study cohort.

INTRODUCTION

Atrial septal defect (ASD) is the second most common congenital heart defect with an estimated worldwide birth prevalence of 1.6 per 1000 live births.[1] ASDs are usually detected and closed during childhood, but can also be discovered at adult age. When left untreated, chronic volume overload of the pulmonary vasculature can cause structural and mechanical changes in the pulmonary vascular bed. Eventually, patients may develop pulmonary arterial hypertension (PAH), accompanied by progressive right ventricular dilatation and dysfunction.[2] Therefore, it has been shown that ASD closure of a hemodynamically significant shunt is indicated at all ages.

Patients with PAH related to congenital heart disease suffer from substantial morbidity and mortality, with five-year mortality rates ranging from 5 to 23%.[3, 4] After ASD repair, it is unclear what proportion of patients continues to have high pulmonary pressures and which factors are associated with the persistence of PAH. It is also still under debate whether very high pulmonary artery pressures (PAP) are reversible after closure.[5-7] In addition, it has been reported that even patients with low pulmonary pressures before ASD repair may develop PAH after the procedure.[5, 8]

The objective of this systematic review was to investigate the prevalence of PAH both before and after ASD repair. In addition, we aimed to identify factors that are associated with the development or persistence of PAH after ASD closure.

METHODS

Protocol and registration

The systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement.[9] A pre-defined review protocol of this study can be accessed through PROSPERO (registration number: CRD42016034199). No extramural funding was used to support this work.

Information sources and search strategy

We performed a literature search on the 20th of March 2017 in EMBASE and MEDLINE online databases. The following search terms (including their synonyms and MeSH terms) were combined: ("pulmonary arterial hypertension" or "right ventricular pressure" or "tricuspid valve regurgitation") and "atrial septal defect" and "closure". The exact search syntax is shown in Supplemental File 1. Duplicates were identified and removed using Endnote X7.1 (New York, Thomson Reuters, 2013), which was manually checked.

Eligibility criteria

We included articles that reported the prevalence of PAH or data on PAP both before and after percutaneous or surgical ASD closure (both secundum ASD and sinus venosus defect) in an adult population (≥ 16 years of age at ASD closure). We only focused on the adult population, because these patients have a more long-term volume overload and are therefore more at risk of developing PAH. When the data for children and adults were separately described, the study was included and only the data of the adult population was used in this review. The following exclusion criteria were used: non-original data (reviews and comments) or non-clinical data (animal and in vitro studies), case series (study population of less than five participants), less than 95% secundum ASD or sinus venosus defect, follow-up shorter than three months, pulmonary pressure before and after ASD closure measured on different modalities, and articles written in languages other than English or Dutch. In addition, studies that only investigated patients with patent foramen ovale (PFO) closure were excluded.

Study selection

A flow diagram of the selection procedure is shown in Figure 1. Three authors (R.Z., L.G., K.V.) independently performed screening on title and abstract. In case of disagreement, a fourth author was consulted to achieve consensus. All references in reviews and in the remaining papers were crosschecked to identify possible relevant papers missed in the original search syntax. Subsequently, two authors (R.Z., V.B.) performed full-text screening based on the eligibility criteria as described above.

Methodological quality assessment

For the methodological quality assessment of the included studies we applied a modified version of the Newcastle-Ottawa Scale.[10] We used the items that were applicable for this review as described in Figure 2. The quality of the studies was assessed on the following main groups: selection of the study cohort, ascertainment of PAH before and after ASD closure, comparability of the cohort, and missing data.

Data extraction and analysis

Data was collected using a standardized form. This included study design, age, sex, type of procedure (surgical or percutaneous), New York Heart Association (NYHA) functional class, ASD diameter, prevalence of PAH and/or mean pulmonary artery pressures before and after ASD closure, modality and cut-off point used for the diagnosis of PAH, mean ASD diameter, mean follow-up time, and missing data.

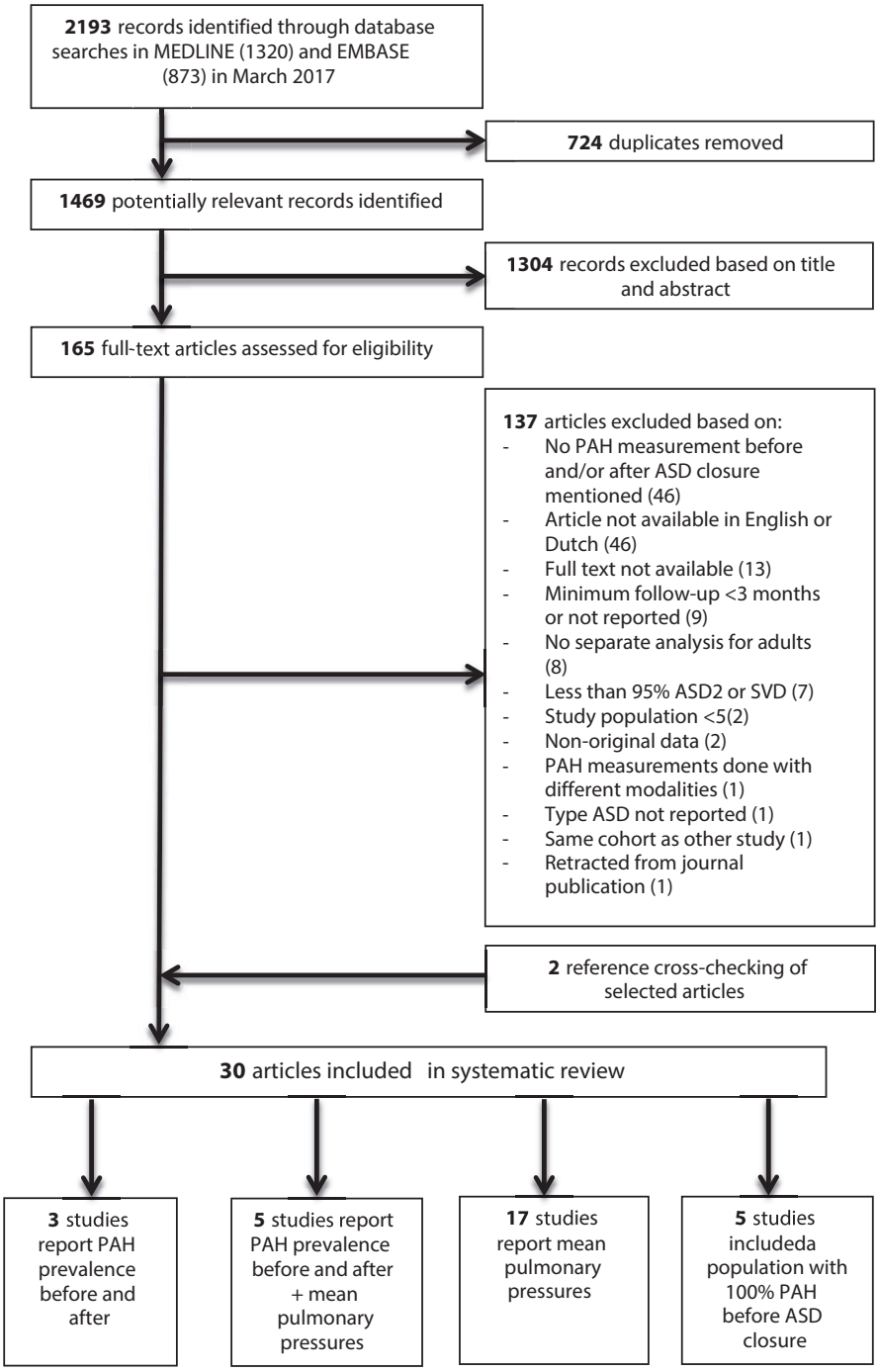

FIGURE 1 - Flow diagram of literature search and selection of studies.

To explore which factors could be associated with the development or persistence of PAH, we plotted the average age at closure, percentage women, and percentage NYHA class III-IV of the separate studies against the pulmonary pressures. Other study baseline characteristics were overall insufficiently reported to aggregate the results in plots. Because of the heterogeneity in modality of PAH measurement and result presentation, a formal meta-analysis was not conducted.

The pooled pulmonary pressure was calculated by the following formula: $\sum N * \text{pulmonary pressure} / \sum N$. The pooled standard deviation (SD) was calculated by the following formula: $\sum N - 1 * SD / \sum N$. Studies that included a patient population with 100% PAH before ASD closure were excluded when calculating the overall prevalence of PAH and mean pulmonary pressures before and after ASD closure, because these studies will cause a false-increased overall prevalence.

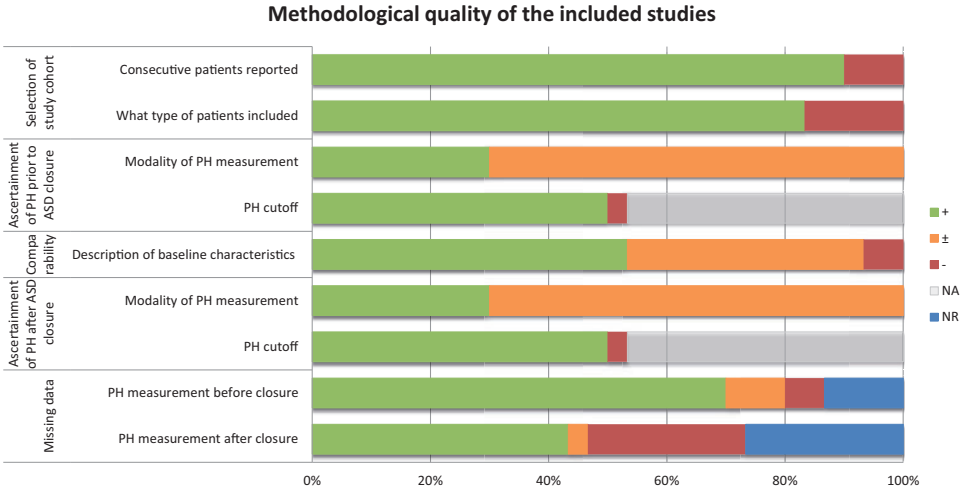

FIGURE 2 - Methodological quality of the included studies.

Selection of study cohort: Consecutive patients reported (+ yes, - no), Type of patients included (+ all patients with ASD, - only PAH patients), *Ascertainment of PAH prior to ASD closure:* Modality of PAH measurement (+ RHC, ± TTE/TEE, - Not defined), PAH cutoff defined (+ yes, - no, NA not applicable (study did not describe the prevalence of PAH), *Comparability of cohort:* Description of baseline characteristics: age, sex, comorbidities (+ all 3 items described, ± 2 items described - ≤ 1 item described), *Ascertainment of PAH after ASD closure:* Modality of PAH measurement (+ RHC, ± TTE/TEE, - Not defined), PAH cutoff defined (+ yes, - no, NA not applicable (study did not describe the prevalence of PAH), *Missing data:* PAH measurement before and after closure (+ < 5%, ± 5–10%, - > 10%, NR not reported).

RESULTS

Search results

The literature search identified 1469 potential relevant records, of which 1304 were excluded based on title and abstract (Figure 1). After comprehensive full-text screening and reference cross-checking, 30 articles were included in this systematic literature review.[5, 6, 11-38] Three studies[15, 17, 25] reported only PAH prevalence before and after ASD closure, five studies[11-14, 30] reported both PAH prevalence and average pulmonary pressures before and after ASD closure, 17 studies[16, 19-21, 23, 24, 26-29, 31-34, 36-38] only reported average pulmonary pressures before and after ASD closure, and five studies[5, 6, 18, 22, 35] included a patient population with 100% PAH before ASD closure. In eight studies, only a subpopulation met the inclusion criteria, which was used for this review.[5, 6, 16, 19, 27, 30, 32, 35] Two studies reported their data for two separate groups instead of for the total cohort, so we maintained these two groups in this review.[24, 33]

Study and patient characteristics of the included studies are shown in Table 1. The total number of study participants varied from 12 to 274, with a mean age ranging from 32 to 76 years (53–91% female). The mean follow-up duration ranged from 13 to 98 months.

Methodological aspects

The quality assessment per category is presented in Figure 2. The results of the quality assessment for the individual studies can be found in Supplemental Table 1. In 90% of the studies patients were included consecutively, in the other 10% this was not reported.

Pulmonary pressures before and after ASD closure were measured using right heart catheterization (RHC) in 9 studies (30%) and using echocardiography in 21 studies (70%). Some studies determined PAP before ASD closure with both RHC and echocardiography, but after closure with only echocardiography. Accordingly, we then also used the echocardiographic measurement of PAP before closure in order to compare these results.

Three studies reported the PAH prevalence both before and after ASD closure assessed by RHC with a cutoff of mean PAP ≥ 20 mmHg[13-15] and one study[5] did not report a cutoff for PAH. According to the current guidelines for the diagnosis of pulmonary hypertension the cutoff of mean PAP ≥ 25 mmHg assessed by RHC should be used.[39] D'Alto *et al.*,[5] Dave *et al.*[14] and Saksena *et al.*[13] presented the data of individual patients, so we were able to adjust the PAH cutoff for these studies to mean PAP ≥ 25 mmHg.

The percentage of patients who were lost to follow-up after ASD closure (i.e., no information available on pulmonary pressures) was between 5 and 10% in one study (3%), more than 10% in eight studies (27%) and not reported in eight studies (27%).

TABLE 1- Study characteristics.

		Study population						Outcome		
First author, year	**Inclusion period**	**Patients included in review (n)**	**Age at closure (years)**	**Sex (% female)**	**ASD diam. (mm)**	**NYHA III-IV (%)**	**Procedure ASD closure**	**Follow-up (months)**	**Modality**	**Cut-off value PH used in article (mmHg)**
Hanlon, 1969	1956–1969	56	32 ± NR	66	NR	16	Surgical	NR [5–138]	RHC	SPAP ≥ 30
Richmond, 1969	1957–NR	26	NR [45–59]	58	NR	58	Surgical	98 (mean) [4–108]	RHC	SPAP > 30
Saksena, 1970	1958–1966	24	51 [38–63]	67	NR	100	Surgical	72 (mean) [24–120]	RHC	MPAP > 20
Dave, 1973	1959– NR	32	42 ± NR	78	NR	35	Surgical	NR [6–144]	RHC	MPAP ≥ 20
Forfang, 1977	1959–1972	93	49 ± NR	74	NR	23	Surgical	NR [22–174]	RHC	MPAP > 20
Thilén, 2000	1958–1968	11	39 ± 9	91	NR	0	Surgical	NR [24–108]	RHC	NA
Veldtman, 2001	1997–1999	40	38 [20–71]	75	13 ± 4	5	Percutaneous	NR [1–12]	TTE	SPAP > 35
De Lezo, 2002	NR	29	56 ± 14	83	26 ± 7*	48	Percutaneous	21 ± 14	RHC	SPAP > 40
Celik, 2004	NR	41	NR [25–NR]	NR	NR	81	Surgical	34 ± 30	TTE	NA
Schoen, 2006	NR	20	43 ± 13	60	24 ± 6*	15	Percutaneous	13 (mean) [11–15]	TTE	SPAP > 30
Suchón, 2006	2000–2002	52	39 ± 15	64	NR	NR	Surgical	14 ± 1	TTE	SPAP > 30
Balint, 2008	1999–2004	54	59 ± 15	76	18 ± 7	NR	Percutaneous	31 ± 15	TTE	SPAP 40–49 (mild), 50–59 (moderate), ≥ 60 (severe)
Mahadevan, 2009	1990–2005	36	46 ± 15	64	20 ± 6*	NR	Percutaneous	30 ± 17	TTE	NA
Yalonetsky, 2009 group 1	1998–NR	23	52 ± 6	74	19 ± 5	NR	Percutaneous	NR [6–NR]	TTE	NA
Yalonetsky, 2009 group 2	1998–NR	23	67 ± 5	70	18 ± 5	NR	Percutaneous	NR [6–NR]	TTE	NA
Yong, 2009	1999–2006	215	54 ± 16	73	19 ± 6	19	Percutaneous	15 [IQR 8–43]	TTE	SPAP ≥ 40
Cohen, 2010	2001–NR	27	69 ± 6	63	NR	NR	Percutaneous	40 ± 26	TTE	NA

TABLE 1- Continued.

		Study population							Outcome	
First author, year	**Inclusion period**	**Patients included in review (n)**	**Age at closure (years)**	**Sex (% female)**	**ASD diam. (mm)**	**NYHA III-IV (%)**	**Procedure ASD closure**	**Follow-up (months)**	**Modality**	**Cut-off value PH used in article (mmHg)**
Mainzer, 2010	2002–2003	16	45 ± 17	63	23 ± 6	NR	Percutaneous	48 ± 5	TTE	NA
Ekim, 2011	2001–2010	20	46 ± NR	70	26 ± 4	NR	Surgical	NR [4–92]	TTE	NA
Humenberger, 2011	NR	236	49 ± 18	70	22 [IQR 19–26]	15	Percutaneous	28 ± 19	TTE	SPAP ≥ 40
Huang, 2012	2007–2010	7	NR	NR	NR	NR	Percutaneous	23 ± 10	RHC	SPAP ≥ 60 (severe)
Kefer, 2012	1999–2009	112	46 ± 17	71	PH: 22±5 no PH: 18 ± 6	NR	Percutaneous	60 ± 34	TTE	SPAP > 40
Nakagawa, 2012	2005–2010	30	76 ± 4	67	20 ± 6	27	Percutaneous	19 ± 11	TTE	MPAP ≥ 25
D'Alto, 2013	NR	12	41 ± 12	NR	NR	NR	Percutaneous and surgical	53 ± 25	RHC	MPAP ≥ 25†
Mangiafico, 2013	2008–2011	20	58 ± 11	NR	NR	0	Percutaneous	28 ± 16	TTE	NA
Jampates, 2014 < 60 years	2007–2012	274	39 ± 12	83	21 ± 7	5	Percutaneous	NR [6–NR]	TTE	NA
Jampates, 2014 ≥ 60 years	2007–2012	59	66 ± 5	78	21 ± 8	8	Percutaneous	NR [6–NR]	TTE	NA
Baykan, 2015	2013–2014	42	36 ± 14	60	22 ± 6	14	Percutaneous	NR [3–NR]	TTE	NA
Kijima, 2015	2006–2014	14	66 ± 13	71	23 ± 8	21	Percutaneous	18 ± 16	TTE	NA
Thilén, 2016	1997–2014	148	72 ± 5	72	16 ± 6	30	Percutaneous	53 ± 31	TTE	NA
Brojeni, 2017	2015	47	32 [29–43]	72	17 ± NR	NR	Percutaneous	NR [6–NR]	TEE TTE (FU)	NA
Dalvi, 2017	2002–2014	87	32 ± 12	66	32 ± 3	13	Percutaneous	44 ± 16	TTE	NA

Values are reported as mean ± SD, otherwise as median [range]. *balloon stretched diameter; † no cutoff reported, based on guidelines PAH. **Abbreviations:** FU, follow-up; IQR, interquartile range; MPAP, mean pulmonary artery pressure; NA, not applicable; NR, not reported; NYHA, New York Heart Association; RHC, right heart catheterization; SPAP, systolic pulmonary artery pressure; TTE, transthoracic echocardiography; TEE, transesophageal echocardiography.

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Prevalence of PAH after ASD closure

Eight out of the 30 studies reported the proportion of patients with PAH before and after ASD closure, according to a cut-off level of pulmonary arterial pressure. As shown in Figure 3, the prevalence of PAH varied between 29 and 73% before ASD closure, and between 5 and 50% after closure. When pooling these results, the overall prevalence was 48% before and 18% after the ASD repair. In all studies, the prevalence of PAH after closure decreased with absolute 16 to 54%.

We conducted a sensitivity analysis in which all studies with a loss to follow-up of > 20% (n = 4) were excluded. This yielded a pooled PAH prevalence of 42% before and 16% after ASD closure.

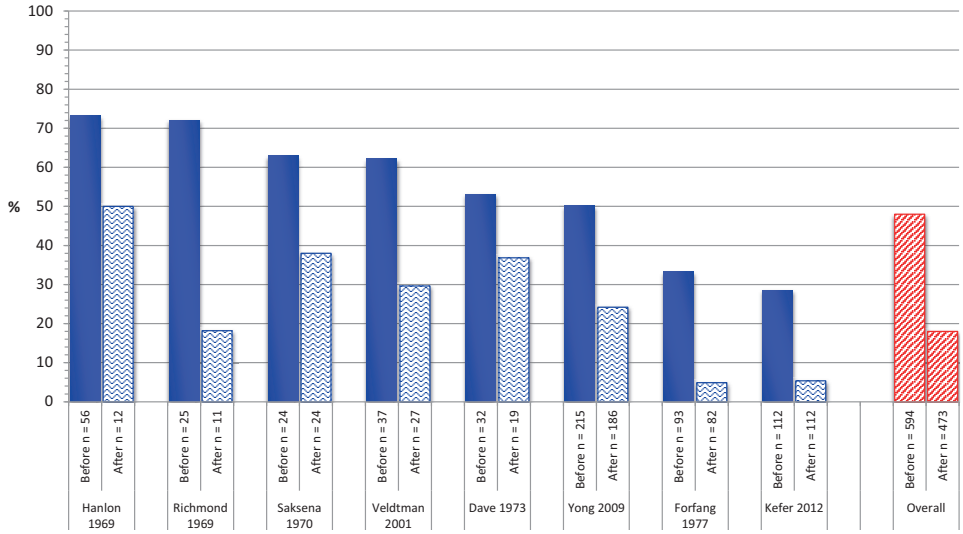

FIGURE 3 - PAH prevalence before and after ASD closure.

PAH prevalence before and after ASD closure presented per study. The number of patients shown in this bar chart is the number that had a measurement of pulmonary pressures (right heart catheterization or echocardiography).

Pulmonary pressures before and after ASD closure

In Figure 4, the pulmonary pressures before and after ASD closure are visualized. In all studies, the average pulmonary pressure of the study cohort decreased after closure. In 16 studies, a statistically significant reduction was found. In the remaining studies, only one described no significant difference[27] and the other five (of which two studies reported the results for two different subgroups[24, 33]) did not report any p-values. Considering that this is paired data, the individual reduction per patient would be

needed to derive the appropriate p-value, and these studies provided only the mean and standard deviation before and after ASD closure in the entire group. Therefore, the p-value could not be calculated from the data provided by the studies.

When pooling these results, the overall systolic PAP was 43 ± 13 before ASD closure and 32 ± 10 after closure. The overall mean PAP was 34 ± 10 before closure, and 28 ± 8 after closure. A sensitivity analysis with the exclusion of studies with loss to follow-up > 20% (n = 3) resulted in an overall systolic PAP of 39 ± 11 before and 31 ± 10 after ASD closure.

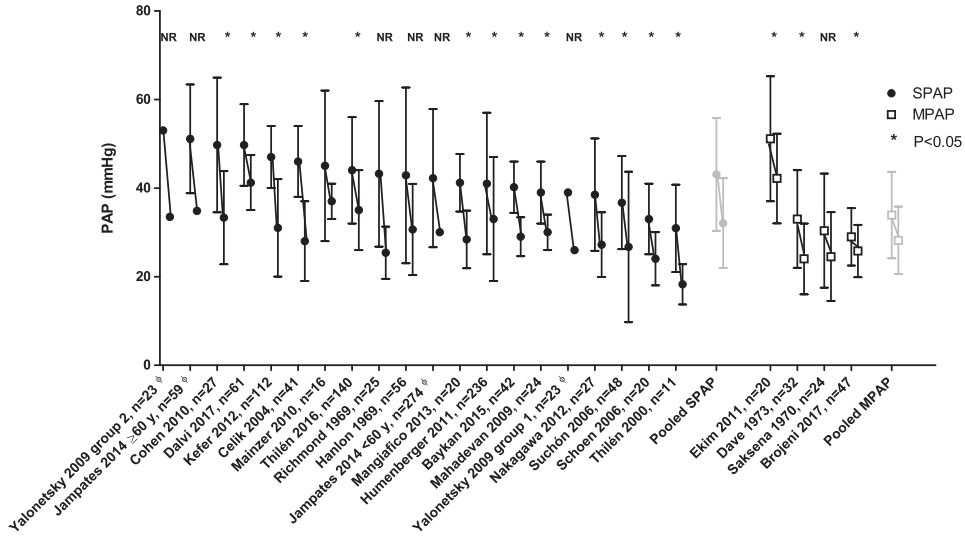

FIGURE 4 - Pulmonary pressures before and after ASD closure.

SPAP and MPAP before and after ASD closure shown as mean±SD. The number of patients displayed is the number that underwent measurement of PAP before closure. *Significant reduction of $p < 0.05$; NR, not reported. †SD before and/or after was not available and therefore also not included in the pooled pulmonary pressures.

Studies that included 100% PAH

Five studies had only selected patients with PAH before ASD closure, resulting in a PAH prevalence of 100% before closure.[5, 6, 18, 22, 35] All five studies presented PAP before and after closure (Supplemental Figure 1). The reduction in PAP after ASD closure was statistically significant in all five studies. Two studies additionally reported a PAH prevalence after ASD closure.[5, 22] Balint *et al.*[22] reported a prevalence of 56% and D'Alto *et al.*[5] 100% after ASD closure. Thus, in the latter study, although the average PAP decreased, all patients continued to have pulmonary pressures that were above the cutoff for PAH; therefore, no reduction in the prevalence of PAH was found. Of note, this study included only 12 patients.

When pooling the results of these five studies, the overall systolic PAP was 60 ± 15 before and 39 ± 13 after ASD closure. The overall mean PAP was 46 ± 8 before and 30 ± 8 after ASD closure.

Increase of PAP after ASD closure among individual patients

Although the average pulmonary pressures in the entire cohort decreased after ASD closure in all studies, five studies reported that the pulmonary pressure increased after ASD closure in a small subset of patients.[5, 13, 14, 22, 35] An increase in PAP was found in one to maximum four patients per study (5 to 17% of the study cohort). In three out of five studies, all patients with a rise in PAP were already diagnosed with PAH before ASD closure.[5, 22, 35] In the two other studies, only a very mild increase in PAP was present: in the study of Saksena *et al*. two patients who did not have pulmonary hypertension before ASD closure had an increase in PAP (mean PAP increased from 14 to 26 mmHg in one patient, and from 18 to 26 mmHg in the other) and another patient had an increase in mean PAP from 17 to 23 mmHg. In the study of Dave *et al*., only one patient had an increase in mean PAP from 21 to 27 mmHg. The patients with an increase in PAP were not significantly older, or could not be clearly distinguished based on other baseline characteristics from the patients with a decrease in PAP.

Three studies described that not any patient had an increase in PAP.[11, 12, 16] Importantly, all other studies have not explicitly stated whether or not there were individual patients with an increase in PAP.

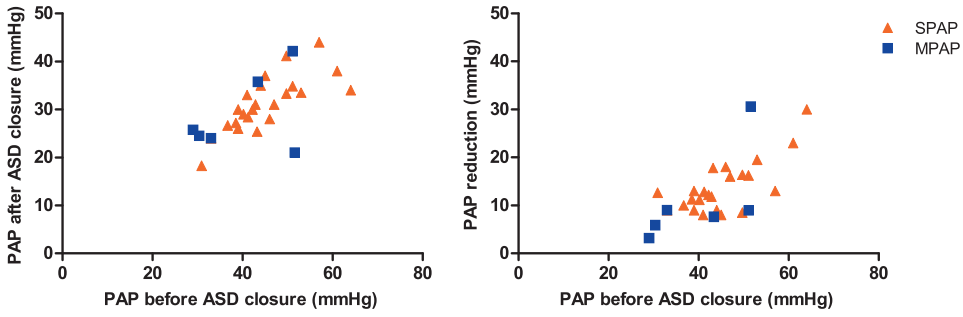

FIGURE 5 - Association of PAP before closure with PAP after closure and reduction in PAP.
Each symbol illustrates one study cohort.

Associations with pulmonary pressures

As shown in Figure 5, studies with a higher PAP before ASD closure also reported greater absolute PAP reductions. It may also be concluded that studies with a higher mean age

also have a higher PAP before ASD closure, with a corresponding larger PAP reduction (Figure 6). We did not observe any associations for sex and NYHA class (Supplemental Figure 2).

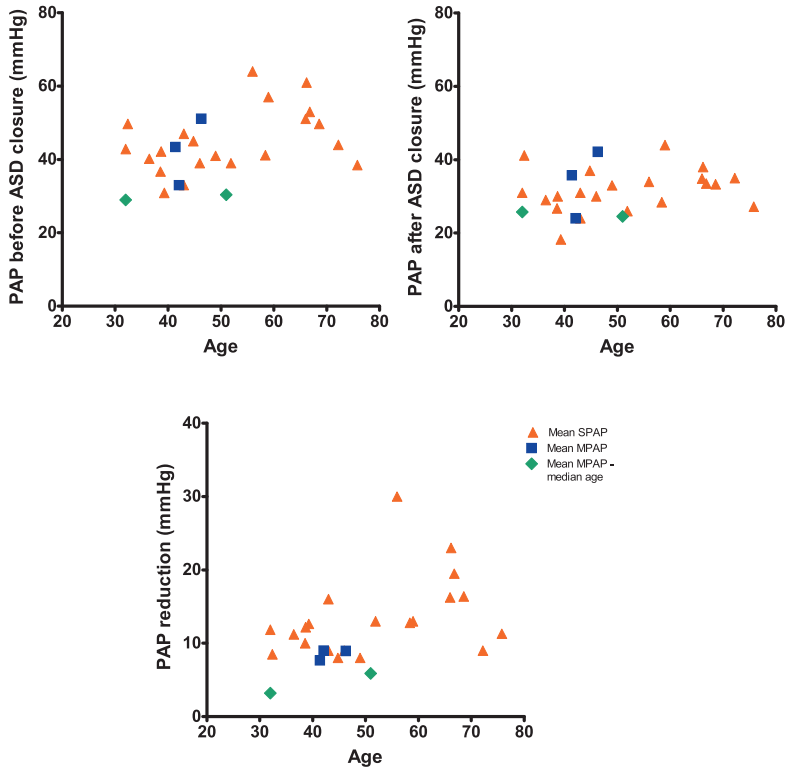

FIGURE 6 - Association of age with PAP before closure, PAP after closure, and reduction in PAP.

Each symbol illustrates one study cohort. The green symbol illustrates studies that reported the median age instead of the mean age.

DISCUSSION

To the extent of our knowledge, this is the first systematic review that provides an overview of all studies that investigated the prevalence of PAH or the pulmonary arterial pressures before and after ASD closure. In addition, we explored factors that were possibly associated with higher pulmonary pressures before or after ASD closure. The most important finding of this review is that the PAH prevalence and the mean pulmonary pressures decreased in all studies after ASD closure, regardless the mean age of the study cohort or pulmonary pressures. In addition, studies with the highest pulmonary pressures before closure generally also reported the largest reduction in pulmonary pressures. The proportion of patients with PAH after ASD closure remained substantial, although widely varying (5–50%).

PAH prevalence

The widely varying prevalence of PAH after ASD closure may be partly explained by different selection criteria of the studies. The main studies with the highest proportion of patients with PAH after closure are published over 30 years ago, and included a small number of patients.[11, 13, 14] Accordingly, studies with the lowest percentage of PAH after ASD closure included the highest number of patients (Figure 3). In addition, a high proportion of patients was lost to follow-up in the studies that reported a high PAP after ASD closure. Hanlon *et al.*[11] and Dave *et al.*[14] reported a proportion of 79% and 41% loss to follow-up after ASD closure, respectively. Because healthy patients without complaints may be more likely to withdraw from follow-up, the prevalence in these studies may be overestimated. The "true" prevalence of PAH after closure is therefore likely to be more towards the lower limit of the reported range (i.e., 5–20%). This is also in line with two other large studies that only assessed the prevalence of PAH after ASD closure, reporting a prevalence of 8%[40] and 12%[3] in 717 and 377 adults after ASD closure, respectively.

Studies with 100% PAH included

Of the five studies that selected only patients with PAH before ASD closure, the study of D'Alto *et al.* additionally selected patients based on whether they had PAH after ASD closure.[5] In addition, the study population existed of patients with a very high pulmonary vascular resistance (PVR) and ratio between PVR and systemic vascular resistance (SVR). Therefore, this is actually a highly-selected group with irreversible PAH. This explains why this study showed no change in the PAH prevalence after ASD closure and thus remained to be 100%, whereas all others did report a reduction. Nonetheless, although the PAH prevalence after ASD closure did not decrease, ASD closure may still be beneficial in these patients because the overall mean PAP significantly decreased.

Increase of PAP after ASD closure

This systematic review shows that the PAP may increase in individual patients after ASD closure. However, this increase in PAP was mainly observed in patients who had already been diagnosed with PAH before the closure. In other patients, there was only a negligible increase in PAP. One study that was not included in this review because they also studied patients with ASD closure in their childhood, did describe a large group of patients (n = 20, 10%) that had a normal PAP before ASD closure, but developed PAH during follow-up.[41] Furthermore, most studies did not report if there were individual patients who had an increase of PAP after closure. This could have resulted in under-reporting and therefore underestimation of this number. For future research, it is

advisable to report individual results of pulmonary pressure changes to provide more insight in this topic.

Association between age and pulmonary pressures

Some previous studies have also investigated which factors were associated with PAH after ASD closure.[25, 41] In the study of Yong and colleagues, age at closure was significantly associated with PAH in a multivariable analysis.[25] Humenberger *et al.* reported that although a reduction in systolic PAP was observed in all age groups, older patients ended up with higher PAP after the ASD closure.[29] Although not included in this review because also children were included, the study of Gabriels *et al.* reported that age at closure was significantly associated with PAH in the multivariable analysis, when adjusted for mean PAP before repair, body mass index and systolic blood pressure.[41] In this review, the mean age of the study cohort seemed to be positively associated with the mean PAP reduction, and possibly with the mean PAP before ASD closure (Figure 6). However, we could not demonstrate a clear association between the mean age of the study cohort and the mean PAP after ASD closure. These differences may be explained by the fact that this review only studied aggregated data (i.e., mean age of the study cohort), as opposed to individual patient data in the original studies.

Clinical implications

In the ESC guidelines for the management of grown-up congenital heart disease,[42] it is recommended that patients with elevated PAP and with ASD closure at adult age (especially over 40 years of age) should be checked periodically. Advised is regular follow-up during the first two years and then, depending on the results, every 2–4 years. Given the substantial percentage of patients with PAH after closure, this review confirms that long-term follow-up with monitoring of RV pressures is mandatory.

A long-standing discussion is whether ASD closure is still useful in elderly patients.[43-45] Importantly, in this review we found a decrease in PAP in all studies, regardless of the mean age of the study cohort. On top of that, the reduction in PAP may even be higher in studies that included older patients. Therefore, the results from this review show that ASD closure leads to decreased pulmonary pressures in all investigated study cohorts and may thus be considered beneficial at any age.

Study limitations

We included studies that measured PAP with either echocardiography or RHC. Although RHC is the reference standard to obtain hemodynamic measurements,[46] echocardiography is non-invasive and easy to use in clinical practice. Including both

modalities in this review is one cause of the heterogeneity of the results. Moreover, different cutoff values for PAH were used, even among studies that measured PAP with the same modality. We have tried to improve this by using the cutoff value for mean PAP ≥ 25 mmHg (measured with RHC) in studies where individual patient data were reported.

Second, there was a high number of patients lost to follow-up in a large subset of studies, which might have introduced selection bias. It is difficult to indicate in what direction this may have biased the results. The prevalence of PAH can be underestimated if patients with the highest pulmonary pressures are lost to follow-up, or overestimated if more healthy people with low pulmonary pressures are being discharged from follow-up and do not receive a second echocardiogram or RHC after ASD closure. We believe that the latter is more likely to have occurred. Nonetheless, in a sensitivity analysis with the exclusion of studies with > 20% loss to follow-up, there was only a slight decrease in the pooled PAH prevalence and pooled systolic PAP, both before and after ASD closure.

CONCLUSIONS

This systematic review shows that the reported prevalence of PAH after ASD closure varies between 5 and 50%, and was highest in older studies with small study cohorts and studies with a large proportion of patients lost to follow-up. The prevalence of PAH and mean pulmonary pressures decreased in all studies, regardless of the mean age or pulmonary pressures of the cohort. In addition, studies with a higher mean PAP before closure and a higher mean age of the study cohort reported greater PAP reductions. Still, long-term follow-up of pulmonary artery pressures is warranted, as the proportion of patients with PAH after ASD remains substantial, and the pulmonary pressure may increase in individual patients.

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FILE 1 - Search syntax used to identify publications of interest. Search date March 20, 2017.

MEDLINE

"pulmonary hypertension"[tiab] OR "pulmonary arterial hypertension"[tiab] OR "pulmonary artery hypertension"[tiab] OR PAH[tiab] OR (pulmonary[tiab] AND (artery[tiab] OR arterial[tiab]) AND (pressure[tiab] OR pressures[tiab])) OR (right[tiab] AND (ventricular[tiab] OR ventricle[tiab]) AND (pressure[tiab] OR pressures[tiab])) OR ((tricuspid[tiab] OR tricuspidalis[tiab] OR tricuspidal[tiab] OR tricuspide[tiab]) AND (regurgitant[tiab] OR regurgitation[tiab] OR insufficiency[tiab] OR velocity[tiab] OR velocities[tiab] OR gradient[tiab] OR gradients[tiab])) OR "Hypertension, Pulmonary"[MeSH Terms]
AND
ASD[tiab] OR ((atrial[tiab] OR atrium[tiab] OR atria[tiab] OR interatrial[tiab]) AND (septum[tiab] OR septal[tiab]) AND (defect[tiab] OR defects[tiab])) OR (ostium[tiab] AND primum[tiab] AND (defect[tiab] OR defects[tiab])) OR (ostium[tiab] AND secundum[tiab] AND (defect[tiab] OR defects[tiab])) OR (sinus[tiab] AND venosus[tiab] AND (defect[tiab] OR defects[tiab])) OR "Heart Septal Defects, Atrial"[MeSH Terms]
AND
closure[tiab] OR closed[tiab] OR surgery[tiab] OR repair[tiab] OR repaired[tiab] OR occlusion[tiab] OR occluder[tiab] OR occluded[tiab] OR "Septal Occluder Device"[MeSH Terms]
NOT
(animals[MeSH] NOT humans[MeSH])

EMBASE

"pulmonary hypertension":ti,ab OR "pulmonary arterial hypertension":ti,ab OR "pulmonary artery hypertension":ti,ab OR PAH:ti,ab OR (pulmonary:ti,ab AND (artery:ti,ab OR arterial:ti,ab) AND (pressurev OR pressures:ti,ab)) OR (right:ti,ab AND (ventricular:ti,ab OR ventricle:ti,ab) AND (pressure:ti,ab OR pressures:ti,ab)) OR ((tricuspid:ti,ab OR tricuspidalis:ti,ab OR tricuspidal:ti,ab OR tricuspide:ti,ab) AND (regurgitant:ti,ab OR regurgitation:ti,ab OR insufficiency:ti,ab OR velocity:ti,ab OR velocities:ti,ab OR gradient:ti,ab OR gradients:ti,ab))
AND
ASD:ti,ab OR ((atrial:ti,ab OR atrium:ti,ab OR atria:ti,ab OR interatrial:ti,ab) AND (septum:ti,ab OR septal:ti,ab) AND (defect:ti,ab OR defects:ti,ab)) OR (ostium:ti,ab AND primum:ti,ab AND (defect:ti,ab OR defects:ti,ab)) OR (ostium:ti,ab AND secundum:ti,ab AND (defect:ti,ab OR defects:ti,ab)) OR (sinus:ti,ab AND venosus:ti,ab AND (defect:ti,ab OR defects:ti,ab))
AND
closure:ti,ab OR closed:ti,ab OR surgery:ti,ab OR repair:ti,ab OR repaired:ti,ab OR occlusion:ti,ab OR occluder:ti,ab OR occluded:ti,ab
NOT
([conference abstract]/lim OR [conference paper]/lim OR [conference review]/lim OR [editorial]/lim OR [erratum]/lim OR [letter]/lim OR [note]/lim OR [animals]/lim)
AND
[embase]/lim

SUPPLEMENTAL TABLE 1 - Methodological quality of the included studies.

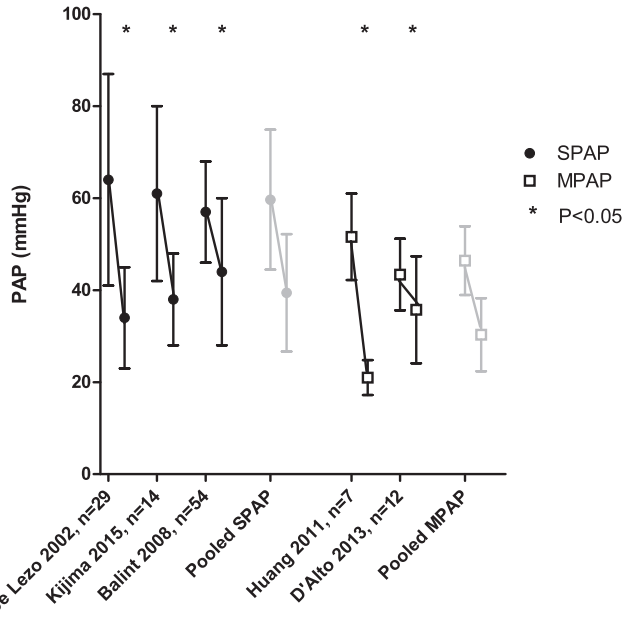
	Selection of the study cohort		Ascertainment of PAH prior to ASD closure		Comparability of the cohort	Ascertainment of PAH after ASD closure		Missing data	
Author, year	**Consecutive patients reported**	**Type of patients included**	**Modality of PH measurement**	**PH cutoff**	**Description of baseline characteristics**	**Modality of PH measurement**	**PH cutoff**	**PH measurement before closure**	**PH measurement after closure**
Hanlon, 1969	+	+	+	+	+	+	+	+	-
Richmond, 1969	+	+	+	+	±	+	+	±	-
Saksena, 1970	+	+	+	+	+	+	+	+	+
Dave, 1973	+	+	+	+	+	+	+	+	-
Forfang, 1977	+	+	+	+	+	+	+	+	±
Thilén, 2000	+	+	+	NA	±	+	NA	+	+
Veldtman, 2001	+	+	±	+	±	±	+	+	±
De Lezo, 2002	+	-	±	+	+	±	+	+	-
Celik, 2004	+	+	±	NA	-	±	NA	+	+
Schoen, 2006	-	+	±	+	±	±	+	+	+
Suchón, 2006	-	+	±	+	±	±	+	±	NR
Balint, 2008	+	-	±	+	+	±	+	+	-
Mahadevan, 2009	+	+	±	NA	+	±	NA	-	-
Yalonetsky, 2009	+	+	±	NA	+	±	NA	+	NR
Yong, 2009	+	+	±	+	+	±	+	+	+
Cohen, 2010	+	+	±	NA	±	±	NA	+	+
Mainzer, 2010	+	+	±	NA	±	±	NA	NR	NR
Ekim, 2011	+	+	+	NA	+	±	NA	+	NR
Huang, 2011	+	-	+	+	±	±	+	+	+
Humenberger, 2011	-	+	+	NA	+	±	NA	+	+

05

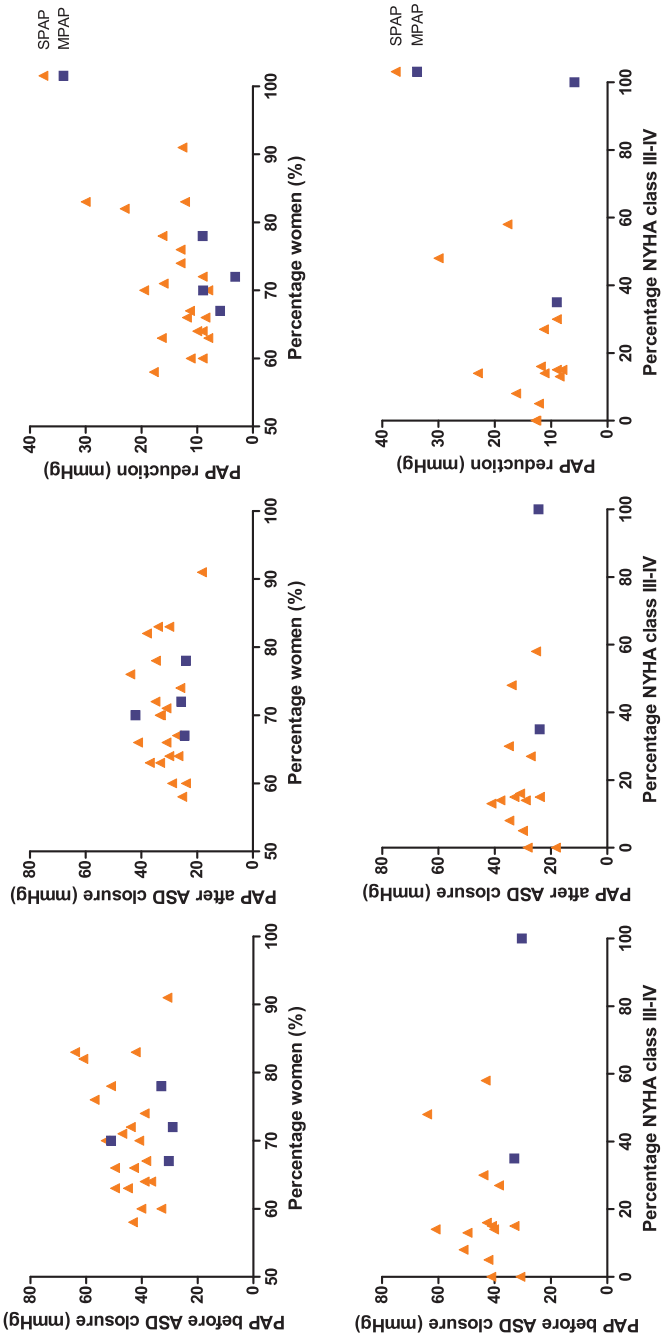
SUPPLEMENTAL TABLE 1 - Continued.

	Selection of the study cohort		Ascertainment of PAH prior to ASD closure		Comparability of the cohort	Ascertainment of PAH after ASD closure		Missing data	
Author, year	**Consecutive patients reported**	**Type of patients included**	**Modality of PH measurement**	**PH cutoff**	**Description of baseline characteristics**	**Modality of PH measurement**	**PH cutoff**	**PH measurement before closure**	**PH measurement after closure**
Kefer, 2012	+	+	±	+	+	±	+	+	+
D'Alto, 2013	+	-	+	+	-	+	+	+	+
Mangiafico, 2013	+	+	±	NA	+	±	NA	NR	NR
Jampates, 2014	+	+	+	NA	+	±	NA	+	+
Baykan, 2015	+	+	±	NA	±	±	NA	NR	NR
Kijima, 2015	+	-	+	+	±	±	+	+	+
Dalvi, 2016	+	+	±	NA	±	±	NA	+	-
Thilén, 2016	+	+	+	NA	+	±	NA	-	NR
Wang, 2016	+	+	+	+	±	±	+	+	+
Brojeni, 2017	+	+	±	NA	±	±	NA	NR	NR

Selection of study cohort: Consecutive patients reported (+ yes, - no), Type of patients included (+ all patients with ASD, - only PH patients), *Ascertainment of PH prior to ASD closure:* Modality of PH measurement (+ RHC, ± TTE/TEE, - Not defined), PH cutoff defined (+ yes, - no, NA not applicable (study did not describe the prevalence of PH), *Comparability of cohort:* Description of baseline characteristics: age, sex, comorbidities (+ all 3 items described, ± 2 items described - ≤ 1 item described), *Ascertainment of PH after ASD closure:* Modality of PH measurement (+ RHC, ± TTE/TEE, - Not defined), PH cutoff defined (+ yes, - no, NA not applicable (study did not describe the prevalence of PH), *Missing data:* PH measurement before and after closure (+ < 5%, ± 5–10%, - > 10%, NR not reported).



SUPPLEMENTAL FIGURE 1 - Pulmonary pressures before and after ASD closure for the studies that included 100% PAH before closure.



SUPPLEMENTAL FIGURE 2 - Association of sex and NYHA class with PAP before closure, PAP after closure, and PAP reduction.

Chapter 06

Risk factors for pulmonary hypertension in adults after atrial septal defect closure

Roxanne D. Zwijnenburg, Vivan J.M. Baggen, Maarten Witsenburg, Eric Boersma, Jolien W. Roos-Hesselink, Annemien E. van den Bosch

Submitted.

ABSTRACT

Background Atrial septal defect (ASD) closure is performed to prevent pulmonary hypertension (PH), which is associated with poor outcome. This study investigates the prevalence of PH in adults before and after ASD closure and explored associations between patient characteristics and PH after ASD closure.

Methods Consecutive adult patients who underwent surgical or percutaneous ASD closure (2000–2014) in the Erasmus MC, the Netherlands, were included. Echocardiograms before and after ASD closure were retrospectively assessed. Patients were categorized into three groups (no, possible, and likely PH) based on tricuspid regurgitation velocity (< 2.9, 2.9–3.4, and ≥ 3.4 m/s) or mean pulmonary arterial pressure (< 20, 20–24, and ≥ 25 mmHg). Cox regression was performed to identify associations between patient characteristics and PH after ASD closure.

Results Of the 244 eligible patients who underwent ASD closure, 198 (81%) had echocardiograms both before and median 15 [IQR 12–35] months after ASD closure (median age at closure 45 [IQR 30–57] years, 75% female). The prevalence of PH was 13.1% (n = 26) before ASD closure and 5.0% (n = 10) after closure. NYHA III–IV (HR 11.07, 95% CI 3.12–39.29, $p < 0.001$), pulmonary disease (HR 10.43, 95% CI 2.12–51.21, $p = 0.004$), cardiac medication use (HR 3.96, 95% CI 1.02–15.34, $p = 0.047$), right ventricular fractional area change (HR 0.87, 95% CI 0.81–0.93, $p < 0.001$), and tricuspid annular plane systolic excursion (HR 0.75, 95% CI 0.59–0.95, $p = 0.018$) were significantly associated with PH.

Conclusions Adults with low pulmonary pressures before ASD closure are not at risk of PH after closure. Especially in patients with risk factors for PH after ASD closure close follow-up is warranted.

INTRODUCTION

Atrial septal defect (ASD) is a common congenital cardiac anomaly and accounts for approximately 10% of congenital cardiac anomalies.[1] Increased pulmonary flow caused by left-to-right shunting can lead to right ventricular failure and/or pulmonary hypertension (PH).[2] Left untreated, the consequent increase in pulmonary vascular resistance can cause progressive deterioration of right ventricular (RV) function, right heart failure, and eventually death.[3, 4] Therefore, in patients with a significant hemodynamic shunt causing RV volume overload, ASD closure is indicated, unless specific contra-indications are present.[5]

The reported prevalence of PH in adult patients with a closed ASD is widely varying from 5% up to 50% in older studies.[6-15] Some studies even suggest that pulmonary pressures can further increase after ASD closure in individual patients.[16] The persistence or development of PH after ASD closure is probably due to less reversible changes in extracellular matrix and endothelial dysfunction, accompanied with increased pulmonary vascular resistance. PH associated with congenital heart disease is associated with high mortality and morbidity.[4] Identification of patient characteristics associated with the persistence or development of PH after ASD closure, as well as further delineation of the clinical course of patients with PH after ASD closure is important, as it affects individual patient follow-up and therapeutic management, and contributes to the general understanding of PH pathophysiology.

The objective of this study is to estimate the prevalence of PH before and after ASD closure. In addition, this study aims to investigate the association between patient characteristics and the presence of PH after ASD closure.

METHODS

Study population

All consecutive adult patients who underwent surgical or percutaneous closure of an ASD ostium secundum or sinus venosus defect between 2000 and 2014 in our center were identified. We excluded patients with a patent foramen ovale (PFO), significant pulmonary stenosis (> 2.0 m/s or requiring surgery), Ebstein's anomaly, left heart surgery or PCI during the procedure, lung transplantation before ASD closure, and patients with other identifiable causes for PH such as chronic thromboembolic PH.

Patient characteristics such as age at ASD closure, sex, height, weight, blood pressure, saturation, New York Heart Association (NYHA) functional class, cardiac medication, known pulmonary disease (chronic obstructive pulmonary disease (COPD) or obstructive sleep apnea syndrome (OSAS)), type of procedure (percutaneous or surgical), residual

lesions, and cardiac (re-)interventions were obtained from the electronic patient charts. The Erasmus MC medical ethics committee approved the study protocol and waived the need for written informed consent.

Echocardiography

All transthoracic echocardiograms before and after ASD closure were retrospectively assessed. Right atrial pressure was estimated from the inferior vena cava size and the response to inspiration.[17] Right atrial (RA) and right ventricular (RV) dimensions and area were primarily measured on a RV focused view. If this view was not available, these measurements were performed on the apical four-chamber view. Cardiac dimensions were measured in agreement with the current recommendations of the American Society of Echocardiography and European Association of Cardiovascular Imaging.[17] Tricuspid regurgitation (TR) and pulmonary regurgitation (PR) were quantified on color Doppler echocardiography and right ventricular systolic pressure; mean and diastolic pulmonary arterial pressures were calculated in accordance with the current recommendations of the American Society of Echocardiography and European Association of Cardiovascular Imaging.

PH assessment

Patients were categorized into three groups based on TR velocity (no PH: < 2.9 m/s, possible PH: 2.9–3.4 m/s, and likely PH: ≥ 3.4 m/s) according to the ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension.[18] When this measurement was not available, categorization was based on mean pulmonary arterial pressure (mPAP) (pulmonary regurgitation maximal velocity and estimated right atrial pressure; < 20, 20–24, and ≥ 25 mmHg, respectively). When both TR velocity and mPAP could not be measured, patients were categorized as possible PH based on evaluation of enlarged RV dimensions, decreased RV function and signs of PH (systolic and diastolic septal flattening on the parasternal short axis image at midventricular level[19]) by a cardiologist expert in the field of echocardiography and pulmonary hypertension (A.E.v.d.B.). RV function was quantified with tricuspid annular plane systolic excursion (TAPSE) and RV fractional area change (FAC).

Mid-term and long-term follow-up

The mid-term follow-up was defined as the first echocardiogram that was performed ≥6 months after the ASD closure and was collected in all patients. We actively invited the high-risk subset of patients (patients categorized as likely PH before ASD closure, or possible or likely PH after closure) to visit our outpatient clinic for a long-term follow-

up visit. Mid- and long-term follow-up was performed by non-invasive examinations (clinical examination, electrocardiography and transthoracic echocardiography). If patients were followed up in another hospital and were not able to come to the Erasmus Medial Center Rotterdam, we contacted the patients and asked for their consent for retrieving their data from the local hospital.

Statistical analysis

Continuous variables were reported as mean ± standard deviation (SD), or as median [interquartile range (IQR)] if data was skewed. Categorical data were presented as frequencies and percentages. Comparisons across the different PH categories (no PH, possible PH, and likely PH) were performed using the Chi-Square Mantel-Haenszel test for trend for categorical variables and linear regression for continuous variables. Cox proportional hazards regression was performed to identify associations between patient characteristics and PH after ASD closure. Proportional hazards assumption was checked in R (version 3.4.1) using log-minus-log plots for categorical variables and Schoenfeld residuals for continuous variables. Because of the limited number of patients with PH after ASD closure, multivariable Cox regression analysis was not performed. Statistical analysis was performed using SPSS version 21.0.0.1 (IBM Corp., Armonk, NY, USA). Two-sided *p*-values of < 0.05 were considered statistically significant.

RESULTS

Study population

We identified 281 adult patients with an ASD closure in our center, of whom 37 patients were excluded based on pre-defined criteria (Figure 1). Of the 244 eligible patients, 6 had missing echocardiographic data on RV pressures before the ASD closure and 40 patients had missing echocardiographic data on RV pressures after the ASD closure. Therefore, 198 patients were included in the final study cohort. The 46 patients with missing RV pressures did not have significant differences in age at closure, body mass index, use of cardiac medication, NYHA class, type of procedure, or PH classification before ASD closure compared to the 198 patients included in the analysis.

Baseline patient characteristics

Of the 198 patients, the ASD was closed percutaneously in 110 patients and surgically in 88 patients. The median age at closure was 45 [IQR 30–57] years and 75% was female. Baseline characteristics of the study population (and for the separate PH classification groups) are further specified in Table 1.

Patients who were classified as likely PH before ASD closure were significantly older, a larger proportion was in NYHA class III–IV, used cardiac medication, had a higher body mass index, a higher systolic blood pressure, a larger proportion had a saturation under 95%, had more often loss of sinus rhythm, a longer QRS duration, a larger defect diameter, more often RA pressure ≥ 10 mmHg, a larger RA end-systolic area, higher TR maximum velocity, decreased RV FAC, and larger RV end-diastolic diameter.

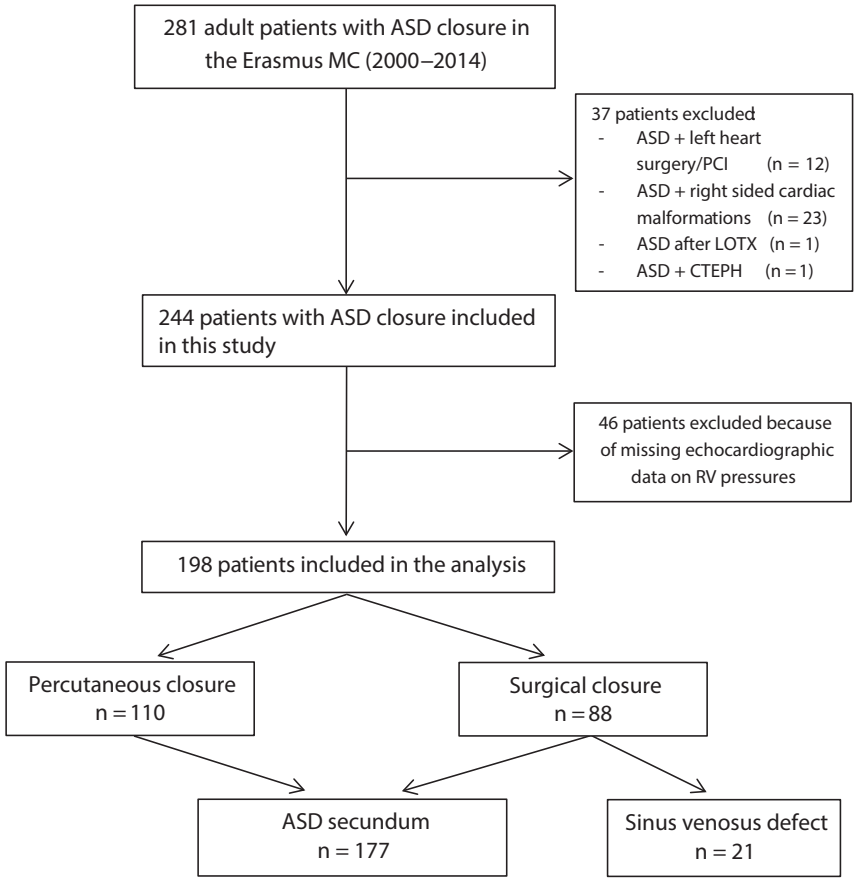


FIGURE 1 - Flowchart of the patient selection.

TABLE 1 - Baseline characteristics of the study cohort (n = 198).

	Valid cases, n (%)	All, n = 198	PH classification before ASD closure			*P* for trend
			No PH (n = 121)	Possible PH (n = 51)	Likely PH (n = 26)	
Clinical characteristics						
Age at closure, years*	198 (100)	45 [30–57]	42 [27–52]	53 [44–64]	50 [36–66]	< 0.001
Sex, female n (%)	198 (100)	148 (75)	91 (75)	39 (77)	18 (69)	0.650
NYHA class III–IV, n (%)	198 (100)	24 (12)	7 (6)	6 (12)	11 (42)	< 0.001
Sinus venosus defect, n (%)	198 (100)	21 (11)	15 (12)	5 (10)	1 (4)	0.207
Surgical repair, n (%)	198 (100)	88 (44)	52 (43)	21 (41)	15 (58)	0.298
Pulmonary disease, n (%)	198 (100)	8 (5)	4 (3)	2 (4)	2 (8)	0.355
COPD	198 (100)	7 (4)	4 (3)	2 (4)	1 (4)	-
OSAS	198 (100)	2 (1)	0 (0)	0 (0)	2 (8)	-
Cardiac medication, n (%)	198 (100)	66 (33)	27 (22)	23 (45)	16 (62)	< 0.001
ACE-inhibitor	198 (100)	12 (6)	5 (4)	4 (8)	3 (12)	-
Beta blocker	198 (100)	49 (25)	19 (16)	20 (39)	10 (39)	-
Diuretic	198 (100)	33 (17)	10 (8)	14 (28)	9 (35)	-
Anti-arrhythmic	198 (100)	12 (6)	2 (2)	6 (12)	4 (15)	-
Body mass index, kg/m^2	198 (100)	25 ± 5	24 ± 4	27 ± 5	25 ± 5	0.016
Systolic blood pressure, mmHg	197 (99)	133 ± 19	123 ± 16	142 ± 21	139 ± 19	< 0.001
O_2 saturation < 95%, n (%)	126 (64)	16 (8)	4 (6)	6 (17)	6 (32)	0.002
Electrocardiography						
Heart rate, beats/min	197 (99)	73 ± 12	73 ± 12	74 ± 14	73 ± 11	0.998
Loss of sinus rhythm, n (%)	198 (100)	30 (15)	13 (11)	9 (18)	8 (31)	0.009
QRS duration, ms	198 (100)	109 ± 19	107 ± 18	110 ± 19	116 ± 24	0.021
PR interval, ms	173 (87)	164 ± 24	169 ± 23	171 ± 25	168 ± 31	0.114
Echocardiography						
Defect diameter, mm	163 (82)	21.5 ± 9.4	20.4 ± 8.6	21.2 ± 9.7	27 ± 11	0.007
RA pressure, mmHg	177 (90)	6.5 ± 2.8	6.1 ± 2.5	6.6 ± 2.8	7.8 ± 3.9	0.012
RA pressure ≥ 15 mmHg	177 (90)	10 (6)	4 (4)	2 (5)	4 (17)	0.025
RA end-systolic area	159 (80)	29 ± 11	26 ± 8	33 ± 13	38 ± 14	< 0.001
RA end-systolic area > 18 cm^2, n (%)	159 (80)	151 (76)	95 (93)	37 (97)	19 (100)	0.144
TR maximum velocity, m/s	187 (94)	2.8 ± 0.5	2.4 ± 0.3	3.0 ± 0.2	3.8 ± 0.4	< 0.001
RV FAC, %	154 (78)	38 ± 8	39 ± 7	39 ± 10	31 ± 8	0.001
RV FAC < 35%, n (%)	154 (78)	55 (28)	27 (27)	14 (38)	14 (78)	< 0.001
RVEDD basal, mm	154 (78)	5.6 ± 0.9	5.4 ± 0.8	5.8 ± 1.0	6.2 ± 0.9	0.001
RVEDD basal > 41 mm, n (%)	154 (78)	146 (95)	94 (95)	34 (92)	18 (100)	0.680

TABLE 1 - Continued.

	Valid cases, n (%)	All, n = 198	PH classification before ASD closure			*P* for trend
			No PH (n = 121)	Possible PH (n = 51)	Likely PH (n = 26)	
Echocardiography						
LV FS, %	187 (94)	38 ± 9	37 ± 9	40 ± 8	39 ± 9	0.205
LV systolic function, n (%)	190 (96)					0.412
Normal	190 (96)	159 (84)	100 (83)	43 (91)	16 (70)	
Mildly impaired	190 (96)	28 (14)	18 (15)	4 (9)	6 (26)	
Moderately impaired	190 (96)	3 (2)	2 (2)	0 (0)	1 (4)	
Severely impaired	190 (96)	0 (0)	0 (0)	0 (0)	0 (0)	
TAPSE, mm	106 (54)	28.4 ± 6.3	28.7 ± 5.7	29.0 ± 6.9	25.4 ± 8.0	0.262
TAPSE < 17 mm	106 (54)	3 (3)	2 (3)	0 (0)	1 (10)	0.503

P-value for trend was calculated using the Chi-Square Mantel-Haenszel test for categorical variables and linear regression for continuous variables. For pulmonary disease en cardiac medication: no comparisons were made between subgroups. **Legend:** *Median [IQ_1–IQ_3], other n (%) or mean ± SD. **Abbreviations:** ASD, atrial septal defect; NYHA, New York Heart Association; COPD, chronic obstructive pulmonary disease; OSAS, obstructive sleep apnea syndrome; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; PH, pulmonary hypertension; RA, right atrial; TR; tricuspid regurgitation, RVFAC, right ventricular fractional area change; RVEDD, right ventricle end-diastolic dimension; LV, left ventricular; FS, fractional shortening; TAPSE, tricuspid annular plane systolic excursion.

Echocardiographic follow-up after ASD closure

Figure 2 provides an overview of the PH classification before and after ASD closure. Before ASD closure, the majority of patients (n = 121, 61.1%) was categorized as no PH. After ASD closure, in 174 patients (87.9%) no signs of PH were present. The prevalence of PH was 13.1% (n = 26) before and 5.0% (n = 10) after ASD closure. Median mid-term follow-up time was 15 [IQR 12–35] months. Of the ten patients with likely PH after closure, seven were suspected to have PAH and the other three were suspected of PH due to left ventricular diastolic dysfunction according to the ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension.[18]

Figure 2 shows that RV pressures decreased in almost all patients after ASD closure. Only one patient (ASD closed at age of 63 years) had an increase in the pulmonary pressure, but this patient also had left ventricular diastolic dysfunction.

Before ASD closure, the classification of PH was based on TR velocity in 187 patients (94%), on PR velocity in 4 patients (2%), and on a combination of RV dimensions, RV function and signs of PH in 7 patients (4%). In 29 patients (15%) the PH classification after ASD closure was based on different variables (i.e., TR before ASD closure, and PR velocity or right ventricular function after ASD closure), because reliable measurements of the TR maximal velocity were not always available.

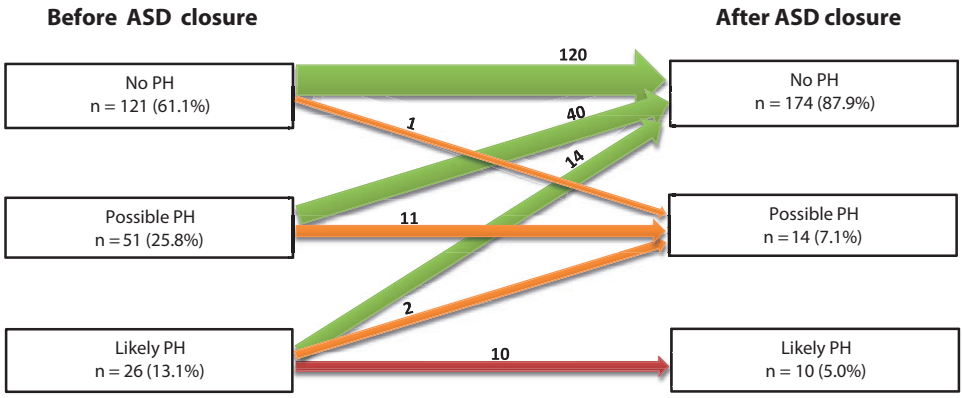


FIGURE 2 - Classification of pulmonary hypertension before and after ASD closure.

No PH: TR velocity < 2.9 m/s or mPAP < 20 mmHg; Possible PH: TR velocity 2.9–3.4 m/s or mPAP 20–24 mmHg; Likely PH: TR velocity ≥ 3.4 m/s or mPAP ≥ 25 mmHg. **Abbreviations:** ASD, atrial septal defect; mPAP; mean pulmonary artery pressure; PH, pulmonary hypertension, TR, tricuspid regurgitation.

PH medication use

Before ASD closure, four patients used PH medication. Three patients used a phosphodiesterase-5 inhibitor and one patient used a combination of a phosphodiesterase-5 inhibitor, an endothelin antagonist, and a prostacyclin analogue. Of the ten patients classified as likely PH after ASD closure, seven patients used a combination of a phosphodiesterase-5 inhibitor and an endothelin antagonist. Two patients only used a phosphodiesterase-5 inhibitor.

Associations between baseline characteristics and PH after ASD closure

In Table 2, the results of the Cox proportional hazards regression are displayed. Of all clinical, electrocardiographic and echocardiographic measurements that were evaluated, we found that NYHA functional class, presence of pulmonary disease, use of cardiac medication, TR maximum velocity, RV FAC, TAPSE and PH before ASD closure were significantly associated with PH at mid-term follow-up. Of note, age at closure and the diameter of the ASD or sinus venosus defect were not found to be associated with PH after ASD closure.

Long-term follow-up

Long-term follow-up was complete in 25 patients (66%) and not available in 13 patients (34%) due to loss to follow-up (n = 10) and death (n = 3). The 13 patients with missing long-term follow-up did not have significant differences in age at closure, body mass index, cardiac medication use, NYHA class, type of procedure, or PH classification before

ASD closure compared to the 25 patients that had completed the long-term follow-up. The median time from ASD closure to long-term follow-up was 52 [IQR 31–95] months. Except for one patient, all patients were in the same PH category as they were at the mid-term follow-up after ASD closure. One patient who initially changed from no to possible PH at mid-term follow-up, changed back to no PH at long-term follow-up.

TABLE 2 - Associations between patient characteristics and classification of "likely PH" after ASD closure.

	Valid cases (n%)	Hazard ratio	95% CI	*P*-value
Clinical characteristics				
Age at closure, years	198 (100)	1.02	0.98–1.07	0.241
Sex, female	198 (100)	1.16	0.30–4.50	0.828
NYHA class III–IV	198 (100)	11.07	3.12–39.3	< 0.001
Sinus venosus defect	198 (100)	0.04	0.00–166	0.450
Surgical repair	198 (100)	1.34	0.39–4.63	0.644
Pulmonary disease, yes	198 (100)	10.43	2.12–51.2	0.004
Cardiac medication, yes	198 (100)	3.96	1.02–15.3	0.047
Body mass index, kg/m^2	198 (100)	1.03	0.92–1.16	0.589
Systolic blood pressure, mmHg	197 (99)	0.99	0.96–1.03	0.601
O_2 saturation < 95%	126 (64)	1.98	0.38–10.3	0.416
Electrocardiography				
Heart rate, beats/min	197 (99)	1.02	0.98–1.07	0.368
Loss of sinus rhythm	198 (100)	1.24	0.26–5.85	0.788
QRS duration, ms	198 (100)	1.01	0.98–1.04	0.600
PR interval, ms	173 (87)	1.00	0.97–1.04	0.863
Echocardiography				
Defect diameter, per mm	163 (82)	1.03	0.96–1.10	0.382
RA pressure, per mmHg	177 (90)	1.10	0.92–1.32	0.293
RA end-systolic area, per cm^2	159 (80)	1.01	0.97–1.06	0.516
TR maximum velocity, per m/s	187 (94)	7.36	2.92–18.5	< 0.001
RV FAC, per %	154 (78)	0.87	0.81–0.93	< 0.001
RVEDD basal, per mm	154 (78)	1.44	0.70–2.94	0.318
LV FS, per %	187 (94)	1.02	0.95–1.10	0.580
LV systolic function, mild/moderately impaired	190 (96)	3.40	0.85–13.7	0.085
TAPSE, per mm	106 (54)	0.75	0.59–0.95	0.018
PH likely before ASD closure	198 (100)	23.03	3.39–157	0.001

CI, confidence interval; others as defined in Table 1.

Clinical outcome

Information on survival was available in all patients using the municipal registry. Overall nine patients died during follow-up, of whom five were categorized as no PH, one patient as possible PH and three patients as likely PH after ASD closure. The median time from ASD closure to death was 69 [IQR 20–92] months. Causes of death were end stage heart failure (n = 4), sudden death (suspected cardiac) (n = 1), malignancy (n = 1), gastro-intestinal bleeding, presumably due to anticoagulants use (n = 1), euthanasia (in a patient with an infaust prognosis due to malignancy) (n = 1), and unknown (n = 1). Re-intervention after the ASD closure because of a significant rest shunt was performed in two patients (1%).

DISCUSSION

The prevalence of PH in adult patients before ASD closure in our study was 13.1% and decreased to 5% after ASD closure. Factors associated with PH after ASD closure were PH before ASD closure, NYHA functional class III–IV, presence of pulmonary disease, cardiac medication use, RV FAC and TAPSE. Patients with one or more of these risk factors need careful follow-up after ASD closure. Patients with low pulmonary pressures before ASD closure have a low risk of developing PH after closure, can be reassured, and require less frequent follow-up.

Prevalence of PH before and after ASD closure

Although pulmonary artery pressure (PAP) decreased after ASD closure in the majority of patients, 10 patients showed no change in PAP resulting in an overall PH prevalence median 15 months after ASD closure of 5%. This number is consistent with some previous studies, who also reported a prevalence of 5% in the adult population after ASD closure.[20, 21] Other studies have reported a higher prevalence ranging from 12 to 50%.[6-8, 10, 11, 22, 23] These studies were mainly published between 1970–1990 and consisted of a small study population with the majority having surgical ASD closure. In these studies, before ASD closure the PH prevalence and NYHA functional class was also higher;[6, 7, 11, 22] therefore, it is likely that this is a selection of more severe cases. There is no documentation on the type of patients who were lost to follow-up, but the percentage of patients lost to follow-up was clearly higher in these older studies (> 25%) and selection bias could have occurred.

Associations between patient characteristics and PH after ASD closure

According to the guidelines of pulmonary hypertension and the literature, the most recognized predictors of poor clinical outcome in patients with PH are TAPSE, pericardial effusion and RA area.[18, 24] In our study, only one patient had significant pericardial effusion so this variable was not further analyzed in our study. RA area was not associated with the presence of PH in adults after ASD closure. Our study did show an association between the presence of pulmonary disease, NYHA class, RV FAC, TAPSE, pulmonary pressures before closure, and PH classification of "likely PH" after ASD closure. These variables have also been reported as predictors for PH in previous studies.[10, 16, 23, 25] Cardiac medication use (ACE inhibitors, diuretics, beta blockers or anti-arrhythmia) has not been described previously as a predictor for PH after ASD closure. This medication is mostly used for problems with the left side of the heart. Because of these left-sided problems, patients can also develop higher pulmonary pressures, which may explain the association with PH after ASD closure in our study population.

Various studies have identified age at ASD closure as a predictor for PH.[16, 23, 26] Interestingly, in our study age at closure was not found to be associated with PH after ASD closure. In the study of Gabriels et al., besides adult patients, also patients with an ASD closure in their childhood were included.[16] Yong et al.[23] and Humenberger et al.[26] only included patients with an ASD repair at adult age, but these patients were much older than in our study cohort (mean age 54 ± 16 and 49 ± 18 years, respectively). The wider age range compared with our study may explain why these studies did find an association between age at closure and PH after ASD closure, in contrast to our study.

Clinical implications

Patients with an ASD repair at adult age, especially those repaired at 40 years of age and older, should be followed on a regular basis during the first 2 years and then depending on the results every 2–4 years, according to the 2010 European Society of Cardiology guidelines for the management of grown-up congenital heart disease.[5] No specific recommendations are made with regard to patient characteristics that further differentiate the need for clinical follow-up. The results of our study show that adult patients without signs of PH before ASD closure are not at risk for the development of PH after the repair and therefore follow-up is needed only with long intervals. In contrast, a substantial proportion of the patients with signs of PH before closure continued to have high right ventricular pressures. Moreover, results of the long-term follow-up demonstrate that the pulmonary pressures do not change after median 15 months after ASD closure. Therefore, it is indeed important to regularly follow-up patients with elevated PAP or signs of PH before closure, while patients without PH can

be checked with a low frequency. Special attention is warranted for those patients who have specific risk factors such as NYHA class III–IV, cardiac medication use, presence of pulmonary disease and right ventricular dysfunction before the ASD closure as they have an increased risk of PH after ASD closure.

Limitations

This study is vulnerable to different types of bias because of its observational and retrospective design. We attempted to avoid selection bias by including all consecutive patients that underwent ASD closure between 2000 and 2014 in our center; however, echocardiographic data on RV pressures was not available in 46 patients. Because the baseline characteristics of these patients were not significantly different from the included study population, we have no indication that a selective loss to follow-up has occurred, but it is important to take this into account when interpreting the results.

Secondly, patients were categorized into PH groups based on echocardiographic measurements, which are less accurate for the diagnosis of PH than the reference standard, right heart catheterization. This was not routinely performed in all patients, because it is unethical to perform these invasive measurements in asymptomatic patients.[18] Some patients have been classified with different echocardiographic variables before and after ASD closure, but these were mostly patients without any signs of PH, and we expect that this has not largely influenced our conclusions.

