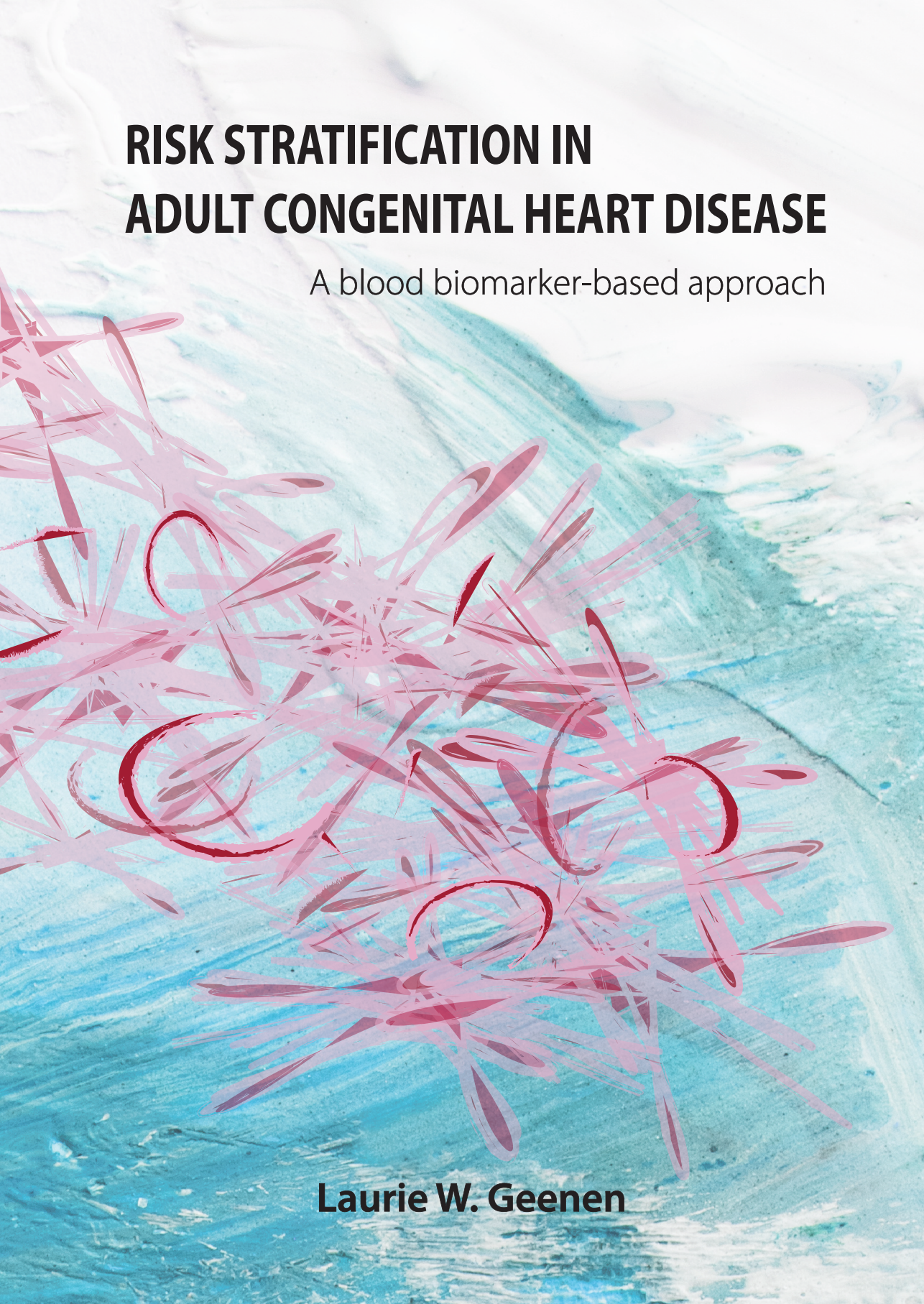
RISK STRATIFICATION IN ADULT CONGENITAL HEART DISEASE

A blood biomarker-based approach



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A blood biomarker-based approach

Risicostratificatie bij volwassenen met een aangeboren hartafwijking

Een benadering gebaseerd op bloedbiomarkers

Proefschrift

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Contents

CHAPTER 1

General introduction and outline of thesis

Adult Congenital Heart Disease

Congenital heart disease is defined as the presence of any structural abnormality present at birth that involves the heart and/or great vessels, and it is the most common type of congenital anomaly with an estimated prevalence of 8.2 per 1000 living births in Europe[1]. Extrapolating this to the Dutch population, this comes down to approximately four new babies with CHD born each day in the Netherlands[2]. CHD can vary from very mild defects that may remain unnoticed and needing no intervention, to very severe defects requiring immediate surgical intervention within the first weeks of life. Nowadays, nearly 90% of the children with CHD survives into adulthood[3], which has led to an increasing number of adult patients with CHD[4].

A historical perspective

Various aspects, to a greater or lesser extent, have contributed to the increasing prevalence of patients with CHD over the last decades[5]. The introduction of the heart lung-machine in 1953 has been of paramount importance for the development of surgical repair of CHD and has resulted in initially lethal circumstances turning into palliated chronic diseases. Improved diagnostic tools such as echocardiography invented in 1953, enabled early detection of CHD as well as detection of mild CHD such as atrial septal defects. Similarly, intra-uterine detection of particularly complex CHD has contributed to better anticipation in post-natal care, improving survival and outcomes[6]. Finally, novel imaging modalities, catheter-based interventions, improved surgical techniques, better post-operative care, and medical therapies over the last century, has further positively affected the survival of these patients.

Challenges in the current era

This reciprocity of numerous medical advances, has resulted in an adult population of patients with CHD that currently outnumbers the children with CHD in Europe[7]. Notwithstanding the great advances in life expectancy, the majority of adult congenital heart disease (ACHD) patients are not cured but rather palliated, and have a lifelong burden of complications[8]. Mild types of ACHD, such as atrial septal defects, have a life expectancy almost comparable to the general population[9], however; the majority of ACHD patients are afflicted by particularly arrhythmias, heart failure, re-interventions, re-operations and sudden cardiac death[10-12]. ACHD patients therefore require permanent specialized care, and close monitoring using routine clinical assessments. The frequency of these assessments is largely based on the complexity of the CHD, and mostly consists of physical examination, electrocardiography, echocardiography, cardiopulmonary exercise testing and imaging techniques. [13, 14]

The outstanding successes in CHD have given rise to new challenges that currently need to be faced in the care for ACHD patients and much attention is now given to the optimization and regulation of special ACHD programs and centers[15]. Besides the increase in the absolute numbers of ACHD patients, ACHD patients nowadays more often have disease of great complexity[4] and survival into advanced age leads to revelation of acquired cardiovascular

disease[16], such as coronary artery disease[17]. As an overall consequence, the healthcare utilization of ACHD is increasing despite improved efficiency in care[18]. Approximately one out of four ACHD patients aged 40 years and older in the Netherlands, is admitted at least once during a 5-year period, and this frequency increases with age[19]. The increased healthcare utilization is accompanied by rising healthcare costs over the past years[20, 21], challenging the ACHD care within countries[22]. Classification of patients into high or low-risk subgroups, preferably using non-invasive techniques to monitor patients over time, are therefore essential for adequate patient management. Improvement in risk stratification of these patients can help to foresee and subsequently anticipate more rapidly to future complications that may occur. For instance, identification of low-risk patients can help to reduce the frequency of follow-up visits and can provide reassurance of their medical condition. Also, earlier recognition of worsening heart failure or requirement of valve interventions, may help to prevent unnecessary hospital visits.

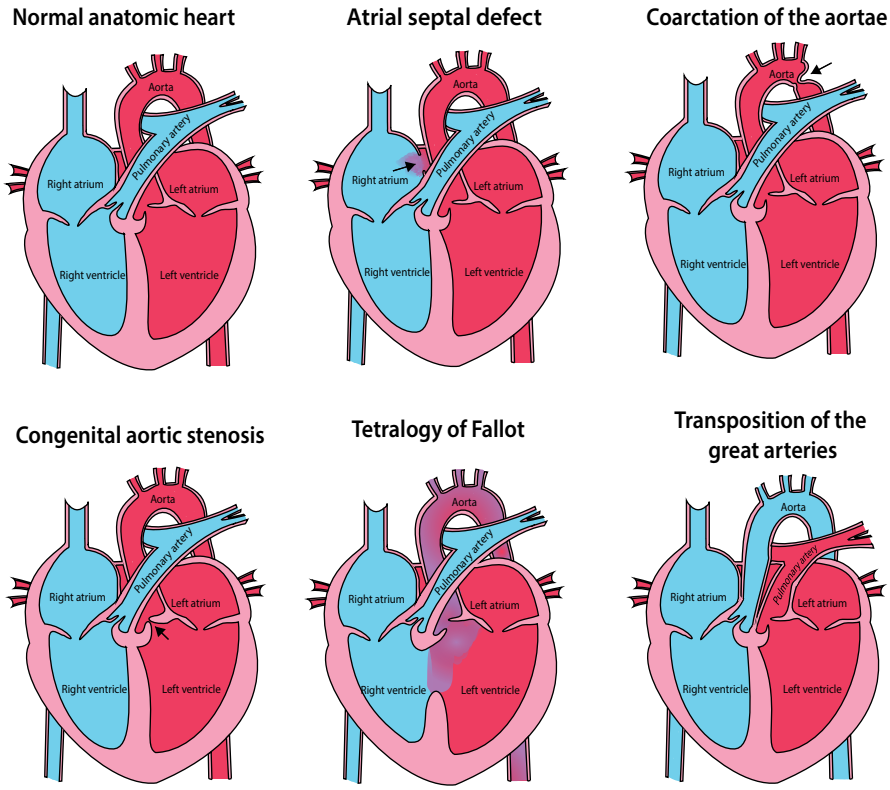

Figure 1. Overview of the main congenital heart defects investigated in this thesis.

Pulmonary Hypertension

Pulmonary hypertension (PH), literally meaning 'high blood pressure within the lungs', is a pathophysiological disorder related to multiple diseases. While the lung vasculature is the main organ affected in patients with PH, the major clinical consequence mostly considers the heart. PH is defined by an elevated mean pulmonary artery pressure of ≥25 mmHg assessed by right heart catheterization, whether or not accompanied by an elevated capillary wedge pressure[23]. PH can develop at any age from early childhood to adulthood, and the prevalence of PH is highly variable among different types of PH, as well as its associated survival prospects[24, 25]. The nonspecific clinical picture together with a relative low prevalence of the disease, often results in a substantial delay between the onset of the disease and the diagnosis[26, 27].

Etiology and classification of pulmonary hypertension

The World Health Organization (WHO) classification of PH distinguishes five groups which is mainly based on the underlying etiology[28] (Figure 2). Pulmonary arterial hypertension (PAH) is one distinctive subgroup, and is a chronic disorder of the pulmonary vasculature leading to an increased vascular resistance in absence of other causes of PH, such as chronic lung embolisms. PAH can be idiopathic, heritable, or associated with underlying conditions. PAH is a rare disease, with an estimated prevalence of 52 cases per million population, and is most prevalently seen in patients with connective tissue disease[29]. PAH can also develop as rare complication in patients with CHD, mainly in heart defects with persistent systemic-to-pulmonary shunts, causing a chronic volume overload affecting the pulmonary vasculature and leading to an increased pulmonary vascular resistance. PAH due to CHD is most frequently seen in the case of atrial- or ventricular septal defects, with an overall estimated prevalence of 3.2% in the ACHD population[30]. Sometimes the pulmonary pressure overrules the systemic pressure leading to an alteration of the shunt direction causing chronic cyanosis, a clinical condition known as Eisenmenger syndrome, which is associated with a considerably reduced life expectancy[31]. Other types of pre-capillary PH concern chronic thromboembolic pulmonary hypertension (CTEPH) and PH due to lung diseases, such as chronic obstructive pulmonary disease or other interstitial lung diseases. Elevated pulmonary artery pressure as a consequence of left-sided heart disease is the most common type of post-capillary PH, primarily seen in the elderly with systolic or diastolic cardiac dysfunction. Furthermore, WHO group 5 comprises of PH due to miscellaneous diseases and/or an unknown pathophysiology. Figure 2 outlines the PH classification in more detail.

The Achilles heel: right ventricular failure

Right ventricular function is the key determinant for outcomes and symptoms in patients with pre-capillary PH [32]. The anatomy of the right ventricle, unlike the left ventricle, is designed for a high-volume low-pressure circulation[33] and supplies the pulmonary circuit of blood flow. Increased pulmonary artery pressures lead to an increased right ventricular afterload, requiring

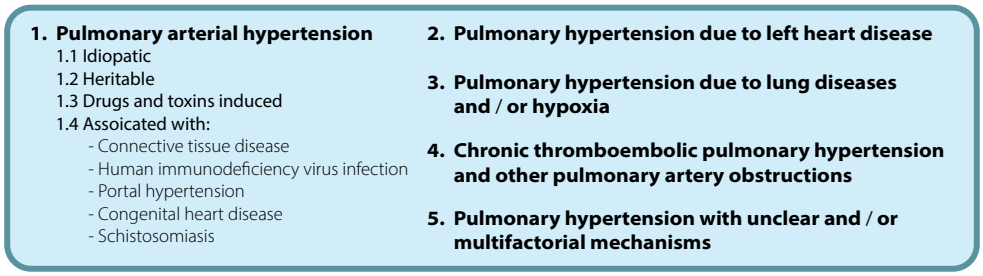

Figure 2. Classification of pulmonary hypertension

adaptation of the right ventricle to the high pressure circulation. Right ventricular adaptation in response to the severe pressure overload in patients with PH, is expressed by ventricular hypotrophy at first, followed by right ventricular dilatation and dysfunction at a later stage[34].

Therapies specifically aiming at the failure of the right ventricle are lacking, since there is no clear understanding of right ventricular failure in general. Current treatment therapies for PAH are mainly focused on lowering of the pulmonary vascular resistance by drugs that mainly act on three pathways involved in the remodeling of the pulmonary vasculature; the endothelin, nitric oxide and prostacyclin pathway[35]. Despite discovery of new PH medications in the last decade, PH remains a chronic disease with still an infaust and poor prognosis[36]. Moreover, estimation of prognosis and adequate risk stratification in the PH remains challenging, especially since there is a high heterogeneity among patients with PH.

Biomarkers and prognostication

The term biomarker originates from 'biological marker', and is often used to refer to an objective, quantifiable substance, structure, or process that is measurable in the body or its products. A biomarker is regarded an indicator of a biologic or disease process that yields predictive, diagnostic and/or prognostic value. In this respect, a biomarker can imply a wide range of measurement techniques and tools, and is a widely-used term in medicine and clinical research.[37] Reproducibility, objectiveness, invasiveness, and costs, can therefore also greatly vary among the type of biomarker used, and therefore its clinical applicability.

Among all types of biomarkers, blood biomarkers have emerged as a promising and favoured modality, due to its advantageous characteristics; substances in blood are relatively easy to obtain and provide an objective measurement. Moreover, the worldwide construction of human biobanks, allow for identification of new blood biomarkers in large cohorts of patients as well as in the general population[38]. In some fields of cardiology, blood biomarkers already serve as diagnostic and prognostic tools in clinical patient care, such as the use of troponin T for the diagnosis of acute coronary syndrome[39]. The best-known heart failure-related biomarker is N-terminal pro B-type natriuretic peptide (NT-proBNP). Current guidelines for the management

of PH, recommend the use of NT-proBNP for risk stratification in PAH patients[23], whereas in the management of ACHD no blood biomarker has yet been recommended by the guidelines[13, 14], although some recent studies have proven their prognostic utility[40-44]. Besides their usefulness in risk stratification, blood biomarkers can help to gain insight in the pathophysiology of diseases, as they are mostly linked to certain molecular or cellular pathways. Therefore, more research is needed to assess how blood biomarkers can be used in the follow-up of patients with ACHD, as well as patients with PH.

Risk stratification

Risk stratification can be referred to as the identification and classification of patients according to their pertaining risk of a certain outcome of interest. Adequately stratifying patients according to their risk of future events, can help to adjust timing of re-interventions or to optimize therapeutic strategies. From the perspective of the patient, risk stratification can help to better inform patients about their prognosis and to manage their expectations.

Apart from blood biomarkers, information retrieved from patient's medical records and features, such as symptoms[45], physical examination, ECG[46], echocardiography, exercise testing[47, 48], cardiac magnetic resonance imaging or cardiac computed tomography, are valuable measurements and tools to stratify ACHD patients according to their risk of adverse cardiac events or survival. Despite all effort made during the past decades to identify prognostic factors, adequate identification of patients at risk of heart failure, or optimal timing of for instance valve replacement, remain mainly based on expert opinion. Therefore there is a clear need for evidence-based modalities that can support risk stratification in patients.

In PH, the guideline recommends to stratify patients with PAH according to their expected 1-year risk of mortality using a combination of determinants; clinical signs and symptoms of heart failure including NYHA functional class, 6-minute walking distance, cardiopulmonary exercise parameters, NT-proBNP level, imaging features and hemodynamic measures.[23] Also, a risk score calculator has been developed to estimate survival at 12 months, which even showed a better discrimination than the risk stratification proposed by the guidelines[49]. For other types of PH, such risk rules, have not been established yet and it therefore remains the question what factors can enhance risk stratification in these patient groups, and whether these may differ from other types of PH. This is important since earlier detection of worsening PH may help to guide optimal therapy in these patients.

Outline and objectives

Outline of this thesis

The chapters in this thesis generally aiming to give answer to the question whether blood biomarkers can enhance risk stratification in ACHD and adults with PH, are mainly based on

data of two prospective observational cohort studies carried out by the Erasmus University Medical Center.

Part I describes the results that are part of the BioCon study; a single center cohort study consisting of 602 patients with moderately to severely complex ACHD patients who were enrolled during a visit the outpatient clinic of the Erasmus Medical Center between 2011-2013. Prior to creation of this thesis, Jannet A. Eindhoven and Vivan J. M. Baggen thoroughly described the prognostic value of various blood biomarkers found in this cohort study: NT-proBNP[40, 50], high-sensitive troponin-T[51], red cell distribution width[44], galectin-3[43] and growth differentiation factor-15[52, 53] in their thesis. This thesis outlines further advances made within the spectrum of blood biomarkers to enhance risk stratification in ACHD. In particular, specific congenital diagnoses are emphasized and the prognostic value of serial repeated measurements is assessed. Moreover, this thesis aimed to establish a clinically useful risk prediction model in ACHD, for which data from an external prospective cohort of ACHD patients was used to externally validate the model. This was based on a risk prediction model developed by Baggen et al. in 2018[54].

A schematic overview of the design of the BioCon study is given in Figure 3. All enrolled patients underwent yearly venous blood sampling, which allowed us to study the prognostic value of single baseline biomarker measurement as well as the temporal evolution of biomarker trajectories over time. We hypothesized that biomarkers involved in the cardiac deterioration of ACHD patients would increase in anticipation to the occurrence of a cardiovascular event, while clinically stable patients were assumed to have constant levels over time. During this thesis, blood stored in the biobank was used to measure baseline soluble suppression of

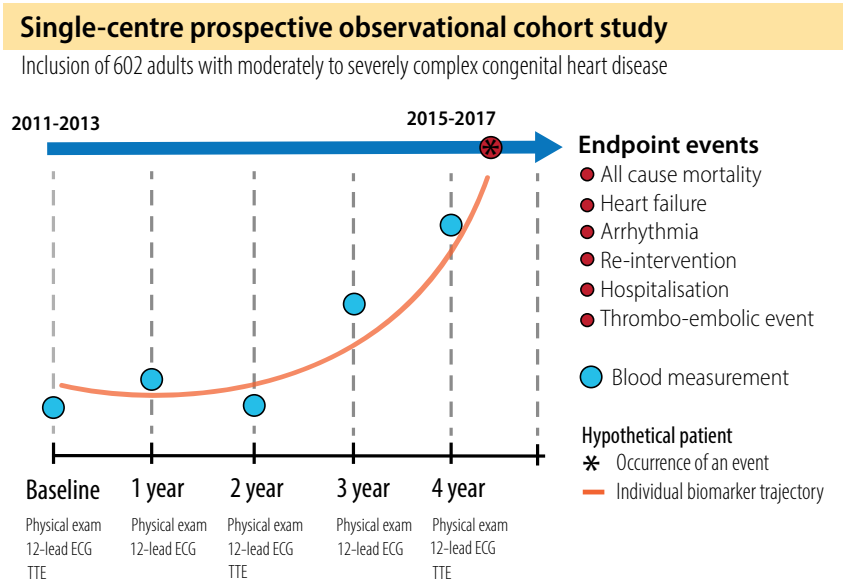

Figure 3. Overview of the design of the BioCon study

tumorigenicity (sST2) and follow-up measurements of high-sensitive c-reactive protein and high-sensitive troponin T.
Part II is mostly based on the BioPulse study, an ongoing prospective observational cohort study enrolling adult treatment naive patients with PH during the diagnostic right heart catheterization. This study consists of a mixed group of pulmonary hypertension patients; only patients with PH due to left heart disease were excluded. The first blood draw is taken during the diagnostic right heart catheterization and thereafter patients return to the outpatient clinic every half-year during which blood sampling is repeated. The results of the first 106 patients enrolled in this study between 2012-2016 are described in this thesis. Baseline biomarkers were determined and related to clinical outcomes and survival in these patients.

Objectives

The main objective of this thesis is to assess the prognostic value and the potential utility of blood biomarkers for enhancement of risk stratification in adult congenital heart disease and adults with pulmonary hypertension.
The following specific objectives are addressed in this thesis;

PART I Blood biomarkers in adult congenital heart disease

- To investigate of the prognostic value of soluble ST2 as novel biomarker in adult congenital heart disease (Chapter 2)
- To observe the temporal evolutions of biomarkers over time and to investigate its relation with prognosis in adults with congenital heart disease (Chapters 3 and 4)
- To investigate the influence of percutaneous atrial septal defect closure on biomarker levels (Chapter 5)
- Determine the prognostic value of certain biomarkers in specific adult congenital heart disease diagnoses (Chapters 6 and 7)

PART II Blood biomarkers in adults with pulmonary hypertension

- To demonstrate the prognostic value of several novel and established biomarkers in adults with pulmonary hypertension (Chapters 8-10)
- To provide an estimate of the prevalence of pulmonary arterial hypertension before and after atrial septal defect closure at adult age (Chapter 11)

PART III Risk stratification and risk prediction

- To develop a clinical useful risk prediction tool for adults with congenital heart disease (Chapter 12)
- To provide an overview of modalities useful for risk stratification of heart failure in adults with congenital heart disease (Chapter 13)

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BLOOD BIOMARKERS IN ADULT CONGENITAL HEART DISEASE

CHAPTER 2

Prognostic value of soluble ST2 in adults with congenital heart disease

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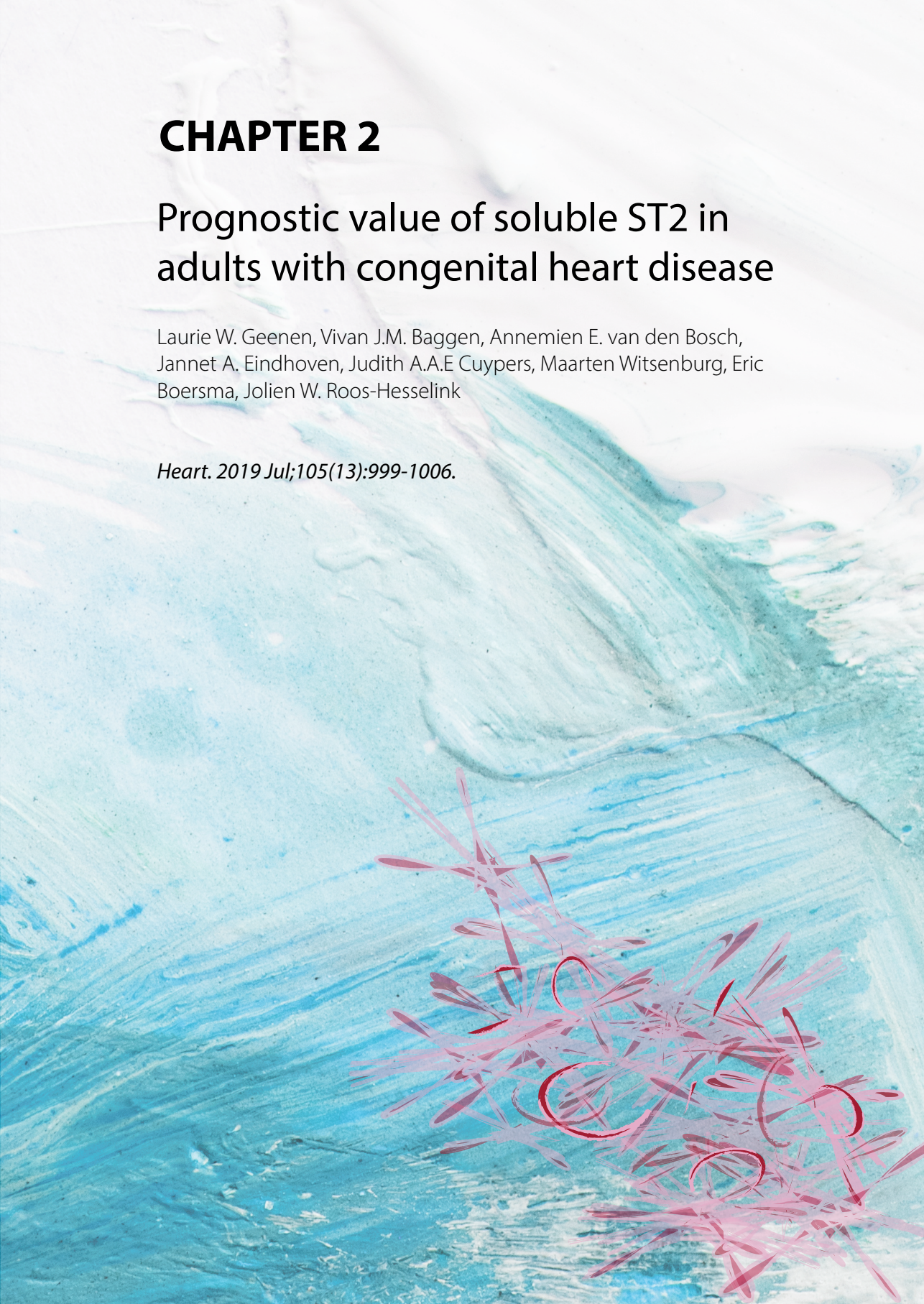

Abstract

Objective: Soluble suppression of tumorigenicity-2 (sST2) is upregulated as response to myocardial stress and may be a potential biomarker for risk stratification in patients with adult congenital heart disease (ACHD). This study aimed to investigate the release of sST2 and its association with cardiovascular events in ACHD.

Methods: In this prospective cohort study, 602 consecutive patients with ACHD visiting the outpatient clinic were included (2011-2013). The association between sST2 and a primary composite endpoint of all-cause mortality, heart failure, hospitalization, arrhythmia, thromboembolic events or cardiac interventions was investigated using multivariable Cox regression.

Results: sST2 was measured in 590 (98%) patients (median age 33 [25-41] years, 42% women). After a median follow-up of 5.8 [IQR 5.1-6.2] years, 225 (38.5%) reached the primary endpoint. sST2 was significantly associated with the primary endpoint when adjusted for age, sex, creatinine and NT-proBNP (HR per twofold higher sST2 1.28, 95% CI 1.03-1.58, p=0.025). This association negated when adjusted for clinical variables and NT-proBNP (HR per twofold higher sST2 1.19, 95% CI 0.96-1.48, p=0.106). Stratified analysis in complex ACHD, did show a significant association between sST2 and the primary endpoint when adjusted for clinical variables and NT-proBNP (HR per twofold higher sST2 1.31, 95% CI 1.01-1.69, p=0.043). Sex-specific analysis showed an association between sST2 and the primary endpoint in women (HR per twofold higher sST2 1.80, 95% CI 1.30-2.49, p<0.001) but not in men (HR per twofold higher sST2 1.19, 95% CI 0.90-1.56, p=0.223).

Conclusions: sST2 is a promising novel biomarker in ACHD patients, specifically in complex ACHD and women. Future research is warranted to elucidate sex- and diagnosis-specific differences.

Introduction

With the improvement of genomic technology, new pathways and biomarkers related to cardiac remodelling are discovered. One of these recently discovered biomarkers is soluble suppression of tumorigenicity-2 (sST2), a member of the interleukin-1 receptor family. The expression of sST2 is upregulated by cardiac myocytes as a response to stress or injury[1]. Higher sST2 levels are found in patients with severe heart failure (HF) and are associated with an increased mortality[2]. sST2 is therefore seen as a promising new biomarker in the ongoing search for the ideal HF biomarker for optimal risk stratification. Adequate risk stratification can contribute to better individualized therapeutic strategies, improving survival and reducing morbidity. Additionally, it can contribute to more detailed tailoring of information about the prognosis of an individual patient.

While sST2 has been extensively investigated in several disease populations such as patients with myocardial infarction[3, 4] and chronic and acute HF[2, 5, 6], only very limited data is available in adult congenital heart disease (ACHD)[7]. The ACHD population is rapidly expanding because of improved therapies during childhood and are characterized by a high burden of HF, arrhythmias and (re-)interventions at adult age[8]. Proper risk stratification is therefore of major importance. It is unknown whether sST2 is associated with more complex ACHD and whether it yields additive prognostic value. Therefore, this study aimed to investigate the release of sST2 among different types of ACHD and its association with cardiovascular events in patients with moderate and complex ACHD, beyond the conventional biomarker N-termiinal pro-B type natriuretic peptide (NT-proBNP).

Methods

Study population and healthy controls

In this prospective observational cohort study, consecutive adults patients with moderate or complex[9] congenital heart disease who routinely visited the out-patient clinic of our centre between April 2011 and April 2013, were included. Exclusion criteria were: age <18 years, pregnancy, mild cardiac lesion (isolated atrial or ventricular septal defect), severe renal dysfunction (creatinine >200 µmol/L) or not capable of understanding or signing informed consent. At the day of study inclusion, all patients underwent physical examination by a cardiologist, 12-lead electrocardiography, transthoracic echocardiography and venous blood sampling. Follow-up was ensured by protocolled structural annual visits to the ACHD out-patient clinic during the first four subsequent years. The study protocol conforms to the principles outlined in the Declaration of Helsinki. All participating patients gave written informed consent. The study protocol and echocardiographic imaging analysis have been described in more detail previously[10, 11].

A healthy control-cohort consisting of self-declared healthy volunteers was recruited between January 2014 and December 2014. All volunteers underwent physical examination, electrocardiography, echocardiography and venous blood sampling on the same day. More details have been described previously[12].

Biomarker assessment

Venous blood samples were taken at the day of study inclusion for study purpose only. No clinical decisions were based on any biomarker. Blood samples were transferred to the clinical chemistry laboratory within 2 hours. NT-proBNP and creatinine were directly determined in fresh blood samples. The rest of the samples were aliquoted and stored at -80ºC until batch analysis was performed. Serum sST2 was measured on the Presage ST2 assay (Critical Diagnostics, San Diego, California, USA), a quantitative sandwich monoclonal enzyme-linked immunosorbent assay (limit of quantitation 2.4 ng/mL). Samples were exposed to two thaw-freeze cycles before analysed. The Presage ST2 assay is not significantly affected by sample freeze-thaw cycles and is stable up to 15 freeze-thaw cycles. sST2 was measured once in study patients and twice in healthy volunteers, in order to assess reproducibility and to obtain reference values.

Definition and assessment of study endpoints

The primary endpoint was defined prior to the collection of data as a composite of all adverse cardiovascular events: all-cause mortality, HF (requiring initiation or change in HF medication or requiring hospitalization), hospitalization for cardiac reasons, arrhythmia (symptomatic and recorded, or requiring treatment), thromboembolic events (ischemic cerebrovascular accident, pulmonary embolism or myocardial infarction) or cardiac interventions (surgical or percutaneous). We defined the secondary endpoint as a composite of all-cause mortality or HF. All patients were annually evaluated at our outpatient clinic according to a standard protocol. Information was retrieved from electronic patient records. Survival status was checked in the Municipal Population Register. Ambiguous endpoint events were adjudicated by two investigators (LWG and JWR-H) without knowledge of any biomarker level. All patients who did not reach one of the endpoints were censored after 1 January 2018.

Statistical analysis

Sample size calculation was performed and has been described previously[10]. Continuous variables are represented as mean ± SD or median (IQR). The χ^2 Mantel-Henszel test for trend or linear regression was performed to compare variables across the different quartiles of sST2. The correlation between sST2 and NT-proBNP was visualized with scatterplots, and the Spearman correlation coefficient was calculated.

Reproducibility of ST2 assay was assessed by Bland-Altman plots with corresponding limits of agreement. The coefficient of variation was determined by the following calculation; SD of the differences of two measurements divided by the mean of two measurements*100%.

The upper limit of normal was determined based on the 97.5th percentile of sST2 levels in healthy volunteers. The 97.5th percentile was estimated using 2log transformed sST2 values and calculated with mean +1.96 SD[13]. Sex specific reference values were calculated.

We used the Kaplan-Meier method to derive the cumulative endpoint-free survival estimates. Survival curves stratified according to the quartile distribution of sST2 were compared with the log-rank test for trend. Cox proportional hazard regression was performed to assess the association between sST2 and the endpoints. Multivariable analyses were performed to adjust for clinical characteristics, creatinine and NT-proBNP. Additivity of sST2 and sex was performed using an interaction term and tested with the log-likelihood-ratio test. Likewise, linearity of sST2 was checked by adding a natural cubic spline with 3 degrees of freedom. Data on NT-proBNP were missing in <1% and were completed by imputation of the mean.

As post-hoc analysis, patients were stratified according to moderate and complex congenital heart disease and the association between sST2 and both endpoints were assessed with Cox regression. Analyses were performed using IBM SPSS Statistics (version 24) and R (version 3.5.1, packages survival). A two-sided p-value below 0.05 was considered statistically significant.

Results

Baseline characteristics

In 590 of the 602 patients with a moderate to complex ACHD who were originally included in this cohort, sST2 was measured (Supplemental Figure 1). The median age of the patients was 33 [IQR 25-41] years, 248 (42%) were women and 90% was in New York Heart Association (NYHA) class I (Table 1).

Median sST2 levels in women and men were 19.5 [IQR 14.5-25.2] ng/mL and 28.5 [IQR 21.9-36.7] ng/mL respectively. sST2 was elevated in seven women (2.8%) and 15 men (4.4%). Higher levels of sST2 were associated with oxygen saturation <90%, higher NYHA class, longer QRS duration, higher left ventricular end-diastolic volume and a larger right ventricular end diastolic annulus diameter (Table 1). Highest sST2 levels were found in patients with Fontan, pulmonary arterial hypertension, Rastelli/reparation à l'etage ventriculaire and univentricular heart (Figure 1). No significant correlation was found between levels of sST2 and NT-proBNP in men, and only a very weak correlation was found in women (r= -0.17, p=0.002). In moderate ACHD, a weak negative correlation was found (r= -0.19, p=0.001). (Supplemental Figure 2)

Reference values and reproducibility

sST2 was measured in 142 healthy volunteers. One healthy volunteer was excluded from analysis because sST2 measurement differed >20 SDs from the mean sST2 value of the healthy cohort and was seen as extreme outlier.

Table 1. Baseline characteristics for all patients and stratified according to the quartile distribution of sST2.

	All (n=590)	sST2 quartiles				P-value for trend
		First <18.0 ng/mL (n=147)	Second 18.0-24.3 ng/mL (n=149)	Third 24.3-32.2 ng/mL (n=149)	Fourth >32.2 ng/mL (n=145)	
Clinical characteristics						
Age, years	33 [25-41]	34 [26-43]	33 [25-41]	33 [25-43]	31 [24-37]	0.039
Sex ,women, n (%)	248 (42)	100 (68)	80 (54)	45 (30)	23 (16)	<0.001
Surgical repair, n (%)	538 (91)	137 (93)	135 (91)	131 (88)	135 (93)	0.769
Age at surgical repair, years	3.8 [0.8-11.9]	4.4 [1.0-13.9]	3.3 [0.8-11.1]	3.6 [0.7-11.7]	3.3 [0.7-12.2]	0.324
Congenital diagnosis, complex*, n (%)	324 (55)	71 (48)	79 (53)	88 (59)	86 (59)	0.033
Cardiac medication use†, n (%)	211 (36)	55 (37)	48 (32)	56 (38)	52 (36)	0.956
Body mass index, kg/m2	24.8 ± 4.4	25.3 ± 4.6	24.6 ± 3.9	24.9 ± 4.7	24.1 ± 4.2	0.040
Heart rate, beats/minute	74 ± 13	74 ± 13	72 ± 14	74 ± 13	75 ± 13	0.332
Systolic blood pressure, mmHg	126 ± 16	126 ± 19	126 ± 16	126 ± 14	127 ± 16	0.682
O2 saturation <90%, n (%)	17 (3)	0 (0)	2 (1)	5 (3)	10 (7)	<0.001
NYHA class, II or III , n (%)	61 (10)	10 (7)	10 (7)	18 (12)	23 (16)	0.004
Electrocardiography						
Rhythm n (%)						0.153
Sinus rhythm	509 (86)	121 (82)	131 (88)	135 (91)	122 (84)	
Paced rhythm	44 (8)	13 (9)	10 (7)	6 (4)	15 (10)	
Other	37 (6)	13 (9)	8 (5)	8 (5)	8 (6)	
QRS duration, ms	113 [100-137]	110 [95-128]	112 [99-136]	115 [101-148]	115 [104-138]	0.002
Echocardiography						
Left atrial volume, mL/m2	21 [16 -29]	20 [16-29]	21 [15-29].5	20 [15-29]	21 [15-30]	0.656
Left ventricular end-diastolic volume, mL/m2*‡	63 ± 19	60 ± 20	63 ± 18	64 ± 17	66 ± 20	0.019

Continue

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	All (n=590)	sST2 quartiles				P-value for trend
		First <18.0 ng/mL (n=147)	Second 18.0-24.3 ng/mL (n=149)	Third 24.3-32.2 ng/mL (n=149)	Fourth >32.2 ng/mL (n=145)	
Left ventricular ejection fraction, %‡	56 ± 8	57 ± 8	56 ± 9	56 ± 7	56 ± 7	0.218
Right ventricular end diastolic annulus, mm	42 ± 8	41 ± 8	41 ± 8	43 ± 8	44 ± 8	0.002
Right ventricular fractional area change, %	38 ± 11	40 ± 12	38 ± 10	37 ± 12	38 ± 11	0.200
Systemic ventricular function, n (%)						0.202
Normal	296 (50)	81 (55)	79 (53)	67 (45)	69 (48)	
Mildly impaired	207 (35)	48 (33)	47 (32)	59 (40)	53 (37)	
Moderately impaired	69 (12)	13 (9)	17 (11)	20 (13)	19 (13)	
Severely impaired	18 (3)	5 (3)	6 (4)	3 (2)	4 (3)	
E/A ratio	1.6 ± 0.7	1.7 ± 0.8	1.6 ± 0.6	1.6 ± 0.6	1.7 ± 0.6	0.910
E' wave, m/s	8.2 ± 2.6	8.3 ± 2.7	8.1 ± 2.5	8.0 ± 2.5	8.5 ± 2.6	0.011
E/E' ratio	11.6 ± 5.1	12.1 ± 5.7	11.7 ± 4.1	11.4 ± 5.0	11.2 ± 5.3	0.220
Severe valvular dysfunction#, n (%)	83(14)	16 (11)	21 (14)	21 (14)	25 (18)	0.136
Laboratory results						
Creatinine, µmol/L	77 ± 18	74 ± 15	73 ± 13	79 ± 17	82 ± 24	<0.001
NT-proBNP§, pmol/L	15 [7-33]	18 [8-36]	13 [7-33]	15 [6-30]	15 [6-29]	0.481
sST2, ng/mL	24.3 [18.0-32.2]	14.0 [11.4-16.1]	21.0 [19.5-22.5]	28.1 [26.1-30.0]	39.9 [34.9-49.6]	-

*Congenital diagnosis of arterial switch operation, aortic stenosis or aortic coarctation (0) versus Tetralogy of Fallot, Rastelli, systemic right ventricle, univentricular heart or pulmonary arterial hypertension (1) †Beta-blocker (=90 ,15 %), ACE inhibitor (n=88 ,15 %), diuretic (n=71,12 %), antiarrhythmic (n=53 ,9 %) angiotensin receptor blocker (n=36, 6 %) ‡ Left-sided volumes were not measured in patients with a systemic right ventricle, univentricular heart, pulmonary hypertension or a poor acoustic window. # Defined as maximal aortic or pulmonary valve velocity > 4.0 m/s; grade 3 or 4 out of 4 aortic, pulmonary or mitral valve regurgitation; or grade 4 out of 4 tricuspid valve regurgitation §Analysis was performed based on 2log transformed values.
NT-proBNP= N-terminal pro-B type brain natriuretic peptide, NYHA= New York Heart Association, sST2= soluble suppression of tumorigenicity-2

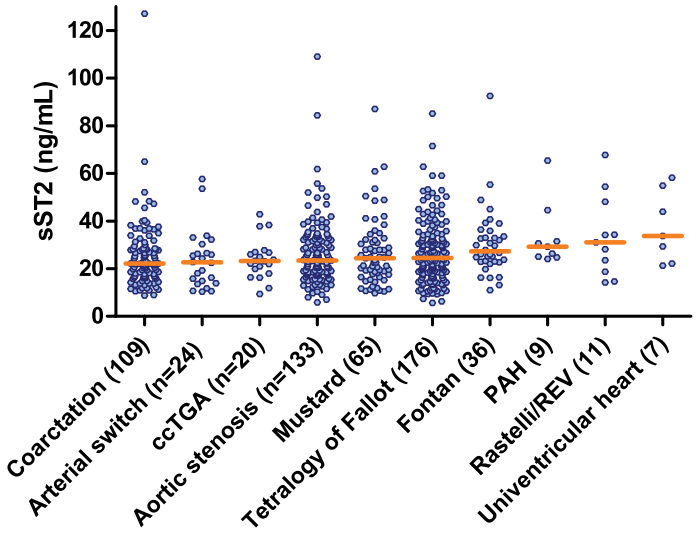

Figure 1. sST2 levels according to the different congenital diagnosis groups.
The median sST2 level in each group is indicated by the horizontal line.
cTGA= congenital corrected transposition of the great arteries, PAH= pulmonary arterial hypertension

sST2 was significantly higher in men than in women (p=0.002) but not associated with age (♂ p=0.138, ♀ p=0.334) (Figure 2). Sex-specific upper limits of normal of sST2 were 44.50 ng/mL for women and 55.85 ng/mL for men. Percentile levels of sST2 in healthy volunteers and patients with ACHD are summarized in Supplemental Table. Reproducibility of the sST2 assay was good, with a coefficient of variation of 7.74% and limits of agreement of -5.59 to 7.61 ng/mL (Supplemental Figure 3).

Follow-up

Survival status was complete in 99.7%. Detailed follow-up data regarding the other endpoints were available in 585 patients (99.2%). After a median of 5.8 [IQR 5.1-6.23] years of follow-up, the primary composite endpoint occurred in 225 patients (38.5%). The secondary endpoint occurred in 69 patients (11.8%). With regard to all separate components of the primary endpoint (ie, patients were not censored at the time of another endpoint than the endpoint of interest), the occurrence of events were: death (n=25), HF (n=59), hospitalization (n=177), arrhythmia (n=127), thromboembolic event (n=29) and cardiac intervention (n=135).

sST2 and associations with study endpoints

Endpoint-free survival stratified according to the quartile distribution of sST2 showed that patients in the lowest sST2 quartile (Q1; sST2 18.0< ng/mL), had a significant better primary and secondary endpoint-free survival than patients in higher quartiles (Figure 3).

Multivariable analysis with continuous sST2 levels and adjustment for age, sex and creatinine showed a significant association between sST2 and both the primary and secondary

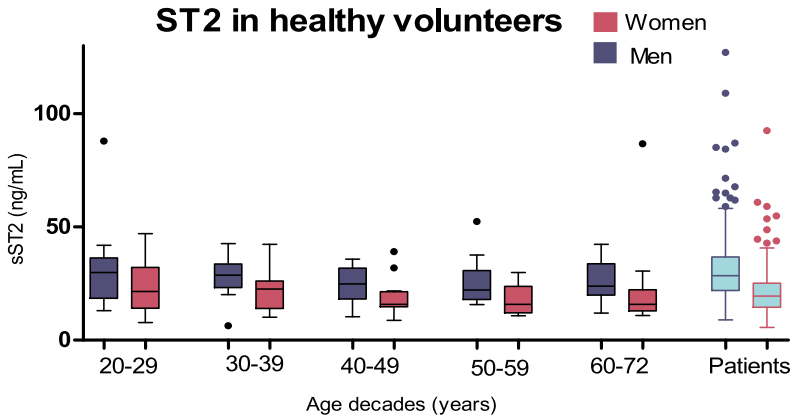

Figure 2. sST2 levels according to age decades and sex in healthy volunteers shown by boxplots.

endpoints (Table 2). Additional adjustment for NT-proBNP showed that a twofold increase in sST2 level was significantly associated with an increased risk of both endpoints. sST2 was also independently associated with the endpoints after full adjustment for age, sex and other clinical characteristics (Table 2). Nevertheless, adjustment for NT-proBNP additional to clinical characteristics, led to non-significant results.

Stratified analysis according to moderate and complex ACHD, showed that sST2 was significantly associated with both the primary and secondary endpoint in complex ACHD. Moreover, sST2 remained significantly associated with the primary endpoint after full

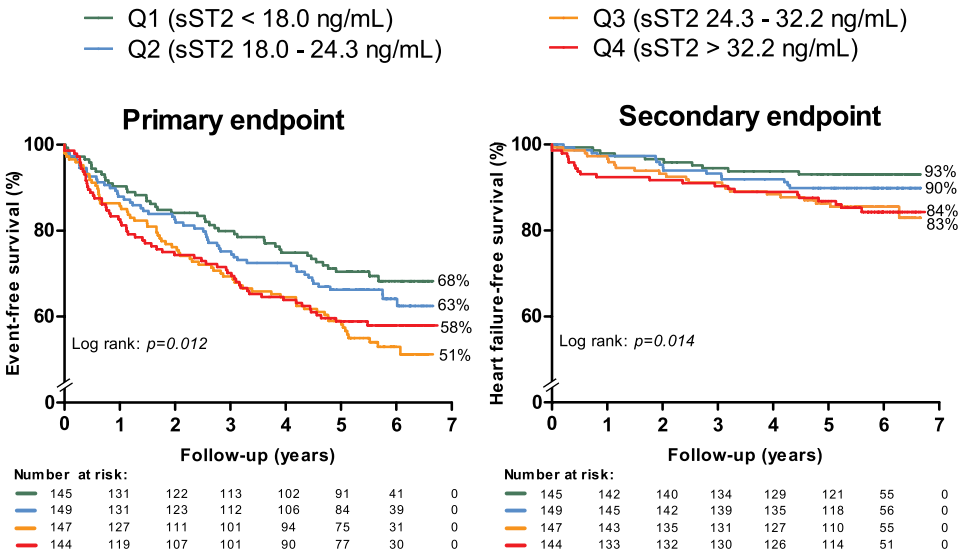

Figure 3. Survival regarding the primary endpoint (any cardiovascular event) and the secondary endpoint (death or heart failure) stratified according to the quartile distribution of sST2.
Q1= quartile 1, Q2=quartile 2, Q3= quartile 3, Q4= quartile

Table 2. Associations between sST2 and the primary (any cardiovascular event) and secondary endpoint (death or heart failure), with adjustment for clinical characteristics.

	HR per two-fold higher value	95% CI	P-value
Any cardiovascular event (n=225)			
sST2 (univariable)	1.30	1.07-1.57	0.007
Adjusted for age and sex	1.47	1.19-1.81	<0.001
Adjusted for age, sex and creatinine	1.44	1.17-1.78	<0.001
Adjusted for age, sex, NT-proBNP	1.28	1.04-1.57	0.022
Adjusted for age, sex, NT-proBNP, creatinine	1.28	1.03-1.58	0.025
Adjusted for age, sex, rhythm, systemic ventricular function	1.46	1.19-1.79	<0.001
Adjusted for age, sex, congenital diagnosis*, NYHA class, cardiac medication	1.29	1.05-1.58	0.016
Full model†	1.28	1.05-1.59	0.017
Full model† and NT-proBNP	1.19	0.96-1.48	0.106
Death or heart failure (n=69)			
sST2 (univariable)	1.48	1.04-2.10	0.029
Adjusted for age and sex	2.23	1.50-3.30	<0.001
Adjusted for age, sex and creatinine	2.16	1.45-3.22	<0.001
Adjusted for sex, age and NT-proBNP	1.48	0.99-2.22	0.059
Adjusted for sex, age, NT-proBNP and creatinine	1.57	1.03-2.39	0.036
Adjusted for age, sex, rhythm, systemic ventricular function	2.13	1.46-3.12	<0.001
Adjusted for age, sex, congenital diagnosis*, NYHA class, cardiac medication	1.60	1.10-2.34	0.015

*Congenital diagnosis of arterial switch operation, aortic stenosis or aortic coarctation (0) versus Tetralogy of Fallot, Rastelli, systemic right ventricle, univentricular heart or pulmonary arterial hypertension (1). †adjusted for age, sex, creatinine, sinus rhythm, systemic ventricular function, congenital diagnosis, NYHA class 2-3 and cardiac medication. Analysis including all covariates (full model) was not performed for the secondary endpoint due to insufficient statistical power.
CI= confidence interval, HR=hazard ratio, NT-proBNP= N-terminal pro-B-type natriuretic peptide, NYHA=New York Heart Association, sST2= soluble suppression of tumorigenicity-2.

adjustment for clinical characteristics and NT-proBNP in complex ACHD. In moderate ACHD, sST2 yielded no prognostic value. (Table 3)

Sex-specific differences of sST2

Sex and sST2 showed a significant interaction; therefore, we performed a stratified post-hoc analysis by sex. Women were older, had a higher heart rate, lower systolic blood pressure and higher NT-proBNP levels than men (Supplemental Table 2). Survival analysis showed that women in the fourth quartile of sST2 (sST2 >25.2 ng/mL), were at higher risk of endpoints. In men, there was no significant difference in endpoint-free survival among sST2 quartiles.

Table 3. Stratified analysis for the association between sST2 and the primary endpoint (any cardiovascular event) and secondary endpoint (death or heart failure) according moderate and complex adult congenital heart disease (ACHD).

	Moderate (n=266)			Complex (n=324)		
	HR*	95% CI	p-value	HR*	95% CI	p-value
Any cardiovascular event		**n=73**			**n=152**	
sST2 (univariable)	0.81	0.57-1.15	0.234	1.61	1.27-2.03	<0.001
Adjusted for age and sex	0.88	0.58-1.34	0.561	1.77	1.37-2.28	<0.001
Adjusted for age, sex and creatinine	0.89	0.59-1.35	0.591	1.73	1.34-2.23	<0.001
Adjusted for age, sex, and NT-proBNP	0.83	0.56-1.24	0.368	1.53	1.19-1.97	<0.001
Adjusted for age, sex, NT-proBNP, creatinine	0.83	0.56-1.24	0.363	1.53	1.18-1.99	0.001
Adjusted for age, sex, rhythm, systemic ventricular function	0.86	0.56-1.31	0.478	1.76	1.37-2.25	<0.001
Adjusted for age, sex, NYHA class, cardiac medication	0.90	0.59-1.37	0.620	1.40	1.10-1.78	0.007
Adjusted for full model†	0.86	0.56-1.30	0.470	1.37	1.07-1.76	0.013
Adjusted for full model† and NT-proBNP	0.80	0.54-1.21	0.294	1.31	1.01-1.69	0.043
Death or heart failure		**n=15**			**n=54**	
sST2 (univariable)	0.52	0.24-1.14	0.100	1.87	1.25-2.80	0.002
Adjusted for age and sex	-	-	-	2.68	1.75-4.11	<0.001
Adjusted for age, sex and creatinine	-	-	-	2.53	1.62-3.95	<0.001
Adjusted for age, sex and NT-proBNP	-	-	-	1.88	1.20-2.95	0.006
Adjusted for age, sex, NT-proBNP, creatinine	-	-	-	1.93	1.20-3.12	0.007

* Hazard ratios are expressed per two-fold higher sST2 level. † adjusted for age, sex, creatinine, sinus rhythm, systemic ventricular function, NYHA class 2-3 and cardiac medication. Moderate ACHD: arterial switch operation, aortic stenosis or aortic coarctation. Complex ACHD: Tetralogy of Fallot, Rastelli, systemic right ventricle, univentricular heart or pulmonary arterial hypertension. Due to only a limited number of events, no further adjustment for clinical characteristics was performed regarding the secondary endpoint.
CI= confidence interval, HR=hazard ratio, NT-proBNP= N-terminal pro-B-type natriuretic peptide, NYHA=New York Heart Association, sST2= soluble suppression of tumorigenicity-2.

(Supplemental Figure 4) Analysis of continuous levels showed that in women sST2 was strongly associated with the endpoints, independent of age, creatinine and NT-proBNP. In men, these associations were absent. (Table 4)

Discussion

This study investigated the prognostic value of sST2 in a large prospective cohort of adults with congenital heart disease. Higher levels of sST2 were found in patients with more complex congenital heart disease. Moreover, levels of sST2 were significantly associated with cardiovascular events, even independent of the established biomarker NT-proBNP. Diagnosis-

Table 4. Stratified analysis for the association between sST2 and the primary endpoint (any cardiovascular event) and secondary endpoint (death or heart failure) according to women and men.

	Women			Men		
	HR†	**95% CI**	**p-value**	**HR†**	**95% CI**	**p-value**
Any cardiovascular event		**n=96**			**n=129**	
sST2 (univariable)	1.80	1.30-2.49	<0.001	1.19	0.90-1.56	0.223
Adjusted for age	1.72	1.22-2.44	0.002	1.30	0.99-1.71	0.063
Adjusted for age and creatinine	1.71	1.22-2.41	0.002	1.28	0.97-1.69	0.080
Adjusted for age and NT-proBNP	1.49	1.04-2.12	0.029	1.16	0.89-1.51	0.274
Adjusted for age, NT-proBNP, creatinine	1.48	1.04-2.11	0.029	1.18	0.89-1.55	0.248
Adjusted for age, rhythm, systemic ventricular function	1.67	1.20-2.33	0.002	1.28	0.98-1.68	0.071
Adjusted for age, congenital diagnosis*, NYHA class, cardiac medication	1.23	0.87-1.75	0.240	1.30	0.99-1.70	0.056
Adjusted for full model‡	1.26	0.89-1.79	0.190	1.28	0.97-1.69	0.076
Adjusted for full model‡ and NT-proBNP	1.17	0.81-1.69	0.407	1.18	0.89-1.56	0.250
Death or heart failure		**n=40**			**n=29**	
sST2 (univariable)	2.72	1.67-4.44	<0.001	1.47	0.81-2.65	0.201
Adjusted for age	2.68	1.55-4.63	<0.001	1.83	1.03-3.23	0.039
Adjusted for age and creatinine	2.68	1.55-4.62	<0.001	1.61	0.88-2.94	0.125
Adjusted for age and NT-proBNP	2.03	1.12-3.69	0.020	1.18	0.69-2.00	0.547
Adjusted for age, NT-proBNP, creatinine	2.04	1.12-3.71	0.020	-#	-	-

* Congenital diagnosis of arterial switch operation, aortic stenosis or aortic coarctation (0) versus Tetralogy of Fallot, Rastelli, systemic right ventricle, univentricular heart or pulmonary arterial hypertension (1) † Hazard ratios are expressed per two-fold increase in sST2 level. P-value of interaction between sex and sST2= 0.047 (primary endpoint) and p= 0.104 for secondary endpoint # Insufficient statistical power to perform analysis. ‡ adjusted for age, creatinine, sinus rhythm, systemic ventricular function, congenital diagnosis, NYHA class 2-3 and cardiac medication. Analysis including all covariates (full model) was not performed for the secondary endpoint due to insufficient statistical power.
CI= confidence interval; HR= hazard ratio, NT-proBNP= N-terminal pro-B-type natriuretic peptide; NYHA= New York Heart Association, sST2= soluble suppression of tumorigenicity-2.

specific analysis showed a significant prognostic value for sST2 in complex ACHD independent of clinical characteristics and NT-proBNP, while in moderate ACHD sST2 yielded no prognostic value. We also revealed important sex-specific differences of sST2; both in healthy controls and patients with ACHD, sST2 was lower in woman throughout all age categories. In addition, whereas sST2 was significantly associated with cardiovascular events in woman, this association was absent in men.

Previous reports

As sST2 has been investigated extensively in HF, it has only been described once in ACHD. Laqqan et al. investigated sST2 in complex ACHD patients with a wide age range(12-70 years)

and identified sST2 as strong prognostic biomarker[7]. The prognostic value of sST2 in patients with chronic and acute HF has been established firmly and is described by Aimo et al. in two meta-analysis. In both populations, sST2 aids the risk stratification.[5,6]. In patients with acute HF, sST2 levels rose in the period prior to readmission for HF or death and serial sST2 measurements better predicted adverse outcomes compared with a single measurement, independent of serial NT-proBNP measurements[14]. Finally, another study showed that higher levels of sST2 were predictive of survival after transcathether aortic valve implantation in patients with aortic stenosis[15].

Pathophysiology of sST2

Soluble ST2 is the circulating form of the transmembrane ST2 ligand, which is the receptor for interleukin-33. sST2 acts as a decoy receptor for interleukin-33 and therefore increased sST2 levels undermine the effects of the interleukin-33/ST2 ligand interaction[16,17]. The interleukin-33/ST2 ligand signalling plays an important role in protecting the myocardium against maladaptive hypertrophy and fibrosis. As sST2 blocks this IL-33/ST2 ligand complex, these cardiac protective effects will be abolished and ventricular failure may develop.[18] In our study, sST2 and NT-proBNP levels were not correlated in complex ACHD. In the first and only study previously investigating sST2 in complex ACHD patients, only a very weak correlation was found between sST2 and NT-proBNP ($r=0.29$, $p<0.001$)[7]. This may suggest that sST2 is involved in another pathophysiological pathway than NT-proBNP regarding myocardial adaptation and dysfunction.

Besides the association with myocardial stress, sST2 is also known for its relation with inflammatory and immune processes[17]. sST2 has been investigated as inflammatory marker in numerous diseases such as asthma, COPD, collagen vascular diseases, trauma and sepsis[19]. Although it is unlikely that sST2 levels were influenced by inflammatory processes in our patients, we cannot preclude that sST2 levels may have been influenced by other unknown processes.

sST2 in healthy individuals

Reference values established in this study were higher for both sexes than reference values described in previous studies using the same ST2 assay[20-22]. However, median/mean sST2 levels were comparable with most values reported in literature[21,22]. A reason for the high reference values may be the relatively limited number of healthy volunteers and therefore the stronger influence of outliers. These high reference values could explain the relatively low number of ACHD patients with an elevated level of sST2 in our study. Identifying patients in our study with elevated sST2 levels based on reference values from the Framingham Heart Study, resulted in 19 women (7.7%) (> 33.2 ng/mL) and 38 men (11.1%) (> 47.6 ng/mL) with an elevated sST2. This would mean that in 9.7% of the patients sST2 was elevated in contrast to the 3.7% we identified.

Although sST2 levels measured in patients with ACHD seemed comparable with the ones found in the healthy volunteers, it is unclear whether sST2 has the same prognostic value in healthy volunteers as in patients with ACHD. A study investigating sST2 in a Finnish healthy

cohort showed that sST2 did not improve long-term prediction of cardiovascular events[23]. In contrast, the Framingham Heart Study found that higher sST2 preceded cardiac adverse events during a mean follow-up of 11.3 years in the general population[24].

Sex-specific differences of sST2

Our study in healthy volunteers found that sST2 levels were significantly lower among women than men; this finding is consistent with previous studies[21, 22]. Normal ranges studied in the Framingham Heart Study found that both sex and age are important determinants of sST2 levels. They described that women taking oestrogen replacement therapy had lower levels of sST2[13], suggesting that hormone release may explain the sex-difference in sST2 levels. However, another study investigating the association between ssST2 and hormones in healthy men and women did not find an independent association[25]. Currently, there has been no clear explanation for these sex-differences and whether sST2-synthesis or secretion is under hormonal control or not.

The patients in our study were much younger compared with the general HF population. Most women presumably are premenopausal and may use hormone replacement as anticonception. This could be an explanation for the interaction between sST2 and sex that we found. Unfortunately, we had no data on hormone levels. Further research is warranted to elucidate the conflicting results on hormonal influences and sST2 in premenopausal women. This is of particular interest in the ACHD population, which is characterized by a relatively young age.

There was no significant association between sST2 and the primary endpoint in men and it seemed that the height of sST2 and the associated risk reached a ceiling, creating a situation in which higher sST2 levels do not reflect higher risks. Although there are no data to support this, a possible explanation might be that men have more fluctuating and extreme levels of sST2. Hormonal influences in women might cause more stable and less extreme sST2 levels leading to a more stable prognostic effect of sST2 over time in women.

Clinical perspectives

sST2 may specifically be useful as prognostic biomarker in stable adults with complex congenital heart disease. Therefore, an sST2 measurement could be considered in these patients, besides an NT-proBNP measurement. Our study was conducted in clinically stable adult patients, and it would be interesting to know whether an increase in sST2 over time reflects clinical worsening. Serial measurements of NT-proBNP have been investigated in ACHD and increased NT-proBNP levels were found before the occurrence of cardiovascular events[26]. sST2 has a narrower biological variation in comparison to NT-proBNP[27], which is an advantage when measuring a biomarker repeatedly. For this reason, sST2 may be a very suitable biomarker in clinical practice to monitor patients over time. Patients with ACHD are characterized by a high need for reinterventions; 23% of the patients in our cohort needed a reintervention during

follow-up. A biomarker that could aid with the right timing of reinterventions is therefore highly desirable in this population.

Limitations

Serum samples were stored for a duration by -80ºC before sST2 was measured. It is unknown whether sST2 levels are affected by this long storage period; however, one studies showed that sST2 is stable in plasma samples for a maximum storage period of 1.5 years at -80 ºC[28]. In our study there was no correlation found between storage time and sST2 levels.

A heterogeneous group of diagnoses are included in this ACHD cohort. sST2 levels were higher in the complex ACHD patients, in which sST2 yielded a strong prognostic value. In contrast, no prognostic value of sST2 was found in moderate ACHD. Unfortunately, diagnoses-specific subgroup analysis were restricted by the limited sample size within each diagnosis group. Patients with isolated repaired atrial or ventricular septal defect were not included in this study due to the expected low number of events. This should be kept in mind when extrapolating the results to other ACHD cohorts.

sST2 is a relatively expensive biomarker and currently not available in standard laboratories. Implementing sST2 in the current risk stratification is therefore challenging. However over the past years sST2 has increasingly been used in patients with HF, potentially expanding the availability of the ST2 assay in the future.

Conclusion

sST2 is significantly associated with adverse cardiovascular events in patients with complex ACHD, independent of the conventional biomarker NT-proBNP. Sex-specific analyses revealed a strong association between sST2 and cardiovascular events in women, however; this association was absent in men. These sex differences of sST2 need further clarification. Nonetheless, sST2 seems to be a potential new prognostic biomarker in patients with ACHD.

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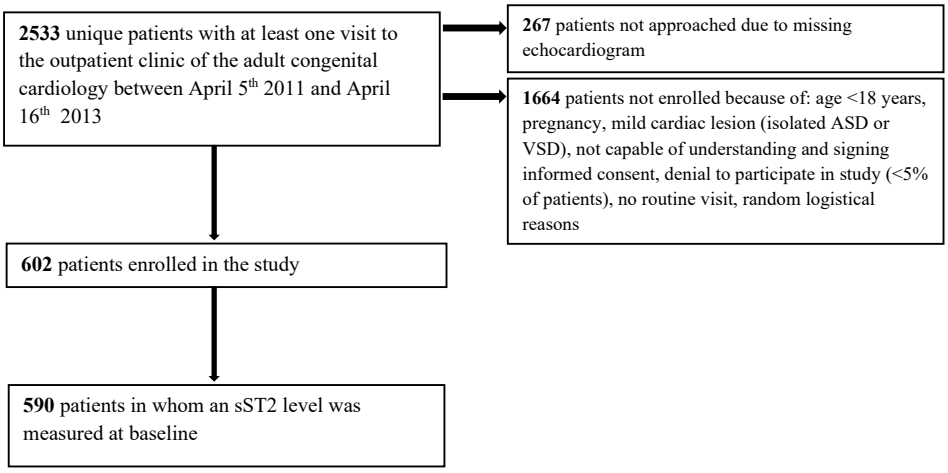
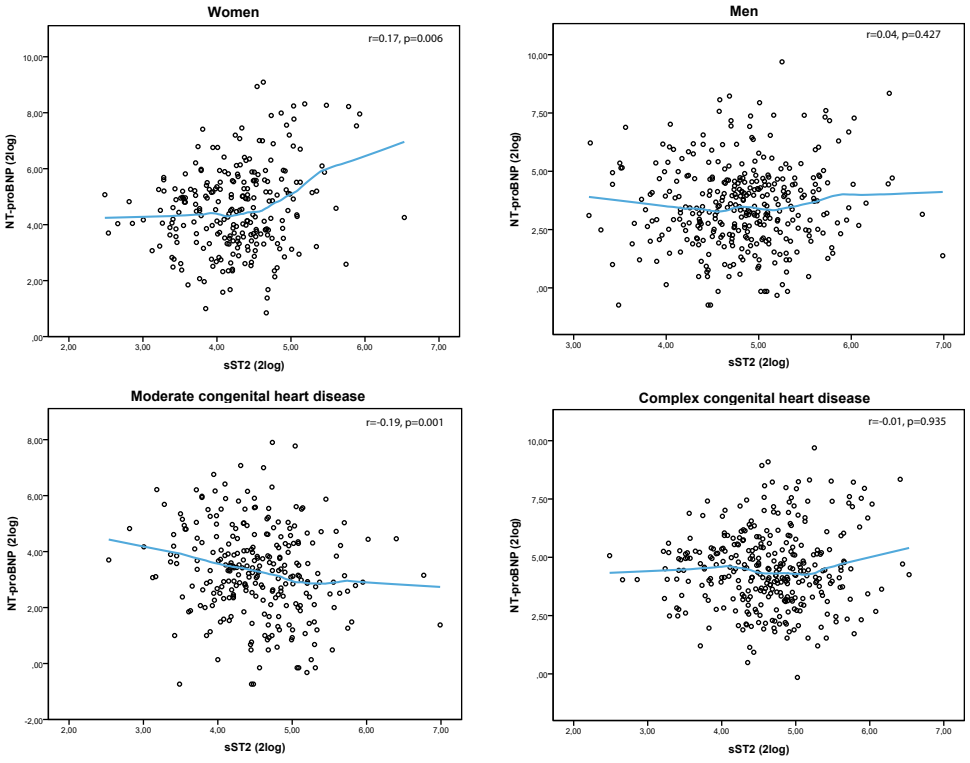


Supplemental Figure 1. Flowchart of the patient selection process.



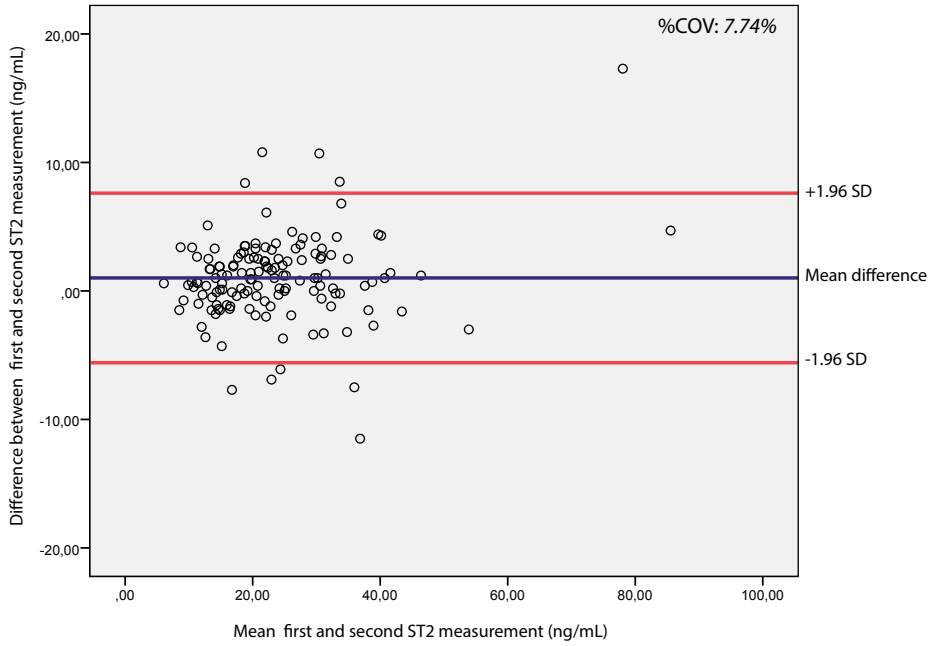
Supplemental Figure 2. Scatterplot showing the correlation between sST2 and NT-proBNP levels, stratified according to women and men and according to moderate and complex congenital heart disease.

The blue line represents the Loess curve for the association between the data points. Moderate congenital heart disease included: arterial switch operation, aortic stenosis or aortic coarctation. Complex congenital heart disease included: Tetralogy of Fallot, Rastelli, systemic right ventricle, univentricular heart or pulmonary arterial hypertension.

Supplemental Table 1. sST2 values according to percentiles found in healthy volunteers and ACHD patients, stratified according to women and men.

sST2 percentiles →	25th	50th	75th	95th	97.5th	*Cut-off**
			Women sST2 (ng/mL)			
Healthy volunteers	13.9	20.1	25.0	40.4	54.9	*44.5*
ACHD patients	14.5	19.5	25.2	37.8	47.9	
			Men sST2 (ng/mL)			
Healthy volunteers	19.4	25.5	32.8	42.4	60.4	*55.9*
ACHD patients	21.9	28.5	36.7	55.2	65.1	

* Cut-off to define elevated levels of sST2, calculated based on the following formula: mean + 1.96 SD (on the 2log scale)
ACHD= adult congenital heart disease, sST2= soluble suppression of tumorigenecity-2



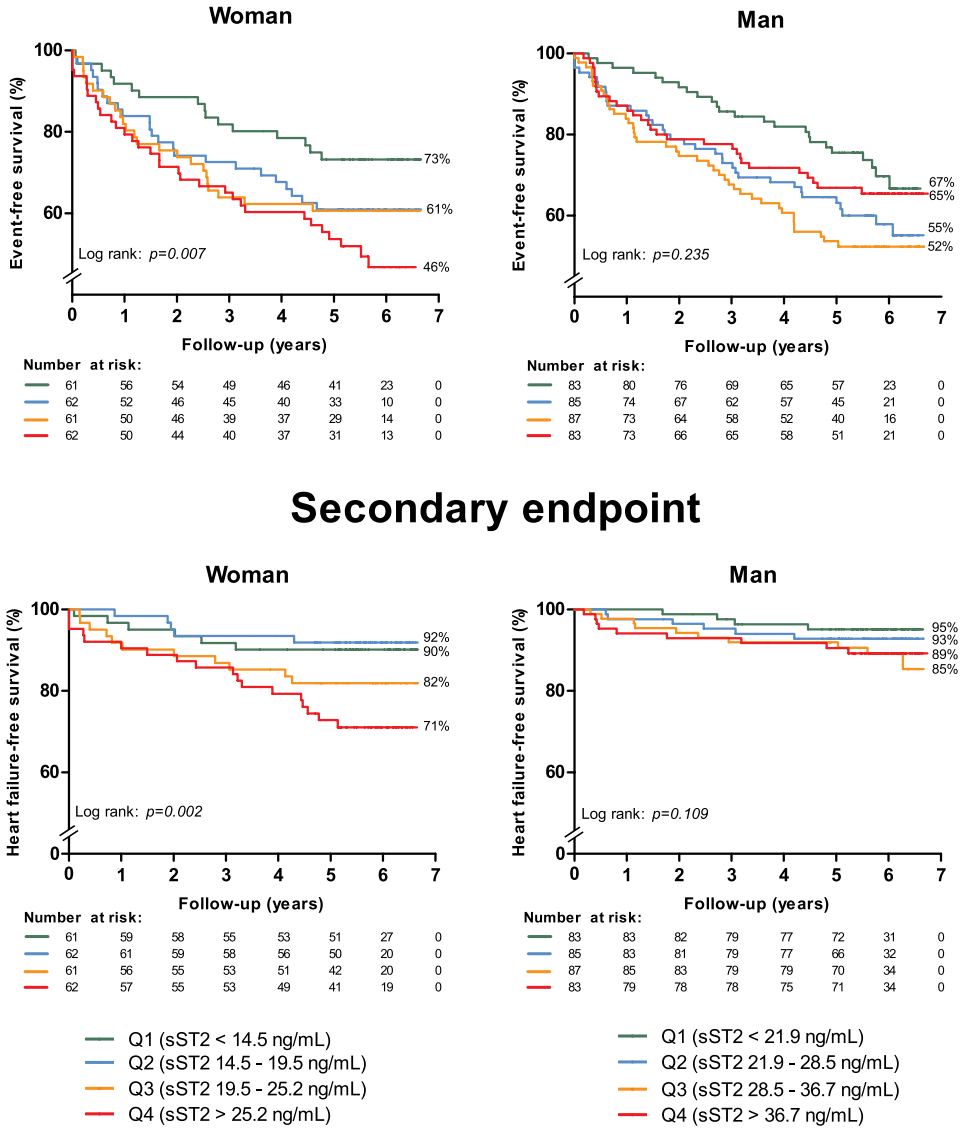
Supplemental Figure 3. Bland-Altman plot showing the reproducibility of the ST2 assay in healthy individuals.
The blue line represents the mean difference of the two sST2 measurements. The red lines indicate the limits of agreement. COV=coefficient of variation, expressed as percentage. SD= standard deviation.

Supplemental Table 2. Baseline characteristics of the adult congenital heart disease cohort, stratified according to sex.