Finally, multivariable Cox regression could not be performed in this study, because of the limited number of patients with PH after ASD closure.

CONCLUSIONS

Adult patients with low pulmonary pressures before ASD closure are not at risk for the development of PH after ASD closure during a follow-up period of 15 months. These patients can be reassured and need less frequent follow-up. Nevertheless, PH is prevalent in approximately 5% of adult patients after ASD closure. Especially those patients with PH before ASD closure, high NYHA functional class, presence of pulmonary disease, cardiac medication use and impaired RV function at baseline are at risk and therefore require close follow-up after ASD closure.

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Chapter 07

Echocardiographic findings associated with mortality or transplant in patients with pulmonary arterial hypertension: a systematic review and meta-analysis

Vivan J.M. Baggen, Mieke M.P. Driessen, Marco C. Post, Arie P. van Dijk, Jolien W. Roos-Hesselink, Annemien E. van den Bosch, Johanna J.M. Takkenberg, Gertjan Tj. Sieswerda

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ABSTRACT

Background Identification of patients at risk of deterioration is essential to guide clinical management in pulmonary arterial hypertension (PAH). This study aims to provide a comprehensive overview of well-investigated echocardiographic findings that are associated with clinical deterioration in PAH.

Methods MEDLINE and EMBASE databases were systematically searched for longitudinal studies published by April 2015 that reported associations between echocardiographic findings and mortality, transplant or clinical worsening. Meta-analysis using random effect models was performed for echocardiographic findings investigated by four or more studies. In case of statistical heterogeneity, a sensitivity analysis was conducted.

Results Thirty-seven papers investigating 51 echocardiographic findings were included. Meta-analysis of univariable hazard ratios (HRs) and sensitivity analysis showed that presence of pericardial effusion (pooled HR 1.70; 95% CI 1.44–1.99), right atrial area (pooled HR 1.71; 95% CI 1.38–2.13) and tricuspid annular plane systolic excursion (TAPSE; pooled HR 1.72; 95% CI 1.34–2.20) were the most well-investigated and robust predictors of mortality or transplant.

Conclusions This meta-analysis substantiates the clinical yield of specific echocardiographic findings in the prognostication of PAH patients in day-to-day practice. In particular, pericardial effusion, right atrial area and TAPSE are of prognostic value.

INTRODUCTION

The ongoing research on pulmonary arterial hypertension (PAH) has led to increased awareness of the pathophysiological, hemodynamic and clinical consequences of this devastating disease.[1] Without intervention, progressive remodeling of the distal pulmonary arteries leads to elevated pulmonary vascular resistance, eventually resulting in right heart failure and death.[1,2] Fortunately, advances in therapeutic modalities have greatly improved the survival and quality of life in patients with PAH.[3] However, the natural course of the disease varies widely between individuals, as some patients live for decades while others die within months of diagnosis.[4] In order to guide optimal clinical management, it is therefore essential to accurately monitor disease progression and estimate prognosis in PAH.

Previously reported predictors of mortality include etiology of PAH, gender and several functional, hemodynamic and biochemical variables.[5-8] Echocardiography is the most readily available cardiac imaging modality and is universally used in the follow-up of patients with PAH. Current literature reports several echocardiographic findings that may provide important prognostic information. The goal of this study is to provide a comprehensive overview of the most thoroughly investigated baseline echocardiographic findings that are associated with adverse clinical outcome in PAH. Separately, this study evaluates the prognostic value of a *change* in echocardiographic findings during a follow-up period.

METHODS

This systematic review was conducted in accordance with the PRISMA statement.[9] A pre-defined review protocol, as adopted by this study, can be accessed through PROSPERO (registration number: CRD42014009231).

Literature search strategy

A comprehensive systematic search was performed in MEDLINE (via PubMed interface) and EMBASE electronic databases on 29 April 2015 using combinations of all synonyms for: PAH, echocardiography and relevant clinical outcomes (components of the Dana Point Time to Clinical Worsening composite endpoint).[1] A validated prognostic search filter with the highest sensitivity (98%) was added to the search syntax.[10] No language or publication period restrictions were applied. The full original search syntax is provided in Supplemental File 1.

Selection of papers

A flow diagram of the selection process is shown in Figure 1.[2] After deduplication, one author performed screening and selection of articles based on title and abstract, using the following exclusion criteria: inappropriate study type (cross-sectional or trial design, reviews, case reports with < 10 patients, editorials or congress abstracts), non-clinical data (technical, animal and in-vitro studies), study population without PAH (e.g. acute pulmonary embolism, exercise-induced pulmonary hypertension), studies that included children < 12 years, and studies that did not relate echocardiographic findings to clinical outcome. Full-text screening was performed by two authors; reasons for exclusion are described in Figure 1. All references of the excluded reviews and included articles were cross-checked to identify possible relevant articles missed in the original search syntax.

Assessment of methodological quality

Study quality was critically appraised using previously developed criteria for prognostic studies.[11] We assessed study design, missing data and loss to follow-up (selection bias), adequate description and measurement of imaging features and outcome (information bias), reported effect size, treatment of continuous risk predictors and multivariable adjustment for possible confounders.

Data extraction and analysis

Study characteristics and hazard ratios (HRs) for all investigated echocardiographic findings with accompanying 95% confidence intervals were extracted using a standardized form. Meta-analysis was performed for all echocardiographic findings that were investigated as continuous parameters by four or more studies, using random effect models. In order to unify the extracted data to allow more studies to be pooled, HRs were recalculated to one uniform clinically applicable number of units change. Heterogeneity was assessed using Cochran's Q test and the I^2 statistic. Imaging findings investigated as dichotomous variables were additionally presented below the corresponding forest plots. For all echocardiographic measurements with significant heterogeneity ($I^2 > 50\%$ or Cochran's Q p-value < 0.10) a sensitivity analysis was performed by excluding specific patient subgroups.

If study data were used in multiple papers and the same echocardiographic findings were evaluated, only the study with the largest sample size was used to exclude the risk of using duplicate data in our meta-analysis. The risk of publication bias was assessed using visual inspection of funnel plots and the Egger's test.

RESULTS

Search results

The systematic literature search in MEDLINE and EMBASE and extensive reference cross-checking retrieved 2733 potentially relevant records, of which 2610 were excluded based on title and abstract (Figure 1). After full-text review of the remaining 123 articles, 37 papers were finally selected.[6, 12-47] Study and patient characteristics of the included studies are shown in Table 1.

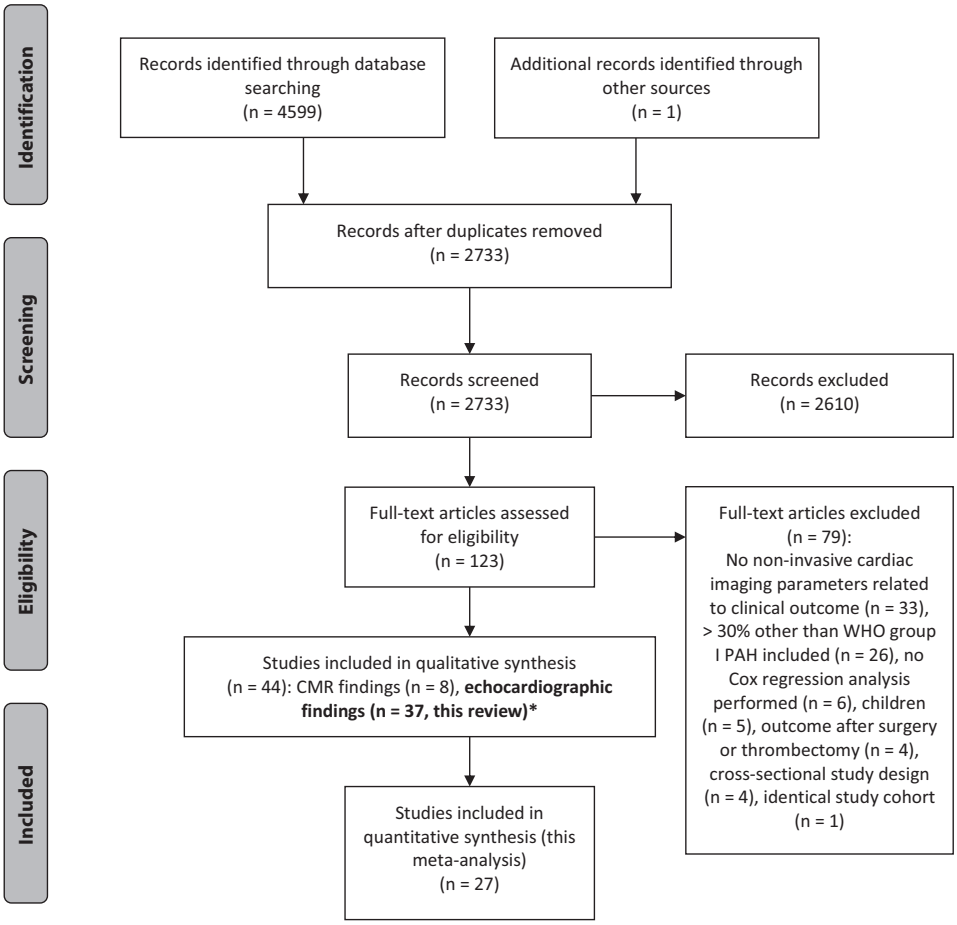

FIGURE 1 - PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 flow diagram.

*, one study investigated both echocardiographic and cardiac magnetic resonance imaging findings.

TABLE 1 - Study characteristics.

Study, ref no.	Size, n	Age, years	Gender, % female	NYHA class III-IV, %	IPAH / hereditary	Drug/toxin	PAH-CTD	Po-PAH	PAH-CHD	WHO I PAH (other/not specified)	WHO III (lung disease)	WHO IV (CTEPH)	Follow-up duration, months	Events, n (%)
[11]	26	41 [16–70]	69	NR	100	_	_	_	_	_	_	_	24 ± 14	16 (62)
[12]	26	43 ± 17	73	58	100	_	_	_	_	_	_	_	NR	6 (23)
[13]	53	45 ± 14	72	70	100	_	_	_	_	_	_	_	35 [NR]	32 (60)
[14]	43	37 [14–67]	70	86	100	_	_	_	_	_	_	_	21 ± 16	12 (28)
[15]	25	38 ± 13	76	100	100	_	_	_	_	_	_	_	12 [0–84]	13 (52)
[16]	81	40 ± 15	73	100	100	_	_	_	_	_	_	_	36 ± 15	41 (51)
[17]	63	55 ± 15	83	70	37	_	38	_	_	_	21	5	19 [10–22]*	23 (37)
[18]	54	52 ± 11	76	76	100	_	_	_	_	_	_	_	50 [NR]	12 (22)
[19]	50	46 ± 13	78	42	46	_	22	_	_	4	_	28	14 [12–18]	19 (38)
[6]	2716	50 ± 17	79	54	49	5	24	5	12	5	_	_	17 [0–24]	340 (13)
[20]	76	61 ± 11	84	53	_	_	100	_	_	_	_	_	36 [NR–113]	42 (55)
[21]	32	53 ± 16	66	91	69	16	6	_	9	_	_	_	21 [NR]	17 (53)
[22]	59	46 ± 16	63	66	100	_	_	_	_	_	_	_	52 [28–79]*	23 (39)
[23]	72	52 ± 16	72	76	100	_	_	_	_	_	_	_	38 [14–71]*	22 (31)
[24]	484	52 ± 15	75	71	56	_	24	11	9	_	_	_	38 [16–60]	264 (55)
[25]	50	61 ± 11	98	70	_	_	100	_	_	_	_	_	16 [9–39]	25 (50)
[26]	80	56 ± 14	76	72	43	_	41	10	_	6	_	_	24 [NR]	33 (41)
[27]	95	31 ± 10	64	56	100	_	_	_	_	_	_	_	21 ± 15	27 (28)
[28]	57	52 ± 14	28	100	63	_	18	11	_	5	_	3	25 ± 29	29 (51)
[29]	181	39 ± 13	67	67	_	_	_	_	100	_	_	_	16 [7–46]	19 (10)
[30]	154	54 ± 9	84	NR	46	1	40	5	6	3	_	_	36 [17–71]*	71 (46)
[31]	61	48 ± 18	84	69	100	_	_	_	_	_	_	_	NR	NR
[32]	142	59 ± 15	65	44	31	9	19	4	9	1	_	27	11 [6–39]	28 (20)
[33]	577	53 ± 15	75	70	_	_	_	_	_	100	_	_	57 ± 50	NR
[34]	406	59 ± 16	65	46	_	_	_	_	_	74	14	12	16 [8–20]*	73 (18)
[35]	32	39 ± 15	69	59	22	_	16	_	53	_	_	9	14 [8–21]	15 (47)
[36]	124	54 ± 16	70	92	_	_	_	_	_	84	_	16	36 ± 22	31 (25)
[37]	71	57 ± 14	76	75	46	_	41	6	_	7	_	_	24 [NR]	20 (28)
[38]	50	56 ± 12	84	72	42	_	38	14	_	6	_	_	48 [NR]	NR
[39]	102	54 ± 16	84	NR	47	_	24	_	_	29	_	_	44 [22–79]*	43 (42)
[40]	37	46 ± 14	76	35	65	_	5	_	24	5	_	_	16 [13–18]*	7 (19)

TABLE 1 - Continued.

Study, ref no.	Size, n	Age, years	Gender, % female	NYHA class III-IV, %	IPAH / hereditary	Drug/toxin	PAH-CTD	Po-PAH	PAH-CHD	WHO I PAH (other/not specified)	WHO III (lung disease)	WHO IV (CTEPH)	Follow-up duration, months	Events, n (%)
[41]	48	44 ± 14	83	100	67	_	21	6	6	_	_	_	53 [21–80]*	18 (38)
[42]	91	42 ± 14	60	73	_	_	_	_	100	_	_	_	46 [4–64]	24 (26)
[43]	79	48 [24–65]	66	92	92	_	8	_	_	_	_	_	NR [12–92]	27 (34)
[44]	121	60 ± 14	66	63	39	_	36	18	_	6	_	_	37 ± 36	49 (40)
[45]	200	54 ± 15	71	50	47	1	33	7	12	_	_	_	43 ± 31	106 (53)
[46]	51	60 ± 15	73	71	33	_	55	6	6	_	_	_	36 ± 24	8 (16)

*interquartile range, otherwise reported as median [range] or mean ± SD.

Abbreviations: CHD, congenital heart disease; CTD, connective tissue disease; CTEPH, chronic thromboembolic pulmonary hypertension; HF, heart failure; IPAH, idiopathic pulmonary arterial hypertension; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; SD, standard deviation; NR, not reported; WHO, World Health Organization.

The total number of patients per study ranged from 25 to 2716, with a mean age ranging from 31–61 years (60–98% female) and 35–100% of patients in New York Heart Association (NYHA) class III–IV. Twelve studies included patients with congenital heart disease (CHD) (6–100% of patients)[6,22,25,30,31,33,36,41-43,46,47] and seven studies included a subset of patients with pulmonary hypertension group III or IV (< 30% of total study population).[18,20,29,33,35-37] The majority of studies used death or transplant as primary outcome; only five studies (14%) used a composite outcome, additionally including hospitalization for heart failure,[20,35,41,47] need for a second vasodilator drug or worsening of functional class.[36] Mean follow-up duration varied between 11 and 53 months, with the primary outcome event occurring in 6–340 patients (10–62% of the study population).

Methodological aspects

In Figure 2, an overview of the methodological quality of all included studies is presented. Individual bias assessment per study is provided in Supplemental File 2. Studies prospectively included consecutive patients diagnosed with the disease (43% of studies) or retrospectively reviewed echocardiographic images. Information on missing values and loss to follow-up was not reported in 16 (43%) and 21 (57%) of the 37 studies, respectively. It is therefore important to recognize the possible impact of selection bias on individual study outcomes.

All studies used right heart catheterization for the diagnosis of PAH in 100% of the included patients, except for one study that used right heart catheterization in 87% and echocardiography in 13% of patients.[35] Definition and measurement of echocardiographic findings and study outcome was appropriate and consistent in the majority of studies; therefore, the impact of information bias is assumed to be small.

Cox regression analysis was performed in all studies; however large differences for predictors included in the multivariable analysis were found. Twenty-nine studies performed some form of multivariable adjustment, of which only 15 adjusted for age and gender. Only nine studies (24%) used more than ten events per predictor. Because of this large variety between studies and overall poor methodological quality of multivariable adjustment, it was chosen to present only the univariable HRs in forest plots.

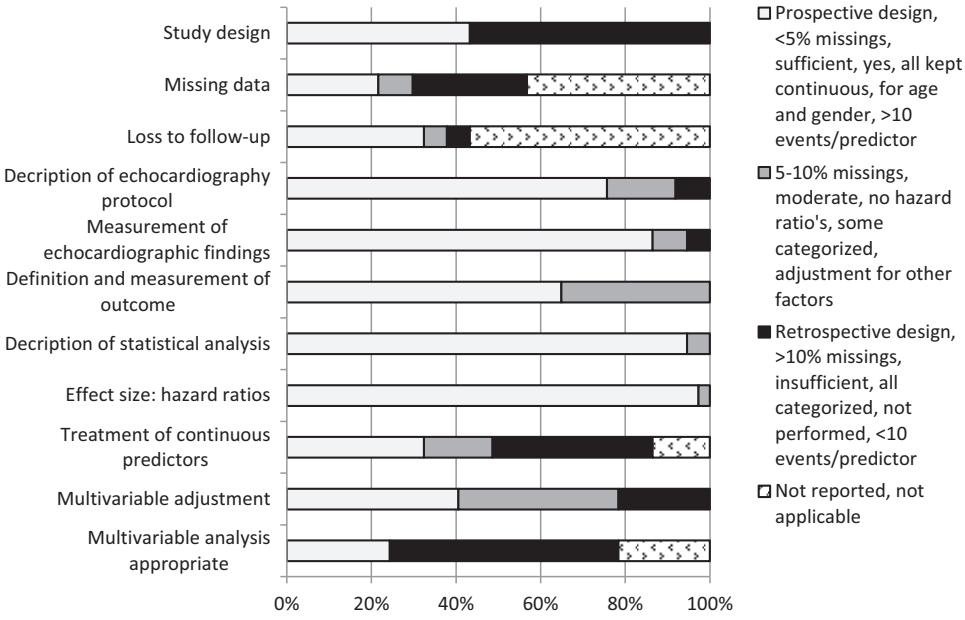

FIGURE 2 - Methodological quality of the included studies.

Methodological quality of the included studies was assessed on the following domains of potential bias: completeness of data (selection bias), standardization of prognostic factors and study outcome (information bias) and statistical calculation of effect size (study outcome).

Prognostic value of baseline echocardiographic findings

In 37 studies, in total 51 echocardiographic findings were evaluated (Supplemental File 3). Meta-analysis was performed for ten echocardiographic findings that were suitable for pooling of results among four or more studies: presence of pericardial effusion, right

atrial area, right ventricular (RV) pressure estimates, severity of tricuspid regurgitation, estimated right atrial pressure, left ventricular (LV) eccentricity index (Figure 3 A), tricuspid annular plane systolic excursion (TAPSE), RV fractional area change, Tei index (RV myocardial performance index) and RV free wall longitudinal peak systolic strain (LPSS; Figure 3 B).

Although not included in the meta-analysis, RV end-diastolic basal dimension[24,30,33,36] or area[17,25,36,37] and tissue Doppler velocity (S') of the tricuspid valve annulus[30,33,35,36,41] were investigated by several studies and could be of prognostic importance. Less investigated echocardiographic measurements such as pulmonary artery capacitance,[19,35] several strain values,[27,38,44] RV diastolic dysfunction,[12,20,30] LV end-diastolic volume,[23] systolic pulmonary artery pressure increase during exercise[37] and RV load adaptation index[44] seem promising but require further evaluation.

Serial echocardiographic evaluation

Five studies included in this review investigated the prognostic value of a *change* in echocardiographic findings during a follow-up period, rather than their absolute baseline values, as indicated in Supplemental File 3.[39,42,44,46,47] Patients with ≥ 5% improvement in RV free wall LPSS on PAH treatment at 6 ± 2 months follow-up had a significantly reduced mortality risk at four years (HR 0.13; 95% CI 0.03–0.50).[39] Tonelli et al. showed that overall mortality was associated with a 10% increase in RV end-diastolic area (HR 1.37; 95% CI 1.08–1.75), tricuspid regurgitation velocity (HR 1.72; 95% CI 1.12–2.70) and difference in qualitative RV function (HR per unit of improvement 0.55; 95% CI 0.31–0.96) at one-year follow-up.[42] Sano et al. recently reported that a mid-term change in RV end-systolic area (HR 0.92; 95% CI 0.86–0.98) and in right atrial area (HR 0.95; 95% CI 0.92–0.99) were significantly related to long-term outcome.[47] In addition, changes in RV load-adaptation index and pericardial effusion have been associated with clinical outcomes in PAH.[44,46]

Risk of bias assessment

Heterogeneity statistics (Cochran's Q and I^2) are presented in the corresponding forest plots in Figure 3. For all echocardiographic measurements with significant statistical heterogeneity, we performed a sensitivity analysis to evaluate possible sources for heterogeneity (Table 2). We excluded specific studies (< 70% in NYHA class III–IV, < 100% PAH, inclusion of CHD, < 50% on PAH medication at baseline, other endpoints than mortality or transplant used) to investigate whether this impacted the pooled HR. For presence of pericardial effusion, right atrial area and TAPSE, sensitivity analysis did not change the overall conclusions. These results can therefore be regarded with a higher degree of certainty (Figure 4).

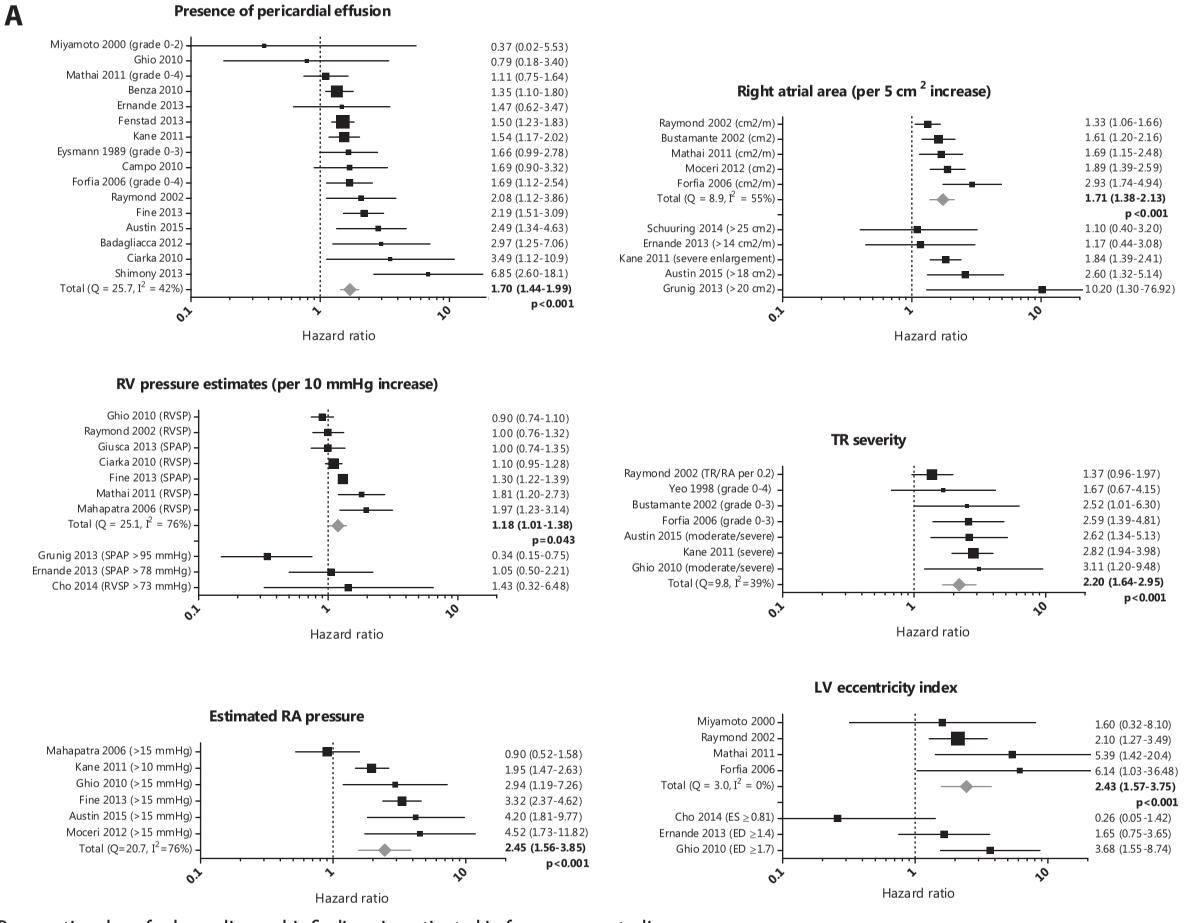

FIGURE 3A - Prognostic value of echocardiographic findings investigated in four or more studies.
General echocardiographic findings

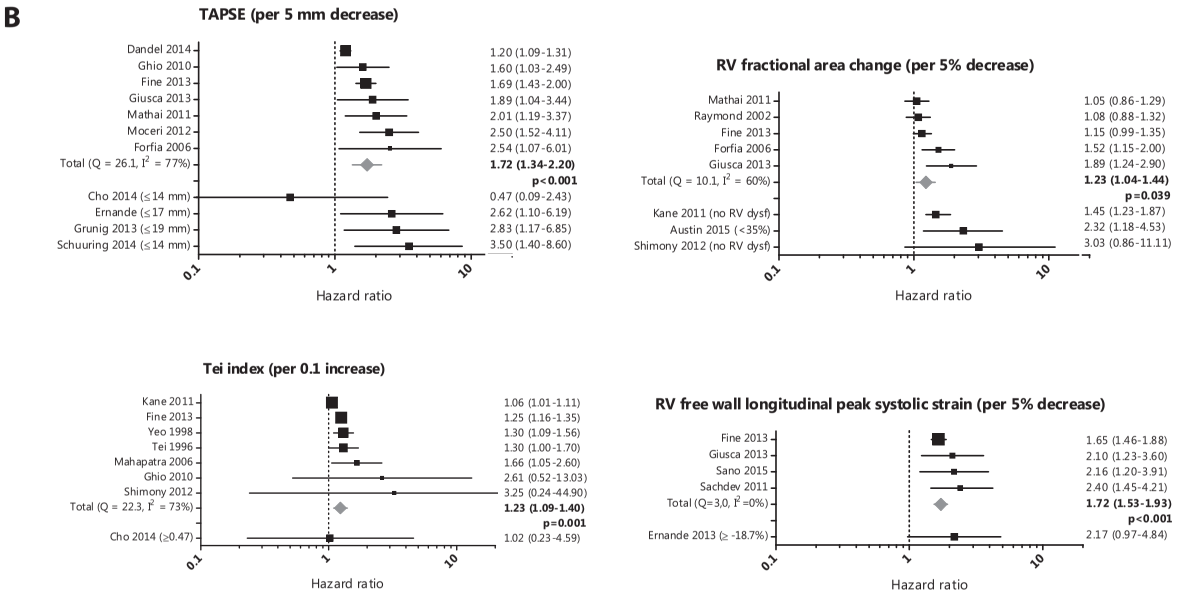

FIGURE 3B - Prognostic value of echocardiographic findings investigated in four or more studies.
Echocardiographic measurements of right ventricular function.

TABLE 2 - Sensitivity analysis for all echocardiographic measurements with significant statistical heterogeneity (I^2 > 50% or p-value < 0.10) in specific study subgroups.

	No. of studies	HR	95% CI	*p*-value	I^2, %	Cochran's *Q* (*p*-value)
Presence of pericardial effusion	**16**	**1.70**	**1.44–1.99**	**< 0.001**	**42**	**25.7 (0.041)**
> 70% NYHA class III-IV	8	1.56	1.31–1.86	< 0.001	23	9.0 (0.249)
100% PAH	12	1.62	1.34–1.96	< 0.001	45	20.1 (0.045)
Exclusion of CHD	12	1.81	1.45–2.25	< 0.001	49	21.5 (0.028)
> 50% on PAH medication / NR	10	1.86	1.40–2.47	< 0.001	60	22.3 (0.008)
Mortality / transplant as outcome	15	1.64	1.39–1.94	< 0.001	38	22.5 (0.069)
Right atrial area, per 5 cm2 increase	**5**	**1.71**	**1.38–2.13**	**< 0.001**	**55**	**8.9 (0.063)**
> 70% NYHA class III-IV	4	1.69	1.29–2.21	< 0.001	61	7.8 (0.051)
100% PAH	4	1.56	1.33–1.84	< 0.001	17	3.6 (0.306)
Exclusion of CHD	4	1.69	1.29–2.21	< 0.001	61	7.8 (0.051)
> 50% on PAH medication / NR	3	1.77	1.17–2.68	0.007	74	7.7 (0.021)
Mortality / transplant as outcome	5	1.71	1.38–2.13	< 0.001	55	8.9 (0.063)
RV pressure, per 10 mmHg increase	**7**	**1.18**	**1.01–1.38**	**0.043**	**76**	**25.1 (< 0.001)**
> 70% NYHA class III-IV	4	1.33	1.00–1.77	NS	72	10.9 (0.012)
100% PAH	5	1.20	0.95–1.52	NS	75	15.8 (0.003)
Exclusion of CHD	5	1.26	1.00–1.59	NS	81	20.6 (< 0.001)
> 50% on PAH medication / NR	5	1.19	1.02–1.38	0.024	66	11.8 (0.019)
Mortality / transplant as outcome	5	1.20	0.95–1.52	NS	75	15.8 (0.003)
Right atrial pressure, >15 mmHg	**6**	**2.45**	**1.56–3.85**	**< 0.001**	**76**	**20.7 (< 0.001)**
> 70% NYHA class III-IV	2	1.38	0.65–2.92	NS	82	5.7 (0.017)
100% PAH	6	2.45	1.56–3.85	< 0.001	76	20.7 (< 0.001)
Exclusion of CHD	4	2.41	1.16–4.98	0.018	82	17.0 (< 0.001)
> 50% on PAH medication / NR	0	-	-	-	-	-
Mortality / transplant as outcome	5	2.28	1.33–3.92	0.003	72	14.2 (0.007)
TAPSE, per 5 mm decrease	**7**	**1.72**	**1.34–2.20**	**< 0.001**	**77**	**26.1 (< 0.001)**
> 70% NYHA class III-IV	3	1.63	1.01–2.63	0.047	69	6.5 (0.039)
100% PAH	4	1.67	1.15–2.44	0.007	77	12.8 (0.005)
Exclusion of CHD	5	1.58	1.22–2.06	< 0.001	79	18.7 (< 0.001)
> 50% on PAH medication / NR	4	3.24	1.92–5.45	< 0.001	0	1.2 (0.756)
Mortality / transplant as outcome	5	1.76	1.22–2.52	0.002	74	15.4 (0.004)
RV FAC, per 5 % decrease	**5**	**1.23**	**1.04–1.44**	**0.039**	**60**	**10.1 (0.039)**
> 70% NYHA class III-IV	3	1.18	0.96–1.44	NS	60	5.0 (0.080)
100% PAH	2	1.06	1.04–1.09	< 0.001	0	0.0 (0.863)
Exclusion of CHD	4	1.16	1.02–1.32	0.026	41	5.0 (0.168)
> 50% on PAH medication / NR	5	1.23	1.04–1.44	0.039	60	10.1 (0.039)
Mortality / transplant as outcome	3	1.18	0.96–1.44	NS	60	5.0 (0.080)

TABLE 2 - Continued.

	No. of studies	HR	95% CI	*p*-value	I^2, %	Cochran's *Q* (*p*-value)
Tei index, per 0.1 unit increase	**7**	**1.23**	**1.09–1.40**	**0.001**	**73**	**22.3 (0.001)**
> 70% NYHA class III-IV	3	1.22	0.99–1.51	NS	76	8.3 (0.016)
100% PAH	6	1.25	1.05–1.48	0.012	58	12.0 (0.035)
Exclusion of CHD	5	1.46	1.24–1.72	< 0.001	0	2.3 (0.677)
> 50% on PAH medication / NR	2	1.25	1.16–1.35	< 0.001	0	0.5 (0.775)
Mortality / transplant as outcome	6	1.25	1.05–1.48	0.012	58	12.0 (0.035)

Abbreviations: CHD, congenital heart disease; CI, confidence interval; FAC, fractional area change; HR, hazard ratio; NR, not reported; NS, non-significant; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; RV, right ventricular; TAPSE, tricuspid annular plane systolic excursion.

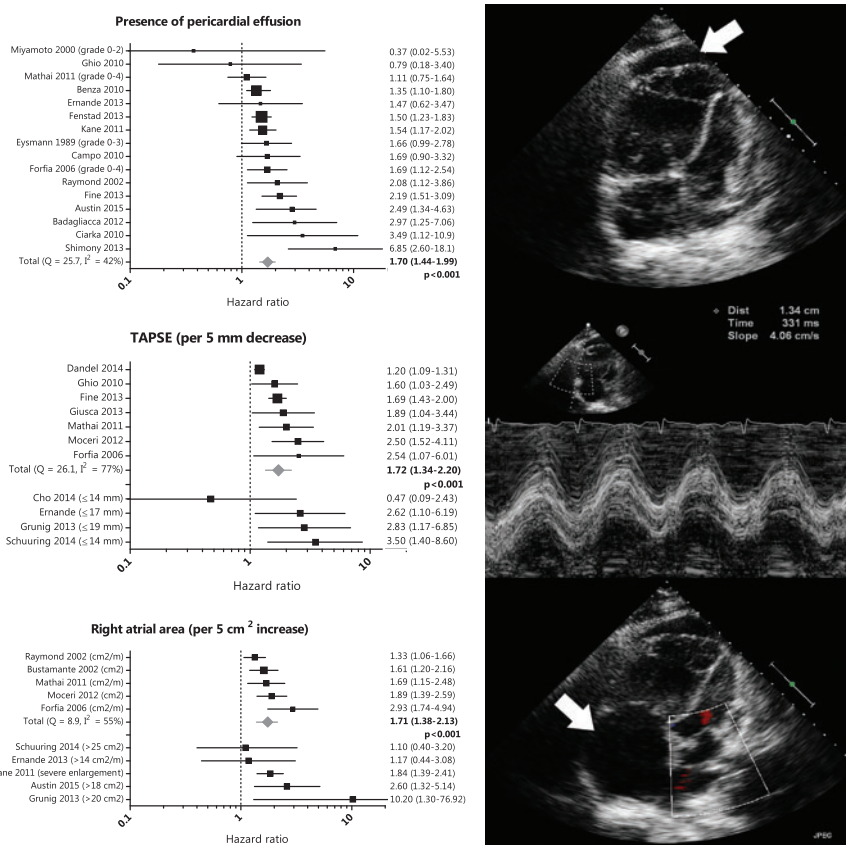

FIGURE 4 - Prognostic value of pericardial effusion, TAPSE and right atrial area.

Key message: Echocardiography is useful in prognostication in pulmonary arterial hypertension. Presence of pericardial effusion, lower TAPSE and enlarged right atrial area are the most robust predictors for mortality and transplant. This is important, since accurate prognostication can aid in adequate expansion of PAH-specific therapy and timely listing for transplantation.

No sensitivity analysis was performed for severity of tricuspid regurgitation, LV eccentricity index and RV free wall LPSS; however, the forest plots show that especially tricuspid regurgitation severity and LV eccentricity index have relatively large standard errors, and thus provide imprecise risk estimations.

A combination of visual assessment of funnel plots and the Egger's test provided statistical evidence of publication bias for TAPSE ($p = 0.026$), right atrial area ($p = 0.027$) and the Tei index ($p = 0.076$ and based on the funnel plot). This may indicate that studies with a positive result are overrepresented, subsequently leading to a relative overestimation of the pooled HR in the meta-analysis.

DISCUSSION

To our knowledge, this is the first systematic review and meta-analysis on the prognostic value of specific echocardiographic findings in patients with PAH. Among 51 echocardiographic findings investigated in 37 studies, meta-analysis and additional sensitivity analysis showed that presence of pericardial effusion, right atrial area and TAPSE were the most robust predictors of mortality or transplant in patients with PAH.

Right ventricular decompensation

Most deaths in patients with PAH are due to right heart failure.[7] Once the right ventricle starts to fail, it is no longer able to overcome the high pulmonary arterial pressures. This will cause a progressive rise in RV diastolic pressure and right atrial pressure, generally accompanied by right atrial enlargement. It is thought that elevated right atrial pressure causes impaired lymphatic and venous drainage, subsequently leading to pericardial fluid accumulation.[17] These insights into the mechanistic course tilting a stable PAH state towards death clearly explain why pericardial effusion, right atrial area, estimated right atrial pressure and RV dysfunction measured on echocardiography are associated with mortality in PAH.

Studies investigating RV function and right atrial pressure as assessed with other diagnostic modalities, such as cardiac magnetic resonance imaging and right heart catheterization, report comparable findings.[6,7,48,49] However, as echocardiography is more widely applied, non-invasive and less expensive, it is more suitable for the evaluation of PAH patients in day-to-day practice.

Serial measurements

The majority of the included studies investigated the prognostic value of baseline imaging findings, evaluated at the time of diagnosis. Complementary information on *changes* in hemodynamic, functional and biochemical variables may better reflect an individual's

response to PAH-targeted therapy – or progression of disease.[50] Interestingly, although the first study included in this review originates from 1989, serial echocardiographic evaluation in PAH has only recently gained scientific attention, as Hardegree and colleagues were the first ones to publish on this topic in 2013.[12,39] Thus far, changes in pericardial fluid accumulation, right atrial area, tricuspid regurgitation velocity, RV free wall LPSS and qualitative RV function, RV dimensions and RV load-adaptation index have been associated with clinical outcomes in PAH.[39,42,44,46,47] This is in line with the conclusions reached by the international working group of Vonk-Noordegraaf et al., who stated that changes in RV imaging parameters after treatment reflect altered exercise capacity and predict subsequent survival.[51] Advantages of echocardiography over more expensive or invasive imaging modalities become especially important in the serial evaluation of individual patients.

Heterogeneity

PAH prognosis depends largely on the underlying etiology, as the right ventricle can show rapid deterioration after initial diagnosis in patients with idiopathic or connective tissue disease-PAH, while it may cope successfully with pressure overload for decades in patients with congenital heart disease.[4,6] Still, most studies in this review investigated the World Health Organization (WHO) group I PAH as a whole. Moreover, some studies in this review *additionally* included small subsets (< 30%) of patients with WHO group III (pulmonary hypertension due to lung disease) or IV (chronic thromboembolic pulmonary hypertension), which further increases the heterogeneity of the study population. The heterogeneity of pulmonary hypertension etiologies among studies in this review likely plays a major role in the observed variation between the reported study results. Along the same line, disease severity and changing available treatment options over time likely contribute to this observed heterogeneity across studies. We therefore conducted a sensitivity analysis in which we excluded studies with other than WHO group I PAH, patients with CHD, < 70% patients in NYHA class III-IV (thus investigating a sicker patient population) or < 50% on PAH medication (representing other available treatment options). Importantly, this clearly reduced the statistical heterogeneity in specific study subgroups; however, the overall conclusions for presence of pericardial effusion, right atrial area and TAPSE remained unchanged.

Study limitations

We presented only univariable HRs in this study, because of the large variety between studies in which multivariable adjustment was performed (regarding the type and number of predictors per event used). Second, formal tests for publication bias retrieved significant results for the variables right atrial area, TAPSE and Tei index. Theoretically,

publication bias may cause underreporting of non-significant HRs, leading to a relative overestimation of the pooled HRs. Exact results of the random effect models as presented in this review should be therefore interpreted with caution.

Clinical implications

In order to adequately expand PAH-specific therapy and timely list patients for transplantation, accurate prognostication is highly important. The data in this review imply that especially pericardial effusion, enlarged right atrial area and decreased TAPSE are useful echocardiographic markers to predict mortality or transplantation. This is largely concordant with the 2015 European Society of Cardiology / European Respiratory Society Guidelines for the diagnosis and treatment of pulmonary hypertension, in which right atrial area and pericardial effusion are recommended as determinants of prognosis.[2] Controversy continues to exist about the use of TAPSE; it has been suggested that progressive RV dysfunction is associated with a decline in TAPSE until a certain floor effect is reached.[52] Of note, considering the multi-faceted nature of this disease, accurate prognostication should always be based on a combination of hemodynamic, functional, biochemical and echocardiographic findings, and should not rely on just one single parameter according to the current guidelines.[2]

CONCLUSIONS

This meta-analysis substantiates the clinical yield of specific echocardiographic findings in the prognostication of PAH patients in day-to-day practice. Although accurate prognostication should not rely on just one single parameter, presence of pericardial effusion, enlarged right atrial area and decreased TAPSE are the most firmly established echocardiographic tools that can be of important additional value.

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43. Schuuring MJ, van Riel ACMJ, Vis JC, et al. New predictors of mortality in adults with congenital heart disease and pulmonary hypertension: Midterm outcome of a prospective study. Int J Cardiol. 2015;181:270-6
44. Dandel M, Knosalla C, Kemper D, Stein J, Hetzer R. Assessment of right ventricular adaptability to loading conditions can improve the timing of listing to transplantation in patients with pulmonary arterial hypertension. J Heart Lung Transplant. 2015;34:319-28
45. Austin C, Alassas K, Burger C, et al. Echocardiographic assessment of estimated right atrial pressure and size predicts mortality in pulmonary arterial hypertension. Chest. 2015;147:198-208
46. Batal O, Dardari Z, Costabile C, Gorcsan J, Arena VC, Mathier MA. Prognostic value of pericardial effusion on serial echocardiograms in pulmonary arterial hypertension. Echocardiography. 2015;32:1471-6.
47. Sano H, Tanaka H, Motoji Y, et al. Right ventricular function and right-heart echocardiographic response to therapy predict long-term outcome in patients with pulmonary hypertension. Can J Cardiol. 2015;31:529-36
48. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007;28:1250-7
49. van de Veerdonk MC, Kind T, Marcus JT, et al. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. J Am Coll Cardiol. 2011;58:2511-9
50. Nickel N, Golpon H, Greer M, et al. The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension. Eur Respir J. 2012;39:589-96
51. Vonk Noordegraaf A, Haddad F, Bogaard HJ, Hassoun PM. Noninvasive imaging in the assessment of the cardiopulmonary vascular unit. Circulation. 2015;131:899-913
52. Mauritz GJ, Kind T, Marcus JT, et al. Progressive changes in right ventricular geometric shortening and long-term survival in pulmonary arterial hypertension. Chest. 2012;141:935-43

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FILE 1 - Search syntax used to identify publications of interest (search date April 29, 2015).

Population #1	"pulmonary hypertension"[tiab] OR "pulmonary arterial hypertension"[tiab] OR "pulmonary artery hypertension"[tiab]
Predictive variable #2	echocardiograph*[tiab] OR echocardiogram[tiab] OR ultrasound[tiab] OR ultrasonograph*[tiab] OR ultrasonogram[tiab] OR ultrasonic[tiab] OR "M-Mode"[tiab] OR "M Mode"[tiab] OR doppler[tiab] OR "cardiac magnetic resonance"[tiab] OR imaging[tiab] OR MRI[tiab] OR "magnetic resonance angiography"[tiab] OR "magnetic resonance angiographies"[tiab]
Outcome #3	death[tiab] OR mortality[tiab] OR survival[tiab] OR fatal[tiab] OR fatality[tiab] OR transplant*[tiab] OR graft*[tiab] OR hospitalization[tiab] OR hospitalisation[tiab] OR admission[tiab] OR readmission[tiab] OR transcatheter[tiab] OR intervention[tiab] OR intravenous[tiab] OR infusion*[tiab] OR "new york heart association"[tiab] OR "functional class"[tiab] OR "functional status"[tiab] OR function[tiab] OR exercise[tiab] OR endurance[tiab] OR (("6-minute"[tiab] OR "6 minute"[tiab] OR "6-min"[tiab] OR "6 min"[tiab] OR "six-minute"[tiab] OR "six minute"[tiab]) AND walk[tiab] AND (test[tiab] OR distance[tiab])) OR VO2max[tiab] OR "VO2 max"[tiab] OR VO2peak[tiab] OR "VO2 peak"[tiab] OR "aerobic capacity"[tiab] OR (oxygen[tiab] AND (uptake[tiab] OR consumption[tiab])) OR symptom*[tiab] OR morbidity[tiab] OR outcome[tiab] OR outcomes[tiab]
Prognostic studies #4	predict*[tiab] OR clinical*[tiab] OR outcome*[tiab] OR risk*[tiab] OR prognos*[tiab]
Search results (#1 AND #2 AND #3 AND #4)	MEDLINE: 2371 EMBASE: 2228 (replace [tiab] by :ti,ab)
Other limits (added search strings): **PubMed:** NOT (animals[MeSH] NOT humans[MeSH]) **Embase:** NOT ([conference abstract]/lim OR [conference paper]/lim OR [conference review]/lim OR [editorial]/lim OR [erratum]/lim OR [letter]/lim OR [note]/lim) AND [embase]/lim	

SUPPLEMENTAL FILE 2 - Risk of bias assessment in individual studies.

	Selection bias			Information bias: defined and measured appropriately?				Statistical calculation of effect size			
Author, Year	**Study design**	**Missing data**	**Loss to follow-up**	**Description of echocardiography protocol**	**Measurement of echocardiographic findings**	**Definition and measurement of outcome**	**Description of statistical analysis**	**Effect size: hazard ratios**	**Treatment of continuous predictors**	**Multivariable adjustment**	**Multivariable analysis appropriate**
Eysmann 1989	+	nr	+	+	+	+	+	±	+	-	NA
Tei 1996	-	+	nr	+	+	+	+	+	-	-	NA
Yeo 1998	-	-	±	+	+	+	+	+	+	+	-
Miyamoto 2000	+	nr	+	±	±	+	+	+	+	+	-
Bustamante 2002	+	+	-	+	+	+	±	+	±	+	-
Raymond 2002	-	-	±	+	+	±	+	+	-	+	-
Forfia 2006	+	+	nr	+	+	+	+	+	+	±	-
Mahapatra 2006	-	-	nr	+	+	+	+	+	+	±	-
Utsunomiya 2009	-	nr	+	+	+	±	+	+	+	-	NA
Benza 2010	+	-	+	-	±	+	+	+	NA	+	-
Campo 2010	-	+	+	-	±	+	+	+	NA	-	NA
Ciarka 2010	+	nr	+	+	+	+	+	+	+	±	-
Ghio 2010	+	nr	nr	+	+	±	+	+	±	-	NA
Ghio 2011	+	nr	nr	+	+	±	+	+	-	±	+
Kane 2011	-	+	+	±	-	+	+	+	-	+	+
Mathai 2011	+	nr	nr	+	+	±	+	+	+	±	-
Sachdev 2011	-	-	nr	+	+	+	+	+	-	+	+
Zeng 2011	-	nr	nr	+	+	+	+	+	-	±	-
Badagliacca 2012	-	nr	nr	±	+	+	+	+	NA	±	-
Moceri 2012	+	nr	+	+	+	+	+	+	±	±	-
Shimony 2012	-	+	nr	-	-	±	+	+	+	-	NA
Tonelli 2012	-	nr	nr	+	+	+	+	+	±	+	-
Ernande 2013	+	nr	nr	+	+	+	+	+	-	+	-
Fenstad 2013	+	+	nr	+	+	±	+	+	NA	+	-
Fine 2013	+	-	+	+	+	+	+	+	-	+	-
Giusca 2013	+	-	nr	±	+	±	+	+	+	±	-
Grunig 2013	+	-	+	+	+	+	+	+	-	+	+

SUPPLEMENTAL FILE 2 - Continued.

	Selection bias			Information bias: defined and measured appropriately?				Statistical calculation of effect size			
Author, Year	**Study design**	**Missing data**	**Loss to follow-up**	**Description of echocardiography protocol**	**Measurement of echocardiographic findings**	**Definition and measurement of outcome**	**Description of statistical analysis**	**Effect size: hazard ratios**	**Treatment of continuous predictors**	**Multivariable adjustment**	**Multivariable analysis appropriate**
Hardegree 2013	-	-	+	+	+	±	+	+	-	±	-
Hardegree 2013	-	±	+	+	+	±	+	+	-	+	+
Shimony 2013	-	+	nr	±	+	±	+	+	NA	±	+
Cho 2014	-	nr	nr	+	+	+	±	+	-	-	NA
Tonelli 2014	-	nr	nr	+	+	+	+	+	±	+	-
Schuuring 2014	+	±	-	±	+	+	+	+	-	±	+
Dandel 2014	-	nr	nr	+	+	±	+	+	±	-	NA
Austin 2015	-	nr	nr	+	+	+	+	+	-	±	+
Batal 2015	-	-	nr	+	+	±	+	+	+	+	+
Sano 2015	-	±	nr	+	+	+	+	+	+	±	-

nr, not reported; NA, not applicable. *Study design*: +, prospective cohort study; -, retrospective cohort study. *Missing data*: +, <5%; ±, 5-10% or <5% selective missing data; -, >10% or >5% selective missing data. *Loss to follow-up*: +, <5%; ±, 5-10% or <5% selective loss to follow-up; -, >10% or >5% selective loss to follow-up. *Echocardiography protocol and statistical analysis*: +, well defined; ±, moderately defined; -, poorly defined. *Echocardiographic findings and outcome*: +, well defined and measured appropriately; ± moderately defined or moderately measured; -, poorly defined or poorly measured. *Effect size*: +, Cox regression model and outcomes presented as HR [95% CI]; ± Cox regression model, outcomes not presented as HR [95% CI]; -, independent-samples T-test or outcomes presented as OR/RR. *Treatment of continuous predictors*: +, all kept continuous; ±, some categorised/dichotomised, some not; -, all categorised/dichotomised. *Multivariable adjustment*: +, yes, at least for age and gender; ± multivariable adjustment for other factors; -, no multivariable analysis performed or not described. *Multivariable analysis appropriate*: +, ≥10 events per predictor used; -, <10 events per predictor used.

SUPPLEMENTAL FILE 3 - Overview of investigated echocardiographic findings per study.

Echocardiographic findings	Total studies(n)	Studies suitable for pooling of results	Eysmann 1989	Tei 1996	Yeo 1998	Miyamoto 2000	Bustamante 2002	Raymond 2002	Forfia 2006	Mahapatra 2006	Utsunomiya 2009	Benza 2010	Campo 2010	Ciarka 2010	Ghio 2010	Ghio 2011	Kane 2011	Mathai 2011	Sachdev 2011	Zeng 2011	Badagliacca 2012	Moceri 2012	Shimony 2012	Tonelli 2012	Emande 2013	Fenstad 2013	Fine 2013	Giusca 2013	Grunig 2013	Hardegree 2013	Hardegree 2013	Shimony 2013	Cho 2014	Tonelli 2014	Schuuring 2014	Dandel 2014	Austin 2015	Batal 2015	Sano 2015
Pericardial effusion	17	16	x			x		x	x			x	x	x	x		x	x				x			x	x	x				x				x	x*	
TAPSE	12	7							x						x		x					x			x		x	x	x			x*	x	x			
RA area	12	5					x	x	x								x	x				x			x				x			x*	x		x		x*
RVSP, SPAP, TR Vmax	11	7						x		x				x	x			x							x		x	x	x			x	x*				
RV fractional area change	9	5						x	x								x	x					x				x	x				x*			x		
Tei index (RIMP)	8	7		x	x					x					x		x						x				x					x					
TR severity	8	7			x		x	x	x						x		x										x					x*		x			
Estimated RA pressure	7	6								x					x		x					x					x					x*		x			
LV eccentricity index	7	4					x		x	x					x			x							x								x					
RV longitudinal peak systolic strain	6	4																	x						x		x	x	x*								x
RV end-diastolic basal diameter	5	3														x						x			x			x					x*					
RV end-diastolic area	5	2							x								x											x	x				x*					
TDI TV lat S'	5	2																				x			x			x	x				x					
LV cardiac output or index	4	3								x															x			x						x				
MV E/A ratio	4	2	x																					x	x								x*					
MV E velocity	3																							x	x								x*					
RVOT time velocity integral	3																					x						x					x*					
PA acceleration time	2			x										x																								
RV ejection time	2							x		x																												
PA capacitance	2									x																	x											
RV free wall thickness	2														x	x																						
RV free wall strain rate	2																		x															x				
RV/LV end-diastolic basal diameter	2																			x				x														
LV ejection fraction	2																					x											x*					
RA/LA area ratio	2																					x												x				
TR deceleration	1		x																																			
TV E/e' ratio	1										x																											
LV end-diastolic volume	1														x																							
RV free wall time to peak syst strain	1																		x																			
RV free wall early diast strain rate	1																		x																			
MV e' velocity	1																				x																	
TV e'/a' ratio	1																				x																	
Total isovolumic time	1																				x																	
Isovolumic contraction peak velocity	1																							x														
Isovolumic acceleration	1																							x														
Isovolumic relaxation time	1																							x														
sPAP	1																										x											
LV free wall LPSS	1																													x								
LV circumferential strain	1																													x								
TR duration (heart rate adjusted)	1																															x						
RV midcavity diameter	1																																x*					
RV longitudinal diameter	1																																x*					
LV end-diastolic diameter	1																																x*					
LV end-systolic diameter	1																																x*					
RV outflow tract acceleration time	1																																x*					

SUPPLEMENTAL FILE 3 - Continued.

Echocardiographic findings	Total studies(n)	Studies suitable for pooling of results	Eysmann 1989	Tei 1996	Yeo 1998	Miyamoto 2000	Bustamante 2002	Raymond 2002	Forfia 2006	Mahapatra 2006	Utsunomiya 2009	Benza 2010	Campo 2010	Clarka 2010	Ghio 2010	Ghio 2011	Kane 2011	Mathai 2011	Sachdev 2011	Zeng 2011	Badagliacca 2012	Moceri 2012	Shimony 2012	Tonelli 2012	Ernande 2013	Fenstad 2013	Fine 2013	Giusca 2013	Grunig 2013	Hardegree 2013	Hardegree 2013	Shimony 2013	Cho 2014	Tonelli 2014	Schuuring 2014	Dandel 2014	Austin 2015	Batal 2015	Sano 2015
abnormal end-diastolic septal curve	1																																x*					
S wave velocity	1																																x*					
D wave velocity	1																																x*					
LV dysfunction (0-1)	1																																	x				
RV load adaptation index	1																																		x*			
RV end-systolic area	1																																					x*

*imaging finding is longitudinally investigated (change between baseline and follow-up).

Chapter 08

Cardiac magnetic resonance findings predicting mortality in patients with pulmonary arterial hypertension: a systematic review and meta-analysis

Vivan J.M. Baggen, Tim Leiner, Marco C. Post, Arie P. van Dijk,
Jolien W. Roos-Hesselink, Eric Boersma, Jesse Habets, Gertjan Tj. Sieswerda

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ABSTRACT

Objectives To provide a comprehensive overview of all reported cardiac magnetic resonance (CMR) findings that predict clinical deterioration in pulmonary arterial hypertension (PAH).

Methods MEDLINE and EMBASE electronic databases were systematically searched for longitudinal studies published by April 2015 that reported associations between CMR findings and adverse clinical outcome in PAH. Studies were appraised using previously developed criteria for prognostic studies. Meta-analysis using random effect models was performed for CMR findings investigated by three or more studies.

Results Eight papers (539 patients) investigating 21 different CMR findings were included. Meta-analysis showed that right ventricular (RV) ejection fraction was the strongest predictor of mortality in PAH (pooled HR 1.23; 95% CI 1.07–1.41, $p = 0.003$) per 5% decrease. In addition, RV end-diastolic volume index (pooled HR 1.06; 95% CI 1.00–1.12, $p = 0.049$), RV end-systolic volume index (pooled HR 1.05; 95% CI 1.01–1.09, $p = 0.013$) and left ventricular end-diastolic volume index (pooled HR 1.16; 95% CI 1.00–1.34, $p = 0.045$) were of prognostic importance. RV and LV mass did not provide prognostic information ($p = 0.852$ and $p = 0.983$, respectively).

Conclusion This meta-analysis substantiates the clinical yield of specific CMR findings in the prognostication of PAH patients. Decreased RV ejection is the strongest and most well established predictor of mortality.

INTRODUCTION

Pulmonary Arterial Hypertension (PAH) is defined as a mean pulmonary arterial pressure ≥ 25 mmHg in the presence of a pulmonary capillary wedge pressure ≤ 15 mmHg as assessed by right heart catheterization, and is characterized by progressive remodeling of the distal pulmonary arteries.[1] PAH includes apparent heterogeneous conditions (idiopathic, heritable, induced by drugs or toxins, associated with connective tissue diseases, HIV infection, portal hypertension and congenital heart disease), but is characterized by similar clinical, hemodynamic and pathological pictures.[1, 2] Left untreated, the resultant increase in pulmonary vascular resistance leads to progressive deterioration of right ventricular (RV) function and eventually death in 45% of incident cases within 3 years.[2, 3] Although recent advances in therapeutic modalities have significantly improved the outcomes of this devastating disease, the course of the disease widely varies between individuals.[4-6] In order to guide optimal clinical management of patients with PAH, accurate prognostication and monitoring of disease progression is therefore of great importance.[5]

Previously reported predictors of mortality include etiology of PAH, sex, and several functional, biochemical and hemodynamic variables.[3, 7-10] Although it is inferred that cardiac magnetic resonance imaging (CMR) could be of important additional value, evidence for the prognostic merit of specific imaging findings is still far from robust. This study therefore aims to provide a comprehensive overview of commonly investigated CMR findings that are predictive of adverse clinical outcome in PAH.

METHODS

This systematic review was conducted in accordance with the PRISMA statement.[11] A pre-defined review protocol as adopted by this study can be accessed through PROSPERO (registration number: CRD42014009231).

Literature search strategy

CMR studies as described in this review were identified through a general search syntax that was designed to aggregate all studies concerning the prognostic value of non-invasive imaging in PAH. A comprehensive systematic search was performed on 29 April 2015 in MEDLINE (via PubMed interface) and EMBASE electronic databases using combinations of all synonyms for: population (PAH), non-invasive imaging and relevant clinical outcomes (components of the Dana Point Time To Clinical Worsening composite endpoint).[2, 12] A validated prognostic search filter with the highest sensitivity (98%) was added to the search syntax.[13, 14] No language or publication period restrictions were applied. The full original search syntax is supplied in Supplemental File 1.

Selection of papers

A flow diagram of the selection process is shown in Figure 1.[11] After deduplication, one author (V.B.) performed screening and selection of articles based on title and abstract, using the following exclusion criteria: cross-sectional study design, study population without PH (e.g. acute pulmonary embolism, exercise-induced PH), > 30% study population with other than WHO group I PAH, inclusion of children < 12 years of age, CMR findings not investigated as potential predictor for clinical outcome, lack of Cox regression analysis. Full-text screening was performed by two authors (V.B., J.H.); exclusion criteria are described in Figure 1. All references of the excluded reviews and included articles were cross-checked to identify possible relevant articles missed in the original search syntax.

Assessment of methodological quality

Study quality was critically appraised using previously developed criteria for prognostic studies.[15] We assessed study design, missing data and loss to follow-up (selection bias), adequate description and measurement of imaging features and outcome (information bias), reported effect size, treatment of continuous risk predictors, and multivariable adjustment for possible confounders.

Data extraction and analysis

Study characteristics and hazard ratios (HRs) for all investigated CMR findings with accompanying 95% confidence intervals were extracted using a standardized form. Meta-analysis was performed for all CMR findings investigated by three or more studies, using random effect models. HRs for specific findings were recalculated to one uniform clinically applicable number of units change. Heterogeneity was assessed using Cochran's *Q* test and the I^2 statistic.[16] CMR findings investigated as dichotomous variables were additionally presented in the corresponding forest plots. To determine the effect of individual study data, sensitivity analyses were performed by recalculating pooled HRs after excluding the results of one study. If study data were used in multiple papers and the same CMR findings were evaluated, only the study with the largest sample size was selected, thus excluding the risk of using duplicate data in our meta-analysis. The risk of publication bias was assessed using visual inspection of funnel plots and the Egger's test.[17]

RESULTS

Search results

Through a systematic literature search in MEDLINE and EMBASE and extensive reference cross-checking, 2733 potentially relevant records were retrieved, of which 2610 were excluded based on title and abstract. After full-text review of the remaining 123 articles, 44 papers were finally selected, of which 8 studies in 539 patients described CMR findings (Figure 1).[18-25]

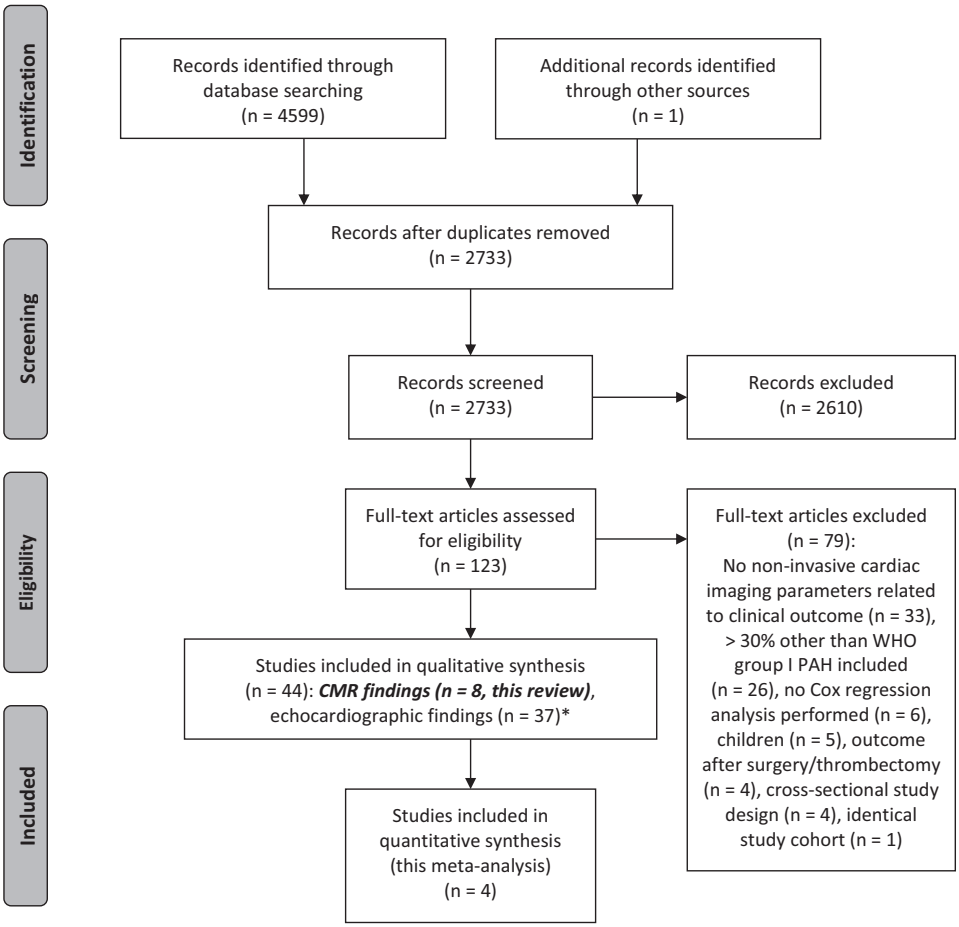

FIGURE 1 - PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 flow diagram.

*, one study investigated both echocardiographic and cardiac magnetic resonance imaging findings. **Abbreviations:** CMR, cardiac magnetic resonance; PAH, pulmonary arterial hypertension; WHO, World Health Organization.

Study and patient characteristics of the included studies are presented in Table 1. The studies were published between 2007 and 2014; study size ranged from 37 to 110 patients, mean age ranged from 39 to 62 years, and 60–79% of the population was female. The majority of patients was diagnosed with idiopathic PAH (41–100%), two studies included a subset of patients with congenital heart disease (9 and 24%, respectively)[23, 25] and one study included patients with PH group III, IV or V (24% of patients).[21] Two studies did not report information on the use of PAH-specific mediation,[24, 25] in all other studies > 60% of patients were on PAH-specific therapy at baseline.

The majority of studies used death (or transplant) as primary outcome; two studies used a composite outcome, additionally including hospitalization for heart failure.[21, 23] Mean follow-up duration varied between 10 and 45 months, and the primary outcome event occurred in 4–25 patients (10–33% of the study population).

Methodological aspects

The individual bias assessment per study is detailed in Table 2. Five out of eight studies had a retrospective study design. Information on missing values and loss to follow-up was not reported in two studies. It is therefore important to recognize the possible impact of selection bias.

Right heart catheterization was used for the diagnosis of PAH in 100% of the included patients. The majority of studies used short-axis segmentation for the measurement of RV volumes;[19-21, 23, 24] one study used a transverse segmenting method[22] and none of the studies used axial slice segmentation. Slice thickness varied between 5 and 10 mm and temporal resolution between 20 and 25 frames/cycle or 35–45 ms. Two studies did not report spatial or temporal resolution.[19, 21] None of the studies explicitly described if the valvular planes were taken into account in the segmentation and how the tricuspid valve was delineated; however, most studies did mention that both ventricles were covered from base to apex. Most studies were not clear about the methodology used for the selection of the trabeculae. The technical details regarding the CMR acquisition and analysis are described in more detail in Supplemental File 2.

Although all studies reported HRs using Cox regression analysis, large differences were found regarding the type and number of predictors per event used in the multivariable analysis. Five studies performed some form of multivariable adjustment, of which only one study adjusted for age and sex. Only one study used ten or more events per predictor. Because of this large variety between studies and overall suboptimal methodological quality of multivariable adjustment, it was decided to present only univariable HRs in the forest plots.

TABLE 1 - Study characteristics.

	Gan 2007[18]	v Wolferen 2007[19]	vd Veerdonk 2011[20]	Freed 2012[21]	Yamada 2012[22]	Cho 2014[23]	Swift 2014[24]	Swift 2014[25]
Study population								
Size, n	70	64	110	58	41	37	80	79
Age, mean ± SD	50 ± 15	43 ± 13	53 ± 15	53 ± 14	39 ± 14	46 ± 14	59 ± 17	62 ± 16
Sex, % female	79	73	76	74	71	76	60	61
NYHA class III–IV, %	nr	89	52	nr	51	35	66	nr
PH subclassification								
Idiopathic PAH	70	100	66	41	100	65	100	44
Hereditary PAH	-	-	6	-	-	-	-	-
Drug- or toxin-induced PAH	-	-	3	-	-	-	-	-
PAH-CTD	23	-	18	-	-	5	-	47
PAH-HIV	3	-	2	-	-	-	-	-
Po-PAH	-	-	5	-	-	-	-	-
PAH-CHD	-	-	-	-	-	24	-	9
WHO I PAH (other/not specified)	4	-	-	34	-	5	-	-
WHO II (left heart disease)	-	-	-	14	-	-	-	-
WHO III (lung disease)	-	-	-	2	-	-	-	-
WHO IV (CTEPH)	-	-	-	3	-	-	-	-
WHO V (unclear/multifactorial)	-	-	-	5	-	-	-	-
PAH medication, %								
Calcium antagonist	4	8	3	31	22	8	nr	nr
Phosphodiesterase 5-inhibitor	6	6	15		88	21	nr	nr
Endothelin receptor antagonist	51	39	35		54	62	nr	nr
Prostacyclin analog	29	47	14	31	34	14	nr	nr
Outcome								
Endpoint	Death	Death, Tx	Death	Death, Tx, HF	Death	Death, HF	Death	Death
Follow-up, months, mean ± SD or median [IQR]	nr	32 ± 16	12 [10–16]	10 ± 6	45 ± 26	16 [13–18]	32 ± 14	nr
Events, n (%)	18 (26)	19 (30)	13 (12)	19 (33)	4 (10)	7 (19)	23 (29)	25 (32)

CHD, congenital heart disease; CTD, connective tissue disease; CTEPH, chronic thrombo-embolic pulmonary hypertension; HF, admission for heart failure; HIV, human immunodeficiency virus; IQR, interquartile range; NYHA, New York Heart Association; nr, not reported; PAH, pulmonary arterial hypertension; Po-PAH, PAH associated with portal hypertension; SD, standard deviation; Tx, transplant; WHO, World Health Organization.

TABLE 2 - Methodological quality of the included studies.

	Gan 2007[18]	van Wolferen 2007[19]	vd Veerdonk 2011[20]	Freed 2012[21]	Yamada 2012[22]	Cho 2014[23]	Swift 2014[24]	Swift 2014[25]
Selection bias								
Study design	–	+	+	+	–	–	–	–
Missing data	nr	+	±	±	+	nr	+	+
Loss to follow-up	+	+	+	+	+	nr	+	nr
Information bias: defined and measured appropriately?								
Description of CMR protocol	+	±	+	±	+	±	±	+
Measurement of CMR findings	+	±	+	+	±	+	+	+
Definition and measurement of outcome	+	+	+	+	+	+	±	±
Description of statistical analysis	+	±	+	+	±	±	+	+
Statistical calculation of effect size								
Effect size: hazard ratios	+	+	+	+	+	+	+	+
Treatment of continuous predictors	+	+	+	+	+	–	+	+
Multivariable adjustment	±	±	±	±	–	–	–	+
Multivariable analysis appropriate	–	–	–	–	NA	NA	NA	+

nr, not reported; NA, not applicable. *Study design*: +, prospective cohort; –, retrospective cohort. *Missing data*: +, < 5%; ±, 5–10% or < 5% selective. *Loss to follow-up*: +, < 5%. *CMR protocol and statistical analysis*: +, well defined; ±, moderately defined. *CMR findings and outcome*: +, well defined and measured appropriately; ± moderately defined or moderately measured. *Effect size*: +, Cox regression model and outcomes presented as HR (95% CI). *Treatment of continuous predictors*: +, all kept continuous; –, all categorized/dichotomized. *Multivariable adjustment*: +, yes, at least for age and gender; ± multivariate adjustment for other factors; –, no multivariate analysis performed or not described. *Multivariable analysis appropriate*: +, ≥ 10 events per predictor used; –, < 10 events per predictor used.

Prognostic value of baseline CMR findings

Eight studies evaluated 21 different CMR findings, as shown in Table 3. Meta-analysis was performed for nine CMR findings that were evaluated by three or more studies. Forest plots and pooled HRs are presented in Figure 2. The strongest predictor of mortality was RV ejection fraction: pooled HR 1.23 (95% CI 1.07–1.41, $p = 0.003$) per 5% decrease. In addition, RV end-diastolic volume index ($p = 0.049$) and end-systolic volume index ($p = 0.013$) and left ventricular (LV) end-diastolic volume index ($p = 0.045$) were of prognostic importance. Notably, measurements of RV and LV mass did not provide prognostic information in PAH ($p = 0.852$ and $p = 0.983$, respectively).

Although RV stroke volume index as measured by the summation of disks method did not have significant prognostic value (pooled HR 1.05, p = 0.242),[21, 22, 24] the pulmonary artery (PA) flow stroke volume index as measured by CMR phase-contrast imaging predicted mortality in two studies.[19, 24] Less investigated CMR measurements such as LV stroke volume,[20, 24] pulmonary artery relative area change,[18] left atrial volume, late gadolinium enhancement at RV insertion points[21] and two novel dynamic contrast-enhanced CMR measurements (full width at half maximum of the bolus passage and pulmonary transit time)[25] seem promising but require further evaluation.

TABLE 3 - Overview of investigated cardiac magnetic resonance imaging findings per study.

CMR finding	Total studies (n)	Gan 2007[18]	van Wolferen 2007[19]	vd Veerdonk 2011[20]	Freed 2012[21]	Yamada 2012[22]	Cho 2014[23]	Swift 2014[24]	Swift 2014[25]
RV ejection fraction	6		x*	x*	x	x	x	x	
RV end-diastolic volume	5		x*	x*	x	x		x	
RV end-systolic volume	4			x*	x	x		x	
RV stroke volume	3				x	x		x	
PA flow stroke volume index	2		x*					x	
PA flow cardiac index	2		x*					x	
RV mass	3		x*		x	x			
LV ejection fraction	5		x*	x*	x	x		x	
LV end-diastolic volume	5		x*	x*	x	x		x	
LV end-systolic volume	4			x*	x	x		x	
LV stroke volume	2			x*				x	
LV mass	3		x*		x	x			
RV wall thickness	1		x*						
LV wall thickness	1		x*						
Ventricular mass index (RV/LV mass)	1							x	
RA volume	1				x				
LA volume	1				x				
PA relative area change	1	x							
RV insertion points late gadolinium enhancement	1				x				
Full width at half maximum of the bolus passage	1								x
Pulmonary transit time	1								x

***additional serial investigation (change between baseline and follow-up). **Abbreviations:** LV, left ventricular; PA, pulmonary artery; RV, right ventricular.

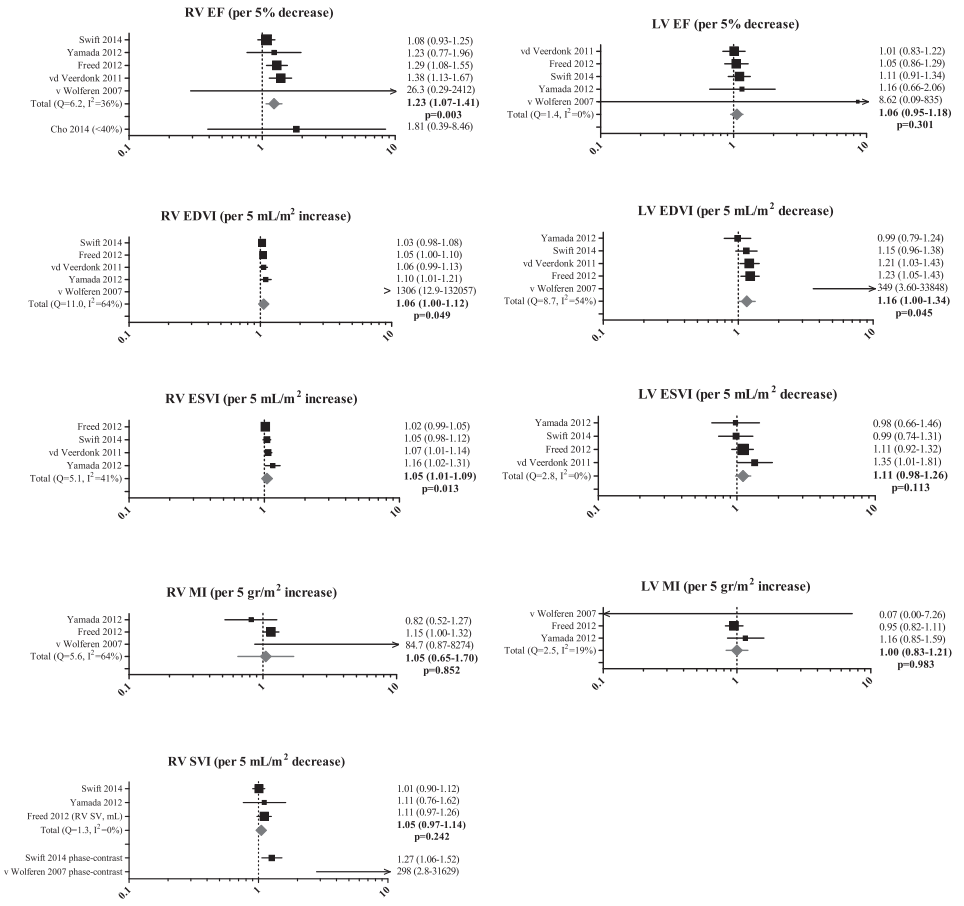

FIGURE 2 - Prognostic value of cardiac magnetic resonance imaging findings evaluated by three or more studies.

Values are presented as mean (95% confidence interval). **Abbreviations:** EDVI, end-diastolic volume index; EF, ejection fraction; ESVI, end-systolic volume index; LV, left ventricular; MI, mass index; RV, right ventricular; SVI, stroke volume index.