	Women (n=248)	Men (n=342)	P-value
Clinical characteristics			
Age, years	33.9 [25.1-44.8]	31.7 [24.2-40.1]	0.033
Surgical repair, n (%)	222 (90)	316 (92)	
Age at surgical repair, years	4.8 [1.0-11.6]	3.1 [0.7-12.2]	0.145
Congenital diagnosis, complex*, n (%)	133 (54)	191 (56)	0.286
Cardiac medication use†, n (%)	93 (38)	118 (35)	0.562
Body mass index, kg/m2	25.1 ± 5.0	24.5 ± 3.9	0.09
Heart rate, beats/minute	75 ± 14	73 ± 13	0.036
Systolic blood pressure, mmHg	124 ± 18	128 ± 14	0.011
O2 saturation <90%, n (%)	12 (5)	5 (2)	0.016
NYHA class, II or III , n (%)	32 (13)	29 (9)	0.082
Electrocardiography			
Rhythm n (%)			0.005
Sinus rhythm	194 (78)	277 (81)	
Paced rhythm	18 (7)	26 (8)	
Other	36 (15)	39 (11)	
QRS duration, ms	106 [94-136]	116 [105-142]	<0.001
Echocardiography			
Left atrial volume, mL/m2	20.8 [16.1-29.9]	20.5 [15.1-28.0]	0.508
Left ventricular end-diastolic volume, mL/m2‡	27.2 ± 4.0	25.3 ± 3.4	<0.001
Left ventricular ejection fraction, %‡	56.6 ± 7.4	55.6 ± 8.0	0.204
Right ventricular end diastolic annulus diameter, mm	39.7 ± 7.6	43.98 ± 8.2	<0.001
Right ventricular fractional area change, %	39.9 ± 10.8	37.1 ± 11.5	0.020
Systemic ventricular function, n (%)			0.04
Normal	128 (52%)	168 (49)	
Mildly impaired	95 (38)	112 (33	
Moderately impaired	21 (9)	48 (14)	
Severely impaired	4 (2)	14 (4)	
E/A ratio	1.7 ± 0.7	1.6 ± 0.6	0.012
E' wave, m/s	7.8 ± 2.5	8.5 ± 2.6	<0.001
E/E' ratio	13.0 ± 5.4	10.6 ± 4.5	<0.001
Laboratory results			
Creatinine, µmol/L	69.9 ± 18.8	82.0 ± 15.2	<0.001
NT-proBNP, pmol/L	22.8 [11.5-45.8]	10.8 [5.1-24.3]	<0.001

Differences between women and men were assessed and expressed by the p-value. A t-test was performed for normal distributed continuous variables otherwise the Mann-Whitney U test was used. For categorical variables, the chi squared test or Fisher Exact test was used, as appropriate. *Congenital diagnosis of arterial switch operation, aortic stenosis or aortic coarctation (0) versus Tetralogy of Fallot, Rastelli, systemic right ventricle, univentricular heart or pulmonary arterial hypertension (1) †Beta-blocker (=90 ,15 %), ACE inhibitor (n=88 ,15 %), diuretic (n=71,12 %), antiarrhythmic (n=53 ,9 %) angiotensin receptor blocker (n=36, 6 %) ‡ Left-sided volumes were not measured in patients with a systemic right ventricle, univentricular heart, pulmonary hypertension or a poor acoustic window.

NT-proBNP= N-terminal pro-B type brain natriuretic peptide, NYHA= New York Heart Association, sST2= soluble suppression of tumorigenicity-2



Supplemental Figure 4. Kaplan-Meier curve showing the event-free (primary endpoint) and heart failure-free survival (secondary endpoint) according to the quartile distribution of sST2 for women and men separately in adults with congenital heart disease.

Sex-specific quartile distributionws of sST2 are specified in the legend.

Q1= quartile 1, Q2= quartile 2, Q3= quartile 3, Q4= quartile 4, sST2= soluble suppression of tumorigenicity-2.

CHAPTER 3

Prognostic value of serial high-sensitivity troponin T measurements in adults with congenital heart disease

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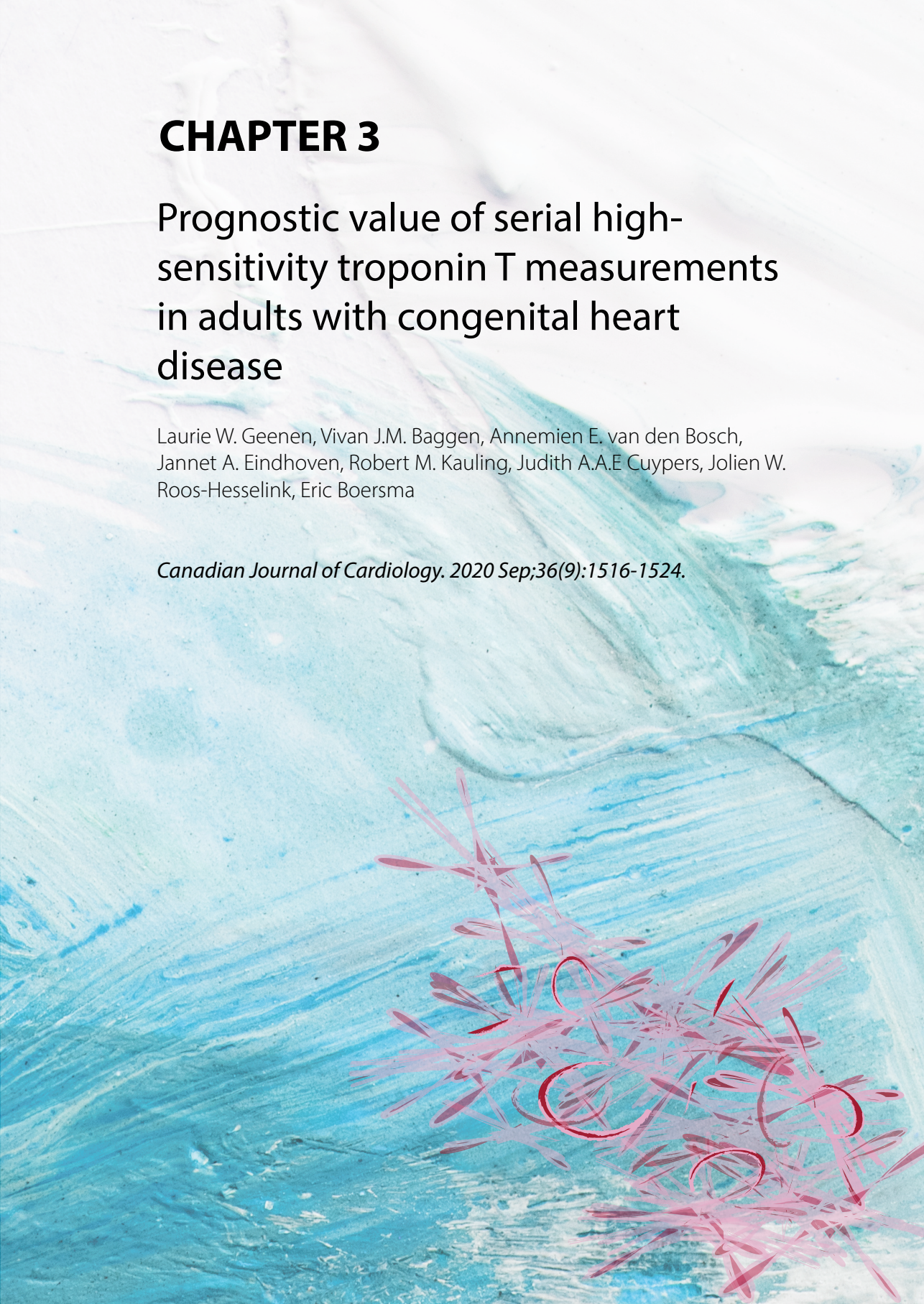

Abstract

Background: Single high-sensitivity troponin T (hs-TnT) measurement is predictive of cardiac events in adults with congenital heart disease (ACHD). We aimed to study the prognostic value of serial hs-TnT measurements in stable ACHD patients.

Methods: In total, 602 consecutive ACHD patients were enrolled in this prospective study (2011-2013). Blood sampling was performed at enrollment and thereafter yearly during scheduled visits, up to 4 years. Hs-TnT, NT-proBNP and eGFR were measured. The composite primary endpoint was defined as all-cause mortality, heart failure, arrhythmia, hospitalization, cardiac (re)interventions or thromboembolic events. The relationship between changes in serial hs-TnT and the primary endpoint was studied by joint models with adjustment for repeated NT-proBNP and eGFR.

Results: In 601 patients (median age 33 [IQR 25-41] years, 42% women, 90% NYHA I) at least one hs-TnT measurement was performed; a mean of 4.3 hs-TnT measurements per patient were collected. After a median follow-up of 5.8 [IQR 5.3-6.3] years, 229 (38.1%) patients reached the primary endpoint. On average, hs-TnT levels increased over time, and more in patients who reached the primary endpoint ($P< 0.001$). A two-fold higher hs-TnT was associated with the primary endpoint (unadjusted HR 1.62; 95% CI 1.44-1.82; $P< 0.001$). The association remained after adjustment for repeated eGFR but not when adjusted for repeated NT-proBNP; repeated NT-proBNP remained associated with the primary endpoint.

Conclusion: In stable ACHD patients, hs-TnT levels increased before the occurrence of an event and repeated hs-TnT was associated with the risk of adverse cardiac events. However, repeated hs-TnT was not superior to repeated NT-proBNP.

Introduction

Adults with congenital heart disease (ACHD) have a lifelong burden of morbidity and mortality[1], and therefore they require attentive follow-up over the course of their lives. The recommended frequency and intensity of follow-up are related to the disease complexity[2]; however, follow-up strategies are mostly based on observational studies or expert opinion. Both the need for life-long monitoring, and the increased prevalence of patients with ACHD[3], have resulted in an increased health care utilization[4]. To be able to maintain an adequate and sustainable management of patients with ACHD, noninvasive, objective, and accurate methods to monitor patients are needed. Easily accessible blood biomarkers may be useful in this respect.

Over the past few years, several prognostic blood biomarkers in the ACHD population have been identified, among which N-terminal pro B-type natriuretic peptide (NT-proBNP) thus far reveals as most relevant[5-7]. We recently demonstrated the relevance of serial NT-proBNP measurements for risk stratification in patients with ACHD[8]. NT-proBNP is secreted by cardiomyocytes in response to myocyte stretch and stimulated by increased wall stress[9]. However, deterioration of cardiac function in ACHD may include more pathophysiologic pathways. High-sensitivity troponin T (hs-TnT), primarily known for its diagnostic ability in acute coronary syndrome[10], is also associated with ventricular dysfunction[11, 12] and with cardiovascular events in patients with ACHD[5]. Secretion of troponin T in chronic heart failure (HF) can be explained by various postulated mechanisms including myocardial and subendocardial ischemia, inflammation, and myocardial apoptosis[13]. In chronic HF, the relative change in hs-TnT between two measurements has been associated with adverse clinical outcomes[14]. Serial hs-TnT measurements may as well be predictive of cardiac events in the ACHD population.

We assessed the temporal evolution of hs-TnT in stable patients with ACHD over a 4-year period, and studied the relation between these longitudinal patterns and the risk of any major adverse cardiac event: all-cause mortality, HF, arrhythmia, hospitalization, cardiac (re) intervention, or thromboembolic event.

Methods

Study design and population

This prospective observational cohort study includes a total of 602 consecutive patients with moderate-to-complex ACHD, who routinely visited the outpatient clinic of the Erasmus MC, a tertiary referral center, between April 2011 and April 2013. We excluded patients aged <18 years, those with mild ACHD (isolated atrial or ventricular septal defect), patients with an impaired renal function (defined as creatinine >200 µmol/L), and pregnant women.

The study protocol was approved by the Erasmus MC medical ethics committee and all research subjects provided written informed consent. The study was performed according to the principles outlined in the declaration of Helsinki.

Patient treatment was according to the discretion of the treating physician, based on current guidelines[2, 15]. At baseline, patients underwent physical examination by a cardiologist, 12-lead electrocardiography, echocardiography and venous blood sampling. Patients returned for yearly follow-up visits during the first 4 subsequent years after study inclusion, in which they received a complete cardiac assessment and venous blood draw. Other aspects of the study protocol have been described previously[5, 16].

Repeated blood sampling and hs-TnT measurements

Venous blood sampling was performed at baseline and all repeated study visits. Blood samples were processed <2 hours after collection and stored at -80 ºC until batch analysis. NT-proBNP was directly measured in fresh serum samples at the clinical chemistry laboratory, using a commercial electrochemiluminescence immunoassay (Roche Diagnostics, Rotkreutz, Switzerland). Hs-TnT measurements were obtained in 2 batches at the clinical chemistry laboratory of our center. A total of 2574 serum measurements were collected, corresponding to a mean of 4.3 measurements per patient. The first batch analysis of 589 (baseline) samples was performed in 2015[12] and the next batch of 1985 (follow-up) samples in 2018. All hs-TnT measurements were performed with a commercial electrochemiluminescence immunoassay (Roche Diagnostics, Rotkreutz, Switzerland). The limit of detection was 5 ng/L and the limit of blank was <3 ng/L. For analytical purposes, hs-TnT levels below <3 ng/L were substituted with a level equal to 1.5 ng/L. Hs-TnT level >14 ng/L was considered elevated. The upper limit of normal for NT-proBNP was 14 pmol/L. Samples had not undergone a prior freeze-thaw cycle. Analysts were blinded to patients' characteristics and endpoints.

Definition and assessment of endpoints

The primary study endpoint was a composite of: all-cause mortality, incident HF (HF requiring initiation or change in HF medication, or requiring hospitalization), hospitalization for cardiac reasons (eg, endocarditis), arrhythmia (symptomatic and recorded, or requiring treatment), thromboembolic events (ischemic cerebrovascular accident, pulmonary embolism, or myocardial infarction), or cardiac (re)interventions (surgical or percutaneous). The secondary study endpoint was composed of all-cause mortality or incident HF. All endpoint events were adjudicated by 2 investigators (L.W.G and J.W.R.H) without knowledge of any biomarker level. Patients who did not reach one of the endpoints were censored after January 1, 2018.

Statistical analysis

Continuous data are presented as mean ± standard deviation for normal distributed variables; otherwise the median and interquartile range (IQR) is presented. Normality of continuous variables was examined by visual inspection of histograms and Q-Q plots. Hs-TnT, estimated glomerular filtration rate (eGFR) and NT-proBNP distributions were skewed and 2log-transformed for further analyses.

Cox proportional hazard regression was used to investigate the association between baseline hs-TnT and study endpoints. We presented crude hazard ratios (HRs) and HRs adjusted for a range of baseline characteristics including age, sex, congenital diagnosis (aortic stenosis, aortic coarctation, or arterial switch operation vs tetralogy of Fallot (ToF), Rastelli, systemic RV, univentricular heart, or pulmonary arterial hypertension), NYHA class (NYHA I vs NYHA II/III), any cardiac medication use (angiotensin-converting-enzyme inhibitors, angiotensin II receptor blockers, beta-blockers, diuretics, calcium blockers, or antiarrhythmic drugs), loss of sinus rhythm, systemic ventricular function (continuous as 0-3), eGFR, and NT-proBNP.

A linear mixed effect model was used to describe the temporal evolution of hs-TnT[17]. Only measurements that were taken before the occurrence of the study endpoints were used. Joint modelling (combining linear mixed effect models with Cox regression models) was applied to assess the association between individual hs-TnT trajectories and occurrence of study endpoints[18]. We report unadjusted HRs as well as HRs adjusted for baseline characteristics, and for repeatedly measured NT-proBNP and eGFR[19]. Furthermore, the absolute change in hs-TnT during the first year was calculated (Δyear 1-year 0), and the Kaplan-Meier method was used to analyse survival according to subgroups based on this change. A subanalysis was performed based on normal vs elevated baseline NT-proBNP levels and also for 3 major diagnosis groups: aortic coarctation, aortic stenosis, and ToF.

Covariates were >99% complete, and missing data were therefore handled by imputation of the mean. SPSS version 24 and R statistical software version 3.5.1 (packages Survival, nlme, JMbayes) were used for the analyses. All statistical tests were 2-tailed, and P-values <0.05 were considered statistically significant.

Results

Baseline characteristics and study endpoints

At least 1 hs-TnT measurement was available in 601 (99.8%) patients, with a median age of 32.5 (IQR 24.7-41.2) years, 253 (42%) women and 90% NYHA class I. (Table 1) In 47 (8%) patients, hs-TnT was elevated at baseline. In 196 (33%) patients, the baseline hs-TnT level was below the limit of blank (<3.0 ng/L), and patients in whom hs-TnT levels remained undetectable at 1 year (n=100) were on average younger, less often NYHA II/III, more often in sinus rhythm, and had a shorter QRS duration. Moreover, volumes of the left atrium and end diastolic left ventricle were lower and the right ventricular fractional area change was higher.

Follow-up data were available in 596 (99.1%) patients. During a median of 5.8 (IQR 5.3-6.3) years of follow-up, respectively, 229 (38.1 %) and 69 (11.6%) unique patients reached the primary and secondary endpoint. Separate components of the endpoint events are shown in Table 2, and median baseline hs-TnT for achievement of each separate event of interest is given in Supplemental Table 1. The occurrence of the primary endpoint was associated with

Table 1. Baseline patients characteristics for all patients and according to the 1-year change in hs-TnT level

		Change in hs-TnT between baseline and 1 year measurement*			
	All ACHD patients	**Decrease**	**Stable (undetectable)**	**Increase**	**P-value**
No. of patients	601	132	100	326	
Clinical characteristics					
Age, years	32.5 [24.7-41.2]	32.5 [24.1-40.4]	25.8 [21.4-33.1]	34.8 [27.2-44.8]	<0.001
Sex, women n (%)	253 (42)	94 (71)	26 (26)	205 (63)	<0.001
Surgical repair, n (%)	540 (90)	118 (89)	90 (90)	296 (91)	0.893
Age at initial surgical repair, years	3.7 [0.8-11.9]	3.1 [0.5-11.0]	1.9 [0.4-6.3]	5.5 [1.2-14.9]	<0.001
Cardiac medication use, n (%)†	212 (35)	50 (38)	18 (18)	134 (41)	<0.001
Body mass index, kg/m^2	24.7±4.4	24.5±4.6	24.3±4.3	25.2±4.3	0.109
Heart rate, beats/minute	74±13	73±14	74±13	74±13	0.586
Systolic blood pressure, mmHg	126±16	127±17	124±16	127±16	0.296
O_2 saturation<90%, n (%)	17 (3)	5 (4)	1 (1)	9 (3)	0.446
NYHA class II/III, n (%)	61 (10)	14 (11)	3 (3)	38 (12)	0.038
Congenital diagnosis, n (%)					
Tetralogy of Fallot	179 (30)	33 (25)	27 (27)	101 (31)	0.398
Aortic stenosis	138 (23)	28 (21)	20 (20)	86 (26)	0.294
Aortic coarctation	112 (19)	26 (20)	27 (27)	51 (15)	0.036
TGA- Mustard operation	65 (11)	14 (11)	6 (6)	38 (12)	0.268
TGA- arterial switch operation	24 (4)	6 (5)	10 (10)	5 (2)	<0.001
Congenitally corrected TGA	20 (3)	8 (6)	1 (1)	11 (3)	0.116
Fontan circulation	36 (6)	7 (5)	8 (8)	20 (6)	0.694
Functionally univentricular heart	7 (1)	2 (1)	0 (0)	5 (2)	0.461
PAH	9 (1)	2 (1)	1 (1)	4 (1)	0.939
REV/Rastelli	11 (2)	6 (5)	0 (0)	5 (2)	0.032
Electrocardiography					
Sinus rhythm, n (%)	520 (87)	108 (82)	94 (94)	282 (87)	0.025
QRS duration, ms	112 [100-137]	118 [105-137]	102 [92-114]	114 [101-145]	<0.001
Echocardiography					
Left atrial volume, mL/m^2‡	21 [15-29]	22 [15-36]	19 [15-23]	21 [17-30]	0.007
LV end-diastolic volume, mL/m^2‡	64±19	68±20	60±16	63±19	0.017
LV ejection fraction, %‡	56±8	55±9	57±6	56±8	0.077
RV end-diastolic annulus dimension, mm	42±8	43±9	39±7	43±8	<0.001
RV fractional area change, %	38±11	38±12	42±10	37±11	0.031
Systemic ventricular function, n (%)					0.067

Continue

Continued

		Change in hs-TnT between baseline and 1 year measurement*			
	All ACHD patients	**Decrease**	**Stable (undetectable)**	**Increase**	**P-value**
Normal	303 (50)	60 (46)	62 (62)	157 (48)	
Mildly impaired	211 (35)	48 (36)	32 (32)	117 (36)	
Moderately impaired	69 (12)	17 (13)	5 (5)	42 (13)	
Severely impaired	18 (3)	7 (5)	1 (1)	10 (3)	
Laboratory results					
eGFR, mL/min/1.73m^2	90 [82-90]	90 [83-90]	90 [85-90]	90 [81-90]	0.125
NT-proBNP, pmol/L	15.2 [6.8-33.3]	17.4 [8.3-43.1]	10.7 [6.1-19.6]	16.4 [6.9-36.7]	0.001
Hs-TnT, ng/L	4.3 [1.5-7.2]	7.7 [5.6-11.79]	1.50 [1.5-1.5]	4.4 [1.5-6.5]	<0.001

*Includes only patients with hs-TnT measurement at both baseline and 1 year. †Beta-blocker (n=90), ACE inhibitor (n=89), diuretic (n=71), antiarrhythmic (n=53), angiotensin receptor blocker (n=36).
‡Left-sided volumes were not measured in patients with a systemic right ventricle, univentricular heart, PAH, or a poor acoustic window. NYHA=New York Heart Association, TGA=transposition of the great arteries, PAH= pulmonary arterial hypertension, REV=Réparation à l'Etage Ventriculaire, eGFR= estimated glomerular filtration rate, NT-proBNP=N-terminal pro B-type natriuretic peptide, hs-TnT= high-sensitivity troponin T.

Table 2. Separate event components of the primary endpoint

Endpoint event	**N (%)**
Death	25 (4.2)
Heart failure	59 (9.9)
Arrhythmia	127 (21.3)
Hospitalization	181 (30.4)
Cardiac (re-)intervention	138 (23.2)
Thromboembolic event	29 (4.9)

Patients were followed until the occurrence of the event of interest and were not censored at the time of another event type.

an older age, cardiac medication use, higher NYHA class, loss of sinus rhythm, worse systemic ventricular function, and higher median baseline hs-TnT (5.7 [IQR, 3.3-9.3] vs 3.8 [IQR, 1.5-6.1] ng/mL) (Supplemental Table 2).

Baseline hs-TnT was significantly associated with the primary and secondary endpoint. After adjustment for baseline characteristics, hs-TnT remained significantly associated with the secondary endpoint. The association between hs-TnT and the study endpoints was no longer statistically significant after adjustment for NT-proBNP (Table 3).

Table 3. Associations between baseline hs-TnT levels and endpoints

	Primary endpoint		Secondary endpoint	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Hs-TnT *(unadjusted)*	1.38 (1.25-1.52)	<0.001	1.92 (1.65-2.24)	<0.001
Adjusted for baseline characteristics*	1.12 (0.99-1.27)	0.061	1.51 (1.22-1.86)	<0.001
Adjusted for baseline NT-proBNP	1.10 (0.99-1.22)	0.084	1.21 (1.00-1.48)	0.050
Adjusted for baseline characteristics* and baseline NT-proBNP	0.99 (0.87-1.12)	0.842	1.23 (0.97-1.56)	0.091

HRs are expressed per two-fold higher hs-TnT level. *age, sex, congenital diagnosis, NYHA class, any cardiac medication, loss of sinus rhythm, systemic ventricular function, eGFR.
HR=hazard ratio, CI=confidence interval, hs-TnT= high-sensitivity troponin T, NT-proBNP = N-terminal pro B-type natriuretic peptide

Evolution of hs-TnT over time and its prognostic value

After omitting measurements that were taken after the occurrence of the study endpoints, 2123 and 2460 hs-TnT measurements were available for analysis concerning the primary and secondary endpoint, respectively. During the entire follow-up period, hs-TnT was on average systematically higher in patients who reached the primary endpoint than those who remained endpoint-free (Figure 1). Hs-TnT tended to increase during follow-up both in patients with and without the primary endpoint, though a higher increase was observed in patients who reached the primary endpoint. Regarding the secondary endpoint, hs-TnT levels increased during follow-up, but the increase did not differ between patients with and without the endpoint (Figure 2).

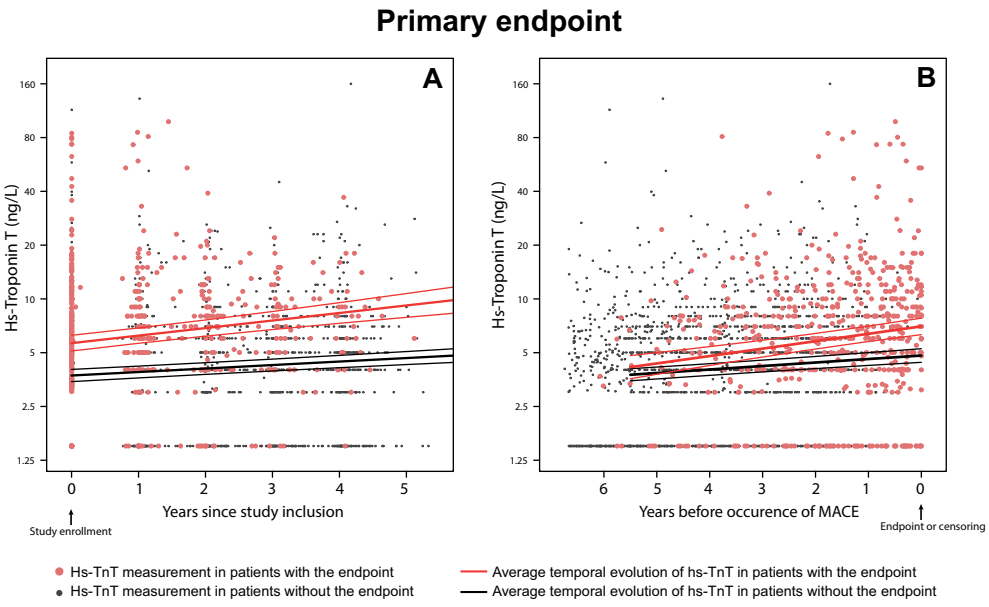

Figure 1. Average evolution of hs-TnT in patients with and without the primary endpoint.
Measurements taken after the endpoint were discarded. Time point zero is denoted as the time of study inclusion (A) or as the time when the event took place (B).

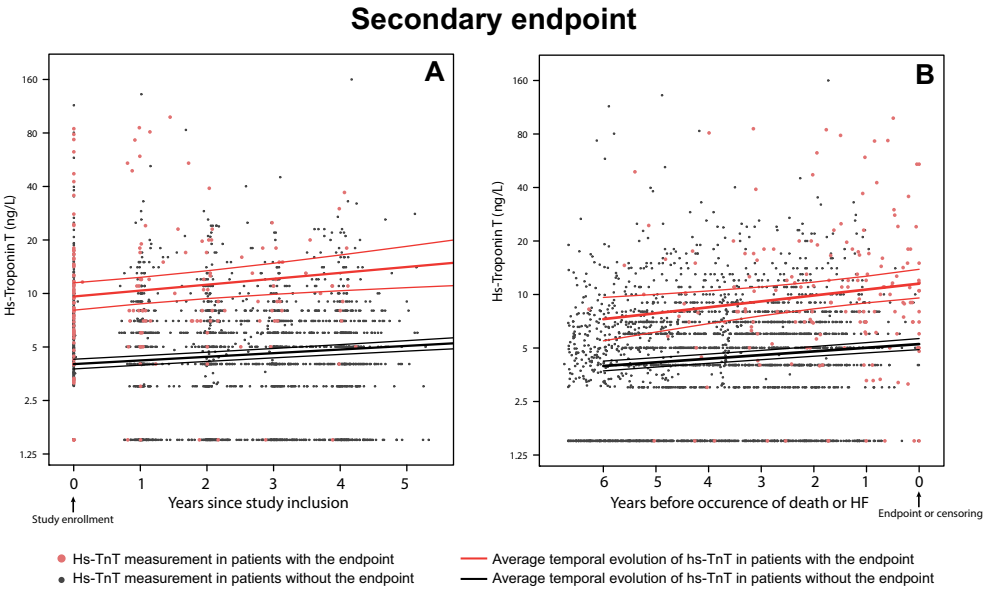

Figure 2. Average evolution of hs-TnT in patients with and without the secondary endpoint. Measurements taken after the endpoint were discarded. Time point zero is denoted as the time of study inclusion (A) or as the time when the event took place (B).

Based on higher HRs obtained from joint models, repeated hs-TnT was more strongly associated with the study endpoints compared with a single baseline hs-TnT measurement (Table 4). The associations between repeated hs-TnT and the study endpoints remained significant after adjustment for baseline NT-proBNP and baseline characteristics separately, but not when these data were combined. In a bimarker model, repeated NT-proBNP, not repeated hs-TnT was associated with the study endpoints. (Table 4)

A stratified analysis based on patients with normal or elevated baseline NT-proBNP showed the absence of prognostic value for a baseline hs-TnT in patients with normal NT-proBNP, while repeated hs-TnT was associated with the primary endpoint (Supplemental Figure 1). Though, in both strata, repeated hs-TnT was no longer associated when adjusted for repeated NT-proBNP.

Hs-TnT yielded a stronger association with the endpoints in patients with ToF and aortic coarctation, and a less strong association in patients with aortic stenosis, when compared with estimates from the entire cohort (Supplemental Figure 2).

Hs-TnT change during the first year

In patients with stable, undetectable hs-TnT levels during the first year of follow-up, the event-free survival was significantly higher, compared with patients who had changing hs-TnT levels during the first year. (Figure 3) Of note, interpretation of absolute decreases or increases of hs-TnT in this analysis, should be done with caution because of regression towards the mean[20].

Table 4. Associations between repeated hs-TnT levels and endpoints.

	Primary endpoint		Secondary endpoint	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Repeated hs-TnT *(unadjusted)*	1.62 (1.44-1.81)	<0.001	2.58 (2.13-3.14)	<0.001
Adjusted for baseline characteristics	1.26 (1.09-1.47)	0.004	1.73 (1.31-2.28)	<0.001
Adjusted for baseline NT-proBNP	1.21 (1.06-1.38)	0.002	1.34 (1.05-1.72)	0.016
Adjusted for baseline characteristics and baseline NT-proBNP	1.07 (0.90-1.26)	0.436	1.31 (0.96-1.80)	0.086
Repeated hs-TnT and NT-proBNP				
Repeated hs-TnT	1.12 (0.98-1.30)	0.102	1.19 (0.89-1.58)	0.262
Repeated NT-proBNP	1.53 (1.38-1.70)	<0.001	2.42 (1.93-3.04)	<0.001
Repeated hs-TnT and eGFR				
Repeated hs-TnT	1.50 (1.32-1.70)	<0.001	2.49 (1.95-3.16)	<0.001
Repeated eGFR	0.59 (0.39-0.94)	0.028	0.69 (0.39-1.38)	0.240

HRs are expressed per two-fold higher biomarker level, at any point in time during follow-up.*age, sex, congenital diagnosis, NYHA, any cardiac medication, loss of sinus rhythm, systemic ventricular function, eGFR
HR=hazard ratio, CI=Confidence Interval, hs-TnT= high-sensitivity troponin T, NT-proBNP=N-terminal pro B-type natriuretic peptide, eGFR=estimated glomerular filtration rate

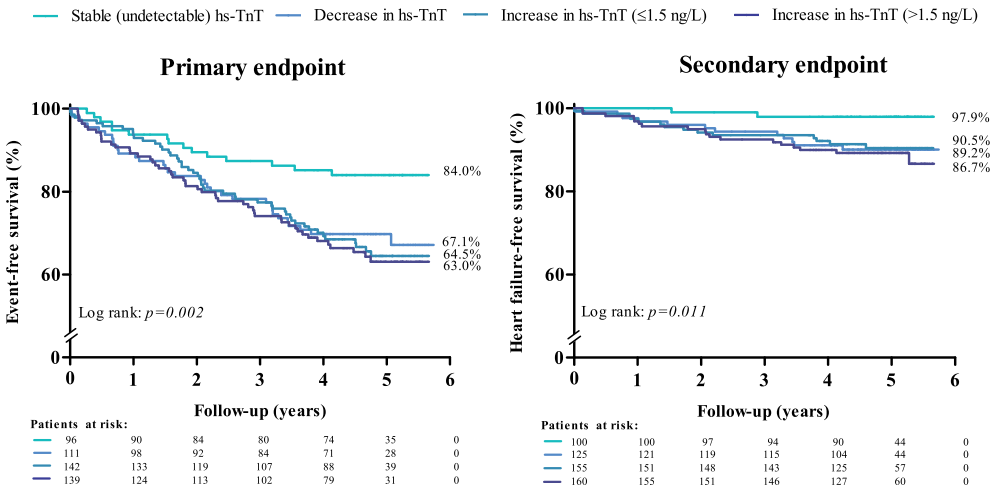

Figure 3. Event-free and heart failure-free survival according to the change in hs-TnT in the first year (Δ year 1-year 0).
Log-rank test represents comparison of survival in stable patients versus the other groups. Of note, a subset of the data was used in this analysis; only patients with both a hs-TnT measurement at baseline and at 1 year could be included (other hs-TnT measurements were discarded), and the time-to event was recalculated from year 1 onwards (patients had to be alive at t=1 year).

Discussion

Clinically stable patients with ACHD who had an adverse cardiac event within 6 years after inclusion had systematically higher hs-TnT at baseline and during follow-up, and values tended to increase before the occurrence of an adverse cardiac event. This seems to reveal a process of ongoing and enhanced cardiomyocyte loss in mostly asymptomatic patients with ACHD. Particularly undetectable, stable hs-TnT levels (<3 ng/L), were present in patients with a more favourable prognosis. Although repeated hs-TnT yielded prognostic value for adverse cardiac events independently of a single baseline NT-proBNP measurement, repeatedly measured hs-TnT did not yield prognostic value independent of repeated NT-pro-BNP measurements.

Value of hs-TnT as prognostic biomarker

The prognostic value of hs-TnT was described for the first time in this same cohort of patients with ACHD[5, 12]. Meanwhile, several other studies have confirmed the prognostic relevance of hs-TnT in some ACHD diagnoses[11, 21-23]. Rybicka et al investigated hs-TnT levels in 131 stable patients with ACHD and found an association with systemic ventricular dysfunction[11]. In adults with congenitally corrected transposition of the great arteries, hs-TnT was associated with systemic right ventricular function and was even superior to NT-proBNP in detecting systemic ventricular dysfunction[22]. Moreover, hs-TnT was predictive of adverse cardiac events in these patients[21]. These studies indicate that hs-TnT release and its prognostic value are not restricted to patients with systemic left ventricles, or certain types of ACHD. Subgroup analysis in our study further supports this; baseline hs-TnT yields prognostic value in adults with ToF, aortic coarctation or aortic stenosis. Especially in ToF, the association with death or HF for both a single and repeated hs-TnT measurements was strong.

To the best of our knowledge, this is the first study that investigated repeated hs-TnT measurements in patients with ACHD. Although data in patients with ACHD are limited, hs-TnT has more extensively been investigated in patients with chronic HF[24]. The relative change between 2 hs-TnT measurements within a 4-month time period was associated with the risk of adverse cardiovascular events in patients with chronic HF[14]. In our study, the absolute change in hs-TnT in the first year was not associated with outcomes. This is most likely due to regression towards the mean; measured values of a random variable fluctuate around a true mean and extreme values therefore tend to regress toward the mean, becoming less extreme[20]. The median hs-TnT level was highest in patients with a decrease in hs-TnT in the first year, supporting the phenomena of regression towards the mean and subsequently the lack of prognostic value found for the absolute hs-TnT change. Joint modelling, as used in this study, solves this problem by adjusting for the within-subject variation[18]. Temporal hs-TnT patterns investigated in chronic HF using joint modelling showed that repeated hs-TnT was associated with adverse cardiovascular events, but not when adjusted for NT-proBNP and C-reactive protein trajectories[25]. Results of our study are in line with results found in patients

with chronic HF[14, 25], suggesting that the role of hs-TnT in the pathophysiology of HF in patients with chronic HF and those with ACHD may be quite similar.

Understanding troponin T release in ACHD

The hs-TnT increase over time found in this study, may indicate the existence of a continuous slow troponin T release from the myocardium, which might reflect ongoing subclinical loss of cardiomyocytes. If we assume that the loss of cardiomyocytes is the result of increased wall stress due to HF progression, the hs-TnT increase will be preceded by an increase in NT-proBNP, as NT-proBNP is secreted in response to increased cardiac wall stress[9]. This could explain the prognostic value of serial NT-proBNP independent of serial hs-TnT and not vice versa. In patients with low baseline NT-proBNP, a steeper hs-TnT increase was observed than in patients with elevated NT-proBNP. However, no independent value for hs-TnT was found, supporting the hypothesis that an increase in hs-TnT is preceded by an increasing NT-proBNP.

Besides myocardial cell death, other mechanisms including myocardial and subendocardial ischemia, inflammation, and infiltrative processes may contribute to release of troponin T[13]. This could explain why hs-TnT showed an association with any adverse cardiac event and not only HF. Contrarily, the absence of troponin T release, reflected by patients with undetectable hs-TnT levels, was associated with a low risk of adverse cardiac events. These patients were also characterized by more favourable baseline clinical characteristics. The absence of troponin T release therefore seems to exclude processes provoking cardiac deterioration and may be helpful to detect low-risk patients.

Hs-TnT levels also increase with a worsening renal function, by diminished renal clearance of troponin T[26, 27]. Although patients with severe renal dysfunction were not included in this study, hs-TnT levels could have been influenced by worsening renal function during follow-up. Nevertheless, serial hs-TnT measurements remained predictive of both endpoints, independent of serial eGFR. Therefore, it is likely that the hs-TnT increase is the result of cardiomyocyte loss rather than the effect of a decreased renal clearance of hs-TnT.

Clinical perspective

A single hs-TnT measurement can be used as prognosticator in patients with ACHD besides NT-proBNP to further discriminate between high- and low-risk patients[5]. In addition, this study showed that serially measuring hs-TnT can enhance precision in estimating prognosis in addition to a single measurement. Particularly, stable, undetectable hs-TnT levels seem to identify low-risk patients whom can be reassured. Nonetheless, repeated hs-TnT may not be the biomarker of first choice; repeatedly measuring NT-proBNP for monitoring and risk assessment in clinically stable patients with ACHD over time seems more valuable. However, clinicians should be aware of the biological and analytical variability of biomarkers[28] and the subsequent effect of regression towards the mean, when interpreting repeatedly measured biomarkers.

As previously described, elevated levels of hs-TnT are not uncommon in asymptomatic patients with ACHD[12]. With the ageing ACHD population[3], coronary artery disease is likely to

become more prevalent and a bigger threat to these patients. In the management of coronary artery disease in patients with ACHD, it should be taken into account that hs-TnT levels are higher in these patients and increase over time.

Limitations

Hs-TnT was measured in thawed serum samples, which had been stored by -80°C. Hs-TnT is known to be stable up to at least one year at -80°C [29] while samples in our study had been stored >1 year. However, we did not find a correlation between storage time within each follow-up moment and hs-TnT levels. Therefore, it is unlikely that levels of hs-TnT have been affected by storage time.

We measured hs-TnT annually, and because of the relatively long time interval between 2 measurements, the time in between biomarker measurement and the onset of a cardiovascular event differs in each case. The last measurement taken before the event may therefore differ from the actual biomarker level prior or at the moment of the actual event. This may also have prevented us to notice more pronounced increases in hs-TnT in anticipation to events. More frequent blood sampling than performed in our study would be needed to more precisely investigate this.

This study consisted of patients with ACHD with different underlying congenital heart defects. Unfortunately, we were restricted by the sample size to perform subgroup analyses for each diagnosis.

Conclusion

In clinically stable patients with ACHD, hs-TnT levels modestly increased over time, indicating loss of cardiomyocytes that might reflect subclinical as well as clinical progression of HF in these patients. Particularly, stable, undetectable hs-TnT levels may identify low-risk patients. However, the additive prognostic value of serial hs-TnT measurements beyond serial NT-proBNP measurements seems limited. Whether hs-TnT could aid guidance of follow-up strategies in specific ACHD diagnoses such as Fontan will require greater sample sizes and needs to be examined in future research.

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Supplemental Table 1. Separate event components of the primary endpoint, with the baseline hs-TnT level for adult congenital heart disease patients with and without the event of interest.

		Baseline hs-TnT level (ng/L)		
Endpoint event	**N (%)**	Without event	With event	P-value
Death	25 (4.2)	4.1 [1.5-6.9]	11.4 [8.6-39.0]	<0.001
Heart failure	59 (9.9)	4.1 [1.5-6.7]	8.6 [4.8-15.6]	<0.001
Arrhythmia	127 (21.3)	3.9 [1.5-6.5]	6.6 [4.1-10.4]	<0.001
Hospitalization	181 (30.4)	4.0 [1.5-6.5]	5.3 [3.2-9.9]	<0.001
Cardiac (re-)intervention	138 (23.2)	4.2 [1.5-6.9]	4.7 [3.1-8.3]	0.020
Thromboembolic event	29 (4.9)	4.2 [1.5-7.1]	6.0 [1.50-9.4]	0.139

Hs-TnT levels are presented as median [inter quartile range]. Patients were followed until the occurrence of the specific event of interest and were not censored at the time of another event type.
Man-Whitney U test was used for comparison of hs-TnT levels in patients with and without the endpoint of interest. Hs-TnT= high-sensitivity troponin T.

Supplemental Table 2. Baseline characteristic according to adult congenital heart disease patients with and without any major adverse cardiac event during follow-up.

	No event	Any event*	
No. of patients	367	229	P-value
Clinical characteristics			
Age, years	30.0 [23.8-37.7]	37.8 [29.7-46.2]	<0.001
Sex, women n (%)	214 (58)	131 (57)	0.790
Surgical repair, n (%)	330 (90)	211 (92)	0.362
Age at initial surgical repair, years	2.7 [0.6-9.6]	5.9 [1.8-14.5]	<0.001
Cardiac medication use, n (%)	91 (25)	120 (52)	<0.001
Body mass index, kg/m^2	24.5 ± 4.1	25.1 ± 4.9	0.094
Heart rate, beats/minute	74 ± 14	73 ± 13	0.867
Systolic blood pressure, mmHg	127 ± 16	125 ± 17	0.324
O_2 saturation<90%, n (%)	5 (2)	11 (5)	0.012
NYHA class II/III, n (%)	10 (3)	51 (22)	<0.001
Congenital diagnosis, n (%)			
Tetralogy of Fallot	104 (28)	75 (33)	0.253
Aortic stenosis	85 (23)	53 (23)	0.996
Aortic coarctation	87 (24)	23 (10)	<0.001
TGA- Mustard operation	37 (10)	27 (12)	0.512
TGA- arterial switch operation	22 (6)	1 (0.4)	0.001
Congenitally corrected TGA	11 (3)	9 (4)	0.538
Fontan circulation	12 (3)	23 (10)	0.001
Functionally univentricular heart	4 (1)	3 (1)	0.808
PAH	2 (1)	7 (3)	0.014
REV/Rastelli	3 (1)	8 (4)	0.018
Electrocardiography			
Sinus rhythm, n (%)	335 (91)	180 (79)	<0.001
QRS duration, ms	111 [99-129]	120 [101-151]	0.004
Echocardiography			
Left atrial volume, mL/m^{2‡}	20 [15-27]	25 [17-36]	<0.001
LV end-diastolic volume, mL/m^{2‡}	63 ± 17	65 ± 21	0.278
LV ejection fraction, %‡	57 ± 7	55 ± 10	0.060
RV end-diastolic annulus dimension, mm	41 ± 8	44 ± 8	<0.001
RV fractional area change, %	39±11	37 ± 12	0.111
Systemic ventricular function, n (%)			<0.001
Normal	212 (58)	90 (39)	
Mildly impaired	115 (31)	92 (40)	
Moderately impaired	31 (8)	38 (17)	
Severely impaired	9 (3)	9 (4)	

Continue

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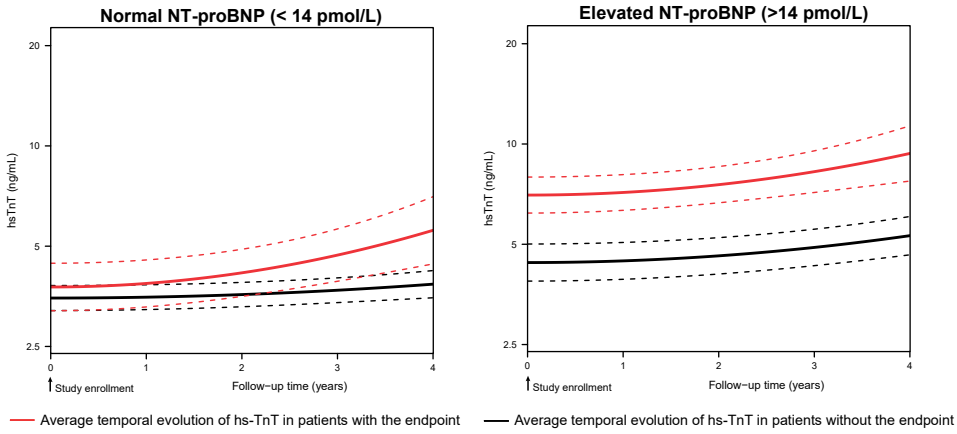
	No event	**Any event***	
No. of patients	367	229	P-value
Laboratory results			
eGFR, mL/min/1.73m^2	90 [85-90]	89[79-90]	<0.001
NT-proBNP, pmol/L	10.9 [5.3- 21.7]	30.7 [11.9-61.2]	<0.001
Hs-TnT, ng/L	3.8 [1.5-6.1]	5.7 [3.3-9.3]	<0.001

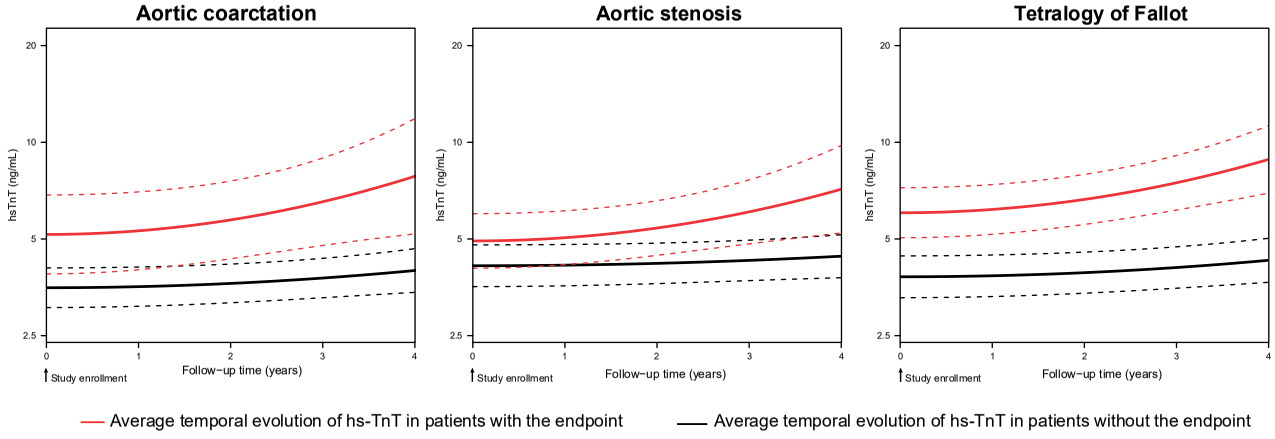
*all-cause mortality, heart failure, arrhythmia, hospitalization, cardiac (re-)intervention or thromboembolic event. ‡Left-sides volumes were not measured in patients with a systemic right ventricle, univentricular heart, pulmonary hypertension or a poor acoustic window. Student T-test or Man-Whitney U test was used to compare normally distributed and non-normally distributed continuous variables respectively, between patients with and without endpoint. For comparison of categorical variables the chi-squared test was used. NYHA=New York Heart Association, TGA=transposition of the great arteries, PAH= pulmonary arterial hypertension, REV=Réparation à l'Etage Ventriculaire, eGFR= estimated glomerular filtration rate, NT-proBNP=N-terminal pro B-type natriuretic peptide, hs-TnT= high-sensitivity troponin T.



	Normal NT-proBNP (n=278)		Elevated NT-proBNP (n=318)	
Primary endpoint, n (%)	63 (23%)		166 (52%)	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Baseline hs-TnT	1.13 (0.88-1.43)	0.338	1.29 (1.16-1.44)	<0.001
Repeated hs-TnT	1.50 (1.11-2.05)	0.009	1.45 (1.28-1.65)	<0.001
Bi-marker model;				
Repeated NT-proBNP	1.56 (1.10-2.22)	0.012	1.58 (1.34-1.88)	<0.001
Repeated hs-TnT	1.27 (0.92-1.71)	0.164	1.08 (0.88-1.32)	0.446

Supplemental Figure 1. Figure showing the average temporal evolution of hs-TnT in ACHD patients according to a normal or elevated NT-proBNP at baseline (derived from linear mixed effect models).

Red line indicates patients with any major cardiovascular event, black line indicates patients without any major cardiovascular event. 95% confidence intervals are indicated by the dotted-lines. Table shows the hazard ratios for the risk of the endpoint per two-fold higher biomarker level, for a single baseline hs-TnT measurement as well as for repeated hs-TnT and repeated NT-proBNP measurements (calculated using Joint Models).



	Aortic coarctation (n=110)		Aortic stenosis (n=138)		Tetralogy of Fallot (n=179)	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Primary endpoint, n (%)	*23 (20.9%)*		*53 (38.4%)*		*75 (41.9%)*	
Baseline hs-TnT	1.62 (1.08-2.42)	0.018	1.15 (0.92-1.42)	0.223	1.48 (1.25-1.76)	<0.001
Repeated hs-TnT	2.35 (1.42-3.99)	<0.001	1.37 (1.07-1.72)	0.011	1.80 (1.46-2.22)	<0.001
Secondary endpoint, n (%)	*3 (2.7%)*		*12 (8.7%)*		*16 (8.9%)*	
Baseline hs-TnT	-*	-*	1.53 (1.03-2.27)	0.037	2.16 (1.63-2.86)	<0.001
Repeated hs-TnT	-*	-*	1.84 (1.16-2.90)	0.009	3.56 (2.33-5.55)	<0.001

Supplemental Figure 2. Figure showing the average temporal evolution of hs-TnT in adult congenital heart disease patients, for three diagnosis groups separately (derived from linear mixed effect models).

Red line indicates patients with any major cardiovascular event (primary endpoint), black line indicates patients without any major cardiovascular event. 95% confidence intervals are indicated by the dotted-lines. Table shows the hazard ratios for the risk of the endpoint per two-fold higher level in hs-TnT, for a single baseline hs-TnT measurement as well as for repeated hs-TnT measurements (calculated using Joint Models). *Not enough statistical power to perform analysis.

CHAPTER 4

Prognostic value of C-reactive protein in adults with congenital heart disease

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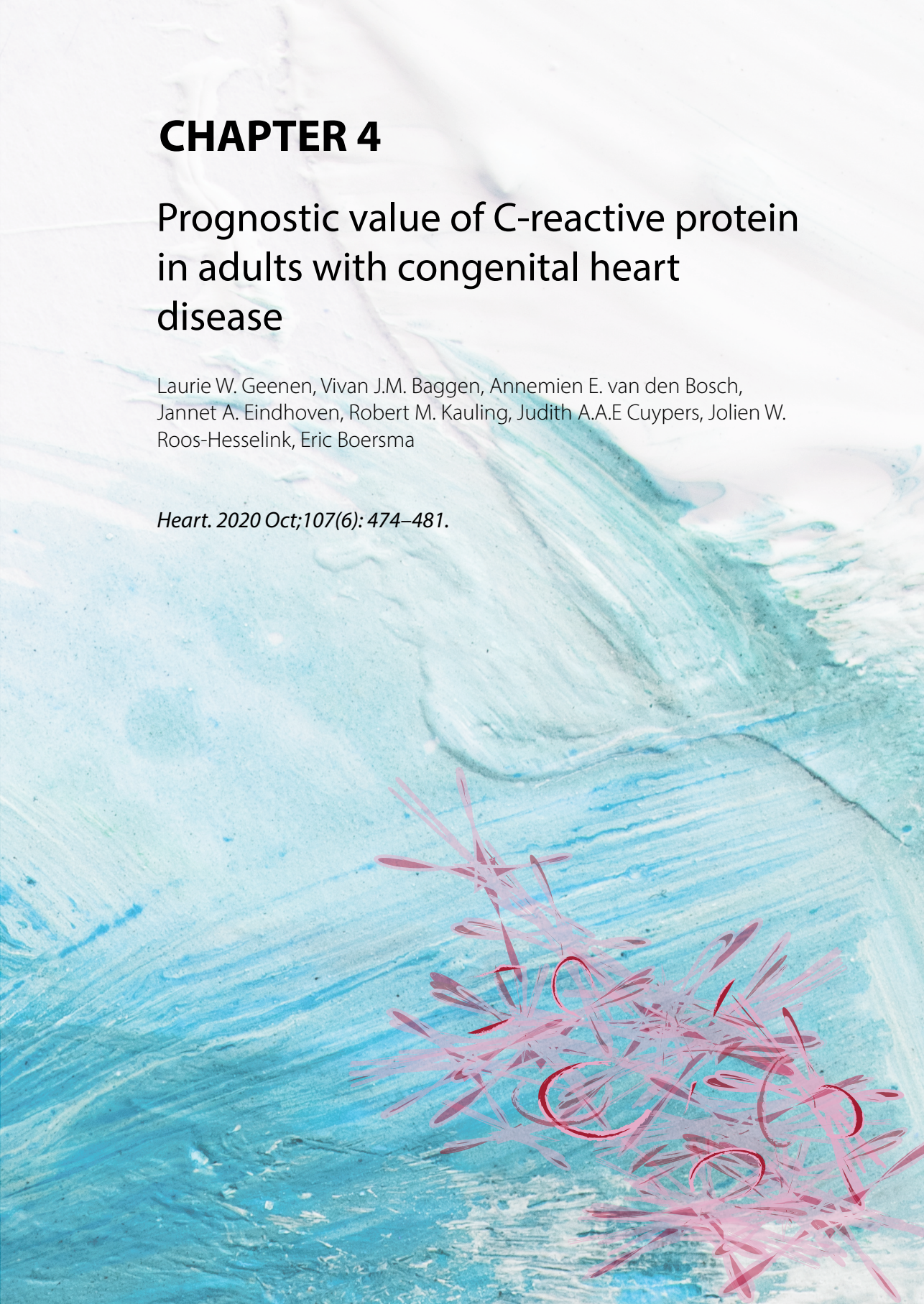

Abstract

Background: High-sensitivity C-reactive protein (hs-CRP) has been associated with outcomes in adult congenital heart disease (ACHD). However, its prognostic value beyond N-terminal pro B type natriuretic peptide (NT-proBNP) or troponin T remains unknown. We studied the temporal evolution of hs-CRP, as well as the relation between hs-CRP and adverse clinical outcomes independent of NT-proBNP and troponin T in patients with ACHD.

Methods: In this prospective cohort study, we enrolled 602 patients with ACHD (2011-2013) who underwent baseline and thereafter annual blood sampling during 4 years. Hs-CRP, hs-troponin T and NT-proBNP were measured. The primary endpoint was composed of death or heart failure (HF). Cox regression and Joint Modelling was used to relate 2log hs-CRP levels with the endpoint, with adjustment for baseline characteristics and (repeated) hs-troponin T and NT-proBNP measurements.

Results: Hs-CRP was measured at baseline in 591 patients, median age 33 years, 58% men, 90% NYHA I with an average of 4.3 measurements per patient. Median follow-up was 5.9 [IQR 5.3-6.3] years (99.2% complete) and 69 patients met the endpoint. Higher baseline hs-CRP was independently associated with higher risk of death or HF (HR 1.36, 95% CI 1.19-1.55). Hs-CRP increased over time prior to death or HF, and repeated hs-CRP measurements were associated with the endpoint, independent of repeated NT-proBNP and hs-troponin T (HR 1.54, 95% CI 1.24-1.98).

Conclusions: Hs-CRP carries incremental prognostic value for the risk of death or HF, beyond NT-proBNP and hs-troponin T. Hs-CRP increased prior to the occurrence of HF or death, supporting the role of inflammation in the clinical deterioration of patients with ACHD.

Introduction

Adults with congenital heart disease (ACHD) are a growing patient population with a high healthcare utilisation[1, 2]. Despite the increased survival prospects, patients with ACHD carry an increased risk of heart failure (HF), arrhythmias, reinterventions and early demise[3-5]. Although mechanisms such as chronic pressure- or volume overload that can lead to cardiac deterioration are well described in patients with ACHD[6], the potential role of chronic inflammation has been investigated to a lesser extent. A recent study by Opotowsky et al. demonstrated that higher high-sensitivity C-reactive protein (hs-CRP) was associated with higher risk of cardiovascular events and mortality in patients with ACHD[7]. These findings suggest that chronic inflammation may contribute to the pathophysiology of deterioration in ACHD, as hs-CRP is a sensitive marker of systemic inflammation.

Currently it is unknown how hs-CRP relates to other biomarkers and echocardiographic measures in ACHD. Besides the great interest in the pathophysiological mechanism of hs-CRP, the additive prognostic value of hs-CRP beyond conventional biomarkers, such as N-terminal pro B type natriuretic peptide (NT-proBNP) and high-sensitivity troponin-T (hs-TnT), has not yet been assessed. It is therefore unclear how hs-CRP could enhance risk stratification. Moreover, it is unknown how hs-CRP evolves over time. Studying these temporal patterns may contribute to better understanding of hs-CRP release in patients with ACHD.

The aim of this study was to investigate the association of hs-CRP with other biomarkers and echocardiographic measurements, as well as its association with long-term cardiovascular outcomes in patients with ACHD. Moreover, we studied the temporal evolution of hs-CRP and the relation of these evolutions with the risk of cardiovascular events.

Methods

Study design and population

This is a prospective observational single-centre cohort study, including 602 clinically stable patients with moderately or severely complex ACHD. Detailed aspects of the study protocol have been described previously[8, 9]. Patients were enrolled during a routine visit at the outpatient clinic (2011-2013). Exclusion criteria were age <18 years, mild ACHD, renal impairment (creatinine >200 µmol/L) or pregnancy. All patients underwent physical examination by a cardiologist, ECG, echocardiography and venous blood sampling at the day of enrolment. Thereafter, yearly outpatient visits were scheduled for 4 years and venous blood sampling was repeated. Patient management was according to the discretion of the treating physician, based on prevailing guidelines[10, 11].

The study was performed according to the principles outlined in the Declaration of Helsinki. All patients gave written informed consent for their participation in the study.

Laboratory testing

During baseline and follow-up study visits, peripheral venous blood sampling was performed. Blood samples were directly used, or stored at -80 ºC in the biobank until batch analysis was performed. NT-proBNP and estimated glomerular filtration rate (eGFR) were directly measured in fresh blood samples in our clinical chemistry laboratory. Serum hs-CRP and hs-TnT were measured batch wise from the same samples (first batches of 591 baseline measurements in 2012; second batches of 1943 follow-up samples in 2018) in thawed serum samples in the same laboratory using Roche immunoturbidimetric assays and electrochemiluminescence immunoassays, respectively (Roche Diagnostics, Basel, Switzerland). Samples had not been exposed to a prior freeze-thaw cycle. On average 4.3 measurements per patient were available. The lower limits of detection of hs-CRP, hs-TnT and NT-proBNP were 0.30 mg/L, 5 ng/L and 0.6 pmol/L, respectively. Lower limit of blank of hs-TnT was 3 ng/L. For analytical purposes, hs-CRP levels <0.30 mg/L were substituted with 0.15 mg/L, and hs-TnT levels <3 ng/L were substituted with 1.5 ng/L. Elevated levels of hs-CRP and NT-proBNP were defined as; >3.0 mg/L and >14 pmol/L, respectively.

Definition and assessment of endpoints

The primary endpoint was defined as all-cause mortality or HF (defined as HF requiring initiation/intensification of HF medication, or requiring hospitalisation). The secondary endpoint was defined as any major adverse cardiovascular event (MACE) and was composed of; all-cause mortality, HF, hospitalisation for cardiac reasons (eg, endocarditis), arrhythmia (symptomatic and recorded, or requiring treatment), thromboembolic events or cardiac (re-)interventions (surgical or percutaneous). Survival status was checked in the Municipal Population Register. Endpoint events were adjudicated by two investigators who were blinded for any study related information.

Statistical analysis

Sample size calculation was performed and has been previously been described[9]. In the current study, the composite endpoint of death or HF was considered the primary endpoint because of its greater clinical relevance than any MACE.

Continuous data are presented as mean±SD or median (IQR). Trends between clinical characteristics and hs-CRP tertiles were tested with the χ^2 Mantel-Haenszel test for trend or linear regression. Skewed biomarker distributions were transformed by taking logarithms to the base 2. The variability of hs-CRP and reference change value (RCV) were assessed in primary endpoint-free patients. The methods are specified in Supplemental File 1.

Survival curves were derived using the Kaplan-Meier method, and were stratified based on elevated NT-proBNP and hs-CRP levels. The log-rank test was used to compare survival curves. Cox regression analyses were performed to investigate the association between baseline hs-CRP and the endpoints. We present crude HRs, and HRs that are adjusted for NT-proBNP, hs-TnT and

baseline characteristics including age, sex, congenital diagnosis, New York Heart Association (NYHA) class, cardiac medication and systemic ventricular function.

The temporal evolution of hs-CRP was described using linear mixed effect models (LME) and Cox models were used to assess the association between hs-CRP and the endpoints. The LME model is described in Supplemental File 2. Parameters of LME and Cox models were estimated jointly in Joint Models to relate biomarker trajectories with endpoints, and avoid bias[12]. The rational and assumptions of Joint Models are outlined in Supplemental File 3. Hs-CRP measurements that were taken after occurrence of endpoints were discarded in the analyses. We reported crude HRs, and HRs adjusted for repeatedly measured NT-proBNP and hs-TnT [13], and baseline characteristics. Subanalyses were performed to assess the association between hs-CRP and endpoints in patients with elevated baseline NT-proBNP. The C-index was calculated to assess the discriminative value of hs-CRP.

Missing baseline covariates were handled by single imputation. SPSS (version 24) and R statistical software (version 3.6.1, packages mice, Survival, nlme, JMbayes) were used. A two-tailed p-value<0.05 was considered statistically significant.

Results

Baseline characteristics

In 591 patients (98.2%) hs-CRP was measured at baseline and was therefore included in the current analysis. Median age was 33 (IQR 25-41) years, 58% of the patients were men and 90% were NYHA class I (Table 1). The most prevalent congenital diagnoses were: Tetralogy of Fallot (n=174, 29%), aortic stenosis (n=136, 23%) and aortic coarctation (n=110, 19%).

Median hs-CRP level was 1.50 (IQR 0.60-3.50) mg/L. Hs-CRP was below the limit of detection in 44 patients (7.4%), and 164 patients (28%) had elevated hs-CRP. Higher hs-CRP was associated with older age, female sex, higher body mass index (BMI), cardiac medication use, and NYHA class. Highest hs-CRP levels were found in patients with a functionally univentricular heart and those with pulmonary arterial hypertension (PAH) (Figure 1). None of the echocardiographic measurements were related to hs-CRP, except for measures of left ventricular diastolic function (E/A ratio, E' wave and E/E' ratio). Hs-CRP showed significant, but weak, correlations with NT-proBNP (Spearman r=0.28, p<0.001) and eGFR (Spearman r=-0.14, p=0.001, but not with hs-TnT (Spearman r=0.04, p=0.323).

Follow-up and associations between baseline hs-CRP and endpoints

Follow-up data were complete in 586 patients (99.2%). Median follow-up was 5.9 (IQR 5.3-6.3) years. Death or HF occurred in 69 patients (11.8%) and MACE in 228 patients (38.9%). Occurrence of individual endpoint events was as follows: 25 deaths, 59 HF, 127 arrhythmias, 180 hospitalisations (of which n=13 endocarditis), 137 (re-)interventions and 29 thromboembolic events.

Table 1. Baseline characteristics of the study cohort for all patients and categorized according to the tertile distribution of hs-CRP.

	All	Hs-CRP <0.80 mg/L	Hs-CRP 0.80-2.50 mg/L	Hs-CRP >2.50 mg/L	p-value	% missing
Number of patients	*591*	*200*	*200*	*191*		
Clinical characteristics						
Age, years	33 [25-41]	31 [23-40]	33 [25-42]	34 [26-42]	0.002	0.0
Sex, men	343 (58)	139 (70)	120 (60)	84 (44)	<0.001	0.0
Surgical repair	537 (91)	184 (92.0)	185 (92.5)	168 (88.0)	0.248	0.0
Age at surgical repair, years	3.9 [0.8-12.0]	2.9 [0.7-11.3]	4.1 [1.0-14.4]	4.2 [0.8-11.2]	0.730	0.0
Mechanical valve	62 (9)	13 (7)	27 (14)	22 (12)	0.063	0.0
Pacemaker	37 (3)	6 (3)	16 (8)	15 (8)	0.065	0.0
ICD	20 (3)	6 (3)	8 (4)	6 (3)	0.837	0.0
Cardiac medication use*	211 (36)	48 (24)	83 (42)	80 (42)	<0.001	0.0
Body mass index, kg/m^2	24.8±4.4	23.3±3.8	24.7±3.8	26.4±5.0	<0.001	0.5
Heart rate, beats/minute	74 ±13.4	73±13	71±13	77±14	0.016	1.4
Systolic blood pressure, mmHg	126±16	126±16	125±15	128±18	0.175	1.9
O2 saturation <90%	17 (3)	4 (2)	4 (2)	9 (5)	0.114	7.4
NYHA class, II or III	61 (10)	15 (8)	19 (10)	27 (14)	0.032	0.0
Electrocardiography						
Rhythm					0.360§	0.0
Sinus	510 (86)	178 (89)	171 (86)	161 (84)		
Paced	44	10 (5)	18 (9)	16 (8)		
Atrial fibrillation	15	3 (1)	6 (3)	6 (5)		
Other	22	9 (5)	5 (2)	8 (4)		
QRS duration, ms	112[100-137]	114 [101-144]	112 [101-134]	111 [97-135]	0.124	7.4
Echocardiography						
Left atrial volume, mL/m^2†	20.7[15.7-29.2]	21.5 [14.9-27.9]	20.2 [16.1-30.8]	20.5 [16.3-28.6]	0.881	28.4
LV end-diastolic volume, mL/m^2†	63.4±18.8	62.3±15.8	66.5±21.8	61.2±18.0	0.737	32.1
LV ejection fraction, %†	56.0±7.8	56.7±7.0	55.7±7.9	55.7±8.6	0.305	32.3
Right ventricular fractional area change, %	38.2±11.3	38.6±10.8	38.4±12.0	37.7±11.2	0.511	35.7
Systemic ventricular function					0.408	0.0
Normal	298 (50)	107 (53)	95 (48)	96 (50)		
Mildly impaired	206 (35)	69 (35)	69 (35)	68 (36)		
Moderately impaired	69 (12)	21 (10)	26 (13)	22 (11)		
Severely impaired	18 (3)	3 (2)	10 (5)	5 (3)		
E/A ratio	1.6±0.7	1.7±0.6	1.7±0.8	1.5±0.6	0.023	27.1

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	All	Hs-CRP <0.80 mg/L	Hs-CRP 0.80-2.50 mg/L	Hs-CRP >2.50 mg/L	p-value	% missing
E' wave, m/s	8.2±2.6	8.5±2.4	8.3±2.8	7.9±2.6	0.039	34.5
E/E' ratio	11.6±5.1	10.8±4.1	11.6±5.3	12.5±5.7	0.007	35.7
Severe valvular dysfunction‡	84 (14.5)	25 (13)	23 (12)	36 (19)	0.068	1.9
Laboratory results						
eGFR, mL/min/1.73m^2	90 [82-90]	90 [86-90]	90 [81-90]	90 [80- 90]	0.002	1.5
Hemoglobin, mmol Fe/L	9.22±0.99	9.39±0.83	9.21±0.94	9.06±1.15	0.001	1.5
NT-proBNP, pmol/L	15 [7-33]	10 [5-22]	15 [7-37]	24 [11-40]	<0.001	0.7
Hs-TnT, ng/L	4.3 [1.5- 7.2]	4.0 [1.5-6.5]	4.6 [1.5-7.6]	4.3 [1.5-7.6]	0.068	0.3

Categorical variables are presented as number (%) and continuous variables as mean ± sd or median [inter quartile range]. *Angiotensin-converting-enzyme inhibitors, angiotensin 2 receptor blockers, beta-blocker, diuretics, calcium blockers or anti-arrhythmic drugs. †Left-sided volumes were not measured in patients with a systemic right ventricle, univentricular heart, pulmonary hypertension or a poor acoustic window. ‡Defined as maximal aortic or pulmonary valve velocity > 4.0 m/s; grade 3 or 4 out of 4 aortic, pulmonary or mitral valve regurgitation; or grade 4 out of 4 tricuspid valve regurgitation. §p-value for comparison of sinus rhythm vs no sinus rhythm.
Hs-CRP= high-sensitivity C-reactive protein, NYHA= New York Heart Association, LV= left ventricular, eGFR=estimated glomerular filtration rate, NT-proBNP= N-terminal pro B-type natriuretic peptide, hs-TnT= high-sensitivity troponin T.

Patients in the first hs-CRP tertile (<0.80 mg/L) had a significant better HF-free and MACE-free survival than patients in higher tertiles (Supplemental Figure 1). Analysed continuously, hs-CRP was associated with death or HF (HR 1.36, 95% CI 1.19-1.55, C-index 0.66), also after adjustment for baseline NT-proBNP, hs-TnT and baseline clinical characteristics (Table 2). Hs-CRP was also associated with death and HF when analysed as separate events (Supplemental Figure 2).

Patients with both elevated baseline hs-CRP and NT-proBNP, had the worst HF-free survival (Figure 2).

Associations between repeated hs-CRP and endpoints

In total, 2421 hs-CRP measurements were available prior to the primary endpoint. Baseline hs-CRP was higher in patients with the primary endpoint (2.53 mg/L) than those who remained endpoint-free (1.29 mg/L) (p<0.001). Hs-CRP further increased in anticipation to death or incident HF, whereas endpoint-free patients showed stable hs-CRP levels throughout follow-up (Figure 3). The average 4-year hs-CRP increase in patients with the primary endpoint was 3.09 mg/L, vs 0.07 mg/L in stable patients. In relation to MACE, no difference was found in hs-CRP evolution.

Any time during follow-up, each twofold higher hs-CRP was associated with a 2.14 times higher hazard of mortality or HF (C-index 0.82) (Table 3). This remained significant after

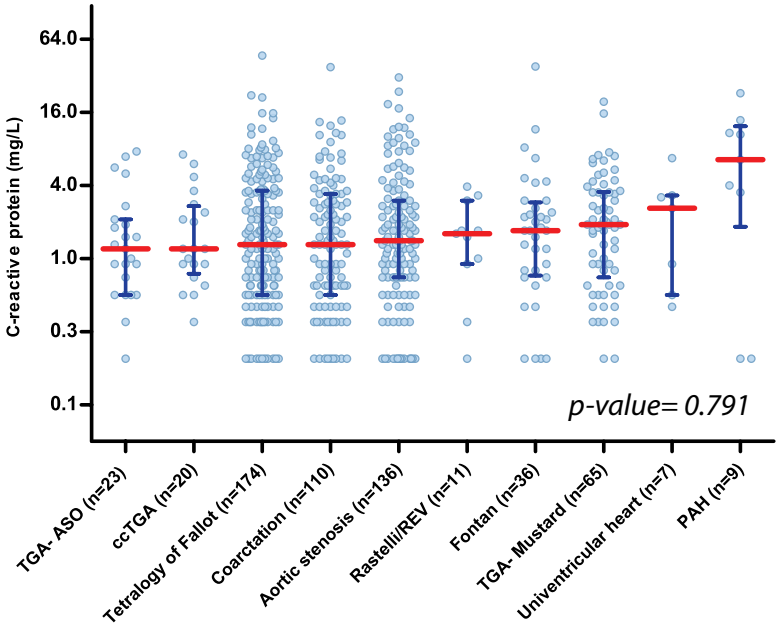

Figure 1. Hs-CRP levels according to congenital diagnosis.
The median hs-CRP level in each diagnosis group is indicated by the horizontal red line together with the 25th and 75th percentile presented by the blue bars. P-value is given for comparison of hs-CRP levels between diagnoses (for the analysis univentricular heart was combined with Fontan, and Rastelli/REV with PAH). TGA-ASO= transpostion of the great arteries corrected by the arterial switch operation, ccTGA= congenitally corrected transpostion of the great arteries, TGA-Mustard= transposition of the great arteries corrected by the Mustard procedure.

Table 2. Association between baseline hs-CRP levels and the endpoints, adjusted for clinical characteristics, NT-proBNP and hs-TnT.

	Death or HF (n=69)		**MACE (n=228)**	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Baseline hs-CRP, mg/L *(unadjusted)*	1.36 (1.19-1.55)	<0.001	1.08 (1.00-1.16)	0.038
Adjusted for:				
Baseline NT-proBNP	1.19 (1.04-1.37)	0.014	1.00 (0.92-1.07)	0.910
Baseline hs-TnT	1.35 (1.18-1.56)	<0.001	1.08 (1.00-1.16)	0.059
Baseline characteristics*	1.21 (1.05-1.40)	0.008	1.02 (0.95-1.10)	0.587
Baseline characteristics* and NT-proBNP	1.18 (1.03-1.35)	0.016	1.00 (0.93-1.08)	0.989
Baseline characteristics* and hs-TnT	1.18 (1.03-1.35)	0.018	1.02 (0.95-1.10)	0.594
Baseline characteristics* NT-proBNP and hs-TnT	1.17 (1.02-1.34)	0.023	1.00 (0.93-1.08)	0.987

Hazard ratios are expressed per two-fold higher biomarker level. *age, sex, congenital diagnosis (aortic stenosis, aortic coarctation, transposition of the great arteries operated by arterial switch procedure vs the other diagnoses), NYHA (NYHA I vs NYHA II/III), cardiac medication use (no vs yes), systemic ventricular function (0-3). hs-CRP= high-sensitivity C-reactive protein, NT-proBNP= N-terminal pro B type natriuretic peptide, hs-TnT= high-sensitivity troponin T, HF= heart failure, MACE= major adverse cardiac event, HR= hazard ratio.

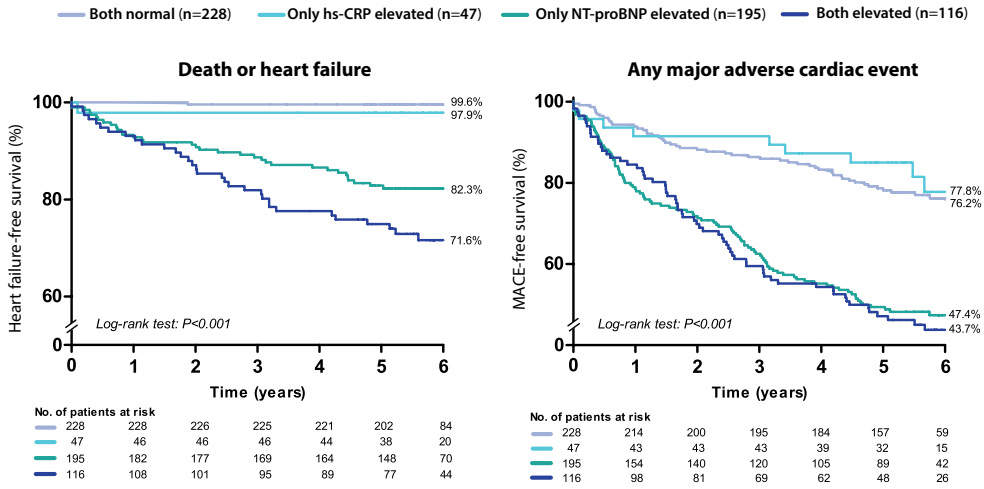

Figure 2. Heart failure-free and MACE-free survival according to normal or elevated baseline levels of hs-CRP and NT-proBNP.
Elevated hs-CRP was defined as >3 mg/L and elevated NT-proBNP as >14 pmol/L.

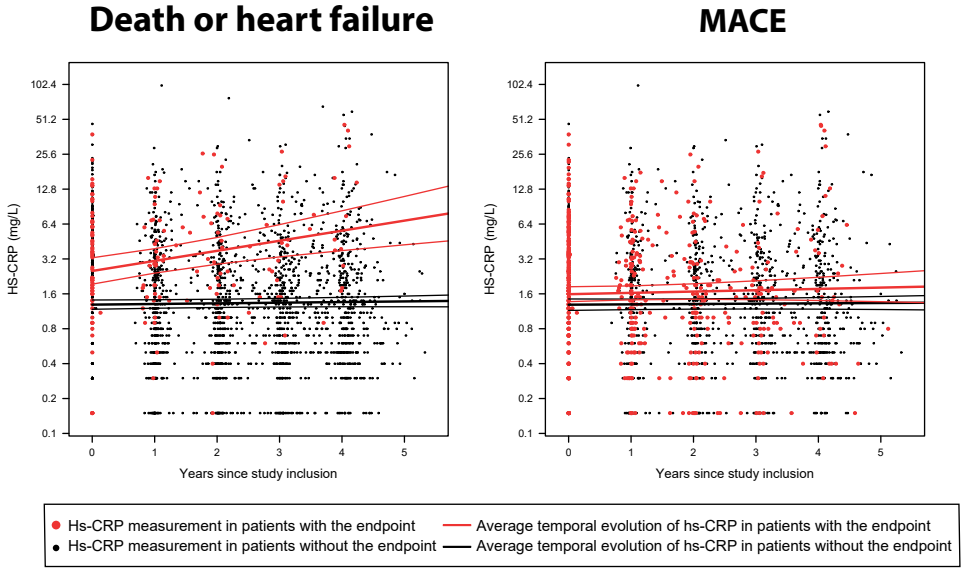

Figure 3. Average evolution of hs-CRP levels in ACHD patients with and without the endpoint.
All measurements taken after the endpoints were discarded. Time point zero is denoted as the time of study inclusion. The average evolution is accompanied by the 95% prediction band (thin lines).

Table 3. Association between repeated hs-CRP levels and endpoints, adjusted for clinical characteristics and (repeated) NT-proBNP and hs-TnT

	Death or HF (n=69)		Any MACE (n=228)	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Repeated hs-CRP, mg/L *(unadjusted)*	2.14 (1.73-2.73)	<0.001	1.22 (1.10-1.37)	<0.001
Adjusted for baseline characteristics*	1.77 (1.38-2.31)	<0.001	1.08 (0.96-1.22)	0.230
Adjusted for baseline NT-proBNP	1.68 (1.35-2.10)	<0.001	1.05 (0.94-1.18)	0.397
Adjusted for baseline characteristics and baseline NT-proBNP	1.60 (1.26-2.05)	<0.001	1.03 (0.92-1.16)	0.585
Repeated hs-CRP and NT-proBNP				
Repeated hs-CRP, mg/L	1.53 (1.20-2.00)	0.002	0.99 (0.88-1.12)	0.916
Repeated NT-proBNP, pmol/L	2.48 (2.04-2.98)	<0.001	1.62 (1.48-1.76)	<0.001
Repeated hs-CRP and hs-TnT				
Repeated hs-CRP, mg/L	1.94 (1.52-2.50)	<0.001	1.13 (1.00-1.27)	0.044
Repeated hs-TnT, ng/mL	2.46 (1.93-3.15)	<0.001	1.57 (1.38-1.78)	<0.001
Repeated hs-CRP, hs-TnT and NT-proBNP				
Repeated hs-CRP, mg/L	1.54 (1.24-1.98)	<0.001	1.00 (0.89-1.12)	0.984
Repeated hs-TnT, ng/mL	1.19 (0.85-1.57)	0.248	1.11 (0.96-1.28)	0.146
Repeated NT-proBNP, pmol/L	2.20 (1.74-2.86)	<0.001	1.53 (1.39-1.69)	<0.001

Hazard ratios are expressed per two-fold higher biomarker level. *age, sex, congenital diagnosis, NYHA, cardiac medication, systemic ventricular function.
hs-CRP= high-sensitivity C-reactive protein, NT-proBNP= N-terminal pro B type natriuretic peptide, hs-TnT= high-sensitivity troponin T, HF= heart failure, MACE= major adverse cardiac event, HR= hazard ratio.

adjustment for repeatedly measured NT-proBNP and hs-TnT, and baseline clinical characteristics. Repeated hs-CRP showed a significant association with MACE, but not when adjusted for baseline characteristics, or other repeatedly measured biomarkers. Associations between repeated hs-CRP and individual endpoints are shown in Supplemental Figure 2.

As posthoc sensitivity analysis, we repeated the analysis after excluding patient with baseline hs-CRP levels >10 mg/L (n=34). This did not result in qualitative meaningful differences in strengths of the associations. Similarly, analysis excluding women who became pregnant during follow-up (n=31) did not lead to different results. Posthoc analysis stratifying for sex showed that repeated hs-CRP was more strongly associated with the risk of death or HF in men than in women (HR 3.95 (95% CI 2.39-8.25) vs 1.55 (95% CI 1.20-2.03), p-value=0.005). This difference was not present when adjusted for NT-proBNP (HR 1.81 (95% CI 1.24-2.78) vs 1.51 (95% CI 1.17-1.99), p-value=0.157).

Subanalysis restricted to patients with elevated baseline NT-proBNP demonstrated that the association between repeatedly measured hs-CRP and the risk of death or HF, is also present in these patients (Supplemental Table 1).

Biological variation of hs-CRP

Based on repeated hs-CRP measurements in primary endpoint-free patients, the within-subject and between-subject variation of hs-CRP were 52% and 113%, respectively. The index of individuality was 0.48, indicating that it may be preferred to have a patient-based reference value based on previously measured hs-CRP levels of the individual patient. The RCV was 149% (lognormal RCV limits -75% to 304%).

Discussion

This study demonstrated that in patients with ACHD higher baseline hs-CRP is associated with a higher risk of death of HF, independent of NT-proBNP and hs-TnT. Furthermore, annually repeated hs-CRP measured up to 4 years, showed that on average hs-CRP levels steadily increased prior to the occurrence of death or HF and remained stable in HF-free patients. As repeated hs-CRP measurements provided prognostic information beyond repeated NT-proBNP and hs-TnT measurements, hs-CRP is a valuable potential biomarker for risk stratification in patients with ACHD.

Comparison with previous studies

In 2018, Opotowsky et al described hs-CRP in a prospective cohort consisting of 707 patients with ACHD[7]. The proportion of patients with elevated hs-CRP (>3 mg/L) in our study was comparable to their study (28% vs 25%), as were study settings on most aspects, except for our cohort not including mild ACHD. This might explain why we found slightly higher hs-CRP levels (median 1.50 vs 1.22 mg/L), despite that our patients were younger, more often NYHA class I and had a lower BMI.

Whereas in our study baseline hs-CRP primarily showed an independent association with all-cause mortality and HF, Opotowsky et al found associations with other adverse cardiac events such as arrhythmia and thromboembolic events. An explanation could be the lower event rate and longer follow-up in our cohort, creating a longer time interval between baseline hs-CRP and event occurrence, probably diluting associations. The fact that repeatedly measured hs-CRP was associated with arrhythmia, reintervention and hospitalisation in our study may support this, as hs-CRP will be measured more closely to the event and therefore better reflects a patients' disease status.

Scognamiglio et al investigated serial CRP measurements retrospectively in 225 adults with PAH due to congenital heart disease. Higher CRP correlated weakly with higher BNP, similar to our study, and was associated a higher mortality risk. While they excluded CRP measurements in patients with signs of active infection, elevated CRP levels (>10 mg/L) were still measured in 54% of the patients at least once during follow-up[14]. Four out of nine patients with PAH in our study had baseline hs-CRP >10 mg/L; one patient had a cold without fever; in the others,

no signs of infection were present. Therefore, elevated hs-CRP seem not restricted to acute infections but may be triggered by their disease state linked to PAH.

Role of inflammation in ACHD

Hs-CRP is an acute phase protein and is commonly used blood test to assess presence of acute inflammation. Improved hs-CRP assays now allow for assessment of chronic, low inflammatory levels in not eminently inflammatory conditions, like ACHD. Findings of our study support evidence regarding chronic inflammation accompanying cardiac deterioration and mortality in patients with ACHD[7, 14, 15]. The question remains whether inflammation induces HF or is rather an 'innocent bystander' and thereon reflects disease severity. Hs-CRP was not associated with measures of right or left ventricular systolic function in our study, while hs-CRP was associated with measures of diastolic dysfunction. This could be explained by age as contributing factor to both increasing hs-CRP and diastolic dysfunction[16]. Nevertheless, other biomarkers that are considered more linked to the HF pathophysiology, such as NT-proBNP and hs-TnT, seem to relate to echocardiographic measurements of cardiac function more closely[9, 17, 18]. In addition, hs-CRP did not correlate with hs-TnT and only weakly with NT-proBNP. Therefore, causal involvement of hs-CRP in the pathophysiology of cardiac deterioration seems less plausible.

Data on whether inflammation are a bystander or a mediator in cardiovascular diseases, in general, remain controversial[19, 20]. In pulmonary arterial smooth muscle cells, known for their role in PAH, it was shown that impediment of inflammation by atorvastatin, reduces hs-CRP-induced expression of inflammatory pathways[21], suggesting causality. Unfortunately, no specific data on ACHD exist. Longitudinal hs-CRP patterns now showed that inflammation increased in patients with ACHD prior to deceasing or HF. Similar patterns have been observed in patients with chronic HF[22]. This at least suggests that progression of HF is associated with increasing inflammatory levels and that stable inflammatory levels are indicative of a stable cardiac status.

Value of hs-CRP as prognostic biomarker in ACHD

There may be several explanations for the independent prognostic value of hs-CRP. First, hs-CRP may reflect other processes of deterioration in ACHD that are not captured by clinical characteristics and biomarkers measured in our study, this makes causal inferences on inflammation and cardiac deterioration difficult, as this requires measurement and adjustment for all other potential confounders. Residual confounding could distort the association between hs-CRP and endpoints, resulting in false conclusions about the relation between inflammation and cardiac deterioration. Nevertheless, hs-CRP can then still be valuable for risk stratification. As hs-CRP is a non-specific inflammatory marker, hs-CRP may identify vulnerable patients in general, and therefore have prognostic value.

The biological variation of hs-CRP found in this study was considerable, which is in line with previous studies [23, 24]. However, both within-subject and between-subject variation were higher and thus the corresponding RCV. This can most likely be explained by differences in

study design: less frequent sampling, less hs-CRP measurements per patient and a different study population.

Clinical implications

Hs-CRP is able to provide prognostic value not captured by other prognosticators. In the absence of another likely cause of inflammation, an elevated hs-CRP (>3 mg/L) may help to detect patients at higher risk of HF or mortality. Hs-CRP may specifically be useful to detect vulnerable patients in copresence of an elevated NT-proBNP. Measuring hs-CRP should therefore be considered in addition to the current practice of NT-proBNP in patients with ACHD. Repeated NT-proBNP can be combined with repeated hs-CRP to further enhance risk stratification. Increasing hs-CRP levels over time can identify high-risk patients who require closer monitoring, while stable levels can identify low-risk patients who can be reassured and less frequently monitored. Nonetheless, the substantial biological variation and high RCV should be kept in mind when interpreting repeated hs-CRP measurements.

If hs-CRP is induced by low-grade inflammation of an extracardiac origin, that subsequently leads to cardiac deterioration, inflammation in general may have substantial negative influences on the prognosis of patients with ACHD. For clinical practice, this would indicate that attention should be paid to inflammatory diseases in ACHD, as this might trigger HF.

Last, inflammation may be a potential therapeutic target; however, further research is needed to elucidate the inflammatory mechanism in ACHD.

Limitations

We were not able to definitely rule out concomitant inflammatory processes which may have led to increasing hs-CRP levels. The association found in our study might therefore be interfered by occurrence of acute infections in some patients. However, we included stable patients with ACHD in who hs-CRP was measured during regular study visits, making the interference of infections with their prognosis and biomarker measurement very unlikely. Also, documented baseline chronic infections and inflammatory conditions were rare; 10 patients carried a rheumatologic diagnosis, 2 patients inflammatory bowel disease, 1 latent tuberculosis, and 1 cystic fibrosis. However, we had no data on depression, while depression has been associated with inflammation and poorer prognosis in ACHD[25].

We could not adjust for oral contraceptive or hormonal replacement use. This may have biased the associations, as hormonal replacement therapy is known to be associated with higher hs-CRP levels[26] and it can be questioned whether oral contraception use could have increased the risk of certain cardiovascular events in women. Peak VO_2 and peak heart rate have been associated with increased hs-CRP levels[7], as we did not have exercise data, we could not assess the prognostic value of hs-CRP beyond these variables.

Conclusion

In clinically stable patients with ACHD, an elevated hs-CRP in combination with an elevated NT-proBNP is associated with an increased risk of death or HF. Hs-CRP levels increased over time prior to the occurrence of HF or death, and serially repeated hs-CRP measurements provided additional prognostic information over repeated NT-proBNP measurements. This study shows that hs-CRP is a potential biomarker for risk stratification in patients with ACHD.

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Supplemental File 1. Method for assessing the variability of repeatedly measured hs-CRP in adults with congenital heart disease.

The variability of hs-CRP was investigated in the subset of the patients that remained free of the primary endpoint en who had at least 2 hs-CRP measurements (n= 495). We first calculated the coefficient of variation (CV) as follows;

$$\mathrm{CV} = 100\% * \mathrm{sd}/\overline{\mathrm{X}}$$

Thereafter, the method described by Fraser and Harris[1] to assess the variation of a set of repeated measurements was used to further investigate the variability of hs-CRP measurements. According to this method, the total variation can be distinguished into three components; the analytical variation (CV_a) due to the analytical measurement imprecision, the within-subject variation (CV_i), and the between-subject variation (CV_g). The CV_a of hs-CRP in our laboratory was estimated at 12% and 8.6% for low and high concentrations, respectively.

$$\mathrm{CV_i} = \mathrm{median}\sqrt{(\mathrm{CV}^2_{\mathrm{subject}}) - \mathrm{CV}^2_{\mathrm{a}}}$$

$$\mathrm{CV_g} = 100\% * \mathrm{sd}_{\overline{\mathrm{x}}_{\mathrm{subject}}}/\overline{\mathrm{X}}_{\mathrm{group}}$$

The index of individuality (II) was calculated accordingly:

$$\mathrm{II} = \sqrt{\mathrm{CV}^2_{\mathrm{i}} + \mathrm{CV}^2_{\mathrm{a}}}/\mathrm{CV_g}$$

Lastly, the reference change value (RCV) was calculated. Because hs-CRP was not normally distributed, the lognormal approach was used to calculate the RCV limits[2]. We used $\alpha = 0.05$ (95% confidence), corresponding to $Z_{0.025} = 1.96$.

$$\mathrm{RCV} = \mathrm{Z}_{\alpha/2} * \sqrt{2(\mathrm{CV}^2_{\mathrm{i}} + \mathrm{CV}^2_{\mathrm{a}})}$$

$$\mathrm{RCV_{low}} = \mathrm{e}^{-\mathrm{Z}_{\alpha/2}*\sqrt{2\ln(\mathrm{CV}^2_{\mathrm{w}}+\mathrm{CV}^2_{\mathrm{a}}+1)}} - 1$$

$$\mathrm{RCV_{up}} = \mathrm{e}^{\mathrm{Z}_{\alpha/2}*\sqrt{2\ln(\mathrm{CV}^2_{\mathrm{w}}+\mathrm{CV}^2_{\mathrm{a}}+1)}} - 1$$

References:

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Supplemental File 2. Specification of the linear mixed effects model.

All variables considered relevant to describe the evolution of hs-CRP over time were included as fixed effects. The fixed effects consisted of repeated hs-CRP, time, diagnosis, sex, age, NYHA class, cardiac medication use, rhythm, systemic ventricular function, body mass index and eGFR. Interactions of time with diagnosis and sex was considered but did not improve model fit. Also, a non-linear term for age was considered using a natural cubic spline with 3 degrees of freedom, but did not improve the model and was therefore left out of the model. The random effects consisted of a non-linear random slope (cubic spline with 3 degrees of freedom) and a random intercept.

Supplemental File 3. Rationale and assumptions of the Joint Model.

The rationale behind the use of Joint Model is based on the fact that blood biomarkers are so-called endogenous time-dependent covariates; they vary dynamically over time, and are directly related to a subject and thus depending on other variables. In addition, the availability of blood biomarkers is related to the survival status of the patient. To account for the special features of these covariates, the use of a Joint Model is recommended for longitudinal and time-to-event data.[1] The concept of a Joint Model is that both models are estimated jointly in such way that the (modelled) value of the longitudinal marker at each point in time is related to the hazard of the endpoint event. One of the features is that the level of the longitudinal marker is not assumed constant between two subsequent visits in this framework.

The key assumption of a Joint Model is Full Conditional Independence. The validity of this assumption depends on whether the censoring and planning of visits is non-informative. This means that patients decision to withdraw from the study or the patient's appearance for the next study visit may depend on observed history of the patients (baseline covariates and observed longitudinal measures), however it may not depend on underlying latent patient characteristics associated with prognosis. There is no statistical test to validate this assumption, but its validity is based on the design of the study. Likewise Linear Mixed Effects models, the residuals of the Joint Model should be approximately normally distributed. After fitting each Joint Model, normality of residuals were checked by QQ-plots. No relevant deviation of normality was observed.

Reference:

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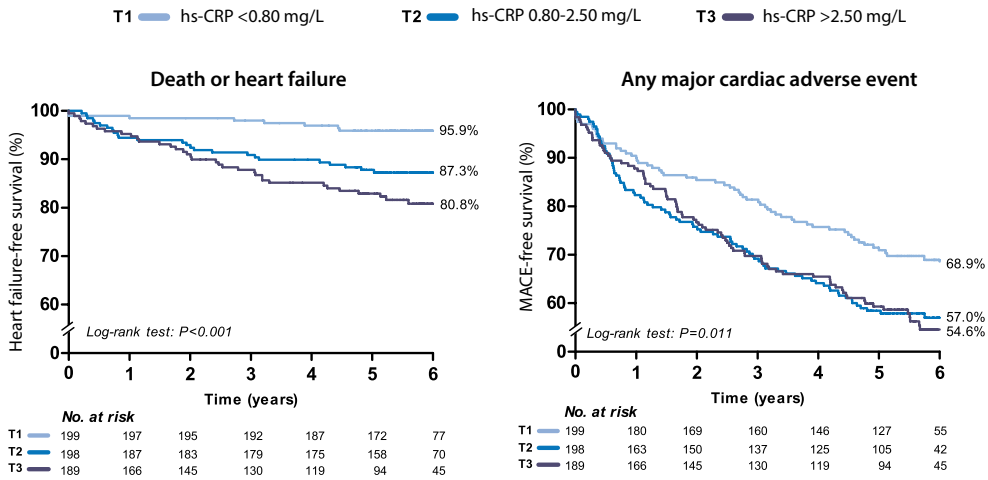
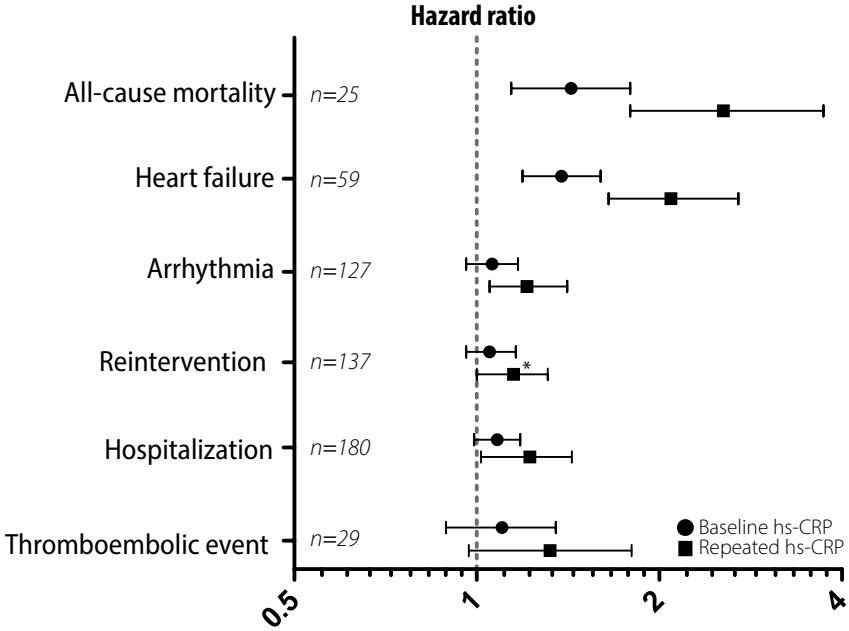


Supplemental Figure 1. Survival according to the tertile hs-CRP distribution for the primary (death or heart failure) and secondary endpoint (any major cardiac adverse event) in adults with congenital heart disease.
Survival curves were compard with the log-rankt test for trend. hs-CRP= high-sensitivity C-reactive protein, T1=tertile 1, T2= tertile 2, T3= tertile 3.



Supplemental Figure 2. Univariable associations of baseline and repeated hs-CRP with the individual event components of the endpoints in adults with congenital heart disease.
*p-value <0.05. Hazard ratios are expressed per two-fold higher CRP level. Occurrence of the number of each individual endpoints reached in the study is given for each event.

Supplemental Table 1. Subanalysis in patients with an elevated NT-proBNP >14 pmol/L (n=312) for the association between hs-CRP and all-cause mortality or heart failure.

	Death or heart failure	
	HR (95% CI)	**p-value**
Baseline hs-CRP	1.22 (1.06-1.42)	0.007
Adjusted for baseline characteristics*	1.16 (1.01-1.34)	0.035
Adjusted for baseline NT-proBNP	1.18 (1.02-1.35)	0.026
Adjusted for baseline characteristics* and baseline NT-proBNP	1.16 (1.01-1.33)	0.034
Repeated hs-CRP *(unadjusted)*	1.69 (1.36-2.11)	<0.001
Adjusted for baseline characteristics*	1.53 (1.20-1.98)	<0.001
Adjusted for baseline NT-proBNP	1.58 (1.26-2.00)	<0.001
Adjusted for baseline characteristics* and baseline NT-proBNP	1.48 (1.18-1.89)	<0.001
Repeated hs-CRP and NT-proBNP		
Repeated hs-CRP	1.51 (1.18-1.94)	<0.001
Repeated NT-proBNP	2.29 (1.90-2.75)	<0.001

*age, sex, congenital diagnosis, NYHA, cardiac medication, systemic ventricular function. The endpoint of death or heart failure was met in 67 (21.4%) patients. HRs are expressed per two-fold higher biomarker level. Hs-CRP= high-sensitivity C-reactive protein, HR=hazard ratio, NT-proBNP= N-terminal pro B-type natriuretic peptide.

CHAPTER 5

Evolution of blood biomarker levels following percutaneous atrial septal defect closure in adults

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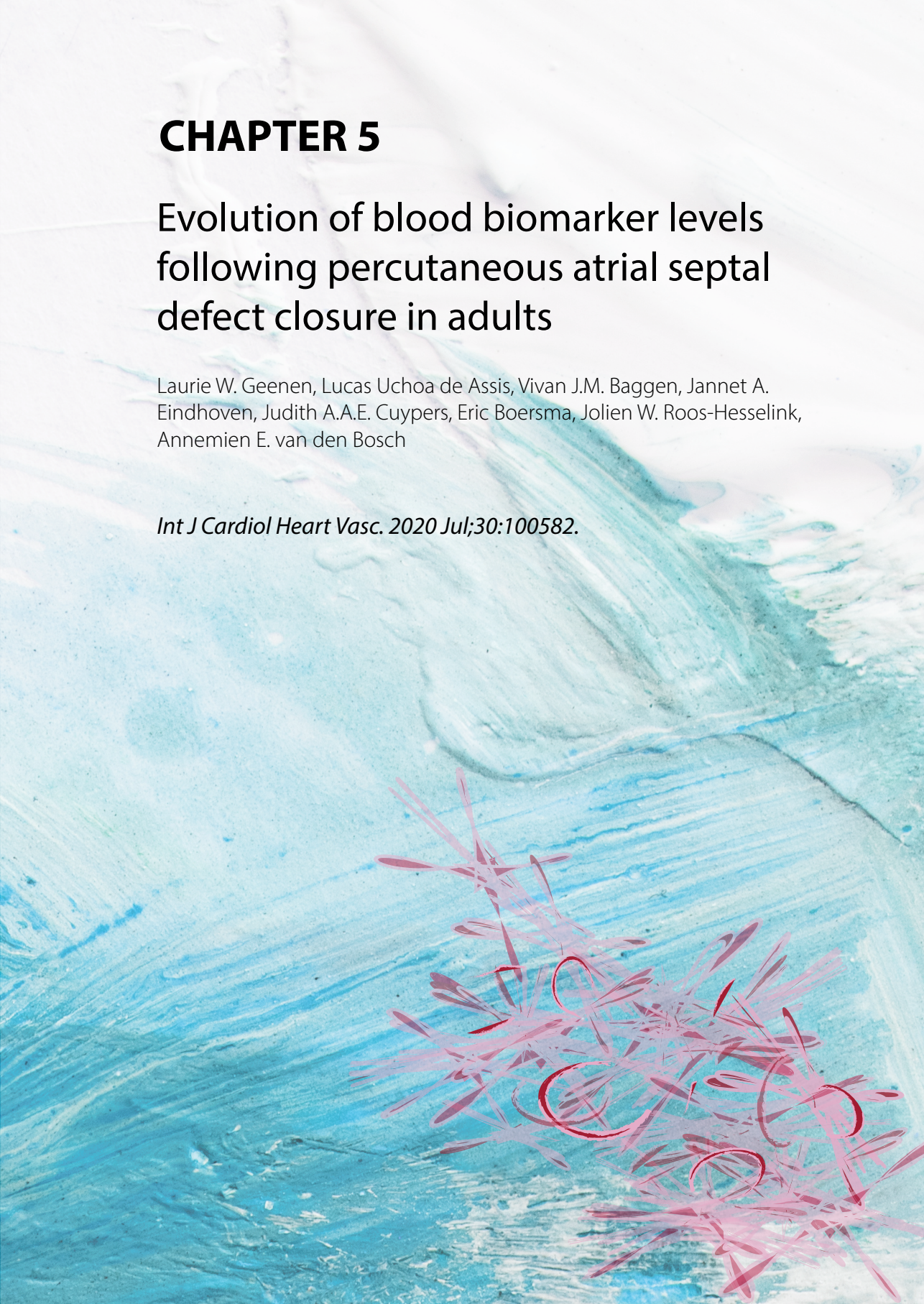

Abstract

Background: We sought to assess the effects of percutaneous atrial septal defect (ASD) closure on blood biomarker levels that possibly reflect reverse cardiac remodeling. Therefore, this study investigated temporal changes in six blood biomarkers following percutaneous ASD closure in adults.

Methods: In this prospective observational cohort study, adults with ASD type II scheduled for percutaneous closure were included (2012-2016). NT-proBNP, high-sensitive troponin-T (hs-TnT), high-sensitive C-reactive protein (hs-CRP), red blood cell distribution width (RDW), growth differentiation factor-15 (GDF-15) and galectin-3 were measured one day prior to ASD closure and one day, three months and one year post ASD closure, and changes were evaluated using paired T-tests. Echocardiographic measurements were obtained.

Results: Fifty patients were included (median age 50 years, 62% women, 32% NYHA II). At baseline, biomarker levels were elevated in a substantial number of patients; NT-proBNP n=22 (45%), hs-TnT n=6 (13%), hs-CRP n=19 (40%), galectin-3 n=5 (11%) and GDF n=10 (23%). One day after ASD closure, significant increases of hs-TnT (median change (Δ) =12 ng/L), hs-CRP (Δ=1.9 mg/L), GDF-15 (Δ=129 pg/mL) and RDW (Δ=0.1%) were observed, and a decrease in galectin-3 (Δ=-1.0 ng/mL). Consequently, 92% had at least one abnormal biomarker directly after closure. At three months biomarker levels returned to baseline, and while echocardiographic measures 1 year post closure were indicative of reverse cardiac remodeling, biomarker levels did not further decrease.

Conclusion: Percutaneous ASD closure in adults leads to a direct increase in most blood biomarkers, in particular hs-CRP and hs-TnT. After three months, biomarkers returned to baseline levels and remained stable up to one year.

Introduction

Atrial septal defect (ASD) accounts for approximately 13% of all congenital heart defects and is the second most prevalent congenital heart defect[1]. Although ASDs are often detected and closed during childhood, it is not uncommon that ASD's remain undiagnosed and clinically manifest at adult age. Long-term persistence of left-to-right shunts can trigger adverse cardiac remodeling including right ventricular dilatation and dysfunction, and increased right-sided cardiac pressures[2]. In some cases, systemic-to-pulmonary shunts affect the pulmonary vasculature leading to pulmonary arterial hypertension (PAH)[3]. PAH is associated with a substantial decrease in life expectancy[4] and ASD closure is therefore indicated in patients with a hemodynamic relevant ASD[5]. However, some of the patients that undergo ASD closure at adult age, still develop PAH[6].

ASD closure can lead to reverse cardiac remodeling, including improvement in left ventricular (LV) function and decreases in right ventricular dimensions and pressures[7], accompanied by an improvement in exercise capacity[8] and NYHA class[9]. However, in older patients, LV diastolic dysfunction may cause development of acute congestive left heart failure following ASD closure as a result of the sudden flow increase[10]. Better comprehension of the pathophysiological consequences and reverse cardiac remodeling after ASD closure at advanced age, may be helpful to adjust monitoring and determine follow-up strategies of patients after ASD closure.

Blood biomarkers can be of help to gain insight in cardiac remodeling after ASD closure by reflection of certain biological processes, such as inflammation or myocardial injury. N-terminal pro-B natriuretic peptide (NT-proBNP) is released in response to increased cardiac wall stress, troponin-T and C-reactive protein (CRP) are indicative of cardiac myocyte injury and inflammation, respectively. Also novel blood biomarkers such as growth differentiation factor-15 (GDF-15) and galectin-3 have been linked to the pathophysiology and progression of heart failure[11]. While NT-proBNP and troponins have been investigated in the light of ASD closure in adults, very limited data is available on other biomarker responses following ASD closure. This study aimed to investigate the temporal changes of NT-proBNP, high sensitivity troponin-T (hs-TnT), high sensitivity CRP (hs-CRP), red cell distribution width (RDW), galectin-3, and GDF-15 following percutaneous ASD closure in adults.

Methods

Study design and population

This is a single-center prospective observational cohort study. Adult patients with an ASD type II scheduled for percutaneous ASD closure at our center were enrolled between March 2012 and December 2016. Patients were excluded if they lived abroad, had renal dysfunction (estimated glomerular filtration rate <30 ml/min/1.73m^2), or if patients had switched to a surgical ASD

closure procedure. The study protocol was approved by the medical ethical committee and written informed consent was provided by all patients. The study was performed in accordance with the principles outlined in the Declaration of Helsinki.

Baseline was defined as the visit scheduled prior to ASD closure. Physical examination, 12-lead electrocardiography, echocardiography and venous blood sampling were performed. Decision for percutaneous ASD closure was at the discretion of the treating physician following a multi-disciplinary discussion by experts, according to the ESC guidelines[5]. ASD closure was performed using the Amplatzer or Gore Septal device. Hemodynamic measurements were taken during the catheter intervention, prior to defect closure. Physical examination, ECG and venous blood sampling were repeated one day after closure at 3 (range 1-5) months and 12 (range 8-20) months after ASD closure. Two-dimensional trans thoracic echocardiography (TTE) was performed at baseline (before ASD closure) and 12 months after closure using a commercially available ultrasound system (iE33 or Epic, Philips Medical Systems, Best, the Netherlands). Routine echocardiographic indexes including right atrial pressure and right ventricular systolic pressures (RVSP), were measured in accordance with the guidelines[12, 13]. Right atrial and right ventricular measurements were primarily performed in RV-focused views if available, otherwise the four chamber view was used. Echocardiography studies were blinded for biomarker levels.

Biomarker measurements

Blood biomarkers were measured for study purposes only and not used for clinical decision-making. Blood samples were transferred to the clinical chemistry laboratory within 2 hours. NT-proBNP and RDW were measured in fresh blood samples. NT-proBNP was measured using a commercial electrochemiluminescense immunoassay (Roche Diagnostics). The rest of the samples were aliquoted and securely stored at -80 °C in the central Biobank. Hs-TnT, hs-CRP and GDF-15 were measured by batch analyses in thawed serum samples using electrochemiluminescence immunoassays (for hs-TnT and GDF-15) or an immunoturbidimetric assay (for hs-CRP) (Roche Diagnostics, Basel, Switzerland). Lower limits of detection were 3 ng/L for hs-TnT, 0.3 mg/L for hs-CRP and 400 ng/L for GDF-15. Galectin-3 levels were measured using the ARCHITECT chemiluminescent microparticle immunoassay (Abbot Diagnostics, Hoofddorp, the Netherlands) and had a lower limit of quantitation of ≤4.0 ng/mL. The upper limits of normal were defined as >15 pmol/L for NT-proBNP, >14 ng/L for hs-TnT, >3 mg/L for hs-CRP and >16% for RDW. For galectin-3 sex specific reference values were applied; 21.3 ng/mL for women and 16.9 ng/mL for men[14]. For GDF-15 an age-specific cut-off was applied; >920 pg/mL for patients aged <50 years and 1330 pg/mL for patients aged >50 years[15].

Statistical analysis

Continuous data are given as mean ± SD or median and interquartile range [IQR]. Baseline characteristics are shown for all patients, and shown for patients below or above the median age in our study separately. Comparison of clinical characteristics between patients below or

above the median age was performed using the unpaired T-test or Mann Whitney U test for continuous variables. For comparison of categorical variables the Fisher Exact test was used. Biomarker levels were 2log transformed for further analysis because of skewed distributions. Correlation between patient characteristics and baseline blood biomarkers were assessed with Pearson or Spearman correlation coefficient, depending on the distribution of the data. Measurements of TTE, ECG and blood biomarkers between two measurement moments (baseline with 1 day post closure, and baseline with 1 year post closure) were compared using the paired T-test if normally distributed, otherwise the related-samples Wilcoxon signed rank test was used. The McNemar test was used to compare paired categorical data between baseline and 1 year post ASD closure. Biomarker levels are presented as boxplots showing median, quartiles and ranges. The median of the differences in biomarker levels in between baseline and one day after ASD closure in each patient is presented as delta (Δ), with the corresponding inter quartile range. A two-sided p-value <0.05 was considered statistically significant. Data was analyzed using SPSS, Version 24.0, for Windows.

Results

Baseline characteristics

A total of 68 patients were screened prior to percutaneous ASD closure, of which 50 patients were enrolled in the study. A flowchart of patient selection is shown in Supplemental Figure 1. Median age was 50 [IQR 38-62] years, 31 patients were women (62%) and 16 patients (32%) patients were NYHA class II (Table 1). Twelve patients (24%) had a history of atrial fibrillation and in 7 of these patients atrial fibrillation was present at baseline ECG. Normal left ventricular systolic function was present in 41 patients (84%). One patient was diagnosed with PAH before ASD closure. Elevated biomarker levels at baseline were present in a substantial proportion of the patients: NT-proBNP in 22 patients (45%), hs-TnT in 6 patients (13%), hs-CRP in 19 patients (40%), galectin-3 in 5 patients (11%), GDF-15 in 10 patients (23%). (Table 1)

Adults above 50 years of age more frequently used cardiac medication, more often had atrial fibrillation and an abnormal left ventricular function, larger left atrial dimensions and higher mean pulmonary artery pressures (mPAP). NT-proBNP, hs-TnT, hs-CRP and GDF-15 levels before closure were significantly higher in patients aged >50 years (Table 1).

Biomarkers and associations with baseline characteristics

Higher NT-proBNP levels were associated with higher NYHA class. Higher levels of NT-proBNP, hs-TnT, RDW and GDF-15 were associated with the presence of atrial fibrillation. Hs-TnT was the only biomarker that showed a significant correlation with QRS duration and PR interval. Most biomarker levels at baseline correlated with the left atrial dimension, right atrial area and RVSP. NT-proBNP, hs-TnT and RDW positively correlated with invasively measured right atrial pressures and mPAP. (Table 2)

Table 1. Baseline characteristics of adult patients before percutaneous closure of the atrial septal defect.

	Complete cases, n (%)	All patients (n=50)	Patients <50 years (n=25)	Patients >50 years (n=25)	p-value
Clinical Characteristics					
Age at closure, years	50 (100)	50 [38-62]	38 [31-46]	62 [58-68]	-
Sex, women	50 (100)	31 (62)	17 (68)	14 (56)	0.561
Body mass index, kg/m^2	50 (100)	26.6 ± 4.8	25.7 ± 5.6	27.5 ± 3.7	0.135
Systolic blood pressure, mmHg	50 (100)	138 ± 20	129 ± 16	147 ± 20	<0.001
NYHA class	50 (100)				0.032
I		34 (68)	21 (84)	13 (52)	
II		16 (32)	4 (16)	12 (48)	
Cardiac medication use*	50 (100)	16 (32%)	0 (0)	16 (64%)	<0.001
Systemic hypertension	50 (100)	9 (18)	0 (0)	9 (36)	0.002
Coronary artery disease	50 (100)	2 (4)	0	2	-
History of atrial fibrillation	50 (100)	12 (24)	1 (4)	11 (44)	0.002
Electrocardiography					
Rhythm	49 (98)				0.023#
Sinus rhythm		41 (84)	24 (96)	17 (71)	
Atrial fibrillation		7 (14)	0 (0)	7 (29)	
Pacemaker rhythm		1 (2)	1 (4)	0	
Heart rate, beats/minute	49 (98)	71 ± 12	71 ± 11	71 ± 14	0.920
PR interval, ms†	41 (100)	171 ± 28	163 ± 26	184 ± 27	0.020
QRS duration, ms	49 (98)	112 ± 22	106 ±20	117 ± 22	0.059
Complete RBBB	49 (98)	10 (20)	2 (8)	8 (32)	
Echocardiography					
Normal LV function	49 (98)	42 (84)	25 (100)	17 (68)	0.004
LV end-diastolic dimension, mm	50 (100)	46.7 ± 6.3	45.9 ± 4.8	47.4 ± 7.5	0.466
E/E'	35 (70)	8.3 [6.7-9.2]	8.3 [7.0-9.9]	8.3 [6.4-8.7]	0.882
E/A	35 (70)	1.1 [1.0-1.4]	1.3 [1.1-1.8]	0.88 [0.78-1.1]	<0.001
Left atrial dimension, mm	49 (98)	40.4 ± 9.6	36.3 ± 5.1	44.6 ± 11.4	0.007
Right atrial area, mm	49 (98)	23.3 [18.7-28.4]	22.3 [18.3-25.5]	25.3 [19.3-37.4]	0.075
RVED area, cm^2	49 (98)	34.9 ± 8.5	33.2 ± 7.2	36.5 ± 9.6	0.242
RVED basal diameter, mm	50 (100)	49.5 ± 6.8	48.6 ± 6.9	50.3 ± 6.8	0.410
RVED apex to base length, mm	49 (98)	85.6 ± 9.2	86.6 ±8.1	84.6 ± 10.3	0.435
RV fractional area change, %	49 (98)	41.0 ± 9.5	42.5 ± 8.5	39.4 ± 10.4	0.280
TAPSE, mm	46 (92)	27.8 ± 5.7	28.7 ±4.4	26.8 ± 6.9	0.316
Right atrial pressure	44 (88)				0.126
3 mmHg (range 0-5 mmHg)		36 (72)	21 (84)	15 (60)	
8 mmHg (range 5-10mmHg)		8 (16)	2 (8)	6 (24)	

Continue

Continued

	Complete cases, n (%)	All patients (n=50)	Patients <50 years (n=25)	Patients >50 years (n=25)	p-value
RV systolic pressure, mmHg	39 (78)	28 [26-38]	26 [24-28]	37 [32-44]	<0.001
Hemodynamics$					
Right atrial pressure, mmHg	41 (82)	7 [6-9]	7 [5-8]	8 [6-12]	0.095
Mean PAP, mmHg	46 (50)	19 [16-24]	17 [16-20]	22 [19-26]	0.007
Biomarker measurements					
NT-proBNP, pmol/L	48 (96)	14 [5-31]	6 [3-15]	26 [10-92]	<0.001
Hs-TnT, ng/L	48 (96)	5 [3-10]	3 [3-5]	7 [5-15]	<0.001
Hs-CRP, mg/L	48 (96)	2.0 [0.9-4.2]	1.2 [0.5-3.7]	2.4 [1.3-5.3]	0.032
RDW, %	48 (96)	12.7 [12.1-13.2]	12.5 [11.9-13.0]	12.9 [12.3-13.4]	0.098
GDF-15, pg/mL	43 (86)	749 [546-1288]	404 [547-622]	1123 [776-1486]	<0.001
Galectin-3, ng/mL	45 (90)	14.2 [11.7-16.2]	13.3 [14.5-14.3]	14.9 [12.3-17.2]	0.082

Values are given in mean ± SD, median [IQR] or n (%). *use of ACE-inhibitor (n=3), angiotensin receptor blockers (n=4), beta blocker (n=13) or diuretics (n=7). #compares sinus rhythm versus any other rhythm †Atrial fibrillation and pacemaker rhythms are not included. $Measured during percutaneous ASD closure procedure.
NYHA= New York Heart Association, RBBB= right bundle branch block, LV= left ventricular, RV= right ventricular, RVED= right ventricular end-diastolic, TAPSE = tricuspid annular plane systolic excursion, PAP= pulmonary artery pressure, NT-proBNP= N-terminal pro-B natriuretic peptide, hs-TnT= high sensitivity troponin-T, hs-CRP= high sensitivity C-reactive protein, RDW= red cell distribution width, GDF-15= Growth differentiation factor-15.

NT-proBNP correlated with all biomarkers except hs-CRP and of all mutual biomarker correlations, the strongest correlation was observed between NT-proBNP and GDF-15 (r=0.74, p<0.001) (Supplemental Table 1).

ASD closure and acute effects on biomarker levels

Percutaneous ASD closure was performed using the Amplatzer device (n=38) or the Gore Septal occluder (n=12), with a median device size of 24 [IQR 18-28] mm. An elevated mPAP (≥25 mmHg) was measured in 11 patients (23%) prior to ASD closure. Eight (16%) patients had a minimal rest shunt after device implantation. One patient underwent device removal due to cardiac tamponade; this patient was excluded from further analysis. No further complications occurred.

One day post ASD closure a significant increase in concentrations of hs-TnT, GDF-15, RDW, hs-CRP and a decrease of galectin-3 was observed (Figure 1 and Supplemental Figure 3). Of note, NT-proBNP was elevated in 26 patients (52%) 1 day post closure, but no significant increase was observed relative to baseline levels. Increases were most pronounced in hs-TnT and hs-CRP; one day after closure 35 (73%) and 30 (63%) patients had an elevated hs-TnT or hs-CRP, respectively. In only 4 patients (8%) none of the biomarkers was elevated one day post closure. No significant differences were found in the acute absolute biomarker changes (levels

Table 2. Correlations between biomarker levels and baseline clinical characteristics as well as ASD closure related characteristics.

	NT-proBNP	hs-TnT	Hs-CRP	RDW	GDF-15	Gal-3
	r	*r*	*r*	*r*	*r*	*r*
Clinical Characteristics						
Age at closure	**0.51*****	**0.74*****	0.25	0.18	**0.75*****	**0.30***
Women	0.16	**-0.55*****	0.07	0.10	-0.09	-0.09
Body mass index	0.14	0.213	0.26	**0.37****	0.12	0.29
Systolic blood pressure	0.09	**0.45****	0.11	**-0.30***	**0.41****	-0.09
NYHA class	**0.37***	0.25	0.21	-0.03	**0.31***	-0.12
Cardiac medication use	**0.52*****	**0.63*****	**0.36***	0.27	**0.57*****	**0.47****
Hypertension	**0.36***	**0.48****	0.25	0.20	**0.41****	0.06
Atrial fibrillation	**0.44****	**0.54*****	0.19	**0.40****	**0.40****	0.26
Electrocardiography						
Heart rate	-0.03	0.00	0.06	-0.11	0.01	-0.27
PR interval †	0.07	**0.40***	0.11	0.07	0.28	0.29
QRS duration	0.01	**0.39****	0.01	0.03	0.25	0.21
Echocardiography						
LV end-diastolic dimension	0.05	0.17	-0.19	0.19	0.09	0.01
E/E'	0.08	0.13	**0.41***	-0.04	0.09	-0.23
E/A	0.12	**-043***	0.00	0.817	-0.35	0.03
Left atrial dimension	**0.54*****	**0.48****	0.15	**0.41****	**0.47****	**0.38****
Right atrial area	**0.45****	**0.35***	0.01	**0.31***	0.25	0.18
RVED area	0.20	0.27	0.05	0.28	0.16	**0.30***
RVED basal diameter	-0.03	0.09	0.15	-0.01	-0.01	0.08
RVED apex to base length	-0.26	0.08	-0.24	-0.15	-.011	-.010
RV fractional area change	-0.20	-0.19	0.19	-0.00	-0.21	0.05
TAPSE	**-0.34***	-0.18	0.14	-0.10	-0.17	0.00
Right atrial pressure of 8 mmHg	**0.47****	0.29	0.09	**0.34***	0.30	0.18
RV systolic pressure, mmHg	**0.55*****	**0.47****	**0.37***	0.21	**0.47****	0.29
Hemodynamics						
Right atrial pressure	0.26	-0.05	-0.04	**0.43****	0.16	0.15
Mean PAP	**0.49****	**0.37***	0.27	**0.37***	0.21	0.29
Procedural characteristics						
Diameter of ASD, balloon	0.27	0.04	0.26	0.31	-0.01	0.23
Diameter of ASD, echo	0.03	-0.02	-0.25	0.08	-0.02	0.04
Device size	0.05	0.07	0.11	-0.16	0.14	0.07
Device size, bsa indexed	0.08	-0.05	0.13	-0.19	0.14	0.14

Significant correlations are printed in bold with the corresponding level of significance shown by the number of asterisks (*p-value < 0.05, **p-value < 0.01, *** p-value <0.001).
NYHA= New York Heart Association, RBBB= right bundle branch block, LV= left ventricular, RV= right ventricular, RVED= right ventricular end-diastolic, TAPSE = tricuspid annular plane systolic excursion, PAP= pulmonary artery pressure, NT-proBNP= N-terminal pro-B natriuretic peptide, hs-TnT= high sensitivity troponin-T, hs-CRP= high sensitivity C-reactive protein, RDW= red cell distribution width, GDF-15= growth differentiation factor-15, gal-3= galectin-3.

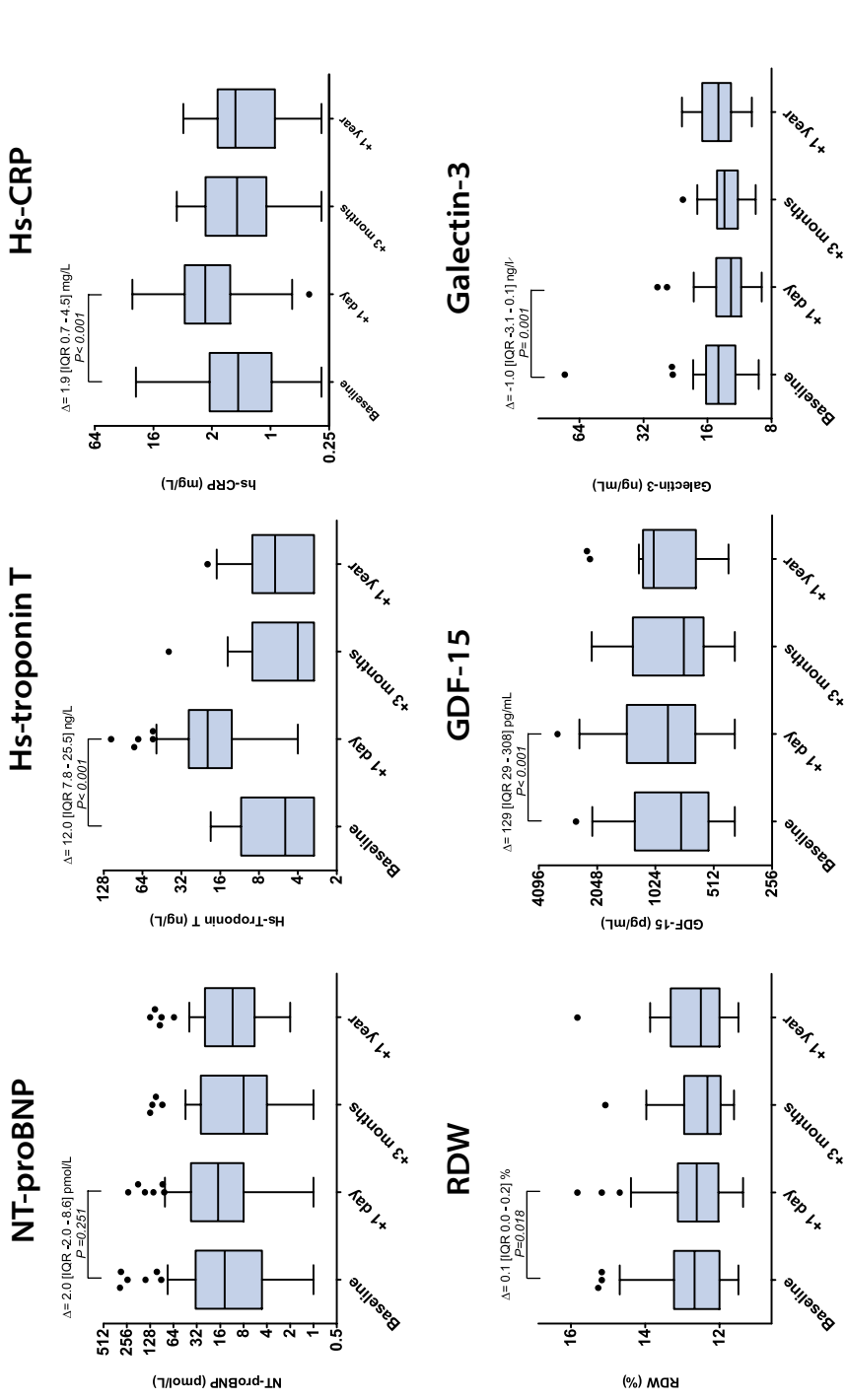

Figure 1. Serial biomarker levels before closure (baseline) and 1 day, 3 months and 1 year after percutaneous ASD closure in adults.
X-axis are on the 2-log scale. Delta (Δ) represents the median of the difference in the biomarker level before and one day ASD after closure in. P-value represents level of significance of the paired t-test.
NT-proBNP= N-terminal pro B type natriuretic peptide, GDF-15= growth differentiation factor 15, RDW= red cell distribution width, hs-CRP= high sensitive c-reactive protein.

one day post ASD closure minus baseline levels) between patients aged <50 years or >50 years (Figure 2).

The acute increase in hs-TnT and hs-CRP showed no significant correlation with mPAP or right atrial pressures before closure and with device disk sizes also after indexing the disk size for BSA. Also no significant differences were observed in the acute biomarker changes and the type of the device used (data not shown).

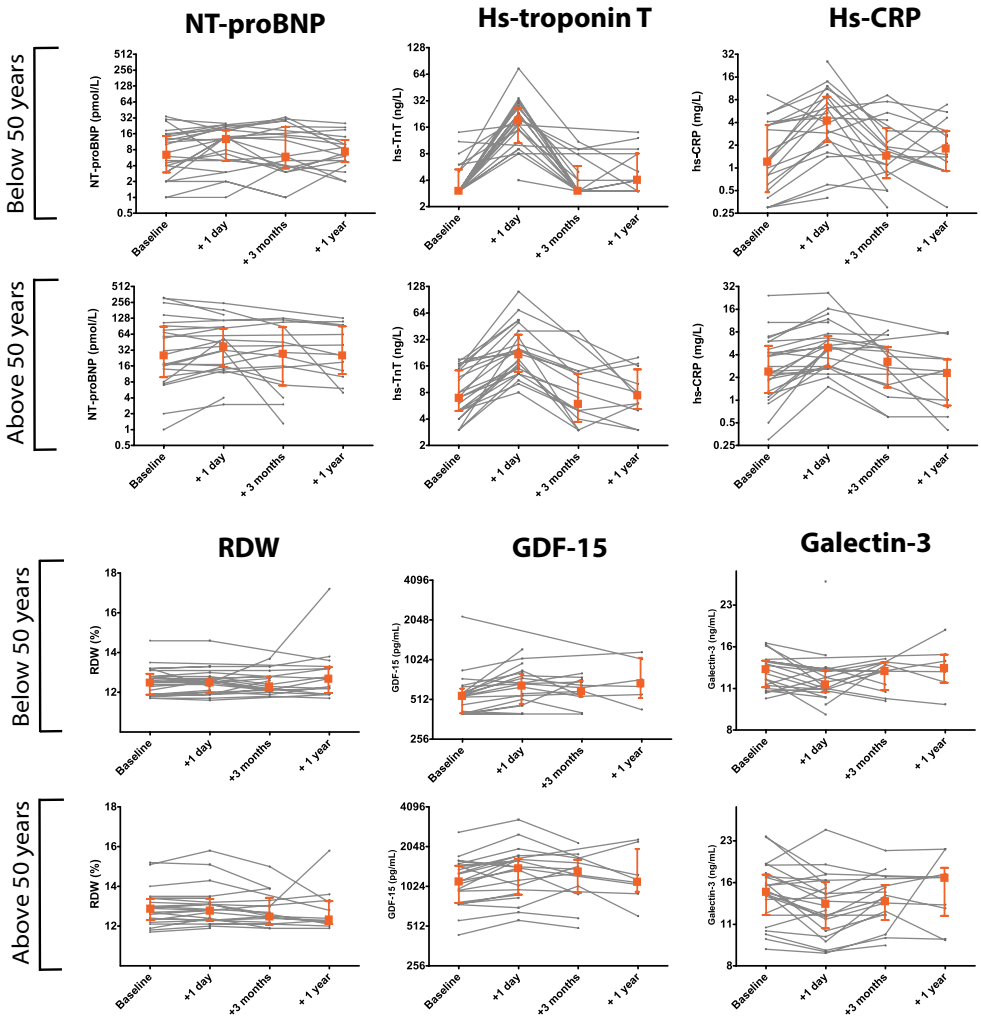

Figure 2. Individual biomarker trajectories of NT-proBNP, hs-troponin T, hs-CRP, RDW, GDF-15 and galectin-3 stratified according to age at ASD closure.

Gray lines represent individual biomarker trajectories over time. Square represents the median biomarker level, together with the 25th and 75th percentile (indicated by the error bars).

NT-proBNP= N-terminal pro B type natriuretic peptide, GDF-15= growth differentiation factor 15, RDW= red cell distribution width, hs-CRP= high sensitive c-reactive protein.

Long-term effects of ASD closure

Three months up to one year post ASD closure blood biomarker levels returned to initial values and thereafter remained stable and no further decreases were observed. An elevated NT-proBNP was still present in 12 patients (40%) one year after ASD closure, while hs-TnT was elevated in only 3 patients (11%) and hs-CRP in 8 patients (30%). Considering echocardiographic changes one year after ASD closure, dimensions of the right ventricle were significantly decreased as well as the tricuspid annular plane systolic excursion, while the left ventricular end-diastolic dimension had increased (Table 3). No differences in baseline characteristics were observed in patients with and without blood biomarker measurements one year post ASD closure, though

Table 3. Echocardiographic and electrocardiographic measurements before percutaneous atrial septal defect closure in adults (baseline) and 1 year after ASD closure.

	Baseline (prior to ASD closure)		**1 year post ASD closure**		**p-value**
	Complete cases, n (%)	Mean ± SD or median [IQR]	Complete cases, n (%)[#]	Mean ± SD or median [IQR]	
Clinical characteristics					
NYHA class	50 (100)		42 (86)		0.020
class I		34 (68)		34 (81)	
class II		16 (32)		8 (19)	
Electrocardiography					
PR-interval[*]	41 (100)	171 ± 28	30 (75)	159 ± 31	0.001
QRS duration	49 (98)	112 ± 22	38 (78)	109 ± 21	0.110
Echocardiography					
LV end-diastolic dimension, mm	50 (100)	46.7 ± 6.3	41 (84)	49.1 ± 4.9	0.009
E/E'	35 (70)	8.3 [6.7-9.2]	31 (63)	8.5 [7.3-10.4]	0.808
E/A	35 (70)	1.1 [1.0-1.4]	33 (67)	1.05 [0.8-1.5]	0.683
Left atrial dimension, mm	49 (98)	40.4 ± 9.6	40 (82)	38.3 ± 10.6	0.084
Right atrial area, cm^2	49 (98)	23.3 [18.7-28.4]	40 (82)	18.0 [14.5-22.5]	<0.001
RVED area, cm^2	49 (98)	34.9 ± 8.5	40 (82)	28.2 ± 7.5	<0.001
RVED basal diameter, mm	50 (100)	49.5 ± 6.8	42 (86)	44.0 ± 6.8	<0.001
RVED apex to base length, mm	49 (98)	85.6 ± 9.2	40 (82)	82.1 ± 9.3	0.001
RV fractional area change, %	49 (98)	41.0 ± 9.5	40 (82)	37.7 ± 7.8	0.155
TAPSE, mm	46 (92)	27.8 ± 5.7	29 (59)	23.2 ± 3.6	<0.001
Right atrial pressure	44 (88)		35 (71)		0.096
Range 0-5 mmHg		36 (72)		32 (91)	
Range 5-10 mmHg		8 (16)		6 (9)	
RV systolic pressure, mmHg	39 (78)	28 [26-38]	29 (59)	27 [23-36]	0.024

[#]One patient was excluded from the 1-year analysis because of cardiac device erosion. [*]Atrial fibrillation, atrial flutter or pacemaker rhythm are not included.
ASD= atrial septal defect, NYHA= New York Heart Association, LV= left ventricular, RVED= right ventricular end-diastolic, TAPSE= tricuspid annular plane systolic excursion, RV= right ventricular.

it should be kept in mind that biomarker measurements were missing in some of the patients at one year (Supplemental Tables 2&3)

As post-hoc sensitivity analysis, we repeated the analysis of the acute and long-term effects of ASD closure on biomarkers levels excluding patients who did not have a 1-year measurement of the biomarker of interest. The results did not differ from the main results.

Discussion

This study investigated temporal changes in the blood biomarkers NT-proBNP, hs-TnT, hs-CRP, RDW, galectin-3 and GDF-15, following percutaneous ASD closure in adults. Percutaneous ASD closure in adults was associated with an acute increase in most blood biomarkers, in particular hs-TnT and hs-CRP, and resulted in at least one elevated biomarker level in 92% of the patients. These findings indicate that hemodynamic changes occurring after ASD closure in adults are likely accompanied by an inflammatory response, cardiomyocyte damage and several pathways of cardiac remodeling. Three months post ASD closure biomarker levels returned to baseline levels and remained stable up to one year after ASD closure. While older patients more often had LV dysfunction and higher right-sided pressures as well as higher biomarker levels before ASD closure, no distinct differences were observed regarding the course of the biomarkers over time in these patients.

Acute effects of ASD closure

The acute increase in hs-TnT following after ASD closure likely reflects cardiomyocyte injury. Two possible explanations can be given; 1) the device insertion and catheter procedure provoke myocardial damage or 2) the acute LV volume overload due to shunt cessation post ASD closure induces myocardial injury. Of note, diagnostic catheterizations itself have not been associated with short-term troponin increases[16, 17]. The increase in hs-TnT is in line with Tárnok et al., who found a short-term cardiac troponin I increases after ASD closure in both children as well as adults[17]. Along with hs-TnT, a pronounced increase in hs-CRP one day post closure was observed, suggesting an inflammatory response following ASD closure. A similar short-term hs-CRP increase has been observed in patients undergoing transradial or transfemoral diagnostic catheterization[18], yet chronic inflammation expressed by elevated hs-CRP has also been proposed to play a role in the pathophysiology of adult congenital heart disease(ACHD)[19]. Whether the increase in hs-CRP is due to shunt termination or attributable to the catheter intervention itself, or a combination of both, is therefore questionable. Nevertheless, it indicates that ASD closure has a significant direct effect on biomarker levels in adults and that elevated levels of hs-TnT and hs-CRP are present in the majority of patients.

Galectin-3 was the only biomarker that decreased in response to ASD closure. This biomarker is known to induce fibroblast proliferation and ventricular dysfunction[20] and inhibition of galectin-3 has shown to terminate further progression of adverse cardiac remodeling in rats[21].

Given the direct galectin-3 decrease after ASD closure, the shunt cessation must have induced a relief in cardiac burden at some point. In a similar manner, levels of GDF-15 increased following ASD closure. GDF-15 is known to protect ventricular cardiomyocytes and induce hypertrophic growth in these cells[22]. Hence, it seems that cardiac protective and adaptive mechanisms are put into operation directly after shunt cessation. To the best of our knowledge, galectin-3, GDF-15 and RDW levels have not been investigated in the context of ASD closure in adults, while all 3 biomarkers have been associated with the prognosis in ACHD[14, 23, 24].

In our study no significant increase in NT-proBNP was found one day after ASD closure, this is in contrast to several studies that did find an increase[25, 26]. An explanation for the absence of an increase in our study may be the presence of already relatively high levels of NT-proBNP before closure. This may be due to a larger proportion of older patients in our study with co-existence of more cardiovascular comorbidities such as hypertension and atrial fibrillation. The diminished left ventricular function in the patients aged over 50 years, may also have contributed to increased NT-proBNP levels. Patients aged below 50 years old at closure had significantly lower NT-proBNP levels before closure; however, no significant increase in NT-proBNP was observed directly after closure.

Long-term effects of ASD closure

Our study did not observe a decrease in biomarker levels between baseline and 3 months to one year post ASD closure. There may be several explanations for the absent decrease in biomarker levels on the long-term. Firstly, reverse cardiac remodeling following ASD closure may have reached a plateau 3 months after closure. Secondly, cardiac remodeling may not be entirely reversible and the molecular or cellular adaptation may have ended after a certain period of time. Thirdly, long-term cardiac remodeling may not be reflected by the biomarkers measured in our study. In addition to these explanations, missing biomarker levels at 3 and 12 months may have prevented us to observe a decrease, as missing biomarker measurements in our study may have more likely been present in in asymptomatic patients in who lower biomarker levels can be expected. However, no differences were observed in baseline characteristics between patients with and without biomarker levels one year post ASD closure.

The direct increase in biomarkers following ASD closure are likely to indicate cardiac adaptation at a molecular or cellular level, however cardiac remodeling after ASD closure visible by echocardiography is considered to take a longer period of time. The significant improvement in NYHA class and decrease in right-heart dimensions as well as right-sided pressures suggests presence of long-term cardiac remodeling after ASD closure in our study. These results are in line with a previous study in 23 patients, who all underwent closure at 50 years or older[9]. Differences in right ventricular end-diastolic dimensions were measured 6 weeks and 1-year after ASD closure, and a significant decrease over time was found. However, similarly to our study, the authors did not find a significant change in BNP in the long-term[9]. In contrast, another study did report a decrease in NT-proBNP 6 and 12 months after ASD closure in adults, which was associated with decreases in RVSP and right ventricular end-diastolic volume[27].

The decrease in TAPSE observed post ASD closure is in line with previous studies [7, 28, 29], and could most likely be explained by recovering of the right ventricular geometry as a result of the reduced right ventricular volume overload after ASD closure. Following the Frank-Starling law, a reduction in volume leads to more efficient pumping of the right ventricle at a lower functional state.

Clinical implications

Findings of this study suggest that ASD closure has an immediate effect on a molecular and cellular level and this change may anticipate perceptible long-term echocardiographic changes in cardiac remodeling. ASD closure in adults leads to acute profound changes in biomarkers, and presence of elevated biomarkers levels post ASD closure seem to be a common reaction. This should be taken into account when measuring biomarkers directly after closure for the interpretation of abnormal levels. The findings of this study warrants further in-depth research whether these biological pathways could be a potential therapeutic target to prevent adverse cardiac remodeling, or enhance reverse cardiac remodeling after closure.

Biomarkers play a role in the prognosis of patients with pulmonary hypertension[30, 31], though it is not clear what role biomarkers play in the development of PAH in adults with an ASD. Whether the biomarker response after ASD closure will be predictive of long-term echocardiographic changes or development of PAH and thus can contribute to the follow-up strategy of these patients, is a highly relevant question that should be further investigated. Based on this study, novel biomarkers like GDF-15 and galectin-3 can, apart from more commonly known biomarkers, contribute to the understanding and future research in this field.

The prognostic value of NT-proBNP, hs-TnT, hs-CRP, GDF-15, RDW and galectin-3 has previously been determined in ACHD patients [14, 19, 23, 24]. Based on biomarker levels found in these studies, the ASD patients in our study had relatively high levels and should, based on the observed biomarker levels, have a far less favorable prognosis than is generally the case in ASD patients[32]. The prognostic value of these biomarkers is therefore likely to be different in ASD patients compared to other ACHD patients and it could be worthwhile to investigate their long-term prognostic value. It would be of great interest to investigate whether biomarker responses following ASD closure, can help to determine the follow-up frequency in patients after ASD closure, which should be addressed in future studies.

Limitations

Because of the number of missing biomarker measurements, deviation in time of blood sampling visits, and the relatively small number of patients, caution should be taken when interpreting the results of this study. The sample size of this study is small, limiting the power to perform statistical analyses, though compared to previous studies on biomarker levels following ASD closure, this can be considered a relatively large study. Differences between two related measurements were investigated using appropriate statistical testing (i.e. paired tests). However these tests do not take into account regression towards the mean; a statistical phenomenon

introduced by random measurement variability, that describes the effect of extreme values becoming less extreme when repeatedly measured based on chance. Regression towards the mean may have biased our results, most likely to an underestimation of the changes in biomarker levels.[33]

As this is an observational study on patient-level, this study cannot confirm any causal relation between biomarker release and cardiac remodeling in adults who undergo ASD closure. This should be kept in mind when interpreting the results of our study.

This study relied on patient blood-sampling for biomarker measurements but logistical or patient-related issues resulted in some patients not having biomarker measurements at certain follow-up moments, in particular 3 months and 1-year follow-up measurements. The missing biomarker measurements were randomly determined by logistical or patient-related issues, but not death. Our observations considering the biomarker trajectories from 3 months to 1-year may still have been biased, in particular the missing data may have prevented us to observe a decrease in biomarker levels on the long-term. Nevertheless, our study was able to show distinct increases in biomarker levels directly after ASD closure.

Conclusion

A substantial number of adults with an unrepaired ASD have elevated blood biomarker levels, even in absence of pulmonary hypertension. Percutaneous ASD closure leads to a direct increase in hs-TnT, hs-CRP, RDW and GDF-15, and a decrease in galectin-3. This suggests that ASD closure is followed by a comprehensive cardiac response including inflammation, cardiomyocyte injury and other pathophysiologic processes. Three months post ASD closure, blood biomarker levels return to baseline levels and remain stable up to one year. While cardiac remodeling was clearly reflected by echocardiographic changes at one year of follow-up, this was not reflected by a distinct decrease in blood biomarker levels on the long-term.

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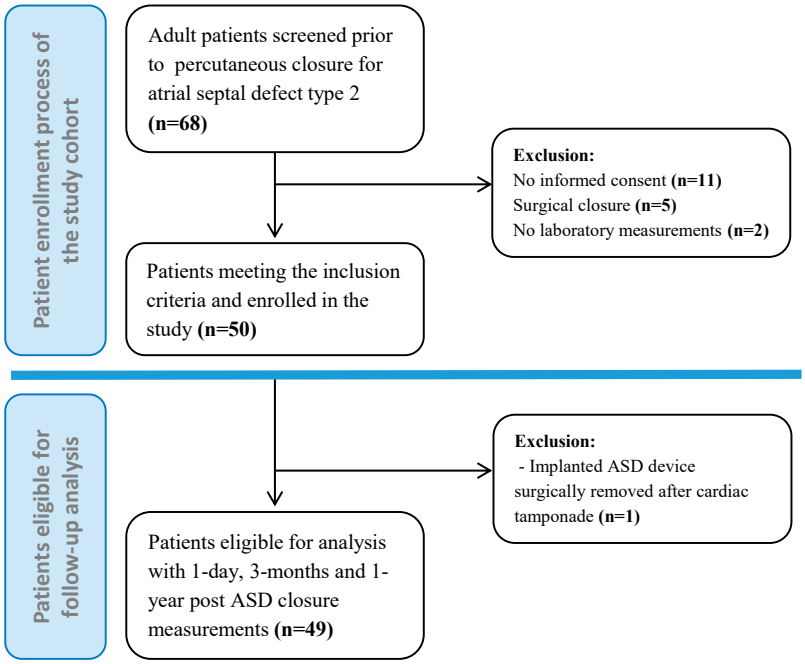
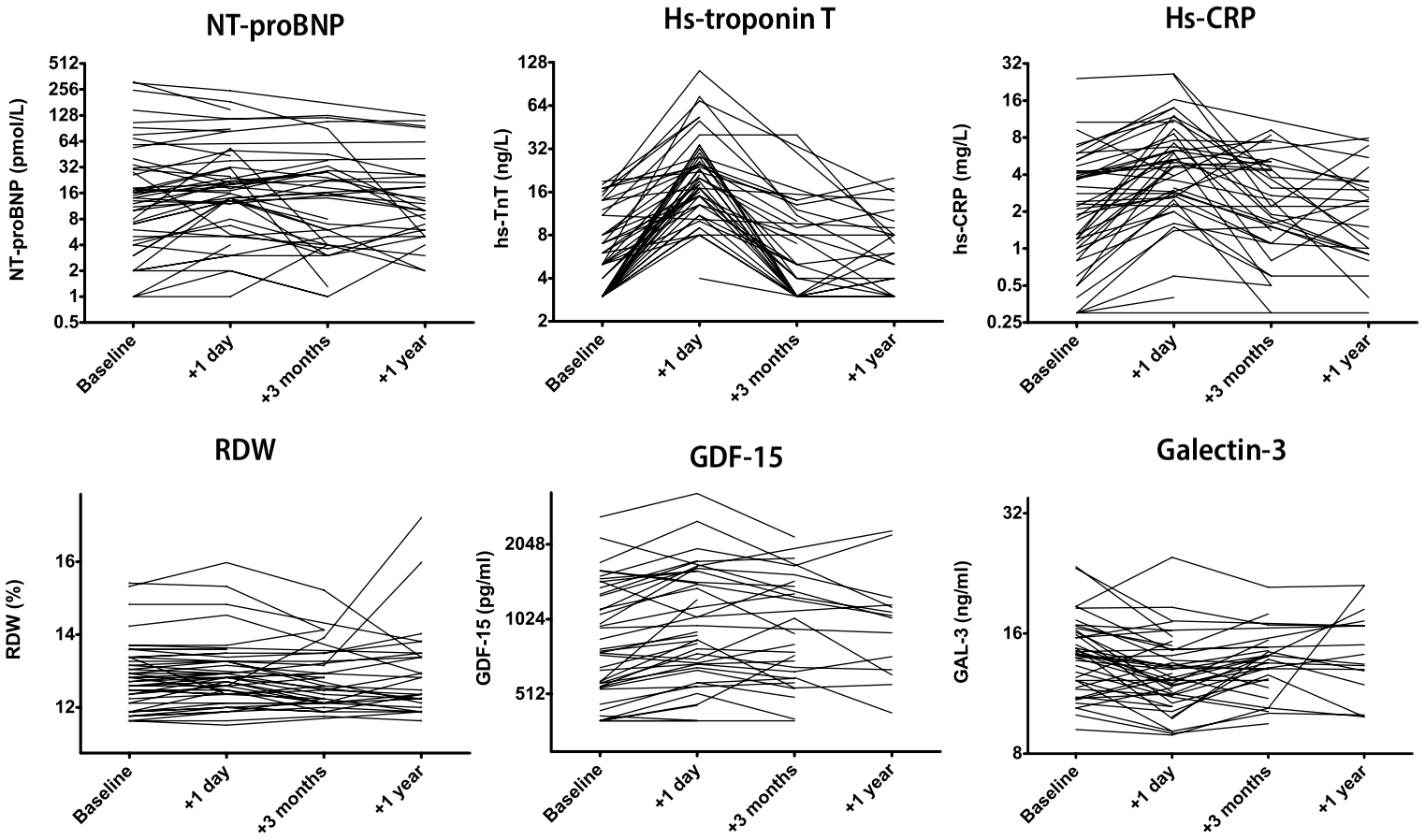


Supplemental Figure 1. Flowchart of the patient selection process.
Both the patient enrollment process of the study cohort and the subsequent number of patients eligible for analysis with follow-up measurements are shown. ASD= atrial septal defect.