Serial CMR evaluation

Two studies included in this review additionally quantified the individual change in specific CMR findings during a follow-up period and directly associated these with patient outcomes. Van Wolferen et al. showed that overall mortality was associated with a decrease in RV ejection fraction ($p = 0.015$) and PA flow stroke volume index ($p = 0.006$) at 1-year follow-up.[19] Van de Veerdonk's group reported a 1-year change in RV ejection fraction ($p = 0.014$), RV end-diastolic and end-systolic volume index (both $p < 0.001$) as significant predictors of long-term outcome.[20]

Risk of bias assessment

A combination of visual assessment of funnel plots and the Egger's test did not provide statistical evidence for publication bias for any of the CMR findings included in the meta-analysis. Statistical heterogeneity among studies was generally low, as presented by Cochran's *Q* and I^2 statistics in Figure 2 in the corresponding forest plots. We noted a discrepancy between the values that were reported in the study of van Wolferen et al. compared with the other studies, as effect sizes and standard errors were extremely large in this study.[19] We therefore conducted sensitivity analyses by excluding the results of this study. This did not result in significantly different HRs and did thus not change our conclusions.

DISCUSSION

To our knowledge, this is the first systematic review and meta-analysis that substantiates the clinical yield of CMR findings in the prognostication of PAH patients. Among eight studies (539 patients) that investigated 21 different CMR findings, RV ejection fraction was found to be the strongest and most well established predictor of mortality in PAH. In addition, increased RV volumes and decreased LV end-diastolic volume at baseline were found to be associated with a higher mortality risk in PAH patients.

Right heart failure

The primary cause of death in PAH is right heart decompensation.[8] This is congruent with the observation that a decrease in RV function is such an important prognostic factor, as it indicates that the right ventricle is no longer able to cope with the high pulmonary pressures, leading to increased RV diastolic pressures and RV dilatation. RV cardiac output as invasively assessed during right heart catheterization has similarly shown to predict mortality in PAH.[7, 8] Studies that have investigated echocardiographic measurements of RV function, such as TAPSE or RV longitudinal strain, report similar findings.[26, 27] RV ejection fraction assessed with equilibrium radionuclide angiography also showed to be of prognostic importance.[28] Nevertheless, CMR is currently considered as the reference standard for the assessment of RV volumes and function.[29] RV function as measured with CMR may therefore be the most accurate and thus most reliable predictor. However, it has to be acknowledged that direct comparisons between the different imaging modalities are not reported yet.

Ventricular-ventricular interaction

Whereas CMR-derived RV volumes are increased in high-risk PAH patients, LV end-diastolic volume is decreased. Similar results have been reported in several echocardiographic studies.[30-32] These findings emphasize once more the inextricable connection between the two ventricles. Although often considered as separate entities, the two ventricles share common myofibers, a noncompliant pericardium and of course the interventricular septum.[33] The pressure-overloaded RV will alter LV geometry by a leftward septal shift, resulting in a D-shaped LV, which is generally considered as one of the hallmarks of pulmonary hypertension. This so-called systolic D-sign is primarily an expression of RV pressure overload, in contrast to a diastolic D-sign which is considered a sign of a volume-overloaded RV.[34] Interestingly, previous studies have shown that the diastolic leftward septal shift is associated with mortality in PAH, in contrast to the systolic leftward septal shift.[35] Accordingly, we found decreased LV end-diastolic volume to be of prognostic value, in contrast to LV end-systolic volume.

The overall observation that systolic LV geometry is not associated with mortality may be explained by the fact that RV systolic pressures are generally high in a stable PAH state. However, once the RV starts to fail, LV preload will decrease due to lower transpulmonary flow, and RV diastolic pressures will rise. The accompanied altered diastolic LV geometry can therefore be seen as a direct expression of RV failure.[35] Subsequently, this altered shape could impair LV filling and function, further leading to increased pulmonary pressures, which may create a vicious circle tilting a stable PAH state towards clinical deterioration and, ultimately, death.

Serial measurements

Apart from baseline findings evaluated at the time of diagnosis, changes in several hemodynamic, functional, biochemical and imaging variables could carry incremental prognostic information, as they conceivably better reflect an individual's course of disease.[19, 20, 36, 37] Thus far, only two studies have directly related subject-specific changes in CMR measurements to clinical outcome using Cox regression.[19, 20] Both studies report the prognostic importance of a decrease in RV ejection fraction over time. In addition, van de Veerdonk et al. recently published the results of repeated CMR measurements during a follow-up period of 10 years, in patients with initially stable idiopathic PAH for 5 years.[38] Although no HRs were presented, this study showed that late disease progression is also accompanied by increased RV volumes and decreased RV ejection fraction.

RV mass

Based on three studies, our results showed that RV mass does not provide prognostic information in PAH.[19, 21, 22] This is probably because concentric RV hypertrophy is an adaptive response to increased RV pressures that serves to maintain wall stress as low as possible, as is also seen in the LV.[39, 40]

RV mass-to-volume ratio allows the distinction between RV concentric and eccentric hypertrophy, which might better reflect the RV's adaptive or maladaptive response. A recently published cross-sectional study in patients with idiopathic PAH showed that eccentric hypertrophy, reflected by a lower RV mass-to-volume ratio, was clearly related to worse RV systolic function, right ventricular to arterial coupling, and clinical impairment.[40] Moreover, in patients with tetralogy of Fallot, lower RV mass-to-volume ratio was predictive of death.[41] Therefore, it may be more prudent to focus on RV mass-to-volume ratios rather than on RV mass alone.

Study limitations

Although we calculated pooled effect estimates in our review, the results of the random effect models should be interpreted with caution, as we included only univariable HRs in the meta-analysis, due to the large variability between studies in which multivariable adjustment was performed (regarding the type and number of predictors per event used). Second, although formal tests for publication bias yielded mainly non-significant results, the relative lack of power of the Egger's test in detecting publication bias for imaging findings investigated in less than ten studies should be recognized. Theoretically, publication bias can cause underreporting of non-significant HRs, leading to a relative overestimation of the pooled HRs. Finally, although differences within studies regarding the CMR acquisition and analysis probably reflect the normal variance in day-to-day practice, they may lead to less precise measurements (independent of the outcome). While the vast majority of the studies used short-axis segmentation, it has been suggested that axial segmentation results in higher reproducibility, as it may decrease the difficulty of valve delineation in the basal slices.[42, 43] Incomplete segmentation of the tricuspid plane, inaccurate delineation of the basal slice and the methodology used to deal with the trabeculae can substantially impact the measurements of RV volumes.[44, 45] The subsequent 'random noise' would dilute the effect and thus could cause a relative underestimation of the actual hazard ratios.

Clinical implications

This review substantiates the clinical yield of specific CMR findings in the identification of patients with PAH at higher risk of clinical deterioration. This is important, as timely

intensification of PAH-specific therapy could prevent further clinical worsening and death. In addition, CMR-derived RV ejection fraction could be of additional value for the longitudinal assessment of PAH. More research is needed to investigate the prognostic value of other serial CMR measurements, and baseline measurements such as and RV mass-to-volume ratio, LV stroke volume, pulmonary artery relative area change and left atrial volume. In addition, it would be worthwhile to directly compare the prognostic value of RV function measured using CMR with RV function measured using other non-invasive imaging modalities, such as echocardiography.

CONCLUSIONS

CMR is useful and accurate in the prognostication of PAH patients. RV ejection fraction at baseline and during follow-up is the strongest and most well investigated predictor of mortality in patients with PAH. In addition, increased RV volumes and decreased LV end-diastolic volume are of prognostic importance.

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FILE 1 - Search syntax used to identify publications of interest (search date 29 April 2015).

Population #1	"pulmonary hypertension"[tiab] OR "pulmonary arterial hypertension"[tiab] OR "pulmonary artery hypertension"[tiab]
Predictive variable #2	echocardiograph*[tiab] OR echocardiogram[tiab] OR ultrasound[tiab] OR ultrasonograph*[tiab] OR ultrasonogram[tiab] OR ultrasonic[tiab] OR "M-Mode"[tiab] OR "M Mode"[tiab] OR doppler[tiab] OR "cardiac magnetic resonance"[tiab] OR imaging[tiab] OR MRI[tiab] OR "magnetic resonance angiography"[tiab] OR "magnetic resonance angiographies"[tiab]
Outcome #3	death[tiab] OR mortality[tiab] OR survival[tiab] OR fatal[tiab] OR fatality[tiab] OR transplant*[tiab] OR graft*[tiab] OR hospitalization[tiab] OR hospitalisation[tiab] OR admission[tiab] OR readmission[tiab] OR transcatheter[tiab] OR intervention[tiab] OR intravenous[tiab] OR infusion*[tiab] OR "new york heart association"[tiab] OR "functional class"[tiab] OR "functional status"[tiab] OR function[tiab] OR exercise[tiab] OR endurance[tiab] OR (("6-minute"[tiab] OR "6 minute"[tiab] OR "6-min"[tiab] OR "6 min"[tiab] OR "six-minute"[tiab] OR "six minute"[tiab]) AND walk[tiab] AND (test[tiab] OR distance[tiab])) OR VO2max[tiab] OR "VO2 max"[tiab] OR VO2peak[tiab] OR "VO2 peak"[tiab] OR "aerobic capacity"[tiab] OR (oxygen[tiab] AND (uptake[tiab] OR consumption[tiab])) OR symptom*[tiab] OR morbidity[tiab] OR outcome[tiab] OR outcomes[tiab]
Prognostic studies #4	predict*[tiab] OR clinical*[tiab] OR outcome*[tiab] OR risk*[tiab] OR prognos*[tiab]
Search results (#1 AND #2 AND #3 AND #4)	MEDLINE: 2371 EMBASE: 2228 (replace [tiab] by :ti,ab)
Other limits (added search strings): **Pubmed:** NOT (animals[MeSH] NOT humans[MeSH]) **Embase:** NOT ([conference abstract]/lim OR [conference paper]/lim OR [conference review]/lim OR [editorial]/lim OR [erratum]/lim OR [letter]/lim OR [note]/lim) AND [embase]/lim	

SUPPLEMENTAL FILE 2 - Technical details regarding the CMR acquisition and analysis.

Author, Year	MR scanner used	Segmenting method for ventricular volumes	Spatial resolution (voxel size)*	Temporal resolution	Valvular planes taken into account?	Software used	Trabeculae in- or excluded from the cavity?
Gan 2007[18]	1.5-T Sonato, Siemens	no volumes measured	N/A	N/A	N/A	N/A	N/A
v Wolferen 2007[19]	1.5-T Sonato, Siemens	short-axis	1.3 x 1.9 x 6.0 mm^3	-	"from apex to base, covering the whole LV and RV"	MASS, Medis	-
vd Veerdonk 2011[20]	1.5-T Sonato, Siemens	short-axis	1.5 x 1.8 x 5.0 mm^3	35-45 ms	"from base to apex of the ventricles"	MASS, Medis	excluded
Freed 2012[21]	1.5-T Achieva, Philips	short-axis	-	~40 ms	"that included both right and left ventricles from base to apex"	ViewForum, Philips	-
Yamada 2012[22]	1.5-T Signa TwinSpeed, GE	transverse	1.6 x 1.8 x 10 mm^3	20 frames/ cycle	-	MASS Analysis Plus version 4.0, Medis	included
Cho 2014[23]	1.5-T Achieva, Philips	short-axis	slice thickness 10 mm	25 frames/ cycle	"encompassed the left and right ventricles in entirety"	ViewForum version 4.1, Philips	-
Swift 2014[24]	1.5-T Signa HDx, GE	short-axis	slice thickness 8 mm	20 frames/ cycle	"fully covering both ventricles from base to apex"	Advantage Workstation 4.1, GE	-
Swift 2014[25]	1.5-T Signa HDx, GE	no volumes measured	N/A	N/A	N/A	N/A	N/A

*, slice thickness if voxel size is not mentioned; -, no information available. **Abbreviations**: N/A, not applicable.

08

PART II

BLOOD BIOMARKERS

Chapter 09

Matrix metalloproteinases as candidate biomarkers in adults with congenital heart disease

Vivan J.M. Baggen, Jannet A. Eindhoven, Annemien E. van den Bosch, Maarten Witsenburg, Judith A.A.E. Cuypers, Jannette S. Langstraat, Eric Boersma, Jolien W. Roos-Hesselink

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ABSTRACT

Background Matrix metalloproteinases (MMPs) are associated with diastolic dysfunction and heart failure in acquired heart disease.

Objective To investigate the role of MMPs as novel biomarkers in clinically stable adults with congenital heart disease.

Methods We measured serum MMP-2, -3, -9 and tissue inhibitor of matrix metalloproteinase-1 in 425 patients and analyzed the association with cardiac function and exercise capacity.

Results MMP-2 was significantly associated with exercise capacity, ventilatory efficiency and left ventricular deceleration time, independently of age, sex, body surface area and NT-proBNP.

Conclusions MMP-2 may provide new information in the clinical evaluation of adults with congenital heart disease.

INTRODUCTION

Since Jerome Gross and Charles Lapiere initially described the enzyme collagenase while observing the metamorphosis of the tadpole in 1962, much research has been performed on what is nowadays known as matrix metalloproteinase-1.[1] Many other members of the family of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), consisting of structurally and functionally related extracellular proteolytic enzymes, have been described since.[2] Together, these enzymes are capable of degrading all kinds of extracellular matrix protein components.[3, 4] A disruption of the balance between MMPs and TIMPs is observed in a wide variety of pathological conditions, such as acute and chronic cardiovascular diseases, cancer progression and different types of arthritis.[4-7] More specific, altered MMP-2, MMP-9 and TIMP-1 levels have been associated with left ventricular (LV) hypertrophy, chronic heart failure and diastolic dysfunction in hypertensive heart disease and coronary artery disease.[8-12] In patients with thoracic aortic aneurysms, specific MMP-2, -9 and TIMP-1 profiles have been described; however, the majority of research in this field is based on histology of the aortic wall rather than circulating blood biomarker levels.[13-15]

To date, little is known about circulating MMP profiles in patients with adult congenital heart disease (ACHD). As biventricular fibrosis, hypertrophy and dysfunction are important causes of morbidity and mortality, the evaluation of MMPs may well prove to be important in this setting. Therefore, the goal of this study was to evaluate MMP profiles in patients with ACHD and to investigate its relation with exercise capacity and echocardiographic findings, in order to explore the potential role of MMPs as new biomarkers.

METHODS

Study design

This is a cross-sectional, observational study. Patients who routinely visited our adult congenital cardiology outpatient clinic between May 2011 and April 2013 were approached and prospectively enrolled. At the same day, patients underwent physical examination by a cardiologist, 12-lead electrocardiography, echocardiography and venous blood sampling. A minority of patients underwent exercise testing for routine clinical follow-up during the same week as the other study investigations. According to the study protocol, these exercise testing results were collected if performed as routine clinical care. The study protocol was approved by the institutional review board of the Erasmus MC and written informed consent was obtained from all subjects.

The following congenital cardiac diagnoses were included: repaired tetralogy of Fallot (including pulmonary atresia with ventricular septal defect), congenital aortic stenosis, aortic coarctation, transposition of the great arteries (TGA) corrected by atrial switch operation (Mustard/Senning), TGA corrected by arterial switch operation, congenitally corrected TGA and functionally univentricular hearts palliated by Fontan procedure. Exclusion criteria were: age < 18 years, severe renal impairment (creatinine > 200 µmol/L), pregnancy, and mild cardiac lesions (isolated atrial or ventricular septal defect).

Data collection

Patients were anonymized using a central online coding system. Data collection was performed using an electronic CRF-based online system (© 2004–2012 OpenClinica, LLC and collaborators, Waltham, MA; www.OpenClinica.com). This included patient demographics, medical history, medication use, symptoms and signs of heart failure (New York Heart Association (NYHA) classification), results of physical examination, electrocardiography, echocardiography, exercise capacity, and laboratory results.

Echocardiography

Patients underwent two-dimensional transthoracic echocardiography using a commercially available ultrasound system (iE33, Philips Medical Systems, Best, the Netherlands) with a 1.5-MHz transducer. Cardiac dimensions and function were measured in accordance with the most recent recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[16] Left ventricular (LV) and right ventricular (RV) dimensions were measured in end-diastolic phase from the parasternal long-axis and RV-focused apical four-chamber view, respectively. LV ejection fraction was calculated using the biplane method of disc summation (modified Simpson's rule). RV systolic function was quantified by tricuspid annular plane systolic excursion (TAPSE) and RV fractional area change. Additionally, LV and RV systolic function was qualitatively graded as normal or abnormal. LV diastolic function was measured using pulsed wave Doppler echocardiography of the mitral valve inflow (E, A and deceleration time) and tissue Doppler imaging of the medial mitral annulus velocity from the apical four-chamber view (E'). Echocardiographic measurements of patients with a systemic (sub-aortic) RV were assessed similar to patients with a sub-pulmonary RV.

Exercise capacity

Exercise testing was performed on an electrically braked cycle ergometer (Ergoselect 200 P, Ergoline GmbH, Bitz, Germany), using a gradually increasing workload of 20 Watts

per minute. It was aimed to complete the test within 8–12 minutes. Minute ventilation, oxygen uptake and carbon dioxide elimination were measured using a computerized breath-by-breath analyzer. Maximum effort was defined as a peak respiratory exchange ratio of > 1.10. Peak workload, heart rate and oxygen uptake were expressed as percentage of the predicted value, based on age, sex, body height and weight. Ventilatory efficiency was expressed as carbon dioxide equivalent (minute ventilation relative to carbon dioxide elimination).

Biomarkers

Venous blood samples were obtained after 30 minutes of rest. Plasma N-terminal-pro-B-type natriuretic peptide (NT-proBNP) was assessed by electrochemiluminescence immunoassays (Roche Diagnostics, Basel, Switzerland) in our clinical chemistry laboratory, as part of routine patient care. Other blood samples were coded, processed and stored at –80 degrees Celsius within two hours, until further analysis. Serum MMP-2, MMP-3 and MMP-9 were measured by batch analysis using the ProcartaPlex™ Multiplex Immunoassay, with sample dilution factor 50 (eBioscience, Vienna, Austria). Serum TIMP-1 was measured using two-site enzyme-linked immunosorbent assays, with sample dilution factor 100 (BMS2018CE, eBioscience, Vienna, Austria). Lower limits of detection were < 34 pg/mL, < 0.83 pg/mL and < 39 ng/mL for MMP-2, MMP-9 and TIMP-1, respectively. As all blood samples were coded, laboratory staff was blinded for patient data.

Statistical analysis

The distribution of data was checked using histograms and the Shapiro-Wilk test. Patient characteristics were presented as mean ± standard deviation or median [interquartile 1-3 (IQ_1–IQ_3)], depending on the distribution of data. Skewed biomarker distributions were log-transformed.

Univariable associations were evaluated using Pearson's correlation. MMP values below the limit of detection were analyzed as the lowest detectable value. We performed a stratified analysis in the three main diagnostic groups (left sided heart disease, right sided heart disease and systemic RV).

Multivariable linear (for continuous outcomes) and logistic (for dichotomous outcomes) regression analyses were only performed for MMP-2, as all other biomarker values yielded non-significant results in the univariable analysis. Unstandardized β-coefficients were adjusted for the potential confounders age, sex and body surface area (BSA). Based on the results of the univariable analysis, β-coefficients were additionally adjusted for other covariates. Data analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). Two-sided *p*-values < 0.05 were considered statistically significant.

RESULTS

We prospectively included 425 patients (median age 33.3 years [IQ_1-IQ_3 25.9–42.1 years], 244 males, 57%). Echocardiography and measurement of serum MMPs was performed in all patients. Bicycle ergometry was performed in 126 patients, of whom 40 patients underwent measurement of oxygen uptake en carbon dioxide elimination. Clinical characteristics and medication use are detailed in Table 1. MMP and TIMP profiles were not different when comparing all patient subgroups based on the congenital cardiac diagnosis (Figure 1).

TABLE 1 - Characteristics of the study population (n = 425).

Variable	Mean ± SD, median [IQ_1–IQ_3] or n (%)	Surgical repair / intervention, n (%)
Age, years	33.3 [25.9–42.1]	
Sex, male n (%)	244 (57)	
Diagnosis, n (%)		
Tetralogy of Fallot	167 (39)	167 (100)
Congenital aortic stenosis	112 (26)	80 (71)
Aortic coarctation	40 (10)	39 (98)
TGA corrected by atrial switch	60 (14)	60 (100)
TGA corrected by arterial switch	18 (4)	18 (100)
Congenitally corrected TGA	18 (4)	9 (50)
Univentricular heart (Fontan)	10 (3)	10 (100)
Age at surgical repair / intervention, years	3.9 [0.8–11.9]	
Systolic blood pressure, mmHg	125 ± 16	
Diastolic blood pressure, mmHg	79 ± 11	
Heart rate, beats/minute	74 ± 14	
Saturation < 90%, n (%)	9 (2)	
Cardiac medication, n (%)		
ACE-inhibitor	61 (14)	
Angiotensin receptor blocker	21 (5)	
Betablocker	58 (14)	
Diuretics	44 (10)	
NT-proBNP, pmol/L	16.6 [7.9–34.3]	
NYHA functional class, II–III n (%)	40 (9)	

Abbreviations: ACE, angiotensin converting enzyme; IQ, interquartile; NT-proBNP, N-terminal brain natriuretic peptide; NYHA, New York Heart Association; SD, standard deviation; TGA, transposition of the great arteries.

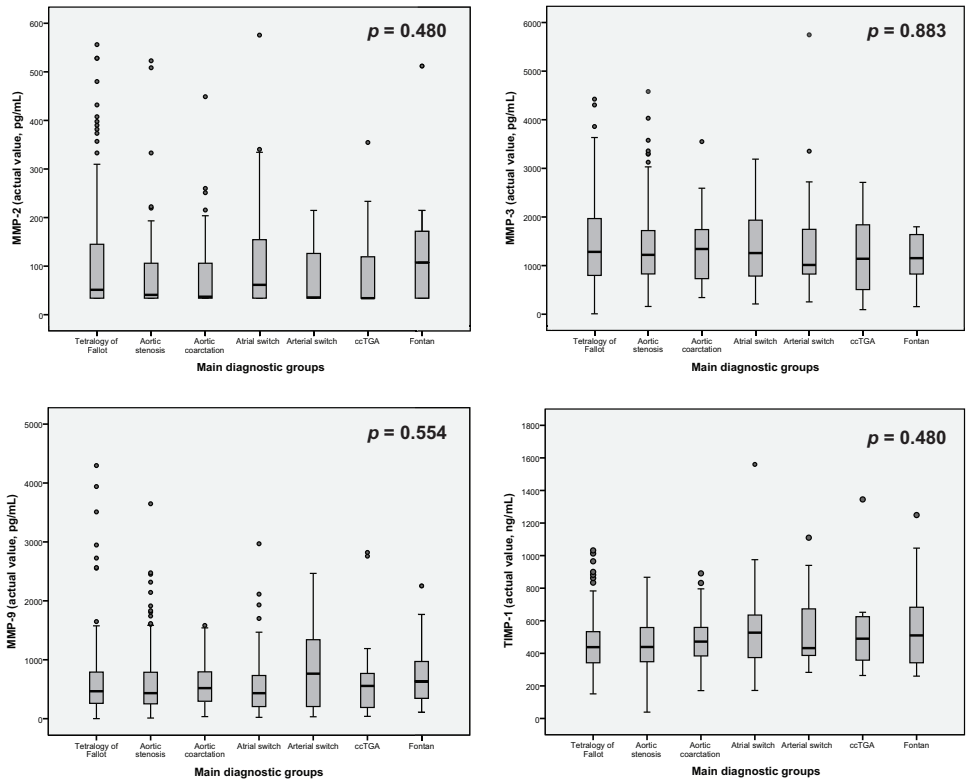

FIGURE 1 - MMP-2, MMP-3, MMP-9 and TIMP-1 values were comparable among the diagnostic groups.

P-values are obtained by comparing the actual MMP-2, MMP-3, MMP-9 and TIMP-1 values across the diagnostic groups using the Kruskal-Wallis test. Comparison of $\log_{10}$ values across diagnostic groups using one-way ANOVA did not yield different conclusions.

Univariable analysis

In Table 2 the correlations of $\log_{10}$ MMP-2, MMP-3, MMP-9 and TIMP-1 with patient characteristics, exercise testing results and echocardiographic data are presented. MMP-2 and MMP-9 were significantly related to BSA, diastolic blood pressure and decreased saturation. MMP-2 significantly correlated with peak workload % ($r = -0.233$, $p = 0.009$), peak oxygen uptake ($r = -0.411$, $p = 0.008$) and carbon dioxide equivalent ($r = 0.396$, $p = 0.034$), of which scatterplots are additionally shown in Figure 2. Furthermore, MMP-2 significantly correlated to NT-proBNP, NYHA functional class, aortic valve peak velocity, LV and RV systolic dysfunction, E' wave velocity < 8 cm/s, E/E' ratio ≥ 13 and LV deceleration time. MMP-9 was only related to peak oxygen uptake ($r = -0.350$, $p = 0.027$). MMP-3, MMP-9, TIMP-1 and MMP-9/TIMP-1 ratios (ratios are not presented in Table 2) were not significantly associated with any other parameters of functional outcome.

TABLE 2 - Univariable associations (Pearson's correlation, n = 425).

Variable	log_{10} MMP-2	log_{10} MMP-3	log_{10} MMP-9	log_{10} TIMP-1
Patient characteristics				
Age, years	0.027	0.093	−0.049	−0.063
Sex, male	0.066	**−0.151****	0.086	−0.011
Body surface area, m^2	**−0.173****	0.073	**−0.137****	0.048
Diagnosis: systemic right ventricle	0.009	−0.018	−0.043	**0.097***
Surgical repair	0.080	−0.039	−0.069	0.005
Systolic blood pressure, mmHg	−0.083	0.067	−0.022	0.077
Diastolic blood pressure, mmHg	**−0.100***	0.017	**−0.111***	0.051
Heart rate, beats/minute	0.042	−0.018	−0.012	0.003
Saturation <90%	**0.136****	0.073	**0.104***	0.074
Use of angiotensin receptor blocker	−0.006	0.054	**0.110***	0.038
log_{10} NT-proBNP	**0.147****	0.035	0.081	−0.004
NYHA functional class, II–III	**0.102***	**0.064**	**−0.018**	**−0.034**
Exercise testing				
Peak heart rate, % of target (n = 126)	**−0.205***	−0.024	0.001	−0.088
Peak workload, % of target (n = 126)	**−0.233****	0.032	−0.080	−0.047
Peak oxygen uptake, mL/min (n = 40)	**−0.411****	0.004	**−0.350***	−0.038
Peak oxygen uptake, % of target (n = 40)	−0.230	−0.141	−0.056	−0.119
Carbon dioxide equivalent (n = 40)	**0.396***	0.214	0.270	0.148
Echocardiography				
LV end-diastolic dimension, mm	−0.100	0.005	−0.072	0.022
Aortic STJ end-diastolic diameter, mm	0.056	0.070	−0.051	−0.085
Aortic valve peak velocity, m/s	**−0.110***	−0.001	0.045	−0.040
Interventricular septum, mm (PLAX)	−0.028	0.092	−0.010	−0.020
RV end-diastolic annulus, mm	0.069	0.071	0.003	0.022
RV end-diastolic area, cm^2	0.061	0.065	−0.009	0.087
RV end-systolic area, cm^2	0.096	0.061	0.002	0.078
Systolic function				
LV ejection fraction, %	−0.065	−0.027	0.050	−0.057
LV systolic dysfunction, 0–1	**0.102***	0.093	−0.073	0.011
RV fractional area change, %	−0.112	−0.017	−0.019	−0.022
RV systolic dysfunction, 0–1	**0.115***	0.047	−0.039	0.018
TAPSE, mm	0.017	0.079	0.081	−0.018

TABLE 2 - Continued.

Variable	log_{10} MMP-2	log_{10} MMP-3	log_{10} MMP-9	log_{10} TIMP-1
LV diastolic function				
E/A ratio	0.012	–0.044	0.049	0.019
E' wave < 8 cm/s	**0.132***	0.050	–0.014	0.002
E/E' ratio ≥ 13	**0.128***	0.103	0.051	–0.033
Deceleration time, ms	**–0.137***	–0.085	0.017	–0.061

*p < 0.05, **p < 0.01. **Abbreviations**: LV, left ventricular; MMP, matrix metalloproteinase; NT-proBNP, N-terminal brain natriuretic peptide; NYHA, New York Heart Association; PLAX, parasternal long-axis view; RV, right ventricular; STJ, sinotubular junction; TAPSE, tricuspid annular plane systolic excursion; TIMP, tissue inhibitor of matrix metalloproteinase.

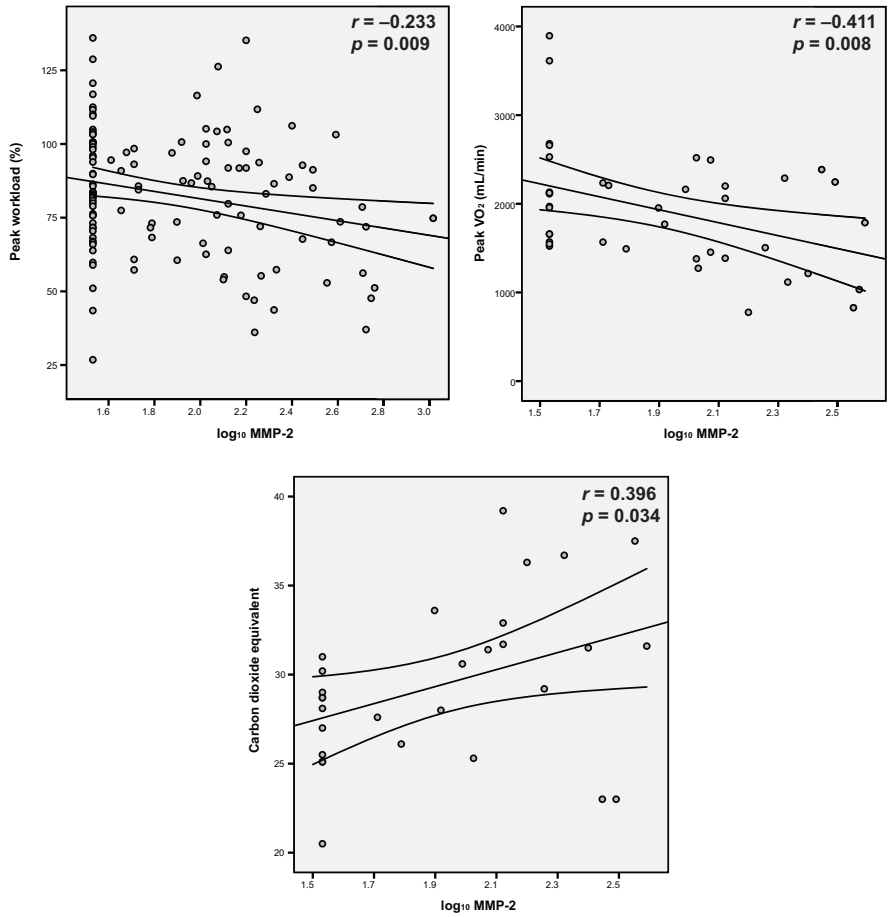

FIGURE 2 - Scatterplots demonstrating the correlation of MMP-2 with peak workload %, peak oxygen uptake and carbon dioxide equivalent.

As 46% of MMP-2 values were < 34 pg/mL (lower limit of detection), all analyses were repeated with exclusion of these values. Also non-parametric tests were performed (Spearman's correlation). These did not yield different conclusions.

TABLE 3 - Unstandardized ß-coefficients of $\log_{10}$ MMP-2 values (n = 425).

	Adjusted for age, sex, BSA	
	β	***P*-value**
NYHA functional class, II–III	0.57	0.152
Exercise testing		
Peak heart rate, % of target (n = 126)	**–5.08**	**0.046**
Peak workload, % of target (n = 126)	**–11.86**	**0.007**
Peak oxygen uptake, mL/min (n = 40)	–238.7	0.175
Peak oxygen uptake, % of target (n = 40)	**–12.79**	**0.046**
Carbon dioxide equivalent (n = 40)	3.69	0.117
Echocardiography		
LV end-diastolic dimension, mm	–0.78	0.362
Aortic STJ end-diastolic diameter, mm	1.80	0.098
Aortic valve peak velocity, m/s	–0.26	0.074
Interventricular septum, mm (PLAX)	–0.00	0.997
RV end-diastolic annulus, mm	2.24	0.051
RV end-diastolic area, cm^2	**3.40**	**0.025**
RV end-systolic area, cm^2	**3.49**	**0.010**
Systolic function		
LV ejection fraction, %	–1.64	0.410
LV systolic dysfunction, 0–1	**1.00**	**0.040**
RV fractional area change, %	**–3.99**	**0.036**
RV systolic dysfunction, 0–1	**0.67**	**0.027**
TAPSE, mm	0.39	0.627
LV diastolic function		
E/A ratio	0.03	0.735
E' wave < 8 cm/s	0.56	0.107
E/E' ratio ≥ 13	0.51	0.129
Deceleration time, ms	**–22.33**	**0.009**

Analysed using linear (for continuous outcome) or logistic regression (for categorical outcome). Significant *p*-values are printed in bold. **Abbreviations:** as defined in Table 2.

Stratified analysis

The associations of echocardiographic measurements with MMP-2 as presented in Table 2 were additionally analyzed in three main diagnostic subgroups: left sided heart disease (congenital aortic stenosis, aortic coarctation and TGA corrected by arterial switch operation), right sided heart disease (repaired tetralogy of Fallot), and systemic RV (TGA corrected by atrial switch operation and congenitally corrected TGA). In patients with repaired tetralogy of Fallot, LV dysfunction (r = 0.184, p = 0.018), RV dysfunction (r = 0.190, p = 0.014), E/E' ratio ≥ 13 (r = 0.165, p = 0.042) and LV deceleration time (r = –0.178, p = 0.028) were significantly associated with MMP-2. These associations were not present in the two other diagnostic groups, and LV diastolic function was not measured in patients with a systemic RV.

Multivariable analysis

In Table 3, the results of the multivariable analysis are presented. ß-coefficients represent the increase in patient characteristic per 10-fold increase in MMP-2, adjusted for age, sex and BSA. Based on the results of the univariable analysis, we additionally adjusted for diastolic blood pressure, decreased saturation and NT-proBNP (not shown in the table). After adjustment for all variables, MMP-2 was significantly associated with peak workload (β = –10.96, p = 0.022), carbon dioxide equivalent (β = –6.86, p = 0.016) and LV deceleration time (β = –22.4, p = 0.012); however, not with RV dilatation and RV or LV systolic function. MMP-2 was not significantly associated with aortic sinotubular junction end-diastolic diameter, although a trend was observed (β = 1.89, p = 0.087).

DISCUSSION

In our cohort of clinically stable adults with congenital heart disease, MMP-2 was significantly associated with decreased exercise capacity (n = 126, peak workload %), decreased ventilatory efficiency (n = 40, carbon dioxide equivalent) and decreased LV deceleration time (n = 425), independently of NT-proBNP and other clinical variables. In addition, MMP-2 was related to LV and RV systolic dysfunction (adjusted for age, sex and BSA) and several echocardiographic markers of diastolic dysfunction in the univariable analysis. MMP-3, MMP-9 and TIMP-1 were not consistently related with exercise capacity or cardiac function. Nor did we observe specific MMP profiles among the varying underlying congenital diagnoses.

Cardiac function

Although this is the first study that reports a modest association of MMP-2 with systolic ventricular function, independently of age, sex and BSA, extracellular matrix remodeling processes have been previously related to systolic ventricular function in other patient groups. In patients with hypertension, serum TIMP-1 levels were independently related to LV longitudinal strain and torsion.[10] Moreover, TIMP-1 was elevated in patients with congestive heart failure.[8]

Increased extracellular matrix remodeling within the myocardium has been more frequently associated with diastolic ventricular dysfunction. In our cohort, diastolic dysfunction (as expressed by E'wave velocity < 8 cm/s, E/E' ratio ≥ 13 and LV deceleration time) was related to MMP-2 in the univariable analysis. Martos et al. showed that in hypertensive heart disease, serum levels of MMP-2 were elevated in those patients with diastolic heart failure, suggesting increased degradation of myocardial collagen and other components of the extracellular matrix.[11] Subsequently, Gardin et al. showed that echocardiographic markers of diastolic dysfunction were independently related to peak oxygen uptake and ventilatory efficiency.[17] In line with these results, Chen et al. recently reported that a serum biomarker of collagen type I synthesis (carboxy-terminal propeptide of procollagen type I, PICP) was related to higher RV late gadolinium enhancement scores (indicating myocardial fibrosis) and lower peak oxygen uptake in patients with repaired tetralogy of Fallot.[18] Diastolic function is often impaired in patients with ACHD.[19] Diastolic dysfunction is especially common in patients with congenital aortic stenosis.[20, 21] In addition, abnormal LV diastolic indices are known to be prevalent in a substantial proportion of patients with tetralogy of Fallot.[22] The most important association found in our study, directly linking increased serum MMP-2 values with decreased exercise capacity and ventilatory efficiency, may therefore be partially explained by increased diastolic dysfunction.

Importantly, the patients in our study were all clinically stable, with 91% of patients in NYHA class I. This probably explains some of the only modest or absent correlations. The stratified analysis in three main diagnostic groups showed that the associations of echocardiographic measurements with MMP-2 as found in the entire study cohort probably largely originate from the group of patients with repaired tetralogy of Fallot. This is possibly explained by a wider variance in ventricular systolic and diastolic function in this patient group.

Aortic stenosis and dilatation

In our study, serum MMP-2, -3, -9 and TIMP-1 levels were similar in patients with aortic stenosis or coarctation compared with all other congenital cardiac diagnoses. As we know patients with aortic stenosis or coarctation often have diastolic dysfunction, we had expected higher MMP-2 levels in these patients.[19] Neither did we find significant relations with aortic dimensions as measured by echocardiography, although a trend was observed for MMP-2 (adjusted *p*-value 0.087). Previous studies have reported altered plasma MMP-2, -9 and TIMP-1 levels in patients with aortic valve stenosis and ascending aorta dilatation, both in bicuspid valves and in chronic thoracic aortic dissection.[13-15] It may therefore be worthwhile to evaluate MMPs in specific patient subgroups with a high risk of aortic dilatation, in combination with more detailed imaging modalities of the aorta, such as CT or MRI.

Matrix metalloproteinases as peripheral markers

As circulating blood biomarkers profiles can be affected by processes throughout the entire body, altered MMP profiles may not entirely reflect the actual histological changes in the myocardium or aortic wall. The interaction between MMPs and other biologically active substrates is highly complex. We constructed a conceptual model of the MMP-TIMP interactions with TGF-β, IL-1β, TNF-α and other substrates as described by the available literature on this topic (Figure 3).[3, 7, 23-25] Due to these complex interactions, it is likely that the underlying disease and patient comorbidities influence MMP levels. In this study, MMP-9 and TIMP-1 were not found to be associated with echocardiographic data, in contrast to previous reports.[8, 11, 12] This might be explained by the difference in patient cohorts, the selection of stable patients visiting the outpatient clinic and the type of MMP analysis performed.

Clinical implications

Previous studies have shown that exercise capacity and ventilatory response to exercise are important prognostic markers in ACHD.[26, 27] Our data provide evidence for a modest association of MMP-2 with several measures of functional outcome, independently of NT-proBNP. Correlations were not strong, indicating that MMP-2 only partially explains the variance in echocardiographic findings and exercise capacity. Nevertheless, these findings indicate that MMP-2 provides new information, incremental to clinical variables and NT-proBNP, even in a clinically stable patient group. MMP-2 may therefore be useful as novel biomarker in patients with ACHD. It would be worthwhile to further evaluate the prognostic value of MMP-2 in longitudinal studies.

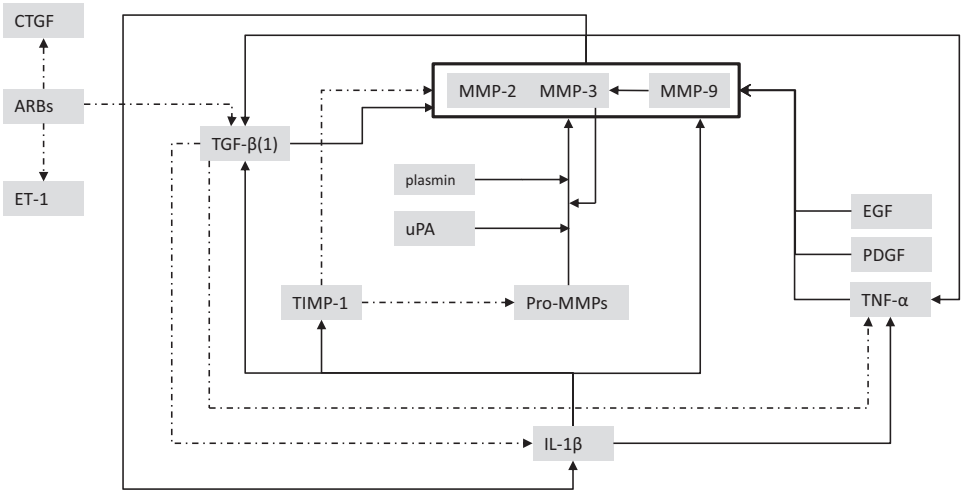

FIGURE 3 - Conceptual model illustrating the highly complex interactions between MMPs, TIMP-1 and other biologically active substrates.

Dashed lines indicate enzyme inhibition. **Abbreviations:** ARBs, angiotensin receptor blockers; CTGF, connective tissue growth factor; EGF, epidermal growth factor; ET-1, endothelin-1; IL-1β, interleukin-1beta; PDGF, platelet derived growth factor; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; uPA, urokinase-type plasminogen activator.

Study limitations

Bicycle ergometry was performed in a subset of 126 patients. Of these, in 40 patients oxygen uptake and carbon dioxide elimination were recorded. The consistency and strength of these data should therefore be further evaluated in multiple studies from different centers and laboratories, in accordance with previously formulated benchmarks for the assessment of novel cardiovascular biomarkers by Morrow and de Lemos.[28] To be clinically useful, the pre-analytical and analytical performance of the MMP assays need to be thoroughly investigated and robust reference values have to be assessed in healthy controls.

CONCLUSIONS

Serum MMP-2 was significantly related to exercise capacity, ventilatory efficiency and LV deceleration time in a diverse cohort of stable adults with congenital heart disease, also after adjustment for NT-proBNP and other clinical variables. In contrast, MMP-3, MMP-9 and TIMP-1 were not consistently related to exercise testing results or cardiac function. Prospective longitudinal studies are warranted to determine the clinical usefulness and prognostic value of MMP-2 as candidate biomarker.

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Chapter 10

Prognostic value of N-terminal pro-B-type natriuretic peptide, troponin-T, and growth-differentiation factor 15 in adult congenital heart disease

Vivan J.M. Baggen, Annemien E. van den Bosch, Jannet A. Eindhoven, Anne-Rose W. Schut, Judith A.A.E. Cuypers, Maarten Witsenburg, Monique de Waart, Ron H.N. van Schaik, Felix Zijlstra, Eric Boersma, Jolien W. Roos-Hesselink

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ABSTRACT

Background The number of patients with adult congenital heart disease (ACHD) is rapidly increasing. In order to optimize patient management, there is a great need to accurately identify high-risk patients. Still, no biomarker has been firmly established as a clinically useful prognostic tool in this group. We studied the association of N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitive troponin-T, and growth-differentiation factor 15 with cardiovascular events in ACHD.

Methods Clinically stable patients with ACHD who routinely visited the outpatient clinic between April 2011 and April 2013 underwent clinical assessment, electrocardiography, echocardiography and biomarker measurement (NT-proBNP, high-sensitive troponin-T, and growth-differentiation factor 15) at the time of study inclusion. Patients were prospectively followed for the occurrence of cardiovascular events (death, heart failure, hospitalization, arrhythmia, thromboembolic events, and re-intervention). Survival curves were derived by the Kaplan-Meier method, and Cox regression was performed to investigate the relation between biomarkers and events with adjustment for multiple clinical and echocardiographic variables.

Results In total, 595 patients were included (median age 33 [IQR 25–41] years; 58% male; 90% New York Heart Association class I). Patients were followed during a median of 42 [IQR 37–46] months. Of the three evaluated biomarkers, NT-proBNP in the upper quartile (> 33.3 pmol/L) was most strongly associated with cardiovascular events (n = 165, adjusted HR 9.05; 95% CI 3.24–25.3, $p < 0.001$) and with death or heart failure (n = 50, adjusted HR 16.0; 95% CI 2.04–126, $p < 0.001$). When NT-proBNP was analyzed as a continuous variable, similar findings were retrieved. The cumulative proportion of patients with death and heart failure was only 1% in the lowest two NT-proBNP quartiles. Elevated NT-proBNP (> 14 pmol/L), elevated high-sensitive troponin-T (> 14 ng/L), and elevated growth-differentiation factor 15 (> 1109 ng/L) identified those patients at highest risk of cardiovascular events (log-rank $p < 0.0001$).

Conclusions NT-proBNP provides prognostic information beyond a conventional risk marker model in patients with ACHD and can reliably exclude the risk of death and heart failure. Elevated levels of NT-proBNP, high-sensitive troponin-T, and growth-differentiation factor 15 identify patients at highest risk of cardiovascular events. These biomarkers therefore may play an important role in the monitoring and management of patients with ACHD.

INTRODUCTION

The surgical treatment of children with moderate and complex types of congenital heart disease has tremendously improved during the past five decades. Today, over 90% of these children survive into adulthood.[1-3] Subsequently, the number of patients with adult congenital heart disease (ACHD) is rapidly increasing, which is estimated to only begin to plateau around the year 2050.[4] The majority of these patients carry residual cardiac abnormalities, which inevitably predispose to complications such as heart failure, arrhythmia, and early demise.[5]

To adequately manage the growing number of patients with ACHD, accurate prognostication is of paramount importance. It enables optimization of patient information, follow-up and diagnostic testing strategies, pharmacological treatment, and timing of re-interventions.[6] Current clinical tools such as echocardiography fail to identify early changes that precede adverse cardiac remodeling and heart failure. Therefore, there is a great need to identify additional measures that can be combined with existing tools to monitor subtle changes in the heart that reflect and possibly predict disease progression before it becomes clinically apparent.[7, 8]

Natriuretic peptides are firmly established diagnostic and prognostic tools in a variety of chronic cardiovascular conditions, such as heart failure and coronary heart disease.[9-11] Cardiac troponins and growth-differentiation factor 15 (GDF-15) also offer prognostic information in these groups.[12-18] Cross-sectional studies in patients with various types of ACHD have shown that natriuretic peptides, high-sensitive troponin-T (hs-TnT), and GDF-15 are related to intermediate prognostic markers, such as ventricular function or exercise capacity.[19-22] Prospective follow-up data are clearly needed in this patient group. The aim of this study was to investigate the association between circulating concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP), hs-TnT, and GDF-15 and a composite endpoint of cardiovascular events in a large cohort of stable patients with ACHD.

METHODS

Study design and population

In this prospective cohort study, we aimed to include all consecutive patients who routinely visited our adult congenital cardiology outpatient clinic with an echocardiogram between April 2011 and April 2013. Moderate and complex types of congenital heart defects[23] were considered eligible for inclusion: congenital aortic stenosis, aortic coarctation, repaired tetralogy of Fallot (including pulmonary atresia with ventricular septal defect), transposition of the great arteries corrected by atrial switch operation (Mustard), transposition of the great arteries corrected by arterial

switch operation, congenitally corrected transposition of the great arteries, complex transposition of the great arteries with ventricular septal defect or double-outlet right ventricle corrected with a Rastelli type repair, functionally univentricular hearts, or pulmonary arterial hypertension after a (corrected) atrial or ventricular septal defect. Exclusion criteria were: age < 18 years, pregnancy, mild cardiac lesion (isolated atrial or ventricular septal defect), not capable of understanding and signing informed consent, or kidney failure (creatinine > 200µmol/L, corresponding to 2.26 mg/dL). The study protocol was approved by the Erasmus MC medical ethics committee and all patients provided written informed consent.

Data collection

At the same day, patients underwent physical examination by a cardiologist, 12-lead electrocardiography, echocardiography, and venous blood sampling. Data collection and storage was performed using an electronic case report form-based online system (© 2004-2012 OpenClinica, LLC and collaborators).[24] This included patient demographics, medical history, medication use, results of physical examination, electrocardiography, echocardiography, laboratory results, and events. New York Heart Association (NYHA) functional class was used to classify symptoms and signs of heart failure. Patients who reported no limitation in ordinary physical activity were considered to be in NYHA functional class I. Other details have been described previously.[19]

Biomarkers

Venous blood samples were obtained after 30 minutes of rest. All biomarker values were measured for research purposes only, and all decisions regarding patient management (including re-interventions) were made independently of any biomarker level. Serum NT-proBNP was directly measured in our clinical chemistry laboratory using a commercial electrochemiluminescence immunoassay (Roche Diagnostics). Other blood samples were left in room temperature during a maximum of two hours. Samples were centrifuged at 1700*g* for 10 minutes at 4°C. Serum and EDTA plasma samples were stored at –80°C until batch analysis. The median storage period at –80°C was 22.0 [IQR 16.4–26.2] months for hs-TnT and 28.7 [IQR 23.3–32.7] months for GDF-15. All samples were subjected to one freeze-thaw cycle. Serum hs-TnT was measured using a commercial electrochemiluminescence immunoassay (Roche Diagnostics). Plasma GDF-15 was measured using a precommercial electrochemiluminescence immunoassay (Roche Diagnostics). Reproducibility of the GDF-15 assay has been described previously.[22] For NT-proBNP, hs-TnT, and GDF-15, the limits of detection were 0.6 pmol/L, 5 ng/L, and 400 ng/L, respectively. For NT-proBNP and GDF-15, all values below the limit of detection

were reported as < 0.6 pmol/L and < 400 ng/L, and for hs-TnT the limit of blank (the highest measured test result likely to be observed for a sample containing no analyte) was 3 ng/L. The upper limit of normal for NT-proBNP was 14 pmol/L (≈ 125 pg/mL), on the basis of the recommended low cutoff for the diagnosis of heart failure in patients presenting with non-acute symptoms.[25] The upper limit of normal for hs-TnT and GDF-15 level were defined as 14 ng/L and 1109 ng/L, on the basis of the 99th and 97.5th percentile value in the normal population, respectively.[18, 22, 26]

Echocardiography

Patients underwent extensive two-dimensional transthoracic echocardiography, using a commercially available ultrasound system iE33 (Philips Medical Systems) with a 1.5-MHz transducer. Images were acquired according to a standardized sequential segmental analysis protocol. Cardiac dimensions were measured in agreement with the current recommendations of the American Society of Echocardiography and European Association of Cardiovascular Imaging and were indexed for body surface area.[27] Left ventricular ejection fraction was measured using the modified Simpson rule. Right ventricular systolic function was quantified with fractional area change. Systemic ventricular function was also visually graded as normal, mildly, moderately or severely impaired. Measurements were performed without knowledge of biomarker levels and before the assessment of events.

Definition and assessment of events

The primary endpoint was defined before the collection of data as a composite of all adverse cardiovascular events: all-cause mortality, heart failure (requiring initiation or change in heart failure medication, or requiring hospital admission), hospitalization for cardiac reasons, arrhythmia (symptomatic and recorded, or requiring treatment), thromboembolic events (ischemic cerebrovascular accident, pulmonary embolism, or myocardial infarction), or cardiac re-interventions (surgical or percutaneous). A secondary composite endpoint of all-cause mortality or heart failure was defined. All patients were prospectively and systematically followed for fatal and nonfatal events by a yearly clinical evaluation at our institution according to a standard protocol. Where necessary, we retrieved information from electronic patient charts and correspondence with referring hospitals. Survival status of all patients was also checked in the Municipal Population Register. Events were adjudicated by two experienced investigators (V.B. and J.R.) without knowledge of biomarker levels. The investigators had direct access to the patient clinical records and the events were independently reviewed by each investigator. For patients with multiple events, event-free survival was defined as the

time from enrollment to the occurrence of the first event. All components of the primary endpoint were also separately assessed as tertiary endpoints for exploratory purposes (in which patients were not censored at the time of another endpoint than the endpoint of interest). Patients without any cardiovascular event were censored at the end of the follow-up duration (August 1, 2015).

Statistical analysis

At the end of the study duration, 25% of the cohort was expected to reach the primary composite endpoint, based on 2.4% cardiovascular deaths and 22.8% major adverse cardiac events such as heart failure, re-intervention, arrhythmia and hospital admission in the Euro Heart Survey.[28] To collect at least 150 events, we therefore estimated that inclusion of ≈ 600 patients would be sufficient to permit appropriate adjustment for potential confounders.

Patient characteristics are presented as mean ± standard deviation or median [interquartile range, IQR], depending on the distribution of data. Comparisons across quartiles of the biomarker distribution were performed using the chi-square Mantel-Haenszel test for trend (for categorical variables) or linear regression (for continuous variables). Associations of baseline characteristics with biomarker levels were also expressed using Pearson or Spearman correlation, depending on the distribution of data. Cumulative end-point free survival estimates and survival curves were derived by the Kaplan-Meier method; groups were compared using the log-rank test. Cox proportional hazards regression was performed to identify associations with the primary and secondary composite endpoint. We analyzed the biomarkers as both categorical and continuous variables (in quartiles, and using a $\log_2$-transformation and expressed per one standard deviation increase in biomarker level, respectively). In the multivariable analysis, we adjusted for potential confounders by selecting age, sex, and other variables that were associated with the primary endpoint in the univariable analysis. The multivariable models were used with a minimum of ten endpoints per degree of freedom.[29] Combinations of biomarkers were investigated by evaluating the number of elevated biomarkers, using the cutoffs as described previously, and as continuous variables in multivariable models. A sensitivity analysis was performed by excluding congenital diagnostic subgroups with the highest NT-proBNP levels. Data analysis was performed by two authors (V.B. and E.B.) using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). Discrimination was described with the concordance (C)-index and the integrated discrimination improvement, calculated using R, Version 3.3.0, packages survC1 and survIDINRI.[30] Two-sided *p*-values of < 0.05 were considered statistically significant. No corrections were made for multiplicity of comparisons.

RESULTS

Baseline measurements

NT-proBNP, hs-TnT, and GDF-15 measurements were performed in 595, 589, and 589 patients, respectively. A flowchart of the patient selection is shown in Supplemental Figure 1. Median age was 33 [IQR 25–41] years and 346 (58%) were men. Baseline characteristics are detailed in Table 1. The vast majority of patients (n = 540, 91%) underwent surgical repair at young age (median 3.7 [IQR 0.8–11.9] years) and was in NYHA functional class I (n = 534, 90%). NT-proBNP was significantly higher in older patients, women, patients with initial repair at older age, patients with a complex type of congenital heart defect (tetralogy of Fallot, Rastelli, systemic right ventricle, univentricular heart or pulmonary arterial hypertension), patients who used cardiac medication, patients with low arterial oxygen saturation, and patients in NYHA class II–III. In addition, NT-proBNP was higher in patients with loss of sinus rhythm, longer QRS duration, higher left atrial volume, higher left ventricular volumes, and impaired ventricular systolic and diastolic function (Table 1). Baseline characteristics stratified according to quartiles of hs-TnT and GDF-15 are further detailed in Supplemental Tables 1 and 2, and the correlation of NT-proBNP, hs-TnT and GDF-15 with baseline characteristics is presented in Table 2. Biomarker values in the diagnostic subgroups are shown in Supplemental Figure 2.

Follow-up

At the end of the follow-up (August 1, 2015), survival status was known in 593 patients (99.7%) and unknown in two patients (0.3%) who emigrated. Detailed follow-up data were available in 587 patients (98.7%). During a median follow-up period of 41.8 [IQR 36.6–46.1] months, the primary composite endpoint of a cardiovascular event occurred in 165 patients (28%) and the secondary composite endpoint of death or heart failure in 50 patients (8%). In total, 12 patients died. Causes of death were end-stage heart failure (n = 6), cardiac arrest (n = 3), sudden death, presumed cardiac (n = 2), and other (n = 1). Forty-six patients developed heart failure requiring hospitalization (n = 23) or medication change or initiation (n = 23). A total of 125 patients were hospitalized 157 times for cardiac reasons: percutaneous (n = 55) or surgical (n = 38) re-intervention, heart failure (n = 23), arrhythmia (n = 11), thromboembolic event (n = 9), endocarditis (n = 8), and other (n = 13). A total of 90 patients had an arrhythmia: supraventricular tachycardia (n = 64), ventricular tachycardia (n = 14), ventricular fibrillation (n = 6), and other (n = 6). Twenty patients had a thromboembolic event: ischemic cerebrovascular accident (n = 11), pulmonary embolism (n = 3), myocardial infarction (n = 3), and other (n = 3). Kaplan-Meier estimates of event-free survival were 87% at year one, 79% at year two and 74% at year three. Heart-failure-free survival was 96% at year one, 94% at year two and 92% at year three.

TABLE 1 - Baseline characteristics of the study population.

			NT-proBNP quartiles (n = 595)				
	Valid cases, n (%)	**All, n = 595**	**Q1 (< 6.8 pmol/ L, n = 150)**	**Q2 (6.8–15.2 pmol/L, n = 148)**	**Q3 (15.2–33.3 pmol/L, n = 148)**	**Q4 (> 33.3 pmol/ L, n = 149)**	***P* for trend**
Clinical characteristics							
Age, years	595 (100)	33 [25–41]	27 [21–34]	30 [23–39]	33 [26–41]	40 [33–49]	< 0.001
Sex, male n (%)	595 (100)	346 (58)	121 (81)	87 (59)	82 (55)	56 (38)	< 0.001
Surgical repair, n (%)	595 (100)	540 (91)	129 (86)	131 (89)	140 (95)	140 (94)	0.005
Age at surgical repair, years	594 (100)	3.7 [0.8–11.9]	2.0 [0.5–8.2]	4.0 [0.8–15.6]	2.4 [0.6–10.3]	6.3 [2.3–16.2]	0.001
Congenital diagnosis, n (%)*	595 (100)	325 (55)	54 (36)	61 (41)	96 (65)	114 (77)	< 0.001
Cardiac medication, n (%)							
ACE-inhibitor	595 (100)	89 (15)	13 (9)	10 (7)	19 (13)	47 (32)	< 0.001
ARBs	595 (100)	36 (6)	13 (9)	7 (5)	2 (1)	14 (9)	0.889
ß-blocker	595 (100)	90 (15)	9 (6)	15 (10)	25 (17)	41 (28)	< 0.001
Diuretics	595 (100)	71 (12)	7 (5)	7 (5)	10 (7)	47 (32)	< 0.001
Body mass index, kg/m^2	592 (99)	24.7 ± 4.4	24.1 ± 3.8	25.0 ± 4.5	25.0 ± 4.5	24.8 ± 4.7	0.222
Heart rate, beats/minute	587 (99)	73 ± 13	75 ± 13	73 ± 14	72 ± 14	74 ± 13	0.478
Systolic blood pressure, mmHg	584 (98)	126 ± 16	128 ± 15	126 ± 15	125 ± 16	126 ± 19	0.308
O_2 saturation < 90%, n (%)	551 (93)	17 (3)	1 (1)	0 (0)	1 (1)	15 (10)	< 0.001
NYHA class, II–III n (%)	595 (100)	61 (10)	2 (1)	8 (5)	11 (7)	40 (27)	< 0.001
Electrocardiogram							
Rhythm, n (%)	595 (100)						< 0.001
Sinus rhythm		514 (87)	142 (94)	142 (96)	131 (89)	99 (66)	< 0.001
Paced rhythm		44 (7)	4 (3)	5 (3)	11 (8)	24 (16)	< 0.001
Other		37 (6)	4 (3)	1 (1)	6 (3)	26 (18)	< 0.001
QRS duration, ms	595 (100)	113 [100–138]	110 [98–126]	108 [97–129]	114 [101–146]	123 [105–156]	< 0.001

TABLE 1 - Continued.

		NT-proBNP quartiles (n = 595)					
	Valid cases, n (%)	**All, n = 595**	**Q1 (< 6.8 pmol/L, n = 150)**	**Q2 (6.8–15.2 pmol/L, n = 148)**	**Q3 (15.2–33.3 pmol/L, n = 148)**	**Q4 (> 33.3 pmol/L, n = 149)**	***P* for trend**
Echocardiogram							
LA volume, mL/m²	427 (72)†	21 [15–29]	18 [14–24]	21 [16–27]	21 [17–30]	30 [19–44]	< 0.001
LV end-diastolic volume, mL/m²	404 (68)†	64 ± 19	62 ± 15	59 ± 16	66 ± 19	70 ± 26	0.002
LV end-systolic volume, mL/m²	403 (68)†	29 ± 13	27 ± 8	26 ± 8	30 ± 13	35 ± 21	< 0.001
LV ejection fraction, %	403 (68)†	56 ± 8	57 ± 6	57 ± 6	55 ± 8	52 ± 12	< 0.001
Interventricular septum, mm	447 (75)	9.2 ± 2.1	8.9 ± 1.7	9.4 ± 2.1	9.2 ± 2.1	9.6 ± 2.5	0.042
Posterior wall, mm	450 (76)	8.8 ± 1.6	8.6 ± 1.4	9.1 ± 1.7	8.8 ± 1.6	8.9 ± 1.7	0.391
RV fractional area change, %	385 (65)	38 ± 11	41 ± 10	40 ± 10	38 ± 12	34 ± 12	< 0.001
Systemic ventricular function, n (%)	595 (100)						< 0.001
Normal		298 (50)	105 (70)	89 (60)	64 (43)	40 (27)	
Mildly impaired		210 (35)	44 (29)	51 (34)	53 (36)	62 (41)	
Moderately impaired		69 (12)	1 (1)	7 (5)	27 (18)	34 (23)	
Severely impaired		18 (3)	0 (0)	1 (1)	4 (3)	13 (9)	
E/A ratio	435 (73)	1.6 ± 0.7	1.7 ± 0.6	1.6 ± 0.6	1.7 ± 0.7	1.6 ± 0.7	0.871
E' wave, cm/s	391 (66)	8.3 ± 2.6	9.0 ± 2.5	8.5 ± 2.2	8.0 ± 2.5	7.0 ± 3.0	< 0.001
E/E' ratio	384 (65)	11.6 ± 5.1	9.7 ± 3.6	10.9 ± 4.0	12.5 ± 5.5	14.6 ± 6.4	< 0.001
Deceleration time, ms	413 (69)	204 ± 58	209 ± 61	208 ± 57	197 ± 50	198 ± 63	0.082
Laboratory results							
Hemoglobin, mmol/L	521 (88)	9.2 ± 1.0	9.4 ± 0.7	9.2 ± 0.8	9.1 ± 0.9	9.2 ± 1.5	0.032
Creatinine, µmol/L	594 (100)	77 ± 18	79 ± 12	76 ± 14	74 ± 14	79 ± 27	0.869

Values are reported as median [IQ_1–IQ_3], otherwise as n (%) or mean ± SD. Differences across NT-proBNP quartiles are analyzed using the Chi-Square Mantel-Haenszel test for categorical variables, otherwise using linear regression. *Congenital diagnosis of aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV, univentricular heart or pulmonary arterial hypertension (1). †Left sided volumes were not measured in patients with a systemic right ventricle, functionally univentricular heart, pulmonary hypertension (n = 137) or a poor acoustic window. **Abbreviations:** ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers; LA, left atrial; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RV, right ventricular.

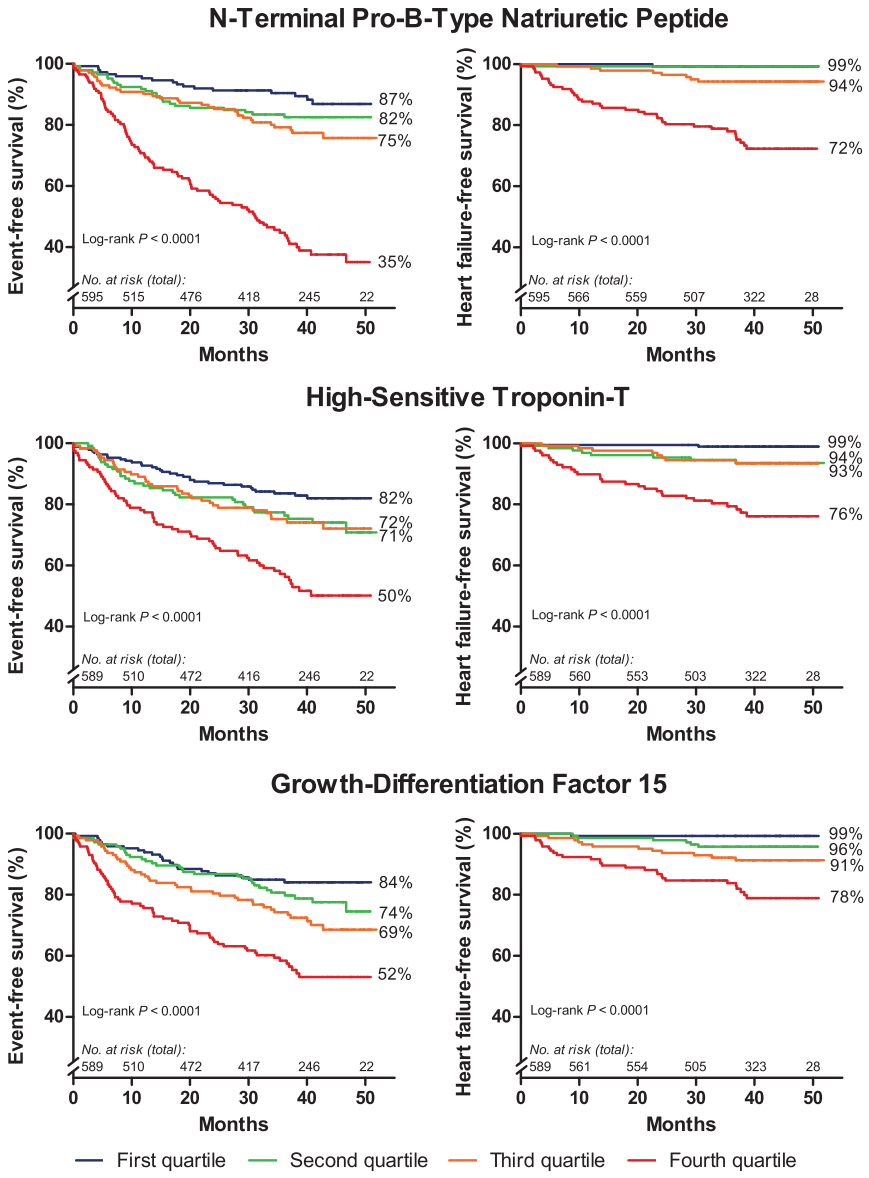

FIGURE 1 - Cardiovascular event-free survival and heart failure-free survival, stratified according to quartiles of NT-proBNP, hs-TnT, and GDF-15.

NT-proBNP: first quartile < 6.8 pmol/L (n = 150), second quartile 6.8–15.2 pmol/L (n = 148), third quartile 15.2–33.3 pmol/L (n = 148), fourth quartile > 33.3 pmol/L (n = 149). *Hs-TnT:* first quartile < 3 ng/L (n = 196), second quartile 3–4.8 ng/L (n = 131), third quartile 4.8–7.7 ng/L (n = 131), fourth quartile > 7.7 ng/L (n = 131). *GDF-15:* first quartile < 487 ng/L (n = 148), second quartile 487–618 ng/L (n = 147), third quartile 618–867 ng/L (n = 147), fourth quartile > 867 ng/L (n = 147).

N-terminal pro-B-type natriuretic peptide

In Figure 1, Kaplan-Meier curves show the cardiovascular event-free survival and heart-failure free survival stratified according to NT-proBNP quartiles. These Kaplan-Meier curves are presented including 95% confidence intervals in Supplemental Figure 3. In patients with a NT-proBNP level below the median (< 15.2 pmol/L) the cumulative proportion of death and heart failure was 1%. Table 3 further details the number of events, person-years and hazard ratios per NT-proBNP quartile. Figure 2 shows the HRs for the primary and secondary endpoint when NT-proBNP was analyzed as a logarithmically transformed continuous variable (per standard deviation increase). These crude estimates did not materially change after multivariable adjustment for potential confounders. Besides age and sex, we considered age at surgical repair, congenital diagnosis, cardiac medication use, saturation, NYHA class, sinus rhythm, left atrial volume, left ventricular end-systolic volume, interventricular septum thickness, systemic ventricular function, and E' wave as potential confounders, because these variables were also associated with the primary endpoint (Supplemental Table 3). Exploratory analysis of tertiary endpoints showed that NT-proBNP was significantly associated with each separate component of the primary endpoint, with the exception of thromboembolic events (Supplemental Figure 4). A sensitivity analysis in which patients with the highest NT-proBNP values were excluded (patients with a systemic right ventricle, functionally univentricular heart or pulmonary arterial hypertension) did not yield different conclusions.

TABLE 2 - Correlations coefficients (*r*) of NT-proBNP, hs-TnT and GDF-15 with clinical characteristics, electrocardiography, echocardiography and other laboratory measurements.

	NT-proBNP		hs-TnT		GDF-15	
	r	***P*-value**	***r***	***P*-value**	***r***	***P*-value**
Clinical characteristics						
Age, years*	0.41	< 0.001	0.36	< 0.001	0.37	< 0.001
Sex, male	–0.30	< 0.001	0.25	< 0.001	–0.23	< 0.001
Surgical repair	0.14	0.001	0.01	0.732	0.04	0.288
Age at surgical repair, years*	0.19	< 0.001	0.21	< 0.001	0.25	< 0.001
Congenital diagnosis†	0.35	< 0.001	0.13	0.002	0.16	< 0.001
Cardiac medication use	0.35	< 0.001	0.29	< 0.001	0.34	< 0.001
Body mass index, kg/m^2	0.05	0.278	0.07	0.072	0.01	0.849
Heart rate, beats/minute	–0.01	0.865	–0.04	0.306	0.10	0.015
Systolic blood pressure, mmHg	–0.06	0.182	0.07	0.09	0.00	0.942
O_2 saturation < 90%	0.19	< 0.001	0.09	0.040	0.13	0.003
NYHA class, II–III	0.37	< 0.001	0.23	< 0.001	0.32	< 0.001

TABLE 2 - Continued.

	NT-proBNP		hs-TnT		GDF-15	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Electrocardiogram						
Sinus rhythm	−0.32	< 0.001	−0.25	< 0.001	−0.25	< 0.001
QRS duration, ms‡	0.15	< 0.001	0.27	< 0.001	−0.00	0.947
Echocardiogram						
LA volume, mL/m²*‡	0.30	< 0.001	0.22	< 0.001	0.15	0.002
LV end-diastolic volume, mL/m²‡	0.18	< 0.001	0.21	< 0.001	0.00	1.00
LV end-systolic volume, mL/m²‡	0.28	< 0.001	0.25	< 0.001	0.06	0.223
LV ejection fraction, %‡	−0.26	< 0.001	−0.19	< 0.001	−0.13	0.012
Interventricular septum, mm	0.11	0.022	0.27	< 0.001	0.04	0.436
Posterior wall, mm	0.02	0.647	0.17	< 0.001	−0.07	0.144
RV fractional area change, %	−0.30	< 0.001	−0.21	< 0.001	−0.10	0.042
Systemic ventricular function, 0–3	0.43	< 0.001	0.24	< 0.001	0.18	< 0.001
E/A ratio	−0.01	0.882	−0.16	0.001	−0.13	0.007
E' wave, cm/s	−0.30	< 0.001	−0.21	< 0.001	−0.24	< 0.001
E/E' ratio	0.36	< 0.001	0.13	0.011	0.17	< 0.001
Deceleration time, ms	−0.09	0.056	0.06	0.266	−0.02	0.713
Laboratory results						
Hemoglobin, mmol/L	−0.11	0.013	0.17	< 0.001	−0.10	0.026
Creatinine, µmol/L	0.07	0.112	0.34	< 0.001	0.26	< 0.001
NT-proBNP, pmol/L			0.42	< 0.001	0.50	< 0.001
hs-TnT, ng/L	0.42	< 0.001			0.31	< 0.001
GDF-15, ng/L	0.50	< 0.001	0.31	< 0.001		

*Spearman's correlation coefficient, other Pearson's correlation coefficient. †Congenital diagnosis of aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV or univentricular heart (1). ‡Left sided volumes were not measured in patients with a systemic right ventricle, functionally univentricular heart, pulmonary hypertension (n = 137) or a poor acoustic window. **Abbreviations:** GDF-15, growth-differentiation factor 15, hs-TnT, high-sensitive troponin-T; LA, left atrial; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RV, right ventricular.

High-sensitive troponin-T

The risk of the primary and secondary composite endpoint for patients in the fourth hs-TnT quartile (> 7.7 ng/L) was significantly higher compared with patients in the first quartile (< 3 ng/L) (Figure 1 and Table 3). Similar results were obtained when hs-TnT was analyzed as a logarithmically transformed continuous variable (Figure 2). When adjusted for potential confounders in the multivariable analysis, hs-TnT, as a categorical and continuous variable, was only predictive of the secondary endpoint.

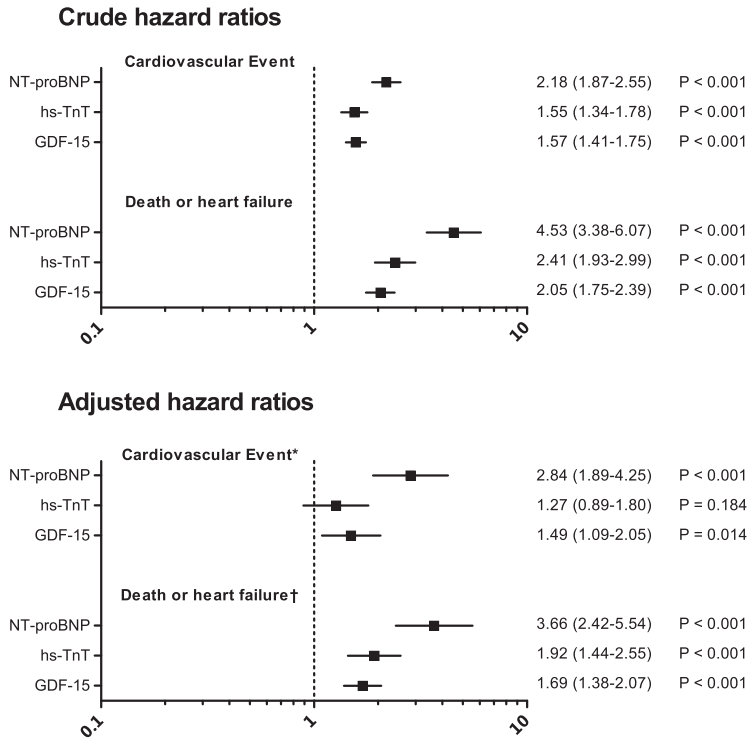

FIGURE 2 - Risk of the primary composite endpoint (any adverse cardiovascular event) and secondary composite endpoint (death or heart failure) for NT-proBNP, hs-TnT, and GDF-15, analyzed continuously.

Skewed biomarker distributions were log transformed. Hazard ratios are expressed per one standard deviation increase of the biomarker. *Adjusted for age (years), sex, congenital diagnosis (aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV, univentricular heart or pulmonary arterial hypertension (1)), systemic ventricular function (0–3), age at surgical repair (years), cardiac medication use (yes/no), saturation < 90%, NYHA class II–III, sinus rhythm (yes/no), LA volume (mL/m^2), LV end-systolic volume (mL/m^2), E' wave (cm/s), and interventricular septum thickness (mm). †Adjusted for age (years), sex, congenital diagnosis (aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV or univentricular heart (1)), and systemic ventricular function (0–3).

Growth-differentiation factor 15

As presented in Figure 1 and Table 3, patients in the fourth GDF-15 quartile (> 867 ng/L) had a significantly higher risk of the primary and secondary composite endpoint compared with patients in the first quartile (< 487 ng/L). Each standard deviation increase in GDF-15 was also associated with a higher risk of the primary and secondary endpoint (Figure 2). Multivariable adjustment yielded similar results, both in the categorical and continuous analysis. GDF-15 was significantly related to each separate component of the primary endpoint, and was the only biomarker that was also associated with thromboembolic events (Supplemental Figure 4).

TABLE 3 - Risk of the primary composite endpoint (cardiovascular event) and secondary composite endpoint (death or heart failure) according to NT-proBNP, hs-TnT and GDF-15 quartiles.