Supplemental Table 1. Mutual biomarker correlations at baseline

	Hs-TnT	Hs-CRP	RDW	GDF-15	Galectin-3
	r	*r*	*r*	*r*	*r*
NT-proBNP	0.38**	0.27	0.53***	0.74***	0.45**
hs-TnT		0.15	0.22	0.69***	0.28
hs-CRP			0.16	0.15	0.05
RDW				0.34*	0.31*
GDF-15					0.24
Galectin-3					

* p-value < 0.05, ** p-value < 0.01, *** p-value <0.001. NT-proBNP= N-terminal pro-B natriuretic peptide, hs-TnT= high sensitive troponin-T, hs-CRP= high sensitive C-reactive protein, RDW= red cell distribution width, GDF-15= Growth differentiation factor-15.



Supplemental Figure 2. Individual biomarker trajectories in adults following percutaneous ASD closure.
X-axis is on the 2-log scale. Biomarker measurements of each patient at each follow-up moment is connected by a line, representing the biomarker evolution over time. NT-proBNP= N-terminal pro B type natriuretic peptide, GDF-15= growth differentiation factor 15, RDW= red cell distribution width, hs-CRP= high sensitive c-reactive protein.

Supplemental Table 2. Baseline characteristics stratified according to patients without biomarker levels 1 year post ASD closure and patients with biomarker measurements 1 year post ASD closure.

	1-year biomarker measurement		
	Absent	**Present**	**p-value**
No. of patients	19	31	
Clinical characteristics			
Age at closure, years	51 [36-62]	48 [38-65]	0.960
Women	14 (74)	17 (55)	0.237
BMI, kg/m^2	26.7 ± 6.3	26.6 ± 3.7	0.952
Systolic blood pressure, mmHg	144 ± 22	134 ± 19	0.103
NYHA class II	7 (37)	9 (29)	0.756
Cardiac medication use*	6 (32)	10 (32)	1.00
Systemic hypertension	4 (21)	5 (16)	0.715
Coronary artery disease	0 (0)	2 (7)	0.485
History of atrial fibrillation	4 (21)	8 (26)	1.00
Electrocardiography			
Sinus rhythm	18 (95)	23 (74)	0.128
Heart rate, beats/minute	69 ± 10	73 ± 14	0.797
PR interval, ms†	178 ± 38	169 ± 27	0.654
QRS duration, ms	116 ± 25	109 ± 19	0.406
Complete RBBB	10 (53)	10 (32)	0.033
Echocardiography			
Normal LV function	18 (95)	24 (77)	0.224
LV end diastolic dimension, mm	44.3 ± 5.8	48.1 ± 6.2	0.080
E/E'	7.1 [6.4-8.7]	8.5 [7.0-11.6]	0.198
E/A	1.06 [0.87-1.33]	1.20 [0.99-1.58]	0.353
Left atrial dimension, mm	37.6 ± 9.2	42.0 ± 9.7	0.188
Right atrial area, mm	23.3 [17.7-25.8]	23.7 [19.2-28.8]	0.407
RVED area, cm^2	32.4 ± 5.6	36.2 ± 9.6	0.147
RVED basal diameter, mm	48.2 ± 5.4	50.2 ± 7.5	0.347
RVED apex to base length, mm	83.6 ± 7.4	86.8 ± 10.0	0.335
RV fractional area change, %	43 ± 10	40 ± 9	0.320
TAPSE, mm	28.2 ± 5.9	27.5 ± 5.7	0.813
RV systolic pressure, mmHg	30 [26-44]	28 [25-37]	0.323
Hemodynamics$			
Right atrial pressure	8 [5-10]	7 [6-9]	0.924
Mean PAP	18 [15-25]	19 [17-24]	0.712

Continue

Continued

	1-year biomarker measurement		
	Absent	**Present**	**p-value**
Biomarker measurements			
NT-proBNP, pmol/L	12 [3-44]	14 [6-31]	0.457
hs-TnT, ng/L	4 [3-8]	5 [3-12]	0.360
hs-CRP, mg/L	1.7 [1.0-4.1]	2.3 [0.8-4.3]	0.657
RDW, %	12.7 [11.9-13.2]	12.7 [12.1-13.3]	0.579
GDF-15, pg/mL	761 [560-1367]	748 [543-1284]	0.848
Galectin-3, ng/mL	14.6 [11.6-16.7]	13.9 [11.7-15.9]	0.337

Values are given in mean ± SD, median [IQR] or n (%). P-value is given for the comparison between patients with and without biomarker levels. Mann Whitney U test was used for comparison of continuous variables and the Fisher exact test for categorical variables. *use of ACE-inhibitor (n=3),angiotensin receptor blockers (n=4), beta blocker (n=13) or diuretics (n=7). #compares sinus rhythm against any other rhythm †Atrial fibrillation and pacemaker rhythms are not included. $Measured during percutaneous ASD closure procedure. BMI= body mass index, NYHA= New York Heart Association, RBBB= right bundle branch block, LV= left ventricular, RV= right ventricular, RVED= right ventricular end-diastolic, TAPSE = tricuspid annular plane systolic excursion, PAP= pulmonary artery pressure.

Supplemental Table 3. Number and % of biomarker measurements for each specific biomarker at each specific endpoint in adults who underwent percutaneous ASD closure.

	Number of available biomarker measurements			
n=50 patients	Prior to ASD closure (baseline)	1 day post ASD closure	3 months post ASD closure	1 year post ASD closure
NT-proBNP	49 (98)	48 (96)	33 (67)	30 (61)
Hs-TnT	48 (96)	48 (96)	31 (63)	27 (55)
Hs-CRP	48 (96)	48 (96)	31 (63)	27 (55)
RDW	49 (98)	45 (90)	32 (65)	28 (57)
GDF-15	43 (86)	44 (86)	25 (51)	14 (29)
Galectin-3	45 (90)	45 (90)	26 (53)	17 (35)

ASD= atrial septal defect, NT-proBNP= N-terminal pro B type natriuretic peptide, hs-TnT= high sensitivity troponin T, hs-CRP= high sensitivity C-reactive protein, RDW= red cel distribution width, GDF-15= growth differentiation factor 15.

CHAPTER 6

Blood biomarkers in patients with bicuspid aortic valve disease

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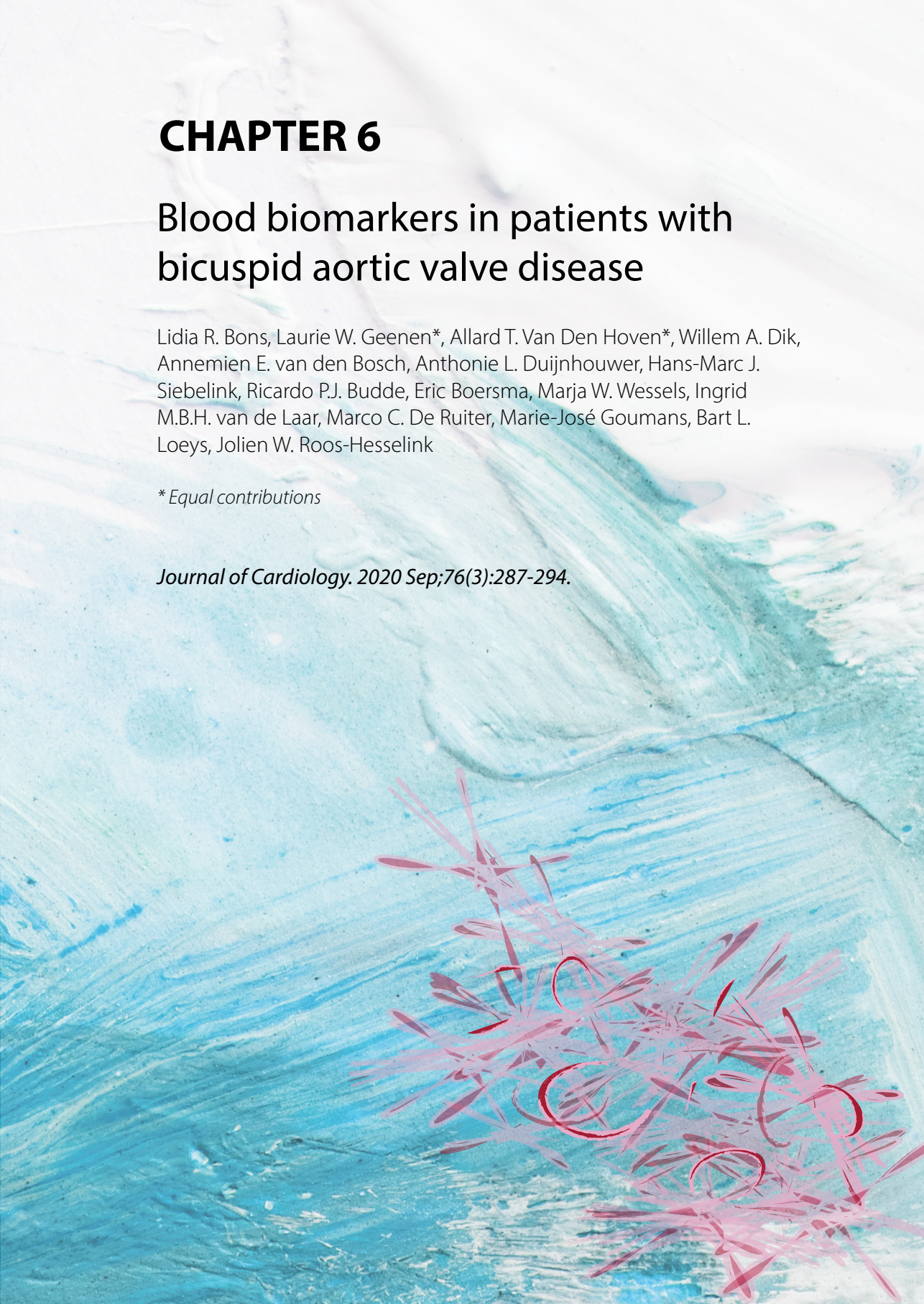

Abstract

Background: Patients with a bicuspid aortic valve (BAV) are at risk of developing valve deterioration and aortic dilatation. We aimed to investigate whether blood biomarkers are associated with disease stage in patients with BAV.

Methods: Serum levels of high sensitivity C-reactive protein (hsCRP), high sensitivity troponin T (hsTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and total transforming growth factor-beta 1 (TGF-ß1) were measured in adult BAV patients with valve dysfunction or aortic pathology. Age-matched general population controls were included for TGFß-1 measurements. Correlation analyses and multivariable linear regression were used to determine the association between (2log-transformed) biomarker levels and aortic valve regurgitation, aortic valve stenosis, aortic dilatation, or left ventricular function.

Results: hsCRP and hsTnT were measured in the total group of 183 patients (median age 34 years, 25^{th}-75^{th} percentile 23-46), NT-proBNP in 162 patients, and TGF-ß1 beta in 108 patients. Elevated levels of NT-proBNP were found in 20% of the BAV patients, elevated hsTnT in 6%, and elevated hsCRP in 7%. Higher hsTnT levels were independently associated with aortic regurgitation [odds ratio per doubling (OR_{2log}) 1.34, 95% CI 1.01-1.76] and higher NT-proBNP levels with aortic valve maximal velocity ($ß_{2log}$ 0.17, 95% CI 0.07-0.28) and aortic regurgitation (OR_{2log} 1.41, 95% CI 1.11-1.79). Both BAV patients with (9.9±2.7 ng/ml) and without aortic dilatation (10.4±2.9 ng/ml) showed lower TGF-ß1 levels compared to general population controls (n=85, 11.8±3.2 ng/ml).

Conclusions: Higher NT-proBNP and hsTNT levels were associated with aortic valve disease in BAV patients. TGF-ß1 levels were lower in BAV patients than in the general population, and not related to aortic dilatation. Longitudinal data are needed to further investigate the prognostic value of biomarkers in these patients.

Introduction

A bicuspid aortic valve (BAV) is an aortic valve with only two cusps instead of three or with three cusps of which two or more are fused with a raphe in between the cusps. It is the most common congenital heart malformation with a prevalence of 0.5-2% in the general population [1, 2]. BAV is accompanied by aortic valve stenosis in about 65% of cases and by aortic valve regurgitation in about 40%, although numbers vary between studies, depending on the age of the patients [3, 4]. Furthermore, approximately 50% of the BAV patients develop aortic dilatation throughout their lifetime [5]. To guide the optimal timing for surgical interventions in patients with BAV, accurate prognostication and monitoring of disease progression of aortic stenosis, aortic regurgitation, or aortic dilatation, is of great importance. Circulating blood biomarkers might provide additional information to determine who is at highest risk for future complications. Currently, there are limited data on the use of biomarkers in patients with BAV and the data that are available mainly focus on aortapathology [6]. It is of great interest to obtain further insight into alterations of growth factors and mediators in aortic valve degeneration, aortic dilatation, or myocardial remodeling. The biomarkers troponin-T and N-terminal pro B type natriuretic peptide (NT-proBNP) may be elevated as a result of pressure overload in aortic stenosis and volume overload in aortic regurgitation, which can help to identify patients with increased left ventricular wall stress who may benefit from earlier treatment. In older patients with degenerative aortic valve stenosis, higher C-reactive protein (CRP) is associated with more severe aortic stenosis [7], but no evidence is available on aortic valve stenosis in patients with a BAV. Finally transforming growth factor-beta 1 (TGF-ß1) is found to be elevated in blood samples of patients with aortic dilatation or syndromes [8-11]. Yet, only small numbers ranging between 9 and 30 patients with a BAV were included in these studies. This cross-sectional study aimed to investigate the association between biomarkers [high sensitivity (hs) CRP, hs TnT, NT-proBNP, and TGF-ß1] and the degree of aortic valve stenosis or regurgitation, left ventricular ejection fraction (LVEF), or aortic diameter in a large cohort of adults with BAV.

Methods

Study design and patient population

For this study we included the data of two cohorts of adults with BAV: the BioCon study, an observational prospective cohort study including consecutive adults with moderate to complex congenital heart disease enrolled between 2011 and 2013 [12], and the BAV study, a multicenter observational cohort study performed between 2014 and 2016 including patients with BAV and/or Turner syndrome[13]. We extracted data from adult patients from both cohort studies with BAV and at least one of the following: [1] aortic valve stenosis (maximal velocity >2.5 m/s), [2] aortic valve regurgitation (at least moderate), [3] aortic dilatation of the sinus of Valsalva or ascending aorta (≥40 mm and/or aortic size index ≥2.1 cm/m^2), [4] aortic coarctation, [5] an

aortic valve intervention (balloon dilatation, resection subvalvular stenosis or valve repair), or [6] Turner syndrome. Patients who previously underwent aortic valve or aortic replacement were excluded. All three types of a BAV according to the Sievers classification were included [14]. This classification is based on the number of raphes, which is a fused area between two cusps. BAV with no raphe are called type 0, valves with one raphe type 1, and valves with two raphes type 2. For research purposes, patients underwent physical examination, two-dimensional thoracic echocardiography (TTE), and venous blood sampling on the same day. Hypertension was defined as current use of antihypertensive medication. The study complied with the Declaration of Helsinki and was approved by the medical ethical committee of the Erasmus Medical Center (MEC10-165 and MEC14-225). Written informed consent was provided by all patients.

TGF-ß1 measurements in general population controls

Between 2014 and 2015, 145 healthy volunteers were prospectively recruited through an advertisement for healthy subjects and stratified into five age groups: 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 to 72 years [15]. TGF-ß1 levels were determined. To create an age-matched reference group, only the participants with an age under 50 years were included for the current study for TGF-ß1 measurements. The inclusion criteria required that subjects had normal results on physical examination, echocardiography, and electrocardiography (ECG). Subjects were excluded when they met any of the following criteria: cardiovascular disease (including aortic dilatation, aortic valve stenosis, aortic valve regurgitation); cardiovascular risk factors consisting of hypertension, diabetes mellitus, hypercholesterolemia; systemic disease or medication known to influence cardiac function; or the finding of cardiac abnormalities during examination. Professional athletes, morbidly obese subjects (body mass index >40 kg/m2), pregnant women, and women with breast implants were also excluded. All participants underwent physical examination, TTE, and venous blood sampling on the same day at the outpatient clinic.

Echocardiography

Standard 2D TTE was performed by an experienced sonographer. All studies were acquired using harmonic imaging on an iE33 or EPIQ7 ultrasound system (Philips Medical Systems, Best, the Netherlands) equipped with a ×5–1 matrix-array transducer (composed of 3040 elements operating at 1–5MHz). The aorta was measured during diastole with the leading edge-to-leading edge method from either the standard parasternal long-axis view or from a more cranial intercostal window to improve visualization of the ascending aorta [16]. Aortic valve stenosis was defined based on a peak aortic velocity of >2.5 m/s [17] and aortic valve regurgitation was classified as no, mild, moderate, and severe according to the guidelines of the European Association of Echocardiography/American Society of Echocardiography [18]. Aortic dilatation was defined as an aortic diameter ≥40 mm or aortic size index ≥2.1 cm/m^2 at the level of the sinus of Valsalva or ascending aorta. All measurements of LVEF based on strain analyses were

performed blinded regarding subject identity using the 2D CPA suite from Tomtec Imaging Systems (Unterschliessheim, Germany).

Laboratory testing

Venous blood sampling was performed for study purposes only. NT-proBNP was measured directly in fresh blood samples with the use of an electrochemiluminesence immunoassay (Roche Diagnostics, Basel, Switzerland) in the clinical chemistry laboratory of the Erasmus MC. The rest of the serum samples were aliquoted and stored at -80°C within two hours after withdrawal. hsTnT and hsCRP were measured in batches in thawed serum samples using electrochemiluminesence immunoassays (Roche Diagnostics). Lower limit of detection was 3 ng/L for hsTnT, 0.3 mg/L for hsCRP, and 0.6 pmol/L for NT-proBNP. TGF-ß1 measurement was only performed in the patients of the BAV study and healthy controls, and was performed by the laboratory medical immunology of the department of Immunology of the Erasmus MC. Serum concentration of human activated TGF-ß1 was measured by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions (Duoset® ELISA, R&D Systems Europe, Ltd., Abingdon, UK). Before the assay, the latent TGF-ß1 contained in patients' serum was activated to the immunoreactive form using acid activation and neutralization. The lower limit of detection was 31.25 pg/ml. For the control subjects, the protocol of TGF-ß1 measurements was exactly the same as for the BAV patients.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD), or median and interquartile range (IQR). Comparison of normally distributed continuous variables was done using the Student's t test or, in case of a skewed distribution, the Mann-Whitney test. Biomarker values below the limit of detection (LoD) were substituted with a value that was equal to 50% of this LoD, for analytical purposes. Because of a skewed distribution, all biomarker levels were 2log transformed for further statistical analysis. The upper limit of normal for NT-proBNP was 14 pmol/L (≈125 pg/mL), on the basis of the recommended cut-off for the diagnosis of heart failure in patients presenting with non-acute symptoms [19]. The upper limit of normal was defined as the 99th percentile of the reference distribution, which corresponded with 14 ng/L for hsTnT [20] and with 10 mg/L for hsCRP [21]. First, the Pearson (r_P) or Spearman (r_S) correlation coefficient between biomarkers, as well as between biomarkers and patient characteristics was determined. Second, regression analysis was performed with biomarker levels as independent variable and disease stage as dependent variable. For disease stage the following variables were used: maximum velocity (Vmax) across the aortic valve, aortic valve regurgitation (no, mild, moderate, and severe), aortic diameter, and LVEF. When the dependent variable was continuous, linear regression analysis was used, and in case of an ordinal variable, ordinal logistic regression analysis was used. Significant univariable associations were further analyzed in multivariable analysis. First, we adjusted for age and sex only and secondly also for Vmax, aortic regurgitation, diameter of the sinus of Valsalva and ascending aorta, and ventricular function. Significant

associations between biomarker levels and disease stage in multivariable linear regression analysis, were further analyzed using categories of disease stages. Aortic valve stenosis was categorized into no aortic valve stenosis (Vmax <2.5 m/s), mild aortic valve stenosis (Vmax 2.5-3.9 m/s), and severe aortic valve stenosis (Vmax ≥4.0 m/s). Aortic regurgitation was analyzed categorical as no, mild, and moderate/severe aortic valve regurgitation. A sensitivity analysis was performed by excluding Turner patient. Third, we compared biomarkers levels between patients with either aortic valve stenosis or regurgitation and patients without both aortic valve stenosis or regurgitation. Also biomarker levels were compared between BAV patients with different Sievers classifications. Missing data were handled by multiple imputation with five iterations [22]. All tests were two-sided and a p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS, version 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The patient selection process is shown in Figure 1. A total of 183 patients were included with a median age of 34 (IQR 23-46) years of which 82 (45%) were female (Table 1). NT-proBNP measurement was available in 162 patients (89%). In a subset of 108 (58%) patients, total TGF-ß1 levels were determined. Patients in whom TGF-ß1 was measured were significantly older, had larger aortic diameters, and fewer patients had or were previously treated for an aortic coarctation compared to the total group of 188 patients. In the total group, a BAV Sievers type 0 was found in 27 (25%) subjects, Sievers type 1 in 144 (79%) subjects, and Sievers type 2 in 12 (6%) subjects. Moderate or severe aortic valve regurgitation was found in 50 (27%) patients, aortic valve stenosis in 80 (44%) patients, and aortic dilatation in 98 (54%) patients. Both aortic valve regurgitation (at least moderate) and aortic valve stenosis was found in 34 (19%) patients. LVEF of less than the lower limit (5th percentile) of the healthy group, which was 45%, was found in 69 (38%) of the BAV patients.

Blood biomarkers

The levels of each biomarker are shown by scatterplots in Figure 2. An elevated NT-proBNP level was found in 37 (20%) patients, an elevated hsTnT level in 10 (6%) patients, and an elevated hsCRP level in 12 (7%) patients. In our reference cohort of 85 volunteers, mean TGF-ß1 levels were 11.8±3.2 ng/mL and not significantly associated with age (r_p=0.13, p=0.227) or sex (11.6±2.7 ng/mL in men vs 12.0±3.6 ng/mL in women, p=0.640) (Supplemental Figure 1). A significantly lower TGF- ß1 was found in both BAV patients with aortic dilatation (9.9±2.7 ng/ml) and BAV patients without aortic dilatation (10.4±2.9 ng/ml) compared to healthy controls with tricuspid aortic valve (TAV) (11.8±3.2 ng/ml) (Figure 3), also after correction for age. We found significant correlations between hsCRP and hsTnT (r_s=0.15, p=0.042), hsCRP and NT-proBNP (r_s=0.35, p<0.001), and NT-proBNP and TGF-ß1 (r_s=-0.24, p=0.025).

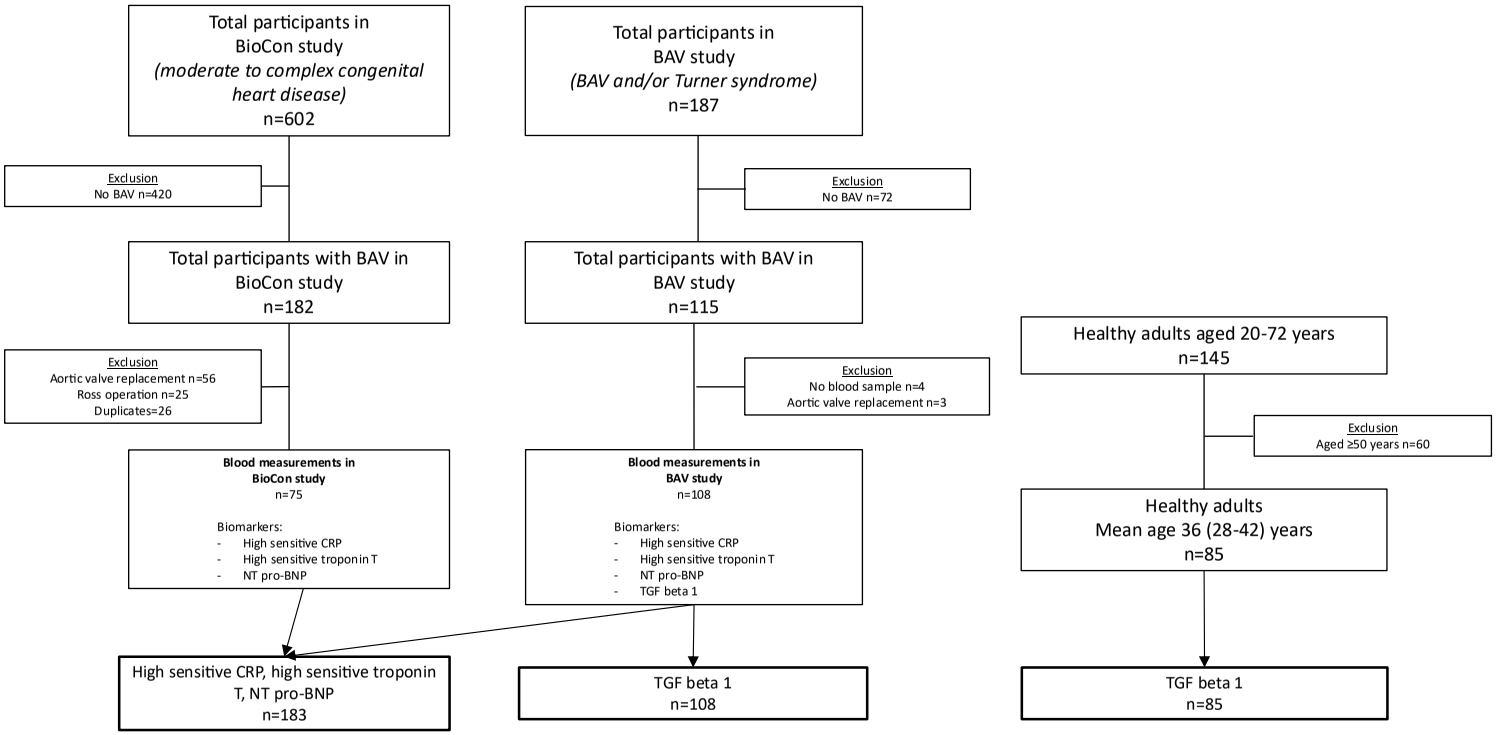

Figure 1. Flow charts of participants from the BioCon and BAV study in blood marker analysis.
BAV, bicuspid aortic valve; hsCRP, high sensitivity C-reactive protein; hsTnT, high sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TGF beta 1, transforming growth factor beta 1.

Table 1. Baseline characteristics

	hsCRP and hsTnT (total group)	NT pro-BNP	TGFß1 (only measured in BAV study)	Healthy controls for TGFß1 measurements
	n=183	n =162	n=108	n=85
Age (years)	34 (23-46)	31 (23-43)	38 (25-52)*	36 (28-42)
Female sex	82 (45%)	72 (44%)	42 (39%)	43 (51%)
Height (cm)	176 (164-183)	176 (165-183)	178 (162-186)	175 (167-182)
Weight (kg)	75 ± 15	75 ± 15	75 ± 15	73 ± 13
Systolic blood pressure (mmHg)	127 ± 17	127 ± 17	126 ± 16	123 ± 12
Diastolic blood pressure (mmHg)	79 ± 11	79 ± 11	78 ± 11	78 ± 8
Aortic diameter, sinus of Valsalva (mm)	35 ± 6	35 ± 6	37 ± 6*	31 ± 3*
Aortic diameter, ascending aorta (mm)	36 ± 8	36 ± 8	38 ± 7*	29 ± 3*
Left ventricular ejection fraction (%)	47 ± 8	47 ± 8	46 ± 8	52 ± 4*
BAV morphology				
Sievers type 0	27 (15%)	22 (14%)	27 (25%)	-
Sievers type 1	144 (79%)	128 (79%)	70 (65%)	-
Sievers type 2	12 (6%)	12 (7%)	11 (10%)	-
Comorbidities				
Aortic coarctation	59 (32%)	57 (35%)	15 (14%)*	0 (0%)
Aortic regurgitation >mild	50 (27%)	46 (28%)	32 (30%)	0 (0%)
Aortic valve stenosis (Vmax≥2.5 m/s)	80 (44%)	70 (43%)	44 (41%)	0 (0%)
Diabetes Mellitus	1 (1%)	0 (0%)	1 (1%)	0 (0%)
Hypertension	40 (22%)	33 (20%)	25 (23%)	0 (0%)
Hypercholesterolemia	10 (6%)	6 (4%)	8 (7%)	0 (0%)
Turner syndrome	22 (12%)	14 (9%)	20 (19%)	0 (0%)
Previous aortic valve intervention				
Surgical aortic valve repair	5 (3%)	4 (3%)	1 (1%)	0 (0%)
Percutaneous balloon dilatation	18 (10%)	18 (11%)	12 (11%)	0 (0%)
Resection subvalvular stenosis	7 (4%)	7 (4%)	0 (0%)	0 (0%)

Values are presented as mean (SD) or median (IQR) for continuous variables and N (%) for categorical variables. Data represent non-imputed values. Missing values were present for blood pressure (1% in BAV patients), aortic diameter at the sinus of Valsalva (1% in BAV patients), ascending aortic diameter (2% in BAV patients and 1% in healthy controls) and LVEF (8% in BAV patients and 14% in healthy controls). *significantly different from the total group, p-value <0.05. Values of 0 (0%) could not be tested for significance.

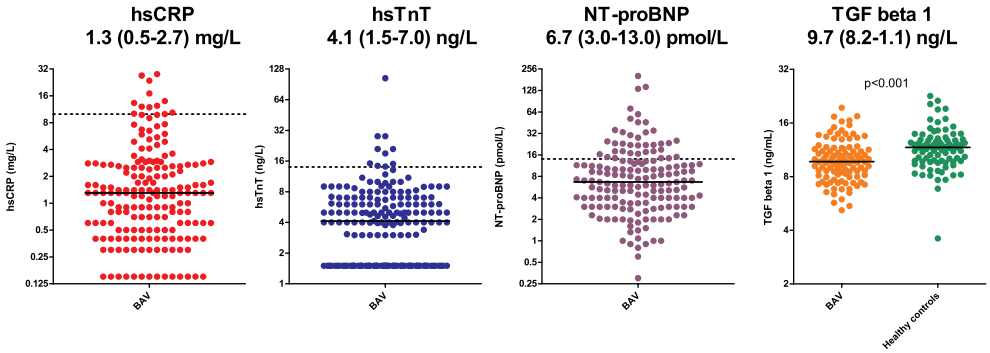

Figure 2. Biomarker levels in adults with BAV.
Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median. The dashed line represents the upper limit of normal, defined as the 99th percentile of the reference distribution for hsCRP and hsTnT, corresponding with 10 mg/L and 14 ng/L. The upper limit of normal for NT-proBNP was 14 pmol/L (≈125 pg/mL), on the basis of the recommended cut-off for the diagnosis of heart failure in patients presenting with nonacute symptoms.
BAV, bicuspid aortic valve; hsCRP, high sensitivity C-reactive protein; hsTnT, high sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TGF beta 1, transforming growth factor beta 1.

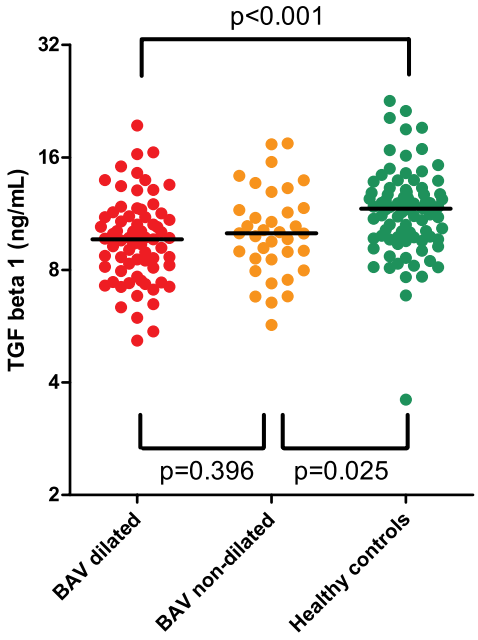

Figure 3. TGF beta 1 levels in BAV patients with and without aortic dilatation (≥40 mm and/or aortic size index ≥2.1 cm/m2) and healthy controls.
Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median.
BAV, bicuspid aortic valve; TGF beta 1, transforming growth factor beta 1.

None of the biomarkers showed an association with aortic diameter or left ventricular function in multivariable analysis (Table 2). A two-fold higher hsTnT level was independently associated with an increased risk of one higher grade of aortic regurgitation by a factor of 1.34 (95% CI 1.01-1.76). When aortic valve regurgitation was categorized as no, mild, or moderate/severe, hsTnT was only significantly higher in the patients with moderate/severe aortic valve regurgitation compared to patients with no aortic valve regurgitation (Figure 4).

A two-fold higher NT-proBNP level was independently associated with a mean 0.17 (95% CI 0.07-0.28) m/s higher Vmax. In addition, a two-fold higher NT-proBNP level was independently associated with an increased risk of one higher level of aortic regurgitation by a factor of 1.41 (95% CI 1.11-1.79). When aortic valve stenosis was categorized into three groups, NT-proBNP levels were only significantly higher in patients with severe aortic valve stenosis (Vmax >4.0 m/s) compared to patients with no aortic valve stenosis (Figure 4). No significant differences in NT-proBNP levels were found between the three categories of aortic valve regurgitation. The comparison between patients with either aortic stenosis or aortic regurgitation and patients without stenosis and regurgitation can be found in Supplemental Figure 2. Supplemental Figure 3 shows the comparison in biomarker level between patients with different Sievers classification. When Turner patients were excluded from the analysis, the results remained the same.

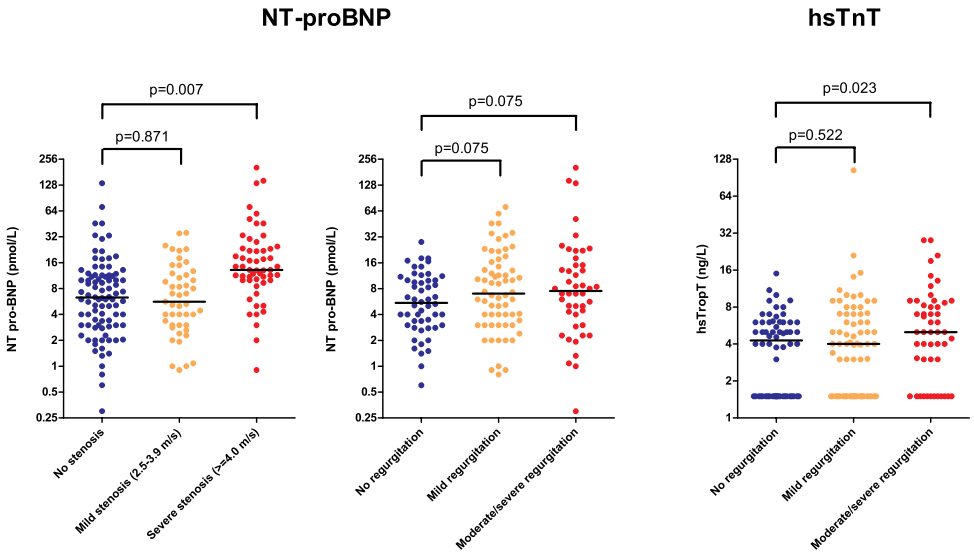

Figure 4. NT-proBNP and hsTnT levels in patients with different stages of aortic valve stenosis and aortic valve regurgitation.

Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median. Abbreviations: NT-proBNP, pro-B-type; hsTnT, high-sensitivity troponin T.

Table 2. Linear or ordinal logistic regression analysis of biomarkers and continuous variables representing disease progression.

		Correlation analysis		Univariable regression analysis		Multivariable regression analysis*	
		Correlation coefficient	p-value	ß or OR (95% CI)†	p-value	ß or OR (95% CI)†	p-value
Log2 hsCRP	Aortic valve stenosis (Vmax in m/s)	0.00	0.981	0.02 (-0.07-0.11)	0.300		
	Aortic valve regurgitation (no, mild, moderate, severe)	-0.02	0.827	0.99 (0.85-1.15)	0.716		
	Aortic diameter at the level of sinus of Valsalva (mm)	0.00	0.996	0.02 (-0.51-0.56)	0.938		
	Aortic diameter at the level of ascending aorta (mm)	0.01	0.855	0.01 (-0.64-0.67)	0.972		
	Left ventricular ejection fraction (%)	0.01	0.191	0.01 (-0.57-0.83)	0.725		
Log2 hsTnT	Aortic valve stenosis (Vmax in m/s)	0.11	0.132	0.09 (-0.04-0.22)	0.164		
	Aortic valve regurgitation (no, mild, moderate, severe)	0.16	0.035	1.29 (1.03-1.57)	0.030	**1.34 (1.01-1.76)**	**0.041**
	Aortic diameter at the level of sinus of Valsalva (mm)	0.29	<0.001	1.56 (1.17-1.95)	<0.001	0.07 (-0.61-0.76)	0.838‡
	Aortic diameter at the level of ascending aorta (mm)	0.19	0.011	1.39 (0.44-2.33)	0.004	-0.40 (-1.40-0.60)	0.431‡
	Left ventricular ejection fraction (%)	0.10	0.260	0.49 (-0.63-1.61)	0.384		
Log2 NT-proBNP	Aortic valve stenosis (Vmax in m/s)	0.19	0.016	0.16 (0.05-0.26)	0.003	**0.17 (0.07-0.28)**	**0.002**
	Aortic valve regurgitation (no, mild, moderate, severe)	0.14	0.070	1.22 (1.01-1.47)	0.035	**1.41 (1.11-1.79)**	**0.005**
	Aortic diameter at the level of sinus of Valsalva (mm)	-0.04	0.593	-0.18 (-0.81-0.46)	0.592		
	Aortic diameter at the level of ascending aorta (mm)	0.03	0.694	0.16 (-0.64-0.95)	0.693		
	Left ventricular ejection fraction (%)	0.17	0.035	0.94 (0.08-0.09)	0.033	0.36 (-0.73-1.44)	0.518‡
Log2 TGFß1	Aortic valve stenosis (Vmax in m/s)	-0.11	0.277	-0.38 (-0.88-0.12)	0.137		
	Aortic valve regurgitation (no, mild, moderate, severe)	-0.01	0.944	0.92 (0.37-2.30)	0.866		
	Aortic diameter at the level of sinus of Valsalva (mm)	0.19	0.045	3.01 (0.10-5.92)	0.043	1.32 (-1.06-3.70)	0.278‡
	Aortic diameter at the level of ascending aorta (mm)	-0.03	0.758	-0.60 (-4.37-3.18)	0.757		
	Left ventricular ejection fraction (%)	-0.13	0.205	-2.60 (-6.59-1.38)	0.200		

Bold = significant in multivariable analysis. * Adjusted for age, sex, Vmax, aortic valve regurgitation, aortic diameters and LVEF. † Beta coefficient was given for linear regression analysis performed with Vmax, aortic diameter or LVEF as dependent variables. Odd ratio was given for ordinal logistic regression analysis performed with aortic regurgitation (no, mild, moderate, sever) as dependent variable. ‡ Not significant after adjustment for only age and sex.

Discussion

This cross-sectional study investigated the levels of hsCRP, hsTNT, NTproBNP, and TGF-ß1 in a large cohort of adults with BAV. We can conclude that a substantial number of patients with BAV have elevated levels of NT-proBNP, hsTnT, and hsCRP. TGF-ß1 levels were found to be lower in patients with BAV than in a healthy age-matched control population with TAV. A higher NT-proBNP was significantly associated with a higher maximum velocity across the aortic valve and more severe aortic valve regurgitation. In addition, higher hsTnT levels were associated with more severe aortic valve regurgitation. Specifically, high levels of NT-proBNP were present in patients with severe aortic stenosis (≥4.0 m/s) and high levels of hsTnT were present in patients with moderate to severe aortic valve regurgitation.

In contrast to imaging biomarkers, blood biomarkers can be obtained in all patients and at relatively low cost, making them very suitable as diagnostic and useful in prediction models. This study already showed that patients with BAV have higher levels of NT-proBNP, hsTnT, and hsCRP and lower levels of TGF- ß1, which can help us to understand the pathophysiology of complications in patients with BAV. In addition, this is the first study that evaluated associations between biomarkers and disease stage in this specific group of patients with BAV and demonstrated that particularly NT-proBNP and hsTNT may have potential prognostic value in adults with a BAV. Whether biomarker levels can predict deterioration of aortic valve pathology and can be used for clinical decision-making in BAV patients should be further investigated in longitudinal studies.

NT-proBNP in BAV patients

NT-proBNP is associated with ventricular dysfunction in patients with congenital aortic valve stenosis [23] and is elevated in older individuals with calcified aortic valve stenosis [24] or aortic valve regurgitation [25]. In older patients with aortic stenosis, the prognostic value of NT-proBNP has also been proven [26, 27]. However, BAV patients differs genetically, histopathologically, and in age from the patient group of aortic valve degeneration represented in literature. BAV patients are prone to develop aortic valve stenosis, aortic valve regurgitation, or aortic dilatation at a relatively young age [28]. We showed that NT-proBNP levels are associated with aortic valve stenosis and regurgitation in BAV patients. NT-proBNP expression is induced by diastolic and systolic myocardial wall stretch [29]. This can be a result of both aortic valve stenosis and regurgitation due to increased left ventricular pressure afterload or volume overload respectively. Two studies [29, 30] that included relatively old patients with aortic valve stenosis, showed that NT-proBNP and BNP are already elevated in patients with normal LV end-diastolic pressure or left atrial pressure, compared to controls. This might suggest that NT-proBNP is already elevated before patients with aortic valve stenosis or regurgitation develop increased pressures and compensated hypertrophy. However, to answer the question whether NT-proBNP could help to identify BAV patients with future LVEF deterioration, longitudinal studies are required.

hsTnT in BAV patients

Troponin T, a marker of myocardial injury, is not only elevated in acute coronary syndrome, but it can also be elevated in chronic heart failure [31] as a result of multiple suspected contributing mechanisms including cardiomyocyte damage from inflammatory cytokines or oxidative stress, apoptosis, or a stretch-related mechanism [32]. Troponin T levels also have a predictive value of long-term outcome in patients with non-ischemic heart failure [33]. Again these studies are performed in older patients compared to the BAV patients included in our study and therefore probably represent another disease etiology. Although we did not find an association between hsTnT levels and LVEF, we did find that hsTnT levels were associated with more severe aortic valve regurgitation in BAV patients. One hypothetical explanation might be the reduced coronary blood flow during diastole found in patients with aortic regurgitation [34, 35], which can result in cardiomyocyte damage and therefore increased hsTnT levels. A reduced coronary blood flow is also found in patients with aortic stenosis [36], while aortic stenosis was not associated with increased hsTnT levels in our cohort.

hsCRP in BAV patients

In older patients with aortic stenosis, Galante et al. found that inflammatory markers, such as CRP, were elevated [37]. Statins have been tested and suggested as a new treatment to reduce the progression of aortic valve stenosis, because they reduce vascular inflammatory processes besides their already known cholesterol-lowering effect [38]. Nevertheless, large randomized controlled trials did not find an effect of statin therapy on cardiovascular events, mortality, or valve dynamics in patients with aortic valve stenosis [39]. In our study, CRP levels did not show an association with the severity of aortic valve stenosis in BAV patients, suggesting that inflammation is less likely to be involved as the underlying process of aortic valve stenosis development in BAV patients. This finding confirms that indeed there is no clear role for statin therapy. A theory of dilatation of the aorta is presumed to be a process of inflammation, in this study we did not find an association between aorta diameter and the level of hsCRP. The reason for this could be that this process only plays a role in late stage of dilatation and the number of patients with a large aortic diameter might have been too small.

TGF-beta 1 in BAV patients

There has been attention for biomarkers in BAV-associated aortic pathology, focusing on matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinase (TIMP), and TGF-ß [6, 40]. Although TGF-ß also seems to be important in thoracic aortic aneurysm development in BAV [41], we did not find an association between serum TGF-ß1 levels and aortic diameter in BAV patients. Our results did show, however, that TGF-ß1 levels are decreased in patients with BAV, both with and without aortic dilatation. This is in line with a study investigating aortic tissue, since they found that a higher proportion of TGF-ß sequestered in the extracellular matrix found in BAV compared to a TAV, probably leads to less free TGF-ß being available to activate the TGFß-pathway [42]. The development of aortic dilatation in BAV patients does not seem to

be associated with an increased TGF-ß signaling, such as in Marfan and Loeys-Dietz syndrome. This is confirmed by another study, which also presented a group of non-dilated BAV patients in whom TGF-ß was less expressed in aortic tissue [43]. This different activation of the TGF-ß pathway in aortic dilatation between BAV patients and patients with Marfan or Loeys-Dietz syndrome needs further attention. Contradictory, other studies presented results with higher TGF-ß1 in BAV patients [10, 11] or no difference in TGF-ß1 between BAV and TAV participants [9], but these studies contained only 30, 24, and 12 BAV patients. Also it is important to mention that platelets are a major source of TGF-ß1 in the circulation. It has been shown that patients with BAV tend to have a higher mean platelet volume [44], indicating increased platelet activation. If this increased platelet activation had affected the TGF-ß1 measurements, BAV patients would have had higher TGF-ß1 levels. However, we found lower levels of TGF-ß1 in BAV patients, but no information on platelet levels was available to substantiate this assumption.

In addition, we provided reference values for TGF-ß1, based on 85 healthy volunteers without aortic dilatation with an age range from 20-50 years. The mean TGF-ß1 levels that we found (11.8±3.2 ng/ml) were both lower [45] or higher [8, 9] than in previously published studies. This could possibly be due to differences in affinity of the antibodies in the immunoassays or inclusion of different reference groups (patient without BAV versus local population). Since the current literature presents a large variation in reference levels of circulating TGF-ß1, reference levels should be measured with the same technique, preferably measured in the same laboratory.

Limitations

Some limitations of our study need to be addressed. The lack of follow-up data prevented us to evaluate the longitudinal prognostic value of these biomarkers. In addition, patients who previously underwent aortic valve or aortic replacement were excluded and therefore BAV patients with the most severe disease stage were less likely to be included in this study. Finally, measurements of LVEF were based on strain analyses in both BAV patients and healthy controls, while the biplane method of disks is the currently recommended 2D method to assess LVEF [46]. A substantial variation between techniques has to be taken into account, with the values found in our study being lower compared to the values referred to as abnormal by the guidelines: LVEF of <52% for men and <54% for women are suggestive of abnormal LV systolic function [16].

Conclusion

A substantial number of patients with BAV have elevated levels of NT-proBNP, hsTnT, and hsCRP and they show lower levels of TGF-ß1 compared to healthy controls with TAV. In BAV patients, higher NT-proBNP was associated with more severe aortic valve stenosis and regurgitation, while higher hsTnT levels were associated with more severe aortic valve regurgitation. No independent association between hsCRP and TGF-ß1 with the degree of aortic valve stenosis or regurgitation, LVEF or aortic diameter was found. This is the first step toward the identification of a biomarker that can be used in prognostic staging and risk prediction.

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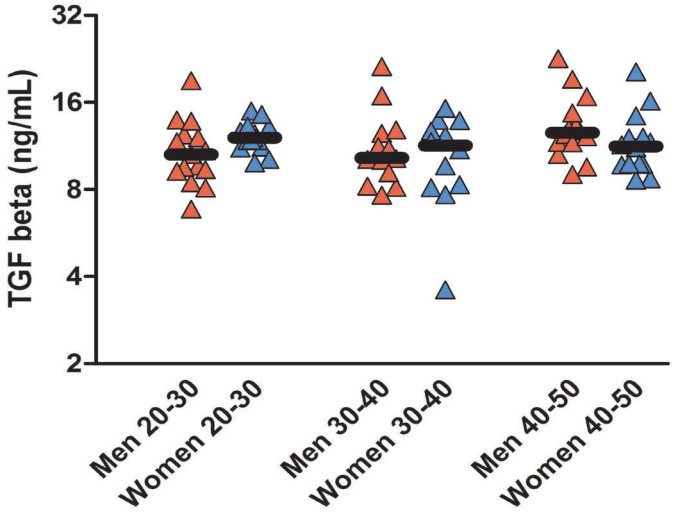
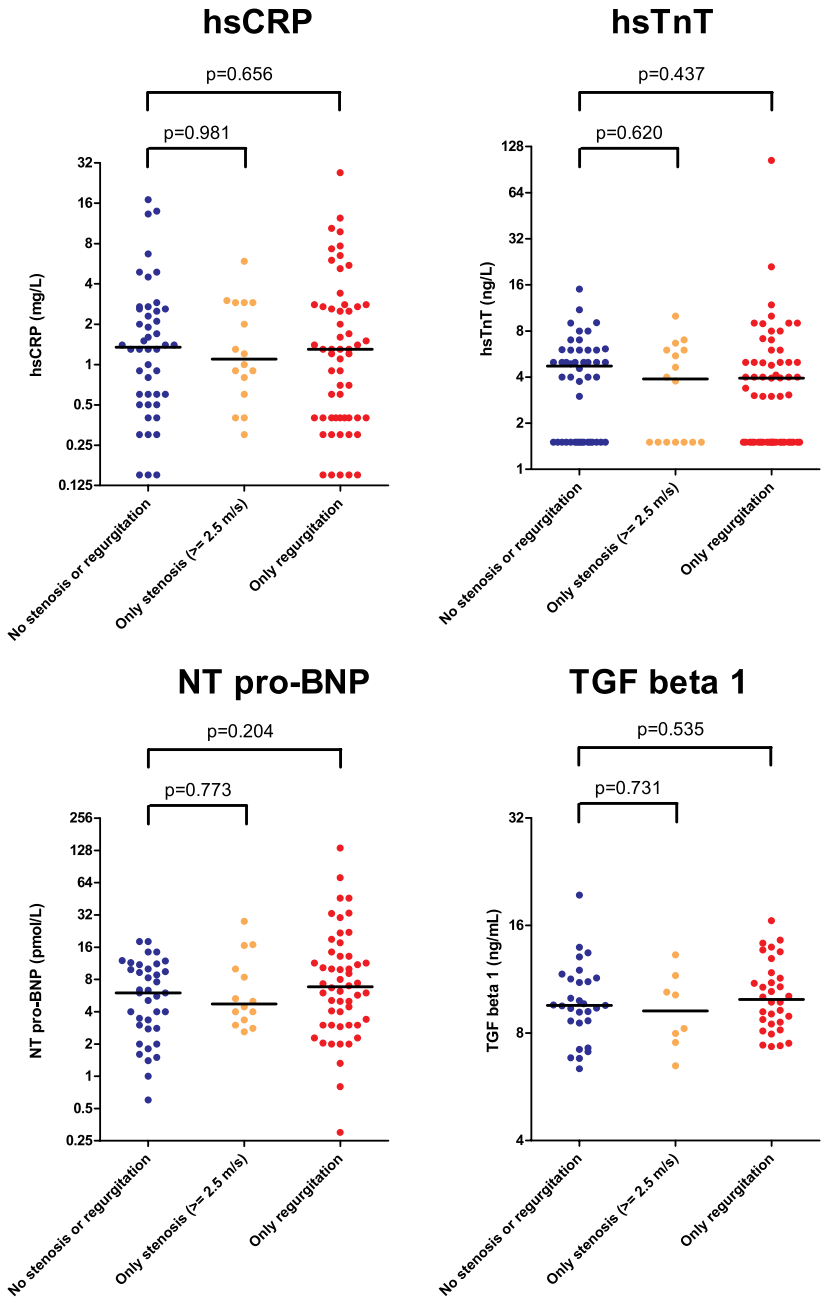
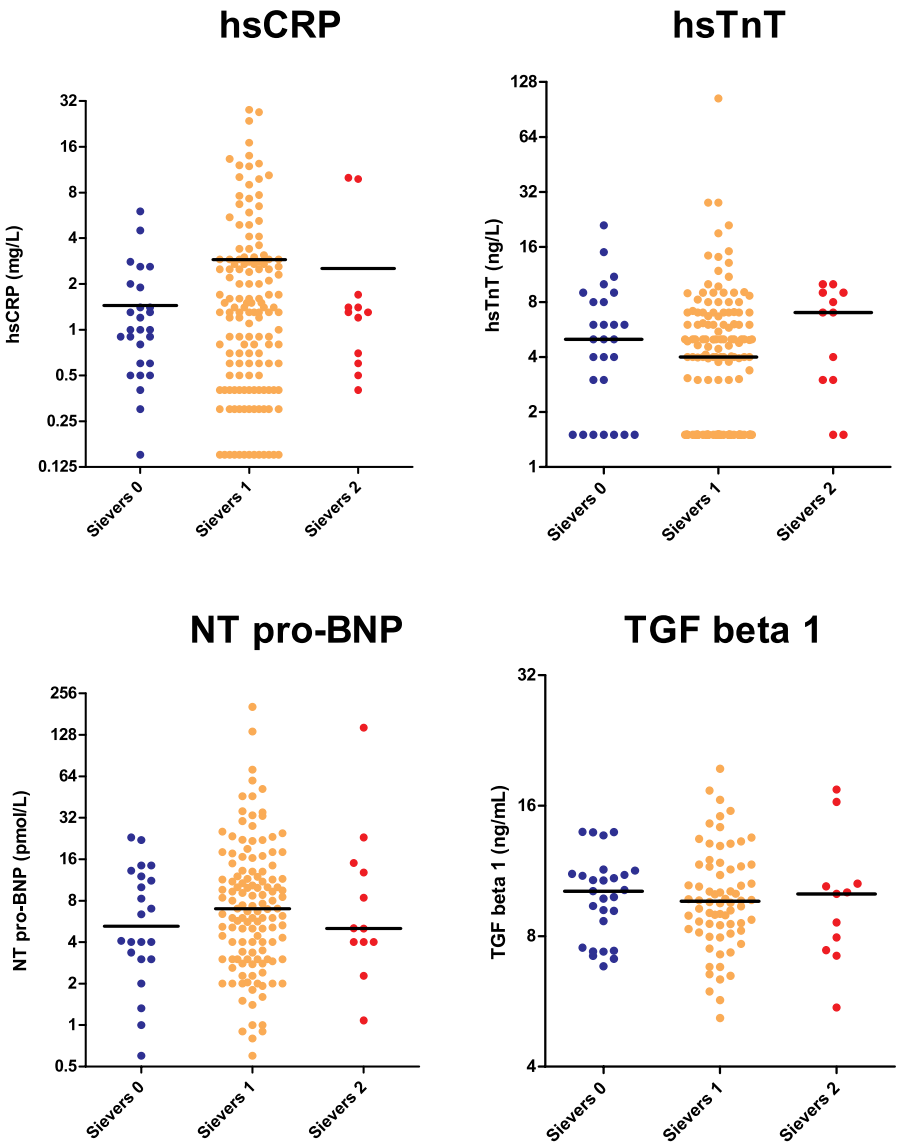


Supplemental figure 1. TGF beta 1 levels according to sex and age groups measured in healthy volunteers (n=85).
Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median. No differences were found between men and women for each age group. TGF beta 1 = transforming growth factor beta 1.



Supplemental figure 2. Biomarker levels in patients without aortic stenosis or aortic regurgitation, patients with only aortic valve stenosis and patients with only aortic valve regurgitation.

Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median. hsCRP = high sensitive C-reactive protein; hsTnT = high sensitive troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; TGF beta 1 = transforming growth factor beta 1.



Supplemental figure 3. Biomarker levels separated by Sievers classification.
Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median. No differences were found between Sievers classifications with use of the One-way anova for NT-proBNP and TGF beta 1 and the Kruskal-Wallis test for hsCRP and hsTnT levels. hsCRP = high sensitive C-reactive protein; hsTnT = high sensitive troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; TGF beta 1 = transforming growth factor beta 1.

CHAPTER 7

Exploring the prognostic value of novel markers in adults with a systemic right ventricle

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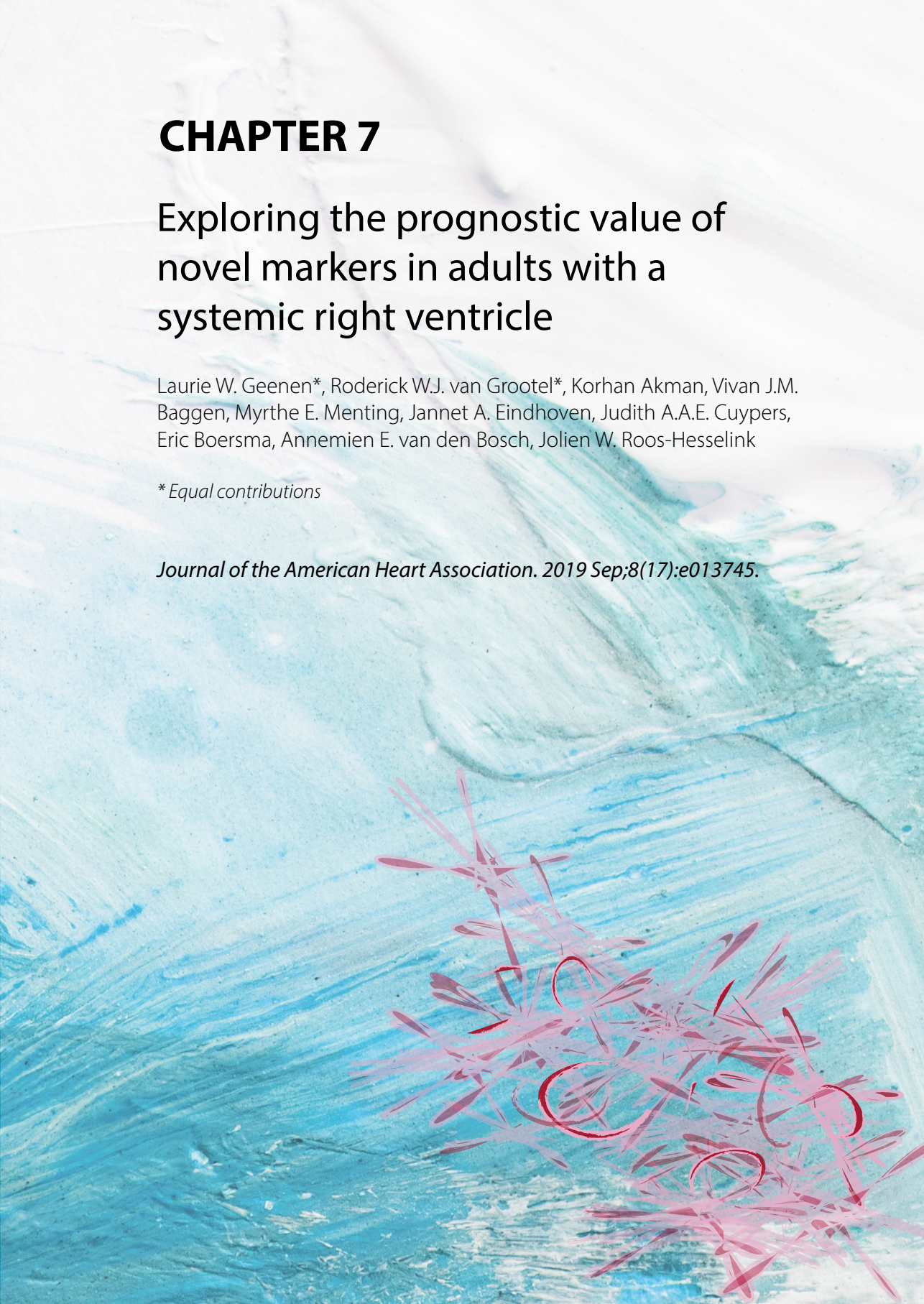

Abstract

Background: Adults with a systemic right ventricle (sRV) have a high risk of cardiac complications. This study aimed to identify prognostic markers in adults with sRV based on clinical evaluation, echocardiography, and blood biomarkers.

Methods and Results: In this prospective cohort study, consecutive clinically stable adults with sRV caused by Mustard- or congenitally corrected transposition of the great arteries (TGA) were included (2011-2013). Eighty-six patients were included (age 37±9 years, 65% male, 83% New York Heart Association functional class I, 76% Mustard-transposition of the great arteries, 24% congenitally corrected transposition of the great arteries). Venous blood sampling was performed including N-terminal pro B-type natriuretic peptide, high-sensitive troponin-T, high sensitive C-reactive protein, growth differentiation factor-15, galectin-3, red cell distribution width, estimated glomerular filtration rate and hemoglobin. Besides conventional echocardiographic measurements, longitudinal, circumferential and radial strain were assessed using strain analysis. During a median follow-up of 5.9 [IQR 5.3-6.3] years, 19 (22%) patients died or had heart failure (primary endpoint) and 29 (34%) patients died or had arrhythmia (secondary endpoint). Univariable Cox regression analysis was performed using dichotomous or standardized continuous variables. New York Heart Association functional class >I, systolic blood pressure and most blood biomarkers were associated with the primary and secondary endpoint (galectin-3 not for primary, N-terminal pro B-type natriuretic peptide and high sensitivity C-reactive protein not for secondary endpoint). growth differentiation factor-15 showed the strongest association with both endpoints (HRs: 2.44 (95% CI 1.67-3.57, p<0.001), 2.00 (95% CI 1.46-2.73, p<0.001), respectively). End-diastolic basal dimension of the subpulmonary ventricle was associated with both endpoints (HR:1.95 (95% CI 1.34-2.85), p<0.001, 1.70 (95% CI 1.21-2.38, p=0.002, respectively). Concerning strain analysis, only sRV septal strain was associated with the secondary endpoint (HR 0.58 (95% CI 0.39-0.86), p=0.006).

Conclusion: Clinical, conventional echocardiographic, and blood measurements are important markers for risk stratification in adults with a sRV. The value of novel echocardiographic strain analysis seems limited.

Introduction

Transposition of the great arteries (TGA) is a complex congenital heart defect, with an incidence of 0.31 per thousand living births per year[1]. Before the arterial switch operation was introduced, TGA patients were operated according to the Mustard or Senning procedure (M-TGA). During this procedure, a baffle is created at the levels of the atria (atrial switch operation) resulting in a right ventricle supporting the systemic circulation (sRV)[2]. Congenitally corrected TGA (ccTGA) is the name given to a morphologic situation where there is both atrioventricular and ventriculoarterial discordance, also resulting in a sRV[3].

Since the right ventricle is designed for volume load and not pressure overload[4], the right ventricle will degenerate earlier, leading to an impaired ventricular function over time. Dysfunction of the sRV may subsequently lead to cardiac complications such as heart failure (HF), arrhythmias, and death [5-8]. Currently, it is not clear which patients are at higher risk of developing complications, nor is it clear how we can best monitor them over time. Identification of high-risk patients is important to enable clinicians to intervene at the right time and also to establish a better expectation of management for patients. However, at the moment prospective longitudinal studies investigating prognostic factors in patients with a sRV are scarce. Studies have investigated prognosis in adult congenital heart disease[6], but rarely in a prospective manner pertaining exclusively sRV patients.[9] Therefore, this study aimed to identify the prognostic value of clinical variables, echocardiographic- and blood biomarkers in adult patients with a sRV.

Methods

Study population and design

Patients with M-TGA and ccTGA were extracted from a prospective cohort of consecutive, clinically stable adults with congenital heart disease who were included during a routine visit to the outpatient clinic of the Erasmus Medical Center, Rotterdam between April 2011 and April 2013. Exclusion criteria were the following: age <18 years, pregnancy, renal dysfunction (creatinine level >200 μmol/L) or patients not capable of understanding and signing informed consent. In our center, the Senning procedure in patients with TGA had not been performed; therefore, only M-TGA patients were included in this study.

All patients underwent clinical examination by a cardiologist, 12-lead electrocardiography, echocardiography and venous blood sampling. The study protocol has been described in more detail previously[10]. This study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committee. Written informed consent was obtained from all participants.

Echocardiography and speckle-tracking analysis

Two-dimensional grayscale harmonic images were obtained with patients in the left lateral decubitus position using a commercially available iE33 or Epic C7 ultrasound system (Philips Medical Systems, Best, The Netherlands) equipped with a transthoracic broadband S5-1 (1–5 MHz) or X5-1 matrix transducer (composed of 3040 elements, with 1–5 MHz extended operating frequency range).

Echocardiographic assessment was performed according to the European Society of Cardiology (ESC) and American Society of Echocardiography (ASE) guidelines for echocardiographic chamber quantification[11, 12].

Speckle tracking analysis was performed (RWG) using TomTec (TomTec 2D CPA using dedicated RV software, TomTec Imaging Systems, Unterschleissheim, Germany) following the current consensus document on strain analysis[13]. Longitudinal strain in the apical 4-chamber view was assessed for all patients, and circumferential and radial strain were only assessed in the short-axis view in M-TGA patients as the appropriate view required for analysis is unobtainable in the ccTGA patients because of the orientation of the sRV in the thorax. Similarly, subpulmonary left ventricular diameters were not measured in the ccTGA group.

Laboratory results

Peripheral venous blood sampling was performed at the day of study inclusion and was for research purposes only. Blood samples were then transferred to the clinical chemistry laboratory of our center within 2 hours after withdrawal. N-terminal pro B-type natriuretic peptide (NT-proBNP), red cell distribution width (RDW) and estimated glomerular filtration rate (eGFR) were directly determined in fresh blood samples whereas the rest of the samples were aliquoted and stored at -80 ºC. Serum levels of growth differentiation factor-15 (GDF-15), high sensitive C-reactive protein (hs-CRP), galectin-3 and high sensitive troponin-T (hs-TnT) were determined in batches. Samples were exposed to only 1 freeze-thaw cycle. The laboratory analyses of all biomarkers have been described in more detail previously[10, 14-17].

Definition of events

Given the relatively low mortality rate in this population, we chose to take combined endpoints. The primary endpoint was composed of all-cause mortality or HF. HF was defined as signs and symptoms of HF requiring hospital admission, or requiring initiation or change in HF medication. The secondary endpoint was a composite of all-cause mortality or arrhythmia. We defined arrhythmia as any symptomatic and registered arrhythmia, or arrhythmias requiring treatment. Presence of premature ventricular complexes or premature atrial complexes were not regarded arrhythmias. HF and arrhythmias were treated as separate endpoints because of potential event-specific predictors because of a probable different mechanism of clinical worsening. Follow-up was assured by yearly scheduled visits to the adult congenital heart disease outpatient clinic during the first 4 years of follow-up and endpoints were systematically

tracked by 2 investigators (LG and JR) until the January 1, 2018. Survival status was checked in the Municipal Population Register.

Statistical analysis

Continuous variables with a normal distribution were presented as mean ± SD, otherwise the median and interquartile range was presented. For blood biomarkers, the geometric mean was given in addition to the median. Patients were stratified according to diagnosis (M-TGA or ccTGA). Continuous variables between these groups were compared using the unpaired t-test or Mann-Whitney U test as appropriate. For frequencies, Fisher exact test was performed. Missing data were addressed by multiple imputation before any further statistical analyses were performed. Multiple imputation was performed in SPSS using 5 imputations and based on all relevant patient characteristics, including the endpoints. Endpoints were not imputed. Biomarker levels were log-transformed to correct for a skewed distribution before further analysis.

Correlations between blood biomarkers and echocardiographic measurements were reflected by Spearman correlation coefficient. The Kaplan-Meier estimator was used to obtain survival curves stratified according to the diagnosis groups. Survival functions were compared using the log-rank test.

Cox regression analysis was performed based on the entire study population. All continuous echocardiographic and blood biomarkers variables were transformed into z-scores to obtain standardized hazard ratios for the associations with the endpoints. Tricuspid regurgitation was treated as continuous variable (0-3), but not standardized. Univariable Cox regression was performed to assess associations between variables and endpoints. Additionally, all univariable associations were alternately adjusted for New York Heart Association (NYHA) functional class (NYHA I versus NYHA II/III) in multivariable analyses. Comparisons of strength of associations were made on the basis of standardized hazard ratios. As post-hoc analysis, a subgroup analysis was performed that was restricted to M-TGA patients only. A 2-sided p-value of <0.05 was considered statistically significant. Statistical tests were performed using SPSS Statistics, version 24.0.

Results

Baseline characteristics

In this prospective cohort study, 86 patients were included: 65 patients with M-TGA and 21 patients with ccTGA. The patient selection process is shown in Supplemental Figure 1. Overall, the mean age was 37 ± 9 years, 56 (65%) were men and most patients were in NYHA class I (83%) (Table 1). Anticoagulants were more often prescribed in ccTGA patients than in M-TGA patients. M-TGA patients were more often in sinus rhythm than ccTGA patients and had an implantable cardioverter defibrillator less often. In patients with ccTGA, significantly higher

Table 1. Baseline characteristics for all patients with a sRV and stratified according to Mustard-TGA and ccTGA patients.

	Complete, n (%)	All patients (n = 86)	Mustard-TGA (n = 65)	ccTGA (n = 21)	p–value
Patient characteristics					
Age, years	86 (100)	37 ± 9	35 ± 6	41 ± 14	0.06
Sex, man, n (%)	86 (100)	56 (65)	43 (66)	13 (62)	0.79
Age at initial repair, years	64 (98)	-	0.7 [0.4-2.2]	-	-
Concomitant heart defect, n (%)	86 (100)				
Ventricular septal defect		27 (31)	21 (32)	6 (29)	1.00
Pulmonary outflow tract obstruction		13	7 (11)	5 (24)	0.16
NHYA class ≥ II, n (%)*	86 (100)	15 (17)	12 (19)	3 (14)	1.00
Body mass index, kg/m^2	84 (98)	24.8 ± 4.0	25.0 ± 4.3	24.2 ± 2.8	0.41
Body surface area, m^2	84 (98)	1.91 ± 0.20	1.92 ± 0.19	1.88 ± 0.21	0.47
Systolic blood pressure, mmHg	83(97)	125 ± 14	125 ± 15	124 ± 13	0.87
Diastolic blood pressure, mmHg	83 (97)	79 ± 12	79 ± 12	78 ± 10	0.64
Heart rate, beats/min	84 (98)	72 ± 13	72 ± 13	71 ± 11	0.86
Oxygen saturation > 96%, n (%)	78 (91)	63 (81)	44 (76)	19 (95)	0.10
Cardiac medication use, n (%)	86 (100)				
ACE-inhibitor		26 (30)	17 (26)	9 (43)	0.18
ARBs		5 (6)	2 (10)	3 (5)	0.59
B-blocker		19 (22)	11 (17)	8 (38)	0.07
Diuretics		17 (20)	11 (17)	6 (29)	0.34
Antiarrhythmic		13 (15)	10 (15)	3 (14)	0.90
Anticoagulants		20 (23)	11 (17)	9 (43)	0.034
Electrocardiography					
QRS duration, msec	69 (80)	114 [105-130]	114 [105-127]	113 [105-137]	0.57
Rhythm, n (%)	86 (100)				0.006†
Sinus rhythm		59 (68)	50 (77)	9 (43)	
Pacemaker rhythm		17 (20)	8 (12)	9 (43)	
Atrial fibrillation		4 (5)	2 (3)	2 (9)	
Other		6 (7)	5 (8)	1 (5)	
Device implantation, n (%)	86 (100)				
Pacemaker		17 (20)	13 (20)	4 (19)	1.00
ICD		9 (10)	4 (6)	5 (24)	0.036
Echocardiography					
sRV dimensions					
End-diastolic basal dimension, mm	57 (66)	59.6 ± 8.4	58.9 ± 8.2	61.1 ± 9.0	0.35
End-diastolic annulus, mm	67 (78)	47.1 ± 8.6	50.4 ± 5.9	34.7 ± 4.6	<0.001
End-systolic area, cm^2	81 (95)	30.7 ± 7.9	30.6 ± 7.5	30.8 ± 9.5	0.93
End-diastolic area, cm^2	81 (95)	41.0 ± 9.2	40.4 ± 8.7	42.9 ± 10.7	0.30
sRV systolic function					
≥Moderately impaired, n (%)	86 (100)	60 (70)	47 (72)	13 (62)	0.42
TAPSE, mm	45 (52)	13 ± 3	12.9 ± 3.1	13.6 ± 2.3	0.64
RV fractional area change, (%)	81 (95)	25.4 ± 8.2	24.3 ± 7.5	28.9 ± 9.5	0.03

Continue

Continued

	Complete, n (%)	All patients (n = 86)	Mustard-TGA (n = 65)	ccTGA (n = 21)	p–value
Tricuspid regurgitation	86 (100)				0.038
None		12 (14)	6 (9)	6 (29)	
Mild		46 (53)	40 (61)	6 (29)	
Moderate		24 (28)	16 (25)	8 (38)	
Severe		4 (5)	3 (5)	1 (4)	
Strain parameters					
LS of the RV free wall, %	77 (90)	-15.5 ± 3.9	-15.0 ± 3.2	-16.7 ± 5.5	0.21
LS of the RV septal wall, %	77 (90)	-12.4 ± 3.3	-12.4 ± 3.3	-12.3 ± 3.4	0.95
Global LS of sRV, %	77 (90)	-13.8 ± 3.4	-13.6 ± 3.1	-14.2 ± 4.1	0.50
Global CS of sRV, %	52 (80)	-	-12.0 ± 3.8	-	-
Global RS of sRV, %	49 (75)	-	-18.9 ± 13.5	-	-
Subpulmonary LV dimensions					
LV end-diastolic diameter, mm/m^2	51 (79)	-	22 ± 4	-	-
LV end-systolic diameter, mm/m^2	49 (75)	-	15 ± 4	-	-
Subpulmonary LV function					
≥ Moderately impaired, n (%)	86 (100)	2 (2)	2 (3)	0	-
Laboratory‡					
Hemoglobin, mmolFe/L	81 (94)	9.5 [9.2-10.0] 9.4	9.5 [9.2-10.0] 9.4	9.3 [9.0-10.0] 9.4	0.96
RDW, %	81 (94)	13.1 [12.6-13.7] 13.2	13.0 [12.6-13.4] 13.1	13.5 [13.2-14.1] 13.5	0.027
eGFR, mL/min/1.73m^2	84 (98)	90 [81-90] 85	90 [83-90] 85	89 [77-90] 83	0.16
NT-proBNP, pmol/L	85 (99)	30.9 [17.7-58.2] 34.5	27.4 [18.2-53.2] 32.7	44.3 [16.4-76.5] 41.1	0.36
Hs-troponin-T, ng/L	85 (99)	6.0 [1.5-9.5] 2.4	5.0 [1.5-8.4] 4.6	8.9 [6.0-15.4] 9.1	0.004
GDF-15, ng/L	84 (98)	623 [501-886] 727	615 [498-862] 698	660 [519-1379] 830	0.29
Hs-CRP, mg/L	85 (99)	1.8 [0.8-3.5] 1.6	1.9 [0.7-3.6] 1.6	1.2 [0.8-2.7] 1.4	0.50
Galectin-3, ng/mL	85 (99)	12.7 [11.2-15.0] 12.7	12.7 [11.4-15.0] 12.7	12.8 [11.0-13.9] 13.0	0.99

*All patients were NYHA II except one patient who was NYHA class III. †Compares sinus rhythm versus all other rhythms. ‡ For the laboratory measurements, the geometric mean is shown on the second line in addition to the median and interquartile range.

NYHA= New York Heart Association, ARBs= angiotensin II receptor blocker, ICD= implantable cardioverter defibrillator, sRV= systemic right ventricle, TAPSE= trans annular plane systolic excursion, LS= longitudinal strain, CS= circumferential strain, RS, radial strain, LV= left ventricular, RDW= red cell distribution width, eGFR= estimated glomerular filtration rate, NT-proBNP = N-terminal pro B-type natriuretic peptide, Hs= high sensitive, GDF-15= growth differentiation factor-15, CRP= C-reactive protein

levels of RDW and hs-TnT were found, compared with M-TGA patients. The sRV end-diastolic annulus was 50.4±5.9 mm in the M-TGA group, which was larger compared with the ccTGA group: 34.7±4.6 mm. Sixty patients (70%) had at least a moderately impaired sRV. Speckle tracking analysis showed that the mean longitudinal strain of the sRV free wall was -15.5±3.9% and the mean sRV circumferential strain was -12.0±3.8%.

Correlations between echocardiographic measurements and blood biomarkers

Both higher levels of NT-proBNP and hs-TnT were weakly correlated with a larger subpulmonary end diastolic dimension. Weak correlations were also found between higher levels of galectin-3 and lower sRV global longitudinal strain and lower sRV longitudinal strain of the septal wall. (Table 2)

Mutual blood biomarker correlations were present, though no strong correlations were found. The strongest correlation was found between NT-proBNP and hs-TnT (r= 0.48, p<0.001). (Table 2)

Table 2. Correlations between blood biomarker and echocardiographic (strain) measurements and mutual biomarker correlations

	HB	RDW	eGFR	NT-proBNP	Hs-TnT	GDF-15	Hs-CRP	Galectin-3
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Echocardiography								
Tricuspid regurgitation	0.08	-0.01	0.09	-0.12	0.18	-0.06	-0.08	-0.01
sRV end diastolic annulus	0.07	-0.11	0.13	-0.04	-0.06	-0.05	0.06	0.21
sRV global LS†	-0.18	-0.09	0.13	-0.21	-0.20	-0.07	-0.02	**-0.25***
sRV GCS†	0.05	-0.07	-0.07	-0.24	-0.10	-0.10	0.01	0.01
sRV LS freewall†	-0.16	-0.03	0.21	-0.19	-0.17	-0.03	-0.03	-.022
sRV LS septal wall†	-0.17	-0.21	-0.01	-0.19	-0.17	-0.11	-0.03	**-0.27***
LV EDD‡	-0.19	-0.08	-0.07	**0.29***	**0.35***	0.09	0.02	0.05
Bloodbiomarkers								
RDW,	-0.09							
eGFR	0.07	**-0.30****						
NT-proBNP	-0.19	**0.34****	**-0.43*****					
Hs-TnT	0.09	**0.44*****	**-0.33****	**0.48*****				
GDF-15	-0.19	**0.40****	**-0.43*****	**0.43*****	**0.32****			
Hs-CRP	-0.14	**0.27***	**-0.22***	**0.39*****	**0.21*****	**0.39*****		
Galectin-3	-0.04	**0.31****	**-0.28****	0.20	**0.26***	**0.37****	**0.31****	

Level of significance is indicated by the following symbols; *p<0.05, ** p<0.01, ***p<0.001. †Variables were transformed to positive numbers for easier interpretation. ‡Indexed for BSA.
HB= hemoglobin, RDW= red cell distribution width, eGFR= estimated glomerular filtration rate, NT-proBNP = N-terminal pro B-type natriuretic peptide, Hs= high sensitive, GDF-15= growth differentiation factor-15, CRP= C-reactive protein, sRV=systemic right ventricle, LS= longitudinal strain, GCS= global circumferential strain, LV EDD= left ventricular end diastolic dimension.

Follow-up

Follow-up was complete in 99% of the patients. After a median follow-up period of 5.9 (inter quartile range 5.3-6.3) years, 19 patients (22%) reached the primary endpoint and 29 patients (34%) reached the secondary endpoint. In 1 patient, an event of HF overlapped with the presence of arrhythmia and therefore reached both endpoints at the same time. Considering all components of the endpoints separately (ie, patients were not censored at the time of another endpoint than the endpoint of interest), the occurrence of events were 5 deaths, 18 HF events, and 26 arrhythmias. Causes of death were end-stage HF (n=2), cardiac arrest (n=1), sudden death, presumed cardiac (n=1), and hemorrhagic shock (n=1). In 9 cases, HF required hospitalization, in the other cases HF medication was initiated or changed. The nature of the arrhythmias were 5 ventricular tachycardia's and 21 supraventricular tachycardias. Supraventricular tachycardias included atrial flutter (n=6), atrial fibrillation (n=4), AV(nodal) re-entry tachycardia (n=2) and other supraventricular tachycardias (n=8). One patient had an AV-block.

Associations with endpoints

Kaplan-Meier curves showed no significant differences in the risk of both endpoints among the M-TGA and ccTGA patients. Cumulative HF-free survival at 6 years, was 81% in ccTGA patients versus 76% in M-TGA patients. Cumulative arrhythmia-free survival was 67% versus 64% respectively (Figure 1).

A lower systolic blood pressure, NYHA class >1, and loss of sinus rhythm were significantly associated with an increased risk of the primary endpoint. All blood biomarkers showed a significant association with the primary endpoint, except galectin-3. GDF-15 showed the

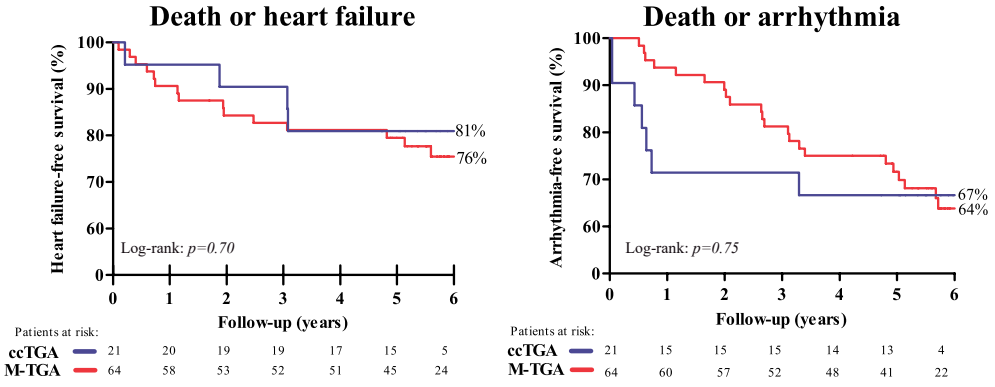

Figure 1. Heart failure-free survival (primary endpoint) and arrhythmia-free survival (secondary endpoint) stratified according to ccTGA and M-TGA patients.
Red line indicates the event-free survival according to M-TGA patients. Blue line indicates the event free survival according to ccTGA patients.
ccTGA= congenitally corrected transposition of the great arteries, M-TGA= transposition of the great arteries corrected by the Mustard operation.

strongest association with the primary endpoint, and a relatively strong association was found between hs-CRP and RDW and the primary endpoint. (Figure 2) (Standard deviations of all variables are presented in Supplemental Table 1).

With regard to the secondary endpoint, systolic blood pressure and NYHA functional class were again significantly associated with the endpoint. Notably loss of sinus rhythm was not associated with the risk of death or arrhythmias. GDF-15, RDW and galectin-3 showed the strongest association of all biomarkers, with the secondary endpoint. NT-proBNP and hs-CRP were the only blood biomarkers not associated with the secondary endpoint. (Figure 3)

Regarding the echocardiographic variables (Figures 2 and 3), end-diastolic diameters of the sRV as well as subpulmonary LV were significantly associated with both endpoints. Tricuspid regurgitation was associated with the primary but not the secondary endpoint. Of all strain variables, solely septal longitudinal strain showed a significant association with the secondary endpoint.

When adjusting associations between blood biomarkers and the primary endpoint for NYHA class, only eGFR was no longer statistically significant. All clinical variables and sRV end diastolic dimension did not remain associated with the primary endpoint, while tricuspid

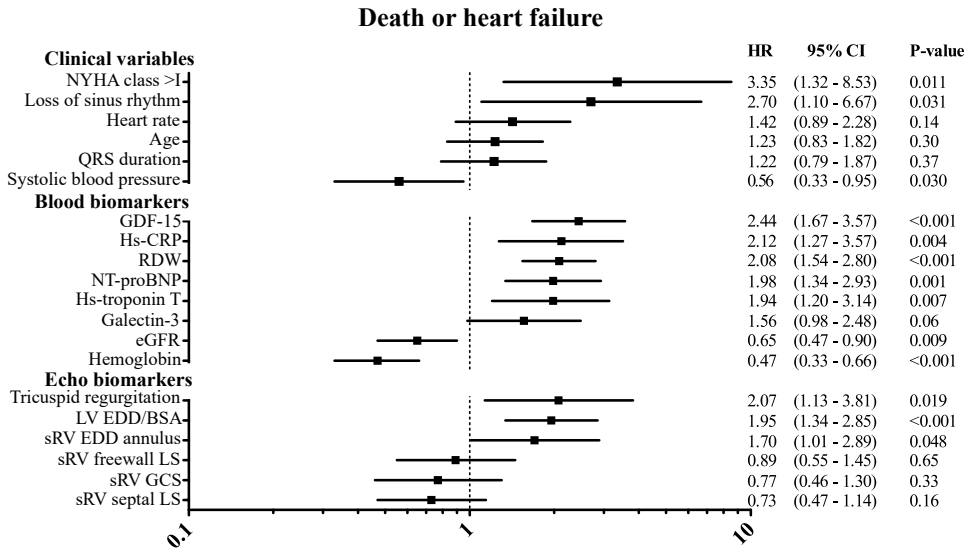

Figure 2. Associations between clinical variables, blood- and echocardiographic biomarkers and the primary endpoint (death or heart failure) in patients with a systemic right ventricle.
Hazard ratios are expressed per increase in standard deviation, except for categorical variables (NYHA class, loss of sinus rhythm and tricuspid regurgitation). All blood biomarker levels were log transformed before standardization. sRV GCS and LV EDD/BSA were only measured in M-TGA patients. Inversed HRs (for comparison purposes); hemoglobin=2.13, eGFR=1.54.
BSA= body surface area, CRP= C-reactive protein, EDD= end diastolic dimension, eGFR= estimated glomerular filtration rate, GDF-15= growth differentiation factor-15, GCS= global circumferential strain, GLS= global longitudinal strain, LS= longitudinal strain, NT-proBNP= N-terminal pro b-type natriuretic peptide, NYHA= New York Heart Association functional class, RDW= red cell distribution width, sRV= systemic right ventricle.

regurgitation did show an independent association with the primary endpoint. GDF-15, RDW, eGFR, systolic blood pressure and heart rate were independently associated with the secondary endpoint as well as 2 echo marker; longitudinal strain of the sRV septal wall and sRV global circumferential strain. (Supplemental Table 2)

Subgroup analysis restricted to M-TGA patients only, showed significant associations with both endpoints for age, systolic blood pressure, NYHA class, and loss of sinus rhythm. Of all blood biomarkers, NT-proBNP yielded the strongest association with the primary endpoint and GDF-15 was most strongly associated with the secondary endpoint. Longitudinal strain of the free wall was significantly associated with the secondary endpoint. (Supplemental Table 3)

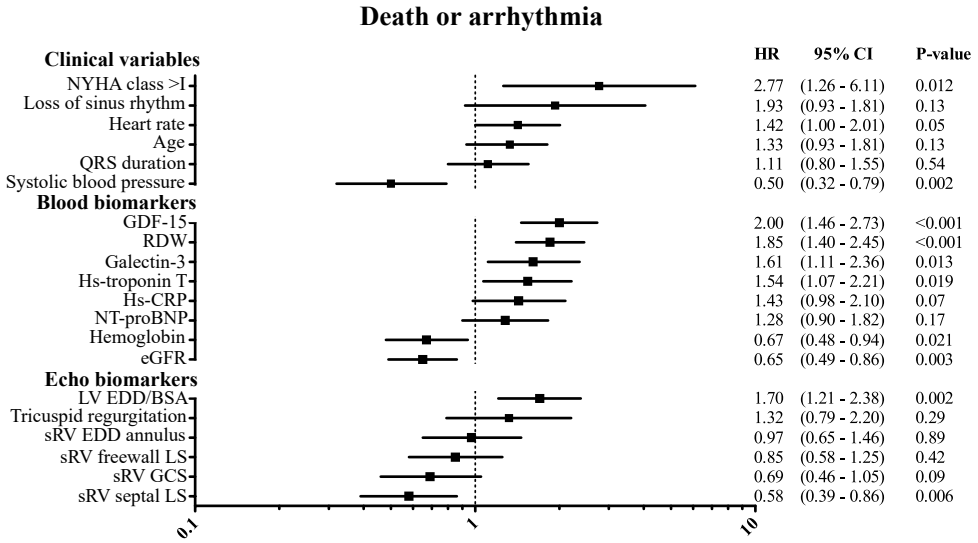

Figure 3. Associations between clinical variables, blood and echocardiographic biomarkers and the secondary endpoint (death or arrhythmia) in patients with a systemic right ventricle.
Hazard ratios are expressed per increase in standard deviation, except for categorical variables (NYHA class, loss of sinus rhythm and tricuspid regurgitation). All blood biomarker levels were log transformed before standardization. sRV GCS and LV EDD/ BSA were only measured in M-TGA patients. Inversed HRs (for comparison purposes); hemoglobin= 1.49, eGFR=1.54.
BSA= body surface area, CRP= C-reactive protein, EDD= end diastolic dimension, eGFR= estimated glomerular filtration rate, GDF-15= growth differentiation factor-15, GCS= global circumferential strain, GLS= global longitudinal strain, LS= longitudinal strain, NT-proBNP= N-terminal pro b-type natriuretic peptide, NYHA= New York Heart Association functional class, RDW= red cell distribution width, sRV= systemic right ventricle.

Discussion

This prospective longitudinal cohort study consisting of 86 adults with a sRV showed that these patients are at high risk of HF, arrhythmias, and death. This emphasizes the importance of adequate risk stratification. Higher NYHA class, lower systolic blood pressure, and loss of sinus rhythm were significantly associated with an increased risk of adverse cardiac outcomes. Concerning echocardiographic measurements, both subpulmonary ventricular end-diastolic dimension and sRV septal strain measurements were found to be associated with endpoints. This study also confirmed tricuspid regurgitation as a risk factor for HF. Most blood biomarkers were associated with adverse cardiac events, of which GDF-15 yielded the strongest association, even stronger than NT-proBNP. Risk stratification in adults with sRV should therefore focus on clinical variables, echocardiographic analysis, and blood biomarkers; however, strain analysis may be less feasible compared with the use of clinical and blood biomarkers.

Previous studies

NT-proBNP is the most widely used and studied blood biomarker for risk stratification in the adult congenital heart disease population[18] and is one of the few blood biomarkers that has previously been investigated in sRV patients. Westhoff-Bleck et al investigated NT-proBNP in a prospective cohort study of 116 patients with TGA corrected by the atrial switch procedure and found that NT-proBNP was an independent prognostic factor for adverse cardiac outcomes. Hemoglobin was also assessed, but showed no significant association.[19] Popelova et al also found a strong independent prognostic value of NT-proBNP in TGA patients after the atrial switch operation[20]. One cross-sectional study showed that hs-TnT was better in detecting patients with sRV dysfunction than NT-proBNP[21]. Surprisingly, our results showed that NT-proBNP was not the strongest prognostic factor. To the best of our knowledge, GDF-15, galectin-3 and RDW have never been described before in this specific ACHD population, although according to our study, these biomarkers may be better predictors for adverse cardiac events than NT-proBNP. In a large prospective cohort study including all types of ACHD patients, higher levels of RDW have been investigated and were shown to be predictive of adverse cardiac outcomes [22]. This same group also demonstrated a significant association between hs-CRP and outcomes in ACHD patients[23].

Although some cross-sectional studies have been published, only a few studies investigated the prognostic value of echocardiographic measures. Conventional measures such as sRV ejection fraction based on magnetic resonance imaging or sRV fractional area change have been linked to clinical outcome[24, 25], with longitudinal strain on magnetic resonance imaging being the most promising. Tricuspid regurgitation is also a known risk factor for adverse outcomes in these patients[26, 27]. Diller et al showed that in patients with a sRV, there is an adverse ventricular-ventricular interaction, similar to tetralogy of Fallot patients[24]. This adverse interaction impedes the subpulmonary left ventricular function in a number of ways; shared myocardial fibers, dyssynchrony and septal shift may all play a role. Another mechanism

is because of backward failure of the sRV, leading to increased stress on the subpulmonary left ventricle. This was also present in our study, demonstrated by the association between left ventricular diameters and clinical events. Current literature is ambiguous regarding the prognostic value of strain analysis in sRV patients[24, 28, 29]. In our study, the prognostic value of strain analysis was limited, though we did find an association between septal longitudinal strain and death or arrhythmia.

Death and HF

The increased risk of HF in patients with a sRV may arise from different mechanisms: myocardial ischemia caused by the single coronary artery that has to supply the right ventricle, and peri-operative damage to the myocardium and the right ventricle which has to deal with a pressure overload. In M-TGA patients, baffle stenosis or leakages can occur and are associated with a worse outcome.[7, 30, 31]

Regarding the clinical variables, NYHA class >1 was associated with death or HF, as well as a lower systolic blood pressure. Lower systolic blood pressure could be a sign of subclinical systemic ventricular failure, though mean systolic blood pressure still was 125 ± 14 mmHg**.** The fact that many blood biomarkers were significantly associated with the primary endpoint, may indicate that the pathophysiology of sRV deterioration is complex and involves multiple processes and cellular pathways. GDF-15 has been suggested as a cardiac biomarker by Wollert et al and is identified as an independent predictor of mortality in many different populations, such as acute and chronic HF, atrial fibrillation, coronary artery disease, and in the community-dwelling elderly.[32] It is therefore thought that GDF-15 is secreted in response to multiple processes, including hypoxia, inflammation and oxidative stress. In contrast, NT-proBNP is primarily induced by myocardial stretch, and this might be only a partial reflection of deterioration of sRV function. This could explain why GDF-15 seems to be even better as predictor for adverse outcomes than NT-proBNP according to our data. As proposed by the study of Opowtowsky et al., inflammation might play a role in the pathophysiology of ACHD[23]. Our study supports this by the associations found between hs-CPR and both endpoints in this study. Mechanisms contributing to hs-TnT secretion in sRV patients might be a combination of loss of myocardiocytes caused by increased wall stress and ischemia caused by failing oxygen supply of the right ventricle by the single coronary artery. However, as mechanisms of hs-TnT secretion in the case of chronic HF are still not fully elucidated, secretion of hs-TnT in sRV patients is even more speculative[33].

Pettersen et al described that the sRV undergoes certain changes in order to be able to support a high-pressure circulation: Longitudinal shortening diminishes, while circumferential shortening significantly enhances[34]. In the present study, we found no association between strain measurements and the primary endpoint. Two recent studies reported absent associations between sRV systolic function on cardiac magnetic resonance imaging and exercise capacity and therefore concluded that the value of sRV imaging at rest seems to be limited[28, 29]. We can

corroborate these findings with this prospective study, though the difficulty in analyzing sRV should also be taken into account.

This study showed that ventricular dilatation measured by the left ventricular end-diastolic dimension was associated with death or HF which can be explained by backward failure of the sRV resulting in subpulmonary ventricular dilatation. A similar process is seen in HF patients with anatomically normal hearts; the deterioration of the contralateral ventricle is an ominous sign of end-stage HF. Enlarged left ventricular end-diastolic dimension could also be the result of a residual shunt or baffle leak. However, patients with severe residual shunt of baffle would have undergone an intervention, so therefore it is less likely the enlarged dimensions are the results of residual shunts or baffle leaks. Nevertheless, former shunts and leaks may still have contributed to the enlarged left ventricular dimension.

Another prevalent problem in sRV patients is tricuspid regurgitation. Although it is still debatable whether tricuspid regurgitation leads to sRV dysfunction or whether sRV dysfunction causes tricuspid regurgitation, tricuspid regurgitation has been associated with the risk of HF[26]. Our data support the presence of tricuspid regurgitation as a risk factor for HF. Notably, tricuspid regurgitation was not associated with the occurrence of arrhythmias.

Arrhythmias

Similar to the primary endpoint, NYHA class >1 and lower systolic blood pressure were significantly associated with an increased risk of the secondary endpoint. Notably, loss of sinus rhythm did not have these increased risks. An explanation for this might be that patients who at baseline already have a pacemaker rhythm or atrial fibrillation, are excluded from the occurrence of some atrial arrhythmias, while patients who are still in sinus rhythm at baseline can lose their sinus rhythm and may therefore be more likely to experience an event during follow-up.

Studies about the prognostic value of biomarkers in relation to arrhythmias are limited in these patients, even though they are characterized by a high burden of arrhythmias[6]. This is confirmed by our study. There are several probable causes for this, the first of which the congenital defect itself. ccTGA patients have an abnormal conduction pathway in which the conduction system takes a longer course to the ventricles. Secondly, perioperative damage may result in myocardial fibrosis, which may lead to arrhythmias. Thirdly myocardial stretch caused by HF may give rise to arrhythmias.

NT-proBNP was not associated with arrhythmias in our study whereas GDF-15 and RDW did show a strong association with the occurrence of arrhythmias. An explanation may be that arrhythmias do not originate solely from myocardial stretch or hypertrophy but may be caused by a combination of myocardial fibrosis, inflammation, hypoxia, and myocardial stress[30]. GDF-15 has been associated with cardiac fibrosis in nonischemic cardiomyopathy[35] and RDW has been suggested as 'being an overall cardiovascular barometer' because of its probable reflection of diverse pathophysiological processes[36]. This might explain the strong prognostic value found for both biomarkers for arrhythmias.

Aggregating the data from our study, and taking into consideration the lack of strong associations between echocardiography and blood biomarkers and the predictive ability of blood biomarkers in particular, it is likely that blood biomarkers are secreted in an earlier onset of subclinical deterioration in sRV patients, while echocardiographic changes may be revealed in a later disease stage and are therefore less predictive of cardiac events. The fact that most blood biomarkers were associated with the endpoints even after adjustment for NYHA class, strengthens the idea that blood biomarkers reflect subclinical sRV failure.

Clinical implications

A wide range of blood biomarkers yielded prognostic value in this study. Besides NT-proBNP, other biomarkers play a pivotal role, and GDF-15 specifically can be a potential biomarker for risk stratification. Some disadvantages of blood biomarkers are the high laboratory costs and the sometimes restricted availability. GDF-15 is a relative expensive biomarker and often not available in standard laboratories. On the other hand, RDW also showed strong prognostic value and furthermore this biomarker is widely available, low cost, and incorporated in a standard complete blood count.

For daily clinical practice findings such as an abnormal NYHA class and lower blood pressures can indicate high-risk patients, and these patients should probably be followed up more closely. While echocardiography plays a key role in the follow-up of patients with complex ACHD, high-quality image acquisition requires skilled sonographers, is hampered by complex anatomy and orientation in the thorax, and is highly user-dependent, which may limit its prognostic value. Newer echocardiography techniques such as strain analysis seem to have little prognostic value in sRV patients and their role in clinical practice is currently unclear. Nevertheless, more easily obtainable conventional echocardiographic measurements such as subpulmonary end-diastolic dimension can still be valuable measures for risk stratification in daily clinical practice.

Limitations

This study is limited by the relatively small sample size, and conclusions drawn from this study should therefore be treated with caution. We did not observe differences in event-free survival in ccTGA and M-TGA patients; however, underlying mechanisms of HF and arrhythmias may be different in these patients, and therefore prognostic biomarkers and echocardiographic measurements could behave differently in each diagnosis subgroup. Unfortunately, the low sample size allowed us to only perform subgroup analysis in M-TGA patients. Also, this study lacked statistical power to adjust for multiple potential confounders and biomarkers in multivariable analysis. Because we used composite endpoints, it should be taken into account that associations could be driven more by 1 of the specific components.

We also did not take into account the concomitant congenital heart defects in both TGA groups, which can influence the prognosis. We acknowledge the considerable limitation concerning the limited sample size of this study, yet to the best of our knowledge this is the

largest study that provided prospective longitudinal data including a wide range of biomarkers and echocardiographic strain analysis in patients with a sRV.

Conclusions

High event rates in adults with a sRV demand adequate risk stratification. Assessing clinical characteristics and blood biomarkers as well as conventional echocardiographic measurements seems most expedient for adequate risk assessment in these patients. However, the contribution of novel echocardiographic strain measurements seems to be limited in this perspective. This study suggests that the underlying pathophysiology of ventricular deterioration in patients with a sRV is complex, requiring a comprehensive clinical approach.

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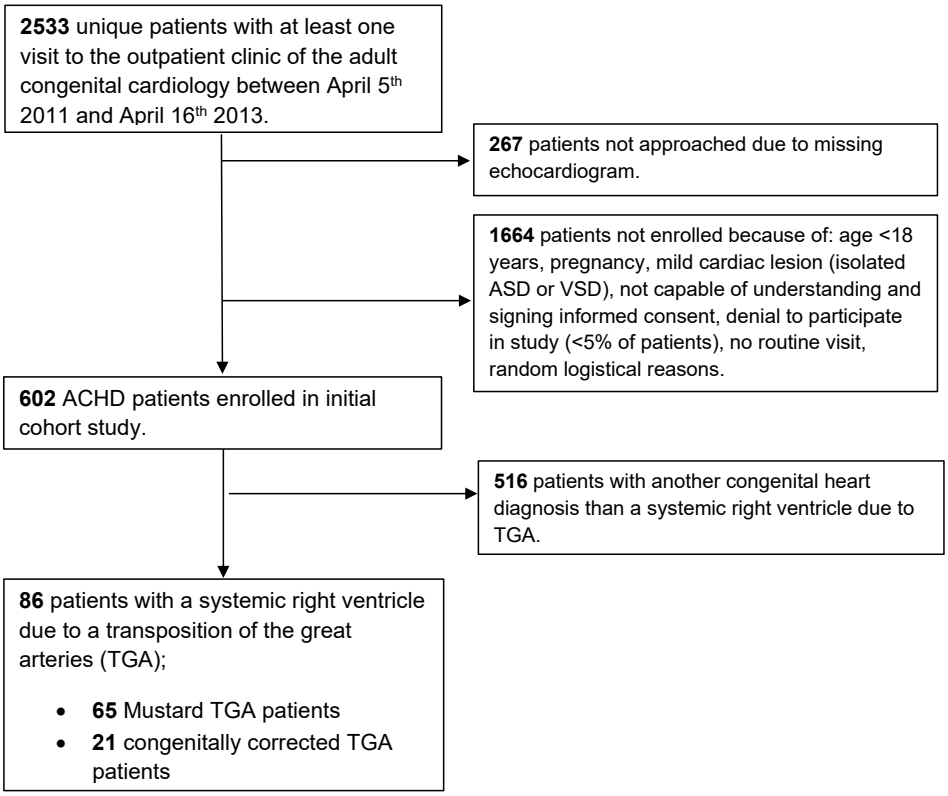


Supplemental Figure 1. Flowchart of the patient selection process of the prospective observational cohort study consisting of adults with a systemic right ventricle.
ASD= atrial septal defect, VSD= ventricular septal defect, ACHD= adult congenital heart disease, TGA= transposition of the great arteries.

Supplemental Table 1. Table showing the standard deviations of variables included in univariable Cox regression, for interpretative purposes of standardized hazard ratios.

	Standard deviation
Clinical variables	
Age, years	9
Heart rate, beats/min	13
Systolic blood pressure, mmHg	14
QRS duration, ms	21
Blood biomarkers	**(log)↓***
NT-proBNP, pmol/L	1.34
Hs-troponin-T, ng/L	1.39
GDF-15, ng/L	0.77
Hs-CRP, mg/L	1.57
Galectin-3, ng/mL	0.39
RDW, %	0.11
eGFR, mL/min/1.73m^2	0.17
Hemoglobin, mmolFe/L	0.15
Echocardiographic markers	
sRV dimensions	
sRV end-diastolic annulus, mm/m^2	8.6
Strain parameters	
LS of the sRV free wall, %	4.0
LS of the sRV septal wall, %	3.3
GCS of the sRV, %	3.8
LV dimensions	
LV end-diastolic diameter, mm/m^2	4.0

*Blood biomarkers were log transformed before standardization and therefore the SD's reported belong to the log transformed blood biomarker levels.
CRP= C-reactive protein, eGFR= estimated glomerular filtration rate, GDF-15= growth differentiation factor-15, GCS= global circumferential strain, Hs= high sensitive, LS= longitudinal strain, LV= left ventricular, NT-proBNP= N-terminal pro b-type natriuretic peptide, RDW= red cell distribution width, sRV= systemic right ventricle.

Supplemental Table 2. Associations between clinical variables, blood- and echocardiographic markers and the primary- (death or heart failure) and secondary endpoint (death or arrhythmia), with adjustment for New York Heart Association functional class in adults with a systemic right ventricle.

	Analysis with adjustment for NYHA functional class					
	Primary endpoint			**Secondary endpoint**		
	HR*	**95% CI**	**p-value**	**HR***	**95% CI**	**p-value**
Clinical characteristics						
Age	1.04	0.38-1.58	0.86	0.95	0.66-1.37	0.80
Heart rate	1.47	0.89-2.44	0.14	1.48	1.02-2.13	0.038
Systolic blood pressure	0.65	0.39-1.09	0.10	0.55	0.35-0.87	0.009
QRS duration	1.23	0.80-1.91	0.35	1.30	0.92-1.83	0.13
Loss of sinus rhythm	2.16	0.85-5.56	0.11	1.61	0.75-3.47	0.23
Blood biomarkers						
NT-proBNP	1.79	1.15-2.80	0.010	1.08	0.73-1.61	0.70
Hs-troponin-T	1.70	1.03-2.78	0.036	1.40	0.96-2.03	0.08
GDF-15	2.24	1.49-3.35	<0.001	1.86	1.35-2.56	<0.001
Hs-CRP	2.02	1.18-3.44	0.010	1.38	0.92-2.06	0.12
Galectin-3	1.30	0.80-2.11	0.29	1.45	0.98-2.14	0.06
RDW	1.91	1.38-2.65	<0.001	1.71	1.26-2.31	0.001
eGFR	0.73	0.52-1.03	0.07	0.72	0.53-0.97	0.032
Hemoglobin	0.52	0.36-0.73	<0.001	0.72	0.53-1.00	0.047
Echocardiographic markers						
Tricuspid regurgitation	1.74	1.11-2.74	0.016	1.23	0.85-1.79	0.28
sRV dimensions						
sRV end-diastolic annulus	1.66	0.97-2.86	0.07	0.94	0.62-1.40	0.74
Strain parameters						
LS of the sRV free wall	1.19	0.73-1.94	0.50	1.06	0.71-1.58	0.74
LS of the sRV septal wall	0.87	0.54-1.41	0.58	0.65	0.43-0.98	0.039
GCS of the sRV†	-‡	-	-	0.64	0.42-0.98	0.038
LV dimensions						
LV end-diastolic diameter†	-‡	-	-	1.41	0.98-2.04	0.07

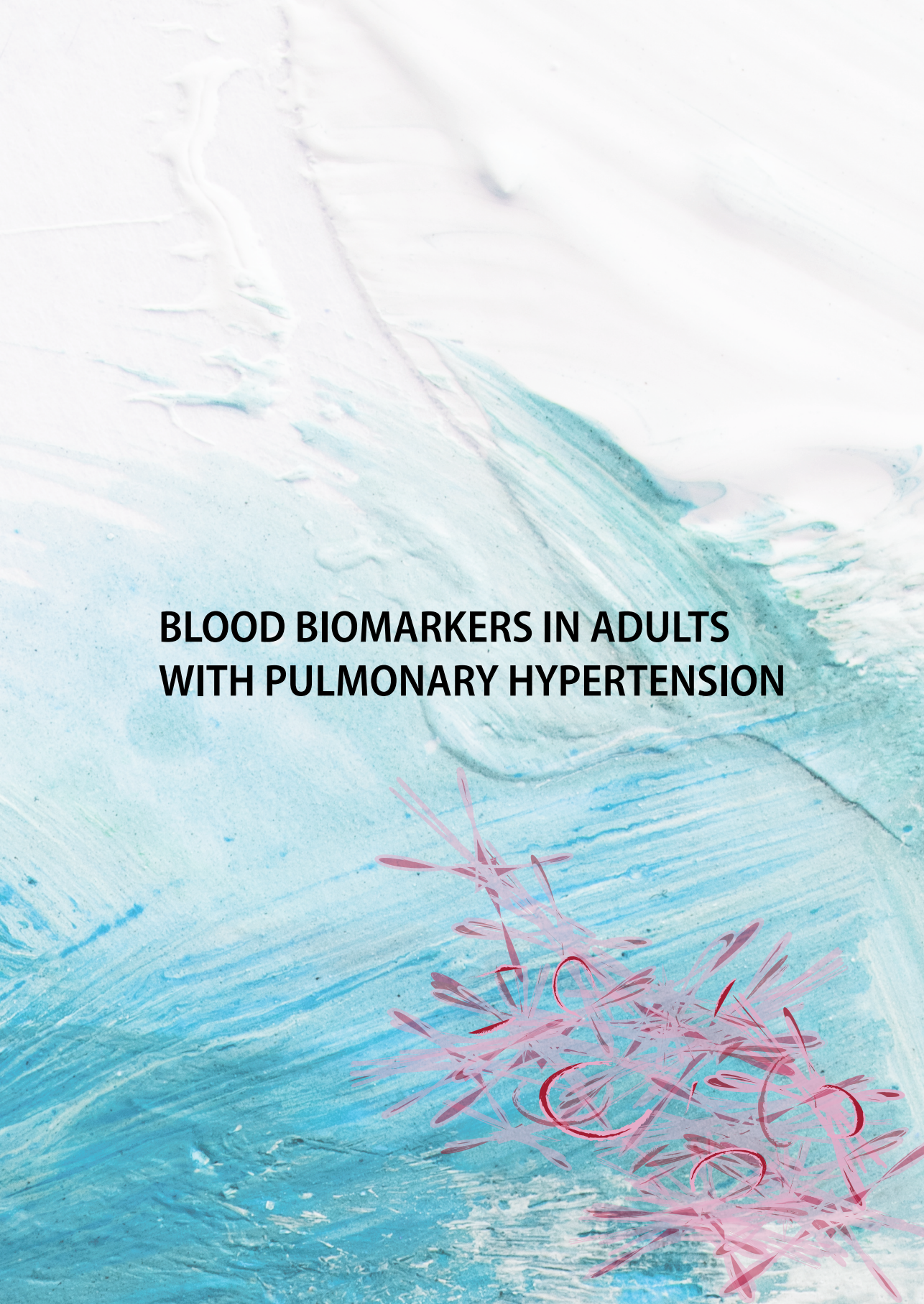
*Hazard ratios are expressed per increase in standard deviation for continuous variables. † In adult patients with transposition of the great arteries operated by the Mustard procedure only. ‡ Not enough power to perform multivariable analysis.CRP= C-reactive protein, eGFR= estimated glomerular filtration rate, GDF-15= growth differentiation factor-15, GCS= global circumferential strain, GLS= global longitudinal strain, Hs= high sensitive, LS= longitudinal strain, LV= left ventricular, NT-proBNP= N-terminal pro b-type natriuretic peptide, NYHA= New York Heart Association, RDW= red cell distribution width, sRV= systemic right ventricle.

Supplemental Table 3. Associations between clinical variables, blood- and echocardiographic markers and the primary and secondary endpoint, restricted to adult patients with transposition of the great arteries operated by the Mustard procedure.

	Mustard TGA patients (n=64)					
	Primary endpoint (n=15)			Secondary endpoint (n=22)		
	HR*	95% CI	p-value	HR*	95% CI	p-value
Clinical variables						
Age	2.54	1.32-4.90	0.005	2.70	1.48-4.93	0.001
Heart rate	1.48	0.87-2.52	0.15	1.53	1.03-2.28	0.036
Systolic blood pressure	0.53	0.30-0.93	0.027	0.46	0.27-0.79	0.005
NYHA >1	5.25	1.89-14.53	0.001	3.87	1.62-9.27	0.002
QRS duration	1.53	0.97-2.42	0.07	1.50	1.02-2.20	0.042
Loss of sinus rhythm	3.53	1.28-9.80	0.015	2.75	1.15-6.62	0.023
Blood biomarkers						
NT-proBNP	2.85	1.75-4.64	<0.001	1.87	1.24-2.83	0.003
Hs-troponin-T	2.58	1.55-4.31	<0.001	2.10	1.40-3.32	<0.001
GDF-15	2.47	1.61-3.78	<0.001	2.81	1.92-4.12	<0.001
Hs-CRP	2.31	1.31-4.07	0.004	1.74	1.13-2.68	0.012
Galectin-3	2.27	1.31-3.93	0.003	2.50	1.51-4.14	<0.001
RDW	2.07	1.48-2.89	<0.001	2.53	1.64-4.04	<0.001
eGFR	0.60	0.44-0.83	0.002	0.52	0.37-0.74	<0.001
Hemoglobin	0.49	0.34-0.72	<0.001	0.65	0.44-0.96	0.030
Echocardiographic markers						
Tricuspid regurgitation	1.60	0.96-2.68	0.074	1.23	0.78-1.95	0.38
sRV dimensions						
sRV end-diastolic annulus	2.00	0.99-4.05	0.05	1.25	0.68-2.31	0.47
Strain parameters						
LS of the sRV free wall	0.62	0.32-1.19	0.15	0.71	0.42-1.20	0.20
LS of the sRV septal wall	0.73	0.44-1.22	0.23	0.62	0.39-0.98	0.039
GCS of the sRV	0.77	0.46-1.30	0.33	0.69	0.46-1.05	0.09
LV dimensions						
LV end-diastolic diameter#	1.95	1.34-2.85	0.001	1.70	1.21-2.38	0.002

*Hazard ratios expressed per increase in standard deviation for continuous variables. #Indexed for body surface area. CRP= C-reactive protein, eGFR= estimated glomerular filtration rate, GDF-15= growth differentiation factor-15, GCS= global circumferential strain, Hs= high sensitive, LS= longitudinal strain, LV= left ventricular, NT-proBNP= N-terminal pro b-type natriuretic peptide, NYHA= New York Heart Association, RDW= red cell distribution width, sRV= systemic right ventricle.

BLOOD BIOMARKERS IN ADULTS WITH PULMONARY HYPERTENSION



CHAPTER 8

The prognostic value of various biomarkers in adults with pulmonary hypertension; a multi-biomarker approach

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Abstract

Background: This study aimed to investigate the prognostic value of six different biomarkers in patients with pulmonary hypertension (PH) and to explore whether a multi-biomarker approach can contribute to a better risk stratification.

Methods: In this prospective study, patients with PH were included at the day of the diagnostic right heart catheterization between May 2012 and October 2016. Venous blood sampling included; NT-proBNP, high sensitive troponin-T, high sensitive CRP, galectin-3, red blood cell distribution width and eGFR. Associations between biomarker levels and the primary endpoint (death or lung transplantation) and secondary endpoint (death, lung transplantation or heart failure) were assessed with Cox regression, adjusted for age and sex. Additionally, adjustment for the REVEAL risk score was performed.

Results: In total, 106 patients were included (median age 58.7 [IQR 47.0-69.2] years, 64% women, 51% pulmonary arterial hypertension). After a median follow-up duration of 23.9 [IQR 15.1-40.0] months, respectively 29 and 37 patients reached the primary and secondary endpoint. All six biomarkers, except eGFR, were significantly associated with the endpoints. 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). However, a single as well as a combination of multiple biomarkers, did not yield prognostic value independent of the REVEAL risk score.

Conclusions: Various biomarkers are associated with the event-free survival in adults with PH. However, risk stratification exclusively based on single or a combination of biomarkers seems not superior to existing risk scores.

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]. Novel biomarkers, such as galectin-3, but also more common blood biomarkers like red cell distribution width (RDW) and C-reactive protein (CRP), have already been associated with heart failure in different PH groups[9-12].

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 with PH at the day of the diagnostic right heart catheterization between the May 2012 and 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: incomplete diagnostic work-up and therefore no confirmed PH diagnosis, not treatment-naïve, age <18 years and not capable of understanding and signing informed consent. In addition, we excluded patients with PH due to left heart disease to be able to study biomarker levels caused by primarily right ventricular failure. 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, 13]: 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, spirometry, 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. Biomarker assessment included NT-proBNP, high sensitive troponin-T (hs-TnT), galectin-3, high sensitive CRP (hs-CRP), RDW and eGFR and is further described in Supplemental Methods 1.

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[14]. The systolic left ventricular function was visually graded as normal, mildly, moderately or severely impaired. 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[15].

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.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) or median [interquartile range]. Biomarker levels were 2-log transformed to correct for skewness. Biomarker values below the limit of detection (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 adjust for age and sex. 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. As post-hoc analysis, we determined for each patient the REVEAL risk score based on the full PAH risk calculator[16]. We defined renal insufficiency as a eGFR <30mL/min per 1.73 m^2. Missing data of the parameters was taken care of with multiple imputation, with 5 imputations. We adjusted every biomarker for the REVEAL score in separate analyses.

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 2-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 Figure 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.

Biomarker levels according to the 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. 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. We found no significant differences in the levels of galectin-3, hs-CRP, RDW and eGFR between the subgroups of PH.

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 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

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

	Valid cases, n (%)	Total (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 3/4, n (%)	106 (100)	58 (55)	32 (60)	8 (53)	9 (43)	9 (56)
Electrocardiography						
Rhythm, n (%)	103 (97)					
Sinus rhythm		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
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

Continue

Continued

	Valid cases, n (%)	Total (n = 106)	PAH (n = 54)	PH-lung disease (n = 15)	CTEPH (n = 21)	WHO 5 / multifactorial (n = 16)
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)	1 (6)
Severely impaired		1(1)	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]
Cardiac index, L/min/m^2	101 (95)	2.6 [2.2-3.3]	2.5 [2.17-3.55]	2.6 [2.09-2.91]	2.7 [2.3-3.0]	2.7 [2.1-3.6]
PVR, Wood units	89 (84)	5.5 [3.3-9.3]	7.1 [5.1-11.8]	4.4 [4.1-5.5]	3.4 [3.0-5.1]	4.7 [2.3-6.8]
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].
RA, right atrial; RV, right ventricular; LV, left ventricular; mRAP, mean right arterial pressure; PA, pulmonary artery; AO, aortic artery.

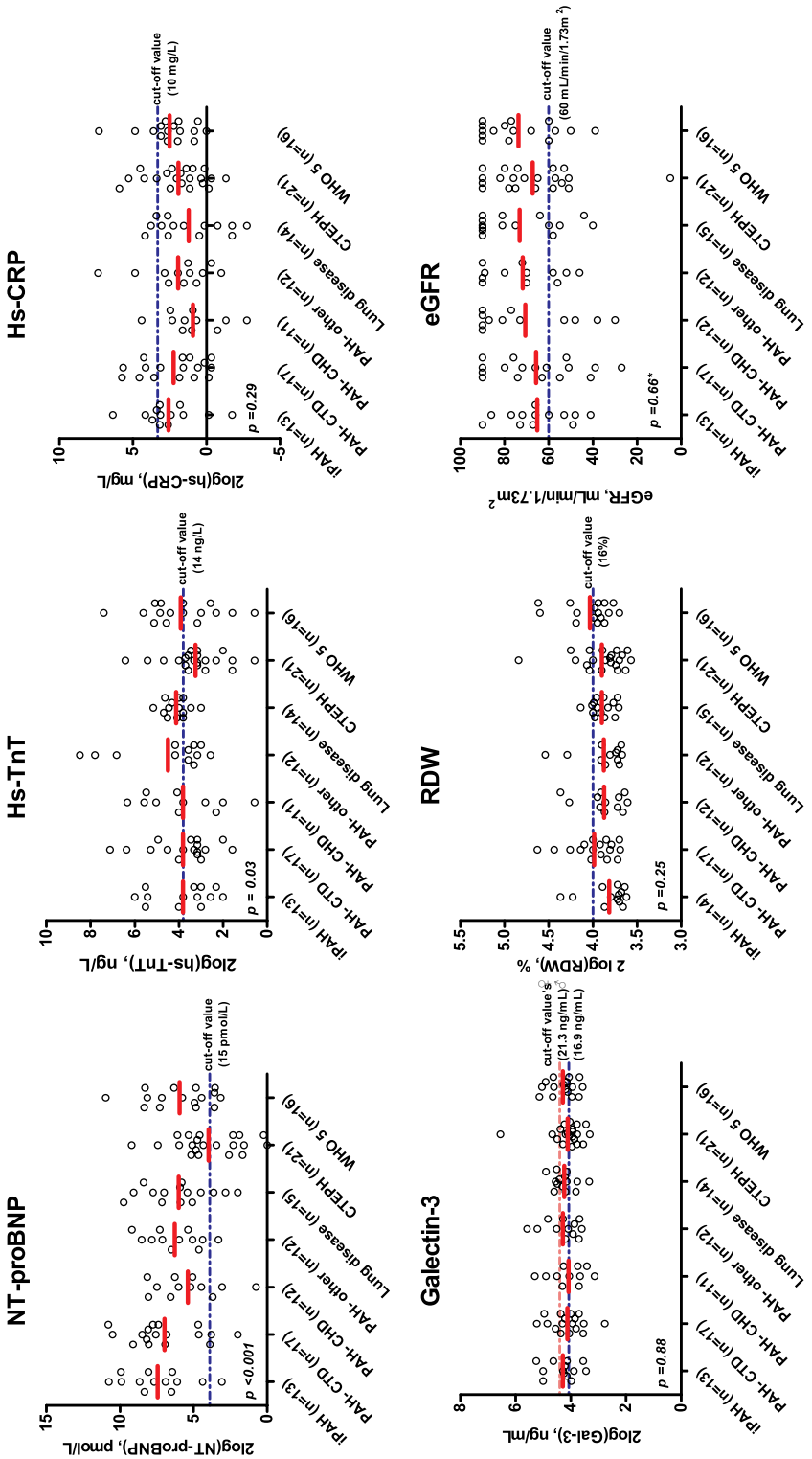

Figure 1. Biomarker levels stratified according to the WHO classification of PH.
Biomarker levels are represented on the 2-log scale (except for eGFR). Blue dotted line represents the biomarker-specific cut-off value. Red line represents the mean biomarker level in each group. For galectin-3, sex-specific biomarker cut-off values are represented (blue dotted line=man, pink dotted line=woman). * Kruskal Wallis test, otherwise one-way ANOVA test.

Table 2. Correlation coefficients of biomarkers with baseline characteristics.

	NT-proBNP	Hs-TnT	Hs-CRP	Galectin-3	RDW	eGFR†
	n=106	n=104	n=104	n=104	n=106	n=106
	r	*r*	*r*	*r*	*r*	*r*
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 3/4	**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

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

correlation was found between hs-TnT and galectin-3 ($r = 0.58$, $p < 0.001$) (Supplemental Table 1).

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 PAH, 49 (91%) patients used any PH medication during follow-up (20% monotherapy, 33% duo therapy, 37% triple therapy). Considering the patients with CTEPH, three patients underwent balloon pulmonary angioplasty, three patients underwent pulmonary endarterectomy surgery and 15 patients used PH medication.

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. For eGFR, patients in the highest tertile of eGFR had a significant better event-free survival than patients in the lower tertiles. (Supplemental Figure 2)

Standardized hazard ratios for the association between biomarker levels and the endpoints are shown (Figure 3). When analysed continuously, all biomarkers except eGFR, 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 0.55-0.78) and galectin-3 (C-index 0.67, 95% CI 0.52-0.83) (Supplemental Table 2). All biomarkers were significantly associated with the secondary endpoint. For RDW, the standardized hazard ratio of the secondary endpoint increased relatively to the hazard ratio of the primary endpoint, as did the C-index.

When biomarker levels were adjusted for the REVEAL risk score, none of the biomarkers remained significantly associated with the endpoints. (Supplemental Table 3)

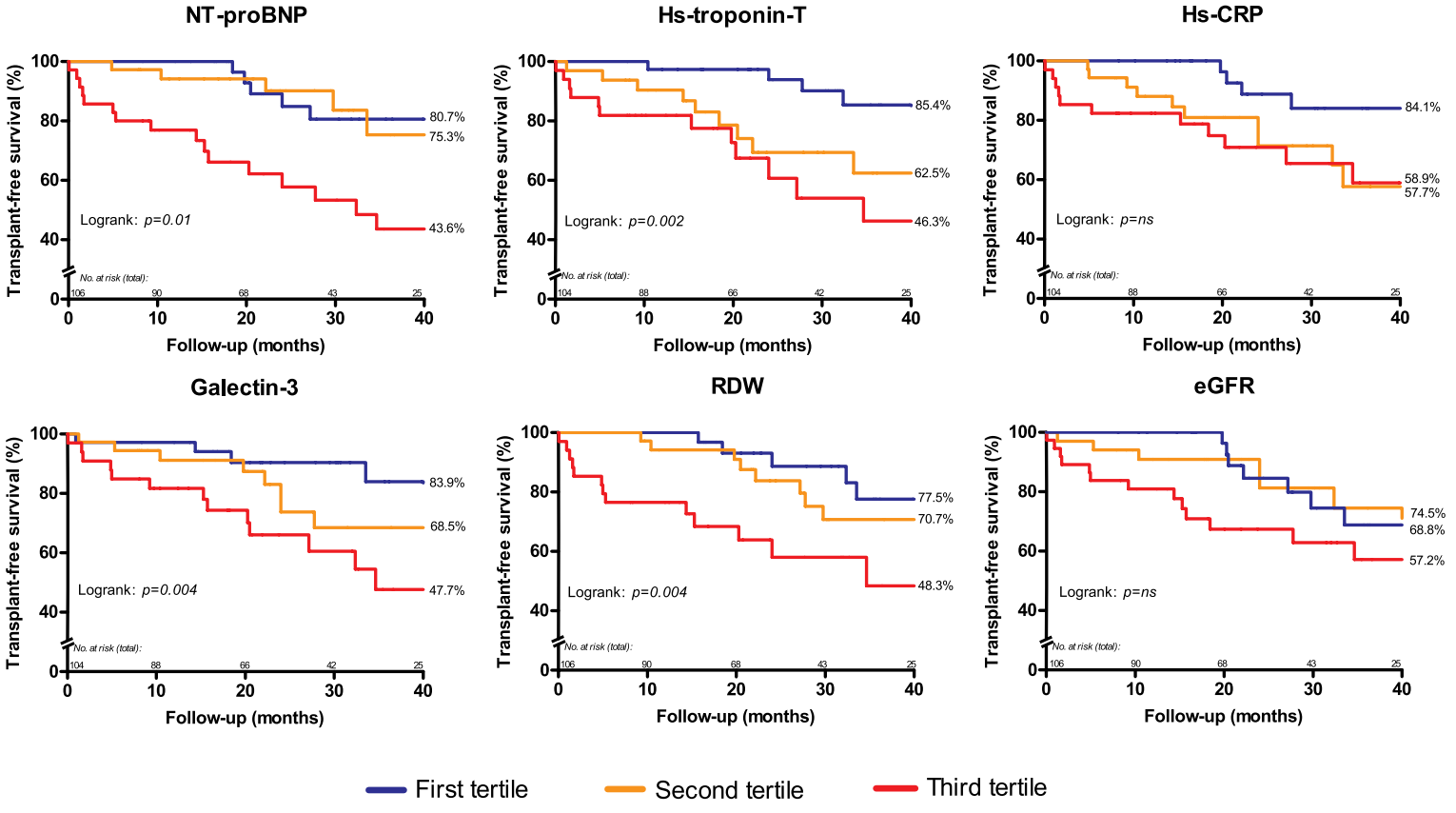

Figure 2. Transplant-free survival according to tertiles of the biomarker levels.
NT-proBNP: first tertile <26.57 pmol/L, second tertile 26.57-150.0 pmol/L, third tertile >150.0 pmol/L. **Hs-TnT**: first tertile <2.0 ng/L, second tertile 2.0-6.3 ng/L, third tertile >6.3 ng/L. **Hs-CRP**: first tertile <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).

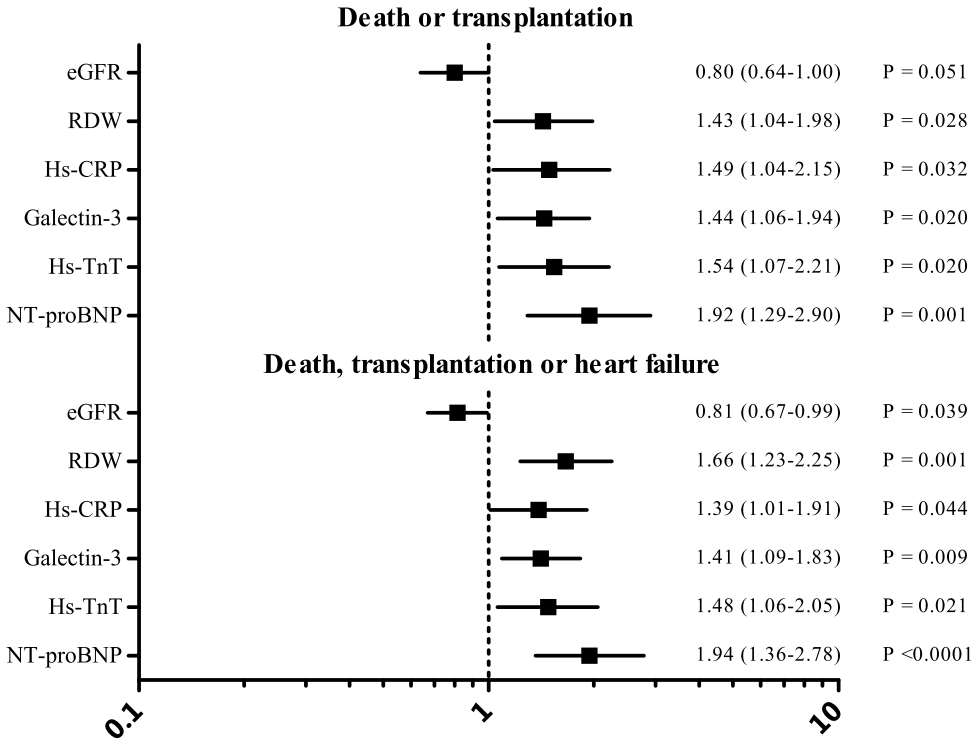

Figure 3. Standardized hazard ratios, reflecting the instantaneous risk of the primary and secondary endpoint per one standard deviation increase in the log_2-transformed biomarker level.
Standardized hazard ratios adjusted for age and sex, with the corresponding 95% CI and p-value for each biomarker.

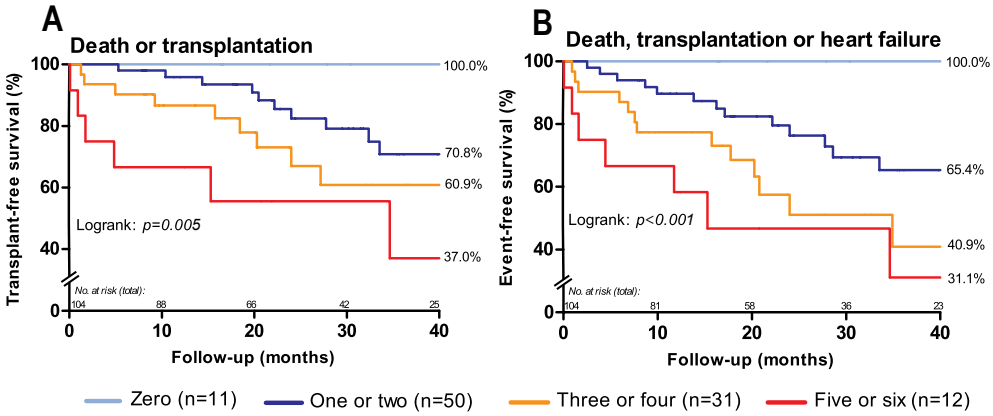

Figure 4. Transplant-free and event-free survival stratified according to the number of elevated biomarkers.
Figure legend shows the number of elevated biomarkers with the corresponding line colour. *for eGFR lowered levels were taken into account. (detailed explanation of the different groups is provided in Supplemental Table 4).

Multi-biomarker approach

Figure 4 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 and sex adjusted hazard ratio for the primary endpoint for one extra elevated biomarker was 1.34 (95% CI 1.08-1.67, p = 0.009). Multi-biomarker analysis concerning the secondary endpoint (Figure 4B) showed similar results (HR 1.33, 95% CI 1.09-1.61, p = 0.004). However, when adjusted for the REVEAL risk score, the number of elevated biomarkers did not yield any independent prognostic value for both endpoints (HR 1.06, 95% CI 0.83-1.36, p = 0.63 and HR 1.09, 95% CI 0.88-1.57, p = 0.44, respectively).

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 levels of NT-proBNP and hs-TnT among the different subgroups of PH was found and only 11% of the patients were free of any abnormal biomarker level. All six biomarkers studied in this prospective cohort were associated with adverse clinical outcomes, independent of age and sex. 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. However, the additive value of individual biomarkers as well as a multi-biomarker model, compared to the existing REVEAL risk score, seems limited. This emphasizes that adequate risk stratification in PH may only be achieved by incorporating multiple clinical characteristics and modalities into a comprehensive risk model.

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[17]. Galectin-3 may particularly be appropriate to monitor right ventricular remodelling[18]. Previous studies investigating galectin-3

mainly focused on patients with PAH only and consisted of smaller cohorts[9, 18, 19]. Calvier et al. found elevated levels of galectin-3 in PAH patients, which correlated with NYHA class[19]. 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[9]. 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 potential 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[11], even independent of NT-proBNP[10]. 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 and sex. 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. Although it did not yield any independent prognostic value besides the REVEAL risk score, it would be interesting to know whether incorporation of RDW in the risk score can improve risk stratification and if it can be a good predictor for heart failure. Specifically since RDW is determined as part of the automated blood count and is therefore inexpensive, easy-accessible and widely available as 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 baseline characteristics of our study showed differences in disease severity among the PH subgroups, it is therefore likely that the prognostic value of the biomarkers in these subgroups also differ. The prognosis may be explained by the interaction between PH and the PH-related comorbidities, which differs between individuals. Causes 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 study shows that NT-proBNP yields the strongest prognostic and discriminative value for adverse cardiac events in adults with PH. NT-proBNP is already incorporated in the REVEAL risk score and PH guidelines. The change of the REVEAL risk score over time has also been investigated[22], however the REVEAL risk score is comprehensive and consists of many invasive as well as less invasive measurements. Invasive hemodynamic measurements may be inconvenient to measure frequently, it would therefore be interesting if biomarkers could be a surrogate marker for one of these variables. The multi-biomarker approach is just a simple reflection of how multiple biomarker can contribute to the risk stratification in PH and should be further investigated in larger studies with the use of continuous levels in a multivariable analysis. Additionally, it would be of interest to investigate whether serial biomarker measurements may improve prognostic precision and can monitor therapy.

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.

During the study, patients were treated goal oriented and in accordance with the ESC guidelines. Due to the long inclusion period, treatment strategies may have changed over time specifically regarding the introduction of combination therapy in PAH patients[23]. This may have introduced differences in prognosis in our study. 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 or HF in adults with PH. However, the additive value of a single biomarker compared to already existing risk scores, is limited. Combining multiple biomarkers may be beneficial in detecting patients at higher risk of events, nevertheless risk stratification based on exclusively biomarkers seems inadequate.

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Supplemental Methods 1.

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 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 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 upper limit of normal 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(1).

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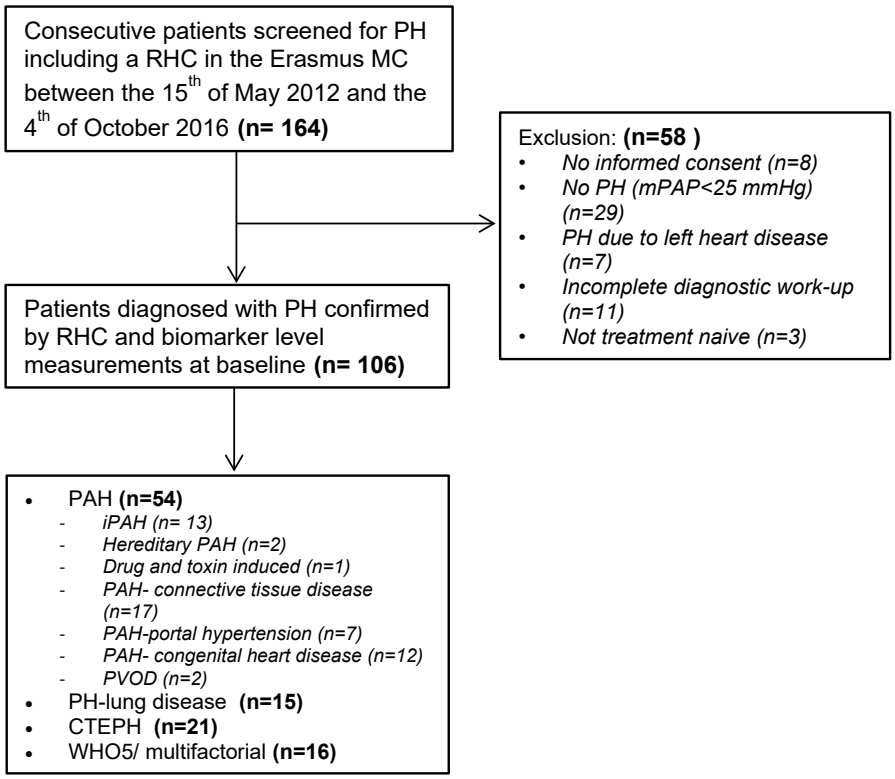
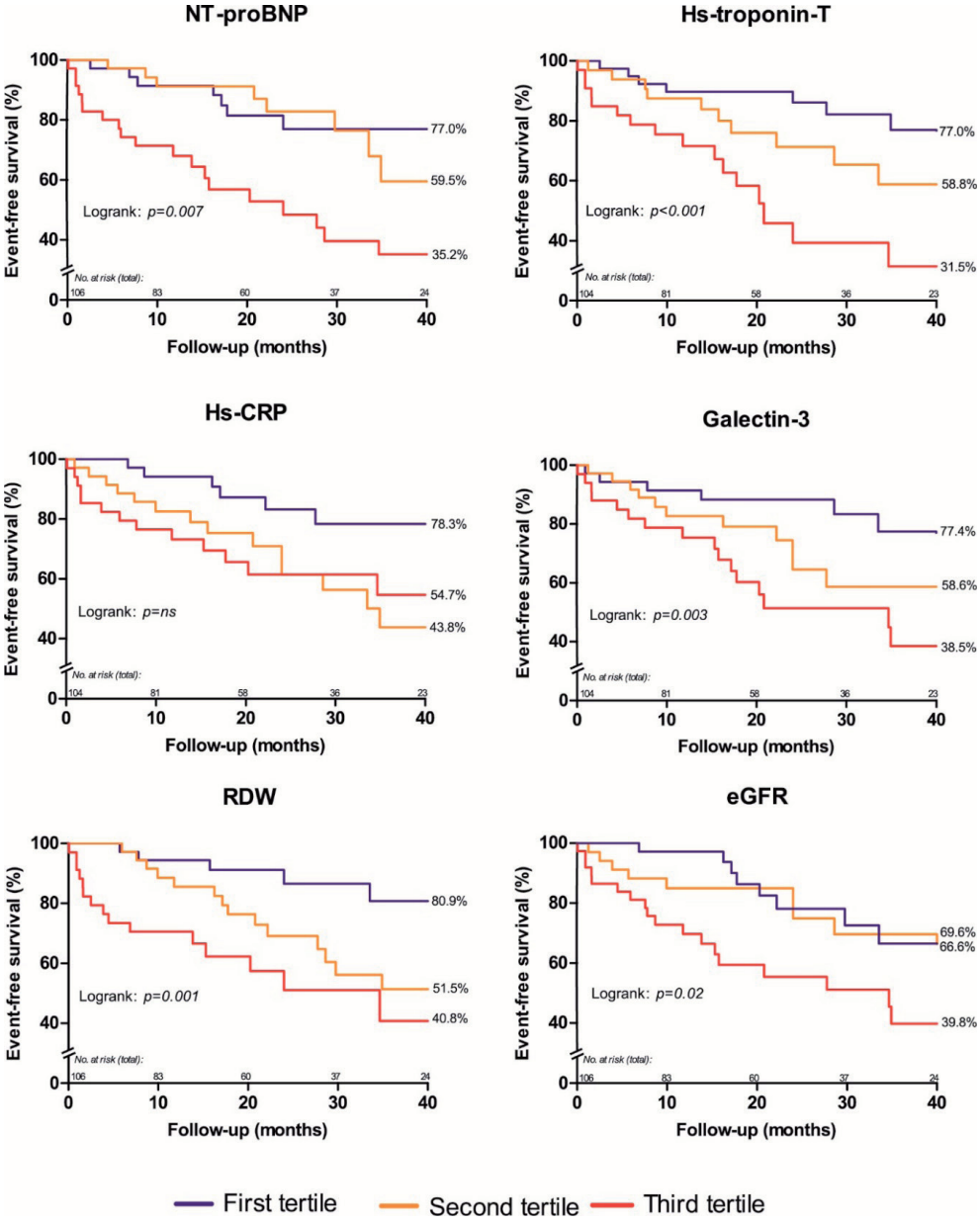


Supplemental Figure 1. Flowchart of the patient selection process

Supplemental Table 1. Mutual correlations between different biomarkers in adults 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 Figure 2. Event-free survival of patients with pulmonary hypertension according to the tertiles biomarker distributions of NT-proBNP, hs-TnT, hs-CRP, galectin-3, RDW and eGFR. **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/1.73m^2, second tertile 60.0-80.3 ml/min/1.73m^2, third tertile ≤60 ml/min/1.73m^2 (for visualization purposes, tertiles of eGFR have been inversed.

Supplemental Table 2. 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 and sex in adults 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)	1.94 (1.29-2.90)	0.001	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)	1.54 (1.07-2.21)	0.020	0.67 (0.55-0.78)	1.48 (1.06-2.05)	0.021	0.65 (0.55-0.76)
Hs-CRP, mg/L	3.6 [1.5-9.0]	24 (23)	1.49 (1.03-2.15)	0.034	0.66 (0.51-0.80)	1.39 (1.01-1.91)	0.044	0.66 (0.53-0.80)
Galectin-3, ng/mL	17.9 [14.0-22.8]	40 (39)	1.44 (1.06-1.94)	0.020	0.67 (0.52-0.83)	1.41 (1.09-1.83)	0.009	0.67 (0.57-0.77)
RDW, %	14.6 [13.2-16.0]	25 (24)	1.43 (1.04-1.98)	0.028	0.62 (0.50-0.73)	1.66 (1.23-2.25)	0.001	0.67 (0.56-0.77)
eGFR, mL/min/1.73m^2	72.5 [55.8-90.0]	33 (31)*	0.80 (0.64-1.00)	0.051	0.62 (0.48-0.77)	0.81 (0.67-0.99)	0.039	0.63 (0.54-0.73)

Biomarker levels in the overall study population expressed in median [IQR] and the corresponding number of patients with an elevated biomarker level. *For eGFR lowered levels were taken into account. †Hazard ratio per one standard deviation increase in the 2-log transformed biomarker level, with the corresponding 95% confidence interval and significance 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/1.73.

Supplemental Table 3. Associations between standardized biomarker levels and the primary (death or lung transplantation) and secondary endpoint (death, lung transplantation or heart failure) in adults with pulmonary hypertension, adjusted for the REVEAL risk score.

	Primary endpoint		Secondary endpoint	
	HR* (95% CI)	**P-value**	**HR* (95% CI)**	**P-value**
NTproBNP, pmol/L	0.99 (0.81-1.24)	0.996	1.01 (0.82-1.23)	0.973
hs- TnT, ng/L	1.12 (0.87-1.44)	0.399	1.10 (0.88-1.37)	0.392
hs-CRP, mg/L	1.05 (1.18-1.62)	0.592	1.01 (0.84-1.21)	0.932
Gal-3 ng/mL	1.19 (0.55-0.67)	0.551	1.21 (0.73-2.00)	0.451
RDW, %	1.55 (0.40-5.97)	0.524	2.30 (0.69-7.70)	0.177
eGFR	0.95 (0.60-1.53)	0.854	0.97 (0.64-1.48)	0.880
Multi-biomarker variable†	1.06 (0.83-1.36)	0.626	1.09 (0.88-1.57)	0.435

*hazard ratio per one standard deviation increase in the 2-log transformed biomarker level with the corresponding 95% confidence interval and significance level, adjusted for the REVEAL risk score calculated with the PAH risk score calculator(1). †variable including the number of elevated biomarker for each patient.

Reference:

1. Benza RL, Gomberg-Maitland M, Miller DP, Frost A, Frantz RP, Foreman AJ, et al. The REVEAL Registry risk score calculator in patients newly diagnosed with pulmonary arterial hypertension. Chest. 2012;141(2):354-62.

Supplemental Table 4. Multi-biomarker approach in adults with pulmonary hypertension, explaining the contribution of each biomarker to the total number of elevated biomarker groups.

	→ Number (%) of patients with elevated biomarker					
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)

CHAPTER 9

The prognostic value of soluble ST2 in adults with pulmonary hypertension

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Abstract

Soluble ST2 (sST2) is upregulated in response to myocardial stress and may serve as biomarker in adults with pulmonary hypertension (PH). This prospective cohort study investigated sST2 levels and its association with echocardiographic and hemodynamic measures, and adverse clinical outcomes in adults with PH of different etiologies. sST2 was measured during the diagnostic right heart catheterization for PH, in adults patients enrolled between May 2012 and October 2016. PH due to left heart failure was excluded. The association between sST2 and a primary endpoint composed of death or lung transplantation and a secondary composite endpoint including death, lung transplantation or heart failure, was investigated using Cox regression with adjustment for NT-proBNP. In total 104 patients were included (median age 59 years, 66% woman, 51% pulmonary arterial hypertension). Median sST2 was 28 [IQR 20-46] ng/mL. Higher sST2 was associated with worse right ventricular dysfunction and higher mean pulmonary and right atrial pressures. Median follow-up was 3.3 [IQR 2.3-4.6] years. The primary and secondary endpoint occurred in 33 (31.7%) and 43 (41.3%) patients, respectively. sST2 was significantly associated with both endpoints (HR per 2-fold higher value 1.53, 95% CI 1.12-2.07, p=0.007 and 1.45, 95% CI 1.10-1.90, p=0.008, respectively). However, after adjustment for NT-proBNP, both associations did not reach statistical significance. In conclusion, higher sST2 levels are associated with more severe PH and right ventricular dysfunction and yields prognostic value in adults with PH, although not independently of NT-proBNP.

Introduction

The soluble form of suppression of tumorigenicity-2 (sST2) is a member of the interleukin-1 receptor family. sST2 is known for its release induced by myocardial strain and stress[1], but also for its involvement in type 2 immune responses[2]. Over the past years, sST2 has arisen as promising biomarker in the heart failure population; sST2 has extensively been investigated in the case of left ventricular failure and has shown a strong prognostic value for mortality in chronic and acute heart failure patients[3, 4]. Its potential relation with right ventricular dysfunction has been investigated to a much lesser extent.

Right ventricular dysfunction is one of the major problems in patients with pulmonary hypertension (PH). Elevated pulmonary arterial pressures caused by an increased pulmonary vascular resistance can lead to progressive right ventricular failure over time[5], contributing to a very poor prognosis in these patients[6, 7]. The etiology of PH is diverse and it is thought that various pathophysiologic pathways play a role in the development of this disease[8]. A previous study showed that the circulating ligand of sST2, interleukin-33, may play a role in the vascular remodeling of the pulmonary endothelium in idiopathic pulmonary arterial hypertension (iPAH)[9]. Moreover, levels of sST2 have been correlated with right ventricular dysfunction in pulmonary arterial hypertension (PAH) patients[10]. As sST2 may thus reflect both cardiac as well as pulmonary vascular remodeling, sST2 could be a valuable biomarker to monitor deterioration in patients with PH.

The aim of this study was to assess sST2 levels between different PH etiologies, to investigate associations between sST2 and hemodynamics and measures of RV dysfunction, and to explore the prognostic value of sST2 for survival in adults with PH.

Methods

Study design

This is a prospective observational cohort study. We aimed to enroll all consecutive adult patients at the day of the diagnostic right heart catheterization for PH between May 2012 and October 2016 in our center. Diagnosis of PH was defined as a mean pulmonary artery pressure (mPAP) of ≥ 25 mmHg, measured by right heart catheterization in accordance with the European Society of Cardiology (ESC) guidelines[11]. Exclusion criteria were: unconfirmed diagnosis of PH due to incomplete diagnostic work-up, not treatment-naive, aged <18 years old or not capable of understanding and signing informed consent. Additionally, patients with PH due to left-heart disease were excluded. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki and was approved by the local medical ethical committee. All patients gave written informed consent.

Patients were classified according to the World Health Organization (WHO) classification of PH; PAH, PH due to lung disease, chronic thromboembolic pulmonary hypertension (CTEPH) and

PH with unclear/multifactorial mechanisms (WHO5). Patients with a mixed clinical picture were grouped under WHO5. WHO 1 patients (PAH) were further stratified in subgroups according to the WHO classification[11, 12]. More details of the study protocol have been published previously[13].