	NT-proBNP (n = 595)				
	Q1 (< 6.8 pmol/L, n = 150)	**Q2 (6.8–15.2 pmol/L, n = 148)**	**Q3 (15.2–33.3 pmol/L, n = 148)**	**Q4 (> 33.3 pmol/L, n = 149)**	***P* for trend***
Cardiovascular event					
No. cases	17	26	33	89	
Person-months	5641	5247	5114	3801	
Crude HR (95% CI)	Reference	1.63 (0.89–3.01)	2.14 (1.19–3.84)	7.31 (4.35–12.3)	< 0.001
Adjusted HR (95% CI)†	Reference	1.63 (0.59–4.54)	2.93 (1.12–7.68)	9.05 (3.24–25.3)	< 0.001
Death or heart failure					
No. cases	1	1	9	39	
Person-months	6053	6004	5741	5121	
Crude HR (95% CI)	Reference	1.00 (0.06–16.1)	9.47 (1.20–74.7)	44.7 (6.14–326)	< 0.001
Adjusted HR (95% CI)‡	Reference	0.82 (0.05–13.1)	5.60 (0.69–45.4)	16.0 (2.04–126)	< 0.001
	hs-TnT (n = 589)				
	Q1 (< 3 ng/L, n = 196)	**Q2 (3–4.8 ng/L, n = 131)**	**Q3 (4.8–7.7 ng/L, n = 131)**	**Q4 (> 7.7 ng/L, n = 131)**	***P* for trend***
Cardiovascular event					
No. cases	34	33	34	61	
Person-months	7070	4513	4391	3697	
Crude HR (95% CI)	Reference	1.52 (0.94–2.45)	1.59 (0.99–2.56)	3.27 (2.15–4.97)	< 0.001
Adjusted HR (95% CI)†	Reference	1.24 (0.54–2.87)	1.11 (0.46–2.69)	1.80 (0.75–4.35)	0.191
Death or heart failure					
No. cases	2	8	9	30	
Person-months	7951	5175	5050	4542	
Crude HR (95% CI)	Reference	6.14 (1.30–28.9)	7.00 (1.51–32.4)	25.3 (6.05–106)	< 0.001
Adjusted HR (95% CI)‡	Reference	7.60 (1.59–36.2)	6.60 (1.39–31.5)	14.4 (3.21–64.4)	< 0.001
	GDF-15 (n = 589)				
	Q1 (< 487 ng/L, n = 148)	**Q2 (487–618 ng/L, n = 147)**	**Q3 (618–867 ng/L, n = 147)**	**Q4 (> 867 ng/L, n = 147)**	***P* for trend***
Cardiovascular event					
No. cases	23	32	41	66	
Person-months	5386	5231	4939	4134	
Crude HR (95% CI)	Reference	1.44 (0.84–2.46)	1.94 (1.17–3.24)	3.63 (2.26–5.84)	< 0.001
Adjusted HR (95% CI)†	Reference	1.03 (0.43–2.49)	1.15 (0.49–2.74)	2.77 (1.12–6.85)	0.007

TABLE 3 - Continued.

	GDF-15 (n = 589)				
	Q1 (< 487 ng/L, n = 148)	**Q2 (487–618 ng/L, n = 147)**	**Q3 (618–867 ng/L, n = 147)**	**Q4 (> 867 ng/L, n = 147)**	***P* for trend***
Death or heart failure					
No. cases	1	6	12	30	
Person-months	5992	5849	5692	5244	
Crude HR (95% CI)	Reference	6.14 (0.74–51.0)	12.6 (1.64–97.2)	33.7 (4.60–247)	< 0.001
Adjusted HR (95% CI)‡	Reference	4.33 (0.52–36.2)	6.58 (0.84–51.7)	12.4 (1.63–94.9)	0.001

*Computed by assigning the median level in each quartile to participants and evaluating this variable continuously. †The primary endpoint was adjusted for age (years), sex, congenital diagnosis (aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV or univentricular heart (1)), systemic ventricular function (0–3), age at surgical repair (years), cardiac medication use (yes/no), saturation < 90%, NYHA class II–III, sinus rhythm (yes/no), LA volume (mL/m^2), LV end-systolic volume (mL/m^2), E' wave (cm/s), and interventricular septum thickness (mm). ‡The secondary endpoint was adjusted for age (years), sex, congenital diagnosis (aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV, univentricular heart or pulmonary arterial hypertension (1)), and systemic ventricular function (0–3). **Abbreviations:** CI, confidence interval; GDF-15, growth-differentiation factor 15; HR, hazard ratio; hs-TnT, high-sensitive Troponin-T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Combination of NT-proBNP, hs-TnT, and GDF-15

To determine the prognostic value of a combination of the three biomarkers, patients were divided into four groups: no biomarkers elevated (n = 256), one biomarker elevated (n = 234), two biomarkers elevated (n = 70), and three biomarkers elevated (n = 22). In Figure 3, Kaplan-Meier curves stratified according to these four groups are presented. These Kaplan-Meier curves are presented including 95% confidence intervals in Supplemental Figure 5. Patients with elevated levels of all three biomarkers were at highest risk of the primary and secondary composite endpoint. The number of events, person-months and hazard ratios in each group are also indicated in Table 4. In Figure 4, the Kaplan-Meier estimates of cardiovascular event-free survival and heart-failure free survival according to more detailed subgroups are presented, also including the number of patients per cell. In patients with a low NT-proBNP level, hs-TnT and GDF-15 were elevated only in a small number of patients. In patients with a high NT-proBNP level, hs-TnT and GDF-15 could further stratify the risk of the primary and secondary endpoint. When all three biomarkers were analyzed as logarithmically transformed continuous variables in a multivariable model, one standard deviation increase in NT-proBNP (adjusted HR 1.85; 95% CI 1.51–2.25, $p < 0.001$) and GDF-15 (adjusted HR 1.20; 95% CI 1.04–1.40, $p = 0.014$) was independently associated with the primary endpoint, but hs-TnT was not (adjusted HR 1.09; 95% CI 0.93–1.29, $p = 0.286$).

TABLE 4 - Risk of the primary composite endpoint (cardiovascular event) and secondary composite endpoint (death or heart failure) according to the number of elevated biomarkers.

	Combination of NT-proBNP, hs-TnT and GDF-15 (n = 582)				
	No biomarkers elevated (n = 256)	**One biomarker elevated (n = 234)**	**Two biomarkers elevated (n = 70)**	**Three biomarkers elevated (n = 22)**	***P* for trend***
Cardiovascular event					
No. cases	33	75	37	15	
Person-months	9522	7668	1795	456	
Crude HR (95% CI)	Reference	2.78 (1.85–4.19)	5.64 (3.52–9.02)	8.48 (4.59–15.7)	< 0.001
Adjusted HR (95% CI)†	Reference	3.15 (1.54–6.43)	4.88 (1.67–14.3)	6.24 (1.57–24.8)	0.001
Death or heart failure					
No. cases	1	18	19	10	
Person-months	10401	9178	2296	611	
Crude HR (95% CI)	Reference	20.4 (2.72–153)	83.4 (11.2–623)	157 (20.1–1230)	< 0.001
Adjusted HR (95% CI)‡	Reference	12.0 (1.56–92.4)	34.0 (4.26–272)	44.4 (5.05–391)	< 0.001

No biomarkers elevated (n = 256): NT-proBNP < 14 pmol/L, hs-TnT < 14 ng/L and GDF-15 < 1109 ng/L. *One biomarker elevated (n = 234):* NT-proBNP > 14 pmol/L (n = 217) or hs-TnT > 14 ng/L (n = 4) or GDF-15 > 1109 ng/L (n = 13). *Two biomarkers elevated (n = 70)*: NT-proBNP > 14 pmol/L (n = 70) and hs-TnT > 14 ng/L (n = 20) or GDF-15 > 1109 ng/L (n = 50). *Three biomarkers elevated (n = 22)*: NT-proBNP > 14 pmol/L and hs-TnT > 14 ng/L and GDF-15 > 1109 ng/L. *Computed by evaluating the number of elevated biomarkers (0–3) as continuous variable. Others as defined in Table 2.

Receiver Operating Characteristic-curves present the discriminatory value of NT-proBNP, hs-TnT, and GDF-15 separately for the primary and secondary endpoint in Supplemental Figure 6. The sensitivity and 1–specificity for cut-offs according to quartiles of distribution are indicated. A clinical risk marker model including only clinical variables (age, sex, age at surgical repair, congenital diagnosis, cardiac medication use, saturation, and NYHA class) yielded a C-index of 0.69 (95% CI 0.64–0.73) for the primary endpoint. Adding electrocardiography and echocardiography variables (sinus rhythm, left atrial volume, left ventricular end-systolic volume, interventricular septum thickness, systemic ventricular function, and E'wave) led to a similar improvement in the C-index in comparison with adding biomarker variables (NT-proBNP, hs-TnT, and GDF-15): 0.74 (95% CI 0.66–0.82) in comparison with 0.74 (95% CI 0.70–0.79), respectively. The full model including all variables yielded the highest C-index: 0.80 (95% CI 0.74–0.87). The differences between the clinical model and the biomarker updated model ($p = 0.001$), the clinical model and the full model ($p < 0.001$), and the echocardiography updated model and the full model ($p = 0.019$) were statistically significant, whereas the difference between the clinical model and the echocardiography updated model

was not (p = 0.057). In comparison with the clinical risk marker model, the integrated discrimination improvement (reflecting the change in discrimination slope) was 0.07 (95% CI –0.01 to 0.17) for the echocardiography updated model, 0.08 (95% CI 0.00–0.21) for the biomarker updated model, and 0.12 (95% CI 0.02–0.27) for the full model.

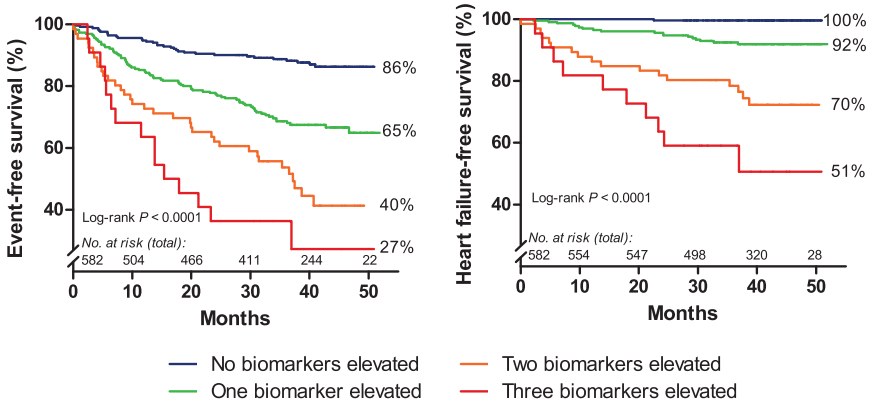

FIGURE 3 - Cardiovascular event-free survival and heart failure-free survival, stratified according to the number of elevated biomarkers.

No biomarkers elevated (n = 256): NT-proBNP < 14 pmol/L, hs-TnT < 14 ng/L and GDF-15 < 1109 ng/L. *One biomarker elevated (n = 234):* NT-proBNP > 14 pmol/L (n = 217) or hs-TnT > 14 ng/L (n = 4) or GDF-15 > 1109 ng/L (n = 13). *Two biomarkers elevated (n = 70)*: NT-proBNP > 14 pmol/L (n = 70) and hs-TnT > 14 ng/L (n = 20) or GDF-15 > 1109 ng/L (n = 50). *Three biomarkers elevated (n = 22)*: NT-proBNP > 14 pmol/L and hs-TnT > 14 ng/L and GDF-15 > 1109 ng/L.

Cardiovascular event-free survival

	NTproBNP low		NTproBNP high	
	hs-TnT low	hs-TnT high	hs-TnT low	hs-TnT high
GDF-15 high	**69** *(n=13)*	**NA** *(n=0)*	**38** *(n=50)*	**27** *(n=22)*
GDF-15 low	**86** *(n=256)*	**75** *(n=4)*	**65** *(n=217)*	**47** *(n=20)*

Heart failure-free survival

	NTproBNP low		NTproBNP high	
	hs-TnT low	hs-TnT high	hs-TnT low	hs-TnT high
GDF-15 high	**100** *(n=13)*	**NA** *(n=0)*	**71** *(n=50)*	**51** *(n=22)*
GDF-15 low	**100** *(n=256)*	**100** *(n=4)*	**91** *(n=217)*	**67** *(n=20)*

Legend:
< 50%
50-85%
>85%

FIGURE 4 - Kaplan-Meier estimates of cardiovascular event-free survival and heart-failure free survival at the end of the follow-up duration, stratified according to elevated versus non-elevated levels of NT-proBNP, hs-TnT, and GDF-15.

The number of patients in each cell is indicated. **Abbreviations:** GDF-15, growth-differentiation factor 15; hs-TnT, high-sensitive troponin-T; NA, not applicable; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

DISCUSSION

This study evaluated the prognostic value of NT-proBNP, hs-TnT, and GDF-15 in clinically stable patients with ACHD. NT-proBNP was strongly associated with cardiovascular events, independently of clinical characteristics and electrocardiographic and echocardiographic measurements. Especially the risk of death or heart failure was extremely low in patients in the lowest two NT-proBNP quartiles (< 15.2 pmol/L). The risk of patients with a high level of NT-proBNP could be further differentiated by the combined use of hs-TnT and GDF-15. Patients with elevated levels of all three biomarkers were at highest risk of cardiovascular events.

Previous reports

This is the first large and prospective cohort study investigating the association of NT-proBNP with a combined endpoint of cardiovascular events in patients with ACHD. Because the detection of early deterioration is of particular interest, it is important that, apart from mortality, other endpoints are also investigated. In this study, NT-proBNP was analyzed both as a categorical and as a continuous variable. Furthermore, the completeness of data due to the prospective design and the adequate event size allowed us to adjust for multiple clinical and echocardiographic variables, which has not previously been performed. All these different analyses yielded similar results with a high degree of certainty.

The results of this study are concordant with the recently published paper by Popelová et al., who reported that a NT-proBNP value of > 630 pg/mL (corresponding to 74 pmol/L)[31] was associated with mortality in a large cohort of patients with stable ACHD (n = 646).[32] B-type natriuretic peptide (BNP) was also associated with mortality in two smaller cohorts of patients with ACHD.[33, 34] In 83 patients with tetralogy of Fallot, Heng et al.[35] recently showed that BNP was a strong predictor of death and sustained arrhythmia in univariable analysis. Westhoff-Bleck et al.[36] and Haberger et al.[37] both found a significant association of natriuretic peptides with a combined endpoint ('hospitalization for any clinical event' and 'heart failure, transplant and death') in patients with a systemic right ventricle (n = 116 and n = 89, respectively). In contrast, two earlier studies in small cohorts of patients with a systemic right ventricle or Fontan circulation did not find a prognostic merit of NT-proBNP and BNP.[38, 39]

What makes our study unique and innovative is that we found a predictive value of hs-TnT and GDF-15 on top of NT-proBNP in patients with ACHD. To our knowledge, hs-TnT has only been reported as a predictor of mortality in patients with pulmonary arterial hypertension due to congenital heart disease.[40] Prospective data on hs-TnT in other types of ACHD and prospective data on GDF-15 are not available.

Comparison of NT-proBNP, hs-TnT and GDF-15

Significant correlations were present between NT-proBNP and GDF-15 ($r = 0.50$), and NT-proBNP and hs-TnT ($r = 0.42$). This reflects a moderate overlap between the biomarkers, and may also explain why hs-TnT was not predictive of the primary endpoint when analyzed in a multivariable model including NT-proBNP and GDF-15. Notably however, the biomarker concentrations were significantly different among specific patient subgroups. For instance, NT-proBNP and GDF-15 were higher in women, while hs-TnT was higher in men. These differences have also been described in the general population.[41, 42] We observed that NT-proBNP and GDF-15 values were high in patients with a Fontan circulation, whereas hs-TnT was much lower. NT-proBNP and hs-TnT were related to QRS duration, left ventricular volumes, and interventricular septum thickness, and GDF-15 was not. Last, GDF-15 was the only biomarker that was also associated with thromboembolic events. Considering NT-proBNP (myocardial wall stress) and hs-TnT (cardiomyocyte damage) primarily as cardiac markers, GDF-15 may therefore also reflect extra-cardiac pathophysiological mechanisms that are involved in the heart failure syndrome.[22] These differences could also explain why these biomarkers seem to provide incremental prognostic information when used in combination.

Clinical implications

Measurement of natriuretic peptides should be considered to diagnose heart failure and to obtain prognostic information, according to the 2012 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic heart failure[25] and the 2013 American College of Cardiology/American Heart Association guideline for the management of heart failure.[43] In the 2010 European Society of Cardiology guidelines for the management of grown-up congenital heart disease, no recommendations are made regarding the use of any biomarker.[44] This is not surprising as no substantial evidence was available at that time. It is important to note that the results of our study imply that NT-proBNP may play an important role in the risk stratification of patients with stable ACHD who are seen at the outpatient clinic, independently of other currently used tools such as echocardiography. Considering the high negative predictive value, NT-proBNP may be particularly valuable as a screening tool. Patients with a low NT-proBNP can be reassured and will need less frequent monitoring and diagnostic examinations at the outpatient clinic. This could lead to important cost reductions in healthcare. High-risk patients may be more accurately identified by the combined use of NT-proBNP, hs-TnT, and GDF-15. These biomarkers therefore may play an important role in the monitoring and management of the rapidly growing population of patients with ACHD.

Future perspectives

In order to be clinically useful, a biomarker should meet three fundamental criteria, according to previously formulated benchmarks for the assessment of novel cardiovascular biomarkers.[45] Analytical methods should allow reliable measurement at a reasonable cost; the biomarker should be consistently and strongly associated with outcomes (independent of, or superior to established tools); and it should help the clinician to manage patients. NT-proBNP and hs-TnT assays are known to have a good analytic performance and precision,[26, 46, 47] and this study provides evidence for a strong association with clinical outcomes in patients with ACHD. To satisfy the last criterion, there should be evidence that the associated risk is modifiable with specific therapy or that biomarker-based therapy enhances care. In non-congenital patients with heart failure, it has been shown that biomarker-guided treatment results in better outcome than clinically guided therapy, although conflicting data have been reported.[48] Such studies have not yet been performed in patients with ACHD and will be the next step to further unravel the value of biomarker-based therapy and to improve the clinical management of these patients. It could also be worthwhile to investigate the value of these biomarkers in other forms of structural heart disease, such as hypertrophic cardiomyopathy. Future research should investigate whether individual changes in biomarkers over time can reflect progression of disease or response to successful interventions, and if serial biomarker assessment can further enhance the prognostication of patients with ACHD.

Study limitations

Patients with ACHD obviously compose a heterogeneous group. Due to the limited number of events, it was not possible to perform a stratified analysis in all diagnostic subgroups. The secondary composite endpoint could not be adjusted for the complete set of potential confounders. Some potential confounders were categorized, such as systemic ventricular function. All of this may have resulted in residual confounding. In addition, patients with mild cardiac lesions were not included in this study, which may affect the external validity of these results. This study reports follow-up data during a median of 42 months, which may be considered relatively short. Therefore, it is important that these results are confirmed in other cohorts with a longer follow-up duration.

CONCLUSIONS

NT-proBNP provides prognostic information beyond a conventional risk marker model in patients with ACHD. Of particular importance, patients with a low risk of death and heart failure can be accurately identified with a high negative predictive value. This can

reassure patients and could lead to important cost reductions in healthcare. In patients with high NT-proBNP levels, hs-TnT and GDF-15 assessment further identifies those patients at highest risk of cardiovascular events. This will enable further optimization of individual follow-up strategies and timely initiation or expansion of therapeutic measures.

CLINICAL PERSPECTIVE

What is new?

- This is the first large and prospective study showing that N-terminal pro-B-type natriuretic peptide (NT-proBNP) is associated with a composite endpoint of cardiovascular events in patients with adult congenital heart disease, independently of multiple clinical and echocardiographic variables.
- Patients with a low risk of death and heart failure can be accurately identified with a high negative predictive value.
- The risk of patients with a high level of NT-proBNP could be further differentiated by adding high-sensitive troponin-T and growth-differentiation factor 15.
- Accordingly, patients with elevated levels of all three biomarkers were at highest risk of cardiovascular events.

What are the clinical implications?

- The results of our study suggest that NT-proBNP may play an important role in the risk stratification of patients with adult congenital heart disease, independently of other currently used tools such as echocardiography.
- The high negative predictive value makes NT-proBNP particularly valuable as a screening tool.
- Patients with a low NT-proBNP can be reassured and will need less frequent monitoring and diagnostic examinations at the outpatient clinic, which could lead to important cost reductions in healthcare.
- The combined use of NT-proBNP, high-sensitive troponin-T, and growth-differentiation factor 15 may further aid in the optimization of follow-up strategies and timing of re-interventions.

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLE 1 - Baseline characteristics of the study cohort according to hs-TnT quartiles.

	hs-TnT quartiles (n = 589)				
	Q1 (< 3 ng/L, n = 196)	**Q2 (3–4.8 ng/L, n = 131)**	**Q3 (4.8–7.7 ng/L, n = 131)**	**Q4 (> 7.7 ng/L, n = 131)**	***P* for trend**
Clinical characteristics					
Age, years	29 [23–34]	33 [24–40]	34 [26–45]	40 [33–51]	< 0.001
Sex, male n (%)	71 (36)	87 (66)	97 (74)	86 (66)	< 0.001
Surgical repair, n (%)	175 (89)	122 (93)	118 (90)	120 (92)	0.616
Age at surgical repair, years	2.0 [0.5–6.6]	4.4 [0.8–15.1]	5.1 [0.8–12.4]	6.3 [1.8–17.0]	< 0.001
Congenital diagnosis, n (%)*	97 (49)	63 (48)	77 (59)	83 (63)	0.005
Cardiac medication use, n (%)	42 (21)	38 (29)	54 (41)	76 (58)	< 0.001
Body mass index, kg/m^2	24.4 ± 4.2	24.8 ± 4.6	25.1 ± 3.8	25.0 ± 5.0	0.126
Heart rate, beats/minute	75 ± 13	74 ± 14	72 ± 12	73 ± 15	0.078
Systolic blood pressure, mmHg	124 ± 14	125 ± 17	128 ± 15	128 ± 19	0.017
O_2 saturation < 90%, n (%)	3 (2)	2 (2)	5 (4)	6 (5)	0.054
NYHA class, II–III n (%)	8 (4)	11 (8)	11 (8)	30 (23)	< 0.001
Electrocardiogram					
Sinus rhythm, n (%)	184 (94)	113 (86)	119 (91)	92 (70)	< 0.001
QRS duration, ms	105 [95–121]	113 [101–133]	121 [105–152]	124 [103–157]	< 0.001
Echocardiogram					
LA volume, mL/m^2†	20 [14–27]	20 [15–27]	21 [16–28]	28 [18–45]	< 0.001
LV end-diastolic volume, mL/m^2†	60 ± 15	62 ± 16	65 ± 22	70 ± 23	< 0.001
LV end-systolic volume, mL/m^2†	26 ± 8	27 ± 12	29 ± 13	34 ± 18	< 0.001
LV ejection fraction, %†	57 ± 6	56 ± 8	56 ± 7	53 ± 11	0.001
Interventricular septum, mm	8.5 ± 1.5	9.1 ± 2.0	9.8 ± 2.4	9.9 ± 2.3	< 0.001
Posterior wall, mm	8.4 ± 1.3	9.0 ± 1.4	9.3 ± 1.8	9.0 ± 1.8	< 0.001
RV fractional area change, %	40 ± 11	40 ± 10	38 ± 11	34 ± 12	< 0.001
Systemic ventricular function, n (%)					< 0.001
Normal	166 (59)	66 (50)	66 (50)	49 (38)	
Mildly impaired	64 (33)	53 (40)	43 (33)	46 (35)	
Moderately impaired	14 (7)	10 (8)	20 (15)	24 (18)	
Severely impaired	2 (1)	2 (2)	2 (2)	12 (9)	
E/A ratio	1.8 ± 0.8	1.6 ± 0.5	1.6 ± 0.6	1.5 ± 0.6	0.001
E' wave, cm/s	8.8 ± 2.5	8.5 ± 2.3	7.8 ± 2.6	7.3 ± 2.7	< 0.001
E/E' ratio	11.0 ± 4.7	11.0 ± 4.3	12.1 ± 5.2	13.0 ± 6.2	0.004
Deceleration time, ms	200 ± 59	202 ± 58	206 ± 56	212 ± 60	0.153

SUPPLEMENTAL TABLE 1 - Continued.

	hs-TnT quartiles (n = 589)				
	Q1 (< 3 ng/L, n = 196)	**Q2 (3–4.8 ng/L, n = 131)**	**Q3 (4.8–7.7 ng/L, n = 131)**	**Q4 (> 7.7 ng/L, n = 131)**	***P* for trend**
Laboratory results					
Hemoglobin, mmol/L	9.0 ± 0.8	9.3 ± 1.0	9.4 ± 0.9	9.4 ± 1.2	< 0.001
Creatinine, µmol/L	71 ± 12	77 ± 13	78 ± 13	85 ± 28	< 0.001

SUPPLEMENTAL TABLE 2 - Baseline characteristics of the study cohort according to GDF-15 quartiles.

	GDF-15 quartiles (n = 589)				
	Q1 (< 487 ng/L, n = 148)	**Q2 (487–618 ng/L, n = 147)**	**Q3 (618–867 ng/L, n = 147)**	**Q4 (> 867 ng/L, n = 147)**	***P* for trend**
Clinical characteristics					
Age, years	28 [22–34]	32 [25–40]	34 [26–42]	40 [30–50]	< 0.001
Sex, male n (%)	98 (66)	106 (72)	81 (55)	59 (40)	< 0.001
Surgical repair, n (%)	135 (91)	129 (88)	134 (91)	137 (93)	0.382
Age at surgical repair, years	1.7 [0.3–7.3]	2.8 [0.8–12.3]	4.0 [0.6–12.0]	7.5 [2.5–15.9]	< 0.001
Congenital diagnosis, n (%)*	71 (48)	72 (49)	85 (58)	93 (63)	0.003
Cardiac medication use, n (%)	20 (14)	49 (33)	55 (37)	85 (58)	< 0.001
Body mass index, kg/m^2	24.3 ± 4.1	25.1 ± 4.3	24.6 ± 4.1	25.0 ± 4.8	0.338
Heart rate, beats/minute	71 ± 14	73 ± 12	74 ± 13	75 ± 13	0.012
Systolic blood pressure, mmHg	125 ± 15	127 ± 15	125 ± 17	128 ± 18	0.332
O_2 saturation < 90%, n (%)	2 (1)	1 (1)	7 (5)	7 (5)	0.024
NYHA class, II–III n (%)	7 (5)	7 (5)	14 (10)	33 (22)	< 0.001
Electrocardiogram					
Sinus rhythm, n (%)	138 (93)	134 (91)	127 (86)	109 (74)	< 0.001
QRS duration, ms	111 [99–130]	117 [103–136]	111 [98–140]	114 [98–144]	0.183
Echocardiogram					
LA volume, mL/m^2†	19 [15–27]	21 [15–27]	22 [16–31]	23 [16–37]	< 0.001
LV end-diastolic volume, mL/m^2†	65 ± 20	63 ± 19	61 ± 18	65 ± 19	0.904
LV end-systolic volume, mL/m^2†	29 ± 13	28 ± 12	27 ± 11	31 ± 15	0.401
LV ejection fraction, %†	56 ± 7	57 ± 8	57 ± 7	54 ± 9	0.045
Interventricular septum, mm	8.9 ± 1.7	9.6 ± 2.2	9.1 ± 2.3	9.2 ± 2.2	0.453
Posterior wall, mm	8.8 ± 1.6	9.1 ± 1.6	8.9 ± 1.8	8.5 ± 1.3	0.130
RV fractional area change, %	39 ± 10	39 ± 11	39 ± 13	36 ± 11	0.205

SUPPLEMENTAL TABLE 2 - Continued.

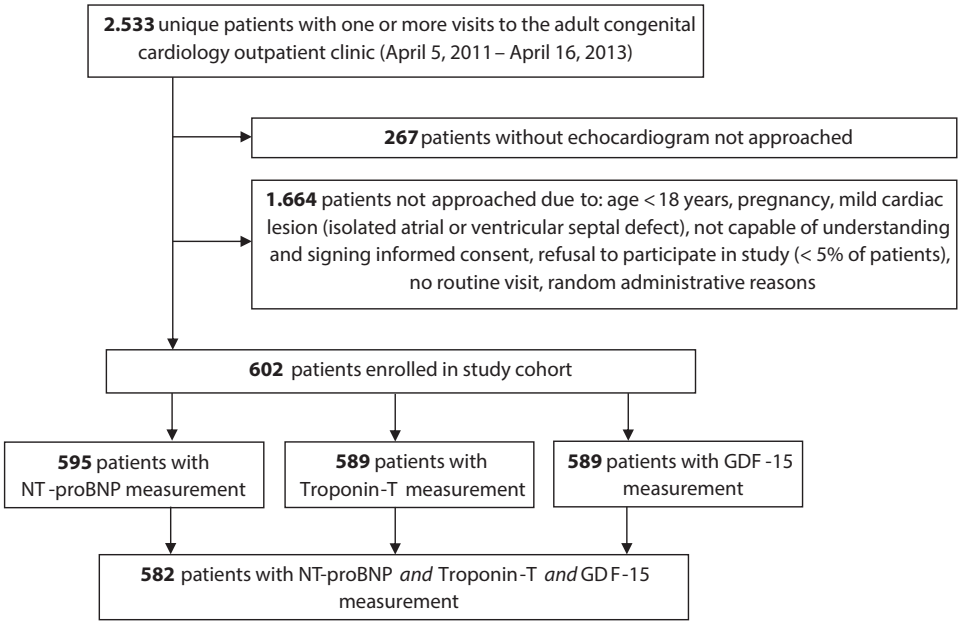
	GDF-15 quartiles (n = 589)				
	Q1 (< 487 ng/L, n = 148)	**Q2 (487–618 ng/L, n = 147)**	**Q3 (618–867 ng/L, n = 147)**	**Q4 (> 867 ng/L, n = 147)**	***P* for trend**
Echocardiogram					
Systemic ventricular function, n (%)					< 0.001
Normal	86 (58)	80 (54)	75 (51)	57 (39)	
Mildly impaired	50 (34)	45 (31)	51 (35)	58 (39)	
Moderately impaired	10 (7)	17 (12)	16 (11)	26 (18)	
Severely impaired	2 (1)	5 (3)	5 (3)	6 (4)	
E/A ratio	1.8 ± 0.7	1.6 ± 0.5	1.7 ± 0.8	1.5 ± 0.6	0.009
E' wave, cm/s	8.9 ± 2.5	8.5 ± 2.4	8.2 ± 2.7	7.1 ± 2.6	< 0.001
E/E' ratio	10.5 ± 4.1	11.2 ± 5.1	11.6 ± 4.5	13.5 ± 6.2	< 0.001
Deceleration time, ms	206 ± 58	202 ± 56	209 ± 64	198 ± 54	0.491
Laboratory results					
Hemoglobin, mmol/L	9.3 ± 0.8	9.3 ± 0.8	9.2 ± 1.1	9.1 ± 1.2	0.083
Creatinine, µmol/L	76 ± 13	76 ± 12	74 ± 12	82 ± 27	0.008

Legend Supplemental Tables 1,2: Values are reported as median [IQ_1–IQ_3], otherwise as n (%) or mean ± SD. Differences across biomarker quartiles are analysed using the chi-square Mantel-Haenszel test for categorical variables, otherwise using linear regression. *Congenital diagnosis of aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV or univentricular heart (1). †Left sided volumes were not measured in patients with a systemic right ventricle, functionally univentricular heart, pulmonary hypertension (n = 137) or a poor acoustic window. **Abbreviations (Supplemental Tables 1,2):** GDF-15, growth-differentiation factor 15, hs-TnT, high-sensitive troponin-T; LA, left atrial; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RV, right ventricular.

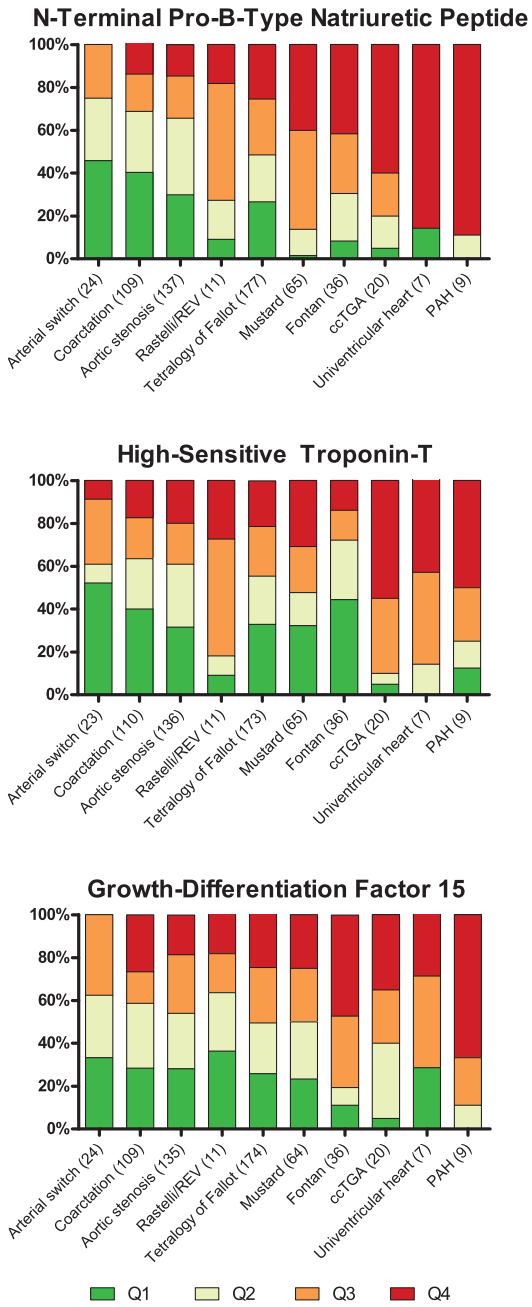
SUPPLEMENTAL TABLE 3 - Risk of the primary composite endpoint (cardiovascular event) and secondary composite endpoint (death or heart failure) for other baseline characteristics.

	Cardiovascular event (n = 165)		Death or heart failure (n = 50)	
	Crude HR (95% CI)	***P*-value**	**Crude HR (95% CI)**	***P*-value**
Clinical characteristics				
Age, years	1.03 (1.02-1.05)	< 0.001	1.07 (1.05-1.09)	< 0.001
Sex, male	1.13 (0.83-1.53)	0.446	1.81 (1.03-3.16)	0.038
Surgical repair, yes	1.01 (0.59-1.72)	0.972	0.90 (0.36-2.28)	0.830
Age at surgical repair, years	1.02 (1.01-1.03)	< 0.001	1.03 (1.02-1.05)	< 0.001
Congenital diagnosis*	1.87 (1.35-2.59)	< 0.001	3.99 (1.94-8.21)	< 0.001
Cardiac medication use	3.41 (2.50-4.66)	< 0.001	15.4 (6.57-36.2)	< 0.001
Body mass index, kg/m^2	1.02 (0.99-1.06)	0.225	1.09 (1.04-1.15)	0.001
Heart rate, beats/minute	1.00 (0.99-1.01)	0.837	1.02 (1.00-1.04)	0.125
Systolic blood pressure, mmHg	0.99 (0.99-1.00)	0.250	1.01 (0.99-1.02)	0.509
O_2 saturation < 90%	2.54 (1.29-4.98)	0.007	4.51 (1.79-11.4)	0.001
NYHA class, II–III	4.63 (3.26-6.57)	< 0.001	9.63 (5.52-16.8)	< 0.001
Electrocardiogram				
Sinus rhythm	0.37 (0.26-0.53)	< 0.001	0.21 (0.12-0.37)	< 0.001
QRS duration, ms	1.01 (1.00-1.01)	0.004	1.01 (1.00-1.02)	0.052
Echocardiogram				
LA volume, mL/m^2	1.03 (1.02-1.03)	< 0.001	1.02 (1.01-1.03)	< 0.001
LV end-diastolic volume, mL/m^2	1.02 (1.01-1.03)	0.002	1.03 (1.01-1.05)	< 0.001
LV end-systolic volume, mL/m^2	1.03 (1.02-1.04)	< 0.001	1.04 (1.03-1.06)	< 0.001
LV ejection fraction, %	0.96 (0.94-0.98)	< 0.001	0.92 (0.89-0.95)	< 0.001
Interventricular septum, mm	1.11 (1.02-1.20)	0.011	1.17 (1.02-1.34)	0.021
Posterior wall, mm	1.06 (0.95-1.19)	0.312	1.13 (0.92-1.39)	0.243
RV fractional area change, %	0.98 (0.96-1.00)	0.024	0.94 (0.91-0.97)	< 0.001
Systemic ventricular function, 0–3	1.60 (1.35-1.89)	< 0.001	2.22 (1.68-2.94)	< 0.001
E/A ratio	0.81 (0.58-1.11)	0.191	0.38 (0.15-0.97)	0.043
E' wave, cm/s	0.77 (0.70-0.85)	< 0.001	0.53 (0.42-0.68)	< 0.001
E/E' ratio	1.06 (1.02-1.09)	0.001	1.10 (1.04-1.16)	0.001
Deceleration time, ms	1.00 (1.00-1.00)	0.504	1.00 (0.99-1.00)	0.260
Laboratory results†				
Hemoglobin, mmol/L	0.94 (0.79-1.12)	0.498	0.74 (0.54-1.01)	0.057
Creatinine, µmol/L	1.16 (1.03-1.31)	0.017	1.20 (1.00-1.44)	0.047

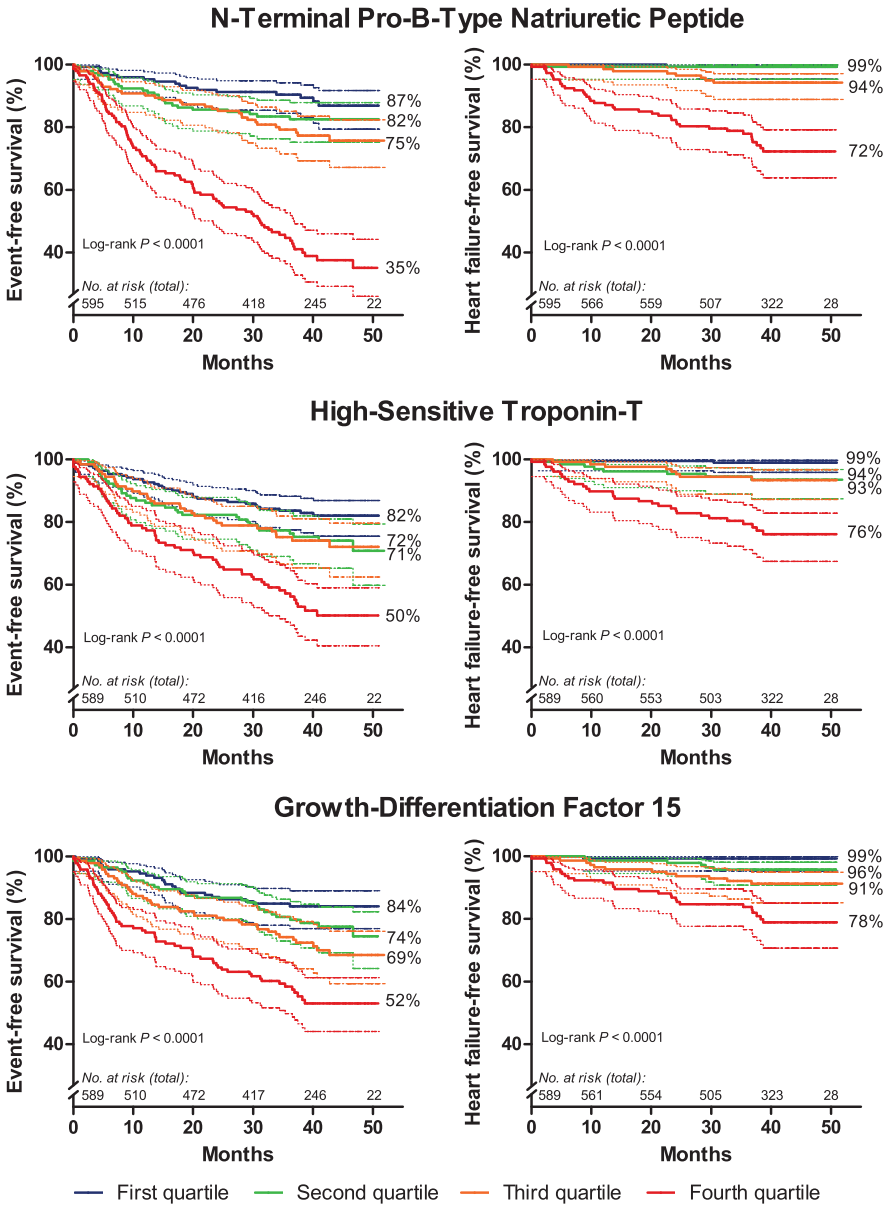
*Congenital diagnosis of aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV or univentricular heart (1). †Expressed per one standard deviation increase in biomarker. **Abbreviations:** as defined in Supplemental Tables 1,2.



SUPPLEMENTAL FIGURE 1- Flowchart of the selection of patients.

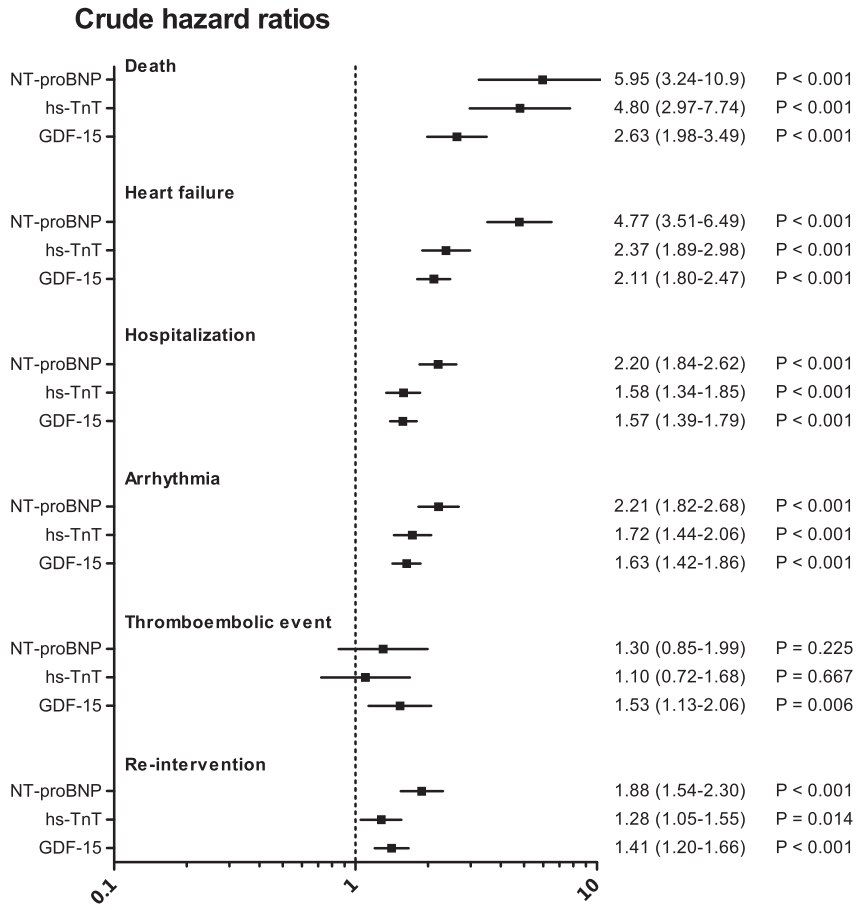


SUPPLEMENTAL FIGURE 2 - NT-proBNP, hs-TnT and GDF-15 levels in the different congenital diagnoses.
Abbreviations: REV, réparation à l'etage ventriculaire; ccTGA, congenitally corrected transposition of the great arteries; PAH, pulmonary arterial hypertension.



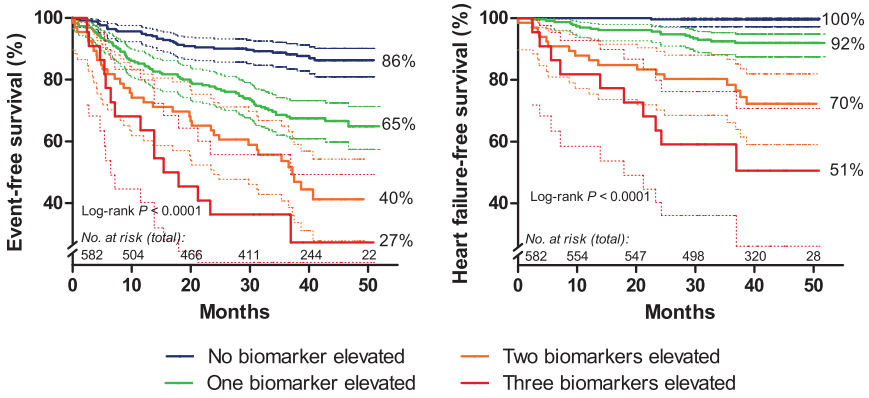
SUPPLEMENTAL FIGURE 3 - Cardiovascular event-free survival and heart failure-free survival, stratified according to quartiles of NT-proBNP, hs-TnT and GDF-15. 95% confidence intervals are indicated.

NT-proBNP: First quartile < 6.8 pmol/L, n = 150, Second quartile 6.8-15.2 pmol/L, n = 148, Third quartile 15.2-33.3 pmol/L, n = 148, Fourth quartile > 33.3 pmol/L, n = 149. *Hs-TnT:* First quartile < 3 ng/L, n = 196, Second quartile 3-4.8 ng/L, n = 131, Third quartile 4.8-7.7 ng/L, n = 131, Fourth quartile > 7.7 ng/L, n = 131. *GDF-15:* First quartile < 487 ng/L, n = 148, Second quartile 487-618 ng/L, n = 147, Third quartile 618-867 ng/L, n = 147, Fourth quartile > 867 ng/L, n = 147.



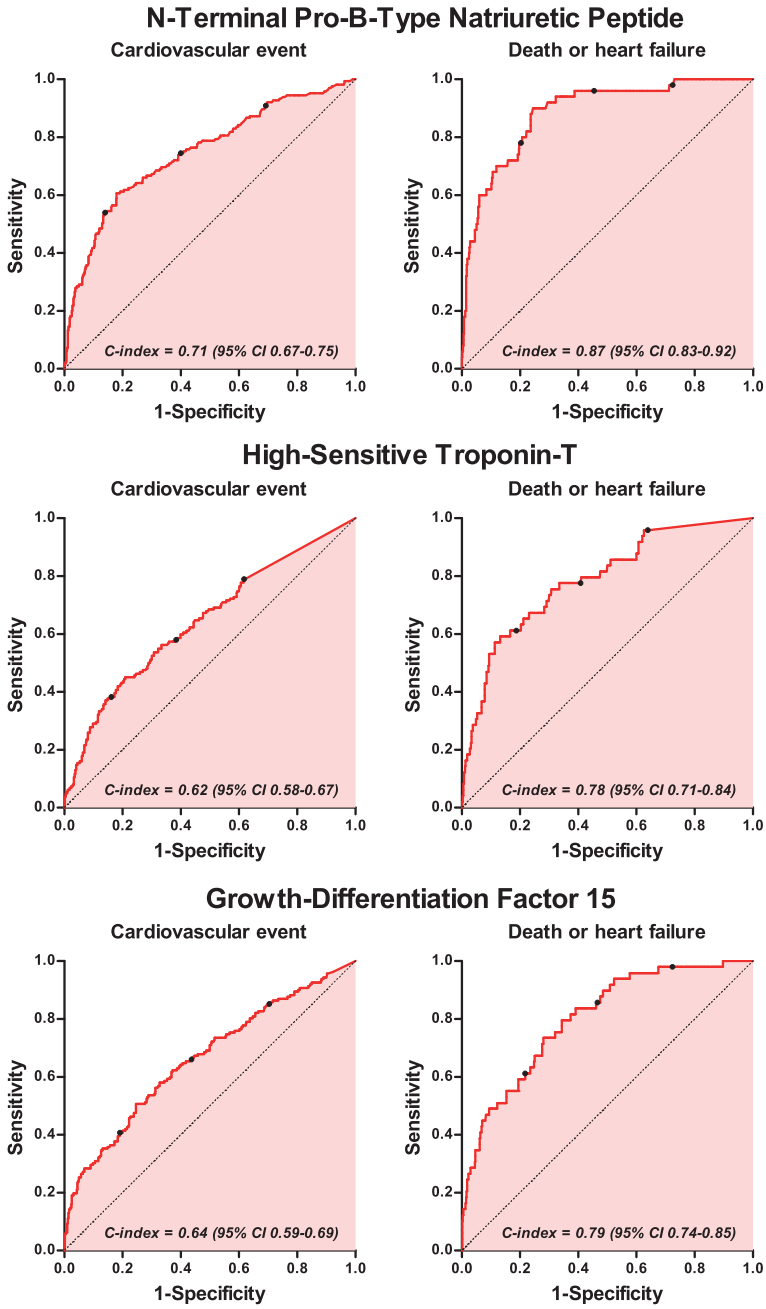
SUPPLEMENTAL FIGURE 4 - Exploratory analysis of tertiary endpoints.

Each component of the primary composite endpoint was separately assessed for NT-proBNP, hs-TnT and GDF-15. Skewed biomarker distributions were logtransformed. Hazard ratios are expressed per one standard deviation increase of the biomarker.



SUPPLEMENTAL FIGURE 5 - Cardiovascular event-free survival and heart failure-free survival, stratified according to the number of elevated biomarkers. 95% confidence intervals are indicated.

No biomarkers elevated (n = 256): NT-proBNP < 14 pmol/L, hs-TnT < 14 ng/L and GDF-15 < 1109 ng/L. *One biomarker elevated (n = 234):* NT-proBNP > 14 pmol/L (n = 217) or hs-TnT > 14 ng/L (n = 4) or GDF-15 > 1109 ng/L (n = 13). *Two biomarkers elevated (n = 70)*: NT-proBNP > 14 pmol/L (n = 70) and hs-TnT > 14 ng/L (n = 20) or GDF-15 > 1109 ng/L (n = 50). *Three biomarkers elevated (n = 22)*: NT-proBNP > 14 pmol/L and hs-TnT > 14 ng/L and GDF-15 > 1109 ng/L.



SUPPLEMENTAL FIGURE 6 - Receiver Operating Characteristic-Curves showing the discriminative ability of NT-proBNP, hs-TnT and GDF-15 for the primary composite endpoint (cardiovascular event) and secondary composite endpoint (death or heart failure). The sensitivity and 1-specificity for cut-offs according to quartiles of distribution is indicated.

Chapter 11

The prognostic value of galectin-3 in adults with congenital heart disease

Vivan J.M. Baggen, Annemien E. van den Bosch, Jannet A. Eindhoven, Myrthe E. Menting, Maarten Witsenburg, Judith A.A.E. Cuypers, Eric Boersma, Jolien W. Roos-Hesselink

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ABSTRACT

Background Galectin-3 is an emerging biomarker for risk stratification in heart failure patients. This study aims to investigate the release of galectin-3 and its association with cardiovascular events in patients with adult congenital heart disease (ACHD).

Methods In this prospective cohort study, 602 consecutive patients with ACHD who routinely visited the outpatient clinic were enrolled between 2011 and 2013. Galectin-3 was measured in thaw serum by batch analysis. The association between galectin-3 and a primary endpoint of all-cause mortality, heart failure, hospitalisation, arrhythmia, thromboembolic events, and cardiac interventions was investigated using multivariable Cox models. Reference values and reproducibility were established by duplicate galectin-3 measurements in 143 healthy controls.

Results Galectin-3 was measured in 591 (98%) patients (median age 33 [25–41] years, 58% male, 90% NYHA I). Median galectin-3 was 12.7 [range 4.2–45.7] ng/mL, and was elevated in 7% of patients. Galectin-3 positively correlated with age, cardiac medication use, NYHA class, loss of sinus rhythm, cardiac dysfunction, and NT-proBNP. During a median follow-up of 4.4 [IQR 3.9–4.8] years, the primary endpoint occurred in 195 patients (33%). Galectin-3 was significantly associated with the primary endpoint in the univariable analysis (HR per two-fold higher value 2.05; 95% CI 1.44–2.93, $p < 0.001$). This association was negated after adjustment for NT-proBNP (HR 1.04; 95% CI 0.72–1.49, $p = 0.848$).

Conclusions Galectin-3 is significantly associated with functional capacity, cardiac function, and adverse cardiovascular events in patients with ACHD. Nevertheless, the additive value of galectin-3 to a more conventional risk marker such as NT-proBNP seems to be limited.

INTRODUCTION

The protein galectin-3 belongs to an ancient lectin family, which is characterized by its binding capacity for beta-galactosides. It is present in numerous cell and tissue types, and plays an important role in cell adhesion, cell activation, cell growth and differentiation, and apoptosis. Given this wide variety of biological roles, galectin-3 is known to be involved in a broad range of pathophysiological processes, including cancer, inflammation, and fibrosis.[1] Currently, galectin-3 is in the spotlight as an emerging biomarker in patients with cardiac disease. Circulating levels have been shown to provide prognostic information in patients with acute and chronic heart failure, independently of other established markers such as natriuretic peptides.[2-4] Galectin-3 has even been included in the ACCF/AHA guideline for additive risk stratification of patients with acute and chronic heart failure.[5]

In contrast to the "tsunami" of biomarker research in the heart failure realm,[6] biomarkers are only making their first steps in the clinical management of patients with adult congenital heart disease (ACHD). Natriuretic peptides are now gaining increasing interest in these patients;[7-9] however, data on emerging biomarkers such as galectin-3 are yet sparse. It is unknown whether galectin-3 provides prognostic information in patients with ACHD and whether it could be useful for risk stratification beyond conventional risk markers. This study therefore investigated the release of galectin-3 and its association with cardiovascular events in patients with moderate and complex ACHD. In addition, we established reference values and reproducibility of galectin-3 in healthy controls.

METHODS

Study design and population

In this prospective cohort study, consecutive adults with moderate or complex congenital heart disease[10] who routinely visited our ACHD outpatient clinic were included between April 2011 and April 2013. Exclusion criteria were: age < 18 years, pregnancy, mild cardiac lesion (isolated atrial or ventricular septal defect), kidney failure (creatinine > 200μmol/L), or incapability of understanding and signing informed consent. According to the study protocol, patients underwent physical examination by a cardiologist, 12-lead electrocardiography, echocardiography and venous blood sampling at the day of study inclusion. All patients were structurally followed-up during four years by annual visits to the ACHD outpatient clinic. The study conforms with the principles outlined in the Declaration of Helsinki, was approved by the Erasmus MC medical ethics committee and all subjects gave written informed consent to participate in the study. The study protocol and the echocardiographic image analysis have been described in more detail previously.[9, 11]

Healthy controls

Self-declared healthy volunteers were prospectively recruited through an advertisement. Between January 2014 and December 2014, all participants underwent physical examination, electrocardiography, echocardiography and venous blood sampling on the same day at our outpatient clinic. Details of the study protocol and exclusion criteria have been published previously.[12]

Sample processing and analysis

Venous blood samples were obtained at the day of study inclusion for research purposes only, and decisions regarding patient management were made independently of any biomarker value. Venous blood samples were transferred to the clinical chemistry laboratory of the Erasmus MC within 2 hours. In study patients, serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) was directly measured in the fresh samples using a commercial electrochemiluminescence immunoassay (Roche Diagnostics, Rotkreuz, Switzerland). In both study patients and healthy controls, other serum was aliquoted and stored according to the same protocol at –80 degrees of Celsius until further analysis. In March and April 2017, samples were thawed in batches. Galectin-3 was directly measured on the ARCHITECT *ci*8200 analyser (Abbott Diagnostics, Hoofddorp, the Netherlands). Samples were subjected to a maximum of one freeze-thaw cycle. In study patients, we performed one galectin-3 measurement per patient. In healthy controls, we performed two galectin-3 measurements from the same aliquot in order to assess reproducibility of the galectin-3 assay in our centre. The ARCHITECT galectin-3 assay is designed to have a limit of quantitation of ≤ 4.0 ng/mL, and an imprecision of ≤ 10% total coefficient of variation for samples with galectin-3 concentrations ranging from 4.0 to 114.0 ng/mL.

Definition and assessment of events

We defined the primary endpoint prior to the collection of data as a composite of the following adverse (cardiovascular) events: all-cause mortality, heart failure (requiring initiation or change in heart failure medication, or requiring hospital admission), hospitalisation for cardiac reasons, arrhythmia (symptomatic and recorded, or requiring treatment), thromboembolic events (ischemic cerebrovascular accident, pulmonary embolism or myocardial infarction), and/or cardiac interventions (surgical or percutaneous). The secondary endpoint was defined as a composite of all-cause mortality and/or heart failure.

Patients were prospectively and systematically followed for fatal and non-fatal events by a yearly clinical evaluation at our ACHD outpatient department until August

1, 2016. Survival status was also checked in the Municipal Population Register. Suspect endpoint events were adjudicated by two experienced investigators (VB and JR) without knowledge of biomarker levels.

Statistical analysis

Continuous patient characteristics are presented for the total cohort and per quartile of galectin-3 distribution as mean ± standard deviation or median [interquartile range], depending on the distribution of data. Comparisons across galectin-3 quartiles were performed using the Chi-Square Mantel-Haenszel test for trend (for categorical variables) or linear regression (for continuous variables). Galectin-3 had a skewed distribution and was therefore $\log_2$ transformed for further analysis.

Reference values for galectin-3 were stratified for sex and calculated as the 97.5th percentile of the distribution in the healthy controls. Reproducibility of the galectin-3 assay was assessed by the R^2, Bland-Altman plots with corresponding limits of agreement (mean difference of two repeated measurements ± 1.96 standard deviation), and coefficient of variation which was calculated as the standard deviation of the differences of two measurements divided by the mean of two measurements *100%.

Primary endpoint-free survival was defined as the time from study inclusion to the occurrence of the first event. Patients without any cardiovascular event were censored at the end of the follow-up duration. Survival curves were derived by the Kaplan-Meier method and stratified for galectin-3 quartile; quartiles were compared using the log-rank test for trend. Cox regression was performed to investigate the association between continuous galectin-3 and the primary and secondary endpoint. We conducted multivariable analyses with adjustment for age, sex, congenital diagnosis, New York Heart Association (NYHA) functional class, cardiac medication use, rhythm, systemic ventricular function, and NT-proBNP. Data on NT-proBNP was 99% complete; imputation of the mean was used to account for missing data. All other covariates were 100% complete. As a post-hoc analysis, we stratified patients according to the number of elevated biomarkers (NT-proBNP and/or galectin-3). The hazard ratios were estimated using Cox regression, by analyzing the number of elevated biomarkers as a categorical variable, with 'no biomarkers elevated' as the reference group.

Data analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Two-sided *p*-values of < 0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Of the 602 patients with moderate to complex ACHD who were included in the cohort, a galectin-3 measurement was available in 591 patients (98%). A flowchart of the patient selection process is provided in Supplemental File 1. The median age was 33 [interquartile range (IQR) 25–41] years and 343 (58%) were men. Surgical repair was performed in 539 patients (91%) at young age (3.8 [IQR 0.8–12.0] years). The majority of patients were in NYHA class I (90%).

Median galectin-3 was 12.7 [IQR 10.9–14.8, range 4.2–45.7] ng/mL. Galectin-3 was elevated (> 97.5th percentile of the reference population) in 16 of the 248 women (6.5%) and in 24 of the 343 men (7.0%). Patients in the highest galectin-3 quartile were significantly older and underwent surgical repair at older age. In the highest quartile, a larger proportion of patients was female, had a complex congenital diagnosis, used cardiac medication, had a low oxygen saturation, and was in NYHA class II–III. In addition, higher galectin-3 was associated with loss of sinus rhythm, worse systemic ventricular function, lower E' wave and higher E/E' ratio, and higher NT-proBNP. Baseline characteristics are further detailed in Table 1. Galectin-3 levels are presented per congenital diagnostic group in Figure 1. This shows that the highest mean galectin-3 levels were present in patients with pulmonary hypertension or a functionally univentricular heart.

Reference values and reproducibility

A total of 147 healthy volunteers were included. Galectin-3 measurements were performed twice in 143 volunteers (Supplemental File 1). In this cohort, galectin-3 was not significantly associated with age ($r = 0.137$, $p = 0.102$) but was significantly higher in women than in men (12.8 ± 3.2 versus 11.5 ± 2.6 ng/mL, $p = 0.007$). Age and sex specific galectin-3 levels are further detailed in Supplemental File 2. The 97.5th percentile of the distribution was 21.3 ng/mL in women and 16.9 ng/mL in men, which was used as a cut-off to describe elevated levels of galectin-3 in the study population. Since galectin-3 was not significantly associated with age in the healthy controls, we did not use age-specific cut-off values. The reference values that were found in this study are compared with previously published studies in Supplemental File 3.[13-17] Reproducibility was excellent, with limits of agreement of –1.1–1.0 ng/mL and a COV of 4.5% (Supplemental File 4).

TABLE 1 - Baseline characteristics of the study population.

		Galectin-3 quartiles				
	All (n = 591)	**Q1 (< 10.9 ng/mL, n = 153)**	**Q2 (10.9–12.7 ng/mL, n = 147)**	**Q3 (12.7–14.8 ng/mL, n = 148)**	**Q4 (≥ 14.8 ng/mL, n = 143)**	***P* for trend**
Clinical characteristics						
Age, years	33 [25–41]	30 [22–38]	31 [24–39]	33 [28–43]	37 [26–49]	< 0.001
Sex, male n (%)	343 (58)	114 (75)	88 (60)	83 (56)	58 (41)	< 0.001
Surgical repair, n (%)	539 (91)	135 (88)	136 (93)	132 (89)	136 (95)	0.099
Age at surgical repair, years	3.8 [0.8–12.0]	2.4 [0.6–9.4]	2.7 [0.7–12.4]	5.0 [1.1–12.2]	5.7 [1.1–14.8]	0.002
Congenital diagnosis, n (%)*	324 (55)	76 (50)	77 (52)	80 (54)	91 (64)	0.018
Cardiac medication use, n (%)†	211 (36)	39 (25)	33 (22)	60 (41)	79 (55)	< 0.001
Body mass index, kg/m^2	24.8 ± 4.4	24.0 ± 4.0	24.7 ± 3.8	25.1 ± 4.5	25.2 ± 5.1	0.009
Heart rate, beats/minute	74 ± 13	74 ± 15	73 ± 12	73 ± 13	74 ± 14	0.719
Systolic blood pressure, mmHg	126 ± 16	127 ± 15	126 ± 16	126 ± 17	126 ± 17	0.571
O_2 saturation < 90%, n (%)	17 (3)	2 (1)	0 (0)	3 (2)	12 (8)	< 0.001
NYHA class, II–III n (%)	61 (10)	7 (5)	13 (9)	13 (9)	28 (20)	< 0.001
Electrocardiogram						
Rhythm, n (%)						0.007
Sinus rhythm	510 (86)	135 (88)	135 (92)	128 (86)	112 (78)	
Paced rhythm	44 (7)	8 (5)	9 (6)	13 (9)	14 (10)	
Other	37 (6)	10 (7)	3 (2)	7 (5)	17 (12)	
QRS duration, ms	113 [100–137]	113 [101–135]	110 [99–130]	112 [101–141]	119 [99–145]	0.351

TABLE 1 - Continued.

	All (n = 591)	Galectin-3 quartiles Q1 (< 10.9 ng/mL, n = 153)	Q2 (10.9–12.7 ng/mL, n = 147)	Q3 (12.7–14.8 ng/mL, n = 148)	Q4 (≥ 14.8 ng/mL, n = 143)	*P* for trend
Echocardiogram						
LA volume, mL/m²‡	21 [15–29]	21 [17–28]	21 [15–30]	19 [14–27]	24 [16–35]	0.069
LV end-diastolic volume, mL/m²‡	63 ± 19	66 ± 19	62 ± 15	59 ± 17	67 ± 24	0.543
LV ejection fraction, %‡	56 ± 8	56 ± 7	57 ± 6	56 ± 9	54 ± 9	0.040
RV end-diastolic annulus, mm	42 ± 8	42 ± 8	42 ± 8	42 ± 9	44 ± 8	0.260
RV fractional area change, %	38 ± 11	40 ± 10	38 ± 11	39 ± 12	36 ± 12	0.093
Systemic ventricular function, n (%)						0.001
Normal	297 (50)	84 (55)	82 (56)	70 (47)	61 (43)	
Mildly impaired	207 (35)	56 (37)	47 (32)	49 (33)	55 (38)	
Moderately impaired	69 (12)	11 (7)	17 (11)	22 (15)	19 (13)	
Severely impaired	18 (3)	2 (1)	1 (1)	7 (5)	8 (6)	
E/A ratio	1.6 ± 0.7	1.6 ± 0.7	1.7 ± 0.7	1.6 ± 0.6	1.7 ± 0.7	0.756
E' wave, cm/s	8.2 ± 2.6	8.8 ± 2.7	8.7 ± 2.5	7.7 ± 2.2	7.5 ± 2.8	< 0.001
E/E' ratio	11.6 ± 5.1	10.5 ± 4.5	10.5 ± 3.3	12.3 ± 5.4	13.4 ± 6.2	< 0.001
Laboratory results						
Creatinine, µmol/L	77 ± 18	76 ± 13	75 ± 13	76 ± 13	81 ± 28	0.018
NT-proBNP, pmol/L	15.3 [6.9–33.3]	11.5 [5.7–23.2]	12.3 [6.2–24.3]	16.1 [6.8–36.0]	28.7 [12.0–60.8]	< 0.001

Values are reported as median [IQR], otherwise as n (%) or mean ± standard deviation. Differences across galectin-3 quartiles are analysed using the Chi-Square Mantel-Haenszel test for categorical variables, otherwise using linear regression. *Congenital diagnosis of aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV, univentricular heart or pulmonary arterial hypertension (1). †ACE-inhibitor (n = 88, 15%), angiotensin receptor blocker (n = 36, 6%), beta blocker (n = 90, 15%), diuretic (n = 71, 12%), anti-arrhythmic (n = 53, 9%). ‡Left sided volumes were not measured in patients with a systemic right ventricle, functionally univentricular heart, pulmonary hypertension (n = 137) or a poor acoustic window. **Abbreviations:** ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LA, left atrial; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RV, right ventricular.

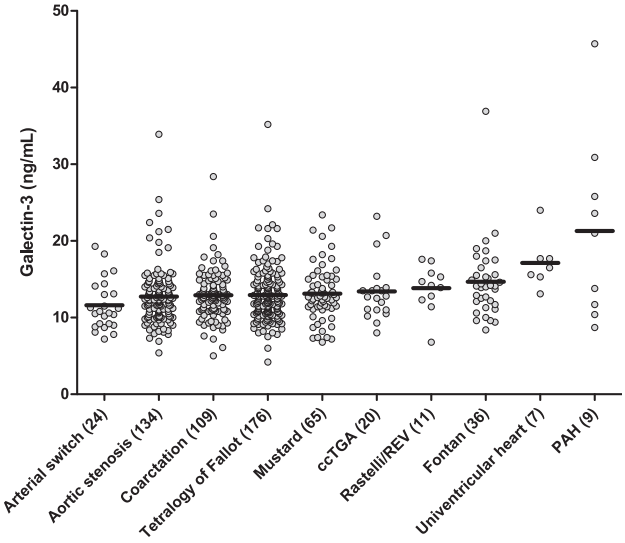

FIGURE 1 - Galectin-3 levels in the different congenital diagnostic groups.
The mean level in each diagnostic group is indicated with a black line.

Follow-up

Survival status and detailed follow-up data were available in 586 patients (99.2%). After a median of 4.4 [IQR 3.9–4.8] years of prospective follow-up, the primary endpoint occurred in 195 patients (33%) and the secondary endpoint occurred in 58 patients (10%). The separate components of the primary endpoint (in which patients were not censored at the time of another endpoint than the endpoint of interest) were all-cause death (n = 16), heart failure (n = 52), hospitalisation for cardiac reasons (n = 148), arrhythmia (n = 110), thromboembolic event (n = 24), and cardiac intervention (n = 111).

Association between galectin-3 and clinical outcomes

Cumulative endpoint-free survival curves stratified per galectin-3 quartile are presented in Figure 2, showing that patients in the fourth quartile were clearly at highest risk of both the primary and secondary endpoint. As is shown in Table 2, higher levels of galectin-3 were also significantly associated with the primary and secondary endpoint when analysed continuously.

Multivariable adjustment for age and sex attenuated the results, but galectin-3 remained significantly associated with the study endpoints. After full adjustment for age, sex, clinical characteristics and NT-proBNP, the associations between galectin-3 and the primary and secondary endpoint were no longer significant. Adjustment for NT-proBNP only yielded similar nonsignificant results (Table 2).

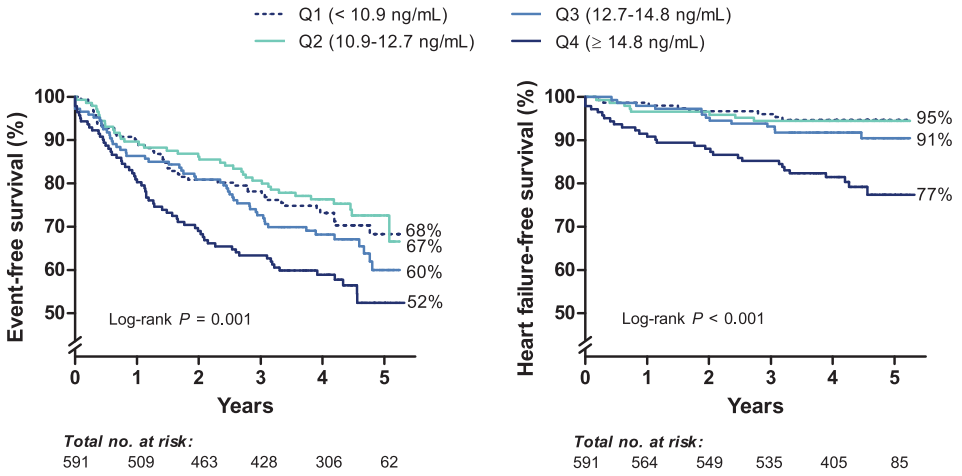

FIGURE 2 - Cardiovascular event-free survival and heart failure-free survival, stratified according to quartiles of galectin-3 distribution.

Q1, first quartile (n = 153); Q2, second quartile (n = 147); Q3, third quartile (n = 148); Q4, fourth quartile (n = 143).

TABLE 2 - Univariable and multivariable associations of galectin-3 with the primary endpoint (cardiovascular event) and secondary endpoint (death or heart failure).

	HR per two-fold higher value	95% CI	*P*-value
Cardiovascular event			
Galectin-3 (univariable)	2.05	1.44–2.93	< 0.001
Adjusted for age and sex	1.59	1.09–2.33	0.017
Adjusted for NT-proBNP	1.04	0.72–1.49	0.848
Full model including all covariates*	0.84	0.58–1.22	0.354
Death or heart failure			
Galectin-3 (univariable)	6.01	3.51–10.3	< 0.001
Adjusted for age and sex	3.19	1.69–6.03	< 0.001
Adjusted for NT-proBNP	1.47	0.79–2.72	0.222
Full model including all covariates*	1.00	0.52–1.91	0.991

*Adjusted for age, sex, congenital diagnosis, cardiac medication use, NYHA class II–III, sinus rhythm, systemic ventricular function, and NT-proBNP. **Abbreviations:** CI, confidence interval; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.

As a post-hoc analysis, we divided patients in three groups: both NT-proBNP and galectin-3 low (n = 265), either NT-proBNP *or* galectin elevated (n = 291), and both NT-proBNP and galectin-3 elevated (n = 31). In Figure 3, the Kaplan-Meier curves stratified according to these three groups are presented. The small group of 31 patients with both elevated levels of NT-proBNP and galectin-3 were clearly at highest risk of adverse events.

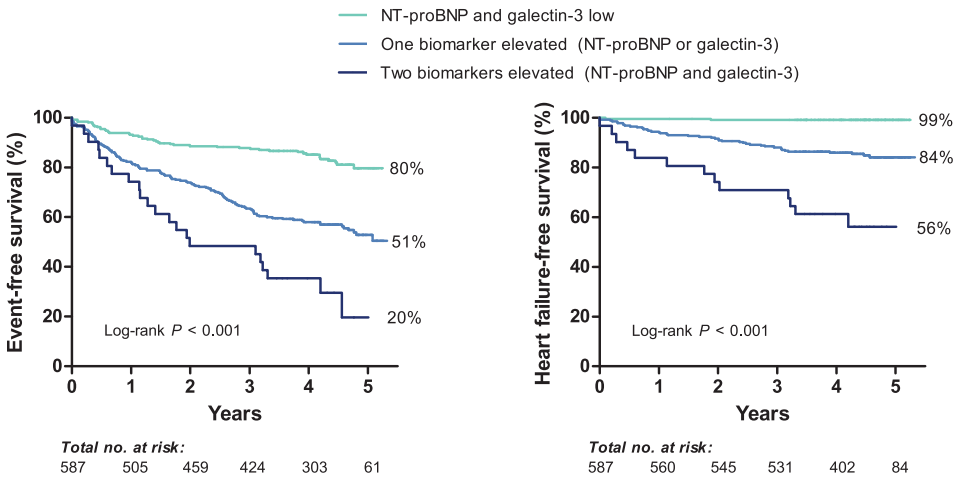

FIGURE 3 - Cardiovascular event-free survival and heart failure-free survival, stratified according to the number of elevated biomarkers.

No biomarkers elevated (n = 265): NT-proBNP < 14 pmol/L and galectin-3 < 21.3 ng/mL in women and < 16.9 ng/mL in men. *One biomarker elevated (n = 291):* NT-proBNP > 14 pmol/L (n = 282) or galectin-3 > 21.3 ng/mL in women and > 16.9 ng/mL in men (n = 9). *Two biomarkers elevated (n = 31):* NT-proBNP > 14 pmol/L and galectin-3 > 21.3 ng/mL in women and > 16.9 ng/mL in men.

TABLE 3 - Risk of the primary endpoint (cardiovascular event) and secondary endpoint (death or heart failure) according to the number of elevated biomarkers.

		Combination of NT-proBNP and galectin-3			
	All (n = 587)	**No biomarkers elevated (n = 265)**	**One biomarker elevated (n = 291)**	**Two biomarkers elevated (n = 31)**	***P*-value***
Cardiovascular event					
No. Cases	195	45	128	22	
Person-years	2025	1022	928	75	
Crude HR (95% CI)		Reference	3.05 (2.17–4.29)	6.14 (3.68–10.2)	0.003
Adjusted HR (95% CI)†		Reference	2.21 (1.51–3.24)	2.25 (1.24–4.08)	0.940
Death or heart failure					
No. Cases	58	2	43	13	
Person-years	2407	1143	1165	98	
Crude HR (95% CI)		Reference	21.0 (5.08–86.6)	70.0 (15.8–310)	< 0.001
Adjusted HR (95% CI)†		Reference	7.80 (1.80–33.8)	9.11 (1.88–44.3)	0.662

Elevated galectin-3 was defined as > 21.3 ng/mL in women and > 16.9 ng/mL in men, based on the 97.5th percentile of the distribution in our healthy controls. Elevated NT-proBNP was defined as > 14 pmol/L. *The last two columns are compared (one versus two biomarkers elevated). †Adjusted for age, sex, congenital diagnosis, cardiac medication use, NYHA class II–III, sinus rhythm, and systemic ventricular function. **Abbreviations:** CI, confidence interval; HR, hazard ratio, NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 3 also shows that patients with two elevated biomarkers (both NT-proBNP and galectin-3) carry a significantly and substantially higher risk of adverse events compared with patients with only one elevated biomarker. However, because of the small size of the group with two biomarkers, the exact numbers should be interpreted with caution. Nevertheless, after adjustment for clinical variables, the difference between these two groups was no longer significant.

DISCUSSION

This is the first large and prospective study that investigates the levels of galectin-3 and its association with clinical characteristics and outcomes in patients with ACHD. Galectin-3 was elevated in only 7% of the study population and was highest in patients with pulmonary hypertension or a functionally univentricular heart. In addition, higher galectin-3 was related to higher age, cardiac medication use, higher NYHA class, loss of sinus rhythm, ventricular dysfunction (both systolic and diastolic), and higher NT-proBNP. Galectin-3 was significantly associated with both the primary endpoint (cardiovascular events) and the secondary endpoint (death or heart failure) in the univariable analysis. However, galectin-3 was not significantly associated with study endpoints after adjustment for NT-proBNP.

Comparison with previous studies

Previous reports have also indicated that galectin-3 probably shares some overlapping information with other variables, especially with NT-proBNP. In two studies in patients with chronic heart failure and a reduced left ventricular ejection fraction, galectin-3 was significantly associated with cardiac events in the univariable analysis. However, this association did not persist after adjustment for other predictors such as NT-proBNP.[18, 19] In contrast, three other studies in patients with heart failure reported that galectin-3 was associated with clinical outcomes independently of natriuretic peptides.[2-4] These differences may depend on the patient population that was investigated. For instance, two of these three studies investigated patients during hospital admission for acute heart failure.[2, 4] The mean left ventricular ejection fraction in two studies was clearly higher (≥ 40%) compared with the studies in which galectin-3 did not provide additional information on top of NT-proBNP.[2, 3] This could be an important difference because de Boer et al.[4] showed that galectin-3 might be more useful in patients with a preserved ejection fraction: while the average galectin-3 levels were comparable between patients with a reduced and a preserved ejection fraction, an identical rise in galectin-3 levels was associated with a much stronger increase in the risk of death or rehospitalisation in patients with a preserved ejection fraction. In addition, differences in sample processing,

storage, and analysis, differences in disease severity (as reflected by varying mortality rates and median galectin-3 levels), and the number and type of other covariates that were included in the multivariable analyses could influence the results.

To our knowledge, there is only one study that previously investigated the association between galectin-3 and clinical outcome in adult congenital patients. In 70 ambulatory adult Fontan patients, Opotowsky et al. showed that galectin-3 levels were associated with adverse outcomes.[20] In this study, NT-proBNP was not measured and the association of galectin-3 with adverse outcomes was adjusted one by one for age, NYHA functional class, estimated glomerular filtration rate, C-reactive protein and the presence of chronic comorbidities.

Galectin-3 release in healthy controls

Whereas galectin-3 was positively associated with age in our patients with ACHD, it was not associated with age in the healthy controls. Higher galectin-3 values may therefore be explained by the disease progression that occurs in the aging ACHD patients, rather than the increase in age itself. This implies that age-specific reference values are probably not required. Both in study patients and in healthy controls we found higher galectin-3 values in women, which was also previously reported in healthy controls[14, 16, 21] and in heart failure patients.[19] However, the data is conflicting with regard to this point as some other studies have reported similar or even higher galectin-3 values in men.[15, 17] Although the age groups in our study were small, the differences between men and women were only significant in the healthy controls aged 30–50 years. A previous study with in-vitro endometrial cells showed that galectin-3 was upregulated by estradiol and progesterone.[22] Because these sex-specific hormonal differences are attenuated after the menopause, differences in baseline characteristics with regard to age may partially explain the conflicting results.

Clinical perspectives

Although most biomarker studies focus on the utility of a single biomarker measurement in diagnosis and risk stratification, other important clinical applications of biomarkers are monitoring of disease progression and guidance of therapy in heart failure management. Galectin-3 is thought to be involved in a different pathophysiological axis than natriuretic peptides. It is mainly related to changes in the extracellular matrix, leading to cardiac and non-cardiac fibrosis.[23] In our study, galectin-3 was significantly associated with several echocardiographic parameters of diastolic dysfunction, which was also found in other studies.[24] Galectin-3 has also been directly related to ventricular fibrosis as assessed by late gadolinium enhancement in patients with non-ischemic dilated cardiomyopathy.[25] In addition, repeated measurements of galectin-3 have

previously been shown to provide prognostic value in patients with heart failure.[26, 27] Interestingly, the normal intra-individual biological variability of galectin-3 is much lower compared with brain natriuretic peptide[28] or NT-proBNP.[29] It may therefore be worthwhile to evaluate if repeated galectin-3 measurements can be used to monitor ongoing ventricular remodelling, and if these are more useful than NT-proBNP to monitor heart failure progression in specific patient subgroups with more ventricular fibrosis and diastolic dysfunction. In addition, galectin-3 might be useful to identify patient groups that possibly benefit from new pharmacologic agents that target fibrosis, as hypothesized previously.[18]

Study limitations

This is a relatively large cohort of patients with ACHD; however, it consists of a heterogeneous group of congenital diagnoses. It is interesting that galectin-3 values are highest among patients with pulmonary hypertension or a functionally univentricular heart, and the prognostic value of galectin-3 may be stronger in these patients subgroups. In addition, although the average galectin-3 levels and the range of the galectin-3 values are roughly comparable between patients with a systemic RV and patients with a systemic LV, the prognostic value of galectin-3 might be different in patients with a systemic RV. Unfortunately, diagnosis-specific analyses are limited by the relatively low sample size within specific diagnostic groups. In addition, in the post-hoc analysis according to the number of elevated biomarkers, the group of patients with both elevated levels of NT-proBNP and galectin-3 was relatively small and the results should therefore be carefully interpreted. Of note, patients with an isolated repaired atrial or ventricular septal defect were not included, because of the expected low number of events. This should be taken into account when extrapolating these results to other cohorts of patients with ACHD.

No data on cardiopulmonary exercise testing was available to include in the current analysis. This may have further attenuated the association of galectin-3 with adverse outcomes, because it is known that exercise testing is of prognostic importance in patients with ACHD.[18]

CONCLUSIONS

In our cohort of patients with ACHD, galectin-3 was elevated in 7% of the study population. Higher galectin-3 level was related to higher age, higher NYHA class, loss of sinus rhythm, ventricular systolic and diastolic dysfunction, and higher NT-proBNP. In addition, galectin-3 was significantly associated with adverse cardiovascular events in the univariable analysis. Nevertheless, the value of galectin-3 incremental to a more conventional risk marker such as NT-proBNP seems to be limited.

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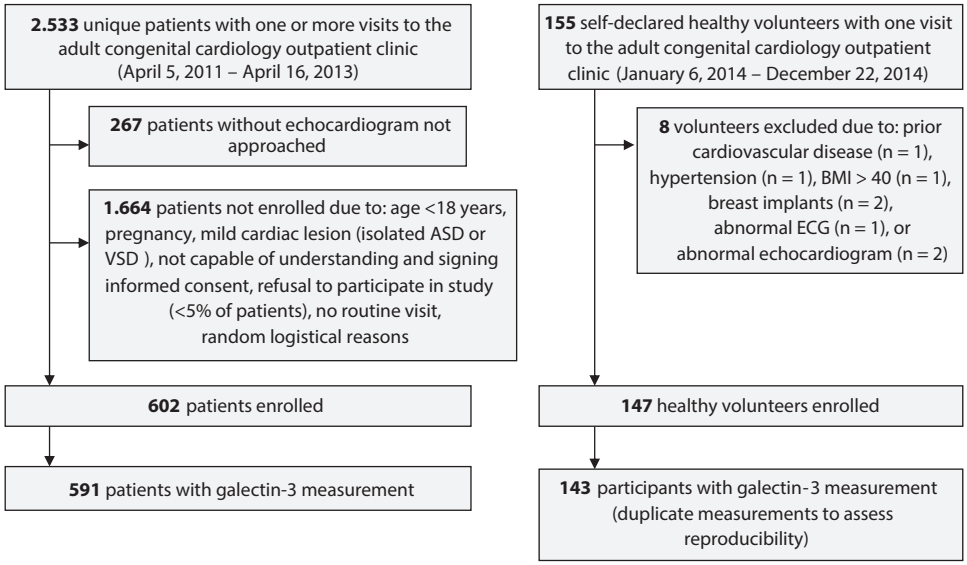
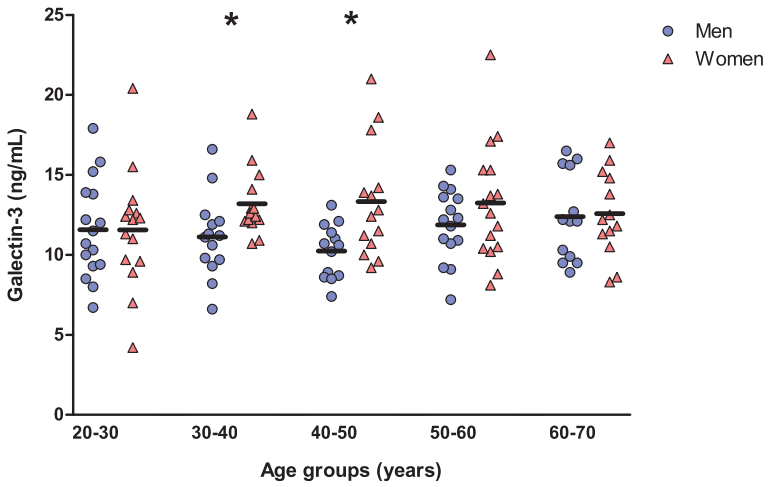
SUPPLEMENTAL MATERIAL



SUPPLEMENTAL FILE 1 - Flowchart of the selection of patients (left panel) and healthy controls (right panel).

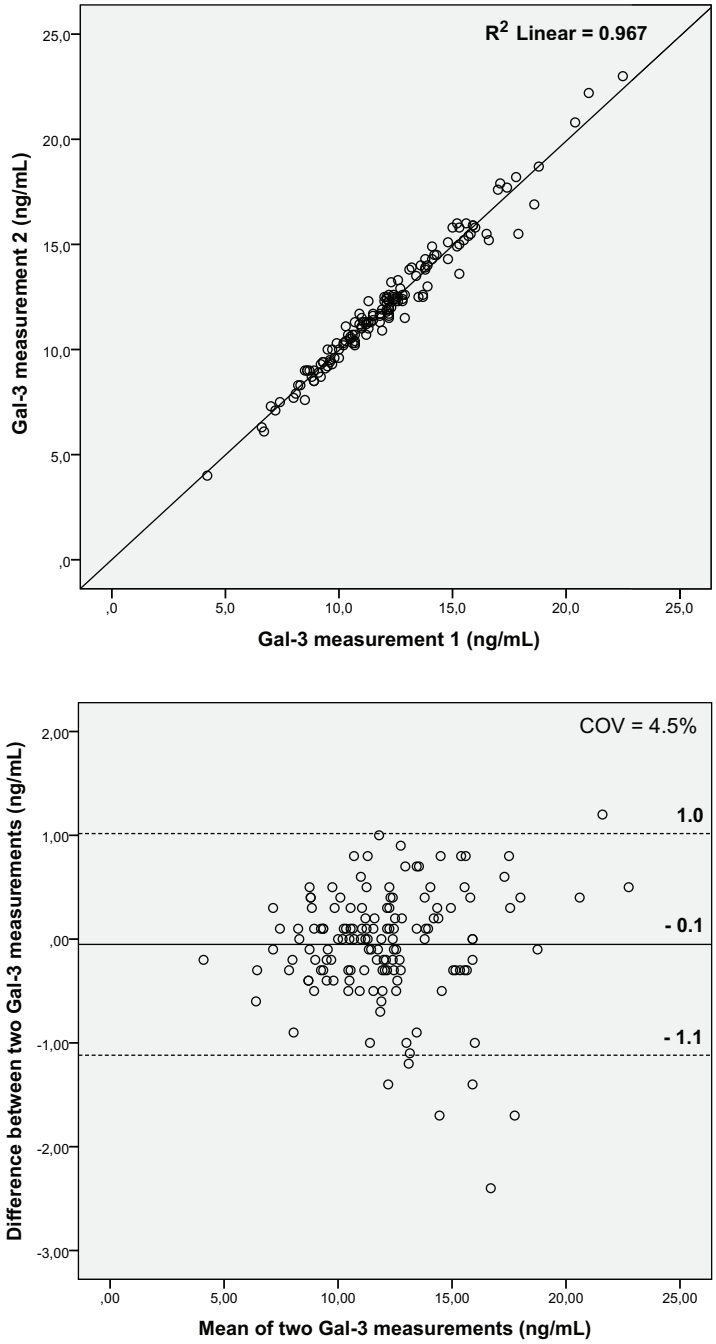


SUPPLEMENTAL FILE 2 - Galectin-3 levels in a healthy control population across different age and sex groups.
The mean level in each subgroup is indicated with a black line. *Two-sided P < 0.05 (Mann-Whitney U test).

SUPPLEMENTAL FILE 3 - Reference values found in this study compared with previously published studies which included > 50 healthy individuals.

Study	n	Analyser	Participants	Specimen	Age, years	Sex, male	97.5% total (ng/mL)	97.5% men (ng/mL)	97.5% women (ng/mL)
Healthy controls (this study)	143	ARCHITECT *ci*8200	Screened healthy volunteers	Frozen serum	44 [33–56]	71 (50)	19.4	16.9	21.3
Krintus 2017[17]	180	ARCHITECT *ci*8200	Screened healthy volunteers	Frozen serum	35 [30–41]	85 (47)	18.1	18.1	18.3
Mueller 2016[16]	402	ARCHITECT *i*2000SR	Blood donors with eGFR >90	Frozen plasma	35 [26–45]	259 (64)	NR	15.8	16.9
Gaze 2014[15]	627	ARCHITECT *i*1000SR, *i*2000SR, *i*4000SR	Apparently healthy individuals	Fresh serum (n = 256) Frozen serum (n = 119) Fresh plasma (n = 122) Frozen plasma (n = 130)	NR	335 (53)	28.4	33.3	27.3
La'ulu 2013[14]	242	ARCHITECT i1000SR, i2000SR	Self-reported healthy individuals	Frozen plasma	19–79 (range)	120 (50)	NR	17.9	19.8
Christenson 2010[13]	1092	two-site ELISA, BG Medicine	Apparently healthy individuals	Frozen plasma	55–80 (range)	520 (48)	22.1	NR	NR

Values are presented as median [IQR] or n (%) unless otherwise indicated. **Abbreviations:** NR, not reported.



SUPPLEMENTAL FILE 4 - Reproducibility of galectin-3 on the ARCHITECT *ci*8200 analyser (Abbott Diagnostics).

Chapter 12

Red cell distribution width in adults with congenital heart disease: a worldwide available and low-cost predictor of cardiovascular events

Vivan J.M. Baggen, Annemien E. van den Bosch, Roland R. van Kimmenade, Jannet A. Eindhoven, Maarten Witsenburg, Judith A.A.E. Cuypers, Frank W.G. Leebeek, Eric Boersma, Jolien W. Roos-Hesselink

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ABSTRACT

Background Red cell distribution width (RDW) is a standard component of the automated blood count, and is of prognostic value in heart failure and coronary heart disease. We investigated the association between RDW and cardiovascular events in patients with adult congenital heart disease (ACHD).

Methods and Results In this prospective cohort study, 602 consecutive patients with ACHD who routinely visited the outpatient clinic were enrolled between 2011 and 2013. RDW was measured in fresh venous blood samples at inclusion in 592 patients (median age 33 [IQR 25–41] years, 58% male, 90% NYHA I) and at four annual follow-up visits. During 4.3 [IQR 3.8–4.7] years of follow-up, the primary endpoint (death, heart failure, hospitalization, arrhythmia, thromboembolic events, cardiac intervention) occurred in 196 patients (33%). Median RDW was 13.4 (12.8–14.1)% versus 12.9 (12.5–13.4)% in patients with and without the primary endpoint ($p < 0.001$). RDW was significantly associated with the endpoint when adjusted for age, sex, clinical risk factors, CRP, and NT-proBNP (HR 1.20; 95% CI 1.06–1.35; $p = 0.003$). The C-index of the model including RDW was slightly, but significantly ($p = 0.005$) higher than the model without (0.74, 95% CI 0.70–0.78 versus 0.73, 95% CI 0.69–0.78). Analysis of repeated RDW measurements (n = 2449) did not show an increase in RDW prior to the occurrence of the endpoint.

Conclusions RDW is associated with cardiovascular events in patients with ACHD, independently of age, sex, clinical risk factors, CRP, and NT-proBNP. This readily available biomarker could therefore be considered as an additive biomarker for risk stratification in these patients.

INTRODUCTION

Red cell distribution width (RDW) is a marker of anisocytosis, which is automatically measured when a complete blood count is requested. RDW is calculated as the coefficient of variation of the red cell volume distribution (standard deviation divided by mean cell volume). A high RDW indicates a greater variation in erythrocyte size, and a low RDW indicates a more homogeneous population of red blood cells.[1] Various distinct pathophysiological mechanisms such as impaired iron mobilization, ineffective erythropoiesis, nutritional deficiencies, decreased hemoglobin level, oxidative stress, and inflammation have been related to an increased RDW.[2-5]

Interestingly, increased RDW has been reported to be closely related to the risk of adverse events in the general population.[6, 7] More specific, it has been shown to be a predictor of cardiovascular morbidity and mortality in patients with acute and chronic heart failure,[2, 8-11] coronary heart disease[12, 13] and pulmonary arterial hypertension.[14] Even an increase in RDW during hospitalization has been related to adverse outcome.[15] Despite these promising data, its current role in clinical practice still pertains to the differential diagnosis of anemia together with the mean cell volume, which was already described in 1983.[16, 17]

The number of patients with adult congenital heart disease (ACHD) is rapidly increasing and although many of these patients have no complaints, the incidence of cardiovascular events and need for (re)interventions is high. Prognostication is an essential component of the routine clinical care of patients with ACHD, and forms the basis of patient information, follow-up management and therapeutic strategies. To our knowledge, it is unknown whether RDW can enhance the prognostication of ACHD patients. The aim of this study was therefore to investigate the association between RDW and cardiovascular events in patients with ACHD. In addition, we evaluated repeated measurements to investigate the changes in RDW level over time.

METHODS

Study design and population

This is a prospective cohort study. We included consecutive adults with a moderate or complex type of congenital heart defect,[18] who routinely visited our ACHD outpatient clinic with an echocardiogram between April 2011 and April 2013. We excluded patients with age < 18 years, pregnancy, a mild cardiac lesion (isolated atrial or ventricular septal defect), not capable of understanding and signing informed consent, or severe kidney disease (estimated glomerular filtration rate < 30 mL/min at baseline). At the day of study inclusion, patients underwent physical examination by a cardiologist, 12-lead electrocardiography, echocardiography and venous blood sampling. All patients were

structurally followed-up during four years by annual visits to the ACHD outpatient clinic, including physical examination by a cardiologist, 12-lead electrocardiography, venous blood sampling, and echocardiography (every two years). During the follow-up, patients were treated in accordance with ESC guidelines.[19] The study protocol was approved by the Erasmus MC medical ethics committee and all participants provided written informed consent. Other details of the study protocol and the echocardiographic image analysis have been described previously.[20, 21]

Laboratory measurements

An automated complete blood count was performed in fresh K2EDTA plasma samples at study inclusion and at the planned yearly follow-up visits in the clinical chemistry laboratory of the Erasmus MC, using a Sysmex XN-1000™ Hematology Analyzer (Sysmex Europe GmbH, Norderstedt, Germany). Up to five subsequent annual measurements per patient were collected. Samples were stored at room temperature and were analyzed within three hours of collection. RDW measurements were performed for research purposes only, and decisions regarding patient management were made independently of RDW measurements. The lower and upper limits of normal for RDW in our lab are 12.0 and 16.0 %, respectively. Anemia was defined as a hemoglobin level of < 139 g/L in men (< 8.6 mmol/L) and < 121 g/L (< 7.5 mmol/L) in women. N-terminal pro-B-type natriuretic peptide (NT-proBNP) was directly measured in fresh serum samples, using a commercial electrochemiluminescence immunoassay (Roche Diagnostics, Rotkreuz, Switzerland). More details have been published previously.[21]

Definition and assessment of events

We defined the primary endpoint prior to the collection of data as a composite of the following (cardiovascular) events: all-cause mortality, heart failure (requiring initiation or change in heart failure medication, or requiring hospital admission), hospitalization for cardiac reasons, arrhythmia (symptomatic and recorded, or requiring treatment), thromboembolic events (ischemic cerebrovascular accident, pulmonary embolism or myocardial infarction), and/or cardiac interventions (surgical or percutaneous). The secondary endpoint was defined as a composite of all-cause mortality and/or heart failure.

According to the study protocol, patients were followed for the occurrence of fatal and non-fatal events by a yearly clinical evaluation at our institution. The end of the follow-up period was set at August 1, 2016. Survival status was also checked in the Municipal Population Register. Suspect endpoint events were adjudicated by two experienced investigators (VB and JR) without knowledge of RDW levels.

Statistical analysis

Patient characteristics were described per quartile of the RDW distribution. Depending on the data distribution, these were presented as mean ± standard deviation or as median [interquartile range (IQR)]. Comparisons across quartiles of the RDW distribution were performed using the Chi-Square Mantel-Haenszel test for trend (for categorical variables) or linear regression (for continuous variables).

For patients with multiple events, event-free survival was defined as the time from enrollment to the occurrence of the first event. Patients without any cardiovascular event were censored at the end of the follow-up duration. Kaplan-Meier endpoint-free survival curves were presented for each RDW quartile separately. Cox regression was performed to investigate the association between baseline RDW and study endpoints. We analyzed RDW both as a categorical variable (in quartiles) and as a continuous variable (RDW was normally distributed and presented per % increase). We also investigated the association of the other components of the automated blood count (hemoglobin, hematocrit and mean cell volume) with the primary and secondary endpoint. Associations were adjusted for age, sex, congenital diagnosis, cardiac medication use, NYHA class, rhythm, and systemic ventricular function. Furthermore, we performed additional adjustment for C-reactive protein and NT-proBNP. Data on NT-proBNP was 99% complete; imputation of the mean was used to account for missing data. All other covariates were 100% complete. We reported crude and adjusted hazard ratios (HR) and their corresponding 95% confidence intervals (CI).

In order to evaluate the potential added value of RDW for risk prediction, we determined C-statistics of models with and without RDW as a predictor. Models were compared using the likelihood ratio test.[22]

We developed linear mixed-effects (LME) models to analyze the temporal pattern of RDW throughout the follow-up, while accounting for the correlation between subsequent RDW measurements within individuals. The correlations in the repeated RDW measurements were modeled using a random intercept and a linear random slopes term. Within-subject variation was expressed as residual variance / total variance * 100%. Between-subject variation calculated as (total variance – residual variance) / total variance * 100%. We evaluated differences in temporal evolvement of RDW between patients with and without the study endpoints by LME models including a time*endpoint interaction term in the fixed part of the model. Because the temporal RDW evolution was similar in patients with and without study endpoints, we did not apply joint modeling to obtain hazard ratios for these relations.

Data analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA) and using the survC1, nlme and JMBayes packages in R statistical software, version 3.3.2 (available at: www.r-project.org).[23, 24] Two-sided *p*-values < 0.05 were considered statistically significant.

RESULTS

Study cohort

Of the 602 patients with moderate to complex ACHD who were included in the cohort, a baseline RDW measurement was available in 592 patients. None of the patients had to be excluded because of severe kidney disease at baseline. The median age at inclusion was 33 [IQR 25–41] years and 342 (58%) were male. Surgical repair was performed in 537 patients (91%) at young age (3.8 [IQR 0.8–11.9] years). The majority of patients were in NYHA class I (90%). Anemia was present in 28 patients (5%). Other baseline characteristics are described in Table 1.

Association of RDW with patient characteristics

Median RDW was 13.1 [IQR 12.7-13.7, range 11.3–20.7]%. The majority of baseline RDW measurements was within the normal range: only 17 patients (3%) had a RDW < 12.0% and only 15 patients (3%) had a RDW > 16.0%. In the highest RDW quartile, patients were significantly older and underwent surgical repair at an older age. Moreover, a larger proportion was female, had a complex congenital diagnosis, used cardiac medication, had a low oxygen saturation and was in NYHA class II–III. In addition, in the highest RDW quartile a larger proportion of patients had loss of sinus rhythm, and patients had a worse systemic ventricular function and higher NT-proBNP levels. Baseline characteristics across quartiles of RDW distribution are further detailed in Table 1. RDW levels were higher in patients with pulmonary hypertension, a functionally univenticular heart or a congenitally corrected transposition of the great arteries (Supplemental File 1).

Follow-up

Survival status according to the Municipal Population Register and detailed follow-up data regarding non-fatal events were available in 588 patients (99.3%). After a median of 4.3 [3.8–4.7] years of prospective follow-up, the primary endpoint occurred in 196 patients (33%) and the secondary endpoint occurred in 57 patients (10%). The components of the primary endpoint are separately displayed in Table 2.

Relation between baseline RDW and study endpoints

Median RDW was 13.4 (12.8–14.1)% versus 12.9 (12.5–13.4)% in patients with and without the primary endpoint, respectively ($p < 0.001$). Median RDW was 13.9 (13.4–15.0)% versus 12.9 (12.5–13.5)% in patients with and without the secondary endpoint, respectively ($p < 0.001$). The cumulative RDW distribution in patients with and without study endpoints is depicted in Supplemental File 2.

TABLE 1 - Baseline characteristics of the study population.

	All (n = 592)	Baseline RDW quartiles (n = 592)				*P* for trend
		Q1 (<12.7%, n=173)	Q2 (12.7-13.1%, n=137)	Q3 (13.1-13.7%, n=149)	Q4 (≥13.7%, n=133)	
Clinical characteristics						
Age, years	33 [25–41]	31 [24–39]	30 [23–39]	33 [26–45]	37 [28–48]	< 0.001
Sex, male, n (%)	342 (58)	112 (65)	85 (62)	88 (59)	57 (43)	< 0.001
Surgical repair, n (%)	537 (91)	156 (90)	123 (90)	133 (89)	125 (94)	0.349
Age at surgical repair, years	3.8 [0.8–11.9]	1.9 [0.5–9.2]	2.8 [0.5–11.7]	4.2 [1.2–12.8]	7.4 [2.6–17.8]	< 0.001
Congenital diagnosis, n (%)[a]	318 (54)	90 (52)	57 (42)	83 (56)	88 (66)	0.004
Cardiac medication use, n (%)	211 (36)	36 (21)	35 (26)	65 (44)	75 (56)	< 0.001
Body mass index, kg/m2	24.8 ± 4.4	24.1 ± 3.7	24.8 ± 4.2	24.6 ± 4.3	25.8 ± 5.2	0.001
Heart rate, beats/minute	74 ± 13	73 ± 14	73 ± 13	73 ± 13	75 ± 13	0.165
Systolic blood pressure, mmHg	126 ± 16	127 ± 15	126 ± 15	126 ± 15	126 ± 20	0.619
O_2 saturation < 90%, n (%)	17 (3)	2 (1)	0 (0)	1 (1)	14 (11)	< 0.001
NYHA class, II–III, n (%)	59 (10)	7 (4)	4 (3)	17 (11)	31 (23)	< 0.001
Electrocardiogram						
Rhythm, n (%)						< 0.001
Sinus rhythm	514 (87)	161 (93)	125 (91)	123 (83)	105 (79)	
Paced rhythm	43 (7)	6 (3)	6 (4)	17 (11)	14 (11)	
Other	35 (6)	6 (3)	6 (4)	9 (6)	14 (11)	
QRS duration, ms	112 [99–137]	112 [102–137]	106 [96–126]	114 [100–147]	118 [98–141]	0.205
Echocardiogram						
LA volume, mL/m2[b]	21 [16–29]	20 [14–27]	20 [15–30]	21 [15–28]	24 [19–40]	< 0.001
LV end-diastolic volume, mL/m2[b]	64 ± 19	63 ± 16	65 ± 18	62 ± 19	63 ± 22	0.733
LV ejection fraction, %[b]	56 ± 8	57 ± 6	56 ± 8	56 ± 8	55 ± 10	0.119

TABLE 1 - Continued.

	All (n = 592)	Baseline RDW quartiles (n = 592)				*P* for trend
		Q1 (<12.7%, n=173)	Q2 (12.7-13.1%, n=137)	Q3 (13.1-13.7%, n=149)	Q4 (≥13.7%, n=133)	
Echocardiogram						
RV end-diastolic annulus, mm	42 ± 8	42 ± 8	41 ± 8	42 ± 9	44 ± 9	0.104
RV fractional area change, %	38 ± 11	39 ± 11	38 ± 11	38 ± 12	37 ± 13	0.338
Systemic ventricular function, n (%)						< 0.001
Normal	299 (51)	94 (54)	81 (59)	66 (44)	58 (44)	
Mildly impaired	208 (35)	63 (36)	39 (28)	58 (39)	48 (36)	
Moderately impaired	67 (11)	15 (9)	15 (11)	17 (11)	20 (15)	
Severely impaired	18 (3)	1 (1)	2 (2)	8 (5)	7 (5)	
E/A ratio	1.6 ± 0.7	1.8 ± 0.7	1.7 ± 0.7	1.5 ± 0.6	1.5 ± 0.6	0.001
E' wave, cm/s	8.3 ± 2.7	8.8 ± 2.8	8.6 ± 2.7	8.1 ± 2.4	7.2 ± 2.5	< 0.001
E/E' ratio	11.6 ± 5.1	11.1 ± 4.4	10.8 ± 4.8	11.3 ± 4.2	13.7 ± 6.7	0.001
Laboratory results						
Hemoglobin, g/L[c]	149 ± 16	151 ± 11	149 ± 12	149 ± 14	145 ± 24	0.003
Hematocrit, L/L	0.44 ± 0.04	0.44 ± 0.03	0.44 ± 0.03	0.44 ± 0.03	0.44 ± 0.06	0.381
Mean cell volume, fL	88 ± 5	88 ± 4	88 ± 4	88 ± 4	87 ± 7	0.077
Creatinine, µmol/L	77 ± 18	77 ± 13	77 ± 15	75 ± 14	79 ± 28	0.432
eGFR, mL/min	90 [82–90]	90 [85–90]	90 [84–90]	90 [82–90]	87 [75–90]	< 0.001
C-reactive protein, mg/L	1.5 [0.6–3.5]	1.0 [0.5–2.5]	1.3 [0.5–3.3]	1.3 [0.5–3.4]	2.5 [1.3–6.3]	< 0.001
Total cholesterol, mmol/L	4.8 ± 1.0	4.6 ± 0.9	4.8 ± 0.9	4.9 ± 1.1	4.8 ± 1.1	0.050
Low density lipoprotein, mmol/L	3.0 ± 0.9	2.8 ± 0.8	3.0 ± 0.8	3.1 ± 1.0	3.0 ± 1.0	0.032
NT-proBNP, pmol/L	15.0 [6.8-33.4]	10.1 [5.5–21.7]	11.7 [5.2–27.3]	16.6 [7.9–31.3]	31.1 [11.9–70.8]	< 0.001

Values are reported as median [IQR], otherwise as n (%) or mean ± SD. Differences across baseline RDW quartiles are analyzed using the Chi-Square Mantel-Haenszel test for categorical variables, otherwise using linear regression. [a]Congenital diagnosis of aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV, univentricular heart or pulmonary arterial hypertension (1). [b]Left sided volumes were not measured in patients with a systemic right ventricle, functionally univentricular heart, pulmonary hypertension (n = 137) or a poor acoustic window. [c]Hemoglobin was converted from mmol/L to g/L by using the conversion factor 0.06202. **Abbreviations:** ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers; eGFR, estimated glomerular filtration rate; LA, left atrial; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RDW, Red blood cell distribution width; RV, right ventricular.

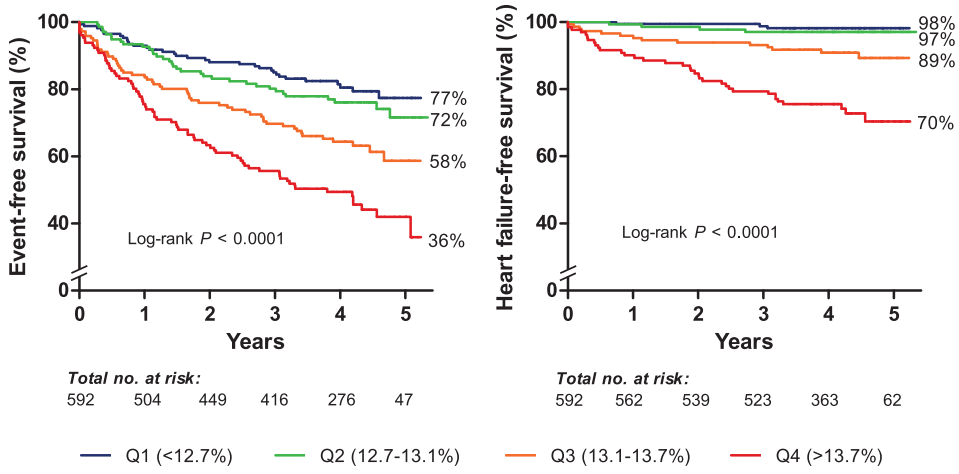

FIGURE 1 - Kaplan-Meier curves stratified per baseline RDW quartile for the primary and secondary endpoint.
Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile.

Patients who were in a higher RDW quartile had a significantly lower primary and secondary endpoint-free survival, as is shown in the Kaplan-Meier curves in Figure 1. When the RDW quartiles were analyzed in a multivariable Cox model including multiple clinical variables, C-reactive protein, and NT-proBNP, the association was partially attenuated but remained statistically significant. Moreover, each separate component of the primary endpoint seemed to occur more frequently in patients in the highest RDW quartile (Table 2). As is shown in Table 3, similar conclusions were found when RDW was analyzed as a continuous variable. None of the other components of the automated blood count (hemoglobin, hematocrit and mean cell volume) were predictive of the primary endpoint; however, a decreased hemoglobin was significantly associated with the secondary endpoint. We therefore conducted sensitivity analyses with additional adjustment of the associations between RDW and study endpoints for hemoglobin, which did not yield different conclusions.

TABLE 2 - Risk of the primary composite endpoint (cardiovascular event) and secondary composite endpoint (death or heart failure) according to baseline RDW quartiles.

		RDW quartiles (n = 592)				
	All (n=592)	**Q1 (< 12.7%, n=173)**	**Q2 (12.7–13.1%, n=137)**	**Q3 (13.1–13.7%, n=149)**	**Q4 (≥ 13.7%, n=133)**	***P* for trend[a]**
Cardiovascular event						
No. Cases	196	34	34	55	73	
Person-years	1966	620	504	473	369	
Crude HR (95% CI)		Reference	1.24 (0.77–1.99)	2.10 (1.37–3.22)	3.48 (2.32–5.23)	< 0.001
Adjusted HR (95% CI)[b]		Reference	1.23 (0.77–1.99)	1.49 (0.99–2.32)	2.13 (1.38–3.30)	< 0.001
Adjusted HR (95% CI)[c]		Reference	1.14 (0.71–1.84)	1.44 (0.92–2.24)	1.76 (1.12–2.76)	0.009
Death or heart failure						
No. Cases	57	3	4	14	36	
Person-years	2338	690	585	590	472	
Crude HR (95% CI)		Reference	1.61 (0.36–7.20)	5.51 (1.58–19.2)	17.4 (5.35–56.5)	< 0.001
Adjusted HR (95% CI)[b]		Reference	1.48 (0.33–6.66)	2.37 (0.66–8.44)	5.34 (1.58–18.1)	< 0.001
Adjusted HR (95% CI)[c]		Reference	1.15 (0.25–5.25)	2.27 (0.64–8.08)	3.48 (1.00–12.1)	0.008
No. Cases (detailed)[d]						
Death	16	1	2	2	11	—
Heart failure	51	2	3	12	34	—
Hospitalization	150	26	27	39	58	—
Arrhythmia	109	18	17	33	41	—
Thromboembolic event	24	2	6	8	8	—
Cardiac intervention	112	23	20	33	36	—

[a]Calculated by assigning the median level in each quartile to participants and evaluating this variable continuously. [b]Adjusted for age (years), sex, congenital diagnosis (aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV or univentricular heart (1)), cardiac medication use (yes/no), NYHA class II–III, sinus rhythm (yes/no), systemic ventricular function (0–3). [c]Additionally adjusted for C-reactive protein and NT-proBNP (both $\log_2$-transformed). [d]All components of the primary endpoint are separately displayed for exploratory purposes (in which patients were not censored at the time of another endpoint than the endpoint of interest). No comparisons are made between subgroups, in order to avoid multiplicity of comparisons.
Abbreviations: CI, confidence interval; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RDW, red blood cell distribution width.

TABLE 3 - Associations between baseline hemoglobin, hematocrit, mean cell volume, and red cell distribution and the primary and secondary endpoint, analyzed continuously.

	Crude HR (95% CI)	*P*-value	Adjusted HR[a] (95% CI)	*P*-value	Adjusted HR[b] (95% CI)	*P*-value
Cardiovascular event						
Hb, g/L	1.00 (0.99–1.01)	0.572	—	—	—	—
Ht, L/L	2.15 (0.06–79.3)	0.678	—	—	—	—
MCV, fL	1.01 (0.98–1.04)	0.551	—	—	—	—
RDW, %	1.47 (1.34–1.62)	< 0.001	1.26 (1.13–1.41)	< 0.001	1.20 (1.06–1.35)	0.003
Death or heart failure						
Hb, g/L	0.98 (0.96–1.00)	0.024	0.98 (0.96–1.00)	0.020	0.98 (0.97–1.00)	0.011
Ht, L/L	0.10 (0.00–13.0)	0.209	—	—	—	—
MCV, fL	0.99 (0.94–1.05)	0.731	—	—	—	—
RDW, %	1.81 (1.59–2.06)	< 0.001	1.45 (1.23–1.70)	< 0.001	1.38 (1.15–1.65)	0.001

[a]Adjusted for age (years), sex, congenital diagnosis (aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV or univentricular heart (1)), cardiac medication use (yes/no), NYHA class II–III, sinus rhythm (yes/no), systemic ventricular function (0–3). [b]Additionally adjusted for C-reactive protein and NT-proBNP (both $\log_2$-transformed). **Abbreviations:** CI, confidence interval; HR, hazard ratio.

A basic risk marker model including age, sex, congenital diagnosis, cardiac medication use, NYHA class, rhythm, systemic ventricular function, and NT-proBNP yielded a C-index of 0.73 (95% CI 0.69–0.78) for the primary endpoint. The augmented model with RDW included as predictor yielded a C-index of 0.74 (95% CI 0.70–0.78), which was a modest but significant improvement ($p = 0.005$).

Repeated RDW measurements

During the follow-up, a total of 2449 RDW measurements were collected. Although the majority of the RDW measurements (n = 2085, 85%) were in the normal range, the RDW was clearly and consequently higher at baseline and throughout the entire follow-up duration in patients with a primary endpoint, compared with patients who remained endpoint-free. This difference was even more pronounced in patients with the secondary endpoint (Supplemental File 3). In Supplemental File 4 and 5, the individual measurements are displayed for each patient separately. Our data did not provide evidence for a steady increase in RDW prior to the occurrence of adverse events. The largest part of variation between the RDW measurements was attributable to between-subject variation (76%) instead of within-subject variation (24%).

DISCUSSION

In this study, we have prospectively followed a large cohort of ACHD patients during 4.3 years with annual repeated RDW measurements. RDW was higher at study inclusion and throughout the entire follow-up period in patients with a cardiovascular event, especially in patients who died or developed heart failure. Increased RDW at inclusion was significantly associated with cardiovascular events during follow-up, independently of NT-proBNP and other prognostic markers. Adding RDW to a prognostic model including established clinical risk factors and NT-proBNP yielded a modest improvement.

Previous reports

To our knowledge, this is the first large and prospective study that investigated the prognostic value of RDW in patients with ACHD. The findings of this study are in line with previous studies that have been performed in patients with acquired cardiac diseases, such as heart failure and coronary heart disease.[2, 8-13] More specific, in a retrospective cohort study of Miyamoto et al. in 144 patients with ACHD who were hospitalized,[25] increased RDW was closely related to the risk of cardiovascular death. Comparable results were found in a retrospective cohort of 109 adult patients with Eisenmenger syndrome.[26] Furthermore, RDW was found to be an indicator of iron deficiency in adult Fontan patients and correlated with lower exercise capacity.[27] The remainder of studies that have been performed in congenital heart disease have only included pediatric patients. In a small cross-sectional study of pediatric patients with a Fontan circulation, increased RDW was related to a higher central venous pressure and a lower mixed venous oxygen saturation.[28] In addition, preoperative RDW has been reported to positively correlate with the length of postoperative stay in children undergoing surgery for congenital heart disease.[29, 30]

Pathophysiological mechanisms

RDW likely reflects a broad range of pathophysiological processes, that could explain the association between RDW and cardiovascular events in patients with ACHD. First of all, RDW has been directly related to markers of impaired iron mobilization, decreased erythropoietin production, and nutritional deficiencies such as iron, folate, or vitamin B12.[2, 3, 31] This may subsequently cause a decreased hemoglobin level, which is associated with a worse prognosis in chronic heart failure patients.[32, 33] Nevertheless, data from previous studies and also from our cohort show that RDW provides prognostic information independent of hemoglobin level.[10] Moreover, only 5% of patients had anemia in our study.

Young red blood cells are relatively large and variable in size, and the erythrocytes shrink and become more equally sized during the natural aging process. Oxidative stress and inflammation are both known to decrease red blood cell survival.[4, 5] The decreased lifespan of the erythrocytes will lead to relatively large amounts of both large and small red cells, which is reflected by an increased RDW.[31] In addition, RDW has been related to endothelial function as assessed with flow-mediated dilatation (as a measure of underlying metabolic derangements), and a high RDW is more frequently present in patients with kidney failure.[34] Although in our study the average C-reactive protein, total cholesterol, low density lipoprotein and estimated glomerular filtration were within the normal range, RDW seemed to be associated with these measures of inflammation, dyslipidemia and kidney function (Table 1).

The heterogeneity of red blood cell size therefore could be an epiphenomenon that reflects several miscellaneous mechanisms, which are related to an individual's prognosis. Previous studies have shown that risk prediction improves when multiple biomarkers are used together, that reflect distinct pathophysiological mechanisms.[21, 35] RDW may combine these different mechanisms in one biomarker, and the red blood cell has therefore been previously proposed as an overall 'barometer' of cardiovascular health.[2]

Clinical implications

The RDW is directly available as part of the automated blood count, which is a routine component of standard blood testing. The measurement is easy, inexpensive, rapid, does not require specific skills or instrumentation, and is readily available in virtually all clinical laboratories worldwide. In light of the accumulating evidence that emphasizes the prognostic value of RDW, the clinical role of RDW may be extended beyond the boundaries of the differential diagnosis of anemia. RDW could be a new tool to enhance the prognostication of ACHD patients, together with other functional, echocardiographic and biochemical markers, and can be easily implemented in routine clinical care. Accurate identification of high-risk patients will enable more intensive follow-up with hopefully prevention of events in the future, and the identification of low-risk patients will lead to reassurance, less intensive follow-up and cost savings.

Study limitations

Changes in RDW during a hospital admission for acute decompensated heart failure have been previously associated with all-cause mortality or readmission for heart failure.[15] In this study with clinically stable ACHD patients, we did not observe an increase in RDW prior to the occurrence of adverse events. However, RDW measurements were

performed only once per year in this study. Therefore, it could be hypothesized that repeated measurements should be performed more frequently in order to find an association between recent changes in RDW and an adverse event.

Imputation of the mean was used to account for the 1% missing NT-proBNP values. Although in general imputation of the mean could be considered as a limitation, it is considered acceptable for covariates with a very low percentage of missing data.

In this cohort, patients with an isolated repaired atrial or ventricular septal defect were not included, due to the expected low number of events in these patients. This should be taken into account when extrapolating these data to other cohorts of patients with ACHD.

CONCLUSIONS

The RDW is significantly associated with cardiovascular events in patients with ACHD, independently of NT-proBNP and other prognostic markers. This readily available biomarker, that can be reliably measured worldwide at a low cost, may therefore be a valuable additional tool in the risk stratification of patients with ACHD.

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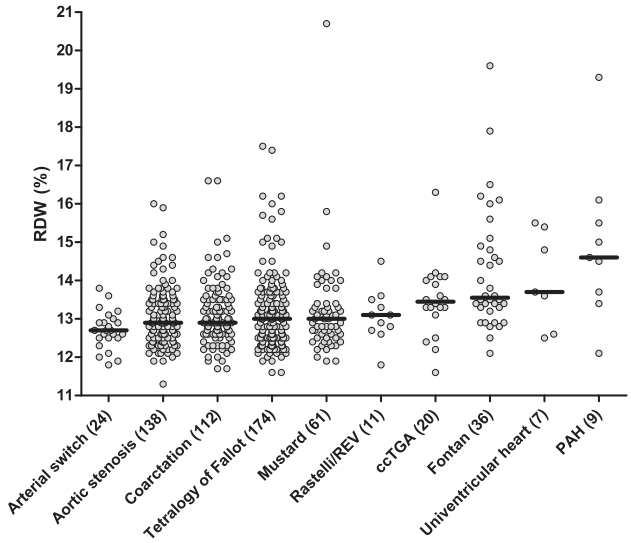
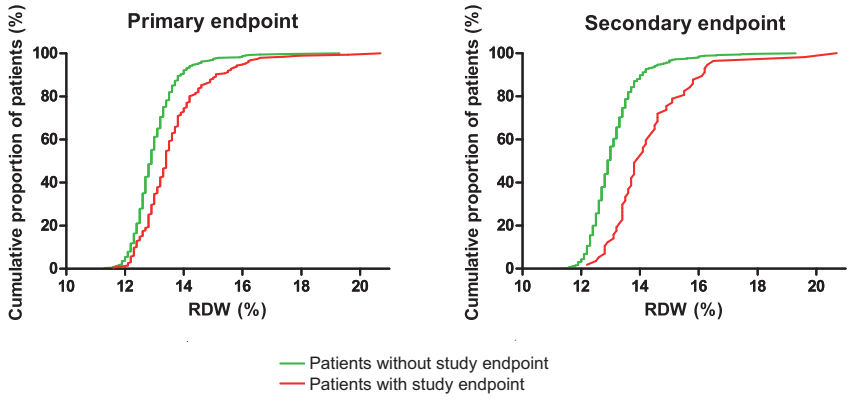
SUPPLEMENTAL MATERIAL

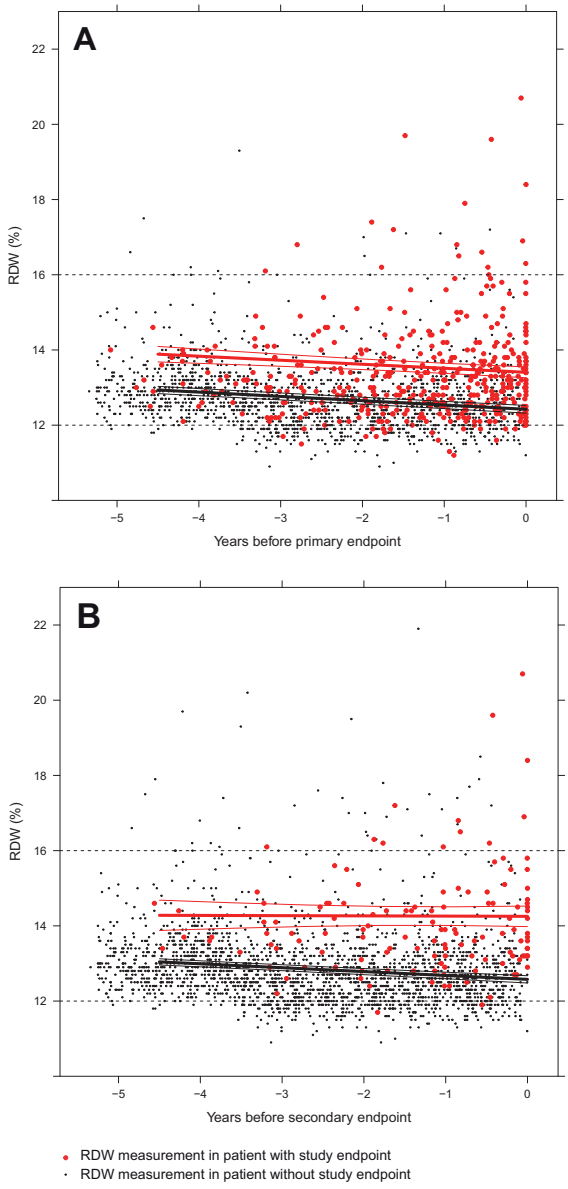


SUPPLEMENTAL FILE 1 - Baseline RDW levels in the different congenital diagnostic groups.

Actual RDW measurements are separately depicted. The median level in each diagnostic group is indicated with a black line.

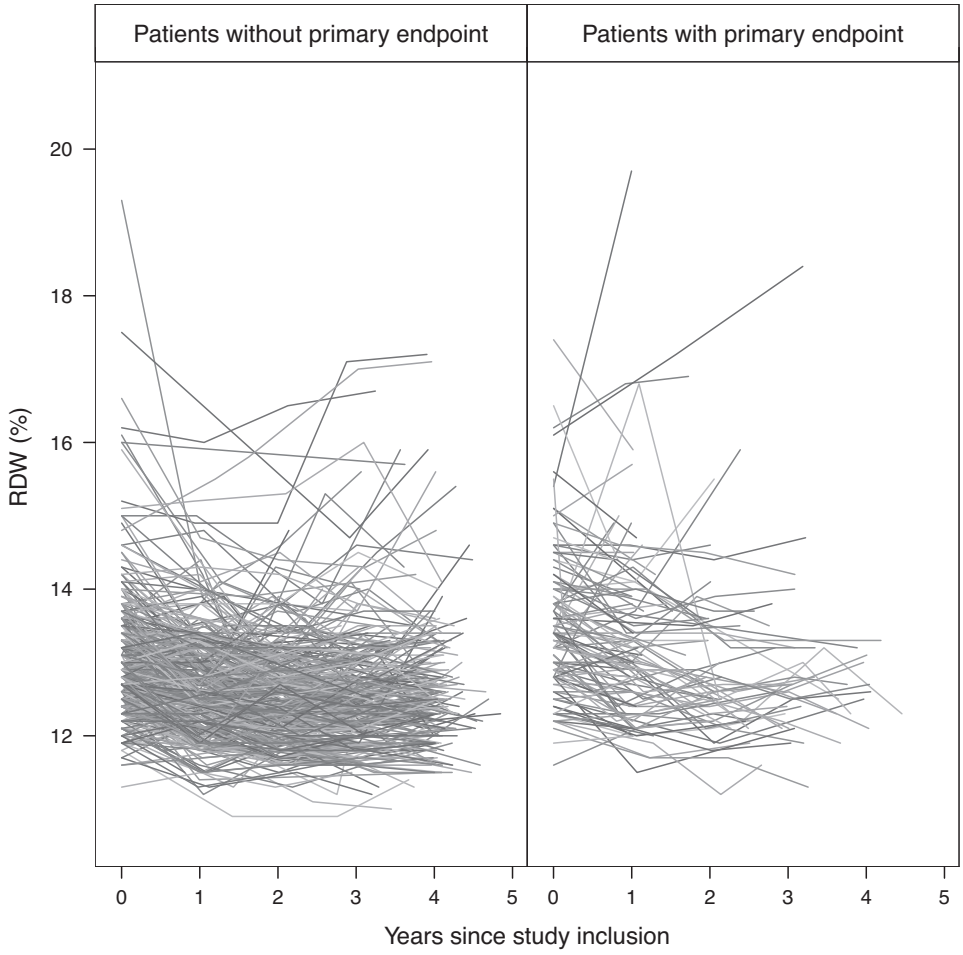


SUPPLEMENTAL FILE 2 - Cumulative distribution of baseline RDW in patients with and without the primary and secondary endpoint.



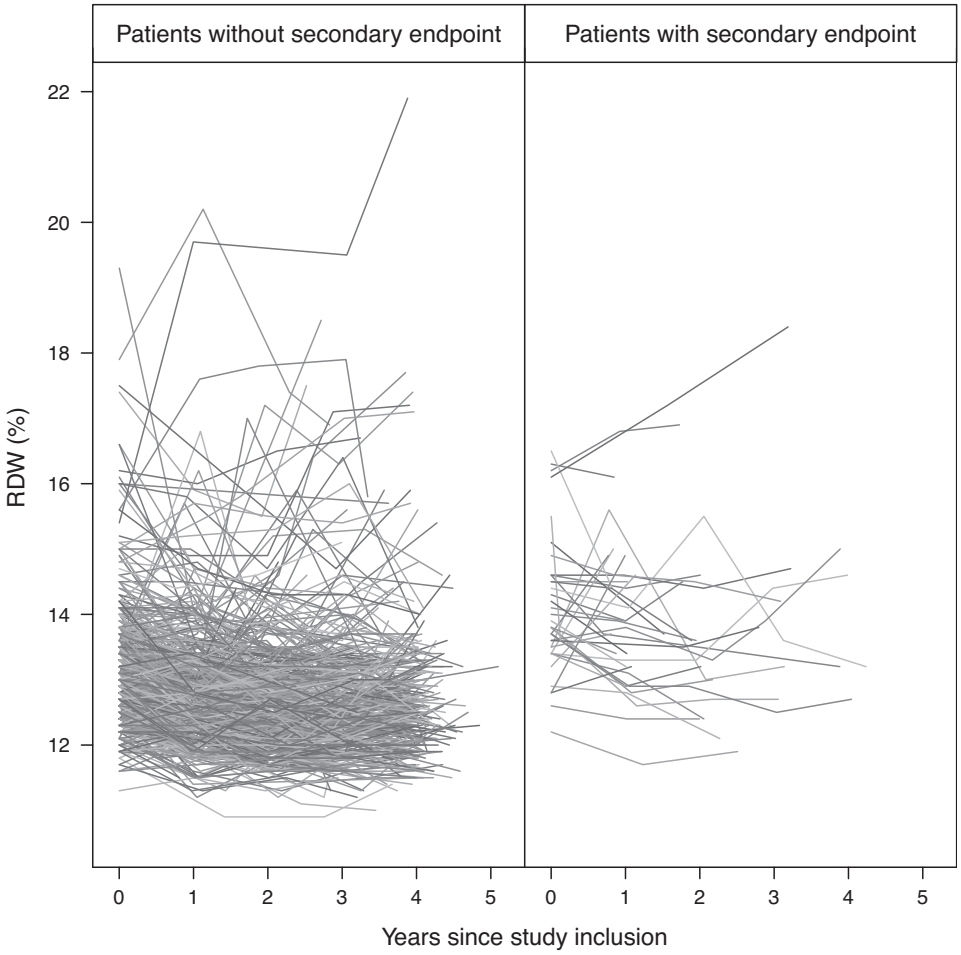
SUPPLEMENTAL FILE 3 - Average RDW evolutions in patients with and without the primary (A) and secondary endpoint (B).

Only the measurements that occurred prior to the primary endpoint (n = 2012) are displayed. One patient with an extreme outlier at year one (28.1%) who did not have an event is not displayed in the graphical illustrations of the results, but was included in all analyses. Lower and upper limits of normal are depicted by dashed lines. Time point zero is denoted as the moment at which patients either experienced an event, or when they were censored because of reaching the end of the follow-up duration without experiencing an event. We displayed all repeated RDW measurements as years before this moment (i.e., on a negative timescale).



SUPPLEMENTAL FILE 4 - Individual RDW evolutions in patients with and without the primary endpoint.

The annual repeated measurements of each individual patient are connected with a separate line. RDW measurements after the event were deleted, therefore only the measurements that occurred prior to the primary endpoint (n = 2012) are displayed in these plots.



SUPPLEMENTAL FILE 5 - Individual RDW evolutions in patients with and without the secondary endpoint.

The annual repeated measurements of each individual patient are connected with a separate line. RDW measurements after the event were deleted, therefore only the measurements that occurred prior to the secondary endpoint (n = 2334) are displayed in these plots.

Chapter 13

Prognostic value of serial N-terminal pro-B-type natriuretic peptide measurements in adults with congenital heart disease

Vivan J.M. Baggen, Sara J. Baart, Annemien E. van den Bosch, Jannet A. Eindhoven, Maarten Witsenburg, Judith A.A.E. Cuypers, Jolien W. Roos-Hesselink, Eric Boersma

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ABSTRACT

Background A single N-terminal pro-B-type natriuretic peptide (NT-proBNP) measurement is a strong prognostic factor in adult congenital heart disease (ACHD). This study investigates NT-proBNP profiles within ACHD patients and relates these to cardiovascular events.

Methods and Results In this prospective cohort, 602 ACHD patients were enrolled at the outpatient clinic (2011–2013). NT-proBNP was measured at study inclusion in 595 patients (median age 33 [IQR 25–41] years, 58% male, 90% NYHA I) and at subsequent annual visits. The primary endpoint was defined as death, heart failure, hospitalization, arrhythmia, thromboembolic event, or cardiac intervention; the secondary endpoint as death or heart failure. Repeated measurements were analyzed using linear mixed models and joint models. During a median follow-up of 4.4 [IQR 3.8–4.8] years, a total of 2424 repeated measurements were collected. Average NT-proBNP increase was 2.9 pmol/L the year prior to the primary endpoint (n = 199, 34%) and 18.2 pmol/L prior to the secondary endpoint (n = 58, 10%), compared with 0.3 pmol/L in patients who remained endpoint-free (*p*-value for difference in slope 0.006 and < 0.001, respectively). In patients with elevated baseline NT-proBNP (> 14 pmol/L, n = 315, 53%), repeated measurements were associated with the primary endpoint (HR per two-fold higher value 2.08; 95% CI 1.31–3.87; $p < 0.001$) and secondary endpoint (HR 2.47; 95% CI 1.13–5.70; $p = 0.017$), when adjusted for the baseline measurement.

Conclusions NT-proBNP increased prior to the occurrence of events, especially in patients who died or developed heart failure. Serial NT-proBNP measurements could be of additional prognostic value in the annual follow-up of ACHD patients with an elevated NT-proBNP.

INTRODUCTION

A congenital heart defect is the most prevalent congenital anomaly, with an occurrence of 9 per 1000 live births.[1] Due to the success of pediatric cardiology and cardiothoracic surgery, the population of patients with adult congenital heart disease (ACHD) is rapidly growing. Currently around 2.3 million patients with ACHD are estimated to be alive in Europe, already outnumbering children with congenital heart disease.[2] Although survival has improved, residual lesions may cause progressive exercise intolerance and late complications such as arrhythmia, heart failure and sudden death.[3, 4] This expanding demographic phenomenon is causing serious issues concerning the optimal management of patients with ACHD. Accurate biomarkers that enable risk stratification and monitoring of disease progression are therefore clearly needed.

Natriuretic peptides are firmly established diagnostic and prognostic tools in a variety of cardiovascular conditions, such as heart failure and coronary heart disease.[5, 6] Previous studies have shown that N-terminal pro-B-type natriuretic peptide (NT-proBNP) is also a strong independent prognostic factor in patients with ACHD.[7-9] However, NT-proBNP is known to have a considerable intra-individual biological variability[10] and progression of disease may lead to substantial changes in the NT-proBNP level over time.[11-14] Repeated NT-proBNP measurements could therefore provide additional prognostic information. The aim of this study was to investigate the temporal evolution of NT-proBNP within individual patients and to relate this to cardiovascular events in a prospective cohort study of patients with ACHD.

METHODS

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure upon reasonable request.

Study design and population

This prospective cohort study was conducted at the outpatient clinic of a tertiary care center between April 2011 and April 2013, that is responsible for the clinical care of all patients with ACHD in the south-west region of the Netherlands. A total of 602 consecutive patients with a moderate or complex type of congenital heart defect[15] who routinely visited the outpatient clinic were enrolled in the study cohort. Exclusion criteria were: age < 18 years, pregnancy, mild cardiac lesion (isolated atrial or ventricular septal defect), not capable of understanding and signing informed consent, or kidney failure (creatinine > 200µmol/L).

According to the study protocol, participants underwent physical examination by a cardiologist, 12-lead electrocardiography, echocardiography and venous blood sampling at the day of study inclusion. During four subsequent years, all patients were structurally followed-up by yearly visits to the ACHD outpatient clinic, including physical examination by a cardiologist, 12-lead electrocardiography, and venous blood sampling. During follow-up, patients were treated in accordance with ESC guidelines.[16] Presence of pulmonary hypertension was defined as a right ventricular systolic pressure of > 40 mmHg, as measured by the tricuspid valve regurgitation maximal velocity (in patients with a systemic left ventricle) or mitral valve regurgitation maximal velocity (in patients with a systemic right ventricle) and the estimated right atrial pressure, in accordance with the guidelines.[17] In patients with pulmonary stenosis we subtracted the gradient across the pulmonary valve from the right ventricular systolic pressure.[18] The study protocol was approved by the Erasmus MC medical ethics committee and all participants provided written informed consent. Other details of the study protocol have been described previously.[9, 19]

N-terminal pro-B-type natriuretic peptide

NT-proBNP was measured at inclusion and at the scheduled yearly follow-up visits. Therefore, a maximum of five subsequent measurements per patient were collected. NT-proBNP measurements were not part of standard clinical care at the time this study was conducted. All measurements were performed for research purposes only, and decisions regarding patient management were made independently of any NT-proBNP level. NT-proBNP was directly measured in fresh serum samples at the clinical chemistry laboratory of the Erasmus MC, using a commercial electrochemiluminescence immunoassay (Roche Diagnostics, Rotkreuz, Switzerland). The limit of detection was 0.6 pmol/L, and all values below the limit of detection were reported as < 0.6 pmol/L. Elevated NT-proBNP was defined as > 14 pmol/L (approximately > 125 pg/mL), based on the recommended low cut-off for the diagnosis of heart failure in patients presenting with non-acute symptoms.[20]

Definition and assessment of events

The primary endpoint was defined prior to the collection of data as a composite of the following adverse (cardiovascular) events: all-cause mortality, heart failure (requiring initiation or change in heart failure medication, or requiring hospital admission), hospitalization for cardiac reasons (including heart failure, arrhythmia, thromboembolic event, cardiac intervention, endocarditis, or other cardiac reasons requiring admission 24 hours or longer), arrhythmia (symptomatic and recorded, or requiring treatment),

thromboembolic event (ischemic cerebrovascular accident, pulmonary embolism or myocardial infarction), or any cardiac intervention (surgical or percutaneous). The secondary composite endpoint was defined as a composite of all-cause mortality or heart failure.

Patients were prospectively and systematically followed for fatal and non-fatal events by a yearly clinical evaluation at our institution according the study protocol until August 1, 2016. We retrieved information from electronic patient charts and also checked the survival status of patients in the Municipal Population Register. Suspect endpoint events were adjudicated by two experienced investigators (V.J.M.B. and J.W.R.-H.) without knowledge of NT-proBNP levels.

Statistical analysis

Variables are presented as mean ± standard deviation or median [interquartile range (IQR)], depending on the distribution of data. NT-proBNP had a skewed distribution and was therefore $\log_2$ transformed for further analysis.

We adjudicated each component of the primary endpoint (death, heart failure, hospitalization for cardiac reasons, arrhythmia, thromboembolic event, and cardiac intervention) separately. In other words, patients were followed until the occurrence of the specific endpoint of interest and were not censored at another endpoint type. This was important because patients frequently experienced multiple event types simultaneously or subsequently. For patients with multiple events, event-free survival was defined as the time from enrolment to the occurrence of the first event. Patients without any cardiovascular event were censored at the end of the follow-up duration. Cox proportional hazards regression was performed to identify associations between baseline NT-proBNP and the study endpoints. We conducted multivariable analyses with adjustment for baseline age (years), sex, congenital diagnosis (moderate vs. complex), NYHA class (I vs II–III), cardiac medication use (angiotensin-converting-enzyme inhibitors, angiotensin II receptor blockers, ß-blockers, diuretics, calcium blockers, or anti-arrhythmic drugs), loss of sinus rhythm, systemic ventricular function (normal, mildly, moderately, or severely impaired), body mass index (kg/m^2), saturation < 90%, ≥ 1 re-intervention after initial corrective surgery, and estimated glomerular filtration range (eGFR; mL/min, $\log_2$-transformed). Data on covariates were 99.3% complete; we used imputation of the mean to account for missing values.

We developed linear mixed-effects (LME) models to describe the temporal NT-proBNP evolution, while accounting for the correlation between subsequent NT-proBNP measurements within individuals. In patients with study endpoints, only NT-proBNP measurements prior to the study endpoint were used. The model building and selection strategy for NT-proBNP is further detailed in Supplemental Methods 1 and Supplemental

Table 1. We developed models with adjustment for the full set of covariates that were used in the Cox models. The proportion of within-subject variation was calculated as the residual variance of the LME model divided by the total variance. To illustrate the average NT-proBNP evolution in patients with and without events, an interaction term in the fixed part of the model was used to allow different slopes in patients with and without events. We additionally developed LME models with time point zero denoted as the moment at which patients experienced an event, or when they were censored because of reaching the end of the follow-up duration without experiencing an event. Repeated NT-proBNP measurements were denoted as years before this moment (i.e., on a negative timescale). These models were used to calculate the increase in NT-proBNP in the year prior to an event. To provide additional insight in the NT-proBNP dynamics over time, we expressed the association between baseline and follow-up NT-proBNP values by the Spearman rank correlation coefficient. In addition, we calculated quartiles of the change in NT-proBNP concentration in the first year (Δ Year 1 - Year 0) and related this to baseline patient characteristics and study endpoints. Hence, a positive value indicates an increase in NT-proBNP in the first year, and a negative value indicates a decrease.

We used joint modeling (JM) to investigate the association between the individual NT-proBNP evolutions over time and the occurrence of study endpoints.[21] JM combines the LME model estimating the NT-proBNP level of an individual patient at time *t*, with a Cox model to relate that estimated value with the incidence of study endpoints after time *t*. Results are presented as hazard ratios (HR) with 95% confidence intervals (CI) for a two-fold higher value of the NT-proBNP level at any time *t* between 0 and 4 years follow-up. HRs were adjusted for the baseline NT-proBNP level by including baseline NT-proBNP as a separate variable in the Cox model. In addition, we constructed multivariate LME models including both serial NT-proBNP and serial eGFR measurements, which were used in a multivariate joint model to investigate the additive value of serial NT-proBNP measurements on top of serial eGFR measurements.

We repeated the JM analyses when stratified for normal versus elevated NT-proBNP level at baseline, and stratified for the absence or presence of pulmonary hypertension. In addition, multiple explorative sub analyses were performed to check the robustness of specific assumptions with regard to endpoint definitions. Because a linear random slopes term did not significantly improve the fit of the model, we only used random intercepts and therefore did not calculate HRs for the slope. Finally, although the present study was not specifically designed to investigate NT-proBNP profiles in patients with a cardiac intervention, we performed explorative post-hoc analyses to evaluate whether we could detect a decrease in NT-proBNP levels after a surgical or percutaneous valve intervention among individual patients.

We used IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA), and the nlme and JMBayes packages in R statistical software, version 3.3.1 (available at: www.r-project.org).[22] Two-sided *p*-values < 0.05 were considered statistically significant.

RESULTS

Patient characteristics

A total of 595 patients underwent NT-proBNP measurement at study inclusion. The median age was 33 [IQR 25–41] years and 346 (58%) were male. Surgical repair of the congenital defect before study entry was performed in 540 patients (91%) at a median age of 3.7 [IQR 0.8–11.9] years), and 534 patients (90%) were in NYHA functional class I. The underlying congenital diagnoses were congenital aortic stenosis (n = 137), aortic coarctation (n = 109), repaired tetralogy of Fallot (including pulmonary atresia with ventricular septal defect, n = 177), transposition of the great arteries (TGA) corrected by atrial switch operation (Mustard, n = 65), TGA corrected by arterial switch operation (n = 24), congenitally corrected TGA (n = 20), complex TGA with ventricular septal defect or double-outlet right ventricle corrected with a Rastelli type repair (n = 11), functionally univentricular heart (n = 43), and pulmonary arterial hypertension after a (corrected) atrial or ventricular septal defect (n = 9). The majority of patients were in sinus rhythm (n = 514, 87%). Systemic ventricular function was graded as normal (n = 298, 50%), mildly impaired (n = 210, 35%), moderately impaired (n = 69, 12%), or severely impaired (n = 18, 3%). Right ventricular systolic pressure was available in 420 patients (71%), of whom 33 patients (7.9%) had pulmonary hypertension. A flowchart of the patient selection and detailed clinical, electrocardiographic and echocardiographic characteristics of the study cohort, also expressed per baseline NT-proBNP quartile, have been previously reported.[9]

Cardiovascular events

Patients were followed-up during a median of 4.4 [IQR 3.8–4.8] years. Survival status according to the Municipal Population Register and detailed follow-up data regarding non-fatal events were available in 590 patients (99.2%). The primary endpoint occurred in 199 patients (34%) and the secondary endpoint in 58 patients (10%). In total, 16 patients died. Causes of death were: end-stage heart failure (n = 8); cardiac arrest (n = 4); sudden death, presumed cardiac (n = 3); other (n = 1). Other components of the primary endpoint were heart failure (n = 52), hospitalization for cardiac reasons (n = 151), arrhythmia (n = 110), thromboembolic event (n = 25), and cardiac intervention (n = 113).

Prognostic value of baseline N-terminal pro-B-type natriuretic peptide

Baseline NT-proBNP was significantly associated with the primary endpoint (crude HR per two-fold higher value 1.55 [95% CI 1.43–1.68], $p < 0.001$) and with the secondary endpoint (crude HR per two-fold higher value 2.30 [95% CI 1.97–2.68], $p < 0.001$). The Kaplan-Meier estimate of primary endpoint-free survival at four years of follow-up was 84.1% in patients with normal baseline NT-proBNP (< 14 pmol/L) and 55.1% in patients with elevated baseline NT-proBNP. Secondary endpoint-free survival at four years of follow-up was 99.3% in patients with normal baseline NT-proBNP (< 14 pmol/L) and 83.2% in patients with elevated baseline NT-proBNP. After complete adjustment for all covariates, the strength of the association was slightly attenuated but remained significant for both the primary endpoint (adjusted HR per two-fold higher value 1.39 [95% CI 1.23–1.57], $p < 0.001$) and the secondary endpoint (adjusted HR per two-fold higher value 1.80 [95% CI 1.42–2.28], $p < 0.001$). The prognostic value of baseline NT-proBNP in this cohort has been previously published (using standardized HRs).[9] Of note, baseline eGFR measurements were not predictive of the primary and secondary endpoint in these multivariable models (adjusted HR per two-fold higher value 1.00 [95% CI 0.58–1.72], $p = 0.993$ and 2.49 [95% CI 0.78–7.89], $p = 0.123$, respectively).

Temporal N-terminal pro-B-type natriuretic peptide evolution

During the follow-up, a median of four yearly repeated NT-proBNP measurements were collected per patient, resulting in a total of 2424 measurements. For the analysis of the primary endpoint 2009 measurements were available, and for the analysis of the secondary endpoint 2319 measurements were available. Figure 1 and 2 describe the average NT-proBNP profiles in patients with and without the primary and secondary endpoint, respectively. Individual NT-proBNP profiles are depicted in Supplemental Figure 1 and 2. Of the total variation in the NT-proBNP level, 89% was attributable to variation between subjects and 11% to variation within subjects.

The average baseline NT-proBNP (intercept) in patients who reached the primary endpoint was 30.6 (95% CI 25.8–36.2) pmol/L, compared with 10.6 (95% CI 9.6–11.8) pmol/L in patients who remained event-free during the follow-up (p-value < 0.001). The average NT-proBNP increase in the last year before the primary endpoint was 2.9 pmol/L, compared with 0.3 pmol/L in patients without the endpoint (p-value for difference in slope 0.006).

In patients who reached the secondary endpoint, the average baseline NT-proBNP was 73.7 (95% CI 56.1–96.8) pmol/L, compared with 12.9 (11.7–14.1) pmol/L in patients without the endpoint (p-value < 0.001). The average NT-proBNP increase in the last year before the secondary endpoint was 18.2 pmol/L, compared with 0.3 pmol/L in patients without the secondary endpoint (p-value for difference in slope < 0.001).

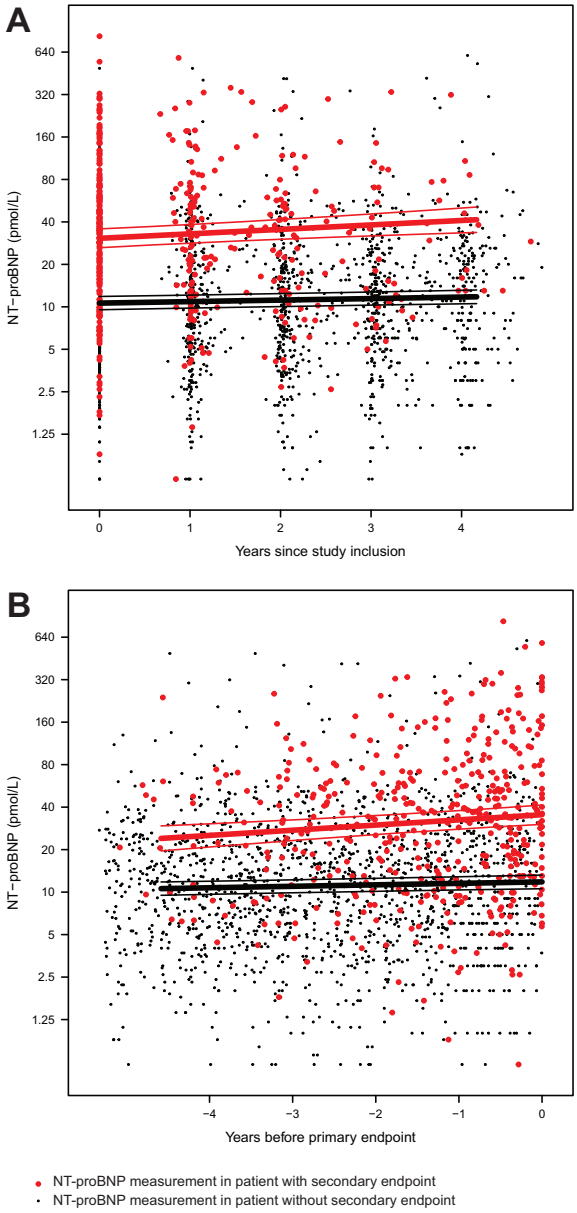

FIGURE 1 - Average NT-proBNP evolution in patients with and without the primary endpoint.

NT-proBNP measurements after the event were deleted, therefore a total of 2009 measurements that occurred prior to the primary endpoint were used in this analysis. A, time point zero is denoted as the date of study inclusion (and first NT-proBNP measurement). B, time point zero is denoted as the moment at which patients either experienced an event, or when they were censored because of reaching the end of the follow-up duration without experiencing an event. We displayed all repeated NT-proBNP measurements as years before this moment (i.e., on a negative timescale).

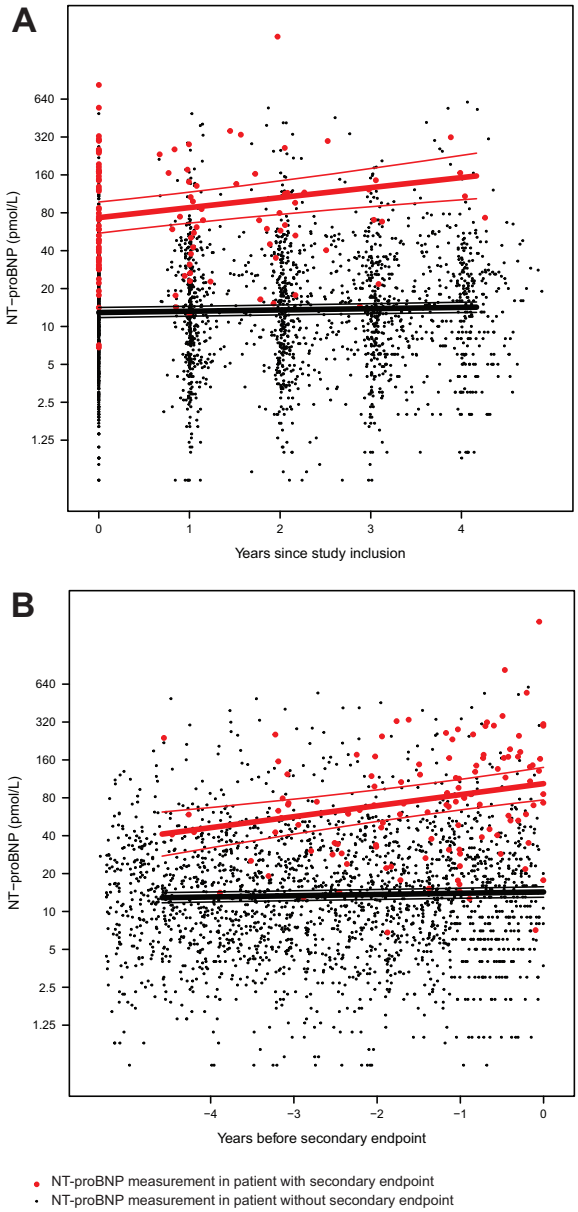

FIGURE 2 - Average NT-proBNP evolution in patients with and without the secondary endpoint.

NT-proBNP measurements after the event were deleted, therefore a total of 2319 measurements that occurred prior to the secondary endpoint were used in this analysis. A, time point zero is denoted as the date of study inclusion (and first NT-proBNP measurement). B, time point zero is denoted as the moment at which patients either experienced an event, or when they were censored because of reaching the end of the follow-up duration without experiencing an event. We displayed all repeated NT-proBNP measurements as years before this moment (i.e., on a negative timescale).