Study procedures

During an inpatient visit, patients underwent physical examination by a cardiologist and pulmonary physician, 6-minute walking test, 12-lead electrocardiography, echocardiography, venous blood sampling, cardiac computed tomography and right heart catheterization, all within the framework of PH screening. Patient characteristics and vital signs were collected. A Swan-Ganz catheter was used to obtain invasive hemodynamic measurements during right heart catheterization; pulmonary arterial pressures, right atrial pressures and capillary wedge pressures were measured. Fick's principle or thermodilution was used to measure cardiac output. When indicated, a fluid challenge was performed to exclude PH due to left heart disease. During follow-up, patient management was according to discretion of the treating physician based on the ESC guideline[11]. Data were collected and stored in an online electronic case report form PAHTool (version 4.3.5947.29411, Inovoltus, Santa Maria da Feira, Portugal).

Echocardiography and cardiac computed tomography analysis

Two-dimensional transthoracic echocardiography was performed with a commercially available ultrasound system (iE33, Philip Medical Systems, Best, the Netherlands). The guideline for echocardiographic cardiac chamber quantification was used for further imaging analysis[14]. The 4 chamber view and parasternal long axis view were used for analysis. We visually graded left ventricular systolic function as normal, mildly, moderately or severely impaired. Presence of pericardial effusion in one of the available views was scored as; mild (<10mm), moderate (10-20 mm) or severe (>20 mm). Cardiac computed tomography was performed according to routine clinical practice. At the level of the pulmonary artery bifurcation, we measured both the central pulmonary artery diameter and the aortic diameter[15].

Definition and assessment of endpoints

The primary composite endpoint was defined as a composite of all-cause mortality or lung transplantation. The secondary endpoint was composed of all-cause mortality, lung transplantation or hospitalization due to heart failure requiring additional treatment with diuretics. Protocolled prospective follow-up visits to the outpatient clinic were scheduled with half yearly intervals. CTEPH patients who were eligible for balloon pulmonary angioplasty or pulmonary endarterectomy, were referred to a specialized center. Patients were not censored after the procedure. We retrieved information from the electronic patient records and the municipal personal records database. Patients were censored at the end of the follow-up period (January 1st 2019) when they did not reach one of the endpoints.

Biomarker assessment

Venous blood sampling was performed during diagnostic right heart catheterization and performed for study purposes only. Blood samples were transferred to the local clinical chemistry laboratory and N-terminal pro b-type natriuretic peptide (NT-proBNP) was directly measured in fresh blood samples. The other serum samples were aliquoted and stored by -80 ºC until batch analysis was performed. sST2 was measured in serum with a quantitative sandwich monoclonal enzyme-linked immunosorbent assay (Presage® ST2 assay, CRITICAL DIAGNOSTICS, San Diego, The United States). sST2 is not significantly affected by sample free-thaw cycles and is reported to be stable up to 15 freeze-thaw cycles. The samples used to measure sST2 levels were exposed to a maximum of 2 freeze-thaw cycles with a median storage time of 3.5 [IQR 2.5-5.0] years. The upper-limit of detection was 170 ng/mL, sST2 measurements reaching the upper limit of detection were further diluted to extent the upper limit.

In addition to the sST2 measurements in PH patients, sST2 was also measured in a healthy cohort in order to assess assay reproducibility and to obtain reference values[16]. This healthy cohort included self-declared healthy volunteers, recruited between January 2014 and December 2014. All volunteers underwent physical examination, electrocardiography, echocardiography and venous blood sampling on the same day. sST2 showed a good reproducibility with a coefficient of variation of 7.75% and limits of agreement of -5.59 – 7.61 ng/mL. This data has previously been published[17]. sST2 measurements were performed twice in each healthy study participant and once in study patients.

Statistical analysis

Continuous variables are presented as mean (SD) or median [inter quartile range (IQR)], depending on the distribution of the data. sST2 and NT-proBNP were 2log transformed because of a skewed distribution. Comparison of sST2 levels across PH subgroups were performed using the Kruskal-Wallis test. Pearson or Spearman correlation coefficient was obtained to describe correlations between sST2 and baseline characteristics. Correlations were visualized using scatterplots. Patients were grouped based on the tertile distribution of sST2 and according to a normal or elevated sST2. Cumulative endpoint-free survival estimates were derived using the Kaplan-Meier estimator and survival between groups was compared with the log-rank test for trend. Univariable and multivariable Cox-proportional hazard regression was used to assess associations between sST2 and study endpoints. We adjusted for sex and age, and NT-proBNP in separate multivariable analyses. For sST2 sex specific reference values were used based on 97.5th percentile in the healthy volunteer cohort; > 44.50 ng/mL for women and > 55.85 ng/mL for men. Statistical analyses were performed using IBM SPSS Statistics (version 24). A 2-sided p-value <0.05 was considered statistically significant.

Results

Baseline characteristics

Of the 106 patients who were originally included in this cohort of adults with PH, sST2 was measured in 104 patients. The patient selection process is shown in Supplemental Figure 1. The median age was 59 [IQR 47-69] years, 64% were women and 54% were in New York Heart Association (NYHA) class III or IV. The cohort consisted of the following PH diagnoses: 53 (51%) PAH, 15 (14%) PH-lung disease, 21 (20%) CTEPH and 16 (15%) WHO5/multifactorial. (Table 1). Median sST2 level was 27.9 [IQR 19.6-44.9] ng/mL. sST2 levels according to the different subgroups of PH are shown in Figure 1. sST2 levels differed significantly between PH subgroups and were the lowest in CTEPH patients. An elevated level of sST2 was found in 14 women (21%) and in 7 men (18%).

Correlations between sST2 and clinical characteristics

Higher levels of sST2 weakly correlated with a higher NYHA class and shorter 6-minute walking distance. Both right ventricular as well as left ventricular measurements on echocardiography correlated with sST2. Regarding invasive hemodynamic measurements, sST2 showed a positive correlation with the mPAP, mean right atrial pressure and pulmonary vascular resistance. Moreover, higher sST2 was associated with lower cardiac output. (Table 2 and Figure 2) As

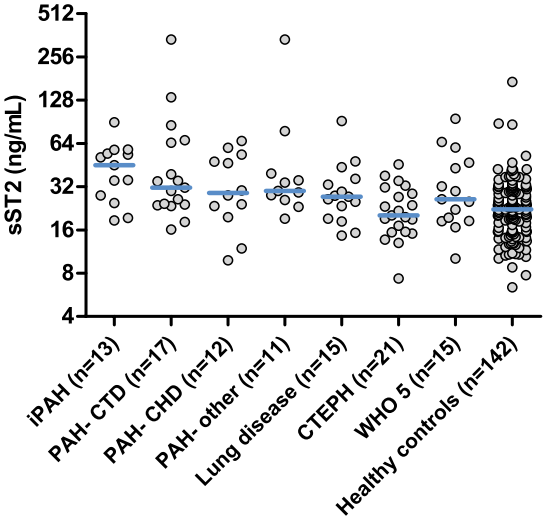

Figure 1. sST2 levels according to the different PH subclasses and sST2 levels in healthy volunteers.
Median sST2 level in each group is indicated by the horizontal line. Y-axis is on the 2log scale.
iPAH= idiopathic pulmonary arterial hypertension, PAH-CTD=pulmonary arterial hypertension due to connective tissue disease, PAH-CHD= pulmonary arterial hypertension due to congenital heart disease, CTEPH= chronic thromboembolic pulmonary hypertension, WHO 5= Pulmonary hypertension classified in group 5, PH due to multifactorial mechanisms.

Table 1. Baseline characteristics for all patients and stratified according to subgroups of PH.

	Complete cases (n, %)	**All** N=104	**PAH** N=53	**PH lung disease** N=15	**CTEPH** N=21	**WHO 5/ mixed** N=15
Clinical characteristics						
Age, years	104 (100)	59 [47-69]	55 [41-66]	63.6 [55-72]	59 [42-73]	65 [57-70]
Sex, women n (%)	104 (100)	66 (64)	35 (66)	8 (53)	12 (57)	11 (73)
Body mass index, kg/m^2	104 (100)	28.1±6.6	26.3±5.8	30.5±6.3	31.4±5.7	27.8±8.5
Heart rate, beats/minute	104 (100)	80 ±16	80±16	81±12	75±17	85±19
Systolic blood pressure, mmHg	104 (100)	127±18	122±15	125±17	131±13	133±27
Oxygen saturation <90%, n (%)	104 (100)	3 (3)	2 (4)	1 (7)	0 (0)	0 (0)
NYHA class III/IV, n (%)	104 (100)	56 (54)	31 (59)	8 (53)	9 (43)	8 (53)
6-minute walking distance	89 (86)	337±139	348±147	309±115	385±130	261±122
Electrocardiogram						
Sinus rhythm, n (%)	102 (97)	90 (87)	46 (87)	13 (87)	18 (86)	13 (87)
QRS duration, ms	100 (96)	98 [90-106]	100 [89-124]	100 [91-111]	94 [88-99]	100 [89-124]
Echocardiogram						
RA area, cm^2	79 (76)	27.5±26.2	28.0±6.9	28.4±8.4	24.6±10.0	28.2±13.1
RV end diastolic basal dimension, mm	74 (71)	51.5±9.6	52.7±8.1	47.8±4.7	51.1±12.1	50.2±13.0
RV fractional area change, %	72 (69)	28.9±8.6	26.7±7.8	31.3±6.2	33.0±4.3	30.7±12.7
TAPSE, mm	72 (69)	20±5	19±5	19±3	21±3	21±7
LV function, n (%):	99 (94)					
Normal		65 (66)	32 (63)	10 (71)	15 (83)	8 (53)
Mildly impaired		29 (30)	17 (33)	4 (29)	3 (17)	5 (33)
Moderately/ severely impaired		4 (4)	2 (4)	0 (0)	0 (0)	2 (14)
LV end diastolic dimension, mm	80 (77)	43.2±7.4	46.7±5.6	46.1±7.3	45.5±5.6	46.8±8.4
Hemodynamics						
mPAP, mmHg	104 (100)	42 [35-52]	46 [39-60]	37 [32-41]	37 [30-48]	42 [34-47]
mRAP, mmHg	104 (100)	10±5	11±6	8±4	8±5	11±6
Capillary wedge pressure, mmHg	90 (87)	13±6	11±5	13±4	14±3	19±9
Pulmonary vascular resistance, WU	87 (84)	5.5 [3.3-9.4]	5.1 [7.1-12.0]	4.4[4.1-5.5]	3.35 [3.0-5.1]	4.1 [2.3-6.8]
Cardiac output, L/min	99 (87)	4.9 [4.0-6.2]	5.0 [3.9-5.8]	5.0 [3.9-5.8]	5.4 [4.9-6.3]	4.9 [4.0-6.8]
Computed tomography						
PA diameter, mm	98 (94)	34.4±5.4	35.3±6.0	34.9±4.1	34.4±5.1	31.1±3.3
PA/AO ratio	98 (94)	1.12±0.24	1.12±0.24	1.03±.012	1.12±0.24	0.98±0.13
Laboratory						
sST2, ng/mL	104 (100)	27.9 [19.6-44.9]	34.2 [24.1-54.1]	27.3 [19.2-36.3]	20.2 [15.5-28.6]	26.2 [18.5-47.1]
NT-proBNP, pmol/L	104 (100)	60 [21-226]	120 [26-280]	60 [23-216]	24 [5-36]	31 [12-282]

NYHA=New York Heart Association, RA= right atrial, RV = right ventricular, TAPSE= trans annular plain systolic excursion, LV= left ventricular, mPAP= mean pulmonary artery pressure, mRAP= mean right atrial pressure, PA= pulmonary artery, WU= Wood-units AO= aorta, NT-proBNP= N-terminal pro B-type natriuretic peptide, sST2= soluble suppression of tumorigenicity-2.

Table 2. Correlations between sST2 and baseline characteristics.

	sST2	
	r	*p-value*
Clinical characteristics		
Age	-0.09	0.346
Sex	0.11	0.260
Body mass index	-0.15	0.124
Heart rate	0.28	0.005
Systolic blood pressure	-0.21	0.036
Oxygen saturation < 90%	0.08	0.417
NYHA class III/IV	0.23	0.018
6-minute walking distance	-0.29	0.007
Electrocardiogram		
Loss of sinus rhythm	0.17	0.095
QRS Duration	0.15	0.142
Echocardiogram		
Right atrial area	0.13	0.269
RV basal dimension	0.17	0.145
RV fractional area change	-0.40	<0.001
TAPSE, mm	-0.35	0.002
LV end diastolic dimension	-0.23	0.044
Hemodynamics		
mPAP	0.40	<0.001
mRAP	0.26	0.008
Capillary wedge pressure	-0.11	0.314
Pulmonary vascular resistance	0.42	<0.001
Cardiac output	-0.27	0.007
Computed tomography		
PA diameter	0.07	0.475
PA/AO ratio	0.08	0.426
Laboratory		
NT-proBNP	0.54	<0.001

Significant correlations are in bold. Abbreviations: sST2= soluble suppression of tumorigenicity-2, NYHA= New York Heart Association, RV= right ventricular, TAPSE= tricuspid annular plane systolic excursion, LV= left ventricular, mPAP= mean pulmonary atrial pressure, mRAP= mean right atrial pressure, PA= pulmonary artery, AO= aorta, NT-proBNP= N-terminal pro B-type natriuretic peptide.

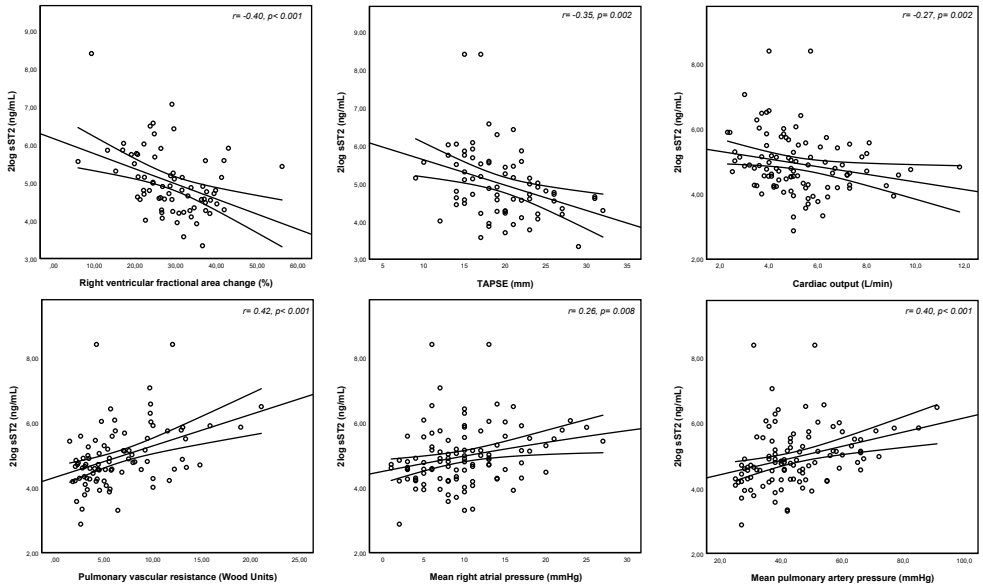

Figure 2. Correlations between sST2 and echocardiographic and hemodynamic measures in adults with pulmonary hypertension.
sST2= soluble suppression of tumorigenicity-2, TAPSE= tricuspid annular plane systolic excursion.

shown in Supplemental Figure 2, there was a moderate positive correlation between sST2 and NT-proBNP levels (r=0.54, p=0.001).

Follow-up

Follow-up data was complete in all patients. After a median follow-up of 3.3 [IQR 2.3-4.6] years, the primary endpoint occurred in 33 patients (31.7%) and the secondary endpoint in 43 patients (41.3%). Considering all endpoints separately, 31 patients died, 4 patients underwent a lung transplantation and 26 patients were hospitalized for heart failure. Causes of death were end-stage heart failure (n=9), sudden death, presumed cardiac (n=4), euthanasia in patients with end-stage pulmonary and cardiovascular disease (n=3), multi organ failure (n=4), kidney and/or live failure (n=2) and other diverse causes (n=9). Regarding the clinical management of the study patients, PH medication was initiated in 92% of the PAH patients and in 71% of the CTEPH patients. In addition, five patients (24%) with CTEPH underwent balloon pulmonary angioplasty and three patients (14%) underwent surgical pulmonary endarterectomy.

Associations between ST2 and clinical outcomes

Figure 3 shows the event-free survival stratified according to patients with a normal and elevated sST2. Patients with an elevated sST2 were at higher risk of both the primary and secondary endpoint (p=0.004 and p=0.001, respectively) compared with patients with a normal sST2. Survival according to the tertile distribution of sST2, showed that patients in the first

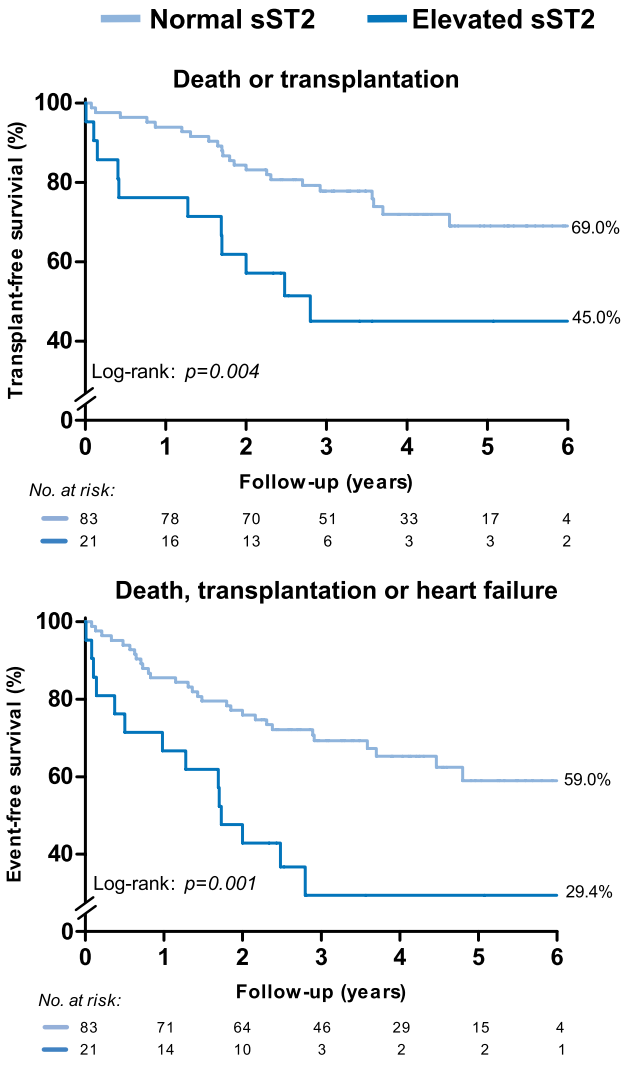

Figure 3. Event-free survival according to a normal sST2 or an elevated sST2 level in adults with pulmonary hypertension.
Elevated sST2 was defined as 44.50 ng/mL for women and 55.85 ng/mL for men, based on the 97.5th sST2 percentile in a healthy volunteer cohort. The percentage shows the cumulative end-point free survival at 6 years of follow-up.

tertile (sST2 <23.5 ng/mL) had the highest event-free survival (Supplemental figure 3). Cox-regression showed a significant association between continuous levels of sST2 and the primary and secondary endpoint, independent of age and sex. However, the association between sST2 and both endpoints did not remain significant when adjusted for NT-proBNP. (Table 3)

Table 3. Associations between sST2 and the primary endpoint (death or lung transplantation) and secondary endpoint (death, lung transplantation or HF).

	Hazard ratio* (95% CI)	p-value
Primary endpoint (n=33)		
sST2 (univariable)	1.53 (1.12-2.07)	0.007
Adjusted for:		
Age and sex	1.59 (1.17-2.16)	0.003
NT-proBNP	1.13 (0.75-1.69)	0.568
Secondary endpoint (n=43)		
sST2 (univariable)	1.45 (1.10-1.90)	0.008
Adjusted for:		
Age and sex	1.50 (1.15-1.95)	0.002
NT-proBNP	1.07 (0.75-1.52)	0.712

*Hazard ratio per 2-fold higher value of sST2. Abbreviations: sST2= suppression of tumorigenicity-2, NT-proBNP= N-terminal pro B-type natriuretic peptide.

Discussion

This is the largest prospective cohort study investigating the prognostic value of sST2 in adults with PH of various etiologies. Higher sST2 was associated with a shorter 6-minute walking distance, higher NYHA functional class, right ventricular dysfunction, higher pulmonary vascular resistance and higher pulmonary- and cardiac pressures. sST2 was elevated in a substantial number of patients (20%) and these patients had a significant worse transplant-free survival. Moreover, higher sST2 was significantly associated with an increased risk for all-cause mortality, lung transplantation or heart failure; however, sST2 yielded no additive prognostic value beyond NT-proBNP.

Previous studies

In 2013 Carlomagno et al. investigated sST2 levels in 25 patients with PAH of different etiologies and 10 controls and found increased levels of sST2 in PAH patients compared with healthy controls. Moreover, levels of sST2 were related to right ventricular dysfunction.[10] Median sST2 level in this study was higher than median sST2 in our study patients with PAH (43.3 ng/mL versus 34.5 ng/mL).To the extent of our knowledge, only 2 studies investigated the prognostic value of sST2 in PH patients. Zheng et al. investigated sST2 levels in 40 patients with idiopathic PAH and found a significant association between sST2 and clinical worsening. These patients were followed for a mean follow-up duration of 14 months in which 12 patients experienced clinical worsening.[18] Another study found a significant association between sST2 in 43 patients with various types of PH except due to left heart disease and found higher levels of sST2 in patients who were hospitalized for heart failure or died during follow-up[19]. This is in line with our study, which demonstrated a significant association between sST2 and the transplant-free

survival in a large cohort of patients with a longer follow-up duration, whom were prospectively followed since their initial diagnosis.

Pathophysiology of sST2 in PH

sST2 is part of the interleukin-33 (IL-33) ST2 ligand interaction, a cardio protective system that is upregulated in cardiomyocytes and fibroblasts as response to myocardial stress or injury. sST2 is the circulating form of ST2 and acts as decoy receptor, blocking the interleukin-33/ST2 ligand interaction.[20] Upregulation of sST2 therefore abolishes the cardio protective effects and causes maladaptive remodeling including myocardial hypertrophy, fibrosis and apoptosis[21]. The exact pathophysiology of PH is currently still not fully elucidated[8]; however, there are 3 processes that play a key role in the development: vasoconstriction, vascular remodeling and micro thrombotic events[22]. Cytokines are identified to have a major contribution in the pathogenesis of PH, of which interleukins are probably the most outspoken cytokines that have been investigated in relation to PAH[23]. It has been proposed that the IL-33/ST2 ligand interaction may also be involved in the development of PAH. A previous study demonstrated that in endothelial cells from iPAH patients a marked loss of nuclear IL-33 is present and that knocking down IL-33 induced and released sST2[9]. Hence, elevated levels of sST2 in this study may partially originate from processes associated with pulmonary vascular remodeling instead of exclusively induced by myocardial stress. This might indicate that the height of sST2 reflects both severity of pulmonary endothelial remodeling as well as progression of right ventricular dysfunction. In our study, sST2 was associated with hemodynamic and echocardiographic measurements, suggesting that patients with more severe PH have more ongoing myocardial and endothelial cell damage reflected by a higher release of sST2. Unfortunately, with data on patient-level we are only able to speculate about the mechanisms potentially inducing sST2 secretion. Further research is needed to elucidate the pathophysiologic involvement of sST2 in PH.

sST2 and etiologic differences of PH

sST2 levels in all subgroups were higher compared to sST2 levels measured in the healthy volunteers, except for patients with CTEPH in whom sST2 levels seemed similar to levels in healthy volunteers. Like PAH, it has been investigated that cytokines also play a role in the pathophysiology of CTEPH[24]. An explanation for the lower sST2 levels in CTEPH patients in our study, could be presence of less severely PH, expressed by the lower pulmonary vascular resistance seen in these patients. However the median pulmonary artery and right atrial pressure in the patients with CTEPH was equal to the median pressure in patients with PH due to lung disease. Another explanation could be the more persevering right ventricular function in these patients, as reflected by a higher fractional area change and a higher trans annular plain systolic excursion. Therefore less sST2 may be secreted due to cardiac stress in these patients.

Limitations

This study is limited by the fact that it includes patients with PH of different etiologies, introducing heterogeneity in our study cohort. sST2 levels differed between PH subgroups, therefore it is presumable that sST2 may yield a different prognostic value in each sub diagnosis of PH. The relatively small sample size restricted further subgroup analysis. Of note, patients with PH due to left heart diseases were not included in this study, this should be kept in mind when extrapolating the results to other studies. Although this is currently the largest cohort that investigated the prognostic value of sST2, it still consists of a relatively low sample size and therefore yields a limited power for multivariable statistical analyses. Blood sampling was performed in treatment naïve PH patients. However in PAH and CTEPH patients, treatment was initiated after diagnosis of PH, during follow-up of the study. In this study we lacked the ability to adjust for treatment effect in the association between sST2 and endpoints. Therefore the associations found in this study, reflect associations between sST2 and outcomes in adults PH patients whom were treated according to the ESC guideline[11]. Serum samples of these patients were stored by -80 ºC until batch analysis took place, this could have affected serum sST2 levels. However, in our study no association was found between the storage duration and serum sST2 levels.

Clinical perspectives

According to our study, patients with an elevated sST2 level have a worse prognosis than patients with a normal sST2 level at the time of diagnosis of PH. sST2 could therefore be used for risk stratification in PH, however the independent prognostic value besides NT-proBNP seems limited, questioning the additive prognostic value sST2 might have in this perspective. sST2 has a lower biological variability than NT-proBNP [25], this could be advantageous when measuring a biomarker repeatedly over time.

Our study suggests that high sST2 levels are seen in different PH etiologies at the time of diagnosis, except for CTEPH patients, in whom sST2 levels seemed similar to the general population. It would be interesting to investigate the influence of PH treatment on sST2 levels over time, as this may elucidate whether sST2 could be a biomarker for assessing treatment effectiveness. Serial repeated measurements of sST2 could also help to investigate secretion of sST2 in anticipation to decompensated heart failure. This study could not provide evidence whether sST2 secretion is induced by myocardial stretch, pulmonary vascular remodeling, or other potentially involved processes such as type 2 immune responses. Future studies are needed to reveal the exact mechanisms of sST2 secretion in relation to PH.

Conclusions

Levels of sST2 are higher in adults at the time of diagnosis with pulmonary hypertension compared to healthy people and differs between PH etiologies. Higher sST2 is associated with a worse exercise capacity, higher pulmonary and cardiac pressures, and with more severe right ventricular dysfunction. Moreover, sST2 is significantly associated with the risk of death or lung

transplantation. Nevertheless, as sST2 yielded no independent prognostic value besides the conventional biomarker NT-proBNP, the usefulness of sST2 as prognostic biomarker in adults with pulmonary hypertension seems to be limited.

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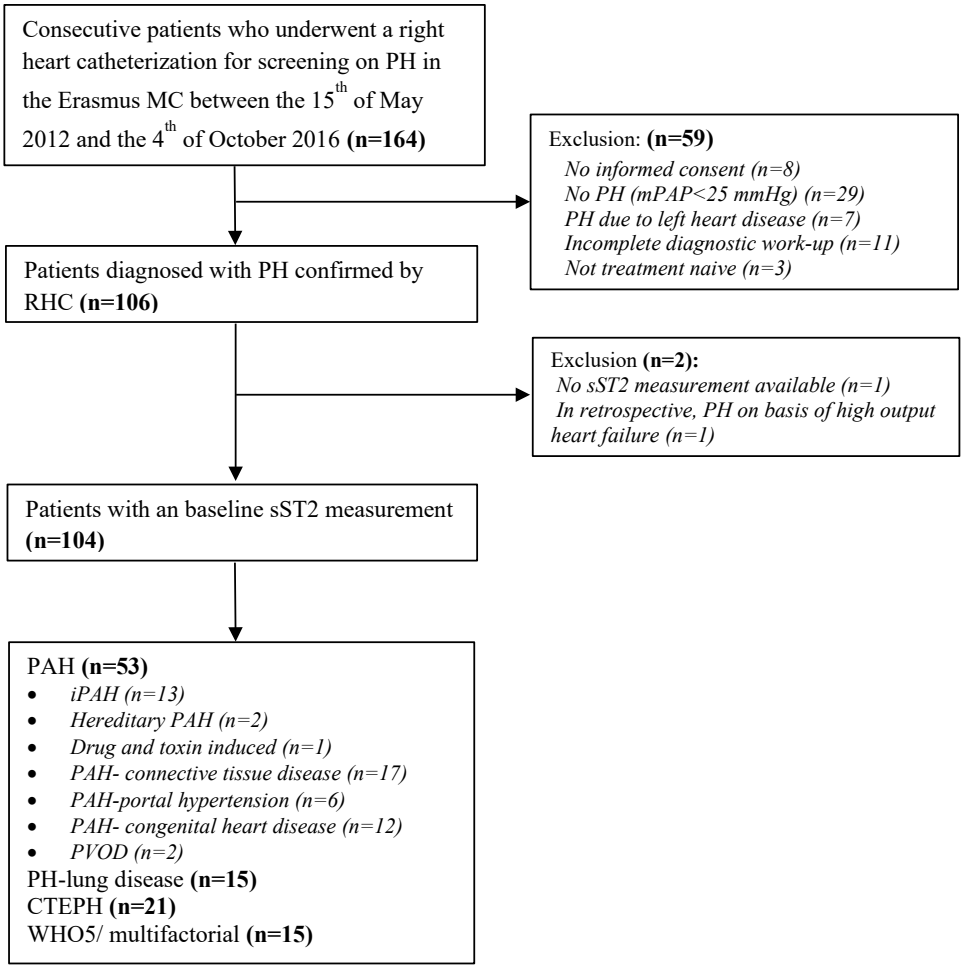
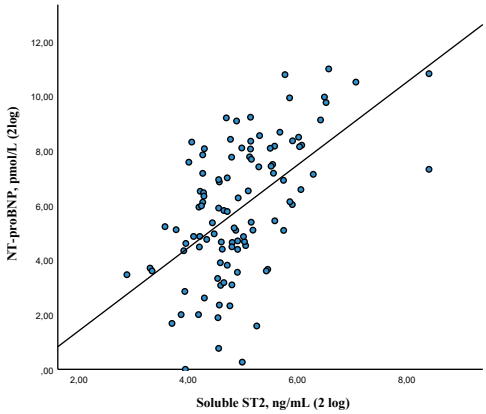
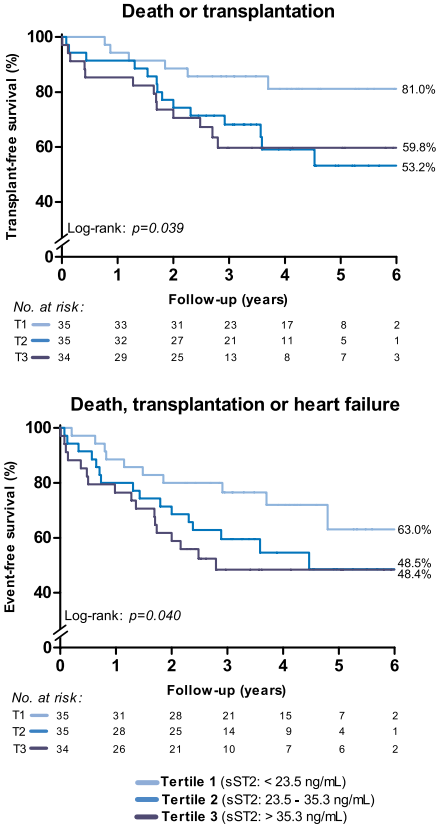


Supplemental Figure 1. Patient selection process of the prospective observational study including adult patients with pulmonary hypertension.
PH = pulmonary hypertension, RHC= right heart catheterization, sST2= soluble suppression of tumorigenicity-2, mPAP= mean pulmonary artery pressure, PAH= pulmonary arterial hypertension, iPAH= idiopathic pulmonary arterial hypertension, PVOD= pulmonary veno-occlusive disease, CTEPH= chronic thromboembolic pulmonary hypertension, WHO5= world health organization class 5.



Supplemental Figure 2. Correlation between 2log transformed sST2 and NT-proBNP levels in adults with pulmonary hypertension.
Spearman correlation coefficient: r=0.54, p=0.001. sST2= soluble suppression of tumorigenicity-2, NT-proBNP= N-terminal pro-B type natriuretic peptide.



Supplemental Figure 3. Event-free survival according to the tertile distribution of sST2 in adults with pulmonary hypertension.
T1= first tertile, T2= second tertile, T3= third tertile. The percentage shows the cumulative end-point free survival at 6 years of follow-up according to each tertile of the sST2 distribution.

CHAPTER 10

Growth differentiation factor-15 as candidate predictor for mortality in adults with pulmonary hypertension

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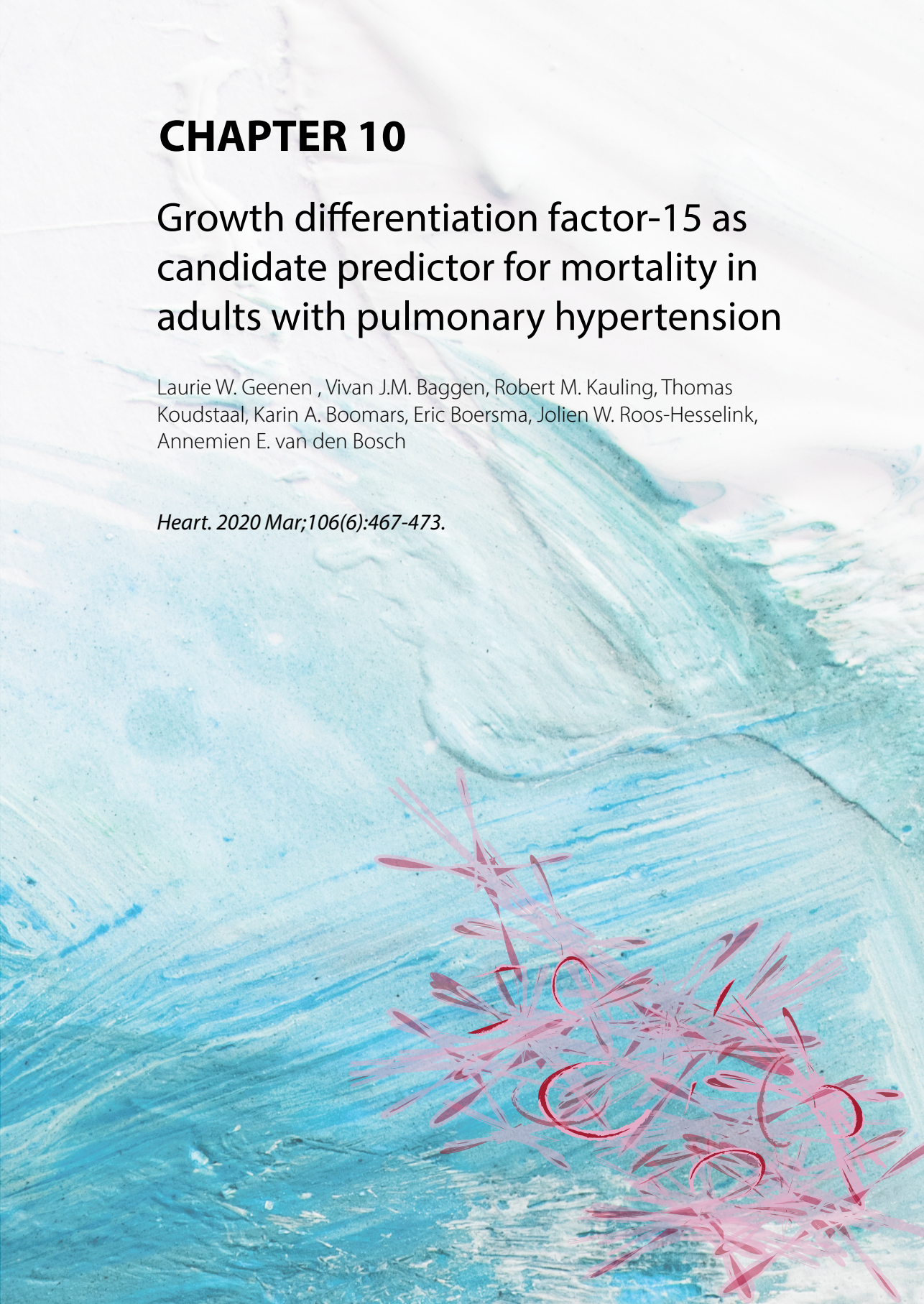

Abstract

Objective: Despite its predictive value for mortality in various diseases, the relevance of growth differentiation factor-15 (GDF-15) as prognostic biomarker in pulmonary hypertension (PH) remains unclear. This study investigated the association between GDF-15 and outcomes in adults with PH.

Methods: This is a single-centre prospective observational cohort study**.** All adults with PH were included at the day of their diagnostic right heart catheterisation between 2012 and 2016. PH due to left heart disease was excluded. Venous blood sampling was performed and included GDF-15 and N-terminal pro-B-type natriuretic peptide (NT-proBNP) measurements. Kaplan-Meier curves and Cox regression analysis were used to investigate the association between GDF-15 and a composite endpoint of death or lung transplantation. We adjusted for age and NT-proBNP in multivariable analysis. Reference values were established by GDF-15 measurements in healthy controls.

Results: GDF-15 was measured in 103 patients (median age 59.2 years, 65% women, 51% pulmonary arterial hypertension). GDF-15 was elevated in 76 patients (74%). After a median follow-up of 3.4 [IQR 2.3-4.6] years, 32 patients (31.1%) reached the primary endpoint. Event-free survival 2 years after diagnosis was 100% in patients with normal GDF-15, versus 72.4% in patients with elevated GDF-15($p=0.007$). A significant association was found between GDF-15 and the primary endpoint (HR per twofold higher value 1.77, 95% CI 1.39-2.27, $p<0.001$), also after adjustment for age and NT-proBNP (HR 1.41, 95% CI 1.02-1.94, $p=0.038$).

Conclusions: High GDF-15 levels are associated with an increased risk of death or transplant in adults with PH, independent of age and NT-proBNP. As non-specific biomarker, GDF-15 could particularly be useful to detect low-risk patients.

Introduction

Growth differentiation factor-15 (GDF-15) is a member of the transforming growth factor-B cytokine superfamily and is known for its role in cell growth and differentiation[1]. Animal models have shown that GDF-15 is induced in response to cardiac pressure overload, ischemia, oxidative stress and reperfusion injury[2, 3]. However, GDF-15 is non-tissue specific; secretion of GDF-15 is produced by a wide variety of cells[4] and is seen in high concentrations in various diseases[5]. It may therefore not be surprising that GDF-15 has been identified as predictor for all-cause mortality in a wide spectrum of diseases; cardiovascular diseases[6, 7], various types of cancers[8, 9] and pulmonary diseases[10, 11]. To that end, the use of GDF-15 in risk stratification has been doubted because of its lack of disease specificity[12]. Paradoxically, non-tissue specific biomarkers could offer a solution in some diseases in which heterogeneity and concomitant disease have a considerable influence on the prognosis.

Pulmonary hypertension (PH) is a disease in which heterogeneity introduces major challenges in risk stratification. PH is defined by an increased pulmonary artery pressure of ≥25 mmHg[13] which eventually leads to right ventricular failure and mortality. However, underlying aetiology of increased pulmonary pressures includes a wide spectrum of diseases. The prognosis of PH strongly depends on the aetiology[14]. A recent study including PH of all aetiologies showed that only in 23.8% of the patients right ventricular failure was the main cause of death and other causes such as respiratory failure, malignancy, sepsis and infection were not uncommon[15]. In this perspective, GDF-15 could be an ideal prognostic biomarker for mortality risk stratification in PH, as it may reflect not only cardiac failure but also incorporate more disease processes.

Therefore, this study aimed to investigate levels of GDF-15 in adults with PH of different aetiologies at the time of diagnosis and to determine its association with prognosis. A cohort of healthy volunteers was used to establish reference values for GDF-15.

Methods

Study design

This prospective observational cohort study aimed to include all consecutive adults diagnosed with PH in our tertiary centre between May 2012 and October 2016. The diagnostic work-up of PH consisted of an inpatient visit during which the following tests were performed: physical examination by a cardiologist and pulmonary physician, 6-minute walking test, ECG, transthoracic echocardiography, venous blood sampling, cardiac CT and right heart catheterisation. Baseline was defined as the day of the diagnostic right heart catheterisation. Diagnosis of PH was defined as a mean pulmonary artery pressure ≥25 mmHg. Exclusion criteria were: unconfirmed diagnosis of PH due to an incomplete diagnostic work-up, not PH treatment-naive, <18 years, not capable of understanding or signing informed consent and

PH due to left heart disease. The study protocol was approved by the local medical ethics committee and is conform to the principles outlined in the Declaration of Helsinki

Classification of PH was done in accordance with the WHO classification: pulmonary arterial hypertension (PAH), PH due to lung disease, chronic thromboembolic pulmonary hypertension (CTEPH) and PH with unclear/multifactorial mechanisms(WHO 5). PAH was further subdivided according to the classification [13, 16].

Self-declared healthy volunteers were recruited between January 2014 and December 2014 to serve as control-cohort. All volunteers underwent physical examination, ECG, echocardiography and venous blood sampling on the same day. More detailed information has been previously published[17].

Data collection

Transthoracic echocardiography was performed using a commercially available ultrasound system (iE33, Philips Medical Systems, Best, the Netherlands). The echocardiographic imaging analysis was performed in accordance with echocardiographic guidelines on cardiac chamber quantification[18]. We used a Swan-Ganz catheter to obtain haemodynamic measurements during the right heart catheterisation. Fick's principle or thermodilution was used to measure cardiac output. On indication, a fluid challenge was performed to distinguish PH due to left heart disease from precapillary PH. Data were collected and stored in an online electronic case report form (PAHTool, version 4.3.5947.29411, Inovoltus, Santa Maria da Feira, Portugal). More details have been described previously[19].

Biomarker assessment

Venous blood sampling was performed during the diagnostic right heart catheterisation and was for study purposes only. N-terminal pro-B-type natriuretic peptide (NT-proBNP) was directly measured from fresh blood samples with the use of an electrochemiluminescence assay (Roche Diagnostics) in the clinical chemistry laboratory. The rest of the serum samples were aliquoted and stored in our biobank by -80 ºC. GDF-15 was determined in thawed serum samples by batch analysis with electrochemiluminescence immunoassay using the Cobas 6000 analyser (Roche Diagnostics, Basel, Switzerland). Limit of detection was 400 pg/mL. GDF-15 was measured once in all patients with PH. In healthy controls, GDF-15 was measured twice to assess reproducibility and to establish reference values.

Definition of endpoints

Endpoints were defined prior to data collection. The primary endpoint was defined as all-cause mortality or lung transplantation. The secondary endpoint was a composite endpoint including the elements of the primary endpoint and heart failure. Heart failure was defined as any hospitalisation due to heart failure with requirement for uptitration or initiation of diuretics. Protocolled half-yearly visits to the outpatient clinic were scheduled to guarantee data on endpoints. Patients were treated in accordance with the European Society of Cardiology

guidelines[13]. PH medication was prescribed when indicated and patients with CTEPH eligible for pulmonary endarterectomy or balloon pulmonary angioplasty were referred to a specialised centre.

Patient records and the municipal personal records database were used to adjudicate the endpoints until 1 January 2019 without the knowledge of any biomarker level.

Statistical analysis

Continuous variables are presented as mean ± SD or median [IQR]. Differences in baseline characteristics between patients with a normal and elevated GDF-15 were investigated with the Student's T-test or Mann-Whitney test ($n < 30$) for continuous variables. Differences in categorical variables were investigated with the χ^2 test or Fisher exact test. Correlations between GDF-15 and clinical characteristics were assessed with Pearson or Spearman correlation.

Reproducibility of GDF-15 assay was visualised by a Bland-Altman plot and the coefficient of variation and intraclass correlation coefficient were calculated. The 97.5th percentile based on 2-log transformed GDF-15 distribution in healthy controls was used to define an elevated GDF-15 level. GDF-15 distributions were visualized using a Kernel density plot.

GDF-15 and NT-proBNP levels were 2log transformed because of skewed distributions. Missing data was taken care of using multiple imputation with 5 imputations considering all meaningful variables as predictor, including the endpoints. Endpoints were not imputed. Pooled estimates were obtained based on Rubin's rule[20]. Survival curves were derived using the Kaplan-Meier estimator and compared with the log-rank test. Cox-proportional hazard regression was used to assess associations with endpoints in uni- and multivariable analysis. The proportional hazard assumption was assessed through Schoenfield residual plots and through proportional hazard tests. We adjusted all analyses for age because we considered this as an important confounder. Due to the limited number of events, we adjusted for only one additional clinical variable at a time in additional analyses. A subgroup analysis was performed restricted to PAH patients, with adjustment for only one clinical variable at a time because of a limited number of events. To evaluate the potential predictive value of GDF-15 beyond age and NT-proBNP, we determined C-indices of models with and without GDF-15. The likelihood ratio test was used to compare models.

Statistical analyses were performed using IBM SPSS Statistics (version 24) and R version 5.5.3. A two-sided p-value <0.05 was considered statistically significant.

Results

Reproducibility of GDF-15 assay and reference values

GDF-15 was measured twice in 145 healthy controls. Reproducibility of the assay was good, with coefficient of variation of 0.68%, limits of agreement of -15.49–23.60 pg/mL and intraclass correlation coefficient of 0.99 (0.99-1.00, p<0.001) (Supplemental Figure 1). Levels ranged

from 400 pg/mL (lower limit of detection; n=15) to 1495 pg/mL. GDF-15 was not significantly different between men and women; however, there was a significant correlation with age (r= 0.48, p<0.001) (Supplemental Figure 2). Consequently, age-specific reference values were established; >920 pg/mL for patients aged <50 years and 1330 pg/mL for patients aged >50 years. Distribution of GDF-15 levels in healthy controls and patients with PH are shown in Figure 1.

Baseline characteristics

GDF-15 was measured in 103 out of 106 patients (97%) who were originally included in this study (Supplemental Figure 3). In three cases, no serum sample was traceable to measure GDF-15. Median age was 59 [IQR 47-69] years, 67 were women (65%) and 52 had PAH (51%). Over half of the patients was in New York Heart Association (NYHA) functional class III (46%) or IV (10%). GDF-15 was above the limit of detection in all patients, with a median GDF-15 of

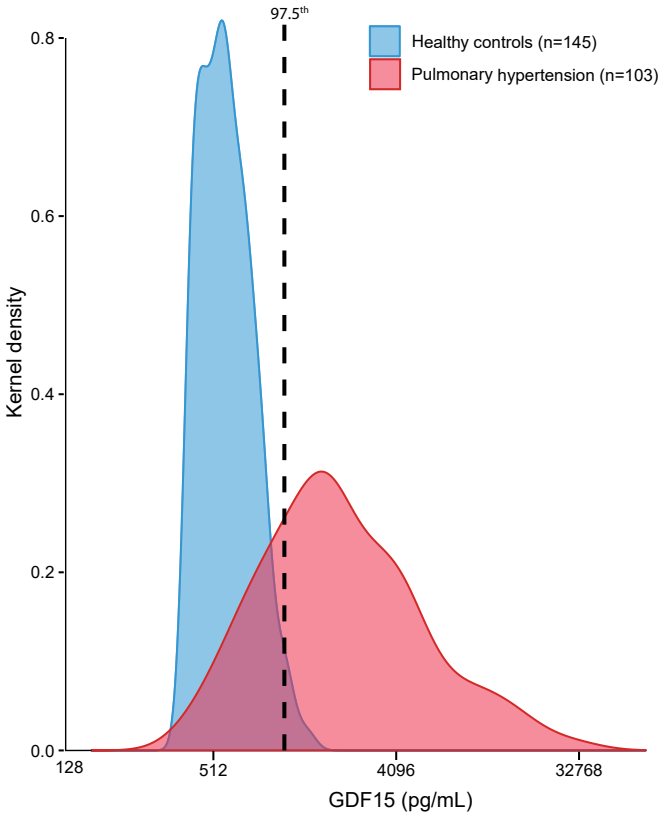

Figure 1. Kernel density plot showing the distribution of growth differentiation factor-15 (GDF-15) in healthy controls and in adults with pulmonary hypertension.
The 97.5th percentile of the GDF-15 distribution in the healthy controls is indicated by the black-dotted line. The x-axis is shown on the 2-log scale.

1974 [IQR 1096-4173] pg/mL. An elevated GDF-15 was found in 76 patients (74%). Patients with normal GDF-15 were significantly younger, had a longer 6-minute walking distance, a lower right atrial area and mean right atrial pressure, a better renal function and a lower NT-proBNP, than patients with elevated GDF-15. (Table 1)

Table 1. Baseline characteristics of all patients and subdivided to patients with a normal GDF-15 and elevated GDF-15 at baseline.

	Valid cases (n,%)	**All** n=103	**Normal GDF-15** n=27	**Elevated GDF-15** n=76	P-value
Clinical characteristics					
Age, years	103 (100)	59 [47-69]	50 [29-63]	63 [53-70]	0.001
Sex, women, n (%)	103 (100)	67 (65)	19 (70)	48 (63)	0.500
Heart rate, beats/minute	103 (100)	80 ± 17	80 ± 19	80 ± 16	0.986
Systolic blood pressure, mmHg	103 (100)	127 ± 18	128 ± 17	126 ± 18	0.611
Oxygen saturation <90%, n (%)	103 (100)	3 (3)	1 (4)	2 (3)	1.00
Body mass index, kg/m^2	103 (100)	28.2 ± 6.5	26.7 ± 6.0	28.8 ± 6.7	0.153
Overweight (25-30 kg/m^2)		29 (28)	10 (37)	19 (25)	
Obesity (>30 kg/m^2)		41 (40)	7 (26)	34 (45)	
Diabetes mellitus	103 (100)	22 (21)	3 (11)	19 (25)	0.130
Systemic hypertension	103 (100)	25 (24)	3 (11)	22 (29)	0.063
NYHA class, n (%)	103 (100)				0.086
Class I		1 (1)	1 (4)	0 (0)	
Class II		45 (44)	13 (48)	32 (42)	
Class III		47 (45)	13 (48)	34 (45)	
Class IV		10 (10)	0 (0)	10 (13)	
Electrocardiography					
Rhythm, n (%)	100 (97)				0.753
Sinus		89 (89)	25 (92)	64 (88)	
Atrial fibrillation, n (%)		7 (7)	1 (4)	6 (8)	
Other, n (%)		4 (4)	1 (4)	3 (4)	
QRS duration, ms	99 (96)	99 [90-106]	94 [88-106]	100 [91-106]	0.311
6-minute walking test					
Distance, m	90 (87)	339 ± 139	398 ± 131	317 ± 137	0.015
Echocardiography					
RA area, cm^2	79 (77)	27.4 ± 8.9	23.3 ± 6.8	28.8 ± 9.2	0.016
RV basal dimension, mm	74 (72)	51.3 ± 9.6	48.8 ± 9.0	52.3 ± 9.7	0.169
RV fractional area change, %	72 (70)	29.0 ± 8.5	28.3 ± 6.5	29.3 ± 9.2	0.672
TAPSE, mm	71 (69)	19.4 ± 5.0	18.2 ± 5.0	19.7 ± 5.0	0.263

Continue

Continued

	Valid cases (n,%)	**All** n=103	**Normal GDF-15** n=27	**Elevated GDF-15** n=76	P-value
LV function, n (%):					0.435
Normal	97 (94)	65 (67)	19 (79)	46 (63)	
Mildly impaired		28 (29)	5 (21)	23 (32)	
Moderately impaired		3 (3)	0 (0)	3 (4)	
Severely impaired		1 (1)	0 (0)	1 (1)	
LV end diastolic dimension, mm		43.4 [37.8-48.0]	44.8 [39.4-47.8]	42.8 [37.5-48.2]	0.751
Right heart catheterization					
mPAP, mmHg	103 (100)	42.0 [35.0-51.0]	42 [32-55]	41 [35-51]	0.546
mRAP, mmHg	103 (100)	10.0 ± 5.4	7.6 ± 3.8	10.8 ± 5.6	0.008
PAWP, mmHg	89 (86)	13.2 ± 6.3	12.7 ± 6.4	13.4 ± 6.3	0.673
PVR, wood units	86 (83)	5.5 [3.3-9.2]	5.7 [3.3-8.5]	5.2 [3.2-9.5]	0.691
Cardiac output, L/min	98 (95)	5.0 [4.0-6.1]	5.0 [4.1-5.7]	4.9 [4.0-6.4]	0.754
Cardiac index, L/min/m^2	98 (95)	2.6 [2.2-3.2]	2.7 [2.3-3.1]	2.6 [2.1-3.4]	0.562
Computed tomography					
PA diameter, mm	98 (95)	34.4 ± 5.4	34.8 ± 6.7	34.2 ± 4.9	0.618
PA/AO ratio	98 (95)	1.12 ± 0.24	1.24 ± 0.35	1.09 ± 0.16	0.035
Laboratory					
NT-proBNP, pmol/L	103 (100)	63[21-218]	24 [7-77]	90 [26-82]	0.001
eGFR	103 (100)	72 [56-90]	78 [72-90]	67 [52-82]	0.002

Table legend: Elevated GDF-15 was defined as: GDF-15 >920 pg/mL (age <50 years) or GDF-15 >1330 pg/mL (age > 50 years).
NYHA=New York Heart Association, RA= right atrial, RV= right ventricular, TAPS= trans annular plane systolic excursion, LV= left ventricular, mPAP= mean pulmonary artery pressure, mRAP, mean right atrial pressure, PVR= pulmonary vascular resistance, PA= pulmonary artery, PAWP= pulmonary artery wedge pressure, AO= aorta, NT-proBNP= N-terminal pro B-type natriuretic peptide, eGFR= estimated glomerular filtration rate.

Higher GDF-15 significantly correlated with higher NYHA class, shorter 6-minute walking distance and the presence of hypertension and diabetes mellitus. GDF-15 did not show any significant correlation with echocardiographic or invasive haemodynamic measures, except for the mean right atrial pressure. GDF-15 did show a significant moderate correlation with NT-proBNP (r= 0.51, p<0.001) and estimated glomerular filtration rate (eGFR) (r= -0.47, p<0.001). (Table 2) There was no statistical difference in GDF-15 distributions across PH subgroups (p=0.061) (Supplemental Figure 4). The distribution of different PH aetiologies across patients with normal versus elevated GDF-15 is presented in Figure 2.

Follow-up

Follow-up data were 100% complete. Median follow-up was 3.4 [IQR 2.3-4.6] years. The primary endpoint was reached in 32 patients (31.1%) and the secondary endpoint was reached in

Table 2. Correlations between GDF-15 and baseline characteristics

	GDF-15	
	r	*p-value*
Clinical characteristics		
Age	**0.39**	**<0.001**
Sex	0.03	0.802
Body mass index	0.02	0.859
Heart rate	0.15	0.128
Systolic blood pressure	**-0.21**	**0.032**
Oxygen saturation < 90%	-0.09	0.380
NYHA class	**0.28**	**0.004**
Diabetes	**0.31**	**0.001**
Systemic hypertension	**0.30**	**0.002**
Electrocardiography		
Loss of sinus rhythm	0.10	0.316
QRS Duration	0.09	0.362
6-minute walking test		
Distance	**-0.44**	**<0.001**
Echocardiography		
Right atrial area	0.16	0.174
RV basal dimension	0.08	0.487
RV fractional area change	-0.05	0.651
TAPSE, mm	-0.04	0.743
LV function	0.15	0.143
LV end diastolic dimension	-0.15	0.187
Right heart catheterization		
mPAP	-0.01	0.899
mRAP	**0.21**	**0.032**
PAWP	-0.06	0.564
Pulmonary vascular resistance	0.18	0.091
Cardiac output	-0.11	0.272
Cardiac index	-0.18	0.083
Computed tomography		
PA diameter	-0.081	0.427
PA/AO ratio	**-0.30**	**0.002**
Laboratory		
NT-proBNP	**0.51**	**<0.001**
eGFR	**-0.47**	**<0.001**

Significant correlations are in bold. 2log transformed GDF-15 levels were used for the analysis.
GDF-15= growth differentiation factor-15, NYHA= New York Heart Association, RV= right ventricular, TAPSE= tricuspid annular plane systolic excursion, LV= left ventricular, mPAP= mean pulmonary artery pressure, mRAP= mean right atrial pressure, PAPW= pulmonary artery wedge pressure, PA= pulmonary artery, AO= aorta.

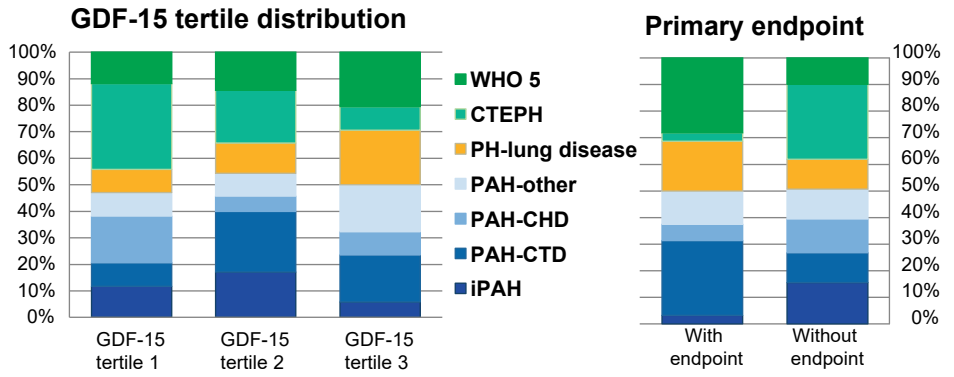

Figure 2. Distribution of pulmonary hypertension subdiagnoses according to a normal or elevated GDF-15 and according to the primary endpoint.

Elevated GDF-15 was defined as; >920 pg/mL (age <50 years), or >1330 pg/mL (age > 50 years). Diagnosis groups are in accordance with the ESC guidelines of PH. PAH-other consisted of; pulmonary veno-occlusive disease(n=2), PAH- associated with portal hypertension (n=7), hereditary PAH (n=2), drug and toxin induced PAH (n=1).

GDF-15 = growth differentiation factor-15, WHO 5= World Health Organization group 5, CTEPH = chronic thromboembolic pulmonary hypertension, PAH-CHD = pulmonary arterial hypertension due to congenital heart disease, PAH-CTD = pulmonary arterial hypertension due to connective tissue disease, iPAH = idiopathic pulmonary arterial hypertension.

41 patients (39.8%). Figure 1 shows the distribution of PH aetiologies according to primary endpoint achievement. Considering all endpoints separately, 30 patients died, 4 patients underwent lung transplantation and 26 patients were hospitalised for heart failure. Causes of death were end-stage heart failure (n=9), sudden death presumed cardiac (n=4), multiorgan failure (n=3), and other diverse causes (n=13) described in detail in Supplemental Table 1. In one patient, cause of death was unknown. PAH specific medication was initiated during follow-up in 90% of the patients with PAH (n=47) and in 71% of the patients with CTEPH (n=15). Five patients (24%) with CTEPH underwent balloon pulmonary angioplasty and three patients (14%) underwent surgical pulmonary endarterectomy.

GDF-15 as prognostic biomarker

Patients with normal GDF-15 had a significant higher transplant-free survival than patients with elevated GDF-15 (p=0.007). This difference was specifically pronounced within the first 2 years of follow-up; patients with normal GDF-15 were all alive and free of transplantation up to 2 years after diagnosis, compared with 72.4% of the patients with elevated GDF-15. Comparable results were found regarding the secondary endpoint. (Figure 3)

Analysed continuously, GDF-15 was significantly associated with the primary endpoint, also after adjustment for several clinical characteristics. After adjustment for age and NT-proBNP, GDF-15 was still significantly associated with the primary endpoint. Addition of GDF-15 to a model with NT-proBNP and age, significantly increased the C-index from 0.73 (95% CI 0.65-0.81) to 0.76 (95% CI 0.69-0.83) (p=0.042).

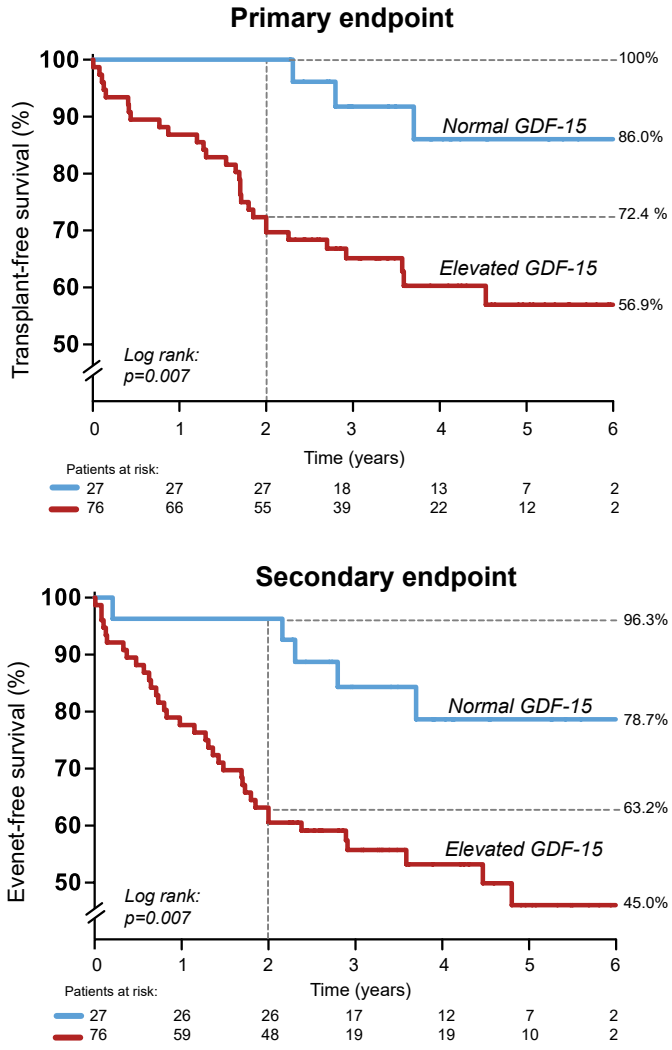

Figure 3. Survival curve demonstrating the cumulative transplant-free survival in patients with pulmonary hypertension with a normal growth differentiation factor-15 (GDF-15) level and patients with an elevated GDF-15 level.
An elevated GDF-15 was defined as; GDF-15 >920 pg/mL (age <50 years), or GDF-15 >1330 pg/mL (age > 50 years).

GDF-15 was significantly associated with the secondary endpoint, although less strong compared with the primary endpoint. This association remained present after adjustment for clinical characteristics, but not after adjustment for age and NT-proBNP. (Table 3)

Subgroup analysis restricted to PAH showed an independent prognostic value of GDF-15 for the primary endpoint when adjusted for clinical variables, including NT-proBNP. Notably, adjustment for eGFR led to a non-significant result in this subgroup. (Supplemental Table 2)

Table 3. Association between GDF-15 and the primary and secondary endpoint

	Primary endpoint (n=32)		Secondary endpoint (n=41)	
	HR*(95% CI)	p-value	HR*(95% CI)	p-value
GDF-15 (univariable)	1.77 (1.39-2.27)	<0.001	1.58 (1.27-1.96)	<0.001
Adjusted for:				
Age and sex	1.74 (1.34-2.26)	<0.001	1.53 (1.21-1.93)	<0.001
Age and NYHA class 3/4	1.60 (1.23-2.08)	<0.001	1.40 (1.11-1.76)	0.005
Age and 6-MWD	1.57 (1.21-2.04)	0.001	1.31 (1.03-1.66)	0.025
Age and right atrial area	1.71 (1.32-2.20)	<0.001	1.49 (1.19-1.87)	0.001
Age and cardiac index	1.77 (1.35-2.31)	<0.001	1.53 (1.21-1.93)	<0.001
Age and mRAP	1.78 (1.37-2.32)	<0.001	1.46 (1.15-1.86)	0.002
Age and eGFR	1.72 (1.28-2.30)	<0.001	1.48 (1.14-1.92)	0.003
Age and NT-proBNP	1.41 (1.02-1.94)	0.038	1.21 (0.91-1.62)	0.192

*HR per two-fold higher value of GDF-15.
HR= hazard ratio, GDF-15= growth differentiation factor-15, NYHA= New York Heart Association, mPAP= mean pulmonary arterial pressure, mRAP= mean right atrial pressure, eGFR= estimated glomerular filtration rate, NT-proBNP= N-terminal pro B-type natriuretic peptide, 6-MWD= 6-minute walking distance.

Discussion

In 76% of the patients with PH, GDF-15 is elevated at the time of the diagnosis. Higher GDF-15 levels were associated with an older age, higher NYHA class, shorter 6-minute walking distance, higher mean right atrial pressure and higher NT-proBNP; however, no association was found with echocardiographic measurements. GDF-15 was significantly associated with mortality or lung transplantation independent of age and NT-proBNP levels, and yielded an incremental predictive value. Moreover, a normal GDF-15 ruled out the risk of death or transplantation in the first 2 years after the diagnosis of PH.

Previous reports

Data on GDF-15 in adults with PH are currently limited and are restricted to some specific PAH diagnoses. Nickel et al. measured GDF-15 in 76 treatment-naive adults with idiopathic PAH (iPAH), 55% of these patients had a GDF-15 level above 1200 ng/L. Higher GDF-15 levels were associated with an increased risk of death or transplantation, independent of NT-proBNP and other clinical variables.[10] Rhodes et al. later confirmed the predictive value of GDF-15 for mortality in 139 patients with iPAH[21]. Zelniker et al. investigated GDF-15 in 96 non-treatment naive patients with PAH. The majority of these patients had iPAH (68%) or PAH due to connective tissue disease (22%). They found that higher GDF-15 levels were associated with the 4-year mortality risk. However, GDF-15 was not a better prognosticator than NT-proBNP, high sensitive troponin-T and proatrial natriuretic peptide in their study.[22]

Cross-sectional studies have shown that GDF-15 is higher in patients with PAH due to systemic sclerosis compared with systemic sclerosis patients free of PAH[23]. Moreover, higher levels of GDF-15 were found in patients with CTEPH compared with patients with a history of acute pulmonary embolism without development of CTEPH[24].

To the best of our knowledge, this is the first study investigating GDF-15 in a group of mixed PH aetiologies. It showed that GDF-15 levels are considerably higher in adults with PH compared with a reference cohort and that a GDF-15 level within the reference range is associated with a low risk of mortality, transplantation or heart failure in adults with PH. GDF-15 could therefore be a promising biomarker. However the price of the GDF-15-assay kit is still high and ideally biomarkers should be easily measured against a low price.

Underlying mechanisms of GDF-15

There are several possible explanations for the high GDF-15 levels in patients with PH and its strong association with mortality found in our study. First, since GDF-15 is involved in the regulation of cell processes and PH is characterised by pulmonary vascular endothelial remodelling [25], GDF-15 could reflect the process of vascular remodelling in PH. This is supported by a study that found higher GDF-15 levels in the vascular endothelial cells of PH[26]. Second, GDF-15 may be induced by myocardial cell stress caused by the increased right ventricular afterload as it has been shown that GDF-15 is induced in the myocardium of mice after exposure to ischaemic injury[2]. Moreover, it has been suggested that GDF-15 is part of a cardioprotective pathway[3]. Third, GDF-15 may have been secreted due to any concomitant disease in these patients such as systemic sclerosis or chronic obstructive pulmonary disease. Finally, it has been shown that GDF-15 levels are higher in patients with diabetes and other unfavourable cardiovascular risk factors[27]. In our study, 21% of the patients had diabetes mellitus and 24% had systemic hypertension and both comorbidities correlated with higher GDF-15 levels.

Considering all of the above-mentioned reasons, it is most presumable that GDF-15 levels were influenced by more disease processes than solely cardiac involvement. This can be further supported by the fact that levels of GDF-15 did show an association with NYHA class and the 6-minute walking distance, while associations with haemodynamic or echocardiographic measurements were mostly absent. Despite limited associations with cardiac pressure and function, GDF-15 did show a moderate positive correlation with NT-proBNP.

The prognosis of adults with PH is still very challenging, mainly due to heterogeneity and accompanied diseases in this population. Paradoxically, as we hypothesised, in this specific setting the low disease specificity of GDF-15 turns out advantageous and could serve as prognostic biomarker to detect patients with low-risk PH. Restricting the survival analysis to only patients with PAH showed approximately the same prognostic value. This strengthens the idea that GDF-15 could be a prognostic biomarker in patients with PH independent of the specific underlying aetiology. Furthermore, GDF-15 showed an independent association with the primary endpoint when adjusted for age and NT-proBNP, and provided an incremental predictive value.

Limitations

Blood sampling was performed during the diagnostic right heart catheterisation in treatment-naive patients with PH. In patients with PAH and CTEPH, treatment was initiated directly after confirmation of diagnosis. We were not able to adjust for treatment effect in the association between GDF-15 and the endpoints. This study therefore reflects the association between GDF-15 and adverse outcomes for patients with PH treated in accordance to the ESC guidelines.

Patients with PH due to left heart disease were not included in this study, and this should be kept in mind when extrapolating the results to other studies. Also our study consisted of a heterogeneous group of PH aetiologies and subgroup analysis could only be performed for PAH. It should be kept in mind that clinical usefulness of GDF-15 might differ among PH aetiologies. Furthermore, this study focused only one a single prognosticator and due to the limited statistical power, the additive value of GDF-15 to existing risk scores could not be assessed. Conclusion with regard to the use of this biomarker in the context of current risk models, specifically concerning patients with PAH, are therefore limited.

Clinical implications

GDF-15 showed a strong association with mortality in our study and specifically identified low-risk patients at the time of diagnosis of PH. Therefore, measuring GDF-15 may be considered in patients with newly diagnosed PH to identify, and subsequently reassure, low-risk patients. In these patients the follow-up frequency could probably be lowered compared with high-risk patients. Since GDF-15 is not tissue-specific and concentrations can rise or decline in response to any disease process, GDF-15 seems not the most optimal biomarker to specifically pursue biomarker guided therapy. Nevertheless, more data are needed to validate these findings before finding its way to daily clinical practice and larger studies with more power are needed to investigate GDF-15 in the light of current existing risk prediction models for PAH.

Conclusions

In adults with PH, a normal GDF-15 level at the time of diagnosis identifies patients with a very low 2-year risk of mortality or transplantation. GDF-15 could therefore be a promising biomarker to identify low-risk patients with normal GDF-15 levels. Due to its lack of tissue-specificity, the role of GDF-15 in future biomarker guided therapy is uncertain and may be limited; however, GDF-15 could potentially serve as predictor for mortality in patients with PH of various aetiologies. Future studies, preferably including a larger cohort of patients with PH, are recommended to further investigate this promising biomarker.

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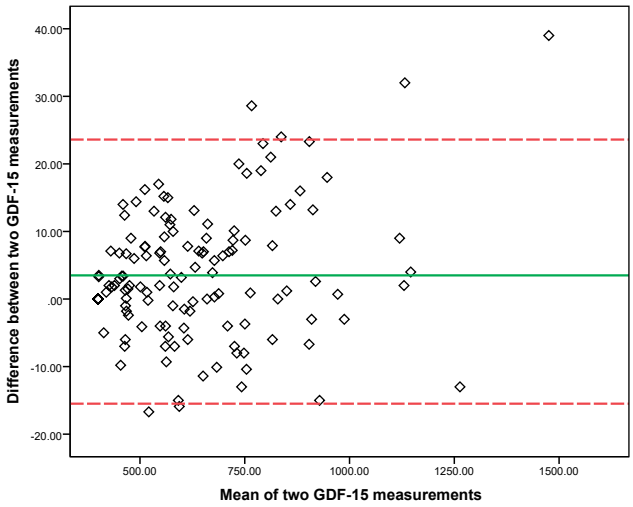
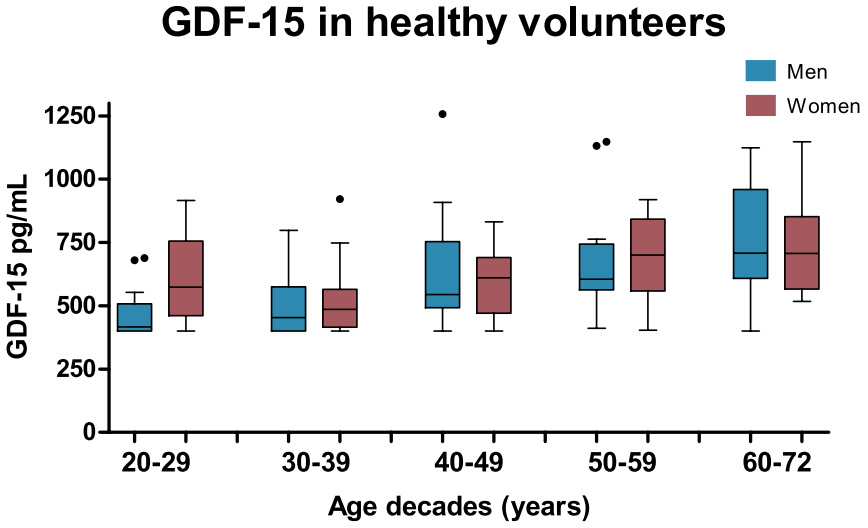
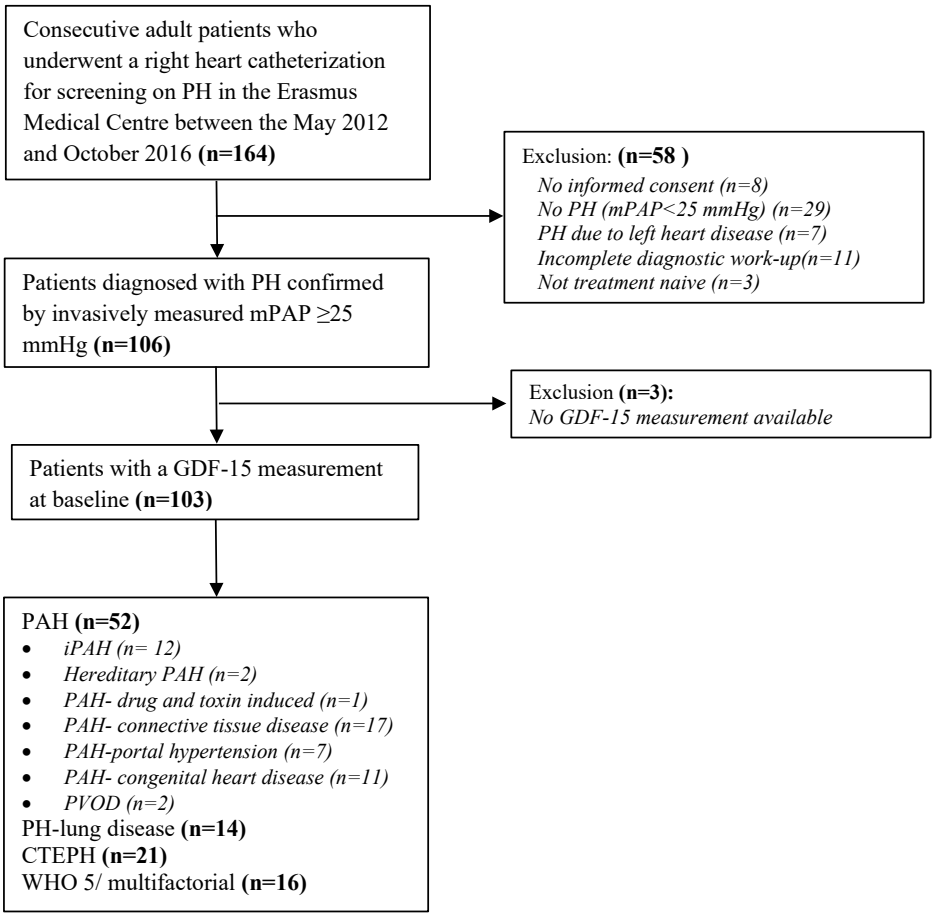


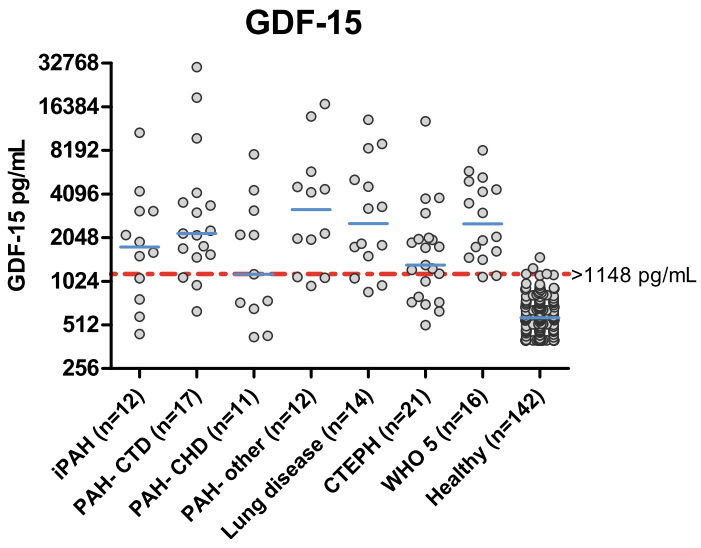
Supplemental Figure 1. Reproducibility of GDF-15 assay in healthy volunteers shown by a Bland-Altman plot.
The green line corresponds with the mean difference between two GDF-15 measurements. The red dotted-lines correspond to the corresponding limits of agreement (+/- 1.96 SD). The coefficient of variation was 0.68%.



Supplemental Figure 2. Levels of GDF-15 in healthy volunteers stratified according to sex and age decades. Each age decade is approximately equally represented by the number of healthy volunteers.
Boxplots showing the median and interquartile ranges. Spread of the data is shown by the whiskers (Turkey boxplot). Outliers are plotted with a dot. The lowest limit of detection of the GDF-15 assay was 400 pg/mL.



Supplemental Figure 3. Flowchart of the patient selection process of the study cohort.
PH= pulmonary hypertension, mPAP= mean pulmonary arterial pressure, GDF-15= growth differentiation factor-15, PAH= pulmonary arterial hypertension, iPAH= idiopathic pulmonary arterial hypertension, PVOD= pulmonary veno-occlusive disease, CTEPH= chronic thromboembolic pulmonary hypertension, WHO 5= World Health Organization group 5.



Supplemental Figure 4. Measurements of GDF-15 in pg/mL according to subgroups in pulmonary hypertension patients and GDF-15 measurements performed in healthy volunteers.
Y-axis is on the 2-log scale. The red line indicates the 97th percentile level of GDF-15 based on measurements in healthy volunteers. The black line indicates the median GDF-15 level in each subgroup. PAH-other consisted of; pulmonary veno-occlusive disease(n=2), PAH- associated with portal hypertension (n=7), hereditary PAH (n=2), drug and toxin induced PAH (n=1). iPAH= idiopathic pulmonary arterial hypertension, PAH-CTD = pulmonary arterial hypertension due to connective tissue disease, PAH-CHD= pulmonary arterial hypertension due to congenital heart disease, CTEPH= chronic thromboembolic pulmonary hypertension.

Supplemental Table 1. Detailed list of death causes in 30 adults with pulmonary hypertension.

Cause of death	Number of cases (%)
End-stage heart failure	9 (30.0)
Sudden death presumed cardiac	4 (13.3)
Euthanasia*	3 (10.0)
Multi-organ failure	3 (10.0)
Kidney/liver failure	2 (6.7)
Malignancy	1 (3.3)
Myocardial infarction	1 (3.3)
Hepatic encephalopathy	1 (3.3)
Progression of systemic sclerosis	1 (3.3)
Sudden death, presumed cerebral	1 (3.3)
End stage lung fibrosis and PH due to polymyositis	1 (3.3)
Occlusion of femoral artery	1 (3.3)
Post lung transplantation	1 (3.3)
Unknown	1 (3.3)

**In patients with end-stage cardiovascular and pulmonary disease.*

Supplemental Table 2. Association between GDF-15 and the primary and secondary endpoint restricted to only adults with pulmonary arterial hypertension.

	Pulmonary arterial hypertension(PAH)			
	Primary endpoint (n=16)		Secondary endpoint (n=20)	
	HR*(95% CI)	p-value	HR*(95% CI)	p-value
GDF-15 (univariable)	1.57 (1.14-2.15)	0.005	1.50 (1.13-1.97)	0.004
Adjusted for:				
Age	1.44 (1.02-2.02)	0.038	1.35 (1.00-1.82)	0.052
Sex	1.57 (1.15-2.15)	0.005	1.49 (1.13-1.97)	0.004
NYHA class 3/4	1.50 (1.10-2.06)	0.012	1.41 (1.08-1.86)	0.013
6-MWD	1.38 (1.01-1.90)	0.046	1.21 (0.91-1.61)	0.183
Right atrial area	1.48 (1.09-2.02)	0.012	1.49 (1.10-1.90)	0.008
Cardiac index	1.61 (1.16-2.25)	0.005	1.56 (1.34-1.81)	0.003
mRAP	1.71 (1.24-2.34)	<0.001	1.55 (1.17-2.05)	0.002
eGFR	1.37 (0.92-2.04)	0.117	1.27 (0.90-1.80)	0.178
NT-proBNP	1.55 (1.07-2.25)	0.020	1.43 (1.03-1.99)	0.031

*HR per two-fold higher value of GDF-15.

CHAPTER 11

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

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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.

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: $\sum \mathrm{N} * \text{pulmonary pressure} / \sum \mathrm{N}$. The pooled standard deviation (SD) was calculated by

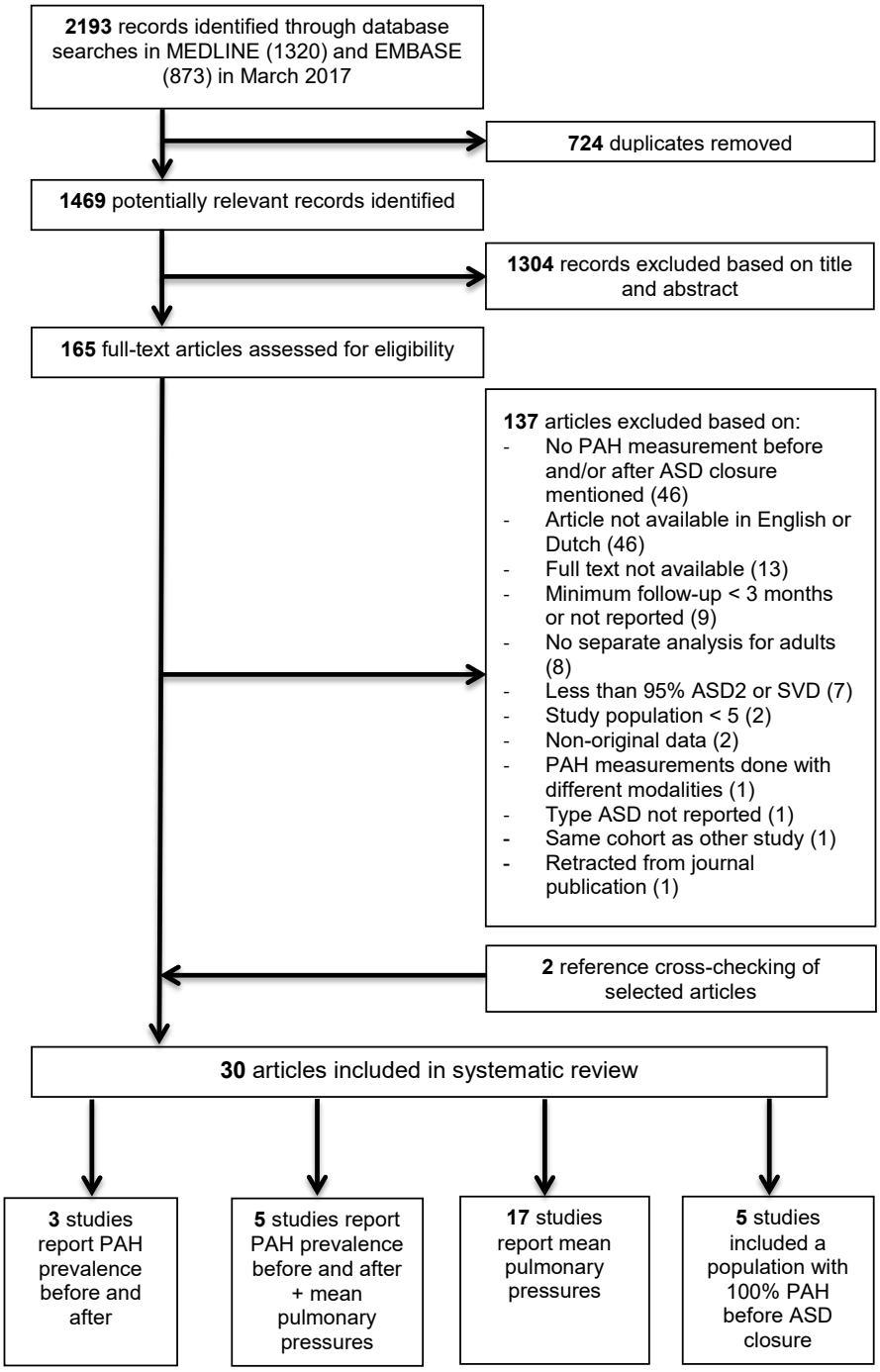

Figure 1. Flow diagram of literature search and selection of studies.

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.

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

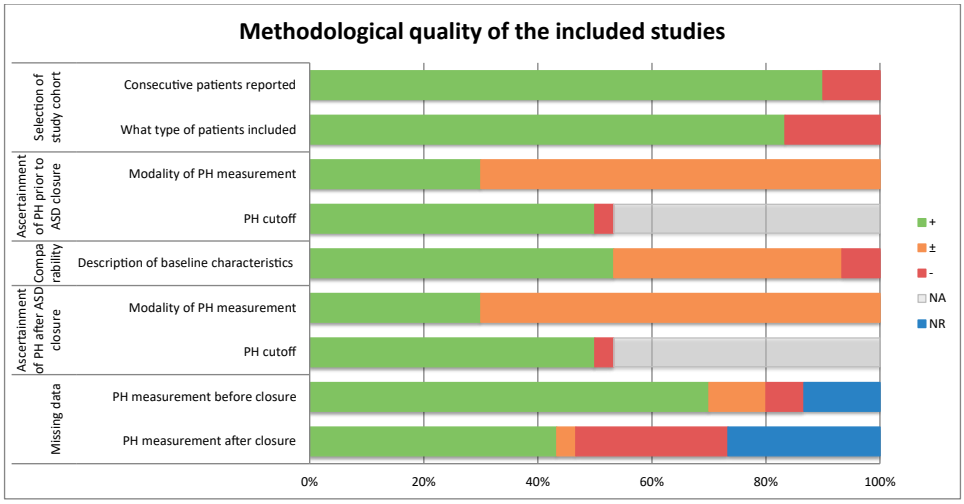

Figure 2. Methodological quality of the included studies.
Selection of study cohort: Consecutive patients reported (+ yes,–no), Type of patients included (+ all patients with ASD,–only PAH patients), *Ascertainment of PAH prior to ASD closure:* Modality of PAH measurement (+ RHC, ± TTE/TEE,–Not defined), 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).

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%).

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.

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

Table 1. Study characteristics.

		Study population						Outcome		
First author, year (ref no.)	**Inclusion period**	**Patients included in review (n)**	**Age at closure (years)**	**Sex (% female)**	**ASD diam. (mm)**	**NYHA class III-IV (%)**	**Procedure ASD closure**	**Follow-up (months)**	**Modality**	**Cut-off value PH used in article (mmHg)**
Hanlon, 1969 (11)	1956 -1969	56	32±NR	66	NR	16	Surgical	NR [5-138]	RHC	SPAP ≥ 30
Richmond, 1969 (12)	1957–NR	26	NR [45-59]	58	NR	58	Surgical	98 (mean) [4-108]	RHC	SPAP > 30
Saksena, 1970 (13)	1958–1966	24	51 [38-63]	67	NR	100	Surgical	72 (mean) [24-120]	RHC	MPAP > 20
Dave, 1973 (14)	1959–NR	32	42±NR	78	NR	35	Surgical	NR [6-144]	RHC	MPAP ≥ 20
Forfang, 1977 (15)	1959 -1972	93	49±NR	74	NR	23	Surgical	NR [22-174]	RHC	MPAP > 20
Thilén, 2000 (16)	1958 -1968	11	39±9	91	NR	0	Surgical	NR [24-108]	RHC	NA
Veldtman, 2001 (17)	1997- 1999	40	38 [20-71]	75	13±4	5	Percutaneous	NR [1-12]	TTE	SPAP > 35
De Lezo, 2002 (18)	NR	29	56±14	83	26±7*	48	Percutaneous	21±14	RHC	SPAP > 40
Celik, 2004 (19)	NR	41	NR [25-NR]	NR	NR	81	Surgical	34±30	TTE	NA
Schoen, 2006 (20)	NR	20	43±13	60	24±6*	15	Percutaneous	13 (mean) [11-15]	TTE	SPAP > 30
Suchón, 2006 (21)	2000-2002	52	39±15	64	NR	NR	Surgical	14±1	TTE	SPAP > 30
Balint, 2008 (22)	1999–2004	54	59±15	76	18±7	NR	Percutaneous	31±15	TTE	SPAP 40-49 (mild), 50-59 (moderate), ≥60 (severe)
Mahadevan, 2009 (23)	1990–2005	36	46±15	64	20±6*	NR	Percutaneous	30±17	TTE	NA
Yalonetsky, 2009 group 1 (24)	1998–NR	23	52±6	74	19±5	NR	Percutaneous	NR [6-NR]	TTE	NA
Yalonetsky, 2009 group 2 (24)	1998–NR	23	67±5	70	18±5	NR	Percutaneous	NR [6-NR]	TTE	NA
Yong, 2009 (25)	1999–2006	215	54±16	73	19±6	19	Percutaneous	15 [IQR 8-43]	TTE	SPAP ≥ 40

Continue

Continued

		Study population						Outcome		
First author, year (ref no.)	**Inclusion period**	**Patients included in review (n)**	**Age at closure (years)**	**Sex (% female)**	**ASD diam. (mm)**	**NYHA class III-IV (%)**	**Procedure ASD closure**	**Follow-up (months)**	**Modality**	**Cut-off value PH used in article (mmHg)**
Cohen, 2010 (26)	2001–NR	27	69±6	63	NR	NR	Percutaneous	40±26	TTE	NA
Mainzer, 2010 (27)	2002–2003	16	45±17	63	23±6	NR	Percutaneous	48±5	TTE	NA
Ekim, 2011 (28)	2001–2010	20	46±NR	70	26±4	NR	Surgical	NR [4-92]	TTE	NA
Humenberger, 2011 (29)	NR	236	49±18	70	22 [IQR 19-26]	15	Percutaneous	28±19	TTE	SPAP ≥ 40
Huang, 2012 (6)	2007–2010	7	NR	NR	NR	NR	Percutaneous	23±10	RHC	SPAP ≥ 60 (severe)
Kefer, 2012 (30)	1999–2009	112	46±17	71	PH: 22±5 no PH: 18±6	NR	Percutaneous	60±34	TTE	SPAP > 40
Nakagawa, 2012 (31)	2005-2010	30	76±4	67	20±6	27	Percutaneous	19±11	TTE	MPAP ≥ 25
D'Alto, 2013 (5)	NR	12	41±12	NR	NR	NR	Percutaneous and surgical	53±25	RHC	MPAP ≥ 25†
Mangiafico, 2013 (32)	2008- 2011	20	58±11	NR	NR	0	Percutaneous	28±16	TTE	NA
Jampates, 2014 < 60 years (33)	2007- 2012	274	39±12	83	21±7	5	Percutaneous	NR [6-NR]	TTE	NA
Jampates, 2014 ≥ 60 years (33)	2007- 2012	59	66±5	78	21±8	8	Percutaneous	NR [6-NR]	TTE	NA
Baykan, 2015 (34)	2013–2014	42	36±14	60	22±6	14	Percutaneous	NR [3-NR]	TTE	NA
Kijima, 2015 (35)	2006–2014	14	66±13	71	23±8	21	Percutaneous	18±16	TTE	NA
Thilén, 2016 (36)	1997–2014	148	72±5	72	16±6	30	Percutaneous	53±31	TTE	NA
Brojeni, 2017 (37)	2015	47	32 [29-43]	72	17±NR	NR	Percutaneous	NR [6-NR]	TEE (baseline) TTE (FU)	NA
Dalvi, 2017 (38)	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.
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.

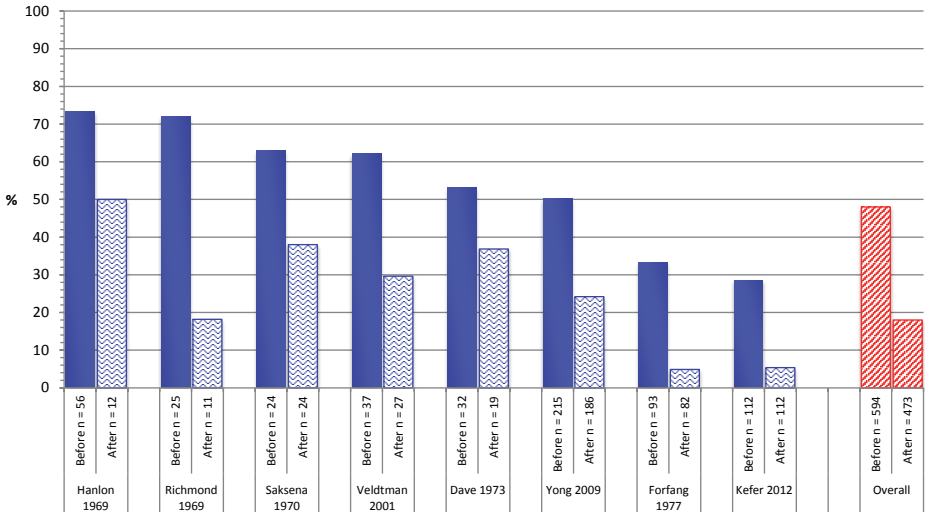

Figure 3. PAH prevalence before and after ASD closure.
PAH prevalence before and after ASD closure presented per study. The number of patients shown in this bar chart is the number that had a measurement of pulmonary pressures (right heart catheterization or echocardiography).

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.

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.

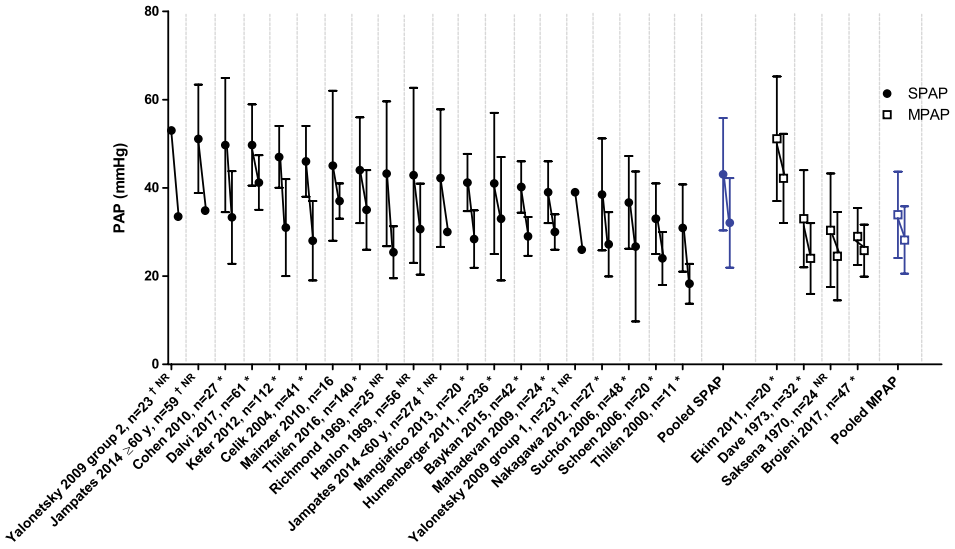

Figure 4. Pulmonary pressures before and after ASD closure.
SPAP and MPAP before and after ASD closure shown as mean±SD. The number of patients displayed is the number that underwent measurement of PAP before closure. *Significant reduction of $p<0.05$; NR, not reported. †SD before and/or after was not available and therefore also not included in the pooled pulmonary pressures.

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.

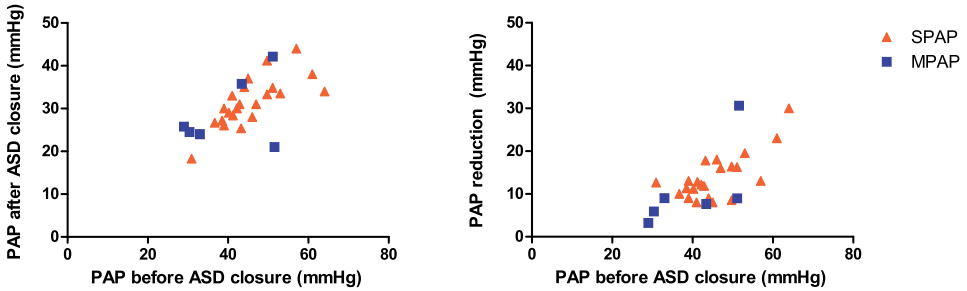

Figure 5. Association of PAP before closure with PAP after closure and reduction in PAP.
Each symbol illustrates one study cohort.

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).

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

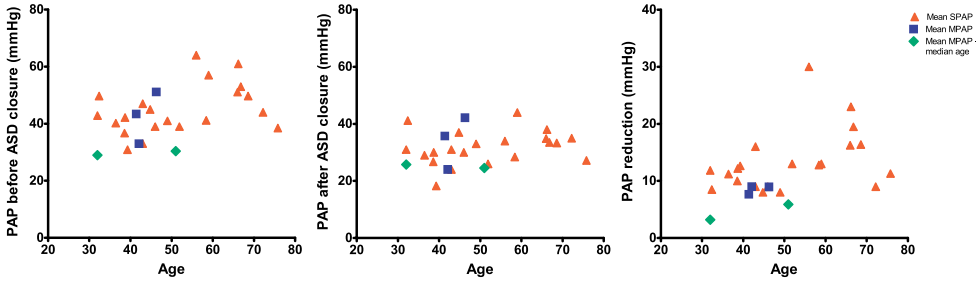

Figure 6. Association of age with PAP before closure, PAP after closure, and reduction in PAP.
Each symbol illustrates one study cohort. The green symbol illustrates studies that reported the median age instead of the mean age.

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 File 1. Search syntax used to identify publications of interest. Search date March 20, 2017.

MEDLINE (via PubMed interface)

"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.

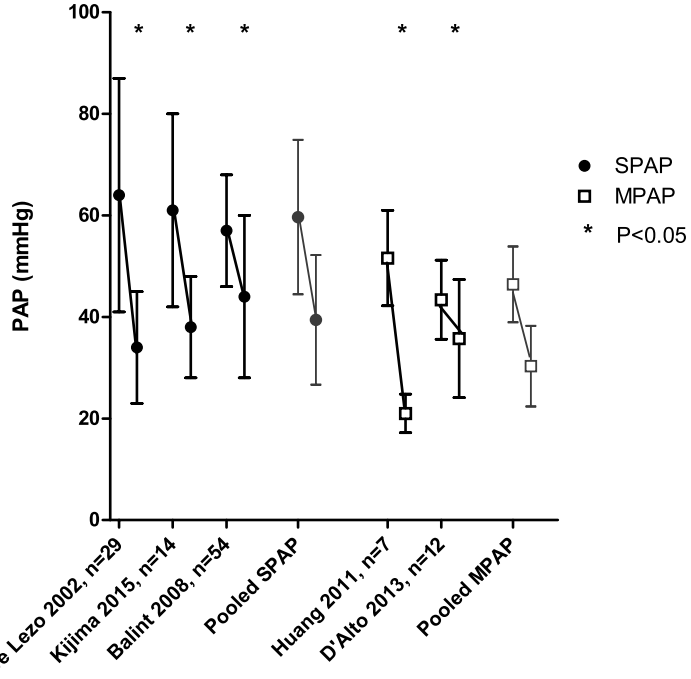
	Selection of the study cohort		Ascertainment of PAH prior to ASD closure		Comparability of the cohort	Ascertainment of PAH after ASD closure		Missing data	
Author, year	**Consecutive patients reported**	**Type of patients included**	**Modality of PH measurement**	**PH cutoff**	**Description of baseline 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	+	+

Continue

Continued

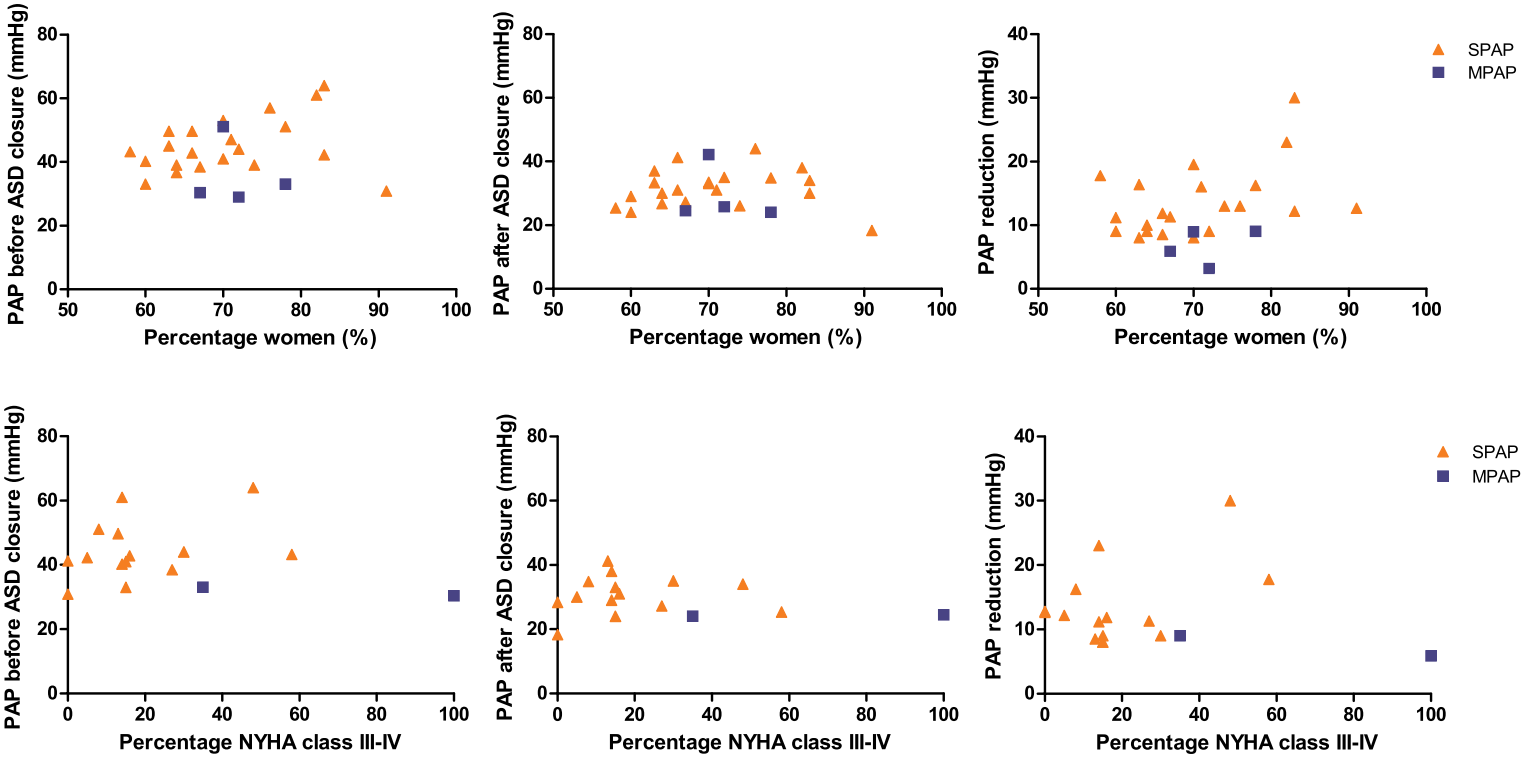
	Selection of the study cohort		Ascertainment of PAH prior to ASD closure		Comparability of the cohort	Ascertainment of PAH after ASD closure		Missing data	
Author, year	**Consecutive patients reported**	**Type of patients included**	**Modality of PH measurement**	**PH cutoff**	**Description of baseline 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.

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 $p<0.05$.



Supplemental Figure 2. Association of sex and NYHA class with PAP before closure, PAP after closure, and PAP reduction.
Each symbol illustrates one study cohort.

RISK STRATIFICATION AND RISK PREDICTION

CHAPTER 12

Tuning and external validation of an adult congenital heart disease risk prediction model

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** Equal contributions*

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Abstract

Aims: Adequate risk prediction can optimize the clinical management in adult congenital heart disease (ACHD). We aimed to update and subsequently validate a previously developed ACHD risk prediction model.

Methods and results: A prediction model was developed in a prospective cohort study including 602 moderately or severely complex ACHD patients, enrolled as outpatients at a tertiary centre in the Netherlands (2011-2013). Multivariable Cox regression was used to develop a model for predicting the 1-year risks of death, heart failure (HF), or arrhythmia (primary endpoint). The Boston ACHD Biobank study, a prospectively enrolled cohort (n=749) of outpatients who visited a referral centre in Boston (2012-2017), was used for external validation. The primary endpoint occurred in 153 (26%) and 191 (28%) patients in the derivation and validation cohorts over median follow-up of 5.6 and 2.3 years, respectively. The final model included 5 out of 14 pre-specified predictors with the following HR's; New York Heart Association class ≥II: 1.92 (95% CI 1.28-2.90), cardiac medication 2.52 (95% CI 1.72-3.69), ≥1 reintervention after initial repair: 1.56 (95% CI 1.09-2.22), body mass index: 1.04 (95% CI 1.01-1.07), $\log_2$ NT-proBNP (pmol/L): 1.48 (95% CI 1.32-1.65). At external validation, the model showed good discrimination (C-statistic 0.79, 95% CI 0.74-0.83) and excellent calibration (calibration-in-the-large=-0.002; calibration slope=0.99).

Conclusion: These data support the validity and applicability of a parsimonious ACHD risk model based on five readily available clinical variables to accurately predict the 1-year risk of death, HF or arrhythmia. This risk tool may help guide appropriate care for moderately or severely complex ACHD.

Introduction

Improvement of surgical techniques and medical treatments over the last 70 years has improved the survival of children with a congenital heart defect (CHD)[1]. Consequently, the population of adults with congenital heart disease (ACHD) is growing, with associated high healthcare utilization[2]. In this context, there is increased focus on optimizing the clinical management of these patients[3].

In addition to improvement in medical techniques over the past decades, advancements in technology, and subsequently the development of software programmes, have enabled big data analysis and advanced statistical modelling. One way this has been applied in the current medical era is the development of risk prediction models, which estimate an individual's risk of a certain outcome given a set of risk factors[4]. This information can help clinicians to inform patients about their prognosis and aids clinical decision making.

In 2018, our group developed an ACHD risk prediction model, incorporating clinical characteristics including echocardiographic and blood biomarkers, that estimated the 4-year risk probability of death, heart failure (HF), or arrhythmia[5]. However, validation of a prediction model is needed to establish its generalizability, and its usefulness in clinical practice. Initially, no prospective data was available to validate the prediction model and retrospectively collected clinical data from a Czech cohort was used for external validation. Moreover, a model providing estimations on a short time horizon may be considered more clinically relevant, as decision-making (e.g. timing clinical follow-up) tends to occur on a time scale of 1-2 years rather than 4 years. In 2012, the Boston ACHD BioBank was established; this large prospective cohort study enrolls ACHD of any type seen at a large referral centre[6].

To further optimize the clinical usefulness of the previously derived prediction model, we used data from the initial cohort to tune our original ACHD risk prediction model towards 1-year risk estimations of death, HF or arrhythmia, and subsequently externally validated the newly developed model in prospective data from the Boston ACHD BioBank.

Methods

Derivation cohort

A risk prediction model was developed in 2018,[5] using data from a prospective observational cohort study consisting of 602 adults with moderately or severely complex CHD from a tertiary centre in the Netherlands. For this cohort study, consecutive adults were enrolled during routine visits to the outpatient clinic of the Erasmus Medical Centre between 2011 and 2013. Patients with mild CHD (isolated atrial or ventricular septal defect), creatinine >200 µmol, age <18 years-old, or who were currently pregnant were excluded. At the baseline visit, patients underwent a physical examination, electrocardiography, transthoracic echocardiography, and venous blood

sampling. Moderately and severely complex ACHD was defined in accordance with the 32nd Bethesda conference classification[7].

Patients were prospectively followed for the occurrence of adverse cardiovascular events and had yearly scheduled visits to the outpatient clinic for the first four subsequent years after study enrolment. Events were adjudicated until the 1st of January 2018 by two researchers, who were blinded to patient characteristics. Written informed consent was obtained from all patients and the study was performed according to the principles outlined in the Declaration of Helsinki. The study was approved by the medical ethics committee of the Erasmus MC. Details have been published previously[8]. This study was conducted in accordance with the TRIPOD statement.

External validation cohort

External validation of the model used data from the Boston ACHD BioBank study, a prospective observational cohort study enrolling patients ≥18 years-old with CHD during an outpatient visit to a tertiary referral centre (The Boston Adult Congenital Heart Service at Boston Children's and Brigham and Women's Hospitals). Details of the implementation and design of this study have been published[6]. The current cohort includes patients enrolled between 2012 and 2017, with a N-terminal pro B-type natriuretic peptide (NT-proBNP) measurement from the baseline visit (n=921). Only patients with moderately or severely complex ACHD were included in the current study, resulting in a total of 749 patients. The study was approved by Boston Children's Hospital's Institutional Review Board with reliance on this board by Partners HealthCare/Brigham and Women's Hospital, and written informed consent was obtained from all participants or their legally authorized representative.

Model development and modifications

The primary endpoint was a composite of all-cause mortality, HF (associated with hospitalisation or initiation/intensification of cardiac medication), or clinically relevant arrhythmia (symptomatic and recorded, or associated with treatment). The original developed model was based on the 4-year risk of all-cause mortality, HF, or arrhythmia using multivariable logistic regression. We modified the logistic regression model to a Cox proportional hazards regression model to enable 1-year risk predictions.

Model development was performed using the 14 initial candidate predictors that had been selected by an expert panel of senior cardiologists at the Erasmus Medical Centre[5] (Table 1). Because missing data were limited (1.5%), missing data were imputed using single multivariate imputation by chained equations in R (package mice). Supplemental Table 1 lists the % of missing data of each variable. Univariable and multivariable Cox regression was used to obtain crude and adjusted predictor effects. Variables were then selected for multivariable analysis based on Akaike's Information Criteria ($p<0.157$) and stepwise backward selection method was used to obtain the final model ($p<0.157$). The proportional hazard assumption was assessed through Schoenfeld residual plots and proportional hazard test, and there was

no indication that the final model violated this assumption. Non-linear and interaction terms were not considered because of limited statistical power. Bootstrap resampling was used to internally validate the model and the slope of the linear predictor obtained from the bootstrap resampling was used for uniform shrinkage of coefficients to adjust for overfitting and to improve external calibration. The cumulative baseline hazard at 1 year was extracted from the Cox regression model to enable prediction of 1-year risks of death, HF, or arrhythmia.

As a secondary analysis, to assess the predictive ability of the score over a longer time horizon, we also used the cumulative baseline hazard at 2 years to predict 2-year risks of the combined endpoint.

External Validation

Predictions were calculated by applying the development model to the validation data. Calibration and discrimination of the final model for the 1-year risk of the composite endpoint was assessed using a calibration plot and the C-statistic, respectively. Calibration-in-the-large was assessed by the difference in the observed mean risk minus the predicted mean risk. The calibration slope was estimated to assess whether the model was overfitted (slope <1) or underfitted (slope >1). The same methodology was used to externally validate the model for predicting the 2-year risks of the composite endpoint. When the external performance of the model is adequate, the model will be refitted on a merged dataset consisting of both the derivation and validation data. Statistical analyses were performed using R version 3.6.1 (packages rms, survival, mice).

Results

Characteristics of derivation and validation cohorts

A schematic overview of the study is shown in Figure 1. Baseline characteristics of both the derivation cohort (n=602) and the validation cohort (n=749) are listed in Table 1. Patients in the validation cohort tended to have characteristics consistent with higher risk. Severely complex CHD was more common in the validation cohort (39%) than in the derivation cohort (29%), and the patients in the validation cohort were also more symptomatic (New York Heart Association (NYHA) functional class > I), were more commonly prescribed cardiac medication, and had a higher prevalence of reintervention after initial repair. Despite these differences, NT-proBNP levels did not differ substantially between cohorts. Age distributions of both studies were different, with Boston having a slightly older study cohort (supplemental Figure 1).

Follow-up data on endpoints were available for 597 out of 602 patients (99.2%) in the derivation cohort and for 692 out of the 749 patients (92.4%) in the validation cohort. No significant differences were observed with regard to the age, sex, body mass index (BMI), heart rate, complexity of CHD, or NT-proBNP between those patients with and without follow-up in the validation cohort. However, those without follow-up were less often prescribed cardiac

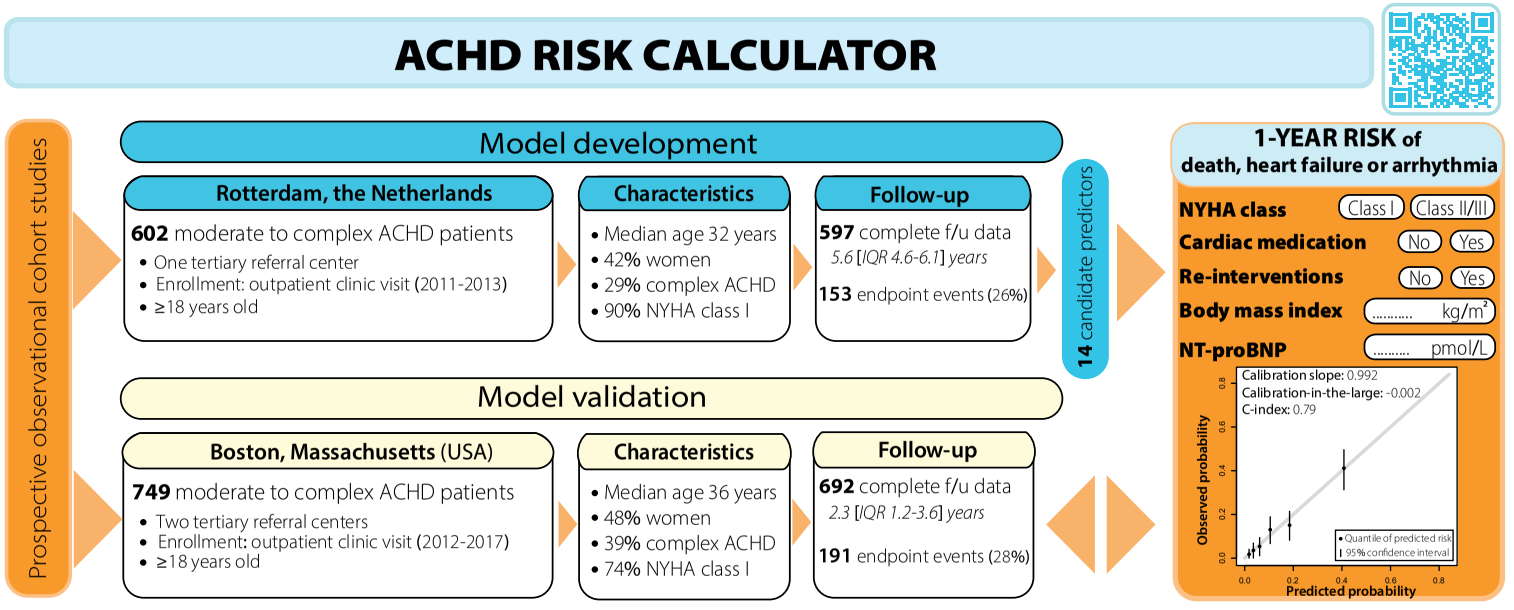

Figure 1. Schematic overview of the development and validation process of the ACHD risk prediction model.
The ACHD risk prediction model provides estimates of the 1-year risk of death, heart failure or arrhythmia for moderately and severely complex ACHD patients based on 5 clinical variables. Online web application of the prediction model is accessible at https://achdwebcalculator-updated.shinyapps.io/achdshinyweb/, or scan the QR code.
ACHD= adult congenital heart disease

Table 1. Baseline clinical characteristics of included ACHD patients in the derivation cohort (Rotterdam) and the validation cohort (Boston)

	Derivation cohort	**Validation cohort**	
Clinical characteristics	Rotterdam (n=602)	Boston (n=749)	**p-value**
Age, years	32 [25-41]	36 [27-48]	<0.001
Woman	254 (42)	361 (48)	<0.001
Congenital diagnosis			
Moderate	429 (71)	458 (61)	<0.001*
Tetralogy of Fallot or DORV	179 (30)	175 (23)	
Aortic coarctation	112 (19)	79 (11)	
LV obstructive disease	138 (23)	78 (10)	
AVSD	0 (0)	37 (5)	
Ebstein anomaly	0 (0)	26 (3)	
Other	0 (0)	63 (8)	
Complex	173 (29)	291 (39)	<0.001*
TGA- arterial switch	24 (4)	29 (4)	
TGA- Mustard or Senning	65 (11)	53 (7)	
TGA- congenitally corrected	21 (3)	26 (3)	
Fontan	36 (6)	133 (18)	
Pulmonary arterial hypertension/Eisenmenger	9 (1)	16 (2)	
Rastelli/REV procedure	11 (2)	10 (1)	
Univentricular heart, palliated or unoperated	7 (1)	9 (1)	
Pulmonary atresia with intact ventricular septum	0 (0)	14 (2)	
Other	0 (0)	1 (0)	
NYHA class			<0.001
Class I	541 (90)	551 (74)	
Class II	56 (9)	166 (22)	
Class III/IV	5 (1)	28 (4)	
Cardiac medication use	212 (35)	433 (58)	<0.001
≥1 reintervention after initial repair	317 (53)	447 (63)	<0.001
Heart rate, beats/minute	74 ± 13	71 ± 13	0.013
Systolic blood pressure, mmHg	126 ± 16	120 ± 14	<0.001
Oxygen saturation <90%	17 (3)	38 (6)	0.037
Body mass index, kg/m^2	24.7 ± 4.4	26.9 ± 5.6	<0.001
Current tobacco use	56 (10)	36 (5)	<0.001
Sinus rhythm on baseline ECG	521 (87)	-	-
Systemic ventricular function			<0.001
Normal	303 (50)	497 (71)	
Mildly impaired	212 (35)	142 (20)	
Moderately/severely impaired	87 (15)†	63 (9)	
NT-proBNP, pmol/L	15 [7-33]	16 [7-40]	0.193

*p-value for comparison moderate versus complex ACHD. †n=18 patients severely impaired systemic ventricular function. Data are presented as mean±SD, median [25th -75th percentile] for continuous variables (normally and non-normally distributed, respectively), and n (%) for categorical variables.
AVSD= atrioventricular septal defect, DORV= double outlet right ventricle, LV= left ventricular, NT-proBNP= N-terminal pro B-type natriuretic peptide, NYHA= New York Heart Association, REV= Réparation à l'Etage Ventriculaire, TGA= transposition of the great arteries.

medication (42 vs 59%, p=0.017) (Supplemental Table 2). Median follow-up in the derivation cohort was 5.6 [IQR 4.6-6.1] years, by which time the primary endpoint had occurred in 153 patients (25.6%). In the validation cohort the median follow-up was 2.3 [IQR 1.2-3.6] years and 191 patients (27.6%) reached the primary endpoint. After 1 year of follow-up, the endpoint had occurred in 52 patients (8.7%) in the derivation cohort and in 91 patients (13.1%) in the validation cohort. Table 2 outlines the specific components of the primary endpoint in each cohort.

Model development

Ten of the 14 pre-specified candidate predictors were associated with the probability of developing the primary endpoint in univariable Cox analysis; these variables were considered for inclusion in multivariable analysis (Table 3). Multivariable analysis identified 5 independent predictors of the primary endpoint; NYHA class [NYHA I (0) versus NYHA II/III (1)], cardiac medication use [no (0) versus yes (1) including: angiotensin-converting-enzyme inhibitor, angiotensin receptor blocker, beta-blocker, diuretics including loop/thiazide/potassium sparing, calcium channel blocker or anti-arrhythmic drug]; ≥ 1 cardiovascular reintervention [no (0) versus yes (1)] BMI (kg/m^2); and $\log_2$ NT-proBNP (pmol/L). Age and congenital diagnosis

Table 2. Proportion of patients experiencing each of the individual components of the composite endpoint in the derivation and validation cohorts.

Variable	Derivation cohort (n=597)	Validation cohort (n=692)
Follow-up, years	5.6 [4.6-6.1]	2.3[1.2-3.6]
Primary composite endpoint		
During entire follow-up duration	153 (25.6)	191 (27.6)
Reached after 1 year of follow-up	52 (8.7)	91 (13.1)
Death	25 (4)	44 (6)
End-stage heart failure	10 (2)	9 (1)
Sudden death / cardiac arrest	10 (2)	6 (1)
Other or unknown	5 (1)	29 (4)
Heart failure	59 (10)	120 (17)
Hospital admission	25 (4)	37 (5)
Initiation or intensification in diuretics	34 (6)	83 (12)
Arrhythmia	128 (21)	109 (16)
Ventricular tachycardia/fibrillation	31 (5)	10 (1)
Supraventricular tachycardia	84 (14)	92 (13)
Other	13 (2)	7 (1)

N (%) are shown for individual event components. Continuous variables are presented as median [25th–75th percentile]. Separate event components of the primary endpoint are shown (ie, patients were not censored at the time of another endpoint event than the endpoint of interest). For heart failure and arrhythmia, only the earliest occurrence is listed (e.g. a patient who had intensification of diuretics and was later hospitalized for heart failure).

(moderately versus severely complex) were included as predictors in the originally developed logistic regression prediction model but were not independent predictors in the re-developed Cox model. After backward selection the model included: NYHA class, cardiac medication use, ≥1 reintervention, BMI and $\log_2$ NT-proBNP (Table 3). The optimism-adjusted C-statistic for predictions at 1 year was 0.81. After application of the bootstrap slope for shrinkage (0.914), the hazard ratios of the final predictors were: NYHA class ≥II 1.82, cardiac medication 2.33, ≥1 reintervention 1.50, BMI 1.03 /kg/m^2, and $\log_2$ NT-proBNP 1.43/pmol/L. The baseline hazard at 1-year was estimated at 1.06.

External validation

The C-statistic of the derived model in the external validation dataset was 0.79 (95% CI 0.74-0.83). Calibration-in-the-large, calculated as the mean predicted probability (=0.135) minus the mean observed probability (=0.133), was excellent (-0.002, ideally equal to zero); this indicates

Table 3. Cox regression analysis for the 14 pre-specified candidate predictors in the derivation cohort.

Variable	Univariable HR (95% CI)	*P*-value	Multivariable HR (95% CI)	*P*-value	Final model HR (95% CI)	p-value
Age, per year	1.05 (1.04-1.07)	<0.001	1.01 (0.99-1.02)	0.360		
Sex, male vs. female	0.80 (0.58-1.09)	0.159	-	-		
Congenital diagnosis complexity, severely vs moderately	2.26 (1.64-3.11)	<0.001	1.20 (0.78-1.84)	0.407		
NYHA class, II-III vs I	5.62 (3.95-8.00)	<0.001	1.90 (1.22-2.95)	0.004	1.92 (1.28-2.90)	0.002
Cardiac medication use, yes vs no	5.14 (3.66-7.21)	<0.001	2.41 (1.60-3.62)	<0.001	2.52 (1.72-3.69)	<0.001
≥1 reintervention after corrective repair, yes vs no	2.29 (1.62-3.23)	<0.001	1.64 (1.13-2.40)	0.010	1.56 (1.09-2.22)	0.015
BMI, per kg/m^2	1.05 (1.02-1.09)	0.003	1.03 (1.00-1.07)	0.044	1.04 (1.01-1.07)	0.020
Heart rate, per beat/min	1.00 (0.99-1.01)	0.953	-	-		
Current tobacco use, yes vs no	0.82 (0.48-1.39)	0.460	-	-		
Oxygen saturation <90% vs ≥90%	2.59 (1.32-5.07)	0.006	0.76 (0.37-1.56)	0.449		
Loss of sinus vs sinus rhythm at baseline ECG	3.24 (2.28-4.61)	<0.001	0.90 (0.59-1.38)	0.633		
Systemic ventricular function, 0-3*	1.85 (1.57-2.19)	<0.001	0.99 (0.81-1.22)	0.952		
Severe valvular dysfunction, yes vs no	1.19 (0.78-1.83)	0.427	-	-		
NT-proBNP, per $\log_2$ pmol/L	1.80 (1.64-1.98)	<0.001	1.44 (1.27-1.64)	<0.001	1.48 (1.32-1.65)	<0.001

*Visually graded based on echocardiography as normal(0), mildly (1), moderately (2), and severely (3) impaired systemic ventricular function (analyzed as continuous variable 0-3). BMI= Body Mass Index, ECG= electrocardiogram**,** NT-proBNP= N-terminal pro B-type natriuretic peptide, NYHA= New York Heart Association.

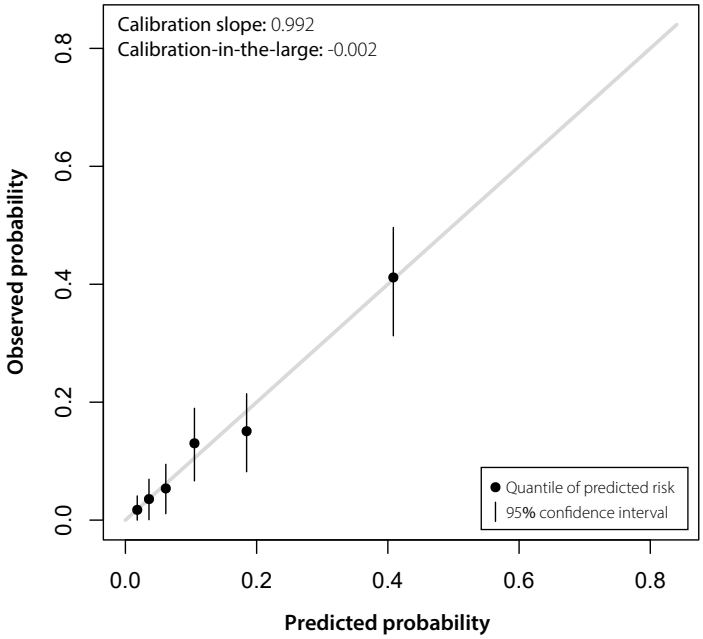

Figure 2. Calibration plot of the external validation of the prediction model by the plotted probabilities of the 1-year risk of death, heart failure or arrhythmia.
Diagonal line shows ideal calibration where predicted probability is equal to the observed probability.

that the predicted probabilities neither systematically overestimated or underestimated the true probability of an event. The calibration slope was 0.99 (95% CI 0.85-1.14), suggesting minimal model overfitting (ideally 1). (Figure 2) Patients categorized in the highest sextile of predicted risk had an observed risk at 1-year of 41.2%, compared with 1.7% in the lowest sextile. The distributions of the predicted probabilities for the derivation and validation cohort are shown in Figure 3; 1-year predicted probabilities were higher in the validation cohort compared to the derivation cohort, in agreement with the higher 1-year observed risk. The endpoint-free survival according to sextile of the 1-year predicted risk for both the derivation and the validation cohort is shown in Supplemental Figure 2. The excellent calibration allowed us to refit the model based on data from both the derivation and validation cohorts.

Hazard ratios of the model variables in the merged dataset were very similar to those seen in the derivation cohort: NYHA class ≥II = 1.91 (95% CI 1.50-2.43), cardiac medication use = 2.53 (95% CI 1.92-3.35), ≥1 reintervention = 1.55 (95% CI 1.20-2.00), BMI = 1.03 (95% CI 1.01-1.05) kg/m^2; and $\log_2$ NT-proBNP = 1.44 (95% CI 1.35-1.54) pmol/L. The risk calculator is available online at https://achdwebcalculator-updated.shinyapps.io/achdshinyweb/.The formula for calculating 1-year risk predictions is provided in Supplemental Table 3, to allow validation of the model by other investigators. The 1-year risk predictions based on the combined data likewise discriminated well which patients would have endpoint-free survival over a 6-year

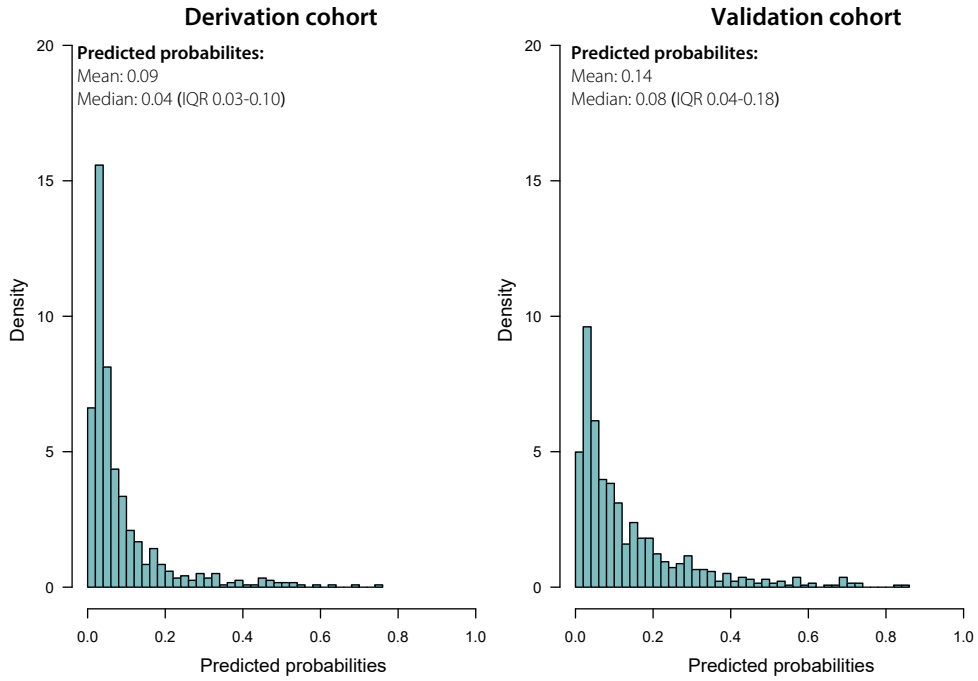

Figure 3. Density plot showing the distribution of the 1-year predicted probabilities for the composite outcome of death, heart failure or arrhythmia in the development cohort and validation cohort.
Mean and median [25th – 75th percentile] predicted probability for each cohort is given. The predicted probabilities of adverse outcome are higher in the validation cohort, which aligns with the higher observed probability.

horizon (Figure 4). The observed risk for patients in the highest sextile of predicted risk was 37.7% versus 0.5%, for patients in the lowest sextile (Supplemental Table 4).

Model performance for the 2-year predicted risks of the endpoint was similar to the 1-year fitted risk prediction model; calibration slope= 0.992, calibration-in-the-large= 0.004, C-index=0.77 (Supplemental Figure 3).

Discussion

This study leveraged data from two large prospectively enrolled cohort studies of adults with CHD to re-develop and subsequently validate a parsimonious ACHD risk prediction model. The final model consisted of five predictors: NYHA class, cardiac medication use, reintervention, BMI, and NT-proBNP. The external validation in 749 patients from the Boston ACHD BioBank study suggests the model accurately predicts the 1-year risk of arrhythmia, HF, or death, and discriminates well between patients who suffer a clinically important cardiovascular outcome in different clinical contexts. The excellent calibration of this model in a large, independent,

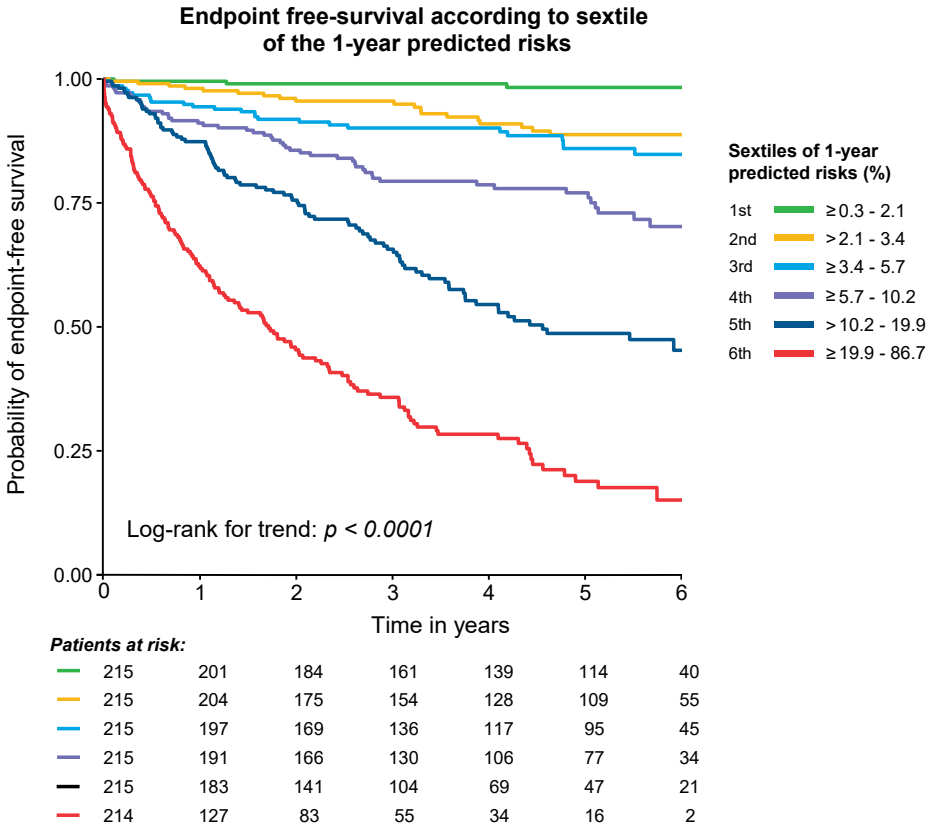

Figure 4. Cumulative endpoint-free survival in 1,289 adults with congenital heart disease according to sextile of the ACHD prediction model 1-year predicted risk of the composite endpoint.
The endpoint was a composite of all-cause mortality, heart failure (associated with hospitalisation or initiation/intensification of heart failure medication) or arrhythmia (symptomatic and recorded, or associated with treatment).
ACHD= adult congenital heart disease.

prospective cohort study, strengthens its validity and generalizability to a diversity of moderately and severely complex ACHD diagnoses.

Comparison with previous model development and validation

The broad spectrum of CHD diagnoses with variable disease severity make it challenging to apply a single prediction model to all adults with CHD. However, in the current study, moderately versus severely complex ACHD was not an independent predictor for the occurrence of HF, arrhythmia or all-cause mortality. There may be several explanations; first, the complexity of the CHD may be partially captured by other variables included in the model, minimizing additive information. Severely complex ACHD patients may tend to use cardiac medication more frequently[9], for example, and are more likely to have NYHA II or III symptoms, and to undergo

reinterventions. Second, the distinction between moderately and severely complex diagnoses could be insufficient to account for heterogeneity across congenital diagnoses as disease severity can vary greatly within moderately and severely complex ACHD patients. The American College of Cardiology/American Heart Association recently published ACHD guidelines which propose a new ACHD classification, comprised of both CHD anatomic class and variables reflecting the patient's "physiological stage"[10]. This was done to create better risk stratification in ACHD and better guide follow-up; recently this proposed classification has been assessed by Ombelet et al. which showed that addition of the physiological stage of patients to the anatomic class improves long-term prediction of cardiac mortality[11]. The results of our study support this approach conceptually, and the need for differentiation based on more characteristics than the severity of the CHD itself. Nevertheless, these results do not undermine the need for diagnosis-specific predictors, as each ACHD diagnosis may have idiosyncratic predictors for outcomes (e.g., hypertension in coarctation of the aorta). Another study by Ombelet et al. showed that NYHA class and other functional indices can predict 15-year mortality in ACHD[12]**.** Our study shows that cardiac medication, prior reinterventions, BMI and NT-proBNP provide additional prognostic information on top of NYHA class for a combined endpoint. While NYHA class is a key clinical variable strongly associated with prognosis, combining functional indices with other clinical variables and more nuanced measurements such as NT-proBNP, seems to improve risk prediction. After all NYHA functional class is limited to 4 possible risk strata and in practice 3; almost all patients at a given time will be classified as NYHA class 1, 2, or 3.

Cohen et al. developed a prediction model to predict the 1-year risk of ACHD HF hospitalization based on a large administrative database in Quebec[13]. That cohort had substantially lower risk: 0.72% 1 year risk of HF hospitalization, as compared with 3.5% in the current cohort. The presence of lifetime comorbidities among which diabetes mellitus, coronary artery disease, and chronic kidney disease, increased the 1-year risk of HF hospitalization. In the current study, comorbidities were not considered as predictors because of the relatively low prevalence; but presumably the presence of these diagnoses are also associated with worse prognosis in this sort of patient.

Age was not included in the final model in the current study. One may argue that, specifically for mortality, age should always be included as a predictor, since it is related to survival prospects. In a meta-analysis of HF from acquired causes, age was an independent predictor for mortality[14]. Age may have lacked predictive value in our study because of the relatively narrow age range, the inclusion of younger patients who were sicker based on timing of clinical visits, or due to the use of a composite endpoint. However, this finding is not unique to our study. Yap. et al, for example, found that age was not a predictor of mortality in a study of 378 patients with ACHD and atrial arrhythmia, even in univariable analysis[15]. In addition, a systematic review assessing predictors for HF in ACHD, reported that age was a significant predictor in only 25% of the studies [16]. Age may therefore be less relevant for prognosis in ACHD compared to variables more directly reflecting clinical condition, such as NYHA functional class[17] or cardiac medication use[9].

The higher predicted risks in the validation cohort parallel the higher observed risks, indicate that the predictive value of the variables behave similarly in cohorts with different overall disease severity. The C-index was 0.79, indicating the model discriminates well between patients who do and do not experience the composite outcome. Further improvement would be desirable, and additional predictors, both general and diagnosis-specific, not considered in the initial derivation, should be explored to enhance discrimination of high- and low-risk patients.

External validation and generalizability

Both cohort studies are likely realistically representative of the overall population of ambulatory, moderately to severely complex ACHD patients engaged in specialist care, since few exclusion criteria were applied in either study. This is important for the generalizability of a model. The observed and the predicted probabilities in the Boston cohort were considerably higher compared to the Rotterdam cohort. A likely explanation is referral selection bias of more complex and ill patients to Boston Children's Hospital/Brigham and Women's Hospital, as suggested by the higher proportion of patients on cardiac medication and NYHA functional class >1. Low-risk patients may be less likely to have scheduled follow-up over a short-to-medium time period compared to high-risk patients, leading to a relatively sicker cohort of ACHD patients; there was no defined follow-up period, in distinction to the annual follow-up for all patients in Rotterdam. The vastly different healthcare systems may in part explain differences in follow-up including regionalization of care in the Netherlands, as might the more expansive geography of referral in the USA and Boston.

Generalizability of this model should be evaluated in low- and middle-income countries or in other situations where ACHD care and therapeutic strategies may differ[18]. Moreover, this model depends on measurement of NT-proBNP, restricting the use of this model to medical centres able to perform this test.

Clinical usefulness

The ACHD risk calculator provides reliable estimations of the 1-year risk of death, HF or arrhythmia, and can provide context to help guide the follow-up strategy of ACHD patients. For instance, follow-up timing may be adjusted based on the anticipated medium-term risk (e.g., if 2-year risk is <5%, it may be reasonable to defer the next regular annual visit unless there is a change in symptoms). Ultimately, the usefulness of the tool depends on being able to identify interventions or other diagnostic tests that will improve a given patient's care, whether in terms of avoiding clinical events or enhancing the quality of life. This might not only take the form of increased surveillance or intervention; avoiding unnecessary clinical visits for patients at very low risk may also substantially enhance patient satisfaction and quality of life.

The Cox model assesses relative risk, and therefore the predicted probabilities depend on the population in which the model is developed. Therefore, absolute risk estimates should be interpreted cautiously when applied to other clinical contexts. Nevertheless, this study shows

that the risk prediction model can accurately identify high and low-risk patients and that the predicted risks were calibrated well in an external cohort. The question remains whether the model improves clinical decision-making. This can be assessed using a decision curve analysis with specified cut-offs for high- versus low-risk and their treatment indication. A randomized controlled trial of physicians using the risk model to aid clinical decision making versus physicians not using the model would be needed to objectively assess its clinical effectiveness and cost-efficiency.

Limitations

Due to insufficient power, additivity and linearity assumption could not be relaxed by including interaction terms and non-linear terms. This may have hindered the discriminative ability, though it has been shown that these terms do not often have considerable influence on the model performance, as interaction- and non-linear terms are likely to induce model overfitting[19].

This model was developed using a composite endpoint. While all three components are substantive, "hard" clinical events, their importance is not equivalent and the strongest risk factors may vary between these three events. Furthermore, the diagnostic and therapeutic strategies relevant to a patient at high risk for arrhythmia may differ from a patient at high risk of developing HF.

We tested 14 pre-selected variables as candidate predictors. Other predictors may be strong predictors of outcome but may have been too uncommon to enable adequate power (e.g. severe cyanosis), were not relevant to both cohorts (e.g. health insurance status, race) or were not available in both datasets (e.g. cardiopulmonary exercise test data).

Conclusions

The ACHD risk calculator, based on data from two large prospective cohort studies including a total 1351 moderately to severely complex ACHD patients, provides reliable estimates for the 1-year risk of death, HF or arrhythmia based on five readily available clinical variables; NYHA functional class, cardiac medication use, reinterventions, BMI, and NT-proBNP. The resulting risk prediction tool is suitable for assistance of risk stratification in clinical practice when caring for moderately and severely complex ACHD patients. We encourage other investigators to collaborate on additional validation and improvement of the current risk model or derivation of diagnosis-specific and outcome-specific models to better focus clinical care on specific risks.

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Supplemental Table 1. Percentage of missing data on predictor variables that were imputed using single imputation for the development cohort and validation cohort.

Variable	Derivation cohort (n=602)	Validation cohort (n=749)
Age	0.0	0.0
Sex	0.0	0.0
Congenital diagnosis complexity	0.0	0.0
NYHA class	0.0	0.5
Cardiac medication use	0.0	0.0
≥1 re-interventions after initial repair	0.0	4.5
Body mass index	0.5	0.3
Heart rate	1.3	0.9*
Current tobacco use	9.1	0.0
Oxygen saturation	7.3	9.2*
Loss of sinus rhythm at baseline ECG	0.0	-
Systemic ventricular function	0.0	6.3*
Valvular dysfunction	1.8	-
NT-proBNP	1.2	0.0

*Missing values were not imputed for these variables, since they were not included in the final model.
Abbreviations: NYHA= New York Heart Association, ECG= electrocardiography, NT-proBNP= N-terminal pro B type natriuretic peptide.

Supplemental Table 2. Baseline characteristics in the validation cohort comparing patients with and without follow-up data.

	Validation cohort (Boston data)		
	Without follow-up	**With follow-up**	**p-value**
No. of patients	57	692	
Clinical characteristics			
Age, years	40 [29-53]	35 [27-48]	0.076
Women	30 (53)	331 (48)	0.494
Body mass index, kg/m^2	26.8 ± 5.6	26.9 ± 5.6	0.994
Heart rate, beats/minute	71 ± 13	72 ± 13	0.557
Systolic blood pressure, mmHg	116 ± 16	120 ± 14	0.070
Oxygen saturation <90%	1 (2)	37 (6)	0.721
Current tobacco use	2 (4)	34 (5)	
Congenital diagnosis, severely complex	17 (30)	274 (40)	0.189
NYHA class			0.587
Class I	40 (76)	511 (74)	
Class II	10 (19)	156 (23)	
Class III/IV	3 (6)	25 (4)	
Cardiac medication use	24 (42)	409 (59)	0.017
≥1 re-intervention after initial repair	15 (47)	432 (63)	0.092
Systemic ventricular function			0.467
Normal	38 (70)	459 (71)	
Mildly impaired	9 (17)	133 (21)	
Moderately/severely impaired	7 (13)	56 (9)	
NT-proBNP, pmol/L	14 [6- 43]	17 [7-40]	0.755

Data are presented as mean ± SD, median [25th-75th percentile] for continuous variables (normally and non-normally distributed, respectively), and n (%) for categorical variables. Percentages are calculated based on the number of cases with complete data, and therefore may differ from what could be calculated directly from the figures. NYHA= New York Heart Association, NT-proBNP= N-terminal pro B-type natriuretic peptide.

Supplemental Table 3. Formula for the 1-year predicted risk of death, heart failure or arrhythmia in moderately to severely complex adult congenital heart disease patients, together with the corresponding R-script.

Formula to predict the probability of the 1-year risk of death, heart failure or arrhythmia:

$$1 - e^{-exp[-5.663442 + 0.64719399 * NYHA + 0.92976606 * medication + 0.43814789 * reintervention + 0.02573959 * BMI + 0.36410392 * logBNP]}$$

Centered baseline hazard at t=1 year:

$$-5.663442 = \log\ (Baseline\ hazard\ at\ t = 1) - center$$

Center= sum of each regression coefficient mean value of predictor variable in the data (is equal to the non-centered linear predictor of the average patient in the data)*

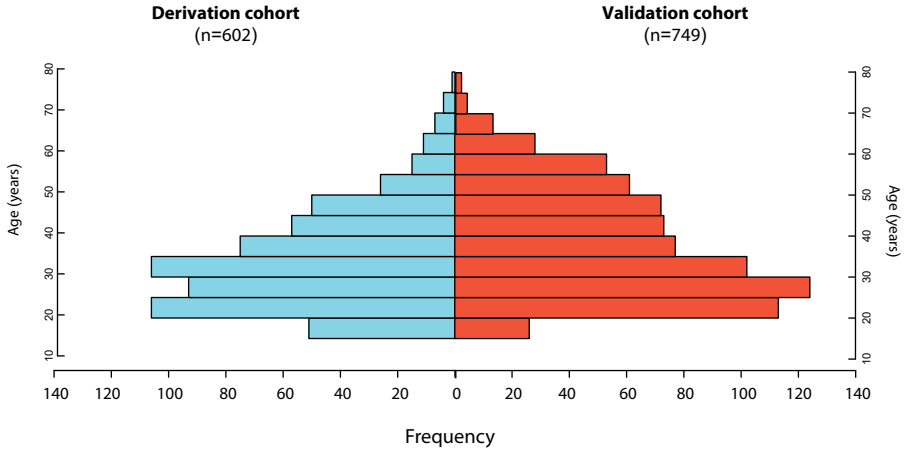
 # r-code #

```
# To calculate the estimated 1-year risk of death, heart failure or arrhythmia for adults with moderate or complex congenital heart disease
#==== variables =====#
# NYHA I =0 versus NYHA II/III =1
# cardiac medication no= 0, versus cardiac medication yes= 1
# ≥1 reintervention no= 0 versus ≥1 reintervention yes= 1
# BMI (kg/m2)
# NT-proBNP (pmol/L)
oneyearrisk <- (1-exp((-exp (-5.663442
                + 0.64719399 * (as.numeric(input$NYHA))
                + 0.92976606 * (as.numeric(input$medication))
                + 0.43814789 * (as.numeric(input$reintervention))
                + 0.02573959 * (as.numeric(input$BMI))
                + 0.36410392 * (as.numeric(log2(input$BNP)))))))
                * 100
```

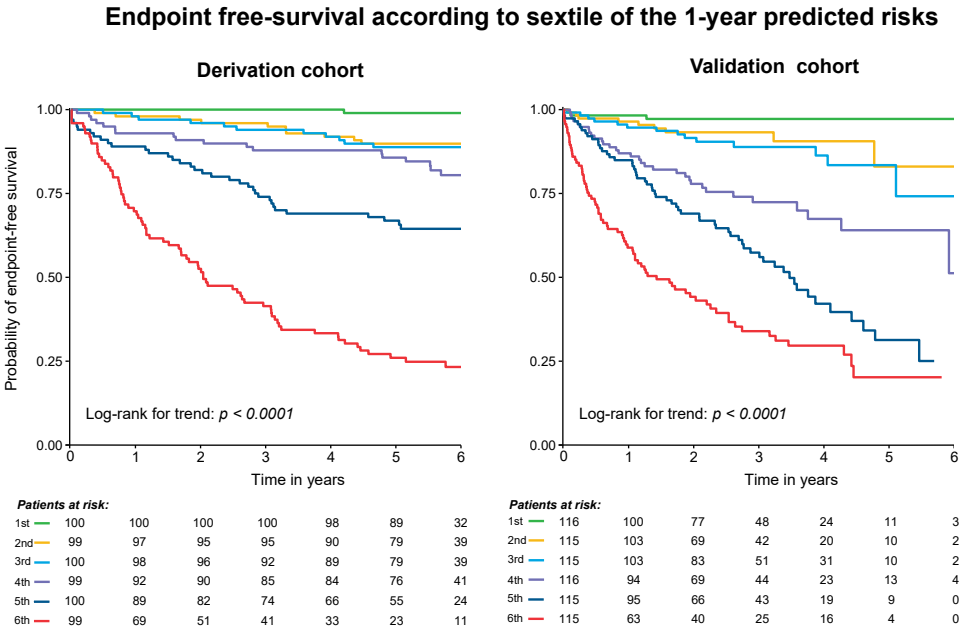
Supplemental Table 4. Sextile distribution of the 1- and 2-year predicted risks of the combined endpoint of death, heart failure or arrhythmia, for the ACHD risk prediction model fitted on the merged dataset of the Rotterdam cohort and Boston cohort.