The Spearman correlation coefficient of the baseline NT-proBNP measurement with Year 1, 2, and 3, respectively, was 0.84, 0.85, and 0.86 in subjects without any event; 0.85, 0.82, and 0.87 in subjects with the primary endpoint; and 0.88, 0.75, and 0.79 in subjects with the secondary endpoint (all $p < 0.001$). In Table 1, quartiles of the change in NT-proBNP concentration in the first year are related to baseline patient characteristics. Patients in the highest quartile (i.e., with the largest increase in NT-proBNP level within the first year) were significantly older, were more likely to be female, had a higher age at initial surgical repair, more frequently used cardiac medication, were in a higher NYHA class and had a worse systemic ventricular function. Of note, in this type of statistical analysis only a subset of the data is used and regression to the mean partly explains the differences in NT-proBNP levels between patient subgroups. The exact results should therefore be carefully interpreted.

Prognostic value of repeated measurements

The HRs of the repeatedly measured NT-proBNP value indicate the risk of the primary and secondary endpoint for a two-fold higher value of NT-proBNP, measured at any time *t* between 0 and 4 years of follow-up (Table 2). These HRs were consequently higher than the HRs of the single baseline NT-proBNP value, calculated using Cox regression (as reported in the text above). This indicates that the repeated NT-proBNP measurements that were taken during the follow-up were more predictive of the primary and secondary endpoint than the single baseline value.

The NT-proBNP level at any point in time during the follow-up was even stronger associated with the study endpoints when adjustment was performed for the baseline NT-proBNP level. I.e., when two patients with the same NT-proBNP level at baseline were compared, higher values during follow-up were strongly predictive of study endpoints (Table 2).

The stratified analysis reported in Table 3 shows that the repeated measurements seem to carry additional prognostic value on top of the baseline value in both patients with a normal NT-proBNP level and patients with an elevated NT-proBNP level at baseline. However, this association is probably underpowered in the group of patients with a normal baseline NT-proBNP, since the event rates of the primary endpoint and especially the secondary endpoint (which may be considered as most clinically relevant in this matter) were very low in these patients. A stratified analysis based on the absence (n = 387) or presence (n = 33) of pulmonary hypertension showed repeated NT-proBNP measurements were associated with the primary and secondary endpoint in both groups ($p < 0.001$ for all analyses). Additional explorative sub analyses excluding cardiac interventions from the primary endpoint, and separately analyzing heart failure requiring hospitalization versus heart failure requiring medication change or initiation did not yield different conclusions.

TABLE 1 - Association between baseline patient characteristics and quartiles of the difference in NT-proBNP (pmol/L) between Year 1 and Year 0.

	Change in NT-proBNP (pmol/L) calculated by Year 1 - Year 0					
	No measurement at Year 1 (n=33)	**Q1 (Δ <-4.1 pmol/L, n=140)**	**Q2 (Δ -4.1-0.0 pmol/L, n=144)**	**Q3 (Δ 0.0-5.1 pmol/L, n=138)**	**Q4 (Δ >5.1 pmol/L, n=140)**	***P*-value (n=562)**
Clinical characteristics						
Age, years	32.8 [25.7–43.4]	32.5 [25.5–41.4]	31.2 [24.1–39.5]	28.8 [22.2–37.6]	37.0 [28.7–45.2]	< 0.001
Sex, male n (%)	20 (61)	69 (49)	92 (64)	93 (67)	72 (51)	0.003
Surgical repair, n (%)	30 (91)	132 (94)	124 (86)	123 (89)	131 (94)	0.057
Age at surgical repair, years	1.7 [0.3–6.6]	3.6 [0.9–13.8]	3.4 [0.4–13.2]	2.9 [0.8–9.4]	6.5 [1.1–15.5]	0.017
Congenital diagnosis, n (%)*	20 (61)	83 (59)	66 (46)	72 (52)	84 (60)	0.054
Cardiac medication use, n (%)	9 (27)	59 (42)	38 (26)	36 (26)	70 (50)	< 0.001
Body mass index, kg/m2	24.2 ± 5.4	24.9 ± 4.5	24.9 ± 4.6	24.2 ± 4.2	24.9 ± 4.0	0.423
Heart rate, beats/minute	72 ± 17	73 ± 13	73 ± 13	73 ± 13	76 ± 13	0.261
Systolic blood pressure, mmHg	122 ± 12	127 ± 18	128 ± 16	126 ± 16	125 ± 16	0.531
O_2 saturation < 90%, n (%)	1 (3)	4 (3)	1 (1)	6 (4)	5 (4)	0.309
NYHA class, II-III n (%)	4 (12)	16 (11)	7 (5)	9 (7)	25 (18)	0.001
Electrocardiogram						
Sinus rhythm, n (%)	28 (85)	117 (84)	132 (92)	121 (88)	116 (83)	0.108
QRS duration, ms	111 [97–132]	115 [101–142]	109 [99–126]	112 [99–140]	118 [100–152]	0.069
Echocardiogram						
LA volume, mL/m²†	20 [14–27]	22 [17–35]	20 [15–25]	20 [15–27]	23 [16–34]	0.009
LV end-diastolic volume, mL/m²†	57 ± 13	67 ± 20	61 ± 17	65 ± 19	62 ± 20	0.125
LV end-systolic volume, mL/m²†	24 ± 7	30 ± 12	27 ± 10	29 ± 11	30 ± 18	0.421
LV ejection fraction, %†	58 ± 6	56 ± 7	56 ± 7	57 ± 7	54 ± 11	0.237
RV fractional area change, %	35 ± 12	38 ± 11	39 ± 12	41 ± 10	36 ± 11	0.019

TABLE 1 - Continued

	Change in NT-proBNP (pmol/L) calculated by Year 1 - Year 0					
	No measurement at Year 1 (n=33)	**Q1 (Δ <-4.1 pmol/L, n=140)**	**Q2 (Δ -4.1-0.0 pmol/L, n=144)**	**Q3 (Δ 0.0-5.1 pmol/L, n=138)**	**Q4 (Δ >5.1 pmol/L, n=140)**	***P*-value (n=562)**
Echocardiogram						
Systemic ventricular function, n (%)						0.001
Normal	20 (61)	67 (48)	74 (51)	86 (62)	51 (36)	
Mildly impaired	10 (30)	45 (32)	53 (37)	44 (32)	58 (42)	
Moderately impaired	3 (9)	21 (15)	13 (9)	7 (5)	25 (18)	
Severely impaired	0 (0)	7 (5)	4 (3)	1 (1)	6 (4)	
Laboratory results						
eGFR, mL/min	90 [89–90]	90 [81–90]	90 [83–90]	90 [84–90]	90 [79–90]	0.048
NT-proBNP, pmol/L (Year 0)	14.4 [5.9–26.0]	32.5 [18.7–59.0]	8.9 [5.5–16.1]	7.3 [3.7–15.3]	24.2 [9.7–56.9]	< 0.001

Values are reported as n (%), mean ± SD or median [IQ_1–IQ_3]. Differences across quartiles of change are analyzed using the Chi-Square test for categorical data, One-Way ANOVA or Kruskal-Wallis for continuous data (depending on the distribution). No trend tests were used, because not necessarily a linear relationship is assumed. *Congenital diagnosis of aortic stenosis, aortic coarctation or arterial switch operation (0) versus tetralogy of Fallot, Rastelli, systemic RV, univentricular heart or pulmonary arterial hypertension (1). †Left sided volumes were not measured in patients with a systemic right ventricle, functionally univentricular heart, pulmonary hypertension (n = 137) or a poor acoustic window. **Abbreviations:** eGFR, estimated glomerular filtration rate; LA, left atrial; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RV, right ventricular.

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Finally, in Figure 3, the association between the change in NT-proBNP level in the first year (Δ Year 1 - Year 0) and the study endpoints is depicted.

N-terminal pro-B-type natriuretic peptide response after cardiac intervention

Of the 113 patients with a cardiac intervention, 41 patients underwent a surgical intervention (valvular replacement, n = 35; valvular repair, n = 4; aortic surgery, n = 1; and defect closure, n = 1) and 72 patients underwent a percutaneous intervention (ablation, n = 21; pacemaker or ICD implantation, n = 20; valve replacement, n = 16; coronary intervention, n = 5; defect closure, n = 4; valvular balloon dilatation, n = 4; aortic stenting, n = 2). To explore whether interventions with a volume or pressure load reduction on the heart were followed by a decrease in NT-proBNP level among individual patients, we plotted the NT-proBNP profiles of all patients with a surgical valve intervention (valve replacement or valve repair) and with a percutaneous valve intervention (valve replacement or balloon dilatation) in Supplemental Figure 3. Within individual patients, large reductions in the NT-proBNP level were observed after the valvular intervention, especially in the surgical valve intervention group. Other exploratory analyses are shown in Supplemental Figure 4 (NT-proBNP profiles in patients with elective versus non-elective cardiac interventions) and in Supplemental Figure 5 (NT-proBNP profiles in patients with sudden cardiac death).

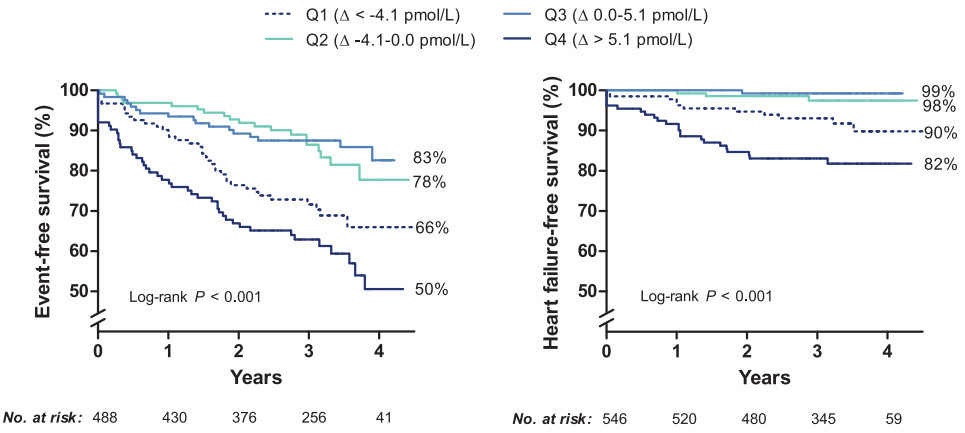

FIGURE 3 - Association of the change in NT-proBNP concentration in the first year (Δ Year 1 - Year 0) with the primary and secondary study endpoint.

Only a subset of the data could be used for this conventional type of analysis, because all measurements after Year 1 were discarded, the time-to-event was recalculated from Year 1 onwards and patients with a study endpoint in the first year were excluded. Hence, for the analysis of the primary and secondary endpoint, 488 and 546 patients were available, respectively.

TABLE 2 - Hazard ratios per two-fold higher value of the biomarker, calculated using a joint model.

	HR (95% CI)	*P*-value
Primary endpoint		
Repeated NT-proBNP measurements*	1.63 (1.49–1.76)	< 0.001
Adjusted for baseline characteristics*†	1.55 (1.35–1.79)	< 0.001
Additionally adjusted for baseline NT-proBNP†‡	2.05 (1.23–3.66)	< 0.001
Repeated NT-proBNP and eGFR measurements§		
Repeated NT-proBNP measurements	1.60 (1.50–1.71)	< 0.001
Repeated eGFR measurements	0.88 (0.67–1.16)	0.266
Secondary endpoint		
Repeated NT-proBNP measurements*	2.46 (2.04–2.87)	< 0.001
Adjusted for baseline characteristics*†	2.10 (1.64–2.75)	< 0.001
Additionally adjusted for baseline NT-proBNP†‡	4.44 (1.50–13.7)	< 0.001
Repeated NT-proBNP and eGFR measurements§		
Repeated NT-proBNP measurements	2.51 (2.20–2.92)	< 0.001
Repeated eGFR measurements	1.30 (0.87–2.11)	0.166

*HR for a patient with a two-fold higher NT-proBNP level than another patient at any point in time during the follow-up. †Adjusted for age (years), sex (0–1), congenital diagnosis (moderate vs. complex), NYHA class (I vs II–III), cardiac medication use (0–1), loss of sinus rhythm (0–1), systemic ventricular function (0–3), body mass index (kg/m^2), saturation < 90%, ≥ 1 re-intervention after initial corrective surgery, and eGFR (mL/min, $\log_2$-transformed). ‡HR for a patient with a two-fold higher NT-proBNP level than another patient at any point in time during the follow-up, when two patients with the same baseline NT-proBNP level are compared. §Analyzed using a multivariate joint model (including both repeated NT-proBNP and repeated eGFR measurements). **Abbreviations:** CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

TABLE 3 - Hazard ratios per two-fold higher value of NT-proBNP, calculated using a joint model: stratified analysis in patients with baseline BNP < 14 pmol/L (normal) and > 14 pmol/L (elevated).

	Baseline NT-proBNP < 14 pmol/L (normal, n = 280)			Baseline NT-proBNP > 14 pmol/L (elevated, n = 315)		
	No. Cases	Crude HR (95% CI)	*P*-value	No. Cases	Crude HR (95% CI)	*P*-value
Primary endpoint	50			149		
Repeated NT-proBNP measurements*		1.61 (1.10–2.52)	0.011		1.65 (1.46-1.86)	< 0.001
Adjusted for baseline NT-proBNP†		2.03 (0.88–4.71)	0.098		2.08 (1.31-3.87)	< 0.001
Secondary endpoint	2			56		
Repeated NT-proBNP measurements*		3.67 (0.41–63.2)	0.284		2.30 (1.89-2.86)	< 0.001
Adjusted for baseline NT-proBNP†		6.62 (0.10–1026)	0.435		2.47 (1.13-5.70)	0.017

*HR for a patient with a two-fold higher NT-proBNP level than another patient at any point in time during the follow-up. †HR for a patient with a two-fold higher NT-proBNP level than another patient at any point in time during the follow-up, when two patients with the same baseline NT-proBNP level are compared. **Abbreviations:** CI, confidence interval; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

DISCUSSION

In this study, we describe yearly repeated NT-proBNP measurements in adult patients with congenital heart disease for the first time. Baseline NT-proBNP levels in patients with an event were markedly higher, especially in patients who developed heart failure or died. In addition, NT-proBNP levels generally increased prior to the occurrence of an event, whereas patients without any cardiovascular event exhibited stable and low NT-proBNP levels. Consequently, when two patients with the same NT-proBNP level at baseline were compared, higher values measured during the follow-up were strongly indicative of events. Repeated measurements were especially of value in patients with an elevated NT-proBNP at baseline.

Repeated biomarker measurements

Repeated NT-proBNP sampling allows the quantification of a change in levels over time. Although, in general, NT-proBNP concentrations were relatively stable over time and the average increase in patients with the primary endpoint was modest, the occurrence of death or heart failure was preceded by a substantial change in NT-proBNP level. The annual increase prior to the occurrence of death or heart failure was approximately 25% of the baseline value, and may therefore be considered clinically meaningful. This is in line with previous studies in patients with chronic heart failure, showing that changes in NT-proBNP level over time are associated with an individual's prognosis.[11-14]

Using all repeated NT-proBNP measurements in the JM yielded a higher HR compared with using only the baseline value in the Cox model. This may be explained by the fact that the JM incorporates all NT-proBNP values that were subsequently taken during the follow-up, and were thus also measured closer to the occurrence of adverse events. A measurement close to the occurrence of an adverse event could better reflect an individual's disease status at that time. Accordingly, in a study on chronic heart failure patients by Masson et al., a single NT-proBNP value at 4 months of follow-up was found to have a greater prognostic accuracy than baseline NT-proBNP.[11]

Although the exact results must be interpreted with great caution because only a subset of the data is used and regression to the mean can play a role, the conventional statistical analyses (Figure 3) confirm our main analysis, and show that the 25% of patients with the largest increase in NT-proBNP during the first year (> 5.1 pmol/L) were at highest risk of the primary and secondary study endpoint. Interestingly, the 25% of patients with the largest decrease in NT-proBNP (< -4.1 pmol/L) also had a high risk of the study endpoints. These data suggest that variations in NT-proBNP level are associated with an increased risk of adverse events, and that the observed direction of change is dependent on the coincidental moment on which the biomarker is measured

(note that our patients were clinically stable). Furthermore, patients with more variation in their NT-proBNP level also appear to have higher baseline NT-proBNP levels (Table 1). Comparable findings have been reported by Masson et al. in patients with chronic heart failure, showing that patients with the largest absolute change in NT-proBNP level within the first 4 months had a higher all-cause mortality risk than patients with a relative stable level.[11] Of note, the strength of evaluating more than two measurements per patient with LME models (as applied in this study), is that it becomes possible to adjust for this variation and to quantify gradual increases in the average NT-proBNP level over time.

Regression to the mean

An extreme measurement has the tendency to become less extreme when it is measured again, due to the intra-patient fluctuations around a true mean. This statistical phenomenon is called regression to the mean, and especially occurs when an analyzed variable has a substantial within-subject variability over time.[23-25] NT-proBNP is known to have a substantial intra-individual biological variation.[10] Regression to the mean is therefore an important concept to consider when serial measurements are analyzed, and differences between two subsequent measurements cannot be directly translated into a true increase or decrease. Multiple (> 2) measurements provide more accurate estimations of an individual's true average NT-proBNP level, and better allow analysis of biomarker evolution over time.

Most of the studies that have evaluated repeated NT-proBNP measurements so far, have assessed the difference between two subsequent NT-proBNP measurements and related relative, absolute and/or categorical changes to adverse events.[11-14] This is the first time that multiple repeated NT-proBNP values are investigated, and that JM techniques are applied. An important advantage of JM is that the LME model part takes into account the random within-subject variation (11% of the total variation in our study). Because this allows adjustment for the inherent biological variation and regression to the mean, this sophisticated statistical technique produces more reliable estimates of the true NT-proBNP profile of individual patients.

Clinical implications

Patients with an elevated NT-proBNP level at baseline are clearly at higher risk of adverse cardiovascular events.[9] In these patients, it seems reasonable to regularly check the NT-proBNP level by an annual measurement, because especially the increase in the year prior to the occurrence of death or heart failure was substantial (~25%). Moreover, in this group the repeated measurements were independently associated with study

endpoints when adjustment was performed for the baseline NT-proBNP measurement. Patients with high NT-proBNP levels seem to have more biological variation; therefore, it could be important to perform multiple (> 2) measurements, in order to detect a gradual increase in the NT-proBNP over time. Patients with a high NT-proBNP level and patients with a substantial increase over time may require more frequent follow-up visits or timely initiation or expansion of heart failure medication. The optimal frequency of repeated measurements cannot be determined from these data and warrants future research.

In contrast, in patients with a normal NT-proBNP level at baseline (< 14 pmol/L, 47% of our study population) the NT-proBNP concentration was relatively stable and the event rates were much lower. Especially the risk of death and heart failure was extremely low, which emphasizes the high negative predictive value of NT-proBNP.[9] Due to these low event rates, no significant additional value of repeated measurements could be shown in this group. Our pragmatic interpretation of these results is as follows: because the absolute risk of events is low in this group, even an increased relative risk for higher values during follow-up (which did not reach statistical significance in our study) would add limited information. It therefore seems unnecessary to repeat NT-proBNP measurements annually in these patients to obtain additional prognostic information. The clinical implication of not needing annual repeated measurements in patients with normal NT-proBNP also includes a possible positive cost-effect, because these measurements are relatively expensive. It may be advisable to update information on NT-proBNP levels at a lower frequency, for instance every four or five years. To provide evidence for this, studies with a much longer follow-up duration are needed.

Finally, NT-proBNP might be useful as a biomarker to detect a response to successful interventions, because we observed large NT-proBNP reductions among individual patients, especially after surgical valve interventions. Whether this could actually improve the patient selection and timing for re-intervention warrants future studies including more frequent NT-proBNP measurements that are scheduled at pre-defined time points both before and after valvular interventions.

Study limitations

Although we performed multivariable adjustment for multiple covariates such as age, sex, congenital diagnosis, NYHA class, cardiac medication use, rhythm, systemic ventricular function, body mass index, saturation, re-intervention, and eGFR, this is an observational study and as such subject to residual confounding. For instance, our cohort consisted of a relatively heterogeneous group of multiple congenital diagnoses, and we could only adjust for a dichotomized variable indicating a moderate (0) or complex (1) type of congenital diagnosis. Other variables that were not taken into account in

this analysis may also play a role in the prognostication of patients with ACHD. Of note, patients with mild cardiac lesions such as isolated atrial or ventricular septal defect were excluded in this study, because the expected event rate in these patients is very low. This should be taken into account when these results are compared with other cohorts of ACHD patients.

In this study, only repeated NT-proBNP and repeated eGFR measurements were analyzed. However, also changes in other biomarkers with less day-to-day variations or other patient characteristics could play a role in the prognostication of ACHD patients. We did not take repeated two-dimensional echocardiographic measurements into account, because these are known to have a relatively large measurement error and we therefore did not consider these suitable for accurate quantification of serial changes.[26] Other repeatedly assessed clinical variables were not available. Ideally, updating multiple variables during each follow-up visit could lead to more precise and individualized dynamic predictions.

Finally, we used composite endpoints in this study, and it should be taken into account that the individual components are not all of similar clinical importance to the patient.

CONCLUSIONS

A single measurement of NT-proBNP at any time during the follow-up is of important prognostic value in patients with ACHD who routinely visit the outpatient clinic. In patients with a normal NT-proBNP level, it is not needed to annually repeat NT-proBNP measurements to obtain additional prognostic information. In patients with an elevated NT-proBNP level, yearly repeated measurements were strongly predictive of study endpoints and provided significant prognostic information beyond the baseline NT-proBNP level. In these patients, serial measurements may therefore aid in the further optimization of individual follow-up strategies, medical therapies and timing of re-interventions.

CLINICAL PERSPECTIVE

What is new?

- NT-proBNP is associated with the risk of death or heart failure in patients with adult congenital heart disease (ACHD).
- ACHD patients without cardiovascular events during 5-year follow-up had stable and low NT-proBNP levels.
- NT-proBNP levels generally increased prior to the occurrence of an adverse event in patients with ACHD, especially prior to death or heart failure.

- Repeated NT-proBNP measurements were useful to improve prognostication in patients with elevated (> 14 pmol/L) baseline values, since higher values, as well as (large) variations were associated with an increased risk of adverse cardiovascular events.

What are the clinical implications?

- ACHD patients with low NT-proBNP concentrations (< 14 pmol/L) have a very favorable prognosis, and serial measurements will therefore be of limited additional value.
- ACHD patients with elevated NT-proBNP concentrations (> 14 pmol/L) have an increased risk of death or heart failure.
- ACHD patients with elevated NT-proBNP concentrations that have additional increments over time represent a population that should receive close follow-up.

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SUPPELEMENTAL MATERIAL

SUPPLEMENTAL METHODS 1 - Linear mixed-effects model building and selection.

We used all NT-proBNP measurements that occurred prior to the primary endpoint (n = 2009), with a median of 4 measurements per patient. We started with a LME with linear and quadratic time evolutions, a nonlinear effect of age using natural cubic splines with 3 degrees of freedom, the main effects of sex, diagnosis, NYHA class, medication use, rhythm, systemic ventricular function, BMI, saturation, re-intervention and logeGFR, and the interactions of time with sex and diagnosis (Model 1).

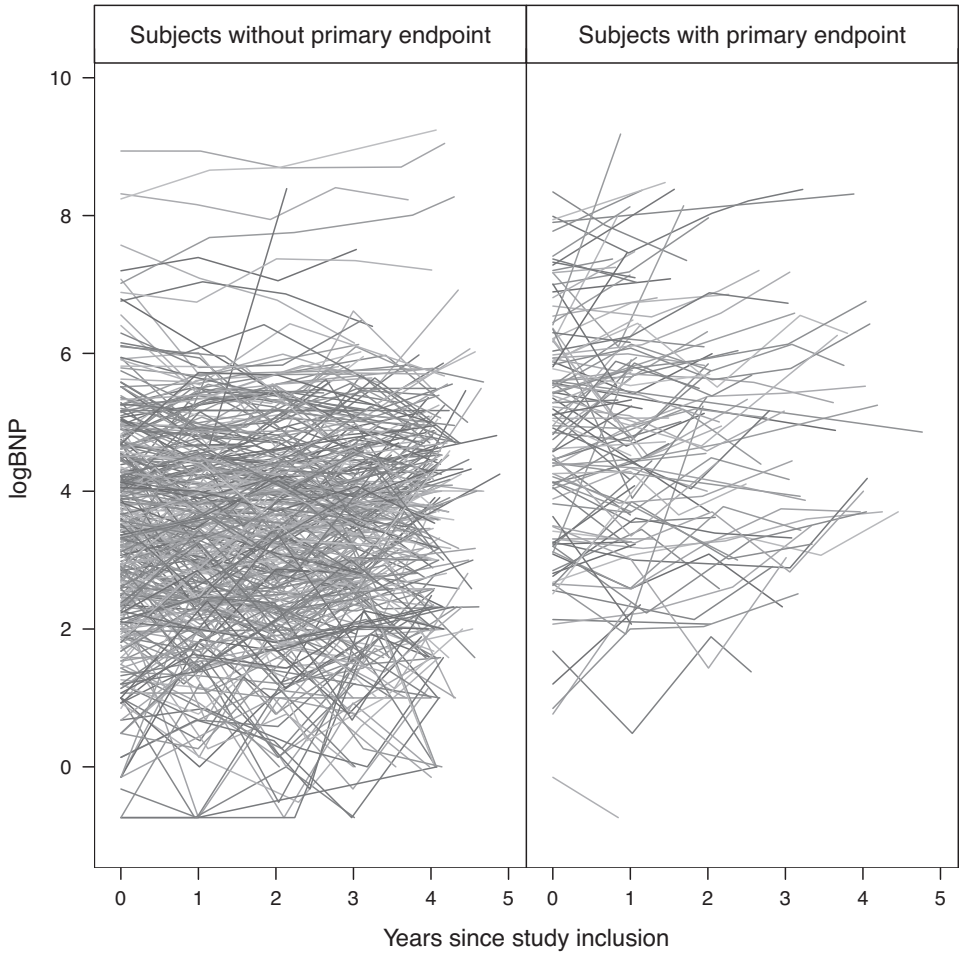
We first expanded the random-effects structure, while keeping the same fixed effects, in order to appropriately model the correlations in the repeated NT-proBNP measurements. We therefore included the linear random slopes term (Model 2) and tested whether this improved the fit of the model by using a likelihood ratio test, which was not the case ($p = 0.323$). Therefore, we continued with a model with only random intercepts.

To define the fixed part of the model, we subsequently tested whether the interaction terms had an important contribution to the fit of the model. To this end, we refitted Model 1 under maximum likelihood (instead of the default restricted maximum likelihood) and then fitted another model without the interaction terms (Model 3). The results suggested that the effects of time do not differ between men and women, and between patients with a moderate or complex diagnosis ($p = 0.249$). We continued by performing the omnibus test for all nonlinear terms in the model. We therefore fitted the model that excluded all nonlinear terms (Model 4) and compared this with Model 3 using the likelihood ratio test. This indicated that the nonlinear terms did not have a significant contribution to the model ($p = 0.096$).

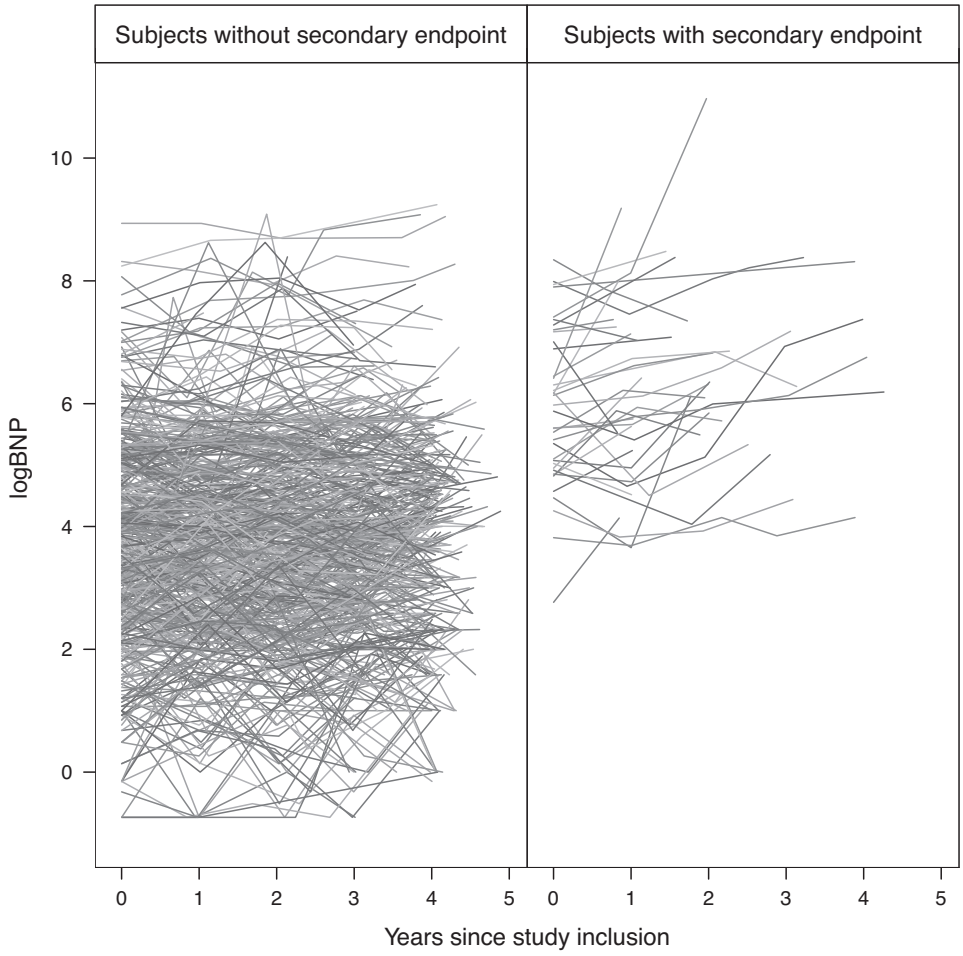
Therefore, the final selected model for the $\log_2$ transformed serial NT-proBNP measurements was a random intercepts LME with a linear time evolution, and main effects of age (linear), sex, diagnosis, NYHA class, medication use, rhythm, systemic ventricular function, BMI, saturation, re-intervention and logeGFR. A schematic overview of the LME model building and selection is presented in Supplemental Table 1. This model was refitted with restricted maximum likelihood and further used for the estimation of the temporal NT-proBNP evolution. Of note, the joint models were estimated using a Bayesian framework.

SUPPLEMENTAL TABLE 1 - Linear mixed-effects model building and selection.

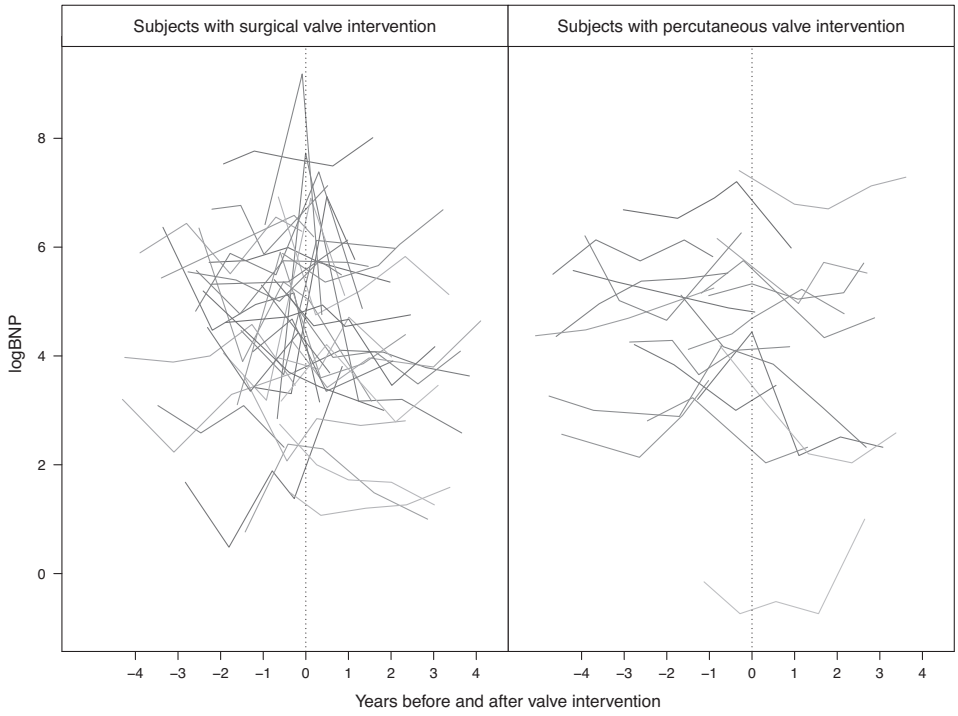
NT-proBNP model	Fixed	Random	LRT	Compared with (method)
1	logBNP ~ (obstimeyr + I(obstimeyr^2)) * (sex + diagnosis) + ns(age,3) + NYHA + medication + rhythm + systfunc + BMI + saturation + reintervention + logeGFR	~ 1 | id		
2	Equal to model 1	~ obstimeyr | id	$p = 0.323$	Model 1 (REML)
3	logBNP ~ (obstimeyr + I(obstimeyr^2)) + sex + diagnosis + ns(age,3) + NYHA + medication + rhythm + systfunc + BMI + saturation + reintervention + logeGFR	~ 1 | id	$p = 0.249$	Model 1 (ML)
4	logBNP ~ obstimeyr + sex + diagnosis + age + NYHA + medication + rhythm + systfunc + BMI + saturation + reintervention + logeGFR	~ 1 | id	$p = 0.096$	Model 3 (ML)



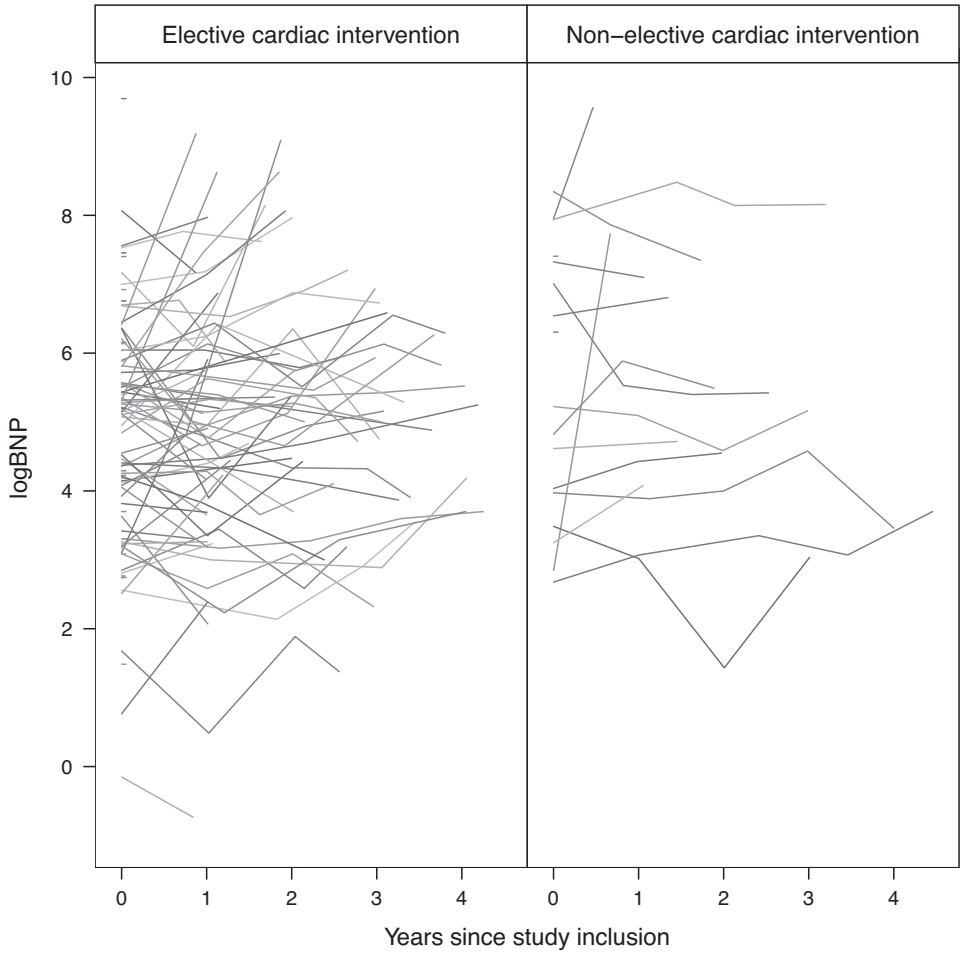
SUPPLEMENTAL FIGURE 1 - Individual NT-proBNP profiles in patients without and with the primary endpoint.



SUPPLEMENTAL FIGURE 2 - Individual NT-proBNP profiles in patients without and with the secondary endpoint.

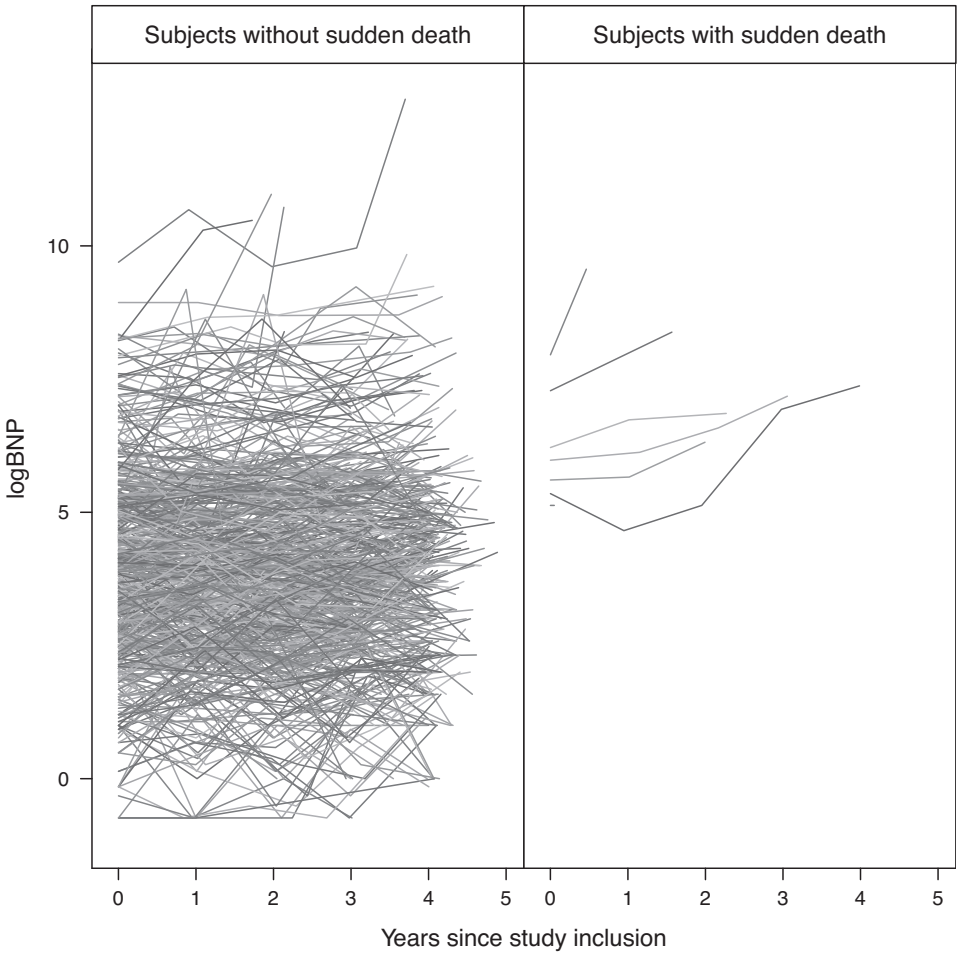


SUPPLEMENTAL FIGURE 3 - NT-proBNP profiles in patients with a surgical or percutaneous valve intervention (at time point 0).



SUPPLEMENTAL FIGURE 4 - NT-proBNP profiles in patients with elective versus non-elective cardiac interventions.

Of the 113 cardiac interventions, 17 were non-elective (pacemaker or ICD implantation, n = 10; surgical aortic valve replacement, n = 4; percutaneous pulmonary valve dilatation, n = 1; coronary intervention, n = 1; ablation, n = 1). No clear differences in the NT-proBNP profiles were observed, probably because the group of patients with a non-elective intervention was relatively small and heterogeneous.



SUPPLEMENTAL FIGURE 5 - NT-proBNP profiles in patients with sudden cardiac death.

The 7 patients with sudden cardiac death had higher NT-proBNP levels at baseline that increased over time in all patients. Because of the low (expected) number of patients with sudden cardiac death, we did not aim to make predictions for this specific endpoint.

Chapter 14

The prognostic value of various biomarkers in adult patients with pulmonary hypertension: a multi-biomarker approach

Vivan J.M. Baggen,* Laurie W. Geenen,* Thomas Koudstaal, Karin A. Boomars, Jannet A. Eindhoven, Eric Boersma, Jolien W. Roos-Hesselink, Annemien E. van den Bosch

*Equal contributions

Submitted.

ABSTRACT

Aims To evaluate diagnosis-specific biomarker profiles in patients with pulmonary hypertension and to investigate the association of these biomarkers with clinical outcomes. Additionally, we explored the prognostic value of a multi-biomarker approach.

Methods and results In this study, 106 consecutive treatment-naïve patients with pulmonary hypertension confirmed by right heart catheterization were prospectively included (median age 58.7 [IQR 47.0–69.2] years, woman, 64%). Venous blood sampling, performed during right heart catheterization, included; NT-proBNP, high-sensitive troponin-T, high sensitive-CRP, galectin-3, red blood cell distribution width and eGFR. During a median follow-up duration of 23.9 [IQR 15.1–40.0] months death or lung transplantation (primary endpoint) occurred in 29 patients and 37 patients reached the secondary endpoint (death, lung transplantation or heart failure). Cox proportional-hazards regression analysis showed significant associations between all biomarkers and the primary and secondary endpoint with adjustment for age. A multi-biomarker approach including the number of elevated biomarkers per patient, demonstrated that patients were at higher risk of adverse events as more biomarker levels were elevated (HR for each extra elevated biomarker; 1.33, 95% CI 1.07–1.64, $p = 0.01$). The absence of any elevated biomarker (n = 11, 11%) ruled out the risk of any event up to 40 months.

Conclusions Various biomarkers are associated with the event-free survival in adult patients with pulmonary hypertension. A single biomarker may not be sufficient to achieve optimal risk stratification. This study provides evidence in favour of a multi-biomarker approach.

INTRODUCTION

Pulmonary hypertension (PH) is a heterogeneous disease, characterized by an increased pulmonary vascular resistance leading to an elevated pulmonary arterial pressure. Eventually, compensatory mechanisms of the right ventricle may fail to cope with the increased afterload resulting in progressive right-sided heart failure (HF) and death.[1, 2] Although treatment options have expanded, morbidity and mortality rates remain high. Risk stratification is crucial to identify patients at high risk and to optimize therapeutic management.

The prognosis of PH varies widely and is besides aetiology,[3] also based on clinical and hemodynamic characteristics including symptoms of HF, 6-minute walking distance, right atrial pressure and cardiac index, according to the European guidelines on PH[4]. Currently, the response to therapy and prognosis is often based on these factors. Biomarkers may provide objective measurements in a relatively non-invasive and easy-accessible manner and the European guidelines on PH advise the use of N-terminal pro B-type natriuretic peptide (NT-proBNP) and troponin-T,[4] which are known to be associated with outcomes in patients with PH.[5, 6] Nevertheless, the search for novel biomarkers in PH is ongoing, resulting in new potential biomarkers reflecting various pathophysiological pathways.[7, 8]

Because heterogeneous conditions involving different pathophysiological pathways can give rise to the development and prognosis of PH, multiple biomarkers may potentially better reflect a patient's condition. In addition, a combination of biomarkers could provide insight in the main pathophysiological mechanisms within PH subgroups. This study aimed to evaluate diagnosis-specific biomarker profiles and to investigate the associations of these biomarkers with clinical outcomes in patients with PH. Additionally, we explored the prognostic value of a multi-biomarker approach.

METHODS

Study design and population

In this prospective observational cohort study, we included all consecutive adult patients diagnosed with PH confirmed by right heart catheterization between the 15th of May 2012 and the 4th of October 2016 in our centre. A mean pulmonary artery pressure (mPAP) of ≥ 25 mmHg measured by right heart catheterization was used as cut-off value for the diagnosis of PH.[4] Exclusion criteria were: patients with incomplete diagnostic work-up and therefore no confirmed PH diagnosis, patients who already used PH-specific medication, patients aged < 18 years and patients not capable of understanding and signing informed consent. In addition, we excluded patients with

PH due to left heart disease because this cause of PH has a fundamental different underlying pathophysiology than other PH groups[9]. The study protocol was approved by the medical ethical committee and written informed consent was provided by all patients. This study was performed conform the principles outlined in the Declaration of Helsinki.

World Health Organization classification

Subgroups of PH were classified in accordance with the World Health Organization (WHO) classification of PH:[4, 10] pulmonary arterial hypertension (PAH), PH due to lung diseases/hypoxia, chronic thromboembolic pulmonary hypertension (CTEPH) and PH with unclear/multifactorial mechanisms (WHO5). Patients with a mixed clinical picture were grouped under WHO5. Group 1 patients (PAH) were further stratified in subgroups according to the WHO classification.

Data collection

During the inpatient screening visit for analysis of PH, all patients underwent physical examination by a cardiologist and pulmonary physician, 6-minute walking test, 12-lead electrocardiography (ECG), echocardiography, venous blood sampling, chest computed tomography scan and right heart catheterization. Patient characteristics and vital signs were collected, including: age, sex, height, weight, blood pressure, heart rate and peripheral oxygen saturation. We used the New York Heart Association (NYHA) functional class to grade the severity of functional limitations by the presence of signs and symptoms of HF.

During right heart catheterization, a Swan-Ganz catheter was inserted in the internal jugular vein. A standardized protocol for the work-up of PH was used to obtain hemodynamic measurements and thermodilution or Fick's principle was used to measure the cardiac output. When the obtained capillary wedge pressure was ambiguous, a fluid challenge was performed to distinguish between pre-capillary PH and PH due to left heart disease.

Data was collected and stored in PAHTool (version 4.3.5947.29411, Inovoltus, Santa Maria da Feira, Portugal), an online electronic case report form.

Echocardiography and cardiac computed tomography

Two-dimensional transthoracic echocardiography was performed using a commercially available ultrasound system (iE33, Philips Medical Systems, Best, the Netherlands). For the imaging analysis, we followed the guidelines for cardiac chamber quantification by echocardiography from the American Society of Echocardiography and the European

Association of Cardiovascular imaging echocardiography.[11] The systolic left ventricular function was visually graded as normal, mildly, moderately or severely impaired. We measured the left ventricular end-diastolic dimension in the parasternal long axis of the left ventricle. The 4-chamber view was used to measure the right atrial area, right ventricular basal dimension and right ventricular fractional area change. M-mode of the 4-chamber view was used to measure the tricuspid annular plane systolic excursion (TAPSE). The presence of pericardial effusion was defined as mild (< 10mm), moderate (10–20 mm) or severe (> 20mm) in one of the views.

Cardiac computed tomography was performed according to routine clinical practice. The central pulmonary artery diameter and the ascending aortic diameter were measured at the level of the pulmonary artery bifurcation.[12]

Clinical follow-up and definition of endpoints

Patients were prospectively followed-up by half-yearly scheduled visits to the outpatient clinic. Specific PH medications were prescribed when indicated in accordance with the ESC guidelines.[4] Patients with CTEPH eligible for pulmonary endarterectomy or balloon pulmonary angioplasty were referred and treated when indicated. Patients who underwent one of the above procedures, were not censored afterwards.

The primary composite endpoint was defined as all-cause mortality or lung transplantation. The secondary endpoint was a composite of all-cause mortality, lung transplantation or HF-related hospital admission that was defined as any hospitalization due to symptoms or signs of HF requiring (additional) treatment with diuretics.

Survival status of all patients was checked in the Municipal Personal Records database. Suspected endpoint events were adjudicated by two independent researchers based on the electronic patient records. When necessary, we contacted referring hospitals and general practitioners to obtain additional information. Patients who did not reach the primary or secondary endpoint were censored at the 1st of June 2017.

Biomarker assessment

Venous blood samples were obtained during right heart catheterization for study purposes only and were at first place not intended for clinical decision-making. Transference of the blood samples to the clinical chemistry laboratory took place within 2 hours from withdrawal. NT-proBNP, estimated glomerular filtration rate (eGFR) and red cell distribution width (RDW) levels were directly determined in the fresh blood samples. The lab-specific upper limits of normal (ULN) to define elevated levels were > 15 pmol/L (≈ 125 pg/mL) for NT-proBNP, > 16% for RDW, and < 60mL/min/1.73m^2 for eGFR.

Other serum samples were aliquoted and stored at -80 degrees Celsius, until batch analysis was performed to determine high sensitive troponin-T (hs-TnT), high sensitive

C-reactive protein (hs-CRP) and galectin-3. Defrosting of the samples took place in batches followed by immediate analysis, to ensure that samples were exposed to only one freeze-thaw cycle. A commercial electrochemiluminescence immunoassay (Roche Diagnostics, Rotkreuz, Switzerland) was used to determine the levels of hs-TnT and hs-CRP with lower limits of detection (LoD) of 3 ng/L and 0.3 mg/L, respectively. The ULN was 14 ng/L for hs-TnT and 10 mg/L for hs-CRP.

Galectin-3 levels were measured with the ARCHITECT *ci*8200 analyser (Abbott Diagnostics, Hoofddorp, the Netherlands). The ARCHITECT assay is designed to have a limit of quantitation of ≤ 4.0 ng/mL. The ULN was based on the 97.5th percentile of a series of healthy volunteers, and was > 16.9 ng/mL for man and > 21.3 ng/mL for woman, as previously described.[13]

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) or median [interquartile range]. Biomarker levels were $\log_2$ transformed to correct for skewness. Biomarker values below the LoD were substituted with a value that was equal to 50% of the LoD, for analytical purposes.

Biomarker release was visualized in scatterplots. Comparisons of biomarker levels across PH subgroups were performed using a one-way ANOVA test or Kruskal-Wallis test. Correlations were reflected by the Pearson or Spearman correlation coefficient, depending on the distribution of the data. Tertiles of the biomarker distributions were determined. The cumulative survival for the tertiles was calculated using the Kaplan-Meier estimator; comparisons between survival tertiles were made using the log-rank test for trend.

To compare the effect sizes of the different biomarkers, biomarker levels were standardized according to the mean and SD of their distribution, and the relation between the thus obtained Z-scores and study endpoints were evaluated by Cox proportional-hazards regression. Multivariable analysis was performed to correct for age as potential confounder in all further analysis. The corresponding C-index, reflecting the discriminative ability of each biomarker up to 40 months, was calculated.

Subsequently, all patients with complete biomarker profiles were included in a multi-biomarker model. Biomarkers were classified as normal or elevated according to the pre-specified cut-off value. The association of the number of elevated biomarkers with the study endpoints was evaluated by Cox regression. Statistical analysis was performed using IBM SPSS software (version 21.0.0.1). The C-index was calculated in R (version 3.3.3), packages SurvC1 and Survival. A two-sided *p*-value of < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

A total of 164 consecutive patients underwent right heart catheterization as part of the screening for PH between 15th of May 2012 and 4th of October 2016, of which 106 patients fulfilled the inclusion criteria and were enrolled in the study (Supplemental File 1). Patients were classified as PAH (n = 54, 51%), PH-lung disease (n = 15, 14%), CTEPH (n = 21, 20%) and WHO5/multifactorial (n = 16, 15%). Baseline characteristics of all patients and specified for each PH diagnosis, are summarized in Table 1. Median age was 58.7 [IQR 47.0–69.2] years, 68 (64%) were woman and 58 (55%) were in NYHA class III or IV. In 8 patients, pericardial effusion was present, of which 7 mild and one moderate.

Follow-up

The median follow-up duration was 23.9 (IQR 15.1–40.0) months. Follow-up data regarding mortality and other endpoints was 100% complete. During follow-up 25 patients died and 4 patients underwent lung transplantation, so the primary endpoint was reached in 29 patients (27.4%). Causes of death included end-stage HF (n = 8), sudden death, presumed cardiac (n = 4), euthanasia in patients with end-stage cardiovascular and pulmonary disease (n = 3), multi-organ failure (n = 3), kidney and/or liver failure (n = 2), myocardial infarction (n = 1), progression of systemic sclerosis (n = 1), hepatic encephalopathy (n = 1), malignancy (n = 1) and sudden death, presumed cerebral (n = 1).

Twenty-two patients were hospitalized for HF requiring (additional) diuretic treatment. This resulted in 37 (34.9%) patients who reached the secondary endpoint. Of the patients with CTEPH, three patients underwent balloon pulmonary angioplasty and three patients underwent pulmonary endarterectomy surgery.

Biomarker release and PH classification

Baseline levels of the six biomarkers stratified according to subgroups of PH are shown in Figure 1. Because of small numbers, heritable PAH, PAH induced by drugs and toxins, PAH associated with portal hypertension and PAH caused by pulmonary veno-occlusive disease were grouped as 'other'.

NT-proBNP significantly differed between the PH subgroups ($p < 0.001$). The highest levels of NT-proBNP were found in the patients with iPAH (median 205.0 pmol/L, IQR 87.9–407.0 pmol/L) and PAH associated with connective tissue disease (median 190.0 pmol/L, IQR 25.9–293.0 pmol/L). NT-proBNP was elevated in 82 (77%) patients. Of the patients with iPAH, all except one had an elevated NT-proBNP.

TABLE 1 - Baseline characteristics of all patients and stratified according to the subgroups of PH.

	Valid cases, n (%)	All patients (n=106)	PAH (n=54)	PH-lung disease (n=15)	CTEPH (n=21)	WHO 5 / multifactorial (n=16)
Clinical characteristics						
Age, years	106 (100)	59 [47–69]	55 [41–66]	64 [55–72]	59 [52–73]	64 [54–69]
Sex, female n (%)	106 (100)	68 (64)	36 (67)	8 (53)	12 (57)	12 (75)
Body mass index, kg/m^2	106 (100)	28.2 ± 6.5	26.3 ± 5.7	30.5 ± 6.3	31.4 ± 5.7	28.0 ± 8.2
Heart rate, beats/minute	106 (100)	80.1 ± 16.5	79.8 ± 16.2	81.1 ± 12.1	75.2 ± 17.2	86.6 ± 19.5
Systolic blood pressure, mmHg	106 (100)	126.6 ± 17.7	123.1 ± 15.5	125.2 ± 16.6	130.9 ± 13.1	134.2 ± 17.7
Oxygen saturation < 90%, n (%)	106 (100)	3 (3)	2 (4)	1 (7)	0 (0)	0 (0)
NYHA class III–IV, n (%)	106 (100)	58 (55)	32 (60)	8 (53)	9 (43)	9 (56)
Electrocardiography						
Rhythm, n (%)	103 (97)					
Sinus rythm		92 (89)	47 (89)	13(87)	18(95)	14(88)
Atrial fibrillation		7 (7)	4 (7)	2(13)	0(0)	1(6)
Other		4 (4)	2 (4)	0(0)	1(5)	1(6)
QRS duration, ms	102 (96)	98 [90–106]	100 [91–106]	100 [91–111]	94 [88–99]	99 [88–114]
6-minute walking test						
Distance, m	90 (85)	338.1 ± 139.1	347.7 ± 147.3	309.0 ± 115.4	685.1 ± 129.8	273.5 ± 126.4
Echocardiography						
RA area, cm^2	81 (76)	27.3 ± 8.9	27.8 ± 6.9	28.4 ± 8.4	24.6 ± 10.0	27.4 ± 13.0
RV basal dimension, mm	76 (72)	51.3 ± 9.5	52.5 ± 8.1	47.8 ± 4.7	51.1 ± 12.1	49.8 ± 12.6
RV fractional area change, %	74 (70)	29.1 ± 8.6	27.0 ± 8.0	31.3 ± 6.2	33.0 ± 4.3	30.8 ± 12.2

TABLE 1 - Continued.

	Valid cases, n (%)	All patients (n=106)	PAH (n=54)	PH-lung disease (n=15)	CTEPH (n=21)	WHO 5 / multifactorial (n=16)
Echocardiography						
RV TAPSE, mm	73 (69)	19.4 ± 4.9	18.6 ± 4.8	18.8 ± 3.1	20.9 ± 3.5	20.4 ± 7.0
LV function, n (%):	100 (94)					
Normal		66 (66)	33 (63)	10 (71)	15 (83)	8 (50)
Mildly impaired		30 (30)	17 (33)	4 (29)	3 (17)	6 (38)
Moderately impaired		3 (3)	2 (4)	0 (0.0)	0 (0.0)	1 (6)
Severely impaired		1 (1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6)
LV end diastolic dimension, mm	82 (77)	43.4 ± 7.5	41.0 ± 7.2	46.1 ± 7.3	45.5 ± 5.6	47.0 ± 8.1
Right heart catheterization						
mPAP, mmHg	106 (100)	42.0 [34.8–51.3]	49.2 [38.0–59.0]	37.0 [32.0–41.0]	37.0 [30.0–48.0]	42.5 [34.0–48.5]
mRAP, mmHg	106 (100)	9.9 ± 5.4	10.6 ± 5.6	8.3 ± 3.8	7.8 ± 4.7	11.7 ± 5.4
Capillary wedge pressure, mmHg	92 (87)	13.2 ± 6.2	11.3 ± 5.6	12.5 ± 4.3	13.7 ± 3.3	18.7 ± 8.2
Cardiac output, L/min	101 (95)	5 [4.0-6.3]	4.9 [3.9-5.7]	5.0 [3.9-5.8]	5.4 [4.9-6.3]	5.1 [4.0-6.8]
Computed tomography						
PA diameter, mm	100 (94)	34.4 ± 5.3	35.3 ± 6.0	34.9 ± 4.1	34.3 ± 5.1	31.1 ± 3.2
PA/AO ratio	100 (94)	1.12 ± 0.23	1.20 ± 0.26	1.03 ± 0.12	1.12 ± 0.24	0.97 ± 0.12

Values are represented as mean ± SD or median [IQR]. **Abbreviations:** RA, right atrial; RV, right ventricular; LV, left ventricular; mRAP, mean right arterial pressure; PA, pulmonary artery; AO, aortic artery.

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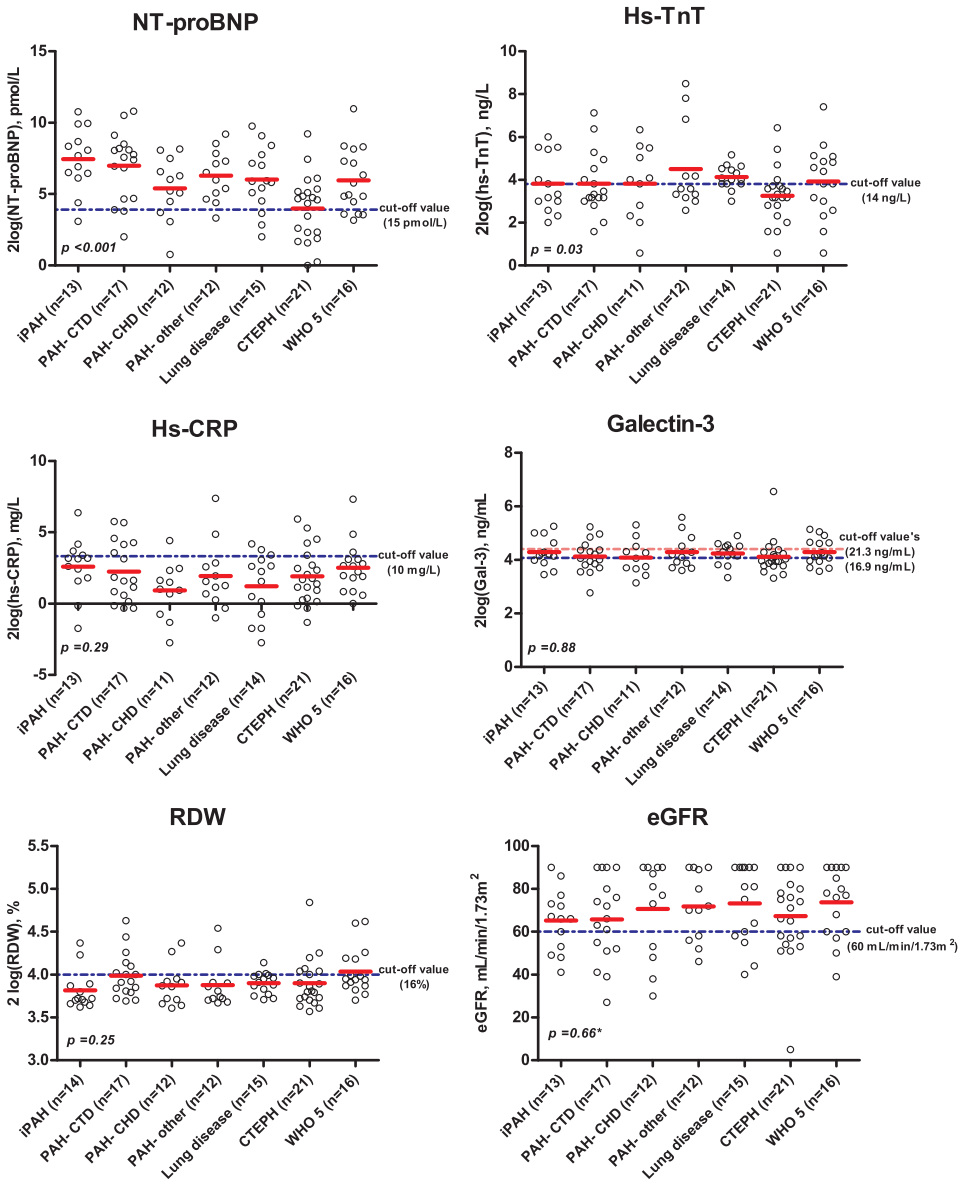

FIGURE 1 - Biomarker release stratified according to the WHO classification of PH.

Biomarker levels are represented on the $\log_2$ scale (except for eGFR; the skewness increased after $\log_2$ transformation). Blue dotted line represents the biomarker-specific cut-off value. Red line represents the mean biomarker level in each group. For galectin-3, sex-specific biomarker cut-off values are represented (blue dotted line = men, pink dotted line = women). *Kruskal Wallis test, otherwise one-way ANOVA test.

Hs-TnT also differed significantly between the PH subgroups ($p = 0.03$) and levels were lowest in CTEPH patients (median 9.0 ng/L, IQR 7.0–13.0 ng/L). In 43 patients (41%), an elevated level of hs-TnT was measured and found in particular in patients with PH-lung disease (64%).

We found no significant differences in the levels of galectin-3, hs-CRP, RDW and eGFR between the subgroups of PH. Overall, 39% of the patients had an elevated level of galectin-3, this percentage was highest in those with PH-lung disease (65%) and iPAH (54%).

Association between biomarkers and baseline characteristics

As shown in Table 2, all biomarkers significantly correlated with the 6-minute walking distance, and all except hs-TnT and RDW showed a significant correlation with NYHA class. NT-proBNP showed the strongest correlation with both the 6-minute walking distance ($r = –0.46$, $p < 0.001$) and NYHA class ($r = 0.40$, $p < 0.001$). NT-proBNP correlated with right ventricular and left ventricular function echocardiographic variables. In contrast, hs-TnT, hs-CRP, galectin-3 and RDW did not correlate with any of the echocardiographic ventricular function variables. In addition to NT-proBNP, only eGFR correlated with right atrial area, right ventricular basal dimension and left ventricular end diastolic dimensions. Hemodynamic measurements exclusively showed a significant correlation with NT-proBNP levels. Mutual correlations between biomarkers were all significant. The strongest correlation was found between hs-TnT and galectin-3 ($r = 0.58$, $p < 0.001$) (Supplemental File 2).

Associations between biomarkers and clinical outcomes

The transplant-free survival according to tertiles of biomarker levels are visualized in the Kaplan-Meier curves (Figure 2). Patients in the highest tertiles of NT-proBNP, hs-TnT, galectin-3 and RDW had a significantly higher risk of death or transplantation compared to patients in the lower tertiles.

Concerning the secondary endpoint, patients in the highest tertiles of NT-proBNP, hs-TnT, galectin-3 and RDW, were at highest risk of death, transplantation or HF-related hospital admission. For eGFR, patients in the highest tertile of eGFR had a significant worse event-free survival than patients in the lower tertiles (Supplemental File 3).

Standardized hazard ratios for the association between the biomarker levels and the endpoints are shown (Figure 3). When analysed continuously, all biomarkers showed a significant association with the primary endpoint, NT-proBNP, hs-CRP and hs-TnT had the highest hazard ratios. According to the C-index, NT-proBNP had the best discriminative power (C-index 0.69, 95% CI 0.58–0.81), followed by hs-TnT (C-index 0.67, 95% CI 0.56–0.78) and galectin-3 (C-index 0.67, 95% CI 0.56–0.79) (Supplemental File 4).

All biomarkers were also significantly associated with the secondary endpoint. For NT-proBNP and RDW, the standardized hazard ratio of the secondary endpoint increased relatively to the hazard ratio of the primary endpoint, as did the C-index.

TABLE 2 - Correlation coefficients of biomarkers with baseline characteristics.

	NT-proBNP n=106	Hs-TnT n=104	Hs-CRP n=104	Galectin-3 n=104	RDW n=106	eGFR# n=106
Clinical characteristics						
Age, years#	0.10	**0.36*****	–0.19	**0.28****	0.15	**–0.43*****
Sex, female	0.05	**0.25***	–0.11	–0.01	0.03	0.01
Body mass index, kg/m^2	–0.15	–0.04	**0.24***	0.13	–0.01	–0.06
Heart rate, beats/minute	0.15	0.08	0.19	**0.26****	0.13	–0.01
Systolic blood pressure, mmHg	**–0.22***	–0.18	–0.04	–0.13	**–0.21***	0.04
Oxygen saturation < 90%	–0.02	–0.06	–0.07	–0.02	0.07	0.13
NYHA class III/IV	**0.40*****	0.18	**0.23***	**0.30****	0.19	**–0.38*****
Electrocardiography						
Loss of sinus rhythm	0.11	0.06	–0.13	–0.08	–0.05	0.01
QRS duration, ms#	0.11	**0.24***	–0.14	0.05	0.07	–0.01
6-minute walking test						
Distance, m	**–0.44*****	**–0.38****	**–0.32****	**–0.44*****	**–0.33****	**0.40*****
Echocardiography						
RA area, cm^2	**0.41*****	0.17	–0.13	–0.03	–0.01	**–0.25***
RV basal dimension, mm	**0.49*****	0.18	0.13	0.03	–0.02	**–0.28***
RV fractional area change, %	**0.34****	–0.17	–0.13	–0.19	–0.05	0.19
RV TAPSE, mm	**0.43*****	–0.19	–0.15	0.00	–0.06	–0.01
LV function, 0–3	**0.35*****	0.14	0.14	0.05	0.19	–0.08
LV end diastolic dimension, mm	**–0.31****	–0.12	–0.16	–0.10	0.02	**0.39*****
Right heart catheterization						
mPAP, mmHg#	**0.42*****	0.03	0.10	–0.01	0.07	–0.07
mRAP, mmHg	**0.30****	0.16	0.18	0.16	0.13	–0.18
Capillary wedge pressure, mmHg	–0.06	–0.03	0.01	0.07	0.03	0.02
Cardiac output, L/min#	**0.46*****	–0.17	–0.06	–0.01	0.03	0.18
Computed tomography						
PA diameter, mm	0.03	0.11	–0.14	–0.05	–0.08	–0.03
PA/AO ratio	–0.10	–0.13	–0.13	**–0.26****	–0.15	0.16

#Spearman, otherwise Pearson correlation coefficient. Significant values are presented in bold. Level of significance is indicated by the number of asterisks. *indicates $p < 0.05$, **indicates $p < 0.01$, ***indicates $p < 0.001$. **Abbreviations:** RA, right atrial; RV, right ventricular; LV, left ventricular; mRAP, mean right arterial pressure; PA, pulmonary artery; AO, aortic artery.

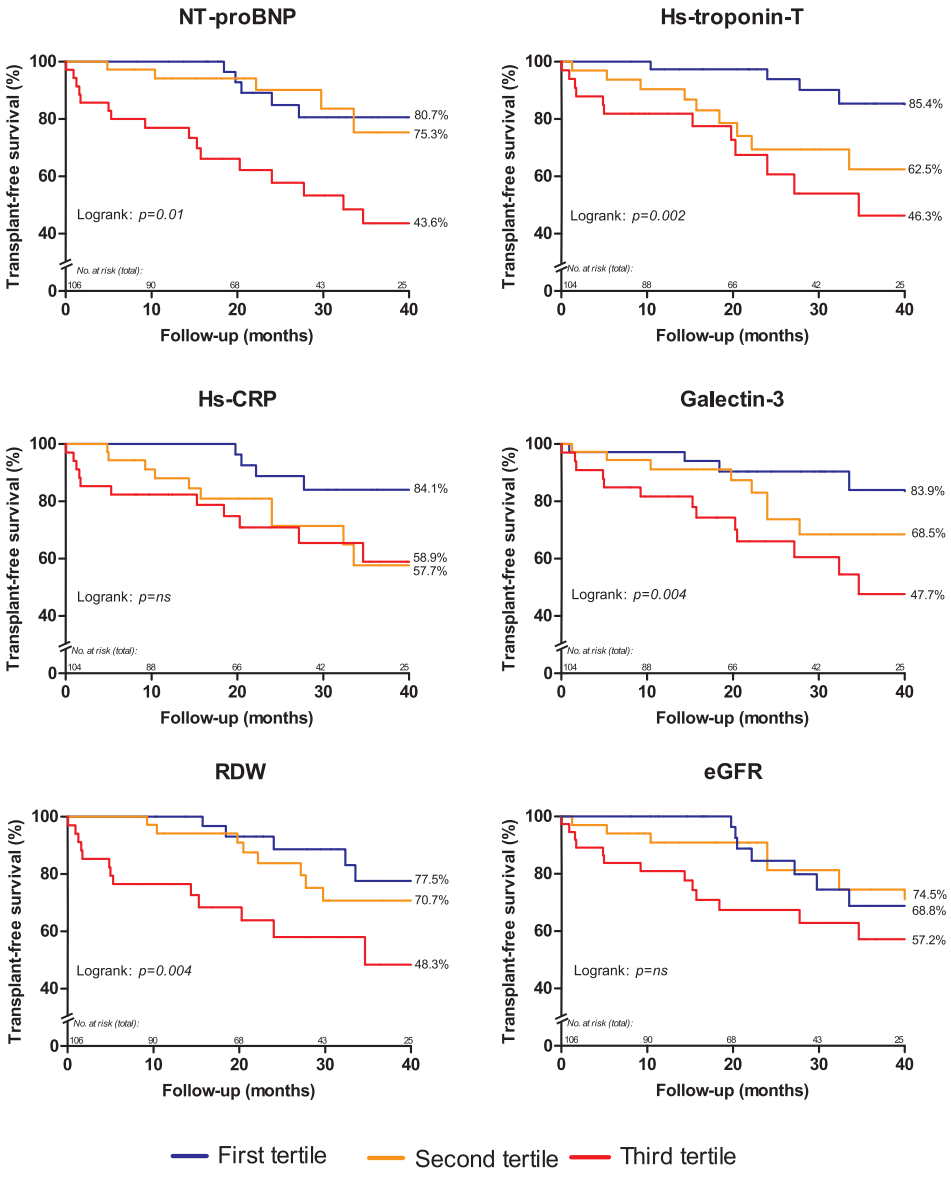

FIGURE 2 - Transplant-free survival according to tertiles of the biomarker levels.

NT-proBNP: first tertile < 26.57 pmol/L, second tertile 26.57–150.0 pmol/L, third tertile > 150.0 pmol/L. *Hs-TnT*: first tertile < 2.0 ng/L, second tertile 2.0–6.3 ng/L, third tertile > 6.3 ng/L. *Hs-CRP*: first tertile < 2.0 mg/L, second tertile 2.0–6.3 mg/L, third tertile > 6.3 mg/L. *Galectin-3*: first tertile ≤ 15.3 ng/mL, second tertile 15.3–19.9 ng/mL, third tertile > 19.9 ng/mL. *RDW*: first tertile ≤ 13.6%, second tertile 13.6–15.5%, third tertile > 15.5%. *eGFR*: first tertile > 80.3 ml/min, second tertile 60.0–80.3 ml/min, third tertile ≤ 60 ml/min (tertiles of eGFR are inversed).

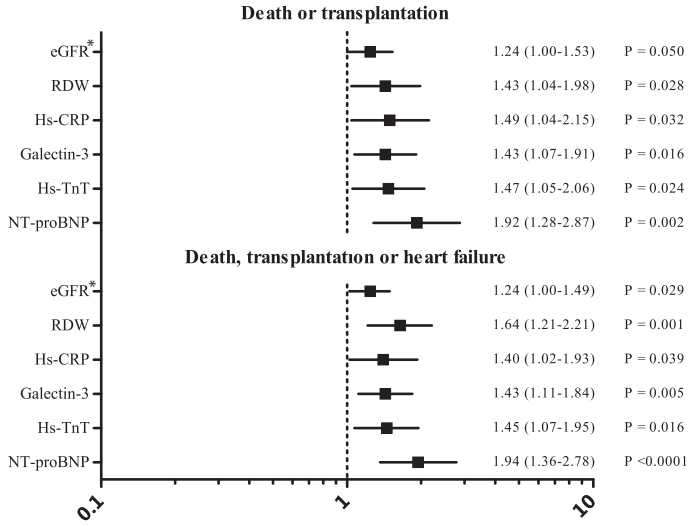

FIGURE 3 - Standardized hazard ratios, reflecting the instantaneous risk of the primary and secondary endpoint per one standard deviation increase in the biomarker level.

Standardized hazard ratios adjusted for age with the corresponding 95% CI and *p*-value for each biomarker. *For eGFR the inverse hazard ratios are shown.

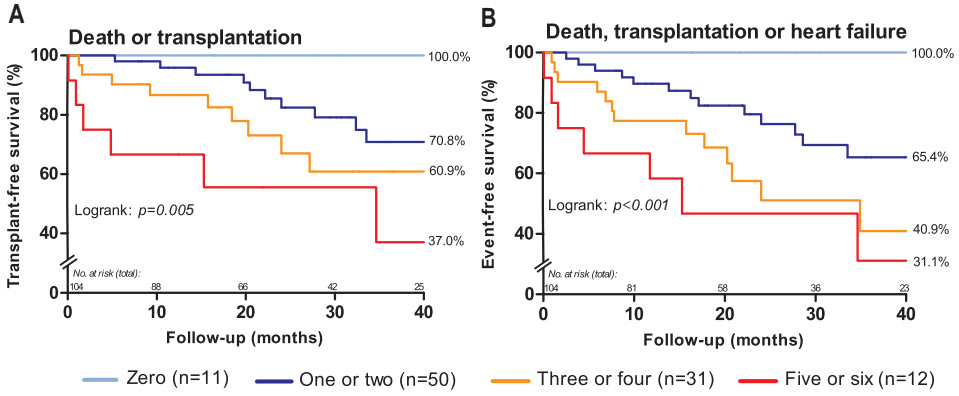

FIGURE 4 - Transplant-free and event-free survival stratified according to the number of elevated biomarkers.

Figure legend shows the number of elevated biomarkers (lowered for eGFR) with the corresponding line colour (detailed explanation of the different groups is provided in Supplemental File 5).

Multi-biomarker approach

Figure 4A shows the Kaplan-Meier curve stratified according to the number of elevated biomarkers. Patients with zero elevated biomarkers were free of events after 40 months whereas of the patients with five or six elevated biomarkers, only 37% was alive and

free of transplantation. The age adjusted hazard ratio for the primary endpoint for one extra elevated biomarker was 1.33 (95% CI 1.07–1.64, $p = 0.01$). Multi-biomarker analysis concerning the secondary endpoint (Figure 4B) showed similar results (HR 1.33, 95% CI 1.10–1.60, $p = 0.003$).

DISCUSSION

This study investigated diagnosis-specific biomarker profiles, the prognostic value of these biomarkers and the potential benefit of a multi-biomarker approach in adult patients with PH.

A significant difference in the biomarker release of NT-proBNP and hs-TnT among the different subgroups of PH was found and only 11% of the patients was free of any abnormal biomarker level. All six biomarkers studied in this prospective cohort were associated with adverse clinical outcomes, independent of age. A multi-biomarker approach demonstrated that patients were at higher risk of events as more biomarkers were elevated, wherein the absence of any elevated biomarker ruled out the risk of any event up to 40 months. This study therefore shows the importance of involving multiple biomarkers in the risk stratification of patients with PH.

Strengths of this study are its prospective design and the inclusion of only treatment naïve patients. The biomarkers therefore reflect a more natural state of disease severity, which is unaffected by treatment status. Additionally, this study evaluated the levels of six biomarkers measured at one point in time reflecting a patients' underlying disease state at a single moment. Patients with PH due to left heart disease were excluded in this study, to prevent any interference in biomarker levels due to myocardial stress primary caused by the left ventricle. We aimed to base our conclusions merely on processes regarding failure of the right ventricle.

New biomarker for PH: galectin-3

To our knowledge, this is the first prospective cohort study investigating the association with galectin-3 and clinical outcomes in patients with heterogeneous types of pre-capillary PH. Galectin-3 belongs to the beta-galactose-binding lectins and induces cardiac fibroblast proliferation, a process associated with the development of HF.[14] Galectin-3 may particularly be appropriate to monitor right ventricular remodelling.[15] Previous studies investigating galectin-3 mainly focused on patients with PAH only and consisted of smaller cohorts.[15-17] Calvier et al. found elevated levels of galectin-3 in PAH patients, which correlated with NYHA class.[17] Mazurek et al. demonstrated that galectin-3 was associated with mortality, however this study included both patients with PAH and patients with PH due to left heart disease.[16] In our current study, we had the

unique opportunity to compare different subgroups of PH. Only 38.5% of the patients with a galectin-3 level of > 19.9 ng/mL was alive and free of transplantation or HF after 40 months. Galectin-3 should therefore be considered as a new promising prognostic biomarker in the pre-capillary PH population.

Cardiovascular health 'barometer': red blood cell distribution width

Previous studies investigating the prognostic role of RDW in patients with PH already demonstrated that levels of RDW are associated with mortality,[18] even independent of NT-proBNP.[19] A variety of mechanisms have been hypothesized to elucidate the prognostic value of RDW in HF patients. Levels of RDW appear to reflect an underlying inflammatory state and an impaired iron metabolism. It has been suggested that RDW can be seen as an overall cardiovascular health 'barometer'.[20, 21]

In our study, elevated levels of RDW were observed in all subgroups of PH and were associated with death or transplantation, independent of age. Patients with RDW > 15%, have a cumulative transplant-free survival of only 48.3% after 40 months. Adding HF-related hospital admission to the endpoints increased the hazard ratio as well as the C-index. This suggests that besides being able to predict mortality or transplantation, RDW may be even better in predicting HF. Since RDW is determined as part of the automated blood count, RDW is an inexpensive, easy-accessible and widely available biomarker.

Heterogeneity across the PH population

A difficulty when studying the PH population is the major diversity in aetiology and the presence of PH-related comorbidities. The prognosis may be explained by the interaction between PH and the PH-related comorbidities, which differs between individuals. Cause of death in our study were diverse, with only 8 patients dying merely due to end-stage HF. It is therefore not surprising that many (potential)biomarkers are suggested for the risk stratification and that there is an increasing demand for a multi-biomarker approach that can capture more pathophysiological axes.[7] Indeed, the different biomarkers in this study demonstrate that the pathophysiology of PH is complex and that one should not merely focus on one all-encompassing biomarker.

Future perspectives

This multi-biomarker approach gives promising results regarding the prognostic value of combining multiple biomarkers. Especially, the negative predictive value was very good. However, a larger cohort of patients is needed to correct for the levels of the other biomarkers in a multivariable analysis and to investigate the diagnosis-specific

prognostic value of all different biomarkers. Additionally, it would be of interest to investigate whether serial biomarker measurements may improve prognostic precision and can monitor therapy. Eventually, a cost-effectiveness study should be performed to determine which biomarkers should be included in the multi-biomarker approach to be most profitable.

Study limitations

NT-proBNP, RDW and eGFR were directly determined in the clinical laboratory and therefore these biomarker results were directly available for the treating physician of a patient. Particularly NT-proBNP is an established biomarker and is used for the risk assessment in PH.[4] It was therefore inescapable and not ethical, to ignore NT-proBNP in the considerations of the clinical management. As a result, this may have diluted the association between NT-proBNP and clinical outcomes.

Six patients with CTEPH underwent pulmonary endarterectomy or percutaneous transluminal pulmonary angioplasty. Patients were not censored after the procedure because this would have introduced bias. However, keeping this patients in the study may also have biased the association between biomarker levels and clinical events, most likely towards the null.

Conclusions regarding the multi-biomarker approach should be treated with caution. This study demonstrates an association between the number of elevated biomarkers and the risk of events.

However, we could not correct for the underlying biomarker correlations in a multivariable-analysis due to limited numbers of events. The biomarkers may share some common prognostic effect; hence the exact additive prognostic value of each biomarker is difficult to compute. Also, the number of elevated biomarkers is analysed continuously instead of categorical due to limited degrees of freedom. Due to the relatively small sample size and limited number of events, the power of this study was not sufficient to correct for the different PH subgroups.

CONCLUSIONS

This study showed that a wide range of biomarkers reflecting different pathophysiological pathways are significantly associated with an increased risk of mortality, lung transplantation and HF in patients with PH. Combining multiple biomarkers is beneficial in detecting patients at higher risk of events.

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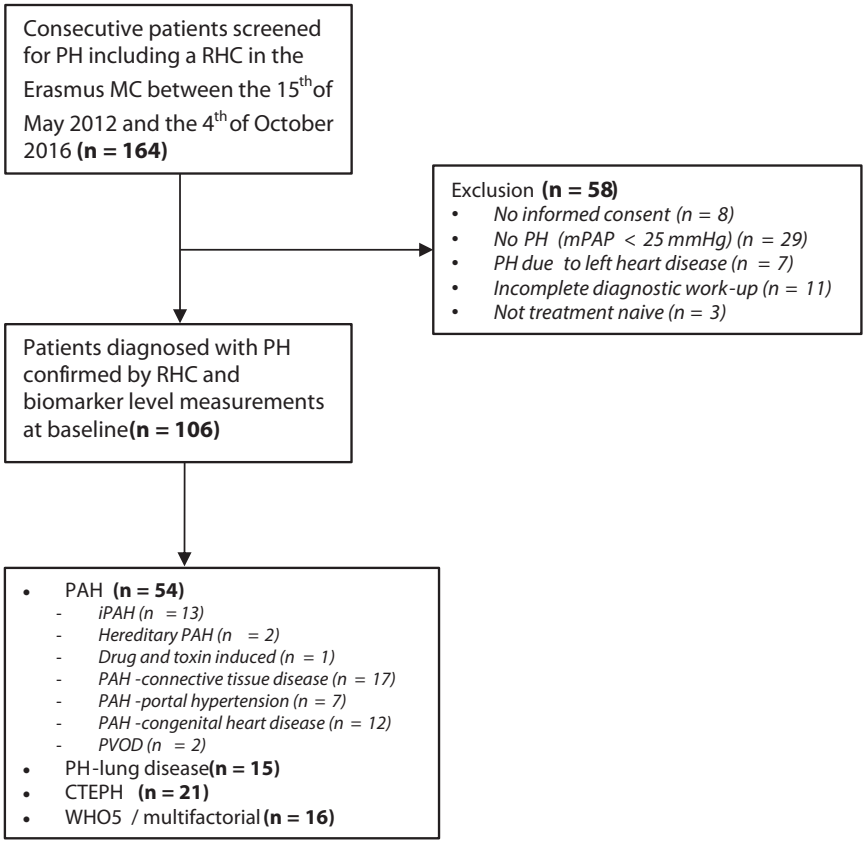
SUPPLEMENTAL MATERIAL

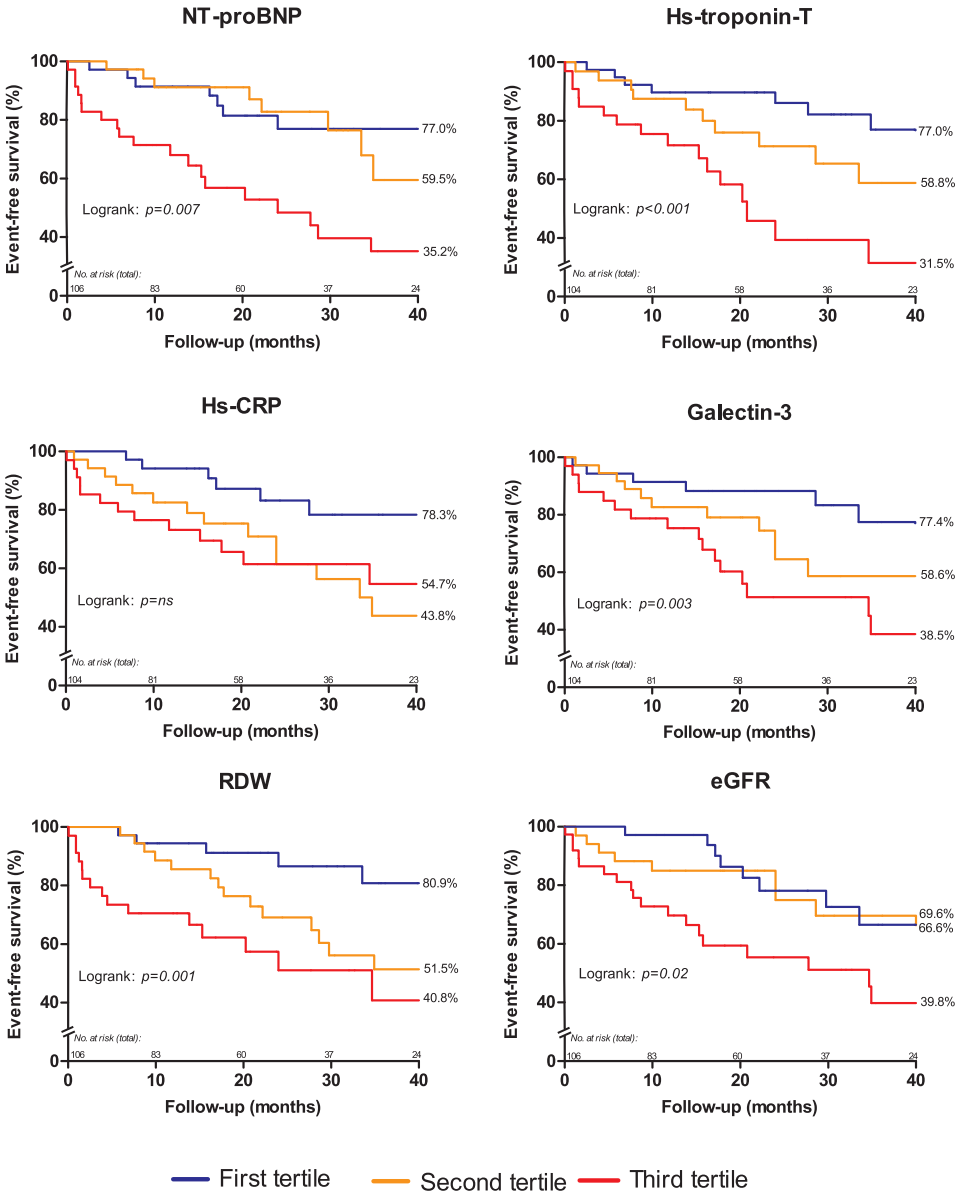


SUPPLEMENTAL FILE 1 - Flowchart of the patient selection process.

SUPPLEMENTAL FILE 2 - Mutual correlations between the different biomarkers in patients with pulmonary hypertension.

	NT-proBNP	Hs-TnT	Hs-CRP	Galectin-3	RDW
	r	*r*	*r*	*r*	*r*
Hs-TnT	0.51***				
Hs-CRP	0.37***	0.43***			
Galectin-3	0.40***	0.58***	0.48***		
RDW	0.28**	0.36***	0.29**	0.26**	
eGFR#	-0.52***	-0.42***	-0.22*	-0.43***	-0.21*

#Spearman correlation coefficient, otherwise Pearson correlation coefficient. Level of significance is indicated by the number of asterisks (*); *indicates $p < 0.05$, **indicates $p < 0.01$, ***indicates $p < 0.001$.



SUPPLEMENTAL FILE 3 -Event-free survival according to tertiles of the biomarker levels.

NT-proBNP: first tertile < 26.57 pmol/L, second tertile 26.57–150.0 pmol/L, third tertile > 150.0 pmol/L. *Hs-TnT*: first tertile < 2.0 ng/L, second tertile 2.0–6.3 ng/L, third tertile > 6.3 ng/L. *Hs-CRP:* first tertile < 2.0 mg/L, second tertile 2.0–6.3 mg/L, third tertile > 6.3 mg/L. *Galectin-3*: first tertile ≤ 15.3 ng/mL, second tertile 15.3–19.9 ng/mL, third tertile > 19.9 ng/mL. *RDW*: first tertile ≤ 13.6%, second tertile 13.6–15.5%, third tertile > 15.5%. *eGFR*: first tertile > 80.3 ml/min, second tertile 60.0–80.3 ml/min, third tertile ≤ 60 ml/min (tertiles of eGFR are inversed).

SUPPLEMENTAL FILE 4 - Biomarker release, associations between biomarkers and the primary (death or lung transplant) and secondary endpoint (death, lung transplant or heart failure) and the corresponding C-index, adjusted for age in patients with pulmonary hypertension.

			Primary endpoint			Secondary endpoint		
	Biomarker value	**Elevated n (%)**	**Hazard ratio** (95% CI)**	***P*-value**	**C-index (95% CI)**	**Hazard ratio** (95% CI)**	***P*-value**	**C-index (95% CI)**
NT-proBNP, pmol/L	62.0 [21.0–220.8]	82 (77)	1.92 (1.28–2.87)	0.002	0.69 (0.58–0.81)	1.94 (1.36–2.78)	< 0.001	0.70 (0.61–0.80)
Hs-TnT, ng/L	13.0 [8.0–25.8]	43 (41)	1.47 (1.05–2.06)	0.02	0.67 (0.56–0.78)	1.45 (1.07–1.95)	0.02	0.65 (0.55–0.76)
Hs-CRP, mg/L	3.6 [1.5–9.0]	24 (23)	1.49 (1.04–2.15)	0.03	0.66 (0.51–0.80)	1.40 (1.02–1.93)	0.04	0.65 (0.53–0.77)
Galectin-3, ng/mL	17.9 [14.0–22.8]	40 (39)	1.43 (1.07–1.91)	0.02	0.67 (0.56–0.79)	1.43 (1.11–1.84)	0.01	0.67 (0.57–0.76)
RDW, %	14.6 [13.2–16.0]	25 (24)	1.43 (1.04–1.98)	0.03	0.61 (0.47–0.75)	1.64 (1.21–2.21)	0.001	0.67 (0.57–078)
eGFR, mL/min/1.73m^{2*}	72.5 [55.8–90.0]	33 (31)	0.81 (0.65–1.00)	0.05	0.62 (0.49–0.76)	0.81 (0.67–0.98)	0.03	0.63 (0.55–0.71)

Biomarker values in the overall study population expressed in median [IQR] and the corresponding number of patients with an elevated biomarker level. Elevated biomarkers are defined as: NT-proBNP > 15 pmol/L, hs-TnT > 14 ng/L, hs-CRP > 10 mg/L, galectin-3 > 16.9♂, > 21.3♀ ng/mL, RDW > 16%. Lowered biomarkers are defined as: eGFR < 60 mL/min/1.73m^2. *For eGFR lowered levels were taken into account. **standardized hazard ratios, with the corresponding 95% confidence interval and significance level.

SUPPLEMENTAL FILE 5 - The number of patients (%) with an elevated biomarker level per group used in the multi-biomarker approach.

No. of total elevated biomarkers	**NT-proBNP**	**Hs-TnT**	**Hs-CRP**	**Galectin-3**	**RDW**	**eGFR**
Zero (n = 6)	-	-	-	-	-	-
One (n = 29)	23 (79)	4 (14)	1 (3)	0 (0)	1 (3)	0 (0)
Two (n = 21)	16 (76)	8 (38)	3 (14)	5 (24)	2 (29)	4 (19)
Three (n = 13)	12 (92)	7 (54)	2 (15)	7 (54)	4 (31)	7 (54)
Four (n = 18)	18 (100)	16 (89)	6 (33)	16 (89)	6 (33)	10 (56)
Five (n = 8)	8 (100)	8 (100)	5 (63)	7 (88)	5 (63)	10 (56)
Six (n = 4)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)

PART III

RISK PREDICTION

Chapter 15

Risk stratification & prognosis

Vivan J.M. Baggen, Laurie W. Geenen, Jolien W. Roos-Hesselink

In: Heart Failure in Adult Congenital Heart Disease (Book chapter).

ABSTRACT

In order to adequately manage the rapidly expanding population of patients with adult congenital heart disease (ACHD) and to optimize patient outcomes, accurate prognostication is of paramount importance. A large part of the risk stratification of patients with ACHD is based on the underlying anatomical defect, concomitant lesions and type of corrective surgery that was performed. In addition, components of the medical history, physical examination and further diagnostic tests (including ECG, echocardiography, cardiac magnetic resonance imaging, exercise testing, and biomarkers) can provide prognostic information. This chapter provides a narrative review of the factors that have been identified as predictors for heart failure and other late complications in the entire cohort of patients with ACHD and within specific congenital subgroups.

INTRODUCTION

The population of adult patients with congenital heart disease is steadily growing and aging, thanks to the major advances in cardiothoracic surgery and pediatric cardiology in the past decades. Although survival has improved and most of these patients have no complaints, today it is widely acknowledged that congenital heart disease is palliated, not cured. Residual or recurrent structural heart defects are common, and may result in late complications such as heart failure and early demise. Therefore, these patients require lifelong surveillance and care in specialized cardiac centers.[1] In order to adequately manage this rapidly expanding population and to optimize patient outcomes, accurate prognostication is of paramount importance.

The etymology of the word 'prognosis' dates back from the ancient Greek civilization, and is literally translated as 'foreknowledge'. As a medical term, it is used to indicate the likely course and outcome of a disease. We aim to determine this forecast by a range of patient characteristics and tests, in order to make individualized risk predictions as accurate as possible. This chapter is therefore structured by a range of components that can be useful to stratify the risk of heart failure and other late complications in patients with ACHD. Although state-of-the-art prognostication and treatment in patients with ACHD is often based on extrapolated data from patients with chronic heart failure, this chapter aims to focus on the evidence that is available from congenital patients.

MEDICAL HISTORY

Congenital defect and corrective surgery

The congenital heart defects can be grouped into mild, moderate and complex lesions.[2] This classification is important, because it is well known that the survival of patients with a complex heart defect is substantially worse than the survival of patients with a mild type of heart defect.[3-6] Patients with repaired patent ductus arteriosus and atrial or ventricular septal defects have the lowest all-cause mortality, being only slightly higher than or even comparable to the general population.[4, 6, 7] In contrast, the long-term survival of patients with a cyanotic defect, Fontan circulation, systemic ventricular heart or other complex congenital heart disease is clearly diminished with a substantial morbidity.[4, 5, 8] The most frequent cause of death in these patients is chronic heart failure.[4] Among patients with the same congenital defect at birth, it is very important which type of corrective surgery was performed. In patients with transposition of the great arteries (TGA), major differences in ventricular function and functional capacity exist between those with a systemic right ventricle after a Mustard or Senning procedure compared with those who underwent anatomical correction by an arterial switch operation in a

more recent surgical era.[9] Consequently, the survival of patients with TGA has markedly improved.[10] Also the pre-operative anatomy may vary in severity, and concomitant congenital lesions in other organs may be present. For instance, in patients with a Fontan circulation, the pre-operative anatomy significantly impacts patient outcomes, with the lowest overall survival in patients with hypoplastic left heart syndrome[11] and heterotaxy syndrome.[12] The presence of concomitant lesions that require more extensive surgical correction, such as atrioventricular valve replacement at the time of Fontan correction, is also related to a higher risk of morbidity and mortality during long-term follow-up.[12]

Among patients with the same congenital defect and type of repair, the practice of repair has undergone major changes over the past decades. Apart from improvement in surgical experience and quality of postoperative care, today's surgical techniques are different from those in the past and are adapted based on the late sequelae we now observe more than three decennia after repair. For instance, in patients with tetralogy of Fallot, later age at initial repair and the use of a palliative shunt have been shown to be associated with worse outcomes.[13-15] Corrective surgery is now seldom performed beyond the first half of infancy, and palliative shunts are almost no longer used. Modern strategies that include the avoidance of the use of a transannular patch and pulmonary valve-sparing approaches may improve patient outcomes,[15] although comparisons are difficult because of era differences and the lack of long-term follow-up data of newer approaches.[16] In patients with a Fontan circulation, the overall survival has also greatly improved in later surgical eras, with the worst outcomes in patients with an atriopulmonary connection (the original technique),[11, 17] and probably the best outcomes in patients with an extracardiac conduit. Fontan patients with a longer bypass time also have an increased risk of mortality.[12]

In conclusion, the severity of the congenital heart disease is not only based on the type of defect, but also strongly influenced by the type of corrective surgery, presence of concomitant lesions, and surgical era. Based on these differences, a suggested modification of the original Bethesda classification is provided in Table 1, which could be useful for the further guidance of follow-up schemes.

Genetics

The identification of a genetic syndrome or mutation is important, not only because it provides implications for future offspring, but also because specific genetic variations are related to the risk of developing associated cardiac complications, such arrhythmias and heart failure. For instance, ASD patients with an associated NKX2.5 syndrome have a higher risk of the development of atrioventricular block and ventricular dysfunction,[18] and may even develop dilated cardiomyopathy. TBX5 is a gene which is involved in Holt-Oram syndrome, which includes atrioventricular node disease, and also

modulates diastolic dysfunction.[19] MYH6 mutations are associated with various forms of congenital heart disease, but also with hypertrophic, dilated and noncompaction cardiomyopathy.[20] In addition, a cardiac congenital abnormality as part of a genetic syndrome (such as Down, Patau, Edward, DiGeorge, Turner, Williams-Beuren, Noonan, or Alagille syndrome) can also involve non-cardiac malformations.[21] These may impact perioperative morbidity, and can have long-standing ramifications on neurodevelopment and overall health.[22]

TABLE 1 - Modified Bethesda classification.

Mild congenital heart disease	Moderate congenital heart disease	Complex congenital heart disease
Unrepaired PFO or small ASD (no associated lesions)	Unrepaired PDA, ASD II, SVD, VSD (with significant shunt)	Congenitally corrected TGA
Unrepaired small VSD (no associated lesions)	AVSD (partial or complete, repaired or unrepaired)	Systemic right ventricle after Mustard/Senning repair for TGA
Repaired PDA	PAPVR with significant hemodynamic shunt or TAPVR	Truncus arteriosus
Repaired ASD II or SVD without residual shunt	Ebstein's anomaly	Conduits, valved or nonvalved
Repaired VSD without residual shunt	Aortic coarctation	Double inlet left ventricle or double outlet right ventricle
Mild congenital aortic valve disease	Moderate/severe congenital aortic disease (subvalvar, valvular or supravalvar)	Hypoplastic left or right heart syndrome
Mild congenital mitral valve disease (no associated lesions)	Moderate/severe mitral valve disease (including parachute valve, cleft leaflet)	Mitral or tricuspid atresia
Mild pulmonary valve disease	Moderate/severe pulmonary valve disease or RVOT obstruction	Fontan procedure
	Arterial switch operation for TGA	PAH-CHD
	Repaired tetralogy of Fallot	Eisenmenger syndrome
	Pulmonary atresia with biventricular repair	Cyanotic congenital heart disease

Abbreviations: ASD, atrial septal defect; AVSD, atrioventricular septal defect; PAH-CHD, pulmonary arterial hypertension due to congenital heart disease; PAPVR, partial anomalous pulmonary venous drainage; PDA, patent ductus ateriosus; PFO, patent foramen ovale; RVOT, right ventricular outflow tract; SVD, sinus venosus defect; TAPVR, total anomalous pulmonary venous drainage; TGA, transposition of the great arteries; VSD, ventricular septal defect.

Age

The presence of chronic pressure or volume overload and cyanosis, as a result of valvular dysfunction, shunts, or other residual lesions, carry long-term effects that steadily increase over time. These effects include atrial and ventricular dilatation, dysfunction, fibrosis, and other forms of disease progression. Accordingly, observational studies

show that the risk of heart failure and death continues to increase with age in patients with ACHD.[4, 23, 24] Apart from disease progression, the oldest patients also originate from an earlier surgical era, with a corresponding median later age at corrective surgery and possibly outdated surgical methods.[5] The oldest patients with ACHD therefore carry multiple risk factors for the development of heart failure. However, the strong positive correlation between age and age at initial corrective surgery makes it difficult to distinguish their separate effects, and to foresee the rate of disease progression with age in the infants that are operated today with newer techniques.

Sex

Within the general population, men have a shorter life expectancy than women. Leading explanations can be classified social/environmental (such as risky behavior, smoking, alcohol, homicide, suicide) and biological (such as effects of estrogen versus testosterone).[25] This sex gap is also observed in patients with ACHD[26] and within specific diagnostic subgroups such as Fontan palliation.[11] It is unknown whether this can be directly translated from the general population, or whether other factors such as medical therapy adherence also play a role.

Previous (re)interventions

Patients with multiple previous surgical or percutaneous (re)interventions are more frequently followed-up in a tertiary referral center. These patients are likely to represent a more complex congenital group and/or are in a worse clinical condition, with a subsequent higher mortality risk.[3] Multiple previous sternotomies may be present in congenital patients and can also be a risk factor on its own, because these increase the risk of complications during surgery. Nevertheless, the absolute risk of reentry injury during repeat sternotomy for congenital heart disease is low.[27]

Previous heart failure or arrhythmia

A history of heart failure is associated with higher mortality rates, for instance in patients with repaired tetralogy of Fallot.[15] A history of arrhythmias is also significantly associated with worse outcomes, such as the occurrence of heart failure, late arrhythmias or mortality in patients with tetralogy of Fallot,[14, 28] Mustard[5] and Fontan palliation.[12, 17] The association between early arrhythmias and heart failure may be explained by surgical damage to the conduction system and post-operative scarring. In addition, the presence of a pacemaker has been identified as a risk factor for mortality.[29] Pacemaker implantation in young adults with congenital heart disease is related to higher NT-proBNP levels, lower peak oxygen uptake, and a longer QRS

duration, indicating that longstanding abnormal ventricular activation in patients with a pacemaker may contribute to progressive ventricular dysfunction and the occurrence of heart failure.[30]

Cardiac medication use

Patients who do not use any cardiac medication such as an ACE-inhibitor, angiotensin receptor blocker, beta blocker, diuretic or anti-arrhythmic are more likely to be in a good clinical condition, and have a much lower risk to develop heart failure.[23] Patients who do use cardiac medication may have a history of heart failure or arrhythmia, with a subsequent higher risk of recurrence. In addition, the chronic use of negative inotropic antiarrhythmic drugs may negatively affect ventricular function. In patients after Fontan correction, diuretic therapy was strongly related to death, transplant[31] or hospitalization for cardiac reasons.[17]

Also, the lack of adequate medical therapy may increase the risk of complications. In many centers, all Fontan patients are routinely treated with systemic anticoagulation in order to manage the high thromboembolic risk due to low flow in the Fontan circuit, reduced cardiac output, possible Fontan obstruction and atrial arrhythmias. Fontan patients lacking thromboprophylactic therapy (warfarin or aspirin) have been shown to carry a higher risk of death or transplant.[31]

Clinical symptoms of heart failure

Most patients with ACHD have no complaints and do not readily report symptoms. Patients often do not recognize subtle changes in functional class and may have no typical symptoms of heart failure. When present, symptoms of heart failure in congenital heart disease include symptoms of systemic ventricular failure (fatigue, dyspnea, dry cough, reduced exercise tolerance, orthopnea, paroxysmal nocturnal dyspnea, wheezing) and symptoms of sub-pulmonary ventricular failure (fatigue, bloating, weight gain, loss of appetite, reduced exercise tolerance, increased abdominal girth).[32] Clinical heart failure based on history, examination and further investigations is documented in 22% of patients with Mustard repair for TGA, in 32% of patients with congenitally corrected TGA, and in 40% of patients after Fontan operation.[32] Early recognition and diagnosis of clinical heart failure is very important. New York Heart Association (NYHA) functional class can be used to classify symptoms of heart failure, with patients who report no limitation in ordinary physical activity considered as NYHA functional class I. NYHA class is known to be significantly associated with adverse outcomes in the overall population of patients with ACHD[4] and also for instance in subgroups of patients with repaired tetralogy of Fallot[33] and Fontan palliation.[34]

PHYSICAL EXAMINATION

Clinical signs of heart failure

Signs of systemic ventricular failure on physical examination in patients with ACHD include a third or fourth heart sound, a laterally displaced apical impulse, basal crackles, absent breath sounds and dull percussion at the lung basal fields. Signs of sub-pulmonary failure are elevated jugular venous pressure, hepatomegaly, ascites, pitting leg, sacral or scrotal edema.[32] Of note, arrhythmias could also be a first clinical manifestation of heart failure. In addition, worsening of cyanosis could be present in patients with intra- or extra-cardiac shunts or fenestrations.

Oxygen saturation

Pulse oximetry is routinely carried out alongside clinical examination mostly for diagnostic purposes. However, it also provides prognostic information. Systemic oxygen desaturation is related to a higher risk of cardiovascular events, death or heart failure in the entire ACHD population[23] and also predicts mortality risk in specific subgroups such as Eisenmenger patients[24] and Fontan patients.[29]

ELECTROCARDIOGRAPHY AND HOLTER MONITORING

Most patients with ACHD have abnormal electrocardiograms (ECGs). Therefore, comparison with previous ECGs in order to detect intra-individual changes in ECG morphology are most relevant to detect underlying disease progression with a higher risk of adverse events.

In concordance with a history of arrhythmia, also the loss of sinus rhythm at standard electrocardiographic evaluation is a strong predictor for clinical outcomes, for instance in patients with Eisenmenger syndrome.[24] Additional evaluation of arrhythmias is primarily performed in symptomatic patients, and may require ambulatory ECG monitoring (Holter), event recorders or implantable loop recorders. A grade II or greater ventricular arrhythmia on ambulatory ECG (which includes ≥ 30 unifocal or multifocal premature ventricular complexes per hour, non-sustained or sustained ventricular tachycardia) has been shown to be a risk marker of sudden death in some cohorts of patients with repaired tetralogy of Fallot,[35] but not in all.[15, 36]

Increased QRS duration has been shown to be associated with the occurrence of heart failure in ACHD patients with a pacemaker.[30] Also in patients with repaired tetralogy of Fallot, a prolonged QRS duration was predictive of ventricular tachycardia and death;[15, 37] however, conflicting data have been reported.[14]

Although the current survival of patients after arterial switch operation for TGA is excellent, the most frequent cause of morbidity and mortality is coronary artery obstruction, which is present in 5 to 7% of survivors. Annual ECG evaluation for signs of ischemia (with advanced imaging if indicated) is therefore recommended in all arterial switch patients with ostial stenosis identified in childhood.[10]

TRANSTHORACIC ECHOCARDIOGRAPHY

Echocardiography is a widely available, portable, cheap, and non-invasive imaging technique that plays a key-role in the clinical follow-up of patients with ACHD. However, the quality of echocardiographic measurements are highly user-dependent and ventricular function and volumes can be challenging to assess in adults with complex congenital heart diseases such as univentricular hearts or systemic right ventricles.[38] An overview of some important echocardiographic predictors that are discussed below is also provided in Figure 1.

Systolic ventricular function

The systolic ventricular function is one of the most important prognostic parameters obtained by echocardiography in the ACHD population. A moderately to severely impaired systemic ventricular function (as expressed by an ejection fraction below 40%) is an independent predictor for sudden cardiac death in the overall ACHD population.[39] This is also the case in specific ACHD subpopulations such as in patients with repaired tetralogy of Fallot[40] and in patients with a systemic right ventricle after Mustard procedure.[41] Also the right ventricular (or subpulmonary) systolic function has been shown to be of prognostic importance, for instance as quantified using fractional area change.[42]

The tricuspid annular plane systolic excursion (TAPSE) quantifies the longitudinal right ventricular function in M-mode. In patients with pulmonary arterial hypertension the TAPSE is frequently reported as an important predictor for adverse clinical outcomes.[43] Accordingly in patients with Eisenmenger syndrome, one study showed that TAPSE was associated with the risk of mortality;[44] however, contradictory results have been published in this patient group.[24] In patients with a systemic right ventricle after the Mustard procedure, the prognostic value of the lateral versus the septal TAPSE may differ.[45] Nevertheless, the TAPSE was not associated with the right ventricular ejection fraction as measured by cardiac magnetic resonance imaging (CMR) in these patients,[46] and the contraction pattern of the systemic right ventricle is thought to shift from longitudinal to circumferential shortening.[47] Therefore, the prognostic value of TAPSE may be limited in this patient group. Although less often used, the mitral annular plane systolic excursion (MAPSE) was found to be associated with sudden cardiac death

and ventricular arrhythmias in patients with repaired tetralogy of Fallot, even beyond the left ventricular ejection fraction.[42]

Other measures of the global ventricular function are the myocardial performance index (Tei index) and systolic to diastolic duration ratio. The systolic to diastolic duration has been reported as independent predictor of mortality in patients with a Fontan circulation and is relatively easy to measure, despite of the complex anatomy of the heart.[48]

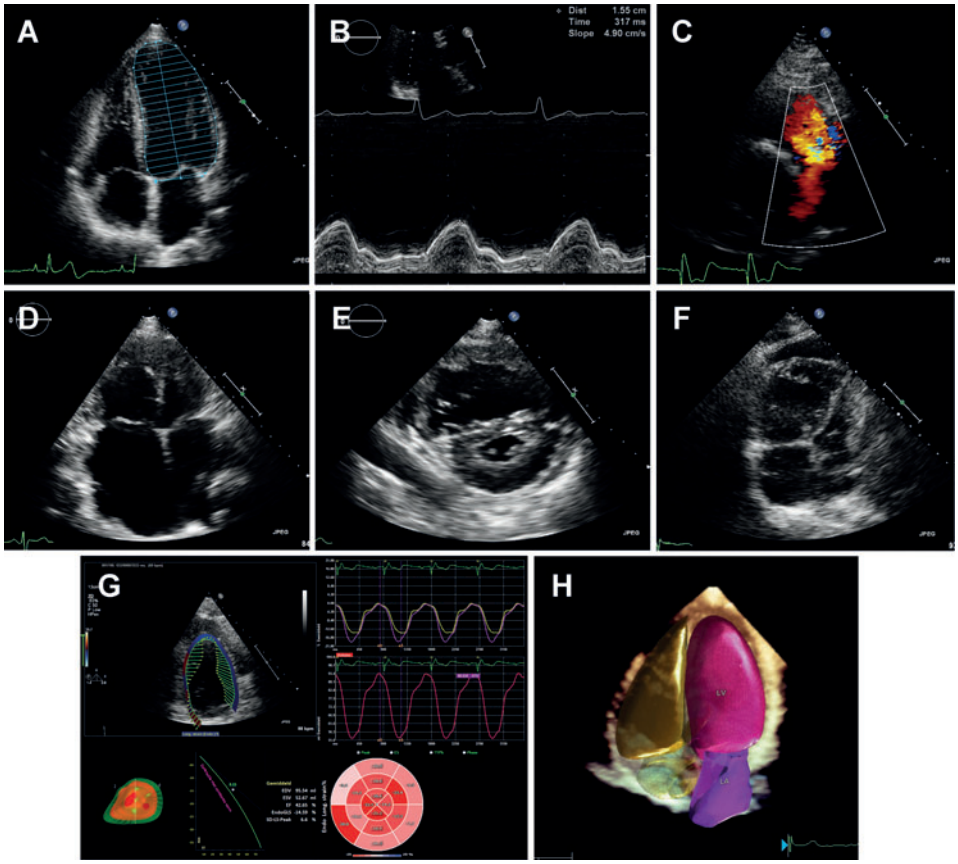


FIGURE 1 - Echocardiographic predictors of adverse clinical outcome in patients with ACHD.

A, measurement of left ventricular ejection fraction using the biplane method of disks (modified Simpson's rule) in a patient with aortic coarctation. B, mildly reduced TAPSE (< 16 mm) in a patient with tetralogy of Fallot. C, severe pulmonary valve regurgitation visualized using color flow Doppler in a patient with tetralogy of Fallot. D, severe biatrial dilatation and E, D-shaped left ventricle in a patient with pulmonary arterial hypertension after surgical repair of a sinus venosus defect and partial anomalous pulmonary venous return. F, pericardial effusion in a patient with pulmonary arterial hypertension. G, global longitudinal left ventricular strain as quantified with speckle tracking echocardiography (courtesy of R.W.J. van Grootel). H, 3D echocardiography analyzed using automated software (Heart Model).

Ventricular and atrial dilatation

In patients with a systemic right ventricle, the ejection fraction may not always be a good indicator for the systolic ventricular function due to the frequent occurrence of atrioventricular valve regurgitation, which paradoxically increases the ejection fraction. One study has reported the systemic right ventricular end-diastolic volume to be a better parameter for adverse outcomes in patients with a systemic right ventricle.[49] In patients with repaired tetralogy of Fallot, right ventricular dilatation is often used to in the timing of pulmonary valve replacement, because it is thought that a severely dilated right ventricle is unable to reverse remodel.

Heart failure may lead to atrial dilatation due to chronic diastolic dysfunction.[50] Several studies have shown that both left and right atrial enlargement is related to a worse clinical prognosis.[51] In patients with pulmonary hypertension and in patients with Eisenmenger syndrome, increased right atrial area was found to be a strong predictor for adverse clinical outcomes.[43, 44, 52] Its association with disease severity can be explained by the failure of the right heart to overcome the high pulmonary pressures. As a result, the pressures in the right ventricle and right atrium will increase, which is often reflected by enlargement of the right atrium.

Shunt lesions and valve disease

Doppler echocardiography can also be used to detect shunt lesions and to grade the severity of valve disease. The presence of a substantial shunt lesion or a hemodynamically significant residual shunt after ASD or VSD repair is important to detect and may require transoesophageal echocardiography to be adequately visualized, because it impacts the classification of the severity of the heart defect and the follow-up strategy.[2] The presence of a pretricuspid shunt has been reported to be an independent predictor of death in Eisenmenger patients.[24]

The grade of valvular stenosis or regurgitation also determines the severity of the heart defect [2], and is important to regularly assess during the routine echocardiographic follow-up of patients with valvular disease. For instance, in patients with repaired tetralogy of Fallot, a moderate or severe tricuspid or pulmonary valve regurgitation has been reported to be associated with an increased risk of sudden cardiac death and arrhythmias.[15]

Pulmonary arterial hypertension

A substantial proportion of patients with ACHD develops pulmonary arterial hypertension and ultimately Eisenmenger syndrome.[53] Doppler echocardiography can be used to estimate pulmonary pressures based on the maximal tricuspid regurgitation

velocity (together with the estimated right atrial pressure, based on inferior vena cava diameter and collapse) or the pulmonary regurgitation maximal and end-diastolic velocity. Long-standing pressure overload of the right ventricle may lead to progressive right ventricular heart failure. Therefore, it is not surprising that elevated pulmonary pressures are strongly indicative of a poor prognosis, and it is important to regularly follow-up right ventricular systolic pressures and function in patients with pulmonary hypertension to timely detect further deterioration.

Pericardial effusion may develop in patients with elevated filling pressures of the right side of the heart.[54] In patients with pulmonary arterial hypertension, pericardial effusion is the most extensively documented parameter that is known to be of prognostic importance.[43] In a multicentre study including patients with Eisenmenger syndrome, in 9.2% of the patients pericardial effusion was present. The presence of pericardial effusion was found to be a strong predictor for all-cause mortality, even after adjusting for other risk factors such as age, NYHA class, pretricuspid shunt, sinus rhythm and oxygen saturation.[24]

Another specific prognostic parameter in pulmonary arterial hypertension is the septal shift during systole due to elevated pressures in the right ventricle,[52] which is also known as the 'D-sign' visible on the echocardiography.

Novel echocardiographic techniques

Speckle tracking echocardiography is a technique which can be used to obtain the ventricular function based on the quantitative assessment of myocardial deformation (strain) and myocardial displacement of displacement rate (velocity) with a high temporal resolution.[55, 56] One study has shown that systemic right ventricular two-dimensional longitudinal strain was associated with adverse clinical outcomes such as death, arrhythmias and an increase in NYHA class in patients with a systemic right ventricle.[45] In patients with tetralogy of Fallot, the left ventricular longitudinal strain was related to sudden cardiac death or life threatening arrhythmias and was associated with a higher NYHA class.[42]

Three-dimensional (3D) echocardiography is an excellent imaging technique to visualize complex congenital anatomies and the technique has improved impressively over the past years. Unfortunately, it is still not widely available and mainly relies on manual input, making it a time consuming echocardiographic technique. New software has been developed to automatically analyze 3D images to shorten analysis time and to make it more feasible for routine practices. This novel software has been shown to measure the left atrial volume, left ventricular volume and the left ventricular ejection fraction in strong agreement with CMR measurements,[57, 58] also within specific subgroups such as patients with bicuspid aortic valve disease.[59] 3D echocardiography

may significantly contribute to the risk stratification of ACHD patients in the future when the image quality has further improved.

CARDIAC MAGNETIC RESONANCE IMAGING

Cardiac magnetic resonance imaging (CMR) is generally accepted as the reference standard when it comes to measurement of the volume, mass and ejection fraction of both the right and the left ventricle. CMR also has its limitations such as higher costs, less availability and limited ability to scan patients with intra-cardiac devices, but it is very useful when echocardiography is unable to provide images of sufficient quality or when echo measurements are borderline or ambiguous.[53, 60] CMR is therefore highly suitable in patients with complex congenital abnormalities in whom it is often difficult to obtain good echocardiographic images of the cardiac anatomy. A graphical illustration of the CMR predictors that are discussed below is provided in Figure 2.

Ventricular function and volumes

In accordance with the studies that evaluated ventricular function in echocardiography, CMR-derived ejection fraction is strongly predictive of mortality or adverse cardiac events in several subgroups of patients with ACHD, including patients with a systemic right ventricle, repaired tetralogy of Fallot, Eisenmenger syndrome and pulmonary hypertension.[45, 61-63] In addition, higher ventricular end-diastolic volumes are independently associated with a higher mortality risk in patients with a Fontan circulation[64] and in patients with repaired tetralogy of Fallot.[62]

Myocardial fibrosis

With the help of gadolinium enhancement technique in CMR, myocardial scarring and fibrosis can be detected. Increased late gadolinium enhancement of the left ventricle in patients with repaired tetralogy of Fallot is related to myocardial dysfunction and is associated with adverse outcomes after correcting for age.[65] The presence of late gadolinium enhancement located at the right ventricular insertion points is also thought to reflect a more advanced disease and poor prognosis in patients with tetralogy of Fallot and in patients with pulmonary arterial hypertension.[66] Therefore, myocardial fibrosis quantified by late enhancement could a valuable additional tool for risk stratification. Myocardial T1 mapping is a relatively novel technique that is able to detect diffuse fibrosis as reflected by prolonged T1 times, and to determine the extracellular volume.[67] In patients with a systemic right ventricle after correction of TGA, higher septal extracellular volumes correlate with other prognostic parameters such as

NT-proBNP levels and the chronotropic index during CPET,[68] suggesting that it may be of prognostic value in these patients.

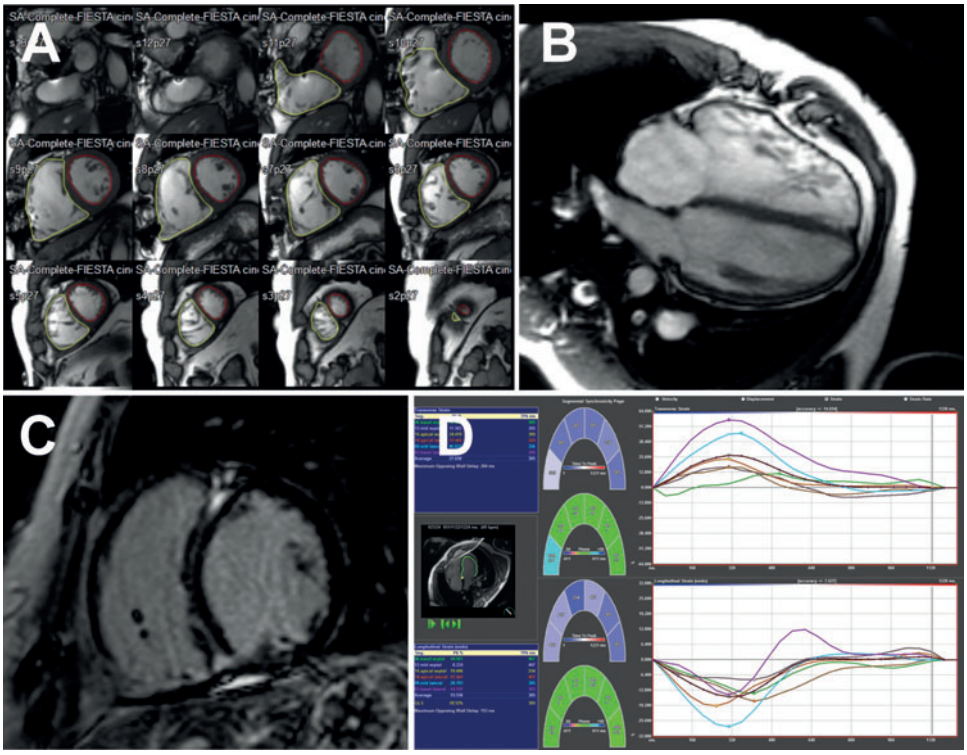


FIGURE 2 - Cardiac magnetic resonance imaging parameters that are reported to be of prognostic importance in patients with ACHD.

A, left and right ventricular ejection fraction as measured by multi-slice short axis cine imaging in a patient with congenital aortic stenosis. B, right ventricular dilatation and pronounced trabecularization as visualized on the four-chamber view in a patient with tetralogy of Fallot. C, late gadolinium enhancement at the right ventricular insertion points. D, feature tracking to derive regional and global left ventricular strain in a patient with tetralogy of Fallot (courtesy of R.W.J. van Grootel).

Deformation imaging

The deformation imaging technique that is available in CMR is known as feature tracking. Longitudinal and circumferential global left ventricular function measured by feature tracking closely agree with the same parameters measured by speckle tracking. Moreover, right ventricular feature tracking parameters showed to be associated with exercise capacity in patients with repaired tetralogy of Fallot, suggesting that it may be useful as prognostic parameter.[69] Also left ventricular dyssynchrony assessed by

myocardial deformation imaging was found to be associated with adverse outcomes in patient with repaired tetralogy of Fallot.[62] However, contradictory results on strain measurements in patients with repaired tetralogy of Fallot have been reported, showing no association between strain measurements and deterioration in left and right ventricular function.[70] Hence, more research is needed to prove the prognostic value of this promising new technique.

CARDIAC COMPUTED TOMOGRAPHY

Computed tomography (CT) angiography has a very high spatial resolution and can therefore reliably evaluate the aortic size and small vasculature of the heart. Therefore, patients with an intrinsic higher risk of aortic dilatation and eventually dissection of the aorta, such as patients with a bicuspid aortic valve, Marfan syndrome or a SMAD3 mutation, require follow-up by cardiac CT to timely detect any progression in aortic dilatation.[71] An ascending aortic area/height ratio of more than 10 cm^2/m is independently associated with in increased cardiovascular mortality risk in patients with a bicuspid aortic valve.[72]

Nevertheless, in the overall population of patients with ACHD, serial cardiac CT measurements are unattractive due to the need of high dosages of ionizing radiation, and are therefore not widely used for the risk stratification in patients with ACHD.[53] However, as the population of adults with congenital heart disease are aging, coronary heart disease may begin to develop in this population, which possibly expands the role for CT angiography in the follow-up and risk stratification of elderly patients with ACHD in the future.

CARDIOPULMONARY EXERCISE TESTING

Cardiopulmonary Exercise Testing (CPET) consists of the assessment of exercise intolerance and ventilatory gas exchange during exercise, and can be considered part of the regular follow-up in ACHD patients. Chronic heart failure is characterized by an impaired cardiac output response to exercise and therefore CPET is widely used in the clinical follow up for the development of heart failure in patients with ACHD.[73]

Peak oxygen uptake

The peak oxygen uptake (peak VO_2) is one of the most important measures to quantify exercise capacity.[74] Peak VO_2 is diminished in symptomatic as well as asymptomatic ACHD patients,[75] with the lowest peak VO_2 found in Eisenmenger syndrome and other cyanotic patients.[76] The majority of patients with a Fontan circulation fail to reach

> 80% of the predicted peak VO_2, independently of the type of Fontan operation.[17] Lower peak VO_2 is associated with a higher risk of hospitalisation or death,[75] the development of heart failure, and independently predicts mortality,[76] indicating that it is a powerful prognostic tool within the entire ACHD population. Studies in specific ACHD populations such as tetralogy of Fallot, transposition of the great arteries (TGA) corrected by atrial switch procedure, and Ebstein's anomaly, report comparable results regarding the prognostic importance of peak VO_2.[37, 77, 78] Peak VO_2 may also be suitable for the assessment of perioperative risk in patients with repaired tetralogy of Fallot undergoing surgical pulmonary valve replacement.[79]

In patients with a Fontan circulation, contradictory results regarding the predictive ability of the peak VO_2 have been reported. Some studies found an increased risk for mortality or morbidity in patients with a lower peak VO_2, even independent of other risk factors.[80] Others suggest that the impaired peak VO_2 in Fontan circulation does not arise from failure of univentricular circulation, but from the intrinsic inability to adequately react to exercise, and therefore has no predictive ability.[17]

Ventilatory efficiency

Ventilatory efficiency can be expressed by the slope of the minute ventilation versus the CO_2 production (VE/VCO_2) assessed during CPET. An elevated VE/VCO_2 slope (> 30) indicates a pulmonary ventilation-perfusion mismatch due to an adequate ventilation but a poor perfusion due to the inability of the heart to adequately increase the cardiac output during exercise. In the overall ACHD population, a higher VE/VCO_2 slope was found to be associated with a higher risk of mortality; however this association was negated after adjustment for peak VO_2.[76] Nevertheless, studies that investigated specific diagnostic subgroups such as tetralogy of Fallot, Fontan circulation and TGA corrected by Mustard or Senning procedure did report that VE/CO_2 slope independently predicted heart failure related hospitalisation or cardiac related death.[37, 78] Combined interpretation of the VE/CO_2 slope together with the peak VO_2 may further increase the accuracy of risk predictions.

Heart rate reserve

The chronotropic response is the ability of the heart to respond to exercise by increasing the heart rate. Most studies use the failure to achieve ≥ 80% of the heart rate reserve as a cut-off for chronotropic incompetence.[81] In a large cohort of 727 ACHD patients, chronotropic incompetence was present in 62% of the patients and was associated with severity of heart failure symptoms as expressed by NYHA class.[82] The heart rate reserve and peak heart rate are both predictors for hospitalisation or mortality in the overall ACHD population.[82] The heart rate reserve in patients with a Fontan circulation is a

strong prognostic parameter to predict mortality or heart transplantation, independent of age, type of Fontan surgery and the use of antiarrhythmic drugs. However, the prognostic value of heart rate reserve in this population seems to be inferior to other non-CPET identified risk factors such as signs and symptoms of heart failure, non- total cavopulmonary connection type of Fontan circulation and a medical history of clinically relevant arrhythmia.[17] Also in patients with Ebstein's anomaly the heart rate reserve was shown to be a significant predictor of adverse outcomes.[77]

Other prognostic parameters

In addition to the clearest prognostic parameters as previously mentioned, there are a couple of other CPET parameters that may be of prognostic importance. The peak load (Watt) as a measure for the exercise capacity is relatively easy to obtain in comparison to the peak VO_2, as no gas exchange measurements are required. Peak load may therefore be easier to use in daily clinical practice. Although less extensively investigated, peak load was shown to be associated with a higher risk of mortality in patients with a systemic right ventricle due to congenitally corrected TGA or TGA corrected by the atrial switch procedure.[49] Furthermore, a saturation drop of more than 5% during exercise is a predictor of all-cause mortality in the overall ACHD population[76] and a lower peak exercise systolic blood pressure is associated with an increased risk of adverse cardiac outcomes in patients with a systemic right ventricle.[49]

BLOOD BIOMARKERS

Standard laboratory testing

Basic laboratory testing should be performed in congenital patients that are suspected of heart failure, which includes a full blood count, iron, kidney function, liver function, protein and albumin, and thyroid function.[32] Anemia is not uncommon in patients with ACHD, and is associated with a 3-fold higher risk of death. Low MCV and diuretic use are related to the presence of anemia in these patients, suggesting that iron depletion and the heart failure syndrome play a role in its pathogenesis. Iron deficiency has also been directly related to adverse outcomes in patients with Eisenmenger syndrome,[83] and iron replacement therapy may even improve exercise capacity in these patients.[84]

Renal dysfunction is more frequently observed in patients with cyanotic heart disease and is related to a worse prognosis. For instance, Fontan patients with post-operative renal insufficiency or a higher creatinine level have a significantly poorer outcome.[12] Liver dysfunction is most frequently reported in patients with a failing Fontan circuit, and is known to have a direct effect on morbidity and mortality. The MELD-XI score, calculated from creatinine and total bilirubin which was originally

developed for patients with end-stage liver disease, also predicted cardiac mortality and transplantation in Fontan patients.[85]

Fontan patients who develop protein-losing enteropathy (PLE), as diagnosed by enteric loss of alpha-1-antitrypsin or the presence of low serum total protein/albumin in addition to persistent or intermittent edema, are especially at high risk of death.[12, 31] PLE is difficult to treat, however with recent advances the five-year survival after the diagnosis of PLE has improved from 50% to 88%.[86]

Natriuretic peptides

In patients with heart failure, natriuretic peptides are firmly established prognostic tools. Neurohormonal activation of the natriuretic, endothelin, sympatho-adrenergic, and renin-aldosterone systems also occurs in all types of congenital heart disease.[87] Accumulating evidence shows that N-terminal pro-B-type natriuretic peptide (NT-proBNP) is related to disease severity and that it is useful for risk stratification in patients with clinically stable ACHD, even beyond conventional risk markers.[23, 88] Importantly, normal levels of NT-proBNP (< 14 pmol/L) can accurately rule out the risk of death and heart failure with a high negative predictive value.[23] Therefore, natriuretic peptides have increasingly gained interest and are currently suggested as a component of the clinical work-up of patients with ACHD.[32] No published data is available yet to confirm that changes in natriuretic peptides over time can be used as a marker for outcome; however, data from acquired heart failure patients suggests that disease progression is related to substantial changes in NT-proBNP over time.[89] A position paper from the working group of grown-up congenital heart disease and the heart failure association of the European Society of Cardiology has therefore suggested that a two-fold increase of baseline NT-proBNP within 6 months is regarded as a significant increase which indicates the need for optimization of heart failure medical therapy.[32]

Novel biomarkers

Biomarkers that reflect other pathophysiological mechanisms which are involved in the heart failure syndrome, such as high-sensitive troponin-T (hs-TnT) and growth-differentiation factor 15 (GDF-15) are also related to the occurrence of heart failure in ACHD patients. In a prospective cohort of 595 patients with moderate and complex ACHD, elevated levels of hs-TnT (> 14 ng/L, 8% of patients) and GDF-15 (> 1109 ng/L, 15% of patients) could predict outcomes in ACHD patients with elevated levels of NT-proBNP, suggesting a potential benefit of a multi-marker approach.[23] Other promising novel cardiac markers are red cell distribution width, galectin-3 and ST-2, but longitudinal studies in patients with ACHD are not available yet.

CARDIAC CATHETERIZATION

In patients with ACHD, cardiac catheterization is usually performed for specific anatomical, diagnostic, or physiological questions or for intervention. Some studies have reported hemodynamic variables as predictors for clinical outcome. As described in more detail above, the presence of pulmonary arterial hypertension is an important predictor of mortality.[90] Also in Fontan patients, elevated preoperative pulmonary artery pressures,[91] elevated Fontan pressure, portal hypertension,[29] and elevated right[31] and left atrial pressure[12] have been identified as risk factors for mortality.

RISK STRATIFICATION IN PREGNANCY

Although many women with heart disease may be in a stable clinical condition, pregnancy is associated with substantial hemodynamic changes that carry an increased risk of cardiac complications. The risk of complications is strongly influenced by the type of heart defect and presence of residual lesions. The modified World Health Organization (mWHO) classification seems to be the most accurate tool in predicting these risks.[92] It stratifies patients based on their underlying diagnosis into four groups from very low risk patients (mWHO I), to high risk patients in whom a pregnancy is thought to be life threatening and therefore contraindicated (mWHO IV).[93] Pregnancy is contraindicated (mWHO IV) in women with pulmonary hypertension, severe cyanosis, reduced left ventricular function, previous peripartum cardiomyopathy with incomplete recovery, symptomatic left ventricular outflow tract obstruction, and Marfan patients with a dilated aortic root.

The most frequently encountered complications are heart failure and arrhythmia. Heart failure occurred in 13% of patients in the Registry Of Pregnancy And Cardiac disease (ROPAC), a large worldwide registry on patients with cardiac disease becoming pregnant. Heart failure occurred more often at the end of the second trimester and around delivery. At higher risk were patients with cardiomyopathy or presented with heart failure before pregnancy.[94] Although supraventricular ectopy and supraventricular tachycardia are seen often in normal pregnant women, atrial fibrillation or flutter are very rare. In ROPAC, atrial fibrillation or flutter occurred in 1.3% of the pregnant women with structural heart disease and was associated with a marked increase in maternal mortality and low birth weight.[95] Ventricular tachyarrhythmia's occurred in 1.4% of pregnant women with cardiovascular disease in ROPAC, mainly in the third trimester, and was associated with heart failure during pregnancy and impacted fetal outcome.[96] Contraceptive advice and careful planning of the pregnancy is essential for women with cardiac disease.[92] Pre-pregnancy counselling should be performed in all women with known cardiac disease in an expertise center. An experienced multidisciplinary team

should be available to provide care, before, during and after pregnancy and should timely discuss the mode of delivery. A vaginal delivery is the preferred mode of delivery in most patients, when needed with epidural anesthesia and assisted second stage. A caesarean section is only preferred in specific high risk groups such as patients with a dilated aorta or severe heart failure.[97]

RISK PREDICTION

Few studies have attempted to develop risk prediction models specifically for patients with ACHD[76, 98] or to validate existing models designed for the general heart failure population.[99, 100] The Seattle Heart Failure Model (SHFM) allows prediction of survival with the use of easily obtained clinical characteristics, such as age, sex, weight, NYHA class, systemic ejection fraction, systolic blood pressure, cardiac medication use, laboratory values, and presence of a device. In patients with heart failure, the model provides an accurate estimate of mean, 1-, 2-, and 3-year survival and allows estimation of effects of adding medications or devices to a patient's regimen. Although the predicted mortality risks by the SHFM do not represent actual ACHD survival, it may help to identify subjects with ACHD at risk for adverse outcome and poor cardiopulmonary efficiency.[100]

CONCLUSIONS AND RECOMMENDATIONS

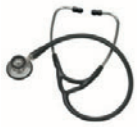
This chapter aimed to provide a comprehensive review of the factors that can be used in day-to-day clinical practice for the risk stratification of patients with ACHD. An overview of the most important reported prognostic factors is provided in Figure 3. Of note, accurate risk stratification does not rely on a single parameter. An integral perspective of the patients' clinical prospects should always be based on a combination of all available information, that is composed of the medical history, physical examination, imaging, exercise testing and biomarkers. The frequency at which individual patients should be monitored at the outpatient clinic and the type and frequency of additional investigations is based on expert opinion in specialist centers. A suggested follow-up scheme of clinically stable patients with ACHD is provided in Table 2. Nonetheless, considering the enormous heterogeneity of the ACHD population, the lack of evidence, and the differences in the availability of additional investigations among centers, it remains challenging to standardize the monitoring and follow-up of individual patients with ACHD.

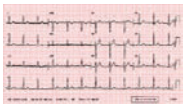
Regular follow-up

Medical History

- Complexity of the congenital heart defect
- Type of corrective surgery
- Pre-operative anatomy and concomitant lesions
- Surgical era and surgical technique
- Genetic syndrome or mutation
- Age and sex
- Previous (re)interventions
- Previous heart failure or arrhythmia
- Cardiac medication use
- Clinical symptoms of heart failure

Physical Examination and ECG

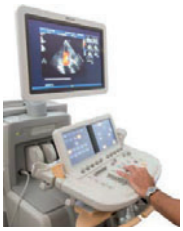




- Clinical signs of heart failure
- Saturation
- Changes in ECG morphology
- Loss of sinus rhythm
- QRS duration

Additional investigations

Echocardiography

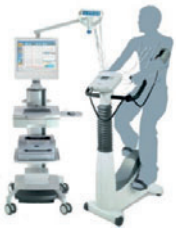


- Systolic ventricular function (LVEF, RVFAC, TAPSE, eyeballing)
- Ventricular and atrial dilatation
- (Residual) shunt lesions
- Severity of valvular disease
- Right ventricular pressure
- Pericardial effusion
- Novel techniques (strain, 3D echocardiography)

Cardiac MRI (CT)

- Systolic ventricular function (LVEF, RVEF)
- Ventricular dilatation
- Novel techniques (late gadolineum enhancement, feature tracking)

Exercise testing



- Peak oxygen uptake (peak VO2)
- Ventilatory efficiency (VE/VCO2 slope)
- Heart rate reserve (chronotropic incompetence)
- Peak load (Watt)

Biomarkers

- Full blood count, iron, kidney function, liver function, protein and albumin, thyroid function.
- NT-proBNP
- High-sensitive Troponin-T
- Growth-differentiation factor 15
- Potential novel biomarkers (galectin-3, ST-2)

FIGURE 3 - Risk factors for heart failure and mortality in patients with ACHD.

TABLE 2 - Outpatient clinic follow-up scheme of clinically stable patients with ACHD.

	Mild congenital heart disease	Moderate congenital heart disease	Complex congenital heart disease
Clinical follow-up	Every 2–5 years, depending on hemodynamic residuals. Patients long-term (> 5 years) after ASD closure without residual shunt or PH may be discharged	Every 1–2 years, depending on severity within subclassification and hemodynamic residuals	At least annually (when in clinically stable condition)
ECG	Routinely (at every check-up)	Routinely (at every check-up)	Routinely (at every check-up)
Chest X-ray	Not routinely advised	Not routinely advised	Not routinely advised
Ambulatory ECG monitoring (Holter)	On indication (palpitations)	On indication (palpitations)	Every 3–5 years and on indication (palpitations)
Ambulatory BP monitoring	On indication	On indication (aortic coarctation)	On indication
Echocardiography	Every 2–5 years, depending on hemodynamic residuals	Every 2 years, annually in case of severe valvular disease	Every 2 years, annually in case of severe valvular disease or on indication
Exercise Testing	At least once (for comparison in case of future clinical deterioration)	Every 3–5 years and pre-pregnancy	Every 3–5 years and pre-pregnancy
CMR	On indication	Consider every 3–5 years	Consider every 3–5 years
CT	Not routinely advised	On indication (evaluation of aortic size/coarctation)	On indication
Full blood count, iron, kidney function, liver function, protein, albumin, thyroid function	Not routinely advised	At least once and when heart failure is suspected	At least once and when heart failure is suspected. Every 2 years in Fontan patients.
NT-proBNP	NT-proBNP every 5 years, annually when > 15 pmol/L (> 125 pg/mL)	NT-proBNP every 5 years, annually when > 15 pmol/L (> 125 pg/mL)	NT-proBNP every 5 years, annually when > 15 pmol/L (> 125 pg/mL)

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Chapter 16

Development and validation of a risk prediction model in patients with adult congenital heart disease

Vivan J.M. Baggen, Esmee Venema, Renata Živná, Annemien E. van den Bosch, Jannet A. Eindhoven, Maarten Witsenburg, Judith A.A.E. Cuypers, Eric Boersma, Hester Lingsma, Jana R. Popelová,* Jolien W. Roos-Hesselink*

*Equal contributions

Submitted.

ABSTRACT

Aims To develop and validate a clinically useful risk prediction tool for patients with adult congenital heart disease (ACHD).

Methods and Results A risk model was developed in a prospective cohort of 602 patients with moderate/complex ACHD who routinely visited the outpatient clinic of a tertiary care centre in the Netherlands (2011–2013). This model was externally validated in a retrospective cohort of 402 ACHD patients (Czech Republic, 2004–2013). The primary endpoint was the 4-year risk of death, heart failure, or arrhythmia, which occurred in 135 of 602 patients (22%). Model development was performed using multivariable logistic regression. Model performance was assessed with C-statistics and calibration plots. Of the 14 variables that were selected by an expert panel, the final prediction model included age (OR 1.02, 95% CI 1.00–1.03, $p = 0.031$), congenital diagnosis (OR 1.52, 95% CI 1.03–2.23, $p = 0.034$), NYHA class (OR 1.74, 95% CI 1.07–2.84, $p = 0.026$), cardiac medication (OR 2.27, 95% CI 1.56–3.31, $p < 0.001$), re-intervention (OR 1.41, 95% CI 0.99–2.01, $p = 0.060$), BMI (OR 1.03, 95% CI 0.99–1.07, $p = 0.123$), and NT-proBNP (OR 1.63, 95% CI 1.45–1.84, $p < 0.001$). Calibration-in-the-large was suboptimal, reflected by a lower observed event rate in the validation cohort (17%) than predicted (36%), likely explained by heterogeneity and different treatment strategies. The externally validated C-statistic was 0.78 (95% CI 0.72–0.83), indicating good discriminative ability.

Conclusion The proposed ACHD risk score combines six readily available clinical characteristics and NT-proBNP. This tool is easy to use and can aid in distinguishing high- and low-risk patients, which could further streamline counselling, location of care, and treatment in ACHD.

INTRODUCTION

Over the past decades, the treatment and intervention strategies for children with congenital heart disease have enormously improved. This had led to the dawning of a new era, in which nowadays the patient population with adult congenital heart disease (ACHD) outnumbers children with congenital heart disease,[1] and we are confronted with new challenges. Today's clinicians face a continuously increasing and aging population of patients with ACHD, who require specialized cardiac care, are at risk of serious adverse events, and in whom monitoring and treatment strategies are largely based on expert opinion.[2] Evidence-based risk stratification would be an important step forward towards individualized management strategies that could lead to better use of health resources, cost savings and improvement of patient care.

To date, many studies have shown that variables such as the complexity of the heart defect,[3-5] age,[5-7] cyanosis,[4, 5, 7] New York Heart Association (NYHA) functional class,[5, 8] ventricular function,[9, 10] or biomarkers (N-terminal pro-B-type natriuretic peptide; NT-proBNP)[6, 11] are related to adverse outcomes in patients with ACHD. The combination of multiple risk predictors into a tool that provides clinically useful risk predictions is most relevant. For instance, in patients with atrial fibrillation[12] or coronary heart disease,[13] risk calculators have been developed which are successfully used in daily clinical practice worldwide. In patients with ACHD, few studies have developed risk models[7, 14] or validated existing prediction models designed for the general heart failure population in patients with moderate or complex ACHD;[15, 16] however, none of these are widely used in daily clinical practice.

The aim of this study was to develop and validate a clinically useful, easy-to-use risk prediction tool that predicts the individualized 4-year risk of death, heart failure or arrhythmia in patients with ACHD. This may aid clinicians in determining the adequate follow-up frequency to monitor patients at the outpatient clinic, to establish the level of care needed for this patient, to provide reliable patient information, and to support clinical judgment when initiating medical therapy.

METHODS

Derivation cohort

We developed a multivariable prediction model by using the data of a prospective cohort of 602 consecutive patients with moderate or complex congenital heart disease.[3] Patients were aged 18 years and older, and routinely visited the outpatient clinic of a tertiary referral centre in the Netherlands (the Erasmus MC, Rotterdam). Patients were enrolled during a 2-year period (April 2011–April 2013) and underwent

clinical assessment, electrocardiography, echocardiography, and venous blood drawing with NT-proBNP measurement at baseline. According to the study protocol, patients were yearly evaluated at our institute during a 4-year period. The primary endpoint was defined as the 4-year risk of all-cause mortality, heart transplant, heart failure (requiring hospital admission or initiation/uptitration of diuretics), or arrhythmia (requiring treatment, or symptomatic and recorded). Follow-up status at year 4 regarding fatal and non-fatal events was complete in 600 patients (99.7%). The institutional review board of the Erasmus MC approved the study protocol and written informed consent was obtained from all patients included in the cohort. A detailed description of the study protocol, participants and data collection has been previously published.[6]

Model development

The primary endpoint was defined prior to the data analysis as a composite of all-cause mortality, heart failure (requiring hospitalization, or initiation or change in heart failure medication), or arrhythmia (symptomatic and recorded, or requiring treatment). In order to prevent overfitting and preserve external validity, we constructed the multivariable models with the use of ≈ 10 events per degree of freedom. Therefore, we evaluated a maximum of 14 variables that were deemed to be of clinical interest by an expert panel of adult congenital cardiologists in our centre. These variables were specified in advance and were 98.6% complete. We used single imputation of covariates based on all covariates considered for the model and based on outcome data to account for missing values; outcome data were not imputed. Continuous variables were analysed continuously, with the exception of oxygen saturation, which was dichotomized at 90 % because we considered this as a clinically meaningful threshold value. Highly skewed variables were $\log_2$-transformed (NT-proBNP). With regard to the categorical variables, we collapsed categories with small numbers (NYHA II–III), we analysed ordinal variables as linear terms, and related variables were grouped (congenital diagnosis, cardiac medication use), in order to use only one degree of freedom per categorical variable.

The main effects were first evaluated using univariable binary logistic regression. Nonlinear effects and interaction terms were disregarded. Variables were selected using Akaike's Information Criterion ($p < 0.157$), and were subsequently entered in a multivariable binary logistic regression model. The final prediction model was retrieved using a stepwise backward selection method ($p < 0.157$). Internal validation (in order to obtain the optimism-adjusted C-statistic of the final model) was performed using bootstrap resampling. Shrinkage of model coefficients to adjust for optimism and to improve external calibration was performed using penalized regression.[17, 18]

Validation cohort

The model was externally validated by using the data of a cohort of 402 patients that routinely visited the outpatient clinic of the Hospital Na Homolce, Prague, Czech Republic (2004–2013). This centre was chosen because it is routine practice to obtain all clinical parameters and also NT-proBNP levels in this hospital. Patients were selected based on retrospective review of medical records. All patients with a moderate or complex type of diagnosis were included. In all patients, NT-proBNP had been determined in fresh serum samples. Exclusion criteria were: referral for hospitalization with manifest heart failure or arrhythmia, age < 18 years, mild cardiac lesion (isolated atrial or ventricular defect), or kidney failure (creatinine > 200 µmol/L). In order to compare the results with the derivation cohort, the occurrence of the primary endpoint as defined above was registered during a period of 4 years from study inclusion.

External validation

Discrimination of the final prediction model was described using the concordance (C)-statistic, which ranges from 0.5 for non-informative models to 1 for perfectly discriminating models.[19] Calibration was visualized by plotting the predicted risks against the observed risks in a validation plot, and further described with the calibration slope (ideally equal to 1) and intercept (ideally equal to zero).[20] Finally, the regression coefficients were refitted on a combined dataset including all patients of the derivation cohort and validation cohort (n = 1004).

Statistical analyses were performed in IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) and using R statistical software, Version 3.3.4, package rms. Development of the web application was performed in the R Shiny package.

RESULTS

Derivation and validation cohorts

The baseline patient characteristics of the derivation cohort (n = 602) and validation cohort (n = 402) are detailed in Table 1. The validation cohort additionally included patients with Ebstein anomaly and atrioventricular septal defect, and no patients after arterial switch operation. Moreover, the validation cohort included fewer patients with congenital aortic stenosis or aortic coarctation and more patients with pulmonary arterial hypertension. In the validation cohort 195 patients (49%) with a complex heart defect were included, compared with 149 patients (25%) in the derivation cohort. Furthermore, in the validation cohort the average NYHA class was higher, and more patients used cardiac medication. Age, sex, body mass index, heart rate, smoking, oxygen

TABLE 1 - Baseline characteristics of the derivation and validation cohort.

Variable	Derivation cohort (n = 602)	Validation cohort (n = 402)
Age, years	32.5 [24.7–41.2]	28.3 [22.5–36.2]
Sex, male	348 (58)	206 (51)
Congenital diagnosis		
Congenital aortic stenosis	138 (23)	23 (6)
Aortic coarctation	112 (19)	21 (5)
Arterial switch operation	24 (3)	0 (0)
Tetralogy of Fallot	179 (30)	106 (26)
Rastelli/REV	11 (2)	7 (2)
Atrial switch operation	65 (11)	87 (22)
Congenitally corrected TGA	21 (4)	22 (5)
Fontan	36 (6)	33 (8)
Functionally univentricular heart	7 (1)	3 (1)
Pulmonary arterial hypertension	9 (1)	43 (11)
Ebstein anomaly	0 (0)	37 (9)
Atrioventricular septal defect	0 (0)	20 (5)
NYHA functional class		
I	541 (90)	109 (27)
II	56 (9)	198 (49)
III	5 (1)	94 (24)
IV	0 (0)	1 (0)
Cardiac medication use	212 (35)	233 (58)
≥1 re-interventions after corrective repair	317 (53)	143 (36)
Body mass index, kg/m^2	24.7 ± 4.4	23.8 ± 4.3
Heart rate, beats/min	74 ± 13	72 ± 14
Current smoking	56 (9)	49 (12)
Oxygen saturation < 90%	17 (3)	21 (5)
Loss of sinus rhythm	81 (13)	43 (11)
Systemic ventricular function		
Normal, 0	303 (50)	224 (56)
Mildly impaired, 1	212 (35)	84 (21)
Moderately impaired, 2	69 (12)	57 (14)
Severely impaired, 3	18 (3)	37 (9)
Presence of severe valvular dysfunction	86 (14)	185 (46)
NT-proBNP, pmol/L*	15 [7–33]	[12-51]

Values are presented as n (%), mean ± standard deviation or median [interquartile range]. *This variable was $\log_2$-transformed for further analysis. **Abbreviations:** NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association functional class; REV, Réparation à l'Etage Ventriculaire; TGA, transposition of the great arteries.

saturation, rhythm, systemic ventricular function and NT-proBNP levels were comparable between both cohorts.

After 4 years of follow-up, the primary endpoint occurred in 135 patients (22%) in the derivation cohort and in 67 patients (17%) in the validation cohort. All individual components of the primary endpoint occurred less frequently in the validation cohort (Table 2).

TABLE 2 - Composites of the primary endpoint in the derivation and validation cohorts.

Variable	Derivation cohort (n = 602)	Validation cohort (n = 402)
Primary endpoint	135 (22)	67 (17)
Death	14 (2)	5 (1)
End-stage heart failure	7 (1)	5 (1)
Sudden death / cardiac arrest	6 (1)	0 (0)
Other	1 (0)	0 (0)
Heart transplant	0 (0)	1 (0)
Heart failure	52 (9)	20 (5)*
Requiring hospital admission	23 (4)	9 (2)
Requiring initiation or change in diuretics	29 (5)	10 (3)
Arrhythmia	112 (19)	55 (14)
Ventricular fibrillation	7 (1)	1 (0)
Ventricular tachycardia	18 (3)	6 (2)
Atrial flutter/fibrillation	51 (9)	22 (6)
Supraventricular tachycardia (unspecified/other)	26 (4)	17 (4)
Other	10 (2)	9 (2)

*Treatment of heart failure was missing in one patient.

Model development

The majority of the 14 variables that were evaluated were predictors of the primary endpoint in the univariable analysis (Table 3). Only heart rate (p = 0.833), smoking (p = 0.410) and severe valvular dysfunction (p = 0.343) were not related with the primary endpoint, and were therefore excluded from the multivariable model. The final prediction model included all variables that were associated with the primary endpoint in the multivariable analysis: age, congenital diagnosis, NYHA class, cardiac medication use, re-intervention, BMI, and NT-proBNP. Of note, the backward selection method resulted in the same final prediction model as a forced entry method of variables with $p < 0.157$ in the multivariable model. The internally validated (optimism-adjusted) C-statistic was 0.85.

TABLE 3 - Main effects in derivation cohort (n = 602).

Variable	Univariable OR (95% CI)	*P*-value	Multivariable OR (95% CI)	*P*-value
Age, years	1.07 (1.05–1.09)	< 0.001	1.03 (1.01–1.06)	0.005
Sex, male	0.73 (0.50–1.07)	0.111	1.27 (0.76–2.13)	0.354
Complex congenital diagnosis	3.28 (2.17–4.95)	< 0.001	1.73 (0.90–3.34)	0.100
NYHA class II–III	8.90 (5.02–15.79)	< 0.001	2.75 (1.33–5.72)	0.007
Cardiac medication use	7.06 (4.62–10.80)	< 0.001	2.52 (1.49–4.24)	0.001
≥ 1 re-interventions after corrective repair	2.30 (1.54–3.45)	< 0.001	1.67 (0.99–2.80)	0.055
BMI, kg/m^2	1.06 (1.01–1.10)	0.009	1.05 (1.00–1.11)	0.059
Heart rate, beats/min	1.00 (0.98–1.01)	0.833	-	-
Current smoking	0.76 (0.39–1.46)	0.410	-	-
Oxygen saturation < 90%	4.67 (1.71–12.80)	0.003	1.12 (0.29–4.29)	0.874
Loss of sinus rhythm	4.35 (2.67–7.10)	< 0.001	1.18 (0.61–2.30)	0.622
Systemic ventricular function, 0–3	2.09 (1.65–2.65)	< 0.001	0.95 (0.66–1.37)	0.787
Presence of severe valvular dysfunction	1.29 (0.76–2.17)	0.343	-	-
$\log_2$ NT-proBNP, per two-fold higher value	2.12 (1.82–2.47)	< 0.001	1.62 (1.34–1.97)	< 0.001

Effect sizes represent the increased 4-year risk of the primary endpoint (death/transplant, heart failure, arrhythmia). **Abbreviations:** OR, odds ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association functional class.

External validation

Similar effects were found for most variables in the validation cohort, except for age and re-intervention. The final prediction model was fitted in the validation dataset to obtain the externally validated C-statistic, which was 0.78 (95% CI 0.72–0.83). Hence, the final model discriminated well between patients with and without a primary endpoint in the validation dataset.

A calibration plot is presented in Figure 1. The calibration-in-the-large was suboptimal: the prediction model systematically overestimated the risk in the validation dataset. The overall proportion of patients with an event in the validation cohort was lower (17%) than would be expected based on the average predicted risk of our model (36%). However, the calibration slope was acceptable, indicating that the predicted risks were not too extreme or too close to the baseline risk. Therefore, when refitting the final coefficients of the model on the combined dataset, we included a correction factor to adjust for the systematic overestimation in the validation dataset (derivation cohort = 0, validation cohort = 1). After shrinkage of the coefficients, the results of the final prediction model were: age (OR 1.02, 95% CI 1.00–1.03, $p = 0.031$), congenital diagnosis (OR 1.52, 95% CI 1.03–2.23, $p = 0.034$), NYHA class (OR 1.74, 95% CI 1.07–2.84, $p = 0.026$), cardiac medication (OR 2.27, 95% CI 1.56–3.31, $p < 0.001$), re-intervention (OR 1.41, 95%

CI 0.99–2.01, $p = 0.060$), BMI (OR 1.03, 95% CI 0.99–1.07, $p = 0.123$), and NT-proBNP (OR 1.63, 95% CI 1.45–1.84, $p < 0.001$). We implemented the first version of our model in a prototype of a web-based ACHD risk calculator (Figure 2). Based on six clinical variables and the NT-proBNP level, it can provide an estimation of the absolute 4-year risk of death, heart failure, or arrhythmia, as defined in this study. The risk calculator is directly available online at https://achdwebcalculator.shinyapps.io/achdwebcalculator/. The absolute risk estimations of this model can be further improved, and we therefore gladly invite other investigators to share their data in order to further improve the current version of the model.

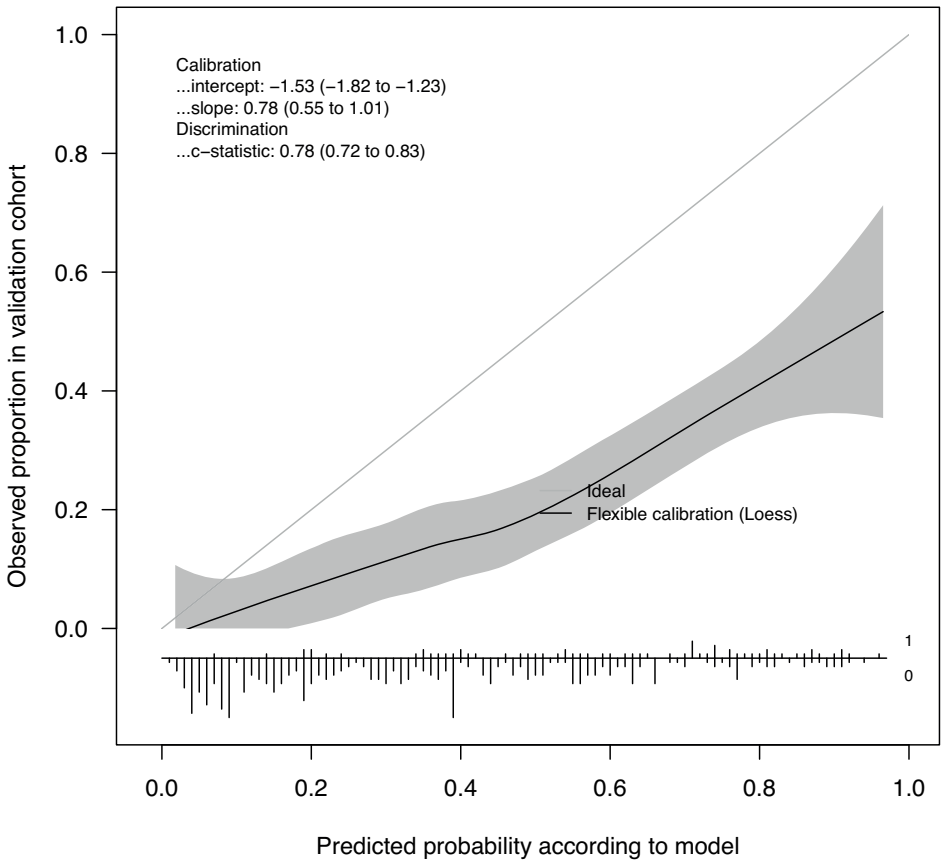

FIGURE 1 - Calibration plot for the prediction of the primary endpoint in the validation cohort (n = 402).

The calibration slope indicates whether the model is overfitted (estimated risks too extreme, value < 1) or underfitted (estimated risks too close to baseline risk, value > 1). The calibration intercept (calibration-in-the-large) indicates whether predicted probabilities are systematically too low (value > 0) or too high (value < 0). The linear bar chart shows the distribution of patients with (1) or without (0) a primary endpoint for each predicted probability.

ACHD Risk Calculator

4-year risk of death, heart failure or arrhythmia

Age (years)

35

Congenital diagnosis

Moderate: e.g. congenital aortic stenosis, aortic coarctation, arterial switch operation, Ebstein anomaly, atrioventricular septal defect, tetralogy of Fallot; Complex: e.g. Rastelli/REV, atrial switch operation (Mustard/Senning), congenitally corrected transposition of the great arteries, fontan, functionally univentricular heart, pulmonary arterial hypertension.

- (●) Moderate
- ( ) Complex

NYHA class

- (●) I: no limitation in ordinary physical activity
- ( ) II-III

Cardiac medication use

ACE-inhibitor, angiotensin receptor blocker, beta blocker, diuretic, aldosterone antagonist, calcium blocker, or anti-arrhythmic drug.

- (●) No
- ( ) Yes

Re-interventions after corrective repair

Any percutaneous and/or surgical re-intervention after initial corrective repair, including interventions in patients without initial corrective surgery. Shunts before the initial correction are not regarded as re-interventions.

- (●) No
- ( ) One or more

Body Mass Index (kg/m2)

25

NT-proBNP (pmol/L)

15

Risk of adverse event within 4 years = 9.1 %

Disclaimer

The presented model is the result of carefully conducted research. This tool is developed and validated based on data of two independent cohorts from tertiary care centers in Rotterdam, the Netherlands (2011-2013) and Prague, Czech Republic (2004-2013). The externally validated C-statistic was 0.78 (95% CI 0.72-0.83), indicating good discriminative ability. Calibration-in-the-large was suboptimal, reflected by a lower observed event rate in the validation cohort (17%) than predicted (36%), likely explained by heterogeneity and different treatment strategies. Other investigators are invited to share their data in order to further improve the absolute risk estimations of the current model.

FIGURE 2 - Web-based ACHD risk calculator, using the coefficients of the final prediction model.

DISCUSSION

To our knowledge, this is the first study that aimed to develop and validate a risk prediction model specifically for patients with ACHD. The final model was developed in 602 patients with ACHD and included six readily available clinical variables (age, congenital diagnosis, NYHA class, cardiac medication use, re-interventions, BMI) and NT-proBNP. External validation in a different ACHD population (n = 402) with an independent treatment strategy showed that the model could discriminate well between patients with death, heart failure, or arrhythmia within 4 years of follow-up and patients without an event during that period. The results are presented in a web-based ACHD risk calculator that is directly suitable for implication in clinical practice.

Comparison with other risk scores

Another risk score for congenital patients was previously developed by Yap et al. in 378 patients with ACHD and atrial arrhythmias at baseline.[14] Of the 9 variables that were found to be of importance in univariable analysis, in a multivariable model NYHA class, single ventricle physiology, pulmonary hypertension, and valvular heart disease remained significantly associated with mortality (n = 40). These variables were combined in a risk score, which was visualized in a Kaplan-Meier curve; however, no model performance characteristics (discrimination, calibration) were evaluated. The definition of the study population and endpoint was slightly different from our study, but the similarity with our study is that we also included NYHA class and congenital diagnosis (where single ventricle physiology and pulmonary hypertension were both defined as a complex diagnosis) in the final model.

Kempny and colleagues recently presented a multivariable mortality risk stratification model that was developed in a retrospective cohort of 1098 adults with Eisenmenger syndrome.[7] The model included age, pretricuspid shunt, oxygen saturation, presence of sinus rhythm and presence of pericardial effusion. In our cohort of ACHD patients, a pretricuspid shunt or presence of pericardial effusion was uncommon, and oxygen saturation and presence of sinus rhythm were not significantly related to study endpoints in the multivariable analysis. Hence, Eisenmenger patients reflect a distinct disease entity in which other variables may be more important. However, NT-proBNP measurements were not included in this study.

The Seattle Heart Failure Model was developed in a cohort of 1125 patients with heart failure, and allows prediction of survival with the use of easily obtained clinical characteristics, such as age, sex, weight, NYHA class, systemic ejection fraction, systolic blood pressure, cardiac medication use, laboratory values, and presence of a device.[21] In patients with heart failure, the model provides an accurate estimate of mean, 1-, 2-, and

3-year survival. This model was validated in a cohort of 153 patients with ACHD, and was able to identify subjects with adverse outcome; however, the predicted mortality risks by the Seattle Heart Failure Model did not represent actual ACHD survival.[15] Still, the type of variables that are included are largely overlapping with our prediction model (age, weight (BMI), NYHA class, cardiac medication use, and laboratory values such as NT-proBNP).

Other variables of potential interest

Inuzuka and colleagues comprehensively demonstrated that a combination of peak oxygen uptake and heart rate reserve are strong predictors of midterm mortality in patients with ACHD, in addition to clinical parameters such as age, low oxygen saturation, and use of negative chronotropic agents.[22] It has also been shown by other studies that exercise testing provides prognostic information in patients with ACHD.[23-25] In addition, various cardiac magnetic resonance imaging measurements have been reported to be valuable for risk stratification. It is known that cardiac magnetic resonance imaging provides the most reliable measurements of right ventricular volumes and function, which are also predictors of clinical outcome.[26]

Because cardiopulmonary exercise testing and cardiac magnetic resonance imaging are relatively expensive and time-consuming, they were not routinely performed in all patients of our cohort and were therefore not investigated in this study. These variables could perhaps further improve the prediction of our model. However, we believe the current set of variables better reflects clinical practice, is readily available and very easy to use. Therefore, this model may be directly suitable for implementation in day-to-day clinical practice.

Calibration

Although the validation cohort included more patients with complex congenital diagnoses, a higher NYHA class, and cardiac medication use (Table 1), they had a better outcome compared with patients in the derivation cohort (Table 2). Because these variables were part of the prediction model, the risk in the validation cohort was systematically overestimated. This resulted in a suboptimal calibration-in-the-large (reflected by a calibration intercept < 0), which is a common problem when externally validating risk models.[19] This may be explained by a difference in the definition and classification of endpoint events. The definition of heart failure included 'initiation or uptitration of diuretics' and the definition of arrhythmia included 'symptomatic and recorded'. As a result, different clinical strategies among centres with regard to treatment with diuretics or holter monitoring could result in a different proportion

of events. Furthermore, misclassification of (non)events may have occurred, although every attempt was made to avoid this by assuring completeness of follow-up in both cohorts and independent review of the events by two investigators. Second, a different classification of baseline covariates may have occurred. The definition for NYHA class I in the derivation cohort was 'no limitation in ordinary physical activity', but this can be subjective and may have been assessed in a stricter way in the validation cohort. The differences in treatment strategies among centres as described above may also impact cardiac medication use and the number of re-interventions. Finally, there may be additional important prognostic factors which explain this difference, and that are not part of the current model.

Clinical implications and future perspectives

In this study, we aimed to combine various risk predictors in order to derive individualized risk predictions. The model provided a good discrimination between high-risk and low-risk patients, and this may serve to optimally inform and, in case of low-risk, reassure patients. High-risk patients should be followed-up in a tertiary care centres, while low-risk patients may be managed in a shared-care model with for instance check-ups every other time in a non-tertiary care centre. In addition, it could support clinical judgment in determining the window in which the next outpatient follow-up visit needs to be scheduled, in assessing the need of initiation or change in medical therapy, planning an intervention, or even considering heart transplantation.

Because the absolute predicted risks were not in accordance with the observed risks in the validation cohort, the absolute risks provided by our model in other cohorts of patients with moderate/complex ACHD must be interpreted with caution. Future research is warranted to evaluate whether the implementation of such a risk prediction tool actually can improve a patients' prognosis, and thus leads to clinical benefit. Finally, it should be investigated how much costs can be saved by implementing such a risk prediction tool.

Study limitations

Although a prospective cohort of 600 patients with ACHD may be considered relatively large in this patient population, it is rather small in comparison with other cohorts in which well-known risk prediction models were developed. Nonlinear terms and interaction terms had to be disregarded in the multivariable prediction model, because we considered it more relevant to analyse the potential additive effects of the highest possible number of different covariates, instead of fully exploring nonlinearity and interactions at the risk of overfitting the model. With larger datasets,

it could be worthwhile to explore these potential effects in order to further improve risk prediction.

CONCLUSIONS

We developed and validated an ACHD risk calculator, which was based on readily available clinical characteristics (age, congenital diagnosis, NYHA class, cardiac medication use, re-interventions, BMI) and NT-proBNP. External validation showed that calibration-in-the-large was suboptimal, which is likely explained by the heterogeneity in patient cohorts and different treatment strategies across centres. Nonetheless, the final prediction model was able to accurately discriminate between patients at high and low risk of death, heart failure, or arrhythmia within 4 years, and could therefore support clinical judgment in day-to-day practice. Other investigators are welcome to share their data in order to further improve the absolute risk estimations of the current model.

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Chapter 17

Summary
General Discussion

SUMMARY

Thanks to the improvements in the medical care of children with congenital heart disease, today's cardiologists are confronted with a growing population of adults with congenital heart disease (ACHD). These patients are mostly young adults, may want to become pregnant or parent, participate in sports, and aim to live their life as close to normal as possible. In order to maximize their quality of life for as long as possible, early detection of late complications and risk stratification is an essential component of routine clinical care. This thesis aimed to establish novel prognostic tools that can be used for the risk stratification of adults with congenital heart disease and/or pulmonary hypertension. In **Chapter 1**, an introduction and overview of the patient populations, potential prognostic tools, outcomes, and study designs is provided.

Part I – Imaging biomarkers

Right ventricular volumes and function are important prognostic parameters in ACHD and pulmonary hypertension patients. Although the most accurate measurements are obtained with cardiac magnetic resonance imaging (CMR), the course trabeculae that are often present in these patients hamper reproducible right ventricular endocardial alignment. **Chapter 2** describes a new method to deal with these trabeculae. Eighty patients (pulmonary hypertension, transposition of the great arteries after arterial switch operation and after atrial switch procedure and repaired tetralogy of Fallot) and 20 controls underwent CMR. Two methods of measuring right ventricular volumes and function were compared. We found that exclusion of trabeculae from the blood volume with a semi-automatic pixel-intensity based software was highly reproducible, and resulted in a substantial change in right ventricular volumes and function, compared with the conventional manual method.

In **Chapter 3**, we describe the cross-sectional association between CMR findings and exercise capacity in 71 adults after arterial switch operation for transposition of the great arteries. Especially main pulmonary artery area was closely related to peak oxygen uptake, indicating that this is an important determinant of limitation in functional capacity and thus a potential prognostic factor. In **Chapter 4**, the association of left atrial size and function with clinical events in 134 adults with repaired tetralogy of Fallot is described. All patients underwent echocardiography with measurement of left atrial maximal, minimal and pre-A wave volume, area and length, and were prospectively followed for the occurrence of cardiovascular events. Higher left atrial volumes, larger left atrial length and decreased total and active emptying fraction were found to be associated with cardiovascular events, indicating that these echocardiographic measurements can provide prognostic information.

The development or persistence of pulmonary arterial hypertension (PAH) after atrial septal defect (ASD) closure at adult age is associated with a poor prognosis. To establish the prevalence of PAH before and after ASD closure and to explore factors that are associated with PAH after ASD closure, we performed a systematic review of all studies that reported the prevalence of PAH or data on pulmonary artery pressures both before and after surgical or percutaneous ASD closure in an adult population in **Chapter 5**; and retrospectively assessed echocardiograms before and after ASD closure in 198 adult patients who underwent ASD closure in our own center between 2000 and 2014 in **Chapter 6**. The reported prevalence of PAH after ASD closure widely varied between 5 and 50%, and was higher in older studies, smaller study cohorts, and studies with a large proportion of patients lost to follow-up. We therefore assumed that the "true" prevalence was more towards the lower limit of the reported range, which was indeed confirmed in our own study (5%). The prevalence of PAH and mean pulmonary pressures decreased in all studies after ASD closure, and in all but one patient in our own cohort. Whereas in previous studies age at closure was reported as predictor for PAH, we found that NYHA functional class, presence of pulmonary disease, cardiac medication use, and impaired RV function were associated with for PAH after ASD closure.

In **Chapter 7** and **Chapter 8**, the echocardiographic findings and CMR findings that provide prognostic information in patients with pulmonary arterial hypertension are systematically reviewed. Among 37 papers that investigated 51 echocardiographic findings, the presence of pericardial effusion, enlarged right atrial area, and decreased tricuspid annular plane systolic excursion were the most well-investigated and robust predictors of mortality or transplant. Of the 21 CMR findings that were described in eight papers, decreased right ventricular ejection fraction was the strongest and most well-established predictor of mortality.

Part II – Blood biomarkers

Chapter 9 presents the cross-sectional association of matrix metalloproteinase (MMP)-2, -3, -9 and tissue inhibitor of matrix metalloproteinase-1 with cardiac function and exercise capacity, in a subset of 425 patients with ACHD from the BioCon study. MMP-2 was found to have a modest correlation with decreased exercise capacity (peak workload), decreased ventilatory efficiency (carbon dioxide equivalent) and decreased left ventricular deceleration time, independently of NT-proBNP. However, peak workload and carbon dioxide were only available in 126 and 40 patients, respectively, and reproducibility and reference values were not assessed in this study.

In **Chapter 10** the most important results of the BioCon study are presented. We investigated the association of NT-proBNP, high-sensitive troponin-T, and growth-differentiation factor 15 with cardiovascular events in 595 patients with ACHD. During a prospective follow-up of median 42 months, the primary endpoint of any cardiovascular event occurred in 165 patients. NT-proBNP was strongly associated with the primary endpoint, independently of multiple clinical, electrocardiographic and echocardiographic variables. The risk of death or heart failure was extremely low (~1%) in patients in the lowest two NT-proBNP quartiles (< 15.2 pmol/L). The risk of patients with an elevated NT-proBNP level could be further differentiated by the combined use of hs-TnT and GDF-15. Patients with elevated levels of all three biomarkers were at highest risk of cardiovascular events. In **Chapter 11**, we describe the galectin-3 measurements that were performed in the BioCon study. Galectin-3 is an emerging biomarker for risk stratification in heart failure patients, and was also significantly associated with functional capacity, cardiac function, and adverse cardiovascular events in our cohort of patients with ACHD. However, the additive value of galectin-3 to a more conventional risk marker such as NT-proBNP was limited. In **Chapter 12**, we propose red cell distribution width (RDW) as an additive biomarker for risk stratification. We found that RDW was significantly associated with cardiovascular events when adjusted for age, sex, clinical risk factors, CRP, and NT-proBNP. In addition, the C-index of the model including RDW was slightly, but significantly higher than the model without (0.74, 95% CI 0.70–0.78 versus 0.73, 95% CI 0.69–0.78). Analysis of repeated RDW measurements (n = 2449) did not show an increase in RDW prior to the occurrence of the primary endpoint; therefore, repeated measurements were not further analyzed in this study.

In **Chapter 13** we investigated repeated measurements of NT-proBNP, as this appeared to be the most promising biomarker of the BioCon study. We analyzed 2424 repeated annual NT-proBNP measurements that were collected during a median follow-up of 4.4 years with linear mixed effect models and joint models. NT-proBNP increased prior to the occurrence of an event, especially death or heart failure, whereas patients without any event exhibited stable and low levels. The high-risk group of patients with an elevated baseline NT-proBNP (> 14 pmol/L, n = 315, 53%) could be further differentiated by repeated NT-proBNP measurements, since these were significantly associated with cardiovascular events when adjusted for the baseline level.

In **Chapter 14**, we describe the first results of the BioPulse study. We prospectively included 106 consecutive treatment-naïve patients with pulmonary hypertension confirmed by right heart catheterization. During a median follow-up duration of 24 months, the primary endpoint of death or lung transplantation occurred in 29 patients

(27%). At baseline, we measured NT-proBNP, high-sensitive troponin-T, high sensitive-C-reactive protein, galectin-3, red blood cell distribution width and estimated glomerular filtration range, all of which were found to be associated with the primary endpoint. In addition, this study supports the idea of a multi-biomarker approach to optimize risk stratification, as an analysis which included the number of elevated biomarker in each patient demonstrated that patients were at higher risk of events as more biomarker levels were elevated. The absence of any elevated biomarker ruled out the risk of any event up to 40 months.

Part III – Risk prediction

Chapter 15 provides a narrative review of the factors that have been identified as predictors for heart failure and other late complications in the entire cohort of patients with ACHD and within specific congenital subgroups. We describe that a large part of the risk stratification of patients with ACHD is based on the underlying anatomical defect, concomitant lesions and type of corrective surgery that was performed. In addition, components of the medical history, physical examination and further diagnostic tests (including ECG, echocardiography, cardiac magnetic resonance imaging, exercise testing, and biomarkers) can provide prognostic information.

Finally in **Chapter 16** we developed and validated a multivariable logistic regression model to predict the 4-year risk of death, heart failure or arrhythmia in patients with moderate or complex ACHD. The model was derived in the BioCon study (n = 602), and externally validated in a retrospective cohort of 402 ACHD patients from a tertiary care center in Prague, Czech Republic. The final prediction model included six readily available clinical characteristics (age, congenital diagnosis, NYHA class, cardiac medication use, re-interventions, BMI) and NT-proBNP. The externally validated C-statistic was 0.78 (95% CI 0.72–0.83), indicating a good discriminative ability. The calibration-in-the-large was suboptimal, reflected by the calibration intercept (< 0) and a lower observed event rate in the validation cohort (17%) than predicted (36%). This is likely explained by the heterogeneity in patient cohorts and different treatment strategies across centers. Nevertheless, the proposed tool could be clinically useful as it aids in distinguishing patients with a high or low risk of death, heart failure, or arrhythmia, which could further streamline care in tertiary care centers.

GENERAL DISCUSSION

Clinical implications of this thesis

Imaging biomarkers

Part I outlines the potential prognostic value of various cardiac magnetic resonance and echocardiographic measurements. Measurements of right and left ventricular volumes and function,[1-3] main pulmonary artery area,[4] left atrial size and function,[5] right ventricular pressure, pericardial effusion, and right atrial area[6] were shown to be associated with patient characteristics, surrogate study endpoints and/or clinical endpoints. Therefore, these measurements can be of prognostic value in specific subgroups of the ACHD population.

When generalizing these results, it is important to take into account that the different studies were performed in various patient cohorts with different congenital diagnoses and a widely varying cardiac anatomy. For instance, precise quantification of right ventricular function is important;[1, 2] however, this cannot be obtained in patients with a single ventricle physiology, and in patients after Mustard correction the anatomic right ventricle serves as the systemic ventricle. In patients after arterial switch operation the main pulmonary artery area seems to be an important determinant in the limitation of functional capacity,[4] as is likely opposed to patients with a congenital aortic stenosis. In patients with tetralogy of Fallot, left atrial area measurements provide prognostic information and this could also be useful for the assessment of diastolic function, because the value of E' is questionable due to the presence of the interventricular patch.[5] In contrast, in patients after Mustard correction, pulmonary venous return is directed towards the systemic ventricle through a noncompliant baffle and the left atrium does basically not exist.[7]

Because of the widely varying cardiac anatomy among the different congenital diagnoses, we did not aim to combine or directly compare the different imaging findings across the entire ACHD population. Instead, the value of these imaging measurements should be appreciated within the investigated specific diagnostic subgroups.

Blood biomarkers

In contrast to imaging biomarkers, blood biomarkers can be obtained in all patients, regardless of the underlying congenital anatomy. We displayed diagnosis-specific biomarker profiles and adjusted the analyses for the complexity of the congenital diagnosis. In Part II, the (potential) value of matrix metalloproteinases,[8] NT-proBNP, troponin-T, GDF-15,[9] galectin-3,[10] and RDW is described in separate chapters. In Table 1, we provide an overview of all biomarkers that were investigated in the BioCon study,

a prospective study of the value of biomarkers in 602 adult patients with congenital heart disease. In order to allow better comparison of the prognostic value of all available biomarkers, we analyzed the biomarkers according to the statistical analysis method and endpoint definition as described in Chapter 16 (4-year risk of death, heart failure, or arrhythmia). Skewed biomarker distributions were $\log_2$-transformed and odds ratios were presented per one standard deviation increase in the biomarker.

From these results, it can be concluded that almost all biomarkers were significantly associated with the endpoint in the univariable analysis, except for MMP-2 and hemoglobin. NT-proBNP has the highest standardized odds ratio, indicating that this biomarker is most strongly associated with adverse events. Furthermore, NT-proBNP has the highest discriminatory value (C-statistic). When all available biomarkers were analyzed together in a multivariable logistic regression model, only NT-proBNP, troponin-T and GDF-15 remained significantly associated with the endpoint. This is explained because although the biomarkers reflect different pathophysiological pathways, one biomarker can also be involved in multiple pathways, so there is a considerable overlap. This is reflected by the correlation coefficients as shown in Table 2. Although the effect of RDW was partly negated when adjustment was performed for all biomarkers (including GDF-15), it was significantly associated with clinical endpoints when only adjusted for NT-proBNP. Because this is a very inexpensive and worldwide readily available biomarker, RDW could also be clinically useful to obtain additional prognostic information.

Of all investigated biomarkers, NT-proBNP was the strongest and most important prognostic marker. It is therefore recommended that at least one measurement of NT-proBNP is performed in all patients with moderate or complex ACHD to obtain prognostic information, preferably when they are in a stable situation when visiting the outpatient clinic for a routine check. Patients with a low NT-proBNP level may be reassured and can be monitored at a lower follow-up frequency, for instance every 2-4 years (also depending on other patient characteristics, such as age and congenital diagnosis) instead of yearly. In patients with an elevated NT-proBNP, it is probably useful to additionally measure other biomarkers, such as troponin-T, GDF-15, or RDW. Furthermore, in these patients annual repeated NT-proBNP measurements should be considered. This can be useful to identify high-risk patients who should be strictly followed-up in a tertiary care center, may benefit from timely initiation or expansion of heart failure medication, cardiac rehabilitation training, or possibly early cardiac interventions in order to pre-empt the onset of late complications.

TABLE 1 - Increased 4-year mortality, heart failure, or arrhythmia risk per one standard deviation increase in the (log_2-transformed) distribution of the biomarker.

Biomarker	Univariable standardized OR (95% CI)	*P*-value	C-statistic	Multivariable standardized OR (95% CI)	*P*-value
MMP-2, ng/L*	1.10 (0.88–1.37)	0.416	-	-	-
NT-proBNP, pmol/L*	3.82 (2.90–5.02)	< 0.001	0.80	2.55 (1.82–3.55)	< 0.001
hs-TnT, ng/L*	2.18 (1.76–2.70)	< 0.001	0.71	1.35 (1.03–1.76)	0.029
GDF-15, ng/L*	2.36 (1.91–2.92)	< 0.001	0.73	1.41 (1.07–1.86)	0.014
RDW, %	1.88 (1.53–2.30)	< 0.001	0.69	1.26 (0.97–1.62)	0.079
Hb, g/L	0.96 (0.79–1.16)	0.660	-	-	-
Galectin-3, ng/mL*	1.77 (1.44–2.18)	< 0.001	0.65	1.01 (0.77–1.32)	0.942
hs-CRP, mg/L*	1.26 (1.03–1.55)	0.023	0.58	0.93 (0.72–1.19)	0.562
Sodium, mmol/L	0.70 (0.57–0.86)	0.001	0.57	0.86 (0.67–1.09)	0.214
Ureum, mmol/L*	1.68 (1.37–2.06)	< 0.001	0.63	1.29 (0.96–1.73)	0.087
eGFR, mL/min*	0.60 (0.48–0.74)	< 0.001	0.61	1.14 (0.83–1.57)	0.419

*Biomarker was log_2-transformed before further analysis because of a skewed distribution.

TABLE 2 - Pearson correlation coefficients representing the correlation between the biomarkers in the BioCon study.

	MMP-2	NT-proBNP	hs-TnT	GDF-15	RDW	Hb	Galectin-3	hs-CRP	Sodium	Ureum	eGFR
MMP-2		0.15	0.07	0.10	0.09	–0.04	0.15	–0.04	–0.03	0.00	–0.07
NT-proBNP			**0.42***	**0.50***	0.37*	–0.13	0.30*	0.24*	–0.15*	0.21*	–0.33*
hs-TnT				0.31*	0.19*	0.14	0.23*	0.05	0.02	0.37*	–0.28*
GDF-15					**0.40***	–0.12	0.40*	0.20*	–0.23*	0.27*	**–0.48***
RDW						–0.20*	0.32*	0.18*	–0.18*	0.18*	–0.27*
Hb							–0.04	–0.12	0.17*	0.08	0.18*
Galectin-3								0.22*	–0.20*	0.21*	**–0.43***
hs-CRP									–0.16*	–0.01	–0.12
Sodium										–0.09	0.18*
Ureum											**–0.52***
eGFR											

*$P < 0.001$. Correlations with $r > 0.40$ are printed in bold.

From risk factor, to risk prediction, to clinical benefit and cost savings

The majority of chapters aims to identify risk factors that are associated with clinical outcome in (specific subgroups of) ACHD, and are therefore regarded as prognostic factor research.[11] A factor's prognostic value over existing prognostic factors should not only be reflected with the effect size and confidence interval (*p*-value) of the multivariable odds ratio or hazard ratio, but preferably also with the improvement in discrimination (C-statistic), and reclassification (net reclassification index, integrated discrimination improvement).

Chapter 16 aimed to combine multiple prognostic factors to predict the absolute risk of future clinical events in individual patients, which is regarded as prognostic model research.[12] We validated the model in an external cohort, showing that it had a good discrimination and could thus well separate low- and high-risk patients. The calibration-in-the-large was suboptimal, indicating that the model systematically overestimated the absolute risk in the validation cohort.[13] Our risk prediction model may therefore be further improved by including larger datasets from other centers.

In addition, more evidence is ideally required beyond external validation before a model can be regarded as clinically useful.[14] Impact studies are needed to assess the effect the implementation of a prediction model on the physician's behavior and patient outcome (net clinical benefit, e.g. the gain in 10-year event-free survival).[15] The reference standard for the evaluation of biomarkers (or prediction models) by clinical outcomes is the assessment in a randomized controlled trial which compares the outcomes of patients for whom clinical decisions are driven by the biomarker (or prediction model) versus usual practice. The challenge of these trials is that a relatively large sample size is required to show a benefit on clinical endpoints. To illustrate this, we calculated the required sample size of a trial in which patients are randomized 1:1 into biomarker decision-based therapy and usual practice. Biomarker decision-based therapy could for instance be that patients with an elevated NT-proBNP (~50%) receive intensive treatment with optimization of heart failure medication, cardiac rehabilitation training, frequent follow-up visits, and/or early re-interventions; while patients with a normal NT-proBNP level receive standard treatment or even less frequent follow-up.

Based on an overall event rate of 22% (the 4-year event rate of death, heart failure, or arrhythmia in Chapter 16) and a 2-sided $\alpha = 0.05$ and $ß = 0.80$, a sample size of ~2800 patients would be needed to demonstrate a hazard ratio of 0.80 for the occurrence of the primary endpoint in the biomarker decision-based treatment group.[16] It may be therefore more feasible to design a biomarker decision-based trial with a lower required sample size by using surrogate endpoints such as the improvement in exercise capacity or ventricular function.

Finally, the cost-effectiveness of the implementation of a biomarker (or risk model) should be studied. The measurement costs of the different biomarkers that are studied in this thesis are widely varying, with most biomarkers ranging from €1 to €20 per measurement (Table 3). Especially GDF-15 is very expensive (€60-80 depending on the costs of pre-clinical assays). Furthermore, NT-proBNP and galectin-3 are relatively expensive, while other routinely available 'standard' biomarkers such as RDW (as part of the automated blood count) and kidney function are almost for free, also because these measurements may be already performed for other clinical reasons. This could be an important reason to consider hs-TnT or RDW as more suitable biomarkers for add-on risk stratification on top of NT-proBNP, in contrast to GDF-15. Cost-effectiveness is typically expressed as a ratio of the gain in health (such as QALY, quality-adjusted life years) and the cost associated with that health gain. In theory, this could be analyzed as part of the trial as proposed above. The average costs per patient in the biomarker decision-based therapy arm should be compared with the average costs per patient in the usual practice arm. However, in order to demonstrate a substantial gain in health as expressed in QALYs in the biomarker decision-based therapy arm, probably a long follow-up and large patient numbers would be required (as shown above). Therefore, it might be a more feasible option to evaluate cost-effectiveness by using microsimulation modeling techniques.[17] These computer modeling techniques are suitable for estimating the effects of proposed interventions before they are implemented, and are for instance used in highway traffic flowing through an intersection, financial transactions, but also in healthcare.[18, 19]

TABLE 3 - Costs for each biomarker measurement in the clinical laboratory of the Erasmus MC (2016–2017).

Biomarker	**Costs**
MMP-2	€ 7,50*
NT-proBNP	€ 19,45
hs-TnT	€ 7,87
GDF-15	€ 60-80*
RDW	€ 0,98
Hb	€ 0,98
Galectin-3	€ 18,31*
hs-CRP	€ 1,54
Sodium	€ 0,86
Ureum	€ 0,89
eGFR (Creatinin)	€ 1,06

*Depending on the costs of pre-clinical assays and measurements in the trial laboratory (incl. BTW).

Bias in observational studies: pitfalls and how to deal with it

All chapters in this thesis are based on observational studies, or a review of observational studies. Although this type of studies can be useful to answer many questions in health research, they are subject to a number of potential limitations that must be acknowledged, in order to appropriately deal with it and/or to justly interpret the obtained results.[20]

Selection and selection bias

Patients with mild ACHD (isolated repaired septal defect without PAH) were not included in the BioCon study. These patients have lower levels of NT-proBNP[21, 22] and much lower event rates;[23] therefore, the association that was found between NT-proBNP and events may be weaker or even absent in these patients. This is due to selection of the study cohort and does not affect the internal validity of the conclusions of the BioCon study for patients with moderate and complex ACHD; however, it does imply that the results may not generalizable to patients with mild ACHD.

Selection *bias* arises when the study participants (i.e., the BioCon study cohort) would not be a representative or random sample of the target population for which a statement is to be made (i.e., clinically stable patients with moderate or complex ACHD).[20] In that case, the relation between the studied exposure and outcome is different for those who participate in the study, compared with all of those who were theoretically eligible for the study. The BioCon study was a single-center study performed at the outpatient clinic of a tertiary care center. Therefore, we cannot exclude referral bias (a type of selection bias); however, almost all patients with moderate or complex ACHD are under tertiary care. Selective non-response or selective loss to follow-up may also result in selection bias, which can be minimized a prospective study design, a high participation rate or 'consecutive' sample (i.e., all eligible patients who are seen within a specific time window are included), and careful study planning. In order to assess the potential impact of selection bias, the rate of non-participants must be reported (e.g. with a flowchart of the study cohort selection process), preferably the most important baseline characteristics of the non-participants should be presented, and sensitivity analyses can be conducted. The BioCon study had a prospective design, aimed to included consecutive patients, and had a high completeness of patient data and follow-up. Therefore, the impact of selection bias on the overall results of the BioCon study (as described in Chapter 4, Chapter 9–13) is probably limited.

In contrast, selection (bias) probably did affect the results of the observational studies that were included in the systematic review in Chapter 5. We found that the reported PAH prevalence after atrial septal defect closure was widely varying (5–50%). This was highest in older studies and in studies which included a small number of patients. It is

likely that the oldest study cohorts are not generalizable anymore to the population of adults with an ASD closure nowadays, because ASD closure is now usually performed percutaneously, and therefore probably with a lower threshold (i.e., in less severe patients). Furthermore, the rate of non-participants was generally not reported and the overall loss to follow-up (i.e., patients with echocardiographic data on pulmonary pressures after ASD closure) was high. Therefore, a probable explanation for the wide range of the reported PAH prevalence is that the inclusion of more severe patients and the selective echocardiographic follow-up of more severe patients has resulted in an overestimation of the PAH prevalence in some studies.

Information bias

Incorrect or inaccurate recording of patient characteristics or study outcomes results in information bias. These variables can be continuous (referred to as measurement error) or categorical (referred to as misclassification). When these errors are independent of other variables, these 'random mistakes' will result in less precise measurements and thus an underestimation of the difference between groups (bias towards the null).[20, 24] An example where non-differential measurement errors may have resulted an underestimation of the effect is Chapter 9. Because we did not assess the reproducibility of the matrix metalloproteinase measurements by duplicate measurements, it could be possible that the detected biomarker levels were not precise enough. This may explain why the correlation between MMP-2 and variables reflecting LV diastolic dysfunction and exercise capacity was only weak, and not related to study endpoints. We have tried to improve this in other studies by first performing duplicate measurements of novel biomarkers in a healthy control cohort. The galectin-3 measurements were found to be highly reproducible and in accordance with normal values that were previously published (Chapter 11). However, the initial duplicate ST-2 measurements with a novel rapid test were varying to such a degree that we decided to cancel the scheduled measurements in the BioCon cohort, and start over with a more conventional ST-2 analysis method (ELISA; results not available yet and therefore not included in this thesis).

When misclassification or measurement errors are dependent of other variables, the true association may be either over- or underestimated. This could for instance occur if patients with a low NT-proBNP level are not as thoroughly assessed for clinical events as patients with a high NT-proBNP level (either during the clinical follow-up by the physician, or during review of the electronic patient record the researcher), or vice versa. We aimed to avoid this by blinding the event adjudication for biomarker levels. Furthermore, not any biomarker was part of the clinical patient work-up during the conduct of the BioCon study. Therefore, we expect that bias due to differential misclassification is minimal.

Confounding

Many epidemiologists, but not all, regard confounding as another type of bias. Confounding occurs when the association between exposure and outcome is (partially) explained by a third variable (the confounder) that is related to both the exposure and the outcome, and is not in the causal chain itself.

Confounding is a causal concept, and can be dealt with in the data-analysis by stratification or multivariable adjustment for confounders.

In prognostic studies, it is not the main question whether biomarkers have a causal relationship with the disease.[25] Biomarkers *can* play a pathophysiological role in the development of disease, but can also be so-called 'innocent bystanders' – surrogates of causal factors that may be easier to measure but do not have a causal association with the disease itself. A clear example of this is NT-proBNP, the biologically inactive terminal fragment of the brain natriuretic peptide prohormone.[26] Because it does not matter if the prognosis is based on causal variables, surrogates of causal variables, or confounders, prognostic research focuses on *association* rather than *causation* and there is strictly speaking no need to 'control for confounders' (although it is often described in this way).[25, 27] Still, in prognostic studies multivariable adjustment is performed, in order to investigate whether specific biomarkers can provide prognostic value incremental to other variables that are known to be of prognostic importance (Chapter 4, Chapter 9-13), and in order to combine multiple predictors to derive clinically useful risk predictions (Chapter 16).

Repeated measurements: regression to the mean

Most studies that investigate serial biomarker measurements only assess the difference between two subsequent measurements, and relate relative, absolute and/or categorical changes to adverse events. This is convenient, because it is easy to analyze and interpret, and requires less data per patient. However, a problem is that these changes cannot be adjusted for the baseline measurements, because this will introduce a bias due to regression to the mean.

Changes between subsequent measurements can be (partially) the result of analytical imprecision and biological variation. Due to this day-to-day variation, every patient has a certain 'normal distribution' of biomarker values that are (hypothetically) measured within a very short time period, with a 'true mean level' and a standard deviation. The standard deviation will be larger for biomarkers with a relatively large biological variation, such as NT-proBNP.[28] In Figure 1, an example is presented in which the actual levels of a patient with a high mean NT-proBNP level (depicted in red), and of a patient with a low mean NT-proBNP level (depicted in blue), do not change between Year 0 and Year 1. When evaluating two subsequent measurements of Year 0 and Year

1, a decrease or increase can be (partially) explained by regression towards the mean NT-proBNP level within a patient. Because regression towards the mean has the same theoretical chance to occur in either direction (upward or downward), it may attenuate the true changes that can be detected, but it does not directly bias the results. However, when the change is adjusted for the baseline measurement as is shown in the right panel, basically two patients with the same baseline NT-proBNP level are compared. Now, the effect of an increase over time is positively biased due to the regression towards the mean effect.

This is a very important concept when analyzing two repeated measurements within the same patient, especially when there is a large intra-individual biological variability, and even more so when adjustment for baseline levels is performed. We believe this concept deserves much more scientific attention, because most studies that have investigated serial measurements do not take this into account. The solution for this problem is the analysis of multiple measurements (> 2) with a linear mixed effects model.[29] We believe this provides a more reliable of the true biomarker evolution over time. Moreover, it adjusts for regression to the mean by including a random effects term for each patient, which takes into account the random within-subject variation.

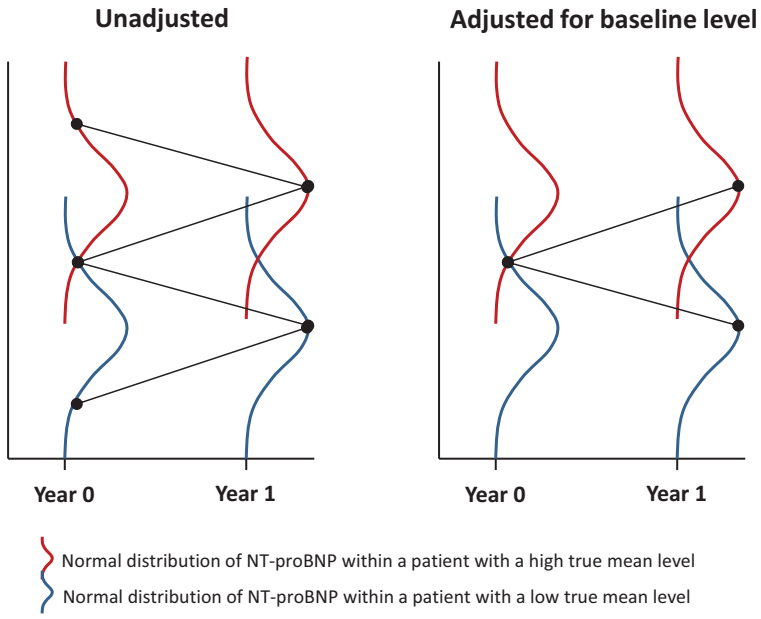

FIGURE 1 - Conceptual model of the bias that is introduced when adjustment is performed for the baseline level, due to biological variation and regression to the mean.

Big data

Today, the calculation of a *p*-value is just a few clicks away in easily accessible point-and-click software such as SPSS. We can hardly imagine that back in the 1970s researchers had to manually calculate the significance of a statistical test with the use of calculators and distribution tables.[30] The computer has greatly accelerated medical research methods by enabling data management and analysis in a more accurate and less time-consuming way.

Along the same line, in one or two decades from now, we can probably hardly imagine that the majority of data harvesting was still manually performed around the year 2017. A large part of the data that is described in this thesis was collected by manually including patients, analyzing echocardiographic images and double-checking electronic patient records. Consequently, cohorts of 600 patients with more than 100 variables per patient such as used in this thesis could be considered as reasonably large datasets nowadays. There are also much larger cohorts such as the Rotterdam study,[31] but these involve major budgets and many researchers to appropriately deal with the amount of work.

An enormous amount of data is continuously generated in all medical fields, from the intensive care unit to the general practice. These data are incredibly valuable, if we would take full advantage of it; however, in medicine we are only at the very beginning stages of this information revolution. Information-power companies such as Google, Amazon, Netflix, and Facebook already know how to predict what kind of shoes you would like to buy or what kind of movies you would like to watch, based on self-learning algorithms and extremely large datasets known as 'big data'. In medicine, big data could be very useful to further improve risk predictions for individual patients. Although all patient information is entered in the electronic patient record at every outpatient clinic visit (or even continuously during hospital admissions), including clinical characteristics, imaging findings and laboratory measurements, we do not have an automatic framework to further take advantage of this data. We already know how to analyze repeatedly collected data in continuously updating dynamic prediction models,[32] but if we can automatically collect all the data that is generated, machine-based learning techniques might be able to further improve the prediction of clinical outcome and to aid in clinical decision making.

The use of big data in medical research is currently hampered by concerns about informed consent; protection of patient privacy, confidentiality, and harm; data quality and re-identification; the reporting of faulty inferences;[33] data sharing and intellectual property rights; and practical issues such as electronic patient records that are not suitable for electronic data collection and the lack of resources to analyze increasingly large datasets.[34] In addition, concern exists that big data in medicine will lead to the (partial) replacement of human doctors by computers.

Nevertheless, artificial intelligence is already transforming the world as we know it and the potential of big data in medicine is huge. Together with wearable devices and mobile health apps this will change how people manage their health. Responsible big data research should take into account the complex ethical issues that are involved, acknowledge that data are people, reduce the change of harm resulting from big data research practices, make sure that the work is sound and accurate, and contribute to building best practices in medicine.[35]

CONCLUSIONS

This thesis aimed to establish novel prognostic tools that can be used for the risk stratification of adults with congenital heart disease and/or pulmonary hypertension. Measurements of right and left ventricular volumes and function, main pulmonary artery area, left atrial size and function, right ventricular pressure, pericardial effusion, and right atrial area are important imaging features that can be of prognostic value within specific diagnostic subgroups. Blood biomarkers can be measured in all patients; especially NT-proBNP should be measured at least once in all patients with moderate or complex ACHD. In patients with an elevated NT-proBNP level it is probably useful to obtain annual repeated NT-proBNP measurements and other biomarkers such as hs-TnT and RDW, while patients with a low NT-proBNP level can be reassured and may require less frequent follow-up. Finally, a simple risk calculation tool consisting of six easy to obtain clinical variables and NT-proBNP is proposed, which could be useful to discriminate high- and low-risk patients. Selection bias and information bias are inherent types of bias to these observational studies and should be acknowledged when interpreting these results. The novel biomarkers that are outlined in this thesis are a next step towards better risk stratification of patients with ACHD. This may serve to optimally inform and reassure patients, to aid clinical judgment in determining optimal monitoring and management strategies, to better utilize medical resources and save costs, and ultimately to improve the clinical outcome of these patients.

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EPILOGUE

NEDERLANDSE SAMENVATTING

Dankzij de vooruitgang van de medische zorg voor kinderen met een aangeboren hartafwijking, wordt de groep volwassenen met een aangeboren hartafwijking alsmaar groter. Deze patiënten zijn met name jongvolwassenen, die misschien moeder of vader willen worden, deelnemen aan sport en streven naar een zo normaal mogelijk leven. De dagelijkse klinische zorg voor deze patiënten is erop gericht om niet alleen de levensverwachting, maar ook de kwaliteit van leven te optimaliseren. Hierbij is het essentieel om het risico op complicaties, zoals het optreden van ritmestoornissen of hartfalen, zo nauwkeurig mogelijk te voorspellen. Het doel van dit proefschrift is het onderzoeken van bekende en nieuwe biomarkers bij volwassenen met een aangeboren hartafwijking en bij patiënten met pulmonale hypertensie. Biomarkers zijn structuren of stoffen die gemeten worden in het lichaam middels beeldvorming (zoals MRI en echocardiografie) en in het bloed, die het risico op complicaties kunnen voorspellen. **Hoofdstuk 1** is de algemene inleiding, waarin de achtergrond en indeling van dit proefschrift wordt beschreven.

Deel I – Beeldvorming

Het hart bestaat uit een rechter- en linker hartkamer, en een rechter- en linker hartboezem. De grootte en functie van de rechter hartkamer zijn sterke voorspellers voor klinische uitkomsten bij volwassenen met een aangeboren hartafwijking en bij patiënten met pulmonale hypertensie. Cardiale MRI wordt beschouwd als de gouden standaard voor deze metingen. Door de hoeveelheid spiervezelbundels ("trabekels") in de rechter hartkamer is een handmatige meting van het volume van de rechter hartkamer echter minder nauwkeurig. In **Hoofdstuk 2** wordt een nieuwe meetmethode beschreven om met deze trabekels om te gaan. Een MRI van het hart werd verricht bij tachtig patiënten (met pulmonale hypertensie, transpositie van de grote vaten met arteriële switch operatie of met atriale switch operatie, of gecorrigeerde tetralogie van Fallot) en 20 gezonde vrijwilligers. Twee methoden om rechter hartkamervolumes en -functie te meten werden vergeleken. De exclusie van trabekels van het bloedvolume met een semi-automatische methode, gebaseerd op de pixel intensiteit, bleek zeer reproduceerbaar te zijn. Tevens resulteerde dit in een substantiële verandering in rechter hartkamervolumes en -functie in vergelijking met de handmatige methode, waarbij trabekels werden geïncludeerd in het bloedvolume. Deze nieuwe methode met exclusie van trabekels lijkt daarom superieur te zijn aan de oude handmatige methode.

In **Hoofdstuk 3** wordt de associatie tussen cardiale MRI-metingen en inspanningscapaciteit beschreven bij 71 volwassenen met een arteriële switch operatie

op de kinderleeftijd. Met name de grootte van de longslagader was sterk gerelateerd aan de maximale zuurstofopname. Dit geeft aan dat een vernauwing van de longslagader waarschijnlijk een sterk beperkende factor is van de inspanningscapaciteit bij deze patiënten, en vermoedelijk ook een prognostische (voorspellende) factor. In **Hoofdstuk 4** wordt de associatie tussen linker hartboezemgrootte en -functie met klinische uitkomsten beschreven in 134 volwassenen met gecorrigeerde tetralogie van Fallot. Middels echocardiografie werden bij alle patiënten het volume, de oppervlakte, de lengte en de functie (ledigingsfractie) van de linker hartboezem gemeten. Grotere linker hartboezemvolumes, -lengte, en verminderde totale en actieve ledigingsfractie waren significant geassocieerd met klinische uitkomsten, wat betekent dat deze echometingen van prognostische waarde kunnen zijn bij deze patiënten.

Volwassenen met een gat in het tussenschot van de hartboezems (atriaal septum defect, ASD) lopen risico op het ontwikkelen van een verhoogde bloeddruk in het longvaatbed (pulmonale arteriële hypertensie, PAH), omdat er door het ASD continue te veel bloed terugstroomt naar de longen. PAH is geassocieerd met een slechte prognose. Het ASD wordt daarom meestal gesloten met een ingreep via de lies of met een hartoperatie. Het is echter niet precies duidelijk hoe vaak er sprake is van PAH bij volwassenen voor en na de ASD-sluiting. In **Hoofdstuk 5** wordt een systematisch overzicht gegeven van alle studies die de bloeddruk in het longvaatbed en/of het percentage volwassenen met PAH voor én na ASD-sluiting hebben beschreven. Vervolgens hebben we in **Hoofdstuk 6** van 198 volwassenen die een ASD-sluiting in ons eigen centrum ondergingen tussen 2000 en 2014, retrospectief alle echo's beoordeeld voor en na de ASD-sluiting. Uit het literatuuronderzoek bleek dat er een grote spreiding was in de prevalentie van PAH na ASD-sluiting: tussen de 5 en 50%. De prevalentie was met name hoog in oudere studies, studies met een klein aantal patiënten en studies waarbij de echo na ASD-sluiting miste bij relatief veel patiënten. We concludeerden in Hoofdstuk 5 daarom dat de "werkelijke" prevalentie waarschijnlijk meer richting de benedengrens van deze range zou liggen, wat inderdaad werd bevestigd in ons eigen onderzoek (5%). Het aantal patiënten met PAH en de gemiddelde drukken in het longvaatbed verminderden in alle studies na de ASD-sluiting en bij (op één na) alle patiënten in ons eigen onderzoek. Tevens hebben we onderzocht welke factoren een risico vormen voor PAH na ASD-sluiting. Hoewel in eerdere onderzoeken de leeftijd ten tijde van ASD-sluiting als belangrijke voorspeller werd gerapporteerd, was in ons onderzoek de NYHA-klasse, aanwezigheid van een longziekte (OSAS of COPD), gebruik van cardiale medicatie en een verminderde rechter hartkamerfunctie gerelateerd aan PAH na ASD-sluiting. Deze patiënten moeten dus na de ASD-sluiting extra goed in de gaten worden gehouden.

In **Hoofdstuk 7** en **Hoofdstuk 8** wordt een systematisch overzicht gegeven van alle echocardiografische en cardiale MRI-metingen, die van prognostische waarde kunnen zijn bij patiënten met PAH. In 37 artikelen werden in totaal 51 verschillende echo-metingen onderzocht. Hiervan bleken de aanwezigheid van pericardeffusie (vocht in het hartzakje), een grotere rechter hartboezem en een lagere TAPSE (maat voor rechter hartkamerfunctie) de meest onderzochte en sterkste voorspellers voor sterfte of longtransplantatie. In 8 artikelen werden 21 cardiale MRI-metingen beschreven: hiervan was een verminderde rechter hartkamer ejectiefractie (maat voor functie) de belangrijkste voorspeller voor sterfte.

Deel II – Biomarkers in het bloed

De BioCon studie is een groot project waarbij 602 volwassenen met een matige of ernstige aangeboren hartafwijking gedurende 5 jaar zijn gevolgd op de polikliniek van het Erasmus MC. Jaarlijks werden deze patiënten gecontroleerd door de cardioloog met een bloedafname, hartfilmpje en (elke 2 jaar) een echo van het hart. In **Hoofdstuk 9** wordt de associatie van vier biomarkers (MMP-2, -3, -9, en TIMP-1) met de hartfunctie en het inspanningsvermogen in een subgroep van 425 volwassenen met een aangeboren hartafwijking uit de BioCon studie onderzocht. Van deze vier biomarkers had alleen MMP-2 een zwakke associatie met enkele metingen van een inspanningstest of echo. Dit zijn echter nog zeer experimentele biomarkers. De nauwkeurigheid van de metingen en de normaalwaarden moeten nog worden onderzocht.

In **Hoofdstuk 10** worden de belangrijkste resultaten van de BioCon studie beschreven. In 595 volwassenen met een aangeboren hartafwijking hebben we de associatie van NT-proBNP, hoog-sensitief troponine-T en GDF-15 met het optreden van complicaties onderzocht. Tijdens een periode van (mediaan) 42 maanden trad er bij 165 patiënten een cardiovasculaire complicatie op (gedefinieerd als overlijden, hartfalen, ziekenhuisopname, ritmestoornis, trombo-embolie of re-interventie). NT-proBNP was zeer sterk gerelateerd aan het optreden van complicaties, onafhankelijk van meerdere klinische gegevens en echocardiografische metingen. Het risico op overlijden of hartfalen was extreem laag (~1%) bij de patiënten met een normale NT-proBNP-concentratie (ongeveer de helft van de totale groep). Het risico van patiënten met een verhoogde NT-proBNP-concentratie kon verder worden bepaald door het gecombineerd gebruik van hoog-sensitief troponine-T en GDF-15: patiënten bij wie alle drie de biomarkers verhoogd waren, hadden het hoogste risico op een complicatie. In **Hoofdstuk 11** worden de galectine-3 metingen van de BioCon studie beschreven. Galectine-3 is een mogelijke nieuwe biomarker voor patiënten met hartfalen. In ons cohort van volwassenen met een aangeboren hartafwijking bleek galectine-3 significant geassocieerd te zijn met de

hartfunctie (gemeten op echocardiografie) en met het optreden van complicaties. De toegevoegde waarde van galectine-3 bovenop een meer bekende biomarker zoals NT-proBNP bleek echter beperkt. In **Hoofdstuk 12** wordt beschreven dat RDW, een meting die standaard wordt verricht bij het bepalen van het hemoglobinegehalte, mogelijk wel van toegevoegde waarde kan zijn bij het voorspellen van cardiovasculaire complicaties, onafhankelijk van leeftijd, geslacht, andere klinische risicofactoren, CRP en NT-proBNP. De C-index (maat voor hoe goed een onderscheid gemaakt kan worden tussen hoog- en laag-risico-patiënten) van het model mét RDW was net iets hoger dan het model zonder (0.74, 95% CI 0.70–0.78 vergeleken met 0.73, 95% CI 0.69–0.78). Uit de analyse van 2449 herhaalde RDW-metingen bleek dat er geen duidelijke stijging was aan te tonen in de RDW-waarde vlak voor het optreden van een complicatie.

In **Hoofdstuk 13** hebben we 2424 herhaalde jaarlijkse NT-proBNP-metingen geanalyseerd, die werden verzameld gedurende 4.4 jaar in de 602 patiënten van de BioCon studie. Hierbij hebben we gebruik gemaakt van gevorderde statistische methoden (liner mixed effect models, joint models). De NT-proBNP-concentratie steeg voorafgaand aan een complicatie, met name bij overlijden of hartfalen. Patiënten zonder een complicatie hadden juist stabiele en lage NT-proBNP concentraties. Binnen de hoog-risicogroep met een verhoogd baseline NT-proBNP (> 14 pmol/L, n = 315, 53%) konden jaarlijks herhaalde NT-proBNP-metingen aanvullende prognostische informatie opleveren, omdat herhaalde metingen (ook na correctie voor de baseline waarde) nog steeds significant geassocieerd waren met het optreden van complicaties.

In **Hoofdstuk 14** worden de eerste resultaten van de BioPulse studie beschreven. De BioPulse studie is een project waarin tot nu toe 106 patiënten met pulmonale hypertensie (een verhoogde druk in het longvaatbed) zijn geïncludeerd. Deze patiënten worden elke 6 maanden gecontroleerd door zowel de cardioloog als de longarts, met een bloedafname, hartfilmpje, longfunctietest, inspanningstest en (jaarlijks) een echo van het hart. Gedurende 24 maanden trad er bij 29 patiënten (27%) een complicatie op (overlijden of longtransplantatie). Verschillende biomarkers op baseline (NT-proBNP, hoog-sensitief troponine-T, CRP, galectine-3, RDW en eGFR) waren significant geassocieerd met het optreden van een complicatie. Hoe meer biomarkers verhoogd waren, hoe hoger het risico was op een complicatie. Deze gegevens pleiten dus voor een aanpak waarbij meerdere biomarkers tegelijk worden gebruikt. Alle patiënten met normale waarden van alle biomarkers waren na 40 maanden nog in leven zonder longtransplantatie.

Deel III – Risico voorspellen

Hoofdstuk 15 geeft een literatuuroverzicht van alle gerapporteerde factoren die mogelijk kunnen bijdragen aan het voorspellen van hartfalen en andere complicaties bij patiënten met een aangeboren hartafwijking in het algemeen of binnen subgroepen met een specifieke aangeboren hartafwijking. Het risico op complicaties is voor een groot deel afhankelijk van de precieze anatomie van de onderliggende hartafwijking en de soort hartoperatie of ingreep die is gedaan. Verder kunnen componenten van de anamnese, lichamelijk onderzoek en aanvullende diagnostische testen (inclusief hartfilmpje, echografie of MRI van het hart, inspanningstest en bloedbiomarkers) informatie opleveren over het risico dat een patiënt heeft op een complicatie.

Tot slot hebben we in **Hoofdstuk 16** een model ontworpen en gevalideerd, waarmee het 4-jaars risico op dood, hartfalen of een ritmestoornis bij volwassenen met een matig tot ernstige aangeboren hartafwijking kan worden voorspeld. Dit model werd ontwikkeld in de Biocon studie (n = 602) en extern gevalideerd in een database van 402 patiënten met een aangeboren hartafwijking uit een tertiair ziekenhuis in Praag, Tsjechië. Het uiteindelijke model bestond uit zes direct beschikbare klinische variabelen (leeftijd, congenitale diagnose, NYHA-klasse, cardiaal medicatiegebruik, re-interventies, BMI) en NT-proBNP. De extern gevalideerde C-index was 0.78 (95% CI 0.72–0.83), wat betekent dat het model een goed onderscheid kan maken tussen hoog- en laag-risicopatiënten. Wel maakte het model een systematische overschatting van het risico in de Tsjechische groep: de kalibratie kan derhalve wellicht nog verder verbeterd worden in de toekomst. Hoe dan ook zou dit model klinisch bruikbaar kunnen zijn bij het maken van een onderscheid tussen patiënten met een hoog of laag risico op complicaties.

CONCLUSIE

Het doel van dit proefschrift was het onderzoeken van bekende en nieuwe biomarkers die gebruikt kunnen worden bij het voorspellen van risico's bij volwassen met een aangeboren hartafwijking en bij patiënten met pulmonale hypertensie. Verschillende MRI en echo-metingen worden beschreven die van prognostische waarde kunnen zijn, zoals rechter hartkamervolumes en -functie, longslagader grootte, linker hartboezemgrootte en -functie, verhoogde drukken in het longvaatbed, pericardeffusie en rechter hartboezemgrootte. Vanwege de verschillen in de anatomie van het hart moet de waarde van deze metingen per diagnose apart worden onderzocht en beoordeeld.
NT-proBNP was de belangrijkste voorspellende biomarker in het bloed en zou bij elke volwassene met een matige of ernstige aangeboren hartafwijking op zijn minst éénmaal gemeten moeten worden. Bij patiënten met een verhoogde NT-proBNP-concentratie is

het waarschijnlijk zinvol om deze meting jaarlijks te herhalen en ook andere biomarkers te meten, zoals hoog-sensitief troponine-T en RDW. Patiënten met een normale NT-proBNP-concentratie kunnen worden gerustgesteld en hoeven waarschijnlijk minder vaak gecontroleerd te worden door de cardioloog. Tot slot wordt een eenvoudige rekentool gepresenteerd, die bruikbaar zou kunnen zijn om onderscheid te maken tussen hoog- en laagrisicopatiënten.

De biomarkers die in dit proefschrift worden beschreven kunnen bruikbaar zijn voor het informeren en geruststellen van patiënten, bijdragen aan het klinische oordeel van de arts bij het bepalen van de optimale behandeling en kunnen mogelijk kosten besparen doordat medische zorg beter kan worden ingezet waar dat nodig is, met als uiteindelijk doel de zorg en klinische uitkomsten van volwassenen met een aangeboren hartafwijking verder te verbeteren.

LIST OF PUBLICATIONS

1. Zwijnenburg RD, **Baggen VJ**, Geenen LW, Voigt KR, Roos-Hesselink JW, van den Bosch AE. The prevalence of pulmonary arterial hypertension before and after atrial septal defect closure at adult age: a systematic review. Am Heart J. 2018;201:63-71.

2. **Baggen VJ**, Baart SJ, van den Bosch AE, Eindhoven JA, Witsenburg W, Cuypers JA, Roos-Hesselink JW, Boersma E. Prognostic value of serial N-terminal pro-B-type natriuretic peptide measurements in adults with congenital heart disease. J Am Heart Assoc. 2018;7. pii: e008349.

3. **Baggen VJ**, van den Bosch AE, van Kimmenade RR, Eindhoven JA, Witsenburg M, Cuypers JA, Leebeek FW, Boersma E, Roos-Hesselink JW. Red cell distribution width in adults with congenital heart disease: a worldwide available and low-cost predictor of cardiovascular events. Int J Cardiol. 2018;260:60-65.

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5. van den Hoven AT, Mc-Ghie JS, Chelu RG, Duijnhouwer AL, **Baggen VJ**, Coenen A, Vletter WB, Dijkshoorn ML, van den Bosch AE, Roos-Hesselink JW. Transthoracic 3D echocardiographic left heart chamber quantification in patients with bicuspid aortic valve disease. Int J Cardiovasc Imaging 2017;33:1895-1903.

6. **Baggen VJ**, van den Bosch AE, Roos-Hesselink JW. Reply: Letter to the editor: Prognostic value of left atrial size and function in adults with tetralogy of Fallot. Int J Cardiol 2017;242:37.

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9. **Baggen VJ**, Driessen MM, Post MC, van Dijk AP, Roos-Hesselink JW, van den Bosch AE, Takkenberg JJ, Sieswerda GT. Echocardiographic findings associated with mortality or transplant in patients with pulmonary arterial hypertension: a systematic review and meta-analysis. Neth Heart J 2016;24:374-389.

10. **Baggen VJ**, Eindhoven JA, van den Bosch AE, Witsenburg M, Cuypers JA, Langstraat JS, Boersma E, Roos-Hesselink JW. Matrix metalloproteinases as candidate biomarkers in adults with congenital heart disease. Biomarkers 2016;21:466-473.

11. **Baggen VJ**, Leiner T, Post MC, van Dijk AP, Roos-Hesselink JW, Boersma E, Habets J, Sieswerda GT. Cardiac magnetic resonance findings predicting mortality in patients with pulmonary arterial hypertension: a systematic review and meta-analysis. Eur Radiol 2016;26:3771-3780.

12. Eindhoven JA, van den Bosch AE, Oemrawsingh RM, **Baggen VJ**, Kardys I, Cuypers JA, Witsenburg M, van Schaik RH, Roos-Hesselink JW, Boersma E. Release of growth-differentiation factor 15 and associations with cardiac function in adult patients with congenital heart disease. Int J Cardiol 2016;202:246-251.

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14. **Baggen VJ**, Chung K, Koole K, Sarneel MH, Rutten FH, Hajer GR. Association of varicosities and concomitant deep venous thrombosis in patients with superficial venous thrombosis, a systematic review. Eur J Gen Pract 2015;21:70-76.

15. **Baggen VJ**,* Driessen MM,* Freling HG, Pieper PG, van Dijk AP, Doevendans PA, Snijder RJ, Post MC, Meijboom FJ, Sieswerda GT, Leiner T, Willems TP. Pressure overloaded right ventricles: a multicenter study on the importance of trabeculae in RV function measured by CMR. Int J Cardiovasc Imaging 2014;30:599-608. *Equal contributions

Submitted manuscripts

16. Zwijnenburg RD, **Baggen VJ**, Witsenburg M, Boersma E, Roos-Hesselink JW, van den Bosch AE. Risk factors for pulmonary hypertension in adults after atrial septal defect closure. Submitted.

17. **Baggen VJ**,* Geenen LW,* Koudstaal T, Boomars KA, Eindhoven JA, Boersma E, Roos- Hesselink JW, van den Bosch AE. The prognostic value of various biomarkers in adult patients with pulmonary hypertension: a multi-biomarker approach. Submitted. *Equal contributions

18. **Baggen VJ**, Venema E, Živná R, van den Bosch AE, Eindhoven JA, Witsenburg M, Cuypers JA, Boersma E, Lingsma H, Popelová JR,* Roos-Hesselink JW.* Development and validation of a risk prediction model in patients with adult congenital heart disease. Submitted. *Equal contributions

Book chapters

Baggen VJ, Geenen LW, Roos-Hesselink JW. Risk stratification and prognosis. In: Heart failure in Adult Congenital Heart Disease. Springer; Swan L, Frogoudaki A (Editors): 2018.

Baggen VJ, Connelly MS, Roos-Hesselink JW. Truncus arteriosus. In: Diagnosis and Management of Adult Congenital Heart Disease. Third edition. Elsevier; Gatzoulis MA, Webb GD, Daubeney PE (Editors): 2018. Page 421-428.

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student	Vivan Baggen	**PhD period**	01 Aug 2014 – 01 Sept 2017
Erasmus MC Department	Cardiology	**Promotor(s)**	
Research School	COEUR	Prof. dr. J.W. Roos-Hesselink	
		Prof. dr. ir. E. Boersma	
		Copromotor: dr. A.E. van den Bosch	

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills		
- Research Integrity	01-2015	0.3
- e-BROK course	09-2015	1.5
Research skills		
- Master in Health Sciences, specialisation clinical epidemiology, NIHES	2014-2017	Total: 70
· Biostatistical Methods I: Basic Principles (CC02)	09-2014	5.7
· Courses for the Quantitative Researcher (SC17)	11-2014	1.4
· Repeated Measurements (CE08)	01-2015	1.4
· Bayesian statistics (CE09)	04-2015	1.4
· Missing values in clinical research (EP16)	05-2015	1.4
· Erasmus Summar Programme 2015	08-2015	4.2
· Study Design (CC01)	09-2015	4.3
· Methodologic Topics in Epidemiologic Research (EP02)	11-2015	1.4
· Advanced analysis of prognosis studies (EWP13)	03-2016	0.9
· Erasmus Summer Programme 2016 (including courses Meta-analysis and Joint Models)	08-2016	1.4
· Clinical Epidemiology (CE02)	10-2016	5.7
· Biostatistical methods II (EP03)	11-2016	4.3

	Year	Workload (Hours/ECTS)
In-depth courses (e.g. Research school, Medical Training)		
- COEUR course 'Vascular clinical epidemiology'	12-2014	1.5
- COEUR course 'Congenital heart disease'	06-2015	1.5
- COEUR symposium 'Right ventricular failure'	04-2016	0.2
Presentations		
- Moderated poster presentation ESC congress Barcelona	08-2014	0.6
- Staflunch 'CMR functie analyse'	10-2014	0.1
- Oral presentation NVVC voorjaarscongres	04-2015	0.6
- Oral presentation AEPC congress Prague	05-2015	0.6
- Journal Club 'Modified Valsalva manoeuvre'	08-2015	0.1
- Moderated poster presentation EuroEcho Sevilla	12-2015	0.6
- Moderated poster presentation PAH symposium Lund	12-2015	0.6
- Oral presentation NVVC voorjaarscongres	03-2016	0.6
- 2x poster presentation ESC congress Rome	08-2016	0.6
- Oral presentation NVVC najaarscongres	11-2016	0.6
- Oral presentation AHA symposium New Orleans	11-2016	0.6
- Oral presentation NVVC voorjaarscongres	04-2017	0.6
- Poster presentation EuroGUCH Lausanne	05-2017	0.3
- 2x poster presentation ESC congress Barcelona	08-2017	0.6
- Rapid fire abstract ESC congress Munchen	08-2018	0.6
International conferences		
- ESC congress Barcelona (5 days)	08-2014	1.5
- PAH symposium GSK Amsterdam (1.5 days)	12-2014	0.4
- REPAIR investigator meeting Vienna (2 days)	01-2015	0.6
- Wintermeeting Davos 2015 (4 days)	03-2015	1.2
- AEPC congress Prague (4 days)	05-2015	1.2
- EuroEcho Sevilla (4 days)	12-2015	1.2
- PAH symposium GSK Lund (1.5 days)	12-2015	0.4
- Wintermeeting Davos 2016 (4 days)	03-2016	1.2
- ESC congress Rome (5 days)	08-2016	1.5
- AHA symposium New Orleans (5 days)	11-2016	1.5
- Wintermeeting Davos 2017 (4 days)	03-2017	1.2
- EuroGUCH congress Lausanne (2 days)	05-2017	0.6
- ESC congress Barcelona (5 days)	08-2017	1.5
- ESC congress Munchen (5 days)	08-2018	1.5

	Year	Workload (Hours/ECTS)
Seminars and workshops		
- OpenClinica course (1 day)	08-2014	0.3
- Junior kamerdag Gouda (1 day)	10-2014	0.3
- NVVC najaarscongres Papendal (1 day)	10-2014	0.3
- Workshop on Photoshop and Illustrator CS6 (1 day)	01-2015	0.3
- NVVC voorjaarscongres Noordwijkerhout (2 days)	04-2015	0.6
- Junior kamerdag Den Haag (1 day)	10-2015	0.3
- NVVC najaarscongres Papendal (1 day)	11-2015	0.3
- Basiscursus Congenitale Echocardiografie (1 day)	01-2016	0.3
- NVVC voorjaarscongres Noordwijkerhout (1 day)	03-2016	0.3
- Junior kamerdag Soestduinen (1 day)	10-2016	0.3
- NVVC najaarscongres Papendal (1 day)	11-2016	0.3
- NVVC voorjaarscongres Noordwijkerhout (1 day)	04-2017	0.3

2. Teaching activities

	Year	Workload (Hours/ECTS)
Lecturing		
- ECG education, minor CHD (5 hours)	11-2015	0.2
- Prevalence of CHD, minor CHD (5 hours)	09-2016	0.2
- Congenitale hartafwijkingen, onderwijs Circulatie 16 (5 hours)	11-2016	0.2
Supervising Master's theses		
- Prognostic value of LA volume in TOF (AR, 3 months)	03-2015	0.6
- PAH after ASD closure (RZ, 12 months)	01-2015	2.4
- Biomarkers in pulmonary hypertension (LG, 6 months)	01-2017	1.2
Other		
- Supervision systematic review, 2nd year medicine (4 weeks)	01-2015	0.1
- Supervision of two students in echocardiographic analysis (AR en CR), 3 and 6 months for 2 days/week	02-2015	0.5
- Supervision minor CHD, 3rd year medicine (4 weeks)	11-2015	0.1
- Supervision systematic review, 2nd year medicine (4 weeks)	01-2016	0.1
- Supervision minor CHD, 3rd year medicine (4 weeks)	10-2016	0.1

ABOUT THE AUTHOR

Vivan Baggen was born in Eindhoven, the Netherlands, on the 18th of August, 1989. After graduating summa cum laude from secondary school in 2007 (Atheneum, Pleincollege Eckart, Eindhoven), she commenced medical school at the University of Utrecht. During her medical study, she worked as a cardiological technician, post processed cardiac magnetic resonance imaging datasets, participated in clinical research at the department of Cardiology of the UMC Utrecht, and spent periods abroad for clinical internships in Malawi, Indonesia and Surinam. She obtained the degree of Bachelor of Medicine cum laude in 2010 and the degree of Medical Doctor cum laude in 2014.

In August 2014, she started her PhD project at the department of Cardiology of the Erasmus MC (promotors: prof. dr. Jolien W. Roos-Hesselink and prof. dr. Eric Boersma). She received a personal research grant of the Dutch Heart Foundation (Dekker grant: physician before specialty training). During this period, she obtained the Master of Health Sciences degree, specialization Clinical Epidemiology (NIHES, Erasmus University, Rotterdam), was engaged in supervising MSc Clinical Research students, and was a committee member of the Juniorkamerdag (Landelijke Assistentendag Cardiologie), an annual symposium for investigators and residents in the field of cardiology in the Netherlands.

From January 2018 onwards she is working as a resident (ANIOS) at the department of Internal Medicine at the Franciscus Gasthuis & Vlietland. Besides her work, she enjoys travelling and sports, including field hockey and skiing.

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