	Merged dataset (n= 1289 ACHD patients)					
	1st sextile	**2nd sextile**	**3rd sextile**	**4th sextile**	**5th sextile**	**6th sextile**
Number of patients	215	215	215	215	215	214
	1-year predicted risks (merged datasets)					
Ranges predicted risks, %	0.3-2.1	2.1-3.4	3.4-5.7	5.7-10.2	10.2-19.9	19.9-86.7
Mean predicted risk, %	1.5	2.7	4.3	7.6	14.4	37.0
Mean observed risk, %	0.5	1.9	5.6	8.9	12.6	37.7
	2-year predicted risks (merged datasets)					
Ranges predicted risks, %	0.5-3.8	3.8-6.0	6.0-9.9	9.9-17.5	17.5-32.6	32.6-97.3
Mean predicted risk, %	2.6	4.8	7.6	13.2	24.2	54.3
Mean observed risk, %	1.0	4.5	8.1	14.4	24.4	54.6
	Predictor values					
Body mass index, kg/m^2	24.1 ± 4.0	24.7 ± 4.2	26.0 ± 4.9	26.0 ± 4.9	26.8 ± 5.4	27.5 ± 6.5
NYHA >1, n (%)	3 (1)	5 (2)	18 (8)	24 (11)	45 (21)	147 (69)
Cardiac medication, n (%)	5 (2)	24 (11)	53 (25)	132 (61)	195 (91)	211 (99)
Re-intervention, n (%)	43 (20)	94 (44)	120 (56)	136 (63)	175 (81)	184 (86)
NT-proBNP, pmol/L	4 [2-6]	10 [6-14]	15 [8-24]	17 [10-33]	30 [19-43]	85 [48-186]

Value of each predictor in each sextile is given and is presented as mean ± SD or median [25th-75th percentile] for continuous variables (for normally and non-normally distributed, respectively), and n (%) for categorical variables.
ACHD= adult congenital heart disease.



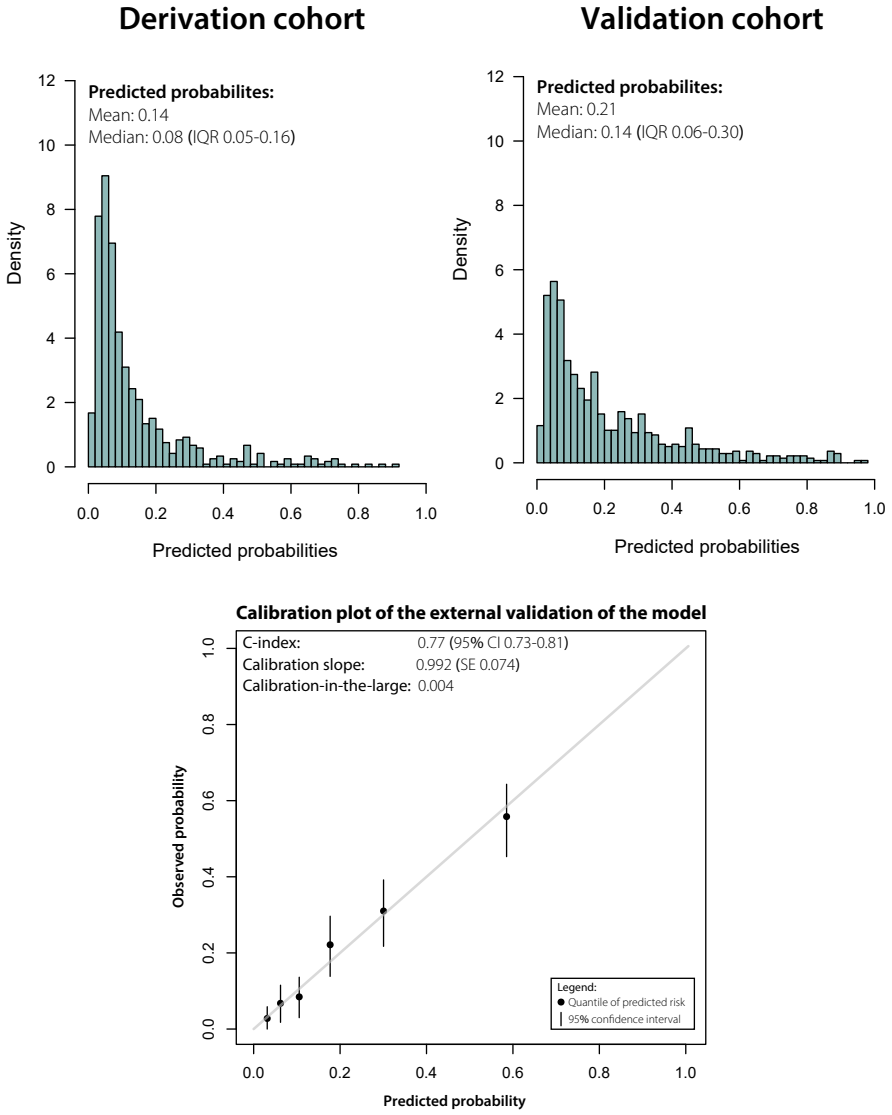
Supplemental Figure 1. Age distribution of participants included in the cohorts from Rotterdam, the Netherlands (derivation cohort) and Boston, United States (validation cohort).



Sextiles of 1-year predicted risks (%)						
	1st	2nd	3rd	4th	5th	6th
Derivation cohort	≥0.5-2.2	≥2.2-3.2	≥3.2-4.6	≥4.6-7.3	≥7.3-14.0	≥14.0-74.0
Validation cohort	>0.3-2.7	≥2.7-4.6	≥4.6-8.1	>8.1-14.3	≥14.3-24.6	≥24.6-84.8

Supplemental Figure 2. Cumulative heart failure and arrhythmia-free survival in adults with moderately or severely complex congenital heart disease, stratified according to the sextile distribution of the 1-year predicted risks in the derivation cohort (Rotterdam) and the validation cohort (Boston).

Prediction model fitted to the 2-year risk of death, heart failure or arrhythmia	
Model development (Rotterdam data, n=597 patients)	
Number of events that occurred within two years of follow-up	83 (13.9%)
Estimated baseline hazard at 2 year	1.10
Model validation (Boston data, n=692 patients)	
Number of events that occurred within two years of follow-up	138 (19.9%)



Supplemental Figure 3. Model performance of the prediction model fitted to the 2-year predicted risk of death, heart failure or arrhythmia in the Rotterdam data and validated using the Boston data.

CHAPTER 13

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]

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

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

These may impact peri-operative morbidity, and can have long-standing ramifications on neurodevelopment and overall health.[22]

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.

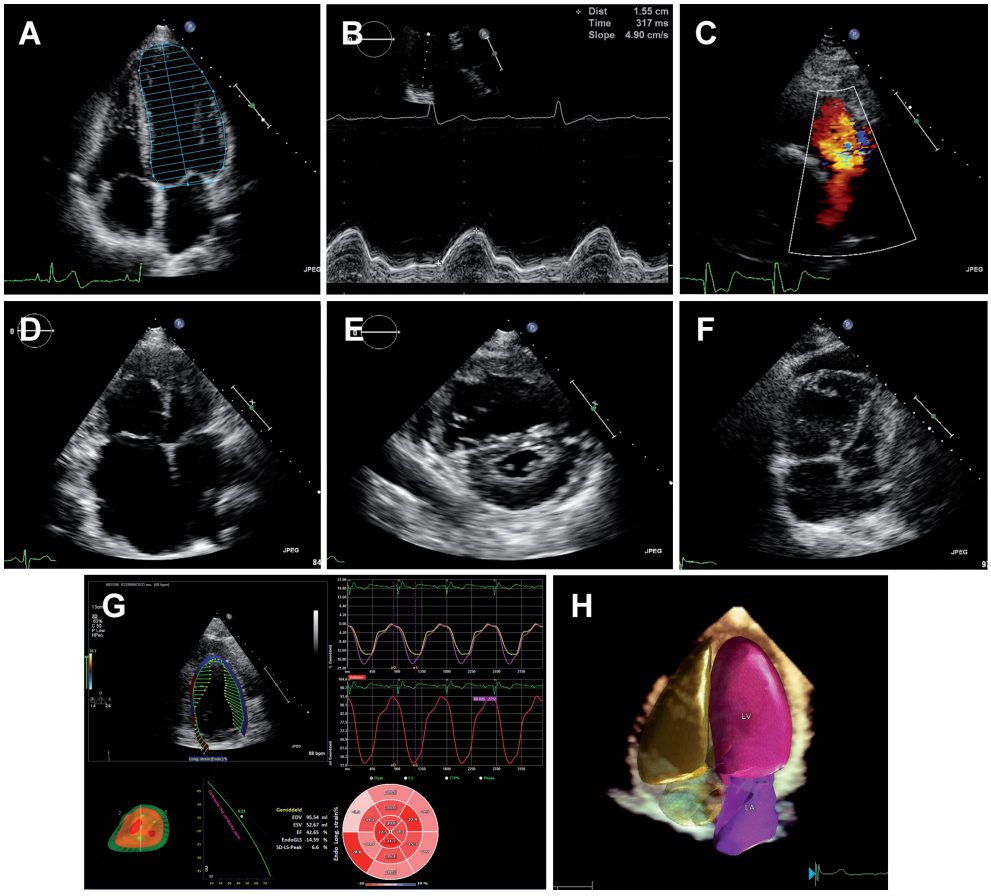


Figure 1. Echocardiographic predictors of adverse clinical outcome in patients with ACHD.
A, measurement of left ventricular ejection fraction using the biplane method of disks (modified Simpson's rule) in a patient with aortic coarctation. B, mildly reduced TAPSE (< 16 mm) in a patient with tetralogy 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).

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]

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

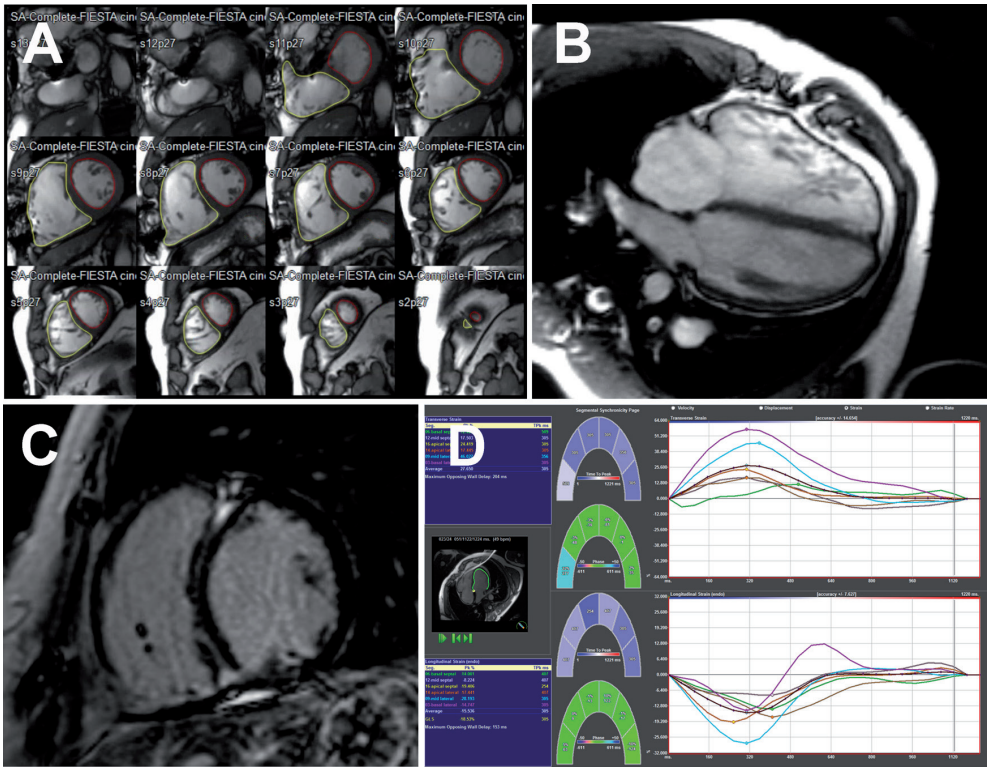


Figure 2. Cardiac magnetic resonance imaging parameters that are reported to be of prognostic importance in patients with ACHD.
A, left and right ventricular ejection fraction as measured by multi-slice short axis cine imaging in a patient with congenital aortic stenosis. B, right ventricular dilatation 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).

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.

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 most clear 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

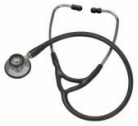
This chapter aimed to provide a comprehensive review of the factors that can be used in day-to-day clinical practice for the risk stratification of patients with ACHD. An overview of the most important reported prognostic factors is provided in Figure 3. Of note, accurate risk stratification does not 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.

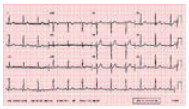
Regular follow-up

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

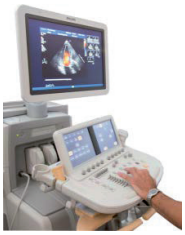




- Clinical signs of heart failure
- Saturation
- Changes in ECG morphology
- Loss of sinus rhythm
- QRS duration

Additional investigations

Echocardiography

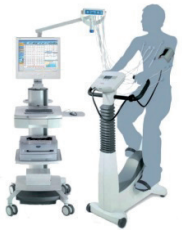


- Systolic ventricular function (LVEF, RVFAC, TAPSE, eyeballing)
- Ventricular and atrial dilatation
- (Residual) shunt lesions
- Severity of valvular disease
- Right ventricular pressure
- Pericardial effusion
- Novel techniques (strain, 3D echocardiography)

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 14

Summary
General discussion

Summary

The contribution of improved medical therapies and care to pediatric congenital cardiology and surgery, has resulted in an improved life expectancy of children with congenital heart disease (CHD). Survival of patients with CHD into adulthood has introduced a new field within cardiology that is characterized by an unique population of relatively young adults with a high incidence of heart failure, arrhythmia, re-operations and premature death. Many of the medical issues in adult patients with CHD are rare, or do not occur in acquired heart disease, the reason why these patients require specialized care. Therefore, this particular field of adult congenital heart disease (ACHD) has received increasing attention and expertise over the last years. Despite the fact that ACHD patients will be confronted with their disease throughout their entire life, and may have concerns about their uncertain longevity, ACHD patients have desires equal to their peers; they want to become pregnant, go on extreme vacations, and participate in sports games. Gaining insight in their disease progression and management in terms of risk stratification and easy monitoring is therefore of great significance, and can contribute to better coping strategies for patients and management of their expectations.

Adults with pulmonary hypertension (PH) are characterized by a rapid disease progression resulting in a very poor prognosis. The etiology of PH is highly heterogeneous and symptoms caused by the disease are nonspecific; therefore, PH is a challenging disease for doctors to recognize resulting in a substantial delay between the first onset of symptoms and the diagnosis. PH is also a complex and rare disease that requires care in tertiary centers specialized in PH. Other than ACHD, PH is an acquired disease that can develop at any age, and ultimately leads to early demise. Therapeutic options have evolved over the last years, improving the life expectancy of patients with PH. As the etiology of the disease is diverse, so is the course of the disease, which makes it difficult to adequately estimate prognosis in these patients. Blood biomarkers have potential to help to monitor disease progression and optimization of follow-up strategies.

This thesis investigated the potential value of blood biomarkers in the risk stratification of adults with CHD and adults with PH. **Chapter 1** provides a general introduction to this topic, outlining the different disease populations and their current challenges, as well as the current position of blood biomarkers in the clinical management of the disease, and risk stratification in a broader sense.

PART I Blood biomarkers in adult congenital heart disease

Part I describes the prognostic value of both novel and established blood biomarkers in ACHD in general, as well as in specific congenital heart defects, among which; systemic right ventricle (sRV), atrial septal defect (ASD) and bicuspid aortic valve (BAV).

Soluble suppression of tumorigenicity-2 (sST2) has recently been discovered as novel heart failure biomarker, and is upregulated by cardiomyocytes in reaction to cardiac damage and stress. In **Chapter 2**, we measured sST2 in thawed serum samples of moderately and severely complex ACHD patients from the BioCon study. In addition, sST2 was measured in samples of healthy controls that were included in the 'Navigator' study, to establish reference values and assess the reproducibility of the sST2 assay. In total, sST2 was measured in 142 healthy controls and 590 ACHD patients. Significantly lower levels of sST2 were found in men than in women, in both healthy controls and patients. Based on the 97.5th sST2 percentile in subjects from the Navigator study, an elevated sST2 was present in 7 women (2.8%) and 15 men (4.4%). Even though higher sST2 was associated with a higher risk of any cardiovascular event in general, stratified analysis showed that sST2 was particularly of prognostic value in women and in severely complex ACHD. The sex-specific differences of sST2 found in this study, had not been previously described in the literature, and future research should elucidate the potential hormonal influence on sST2 levels.

Troponin T is known to be released by the heart into the blood stream in response to cardiomyocyte damage. For this reason, high-sensitivity troponin T (hs-TnT) is an established biomarker for the diagnosis of acute coronary syndrome. While ACHD patients are relatively young and coronary artery disease may not be their first threat, it is remarkable that hs-TnT is elevated in a substantial number of patients with ACHD, which was previously investigated by Eindhoven et al. In **Chapter 3** we studied longitudinal hs-TnT evolutions over time and related this to the risk of cardiovascular events in patients enrolled in the BioCon study. Hs-TnT was annually measured up to 4 year after study enrollment and we found that hs-TnT levels were on average higher in patients that suffered from any major cardiac adverse event during follow-up, and levels tended to increase prior to the occurrence of a major adverse cardiac event. However, also in patients without cardiovascular events, hs-TnT gradually increased over time suggesting loss of cardiomyocytes in ACHD patients most likely due to subclinical progression of heart failure, in absence of coronary artery disease. For use in risk stratification and follow-up assessments, data of our study supported that repeatedly measuring hs-TnT provides additional prognostic value over a single hs-TnT measurement, however repeated hs-TnT does not provide prognostic information beyond repeatedly measuring NT-proBNP. We also observed that patients with stable undetectable hs-TnT levels, were at a very low risk of developing major adverse cardiac events. This could be helpful to identify low-risk patients.

C-reactive protein (CRP) is an inflammatory marker that is widely available and is regularly used in daily clinical practice to trace or rule-out infections. Since the development of high-

sensitivity CRP (hs-CRP), low-graded levels of inflammation can be easily measured. In **Chapter 4**, we investigated the role of hs-CRP in ACHD patients using data from the BioCon study. We assessed the prognostic value of a single baseline hs-CRP measurement, and found that patients with an elevated hs-CRP (>3 mg/L) in combination with an elevated NT-proBNP (>14 pmol/L), had a heart-failure free survival of only 71.6 percent after 6 years, whereas patients with normal levels of both biomarkers had a 6-year heart failure-free survival of 99.6 percent. Hs-CRP remained an independent prognosticator for death or heart failure after adjustment for a wide range of clinical variables, NT-proBNP and hs-TnT. Moreover, longitudinal patterns of hs-CRP revealed that hs-CRP on average increased prior to the occurrence of death or heart failure, while stable levels were observed in patients who were alive and did not experience an episode of heart failure during follow-up. Repeated hs-CRP measurements were associated with the risk of death or heart failure, independent of repeated NT-proBNP measurements. Therefore, high, and increasing hs-CRP levels, seem to identify patients with a poor prognosis and a hs-CRP measurement should be considered in addition to an NT-proBNP measurement in the regular follow-up of ACHD patients. The question whether inflammation may play a causal role in the deterioration of ACHD patients, or that other underlying pathology is responsible, remains to be answered in future research.

Atrial septal defect (ASD) can be diagnosed at adult age may require surgical or percutaneous closure. If the anatomy of the defect permits percutaneous closure and the defect is not too large, this is often the procedure of first choice. In **Chapter 5** we studied the evolution of blood biomarkers following percutaneous ASD closure in adults. Blood sampling was performed prior to ASD closure, and approximately 1 day, 3 months, and 1 year after ASD closure in 50 adults who underwent percutaneous ASD closure. Six different biomarkers were analyzed in this study; NT-proBNP, hs-TnT, hs-CRP, RDW, GDF-15 and galectin-3. We found that in a substantial number of patients at least one of these six investigated biomarkers was elevated before ASD closure, and one day post ASD closure acute increases in most biomarkers were observed, resulting in 92% of the patients who had at least one abnormal biomarker level. Three months post ASD closure, biomarker levels returned to levels present before closure, and remained stable up to 1 year. The biomarker patterns found in this study suggest that ASD closure is directly followed by an extensive cardiac response on a molecular and cellular level. Echocardiography 1 year post closure showed signs of reverse cardiac remodeling, though decreases in blood biomarker levels at 1 year relative to levels before ASD closure, were not observed.

Patients with a bicuspid aortic valve (BAV) have an aortic valve with two instead of three (functional) cusps. The results of a cross-sectional study aiming to investigate whether blood biomarkers are associated with the aortic disease stage were discussed in **Chapter 6**. We investigated NT-proBNP, hs-CRP, hs-TnT and transforming growth factor-beta 1 (TGF-β1). TGF-β1 was also measured in the control subjects of the navigator study, and lower TGF-β1levels were found in BAV patients than in controls. Higher NT-proBNP and hs-TnT were associated with more severe aortic valve regurgitation and NT-proBNP was also positively associated with the degree

of aortic valve stenosis. Hs-CRP and TGF-β1were not associated with aortic valve severity, nor with the aortic diameter or left ventricular ejection fraction. Future studies should investigate whether biomarkers are associated with long-term clinical events such as aortic dissection or worsening aortic stenosis in these patients.

In an anatomical normal heart, the left ventricle pumps blood into aorta, providing the systemic circulation of blood. Before the arterial switch operation, patients with a transposition of the great arteries (TGA) were operated by the Mustard or Senning procedure. This resulted in a right instead of the left ventricle that supports the systemic circulation. In patients with congenitally corrected transposition of the great arteries (ccTGA), the right ventricle also serves as systemic ventricle. In **Chapter 7** we explored the prognostic value of novel echocardiographic measures and blood biomarkers, for risk stratification in adults with a systemic right ventricle (sRV). In total 86 sRV patients from the BioCon study were included in this analysis. A high incidence of heart failure, arrhythmia and death was observed in these patients, pointing out the importance of adequate risk stratification. Several clinical characteristics, blood biomarkers, and conventional echocardiographic measurements, were associated with clinical outcomes. Nonetheless, blood biomarkers seemed better prognosticators for clinical outcomes in adults with a sRV than novel echocardiographic strain measurements. In particular GDF-15 showed a strong association with death or heart failure, even stronger than the more conventional biomarker NT-proBNP. The results of this study suggests that the ventricular deterioration of sRV patients is complex, and risk stratification is challenging. On the basis of this study, we suggested that a comprehensive clinical approach is needed, in which blood biomarkers play a key role.

PART II Blood biomarkers in adults with pulmonary hypertension

In Part II the prognostic value of several established and novel blood biomarkers in adults with pulmonary hypertension (PH) were addressed. These results were all based on the BioPulse study. Additionally, a systematic review is provided that summarizes current literature about the prevalence of pulmonary arterial hypertension (PAH) before and after ASD closure in adults.

In **Chapter 8** the first results of the BioPulse study were presented. In total, 106 adults with pulmonary hypertension were included, and levels of six different biomarkers were measured during the diagnostic right heart catheterization. We described the levels of these six biomarkers, and related them to the risk of clinical outcomes. We measured NT-proBNP, hs-TnT, hs-CRP, galectin-3, RDW and eGFR, and all of the biomarkers except for eGFR, were associated with the risk of death or lung transplantation. NT-proBNP yielded the strongest association of all biomarkers. A multi-biomarker approach showed that patients had a worse prognosis if more biomarkers were elevated. Notably, only eleven patients (11%) had zero abnormal biomarker levels at the time of the diagnosis of PH, and all of these patients were alive after 40 months of

follow-up. The REVEAL Risk Score is a developed risk score used to stratify patients with PAH. In this study, we calculated the REVEAL risk score for each patient and assessed whether any of the six biomarkers had additional prognostic value beyond this risk score. The results showed that a combination of biomarkers, as well as a single biomarker, cannot provide additional prognostic value over the REVEAL risk score. Therefore, risk stratification based on exclusively biomarkers seems inadequate. Nonetheless, this study does suggest that combining multiple biomarkers may be beneficial in detecting PH patients at low and high-risk, and larger studies with preferably continuous biomarker levels instead of dichotomized levels based on cut-offs should further investigate this.

sST2 was also measured in the BioPulse study with the same assay and reference values as described in Chapter 2. The results of sST2 in adults with PH were described in **Chapter 9**. In this study we found that sST2 was elevated in 20% of the patients and higher sST2 was associated with invasively measured right-sided pressures and echocardiographic measures of right ventricular function. Moreover, patients with an elevated sST2 had a significantly lower transplantation-free survival 6 year after their initial diagnosis. However, sST2 yielded no independent prognostic value beyond NT-proBNP, limiting its usefulness as prognostic biomarker for risk stratification in adults with PH. What the exact underlying mechanism is of sST2 in release remains unclarified, and is a highly interesting topic to address in future studies.

Growth differentiation factor-15 (GDF-15) is a nonspecific biomarker that is involved in cell growth, differentiation and apoptosis. We evaluated GDF-15 as predictor for mortality in adults with PH and hypothesized that because of its nonspecific nature, GDF-15 could be a strong prognosticator in PH that is characterized by divergent etiologies. Results of this study were described in **Chapter 10**. GDF-15 was measured in plasma of patients with PH, as well as healthy controls from the Navigator study. Clear differences were observed in the distributions of GDF-15 levels between patients with PH and controls; GDF-15 levels were significantly higher in patients, and based on the 97.5th percentile of GDF-15 levels in healthy controls, we identified abnormal GDF-15 levels in 74% of the patients. It was noted that particularly patients with normal GDF-15 levels had a very good prognosis; these patients were all alive and free of lung transplantation two years after their diagnosis. In contrast, only 72% of patients with elevated GDF-15 was alive and free of a lung transplantation after 2 years. GDF-15 also remained associated with the risk of death or lung transplantation when adjusted for NT-proBNP. This study showed that a GDF-15 measurement should be considered in all patients at the time of the diagnosis of PH, to identify low-risk patients on the basis of normal GDF-15 levels.

Finally, in **Chapter 11** the results of a systematic literature review considering the prevalence of PAH before and after ASD closure in adults were presented. In total 30 papers were include and the prevalence of PAH ranged from 29% to 73% before ASD closure. A decrease in PAH prevalence and mean pulmonary artery pressures were observed after ASD closure in all studies, independent of the age or pulmonary pressures before closure. However, PAH prevalence after ASD closure still ranged from 5% to 50%, warranting long-term follow-up strategies of adults after ASD closure.

PART III Risk stratification

Part III describes the use of other modalities which are helpful to determine prognosis in patients with ACHD. Besides blood biomarkers, a patient's clinical characteristics, demographics and modalities such as electrocardiography, imaging and cardiopulmonary exercise testing can contribute to the risk estimation of certain cardiovascular events.

Validated risk prediction tools are scarce in the ACHD population, and risk stratification in ACHD patients therefore remains largely bases on expert opinion. In **Chapter 12** we described the development and external validation of an ACHD risk prediction model leveraging data from two large prospective cohort studies consisting in total of 1,351 ACHD patients. The BioCon study was used to develop the model and an external cohort of adults with CHD from Boston Childrens and Brigham and Women's hospital was used to validate the model. The final model consisted of 5 readily available variables; NYHA class, cardiac medication use, any re-interventions after initial repair, body mass index, and NT-proBNP. The risk calculator provided reliable estimates for the 1-year risk of death, heart failure or arrhythmia based on these 5 readily available variables, expressed by a good calibration and discrimination in the external cohort. These data support the validity and applicability of this parsimonious ACHD risk model, which may be useful to help guide appropriate care. An online web application has been built to support easy use of the ACHD risk prediction model in daily clinical practice.

Heart failure is a complex clinical syndrome and is one of the major problems in ACHD. In **Chapter 13** we summarized evidence on prognostic factors for heart failure and other late complications in the ACHD population. The contribution of a broad range of factors for risk stratification were discussed on the basis of the current literature, among which the type of the congenital heart defect, genetics, echocardiography, cardiac magnetic resonance imaging, cardiopulmonary exercise testing, and blood biomarkers were identified as important contributors to the risk stratification of ACHD patients.

General discussion

This thesis sought to assess the prognostic value of blood biomarkers as opportunity for enhancing risk stratification in adults with congenital heart disease (ACHD) and adults with pulmonary hypertension (PH). Novel and established blood biomarkers were related to prognosis, and compared with known prognosticators. Also, comparisons were made between different blood biomarkers to allow identification of blood biomarkers that carry the most prognostic value. While biomarker research is often focused on a single biomarker measurement, this thesis also addressed the potential utility of repeated biomarker measurements over time. Besides blood biomarkers, prognostication can be based on various clinical characteristics and measurements. A risk prediction model for risk stratification in ACHD has been established, which was validated in an external cohort of ACHD patients. First, a general evaluation of blood biomarker will be discussed, followed by the main findings and conclusions of this thesis, their possible pitfalls, and their clinical relevance.

Biomarkers and their value: a critical appraisal

New biomarkers emerge at a high pace, however many of them will never become a clinically useful tool. Finding the right biomarker in this jungle of candidate biomarkers is therefore essential, and a critical appraisal of new candidate biomarkers is of pivotal importance. Following the statement of Morrow and de Lemos in 2007, three central questions are of crucial importance for critical evaluation of new biomarkers; I) can the clinician measure the biomarker? II) does the biomarker add new information? III) will the biomarker help the clinician to manage patients? [1]. In the section below, the results of this thesis will be evaluated based on these three questions. Table 1 gives an overview of the biomarkers covered in this thesis.

I. Can the clinician measure the biomarker?

Biomarker measurements should be accurate, precise, reproducible, low in costs, and results should be rapidly available. All these different aspects can influence the ultimate clinical usefulness of a biomarker.

Biomarkers and the invincible measurement uncertainty

Measurement of blood biomarkers, as is the case is for most measurable entities, comes with a certain amount of measurement error, introducing measurement uncertainty. Random measurement error is a natural variability inherent to any measurement process, and represents

Table 1. Overview of the biomarkers covered in this thesis specified by the study population of interest

	ACHD*	ASD closure	Bicuspid aortic valve	Systemic right ventricle	PH	Controls	*Corresponding chapters*
C-reactive protein	•	•	•	•	•		*4-8*
eGFR		•		•	•		5, 7-8
Galectin-3		•		•	•		5, 7-8
GDF-15		•		•	•	•	5, 7, 10
Hemoglobin				•			7
NT-proBNP	•	•	•	•	•		5-8, 12
RDW		•		•	•		5, 7-8
sST2	•				•	•	2, 9
TGF-beta			•			•	6
Troponin T	•	•	•	•	•		3, 5-8

*Includes moderately and severely complex congenital heart disease.
ACHD= adult congenital heart disease, ASD= atrial septal defect, PH= pulmonary hypertension, eGFR= estimated glomerular filtration rate, GDF-15= growth differentiation factor-15, NT-proBNP= N-terminal pro B-type natriuretic peptide, RDW= red cell distribution width, sST2= soluble suppression of tumorigenicity-2, TGF-beta= transforming growth factor beta.

fluctuations around the true value. As random measurement error is insuperable, it can challenge the establishment of valid inferences in clinical research as it may lead to both under and overestimation of associations between exposures and outcomes[2]. Apart from random error which diminishes the measurement precision, systematic error reduces the accuracy of a measurement and can introduce bias. Contrarily to random measurement error, systematic measurement error is preventable but may be hard to track down.

Biological and analytical variation

Apart from measurement error, other sources of variability are present in the case of blood biomarker measurements in human beings[3] and contributes to a substantial variation in some cardiac biomarkers[4]. Natural fluctuations of the body around the homeostatic set point, introduces biological variation in body fluid constituents, leading to inter-individual variation (i.e. between subject variation) and intra-individual variation (i.e. within subject variation). Also laboratory measurements have certain errors leading to an analytical variation in measurements, both within batches as well as between batches.

Several precautions were taken to minimize false inferences due to biological and analytical measurement variability in our blood biomarker studies. For GDF-15, sST2 and galectin-3 (covered in Chapters 2, 5-10), reproducibility of biomarker assays was assessed by duplicate biomarker measurements in healthy controls, in the so-called "Navigator" cohort[5]. The Navigator cohort consists of 155 healthy volunteers, aged between 20-72 years who were

equally distributed per age decade. The agreement between two biomarker measurements was assessed with Bland-Altman plots and the coefficient of variation. Furthermore, in all of our studies, lab analysists were blinded to patient characteristics and outcomes, and unaware of the principal aim of our study. Therefore we can assume the random error to be equally distributed between patients with and without the outcome of interest.

The biological variation in the biomarkers measured in this thesis may not have been optimally reduced in terms of a standardized phlebotomy protocol; while blood sampling in patients was performed after at least 30 minutes of rest, there were no requirements for the fasting state, the moment of the day, or the position of the patient, during the blood sampling procedure. However, the chance that this will have biased the results of our study is low, as it is likely that these variations will have occurred randomly and independent of the outcome of interest. Also sex differences can introduce biological variation in blood measurements[6-8]. In Chapter 2, we observed sex-specific differences in soluble ST2; this biomarker was systematically lower in women compared with men in both healthy subjects form the Navigator study as well as ACHD patients, and yielded a stronger prognostic value in women with ACHD. Sex hormones were not measured at the moment of blood sampling, while this may have helped to explain the sex-differences found in our study. A recent study has shown that cardiac blood biomarker levels are not equal in men and women, and also can have a different diagnostic, prognostic or predictive value among sexes[9]. The last decades more awareness has been raised to sex differences in medical research in general, a topic that should also get more attention in blood biomarker research in particular. Other factors such as changes in diet, medication use, or exercise[10] can introduce variety in blood biomarkers. These factors are hard to keep constant over time in patients and are also difficult to take into account when analyzing repeatedly measured biomarkers over time. However, it is important to keep these factors in mind when studying longitudinal biomarker patterns.

Accessibility and costs

Blood biomarkers are, relative to other forms of biomarkers, such as tissue or cerebrospinal fluid biomarkers, easy to obtain in a relatively non-invasive manner, which makes them a popular and attractive type of biomarker. However, some novel biomarker measurements like GDF-15 and galectin-3, are currently solely measurable by batch analysis which can impede the clinical usefulness of a biomarker, in particularly in acute settings where rapid clinical decision making is key, and test results should be obtained quickly. Biomarkers should also be measurable against a low price. Some of the biomarkers investigated in this study are not commercially available yet, or only against high costs, while others are almost "free".

An example of a biomarker that is low in costs and often measured without specific intentions, is red cell distribution (RDW). RDW is part of the automated blood count, and thus a readily available biomarker measurement that can be measured against a very low price. In both PH (Chapter 9) and ACHD patients[11, 12], RDW has shown to be associated with the risk of adverse cardiac events. This thesis confirmed the prognostic value of RDW in adults with a systemic

right ventricle (Chapter 7). Also CRP is an inexpensive biomarker that is widely available, and since the regular assay has gained sensitivity to measure levels in the low spectrum (high-sensitivity CRP), it can also be useful to detect low-graded inflammatory processes. In Chapter 4 we assessed the potential additive value of hs-CRP to a risk stratification based on NT-proBNP in ACHD patients. We found that low-graded inflammation reflected by a hs-CRP > 3mg/L in combination with an elevated NT-proBNP, can identify patients at high risk of death or HF. Because the low costs and its availability, hs-CRP is an attractive blood biomarker to measure in addition to NT-proBNP in ACHD, augmenting its clinical usefulness in clinical practice. On the opposite, sST2 and GDF-15 are relatively expensive biomarkers, and high biomarker costs may hinder clinical utility as well as realization of thorough studies with large sample sizes, and studies with repeated measurements. However, as described in Chapter 4, the intra- and inter-individual variation of repeated hs-CRP measurements in ACHD patients was substantial, which may hinder its interpretation, and subsequently its clinical utility in daily clinical practice, despite its availability and low costs. The ideal biomarker should not only easy to obtain, but also cheap, and reliable.

II. Does the biomarker add new information?

Blood biomarker investigated in a clinical study are subject to the question whether the blood biomarker adds new information to current established biomarkers and prognosticators. There is no need in introducing the biomarker in clinical practice, if the novel biomarker does not outperform prognostic value of other factors, whether blood biomarkers or other patient-related factors, with equal performances. There is no strict uniform way of how to assess the additive value of blood biomarkers, and several different approaches can be used.

Comparison of biomarkers

To identify the best biomarker, a valid comparison should be made between different blood biomarkers, as well as a comparison with current best practice. First of all, the independent prognostic value of biomarkers can be assessed by using a multivariable regression approach, which enables comparison of multiple prognostic factors. Second, biomarkers can be compared based on the strength of the association found with the endpoint of interest. The differences in units can be overcome by standardization into Z-scores. This approach was used in Chapters 7 and 8 of this thesis. Because the statistical power was limited in these studies, and multivariable analysis was therefore restricted, using Z-scores of each biomarker enabled us to compare the different biomarkers based on the strengths of the associations with the endpoint of interest. The disadvantage of this univariable approach is that it cannot establish the additive prognostic value of one biomarker on top of another. Third, the C-index can be used to assess the predictive value of blood biomarkers. The C-index ranges from 0.5 to 1, where 0.5 indicates that the predictor is non-informative and 1 indicates that the model perfectly discriminates between

patients with and without the endpoint of interest. In survival analysis, the area under the curve for each moment in time can be obtained (time-dependent C-index), or Harrell's C-index can be used for assessing the discriminative value for censored data[13]. In Chapters 7 and 8, we aimed to compare the prognostic strength of various blood biomarkers by expression of the association using standardized hazard ratios as well as by using Harrell's C-index to compare the discriminative value of each biomarker. Although C-indices can be used to assess the discriminative value of a single predictor, its use is more common in the assessment of the discriminative ability of prediction models. In Chapter 12, we obtained the C-index to demonstrate the discriminative value of the prediction model for the 1-year risk of death, heart failure or arrhythmia. The C-index of the final model in the external validation cohort was 0.79, indicating that the model is able to adequately discriminative between patients with and without the defined endpoint at 1-year of follow-up.

In Chapter 10 we studied the predictive value of GDF-15 in adults with PH and found that GDF-15 provided prognostic value beyond NT-proBNP, while sST2 (described in Chapter 9) did not show to be independently associated with outcomes when adjusted for NT-proBNP. NT-proBNP in these two examples was considered the 'gold standard', as the guidelines for the diagnosis and treatment of pulmonary hypertension recommend the use of NT-proBNP for risk stratification[14]. However, the incremental prognostic value of a novel biomarker should ideally be compared with a model that incorporates other established predictors for disease outcome, such as invasive hemodynamics and imaging measurements. Besides the need to measure all these known predictors in the design and execution of the study, a large sample size and an adequate number of endpoints is required to be able to perform multivariable regression including all relevant predictors.

Overlap of biomarkers

Whether biomarkers have additional prognostic information on top of another biomarker, also depends on the common pathophysiologic pathway or disease reflection that two or more biomarkers may share. Too much overlap may result in a lack of additive prognostic value. On the other hand, correlating biomarkers can give a clue about the pathophysiology of biomarkers, and how these are related to each other. In the BioCon, BioPulse and study with ASD patients (Chapter 5), we found a significant correlation between NT-proBNP and troponin T. In Chapter 3, we investigated serial troponin-T measurements in ACHD patients and found that repeatedly measured troponin-T was not an independent predictor when adjusted for repeatedly measured NT-proBNP. We explained this by hypothesizing that in the development of heart failure, increased wall stress may first lead to an increase in NT-proBNP, which will then be followed by an increase in troponin-T because of emerging myocardial damage. This could explain why troponin-T is not an independent predictor beyond NT-proBNP; though this hypothesis has not been investigated. Notably, hs-CRP was identified as independent prognostic biomarker in the BioCon study when adjusted for NT-proBNP (Chapter 4). Hs-CRP did not show a correlation with NT-proBNP, or measures of systolic ventricular function, while

it was associated with NYHA functional class. The absence of these associations may indicate that NT-proBNP and hs-CRP reflect a different aspect of disease severity, however this should be interpreted with caution, as no evidence is currently available to support this.

Based on the existence of mutual biomarker correlations, it is likely that these biomarkers share some common pathway, albeit for some blood biomarkers the exact mechanism of their prognostic value is not clear. Particularly, RDW has been identified as a strong predictor for cardiovascular outcomes in many different diseases, among which ACHD[11, 12] (Chapters 5 and 7) and PH (Chapter 8), but up to now, the mechanism of the increased distribution in the size of erythrocytes in the pathophysiology of cardiovascular disease in general has not been completely elucidated. Because it has been suggested that the increase in size is the result of miscellaneous mechanisms which in conjunction contribute to a reflection of cardiovascular disease severity, RDW has been proposed to be an 'overall cardiovascular barometer'[15]. In Chapter 7, we showed that RDW correlated with all other blood biomarkers studied in patients with a systemic right ventricle, supporting the hypothesis that RDW gives a broad reflection of the cardiovascular disease state.

Consistency with existing literature

A single study is often not sufficient to change clinical practice and replication of findings is therefore of paramount importance for defining whether a biomarker carries prognostic information. In this context, it is also important that associations between a certain biomarker and outcome are consistent between studies, as well as an equivalence in the strength of the associations. Systematic reviews and meta-analysis can help to strengthen the evidence of biomarkers by summarizing results of studies, or even statistically combining estimates in the case of meta-analyses. In 2012, Eindhoven et al. systematically reviewed all current evidence on the usefulness of brain natriuretic peptides (BNP's) in simple and complex CHD[16, 17], however at that time the available literature mostly consisted of cross-sectional and retrospective studies. The fact that the prognostic value of blood biomarkers in ACHD in longitudinal and prospective studies are relatively scarce, may have prevented experts to involve the use of blood biomarkers for risk stratification in the newest ACHD guideline of the American College of Cardiology/American Heart Association[18], albeit that many recent studies have confirmed the prognostic value of NT-proBNP in ACHD[19-24]. Due to heterogeneity in both ACHD and PH, very few meta-analyses have been carried out, which may hinder the application of evidence-based medicine. The BioCon study has provided evidence for a wide variety of blood biomarkers using prospective longitudinal data, but replications of these findings remain important to accumulate evidence for the clinical use of biomarkers in ACHD.

The difficulty to fairly compare studies and justify their conclusions coheres with the uniformity of the study design and methodology, and the reporting hereof. Methodologic guidelines for assessment of prognostic factor studies can contribute to more insight in the study and therefore enabling better interpretation and comparison of published evidence by researchers. In 2005, reporting recommendations for tumor marker prognostic studies (REMARK)

were published to aim for transparent and complete reporting so all available information is available to readers to allow them to judge the usefulness of the data and the generalizability of the study conclusion[25]. Such reporting guidelines could also be of great value in the field of cardiovascular research as well, and development of guidelines should get attention in the near future.

Causation versus association

Besides prognostic value, blood biomarkers can sometimes also add value to the etiology of the disease. There is a discrepancy in 'prognostic' and 'etiologic' research involving blood biomarkers, where prognostic refers to estimation of the likeliness of a disease outcome given the blood biomarker, while etiologic postulates a causal relationship between the blood biomarker and a given disease outcome. For blood biomarkers to be useful for risk stratification, it is not a prerequisite to have causal involvement in the pathophysiology of the disease of interest. Blood biomarkers can be very good predictors, whilst being an 'innocent bystander' in the pathophysiology of the disease. The Biomarker Definitions Working Group has proposed to differentiate between so called 'risk markers' and 'risk factors', where risk markers are referred to as markers which are associated with the disease on statistical grounds, but are not necessarily causally linked, while risk factors are associated with the disease because they are part of the causal pathway.[26] However in practice, the term risk factors and risk markers are often used interchangeable. Demonstrating the causal involvement of a biomarker is not possible based on observational studies, and is therefore hard to prove. Causal inferences are sometimes tried to establish using advanced methods and statistical analysis, such as Mendelian randomization[27]. This technique mimics randomized controlled trial using genes as an instrument for randomization which minimizes confounding, although it can still not prove a definite causal relation between the exposure and a certain disease. The great advantage of identification of biomarkers that are causally related is the potential utility as a target for treatment.

While the aim of the studies described in this thesis were mainly focused on the prognostic value, blood biomarkers can help to gain insight and contemplate about ongoing pathological processes. Troponin T is famous for its role in the diagnosis of acute coronary syndrome, where troponin T is released in response to cardiomyocyte injury. In 2015, Eindhoven et al. cross sectionally studied troponin T in the BioCon cohort, and found elevated troponin T levels in almost 10% of the patients. In Chapter 3 we studied temporal patterns of troponin T in these patients, and we observed that troponin T levels increased over time in all patients. This may reflect ongoing subclinical loss of cardiomyocytes in ACHD patients over time, potentially contributing to the progression of heart failure in the long run. In Chapter 5 we studied blood biomarkers following percutaneous ASD closure in adults and studied levels of six biomarkers before and after closure. We measured the different blood biomarkers before and multiple times after closure of the ASD, which enabled us to study the influence of ASD closure. We observed a significant increase in most blood biomarkers directly after closure, however we

were not able to distinguish biomarker release induced by intervention-associated actions or by a cardiac origin.

Bias in observational studies

The study designs used to retrieve the results and conclusions of this thesis are primarily prospective observational cohort studies. Cohort studies are amenable to several biases that can be subdivided into selection bias, information bias, and confounding bias. Bias distorts the associations found in a study and may subsequently lead to false inferences and conclusions. In that perspective, whether the evidence of a certain biomarker adds prognostic information is valid, depends on potential biases that the study may be subjected to.

Selection bias in cohort studies arises when there are certain patients that are not willing to participate, or when there is exclusion or restriction of certain patients, leading to a distortion in your study sample with respect to the target group of your research. In the BioPulse study (described in Chapters 8-10) patients with PH due to left heart disease were excluded while it could have been interesting to study these patients. However, inclusion of these patients would likely have provoked selection bias. We exclusively enrolled patients who underwent right heart catheterization, and in patients suspected of PH due to left heart disease, there is no indication for a right heart catheterization because the test result will not affect therapeutic decisions. Patients with PH due to left heart disease that would have ended up in our study, would therefore consist of a selection of patients with an unclear clinical picture, only mild or ambiguous echocardiographic images, or any reason that may have driven the decision to perform a right heart catheterization. The BioCon study only included patients with moderately and severely CHD, and therefore the results of this study are not generalizable to the entire ACHD population, in particular patients with mildly CHD. Both the BioCon study and the BioPulse study had a neglectable number of patients that were lost-to-follow-up making bias due to selective follow-up very unlikely.

Information bias occurs when there is inaccuracy in the measures or classifications that are used in the study, and occurs during the data collection of the study. As previously discussed, measurement error is inescapable but does not implicitly introduces bias; this depends on the presence of the type of measurement error, and how the measurement error is distributed. In the cohort studies described in this thesis, information bias was prevented by blinded assignment of event endpoints; events were assigned to patients by two independent researchers without knowledge of biomarker levels. Misclassification in the outcome assessment of our study, is therefore likely to be equally distributed in both patients with high and low biomarker levels.

Confounding bias is mainly distorting conclusions of causal inference and it is therefore discussable whether prognostic biomarker studies are exposed to confounding bias. The main reason for adjustment of variables in our studies was to investigate the independent prognostic value of blood biomarkers, as this helps to determine whether they can be useful in clinical practice on top of measurements, or information that is regularly obtained. However, confounding bias can distort the association found, for instance when women have biologically

higher biomarker levels. This is what we observed in Chapter 2 where we studied the prognostic value of sST2. Analysis adjusted for age and sex lead to a stronger association between sST2 and the study endpoints. This can be explained by lower levels of sST2 found in women than in men, and women also being somewhat older than men, while the risk of the endpoints is approximately equivalent in both sexes. There is confounding bias because age and sex can in this situation lead to an underestimation of the real association between sST2 and adverse clinical outcomes. Adjustment for confounders should therefore also be based logical reasoning, and can be important to obtain unbiased estimates.

III. Will the biomarker help the clinician to manage patients?

One of the final key aspect of a biomarker, and definitely assumed most relevant for clinicians, is whether the biomarker is able to help in the clinical management of patients. This aspect, what can be seen as the final step in the 'biomarker pipeline', is seldomly assessed, as it often requires large randomized controlled trials. Moreover, to prove efficiency of a biomarker in the management of patients, treatment thresholds should first be determined, requiring knowledge of abnormal biomarker levels, or in the case of repeated biomarker measurements, an abnormal change. There may be other methodologic features that hinders interpretation of results and subsequently its associated clinical value.

Defining abnormal biomarker values

Decisions in clinical practice are mostly based on treatment thresholds, which informs clinicians whether they should initiate a certain treatment based on dichotomization of a quantitative test. For biomarkers to aid clinical decision making, it is important that clinicians have knowledge of 'abnormal' levels so a clinical interpretation can be deduced from the biomarker measurement. There as several approaches that can help interpreting and defining abnormal biomarker levels[28].

Levels of a certain laboratory measurement can be studied in the 'healthy' population, which is often referred to as reference values. Reference values for laboratory measurements are based on a representative 'healthy' study sample, ie. a reference group of people that are free of the disease or condition that may cause abnormal levels. Reference values do not imply decision thresholds for a certain medical condition, but are rather a descriptive of a specific (reference) population derived from a reference distribution[29]. The 95th or 97.5th percentile from the reference distribution is often chosen to define abnormal levels[30]. In this thesis, we determined reference ranges for soluble ST2 and GDF-15 based on measurements in healthy individuals from the Navigator study (Chapters 2 and 10). The Navigator study consisting of volunteers who were used as controls to establish reference levels, and to allow comparison of these levels to the ones found in patients. In Chapter 10 the distribution of GDF-15 levels in

these healthy controls was compared with the distribution of GDF-15 in PH patients by a Kernel density plot. The upper reference limit, equal to the 97.5th percentile of GDF-15 in the control subjects, was determined and it was shown that patients with PH with a GDF-15 level within the reference limit, had a significant better survival than patients with GDF-15 levels above the upper reference limit. Based on the GDF-15 measurements in control subjects, we could therefore carefully conclude that GDF-15 levels within the reference range can be helpful to identify PH patients with a good prognosis. However, it should be kept in mind that reference ranges are depending on the reference sample from which they are measured, as well as the laboratory technique, which can limit its generalizability. Besides population-based reference values as described above, reference values can also be based on previous values from the same individual in a certain state of health, which comes down to a subject-based approach[31]. In Chapter 4 where we studied repeated hs-CRP measurements in ACHD patients, the index of individuality indicated that each patient should have its own hs-CRP reference value rather than a population-based approach, because of the large biological variation and the repeatedly measured aspect of laboratory blood biomarkers. Differently from reference values, decision thresholds using a cutoff recommend a certain decision based on whether the value is below or above the threshold, and is not based on a reference population. These discrimination limits can be determined using receiver operating characteristic (ROC) curves, which tells you what the most optimal cut-off is to discriminate between patients with and without the outcome of interest[32].

The use of biomarker cutoffs for prognostication purposes remains a little controversial. The advantage of a cutoff is its clear-cut interpretation for clinicians and the easy applicability in daily clinical practice[33]. However, the downside of dichotomizing continues levels is that 'true' cutoffs may not exist for prognostic factors, as the risk relationship is ultimately almost never discontinuous in blood biomarker research. The practical aspects of cut-off values for clinical interpretation can therefore be conflicting with the desired statistical methodology.

The use of composite endpoints

The use of composite endpoints in clinical studies is often chosen to achieve higher event rates with the need of lower sample sizes, costs, and time. The use of a composite endpoint, is therefore often driven by (statistical) efficiency rather than clinical meaningfulness. Composite endpoints can seriously hinder the interpretation and subsequently the clinical usefulness of studies[34]. For instance, there can be a difference in the importance of the endpoint to the patient (for instance when death is placed alongside non-fatal events), as well as a difference in the frequency of the occurrence of individual event components. Especially in randomized clinical trials this can led to false inference of the effectiveness of therapies[35], but also in observational studies the use of composite endpoints can for instance hinder the clinical implications of the study results[36].

The BioCon study (described in Chapters 2-4, 7) was initially powered on 4 years of follow-up, with an expected event rate in of cardiovascular events and mortality of 25% after 4 years of

follow-up based on the Euro Heart Survey[37], the sample size was estimated at 600 patients to be able to adjust for ≈14 covariables[23]. In this thesis, follow-up on endpoint events was extended, adding up to a median follow-up of approximately 6 years. This resulted in a higher number of any cardiovascular events, and at the same time allowed us to adjust for more potential confounders in the association between blood biomarkers and death or heart failure, which can be considered a more clinically relevant and 'harder' endpoint than any major adverse cardiac event. In Chapter 4 where we studied the prognostic value of hs-CRP, we chose after careful consideration and based on the above-mentioned reasons, to define death or heart failure as the primary endpoint.

The risk prediction model described in Chapter 12, was developed to predict the 1-year risk of death, heart failure, or arrhythmia in adults with moderately and severely complex CHD. This composite endpoint was chosen to have enough statistical power for proper model development; we were able to select 14 candidate predictors for model development based on ≈ 140 events, and thus minimizing the risk of overfitting of our model. However, it must be acknowledged that the importance of the three separate events is not equivalent, and the therapeutic and diagnostic strategies may differ for each the risk of each endpoint. The use of a composite endpoint therefore could limit the clinical utility of the model when used to adjust a patient's care. There are also other solutions to overcome the lack of statistical power for multivariable analysis in setting with a limited number of events[38], for instance shrinkage techniques such as LASSO[39] and ridge regression [40]. With the introduction of online free statistical software programs such as R, these techniques have become more widely available to researchers, lowering the threshold to apply it to medical research.

In contrast, the use of a composite endpoint can help to bypass the problem of competing risks in survival analysis. Competing risks occur when the occurrence of a certain event, precludes the occurrence of the event of prime interest[41]. For example, once a patient has deceased, the patient can no longer be hospitalized for heart failure. Cox regression analysis assumes that patients are at risk of the event of interest till they either get censored or experience the event of interest. This is the reason we decided to adjudicate endpoints independent of each other in the Biocon study, i.e. patients were not censored after the occurrence of their first endpoint event but were still considered at risk for all other endpoints. However, analysis with a single endpoint such as heart failure hospitalization, requires an extended survival analysis method (e.g. competing risk model), but should preferably not, or only with great caution, be analyzed without the inclusion of mortality or other competing events in the form of a composite endpoint.[42]

Defining a clinical relevant change

With the assessment of biomarkers changes over time within an individual patient, it is of paramount importance to distinguish random or biological variation from a true change in biomarker level, which may reflect a change in the clinical disease state of the patient. But how

does a clinician now what a 'real' change is? The assessment of changes in serial longitudinal measurements over time demand attention and wariness, as does its interpretation by clinicians.

First of all, a statistical phenomenon called regression towards the mean can be provoked when studying, or interpreting, the change between two measurements without keeping variability in mind. As measurements fluctuate around a true mean because of random error, extreme values tend to regress towards the mean when repeatedly measured[43]. The observed decrease or increase is not reflecting a real change, but is rather due to statistical chance. Regression towards the mean can therefore bias study results and can result in wrong conclusions about the effect of a treatment, if it is actually due to chance[44]. In Chapter 3, the phenomena of regression towards the mean is clearly visible by the high baseline troponin-T level in the group of patients who showed a decrease in troponin T in the first year, while median baseline troponin-T levels were lowest in the patients with an increase in troponin T. Therefore, no interpretation and emphasis was given to the 1-year change in troponin-T in this study.

Second, the advanced statistical analysis used to investigate repeated measurements to overcome problems like regression towards the mean, are often difficult to interpret by clinicians. Linear Mixed Effects models are able to account for both the within and between-subject variability, by capturing the correlation within the random error terms. In Chapters 3 and 4, we dealt with the biological variation and regression towards the mean using Linear Mixed Effects models to describe the temporal evolution of repeated troponin T and CRP measurements. While this technique offers opportunity in medical research to investigate repeated measurements, nowadays a clinician is just facing 'two measurements' in daily clinical practice. In a non-research setting, it is therefore hard to distinguish a real and clinical relevant change, from random fluctuations not reflective of a change in the disease of the patient. The Reference Change Value (RCV) can be helpful to distinguish a real change, from a change owing to random and biological variation[45]. However, whether the real change also can be considered a clinically meaningful change that can help decision making, is a next step that should be considered. In Chapter 4 we calculated the RCV for hs-CRP based on repeated hs-CRP measurements in ACHD patients free of death or heart failure. The RCV was relatively large, indicating that the difference between two subsequent hs-CRP measurement should be substantial to reflect a change in the disease status of the patient. However, it should be acknowledged that the BioCon study was not designed for this specific purpose. While repeated biomarker measurements seem to be of additive value and can help to determine prognosis in ACHD patients, much more attention should be given to techniques how to interpret and use this in daily clinical practice.

Repeated biomarkers and survival outcomes

Studying longitudinal outcomes, like repeated blood biomarker measurements over time from the same individual, alongside time-to-event outcomes such as death, requires some additional precautions. As blood biomarkers are endogenous time varying variables (ie. internal;

measurements are directly related to a patient and thus depending on other variables) and not exogenous time varying variables (such as the weather; a variable not affected by other variables in the model), the measurements are measured with error (biological variation) and the availability of the measurement is depending on the failure status of the patient; no measurements can take place once the patient has died and therefore the presence of a blood biomarker measurement implicitly states that the patient must be alive at that time. That is why, contrary to exogenous variables, endogenous variables cannot be analyzed with a time-varying covariate in an extended Cox regression analysis. Joint modelling has been proposed as a relatively new statistical technique to relate endogenous longitudinal outcomes to a survival analysis[46, 47]. The downside of using advanced statistical techniques can be the interpretation of these advanced statistical analyses in the context of clinical research. Clinicians are often the main audience of clinical studies and interpretation of these analyses can be difficult as it requires a statistical background. Also, equivalent to most analysis, the results of this analysis represent the average patient in the study, and inferences for individual patients should therefore be made cautiously.

While the results of this thesis support the additional value of repeatedly measuring blood biomarkers over time in ACHD patients, the most optimal frequency of measuring blood biomarkers by clinicians is difficult to determine and should be investigated in further studies. It has been proposed to measure NT-proBNP annually in ACHD patients[23, 48].Yet, individualized instead of predefined measurement intervals, may lead to a more efficient use of blood biomarker measurements. Joint Modeling can be used to incorporate information from prior measurement to estimate the most optimal timing of the next measurement[49]. It has been demonstrated that individualized screening intervals for measurement of NT-proBNP in chronic heart failure patients, can lead to a more efficient use of NT-proBNP measurements; the information gained by a new measurement is optimized and so fewer measurements are required, saving costs, and reducing burden for patients[50]. While these are all very promising findings, it will require a lot of effort in order to be suitable and usable by clinicians in daily clinical practice.

Integration of biomarkers into risk prediction models

A prognostic factor can be any variable that is associated with the risk of a certain outcome, and various routinely collected patient characteristics can therefore serve as prognostic factor, such as; age, heart rate, symptoms, medication use, and a patient's medical history. A single prognostic factor is rarely adequate to accurately estimate prognosis and therefore integration of blood biomarkers in prediction models is essential. Prognostic risk prediction models enable incorporation of multiple prognostic factors to estimate the risk probability of a certain outcome to occur within a specified time period, given the predictor profile of an individual patient.

In Chapter 12 the development of a risk prediction model for moderately to severely complex ACHD patients was described. The final prediction model included 5 out of 14 pre-specified predictors; NYHA functional class, cardiac medication use, re-intervention after initial

corrective repair, body mass index, and NT-proBNP. This prognostic risk prediction model showed to be valid in an external cohort of ACHD patients, and provided accurate estimates for the 1-year risk of death, heart failure or arrhythmia. As a Supplemental file to the main manuscript, we provided sextiles of predicted risks together with the predictor values in each of these groups. This obviously showed that patients in the highest sextile of predicted risk, had a less favorable patient profile; they were more often NYHA class >I, used more often cardiac medication and had more often had a reintervention, and they had on average a higher body mass index and NT-proBNP level. The advantage of a risk prediction model is that it provides absolute probabilities of a certain outcome, and integrates individual prognostic factors (ie predictors) to estimate prognosis. This may help clinicians to get a more complete clinical picture and to tailor information to the individual patient. Whether a risk prediction model improves clinical decision making and patient outcomes, requires implementation and thorough investigation of use of the model in daily clinical practice, most preferably investigated in a randomized-controlled trial setting.

In Chapter 8, we aimed to establish the prognostic value of six biomarkers and investigated a prognostication solely based on blood biomarkers. In this multi-biomarker approach, we calculated how many biomarkers in each patient were elevated. Besides information that is lost by dichotomization of biomarker values, this pragmatic approach also indirectly assumes that each elevated biomarker has an equal contribution to the accumulating risk, which is highly unlikely. It should therefore be acknowledged that this analysis has its flaws; however, the statistical power was insufficient to allow us to adjust for all continuous blood biomarkers in multivariable Cox regression, which would be the preferred technique. As an additional analysis, we assessed the prognostic value of each blood biomarkers independent of the so called "REVEAL risk score". This risk score has been developed in a large cohort of PH patients based on demographics and clinical characteristics, to identify patients at low, intermediate, and high risk of death[51]. A risk score is a simplification of a risk prediction model, where a numerical value is assigned to each risk factor and accumulated into a final score, which is then linked to a certain risk category. The advantage of a risk score is its more pragmatic approach, allowing clinicians to easily derive an estimation of the prognosis of a patient with a certain risk profile. None of the biomarkers in Chapter 8 showed to have an independent prognostic value beyond the REVEAL risk score. However, this does not imply that blood biomarkers are worthless; the REVEAL risk score consists of invasive hemodynamic measures, and the inconvenience associated with the assessment of these invasive measurement, may hinder the usefulness of the REVEAL risk score in the clinical follow-up of patients. Moreover, each separate blood biomarker investigated in Chapter 8 was associated with the risk of death or lung transplantation, indicating that these biomarkers do contain prognostic information in adults with PH of various etiologies. It would be interesting whether blood biomarkers can be surrogate markers for more invasive markers, which can reduce patient's burden.

Adult congenital heart disease

Blood biomarkers in specific CHD diagnoses

As CHD diagnoses have certain clinical and hemodynamic features, CHD may pertain differences on a cellular or molecular level and therefore differences in the prognostic value of blood biomarkers across the spectrum of ACHD diagnoses may exist. In addition, patients with the same CHD diagnosis, still differ greatly due to for instance the surgical technique and outcome, or the concomitant presence of other anomalies. Each type of CHD induces specific loading conditions, myocardial adaptations, and may trigger other pathways of cardiac remodeling. Although, these are merely suppositions, as little is really known about these pathways.

Subgroup analyses were performed in the BioCon study to investigate differences in blood biomarkers per CHD type. In Chapter 7, we explored the prognostic value of several blood biomarkers in adults with a systemic right ventricle because of a transposition of the great arteries. We found that growth differentiation factor-15 (GDF-15) was the strongest prognosticator for the risk of heart failure or death, even stronger than NT-proBNP. The prognostic value of GDF-15 had not been specifically addressed in adults with a systemic right ventricle before, however in a previous publication from the BioCon study including moderately and severely complex ACHD, GDF-15 was associated with the heart failure-free survival, but this association was less strong than for NT-proBNP[23]. NT-proBNP is known to be released in response to pressure and volume overload of both the left and right ventricle[52]. Patients with a systemic right instead of left ventricle, may have another biomarker expression of NT-proBNP, since the right ventricle will be permanently exposed to a severe pressure overload. Only 11 sRV patients (13%) had a NT-proBNP ≤14 pmol/L at baseline, which is a much lower percentage than found in the overall BioCon study (47%). The already abnormal NT-proBNP levels present in most patients with this severe type of CHD may account for the limited prognostic value of NT-proBNP in sRV patients. In sRV patients, GDF-15 may therefore be a more suitable prognostic biomarker, and in general, clinicians should consider the use of blood biomarkers in the regular follow-up of sRV patients. Currently echocardiography is the key tool in the routine follow-up of these patients, while on the basis of our results blood biomarkers seem to perform better, while also being cheaper and less time-consuming than echocardiography. In Chapter 2, that described the prognostic value of soluble ST2 in all ACHD patients from the BioCon study, a difference in prognostic value of soluble ST2 was noticed between moderately and severely complex ACHD; soluble ST2 seemed especially to be a good prognosticator in severely complex ACHD patients.

Confirmed by a study of Baggen et al. an elevated NT-proBNP identifies ACHD patients with a high risk of adverse cardiac outcomes, while a low NT-proBNP identifies patients with a very good prognosis[23]. This cut-off of >14 pmol/L is based on a recommendation by the guideline for chronic heart failure patients presenting with non-acute symptoms[53]. Figure 1 shows the cumulative incidence of death or heart failure in the BioCon study according to a

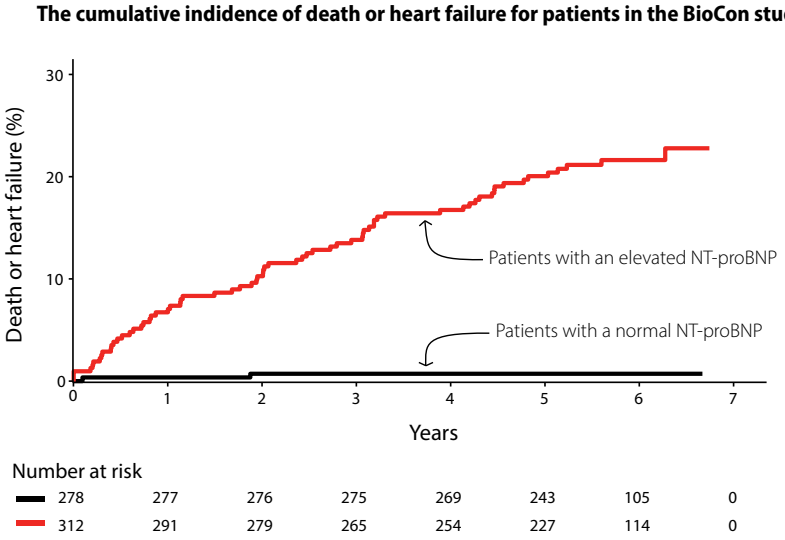

Figure 1. The cumulative incidence of death or heart failure in the BioCon study for patients with an elevated NT-proBNP (> 14 pmol/L) at the baseline visit of the study versus patients with a normal NT-proBNP at baseline.

normal or elevated NT-proBNP over a follow-up of 6 years, which clearly shows the increased risk for death or heart failure in patients with an elevated NT-proBNP. In Chapter 6, levels of NT-proBNP and high sensitive CRP (hs-CRP) were studied in 183 adults with a bicuspid aortic valve (BAV). Isolated BaV is considered a CHD of mild complexity, and was therefore not explicitly included in the BioCon study. While adults with BAV have a life-expectancy almost comparable to the general population[54], 20% of the patients in the BAV study had an elevated (>14 pmol/L) NT-proBNP. Similarly, in Chapter 5, where we studied biomarker levels in adults undergoing percutaneous atrial septal defect (ASD) closure, a relatively high proportion of patients had an elevated NT-proBNP level, whereas patients who undergo ASD closure at adult age have a survival comparable to the general population[55]. In detail, 22 ASD patients (45%) in this study had elevated NT-proBNP level before closure, and 1 year after percutaneous closure NT-proBNP was still elevated in 40% of the patients. Eventhough we did not collect long-term follow-up data from the studies described in Chapters 5, it seems unlikely that an elevated NT-proBNP in patients with an isolated ASD is associated with a prognosis similar to patients with moderately to severely complex ACHD in the BioCon study. Hence, cut-off levels of biomarkers for clinical decision making may require a diagnosis-specific approach rather than a population broad-approach. Ultimately, the results of this thesis support the clinically usefulness of blood biomarkers for risk stratification in ACHD, but does not undermine the clear need for diagnosis-specific studies to further adjust the usefulness of biomarkers to the individual patient (group). Moreover, clinicians should nog stare blindly on normal versus elevated levels, but should take into account the actual level of the biomarker, since the risk increases with increasing levels of the biomarker on a continuous scale, as previously discussed. For patients with low NT-proBNP

levels (<14 pmol/L), there seems no need to measure additional biomarkers as these patients already have a very low risk of death or heart failure. The identification of low-risk patients is very valuable and important, as this information can be used to lower the frequency of follow-up visits lowering patient burden, as well as to reassure patients helping them to decrease disease-associated stress and anxiety.

Single versus repeated biomarker measurements

Most studies focus on a single biomarker measurement when investigating the prognostic value of blood measurement in patients. In clinical practice, blood biomarkers may repeatedly be measured during each regular follow-up visit, supplying new information about a patient's disease status. It is presumable that the course of the biomarker over time provides additional information to the actual level of a single measurement. Baggen et al. demonstrated the additive value of repeatedly measuring NT-proBNP in the ACHD patients of the BioCon study[48]. With the use of the BioBank, we were able to investigate annually repeated measurements of high-sensitivity (hs) troponin-T and hs-CRP. Based on the studies described in Chapters 3 and 4, we found that both repeatedly measured hs-troponin T and hs-CRP provide additional information compared to a single measurement for the risk of cardiovascular events. While repeated hs-CRP measurements were significantly associated with death or heart failure independent of repeated NT-proBNP, repeated hs-troponin T did not provide prognostic information beyond repeated NT-proBNP. Thus far NT-proBNP seems to be the most promising blood biomarker in ACHD, and clinicians should strongly consider measuring NT-proBNP in the regular follow-up of ACHD patients. Defining the most optimal timing of the frequency of repeated NT-proBNP remains to be answered, however based on the studies thus far provided, an annual measurement interval for patients with a high NT-proBNP level (14 pmol/L) seems appropriate. As discussed above, attention should be given to the interpretation of repeated blood biomarker measurements and the biological variation that may impede the interpretation of a clinical relevant change in an individual patient.

Risk stratification beyond blood biomarkers

This thesis mainly focused on the prognostic value of blood biomarkers, and how these markers can contribute to risk stratification in ACHD. However, besides blood measurements other measures such as echocardiography, cardiopulmonary exercise testing, and ECG characteristics, are of major importance in the follow-up of patients to monitor disease severance and prognosis. In Chapter 13 an overview has been given of factors that could contribute to risk stratification in ACHD and how these different modalities should be included in the regular outpatient clinical follow-up mildly, moderately and severely ACHD patients. The frequency of these assessments is proposed, and it is notable that no distinction has yet been given to the difference in the complexity of the CHD and the frequency of NT-proBNP measurements. This is mainly because of absence of evidence to distinguish between the different diagnoses, rather than the absence of a difference in its clinical usefulness. In the BioCon study we did not

have access to all data relevant to risk stratification of ACHD patients, such as social economic status, and modalities like cardiac MRI and cardiopulmonary exercise testing, and therefore we could not assess the independent prognostic value of NT-proBNP in the scope of these entities. However, there lies a complementary role for blood biomarkers, in particular NT-proBNP, to contribute to the regular multimodality assessment of ACHD patients.

Classification of CHD types and risk stratification

ACHD covers the complete variety of cardiac anatomies and therefore consists of a divergent patient population. It is known that the prognosis varies with the type of ACHD and the Bethesda classification of congenital heart defects (CHD) recommended by the guidelines into mildly, moderately or severely complex CHD may help to define the clinical management and may give some sort of prognostic estimation, although it is not specifically designed for this purpose[18]. The (modified) Bethesda classification has been described in Chapter 13. The distinction of these three subgroups may not be sufficient to cover the real heterogeneity in disease severity and subsequently prognosis, determination of follow-up frequency, and treatment strategies. This is supported by the findings of our study considering the development of a risk prediction model described in Chapter 12. The developed ACHD risk prediction model did not include the complexity of the CHD itself, as this was not an independent predictor of the occurrence of arrhythmia, heart failure or mortality in our study. This may support a lack of accuracy in this subdivision for risk stratification purposes. Reflection of disease severity given by variables that vary more with a patients' clinical status, such as NYHA functional class, or other indices for the perceived disease status of a patient, may be more useful for risk stratification. This is supported by a study from Ombelet and colleagues in which they compared serval disease severity and functional indices, among which the Bethesda classification, NYHA functional class and Congenital Heart Disease Functional index (CHDFI). They found that NYHA functional class as well as the CHDFI are better predictors of all-cause mortality than the Bethesda CHD anatomy classification[56]. The new guidelines for the management of adults with CHD published by the American College of Cardiology in 2018 suggest a classification based on a combination of both the type of CHD and the physical state of the patient[18]. This classification is based on expert consensus rather than evidence-based. Recently, the predictive value of this new classification has been assessed by Ombelet et al. and showed that combining the CHD severity with the physical state of a patient indeed improves its predictive value for long-term survival, however it did not perform better than the CHDFI[57]. Further studies should assess whether the prediction model developed in this thesis performs better than these indices, which can further point out the usefulness of this tool for risk stratification in the routine follow-up of ACHD patients.

Implications for the clinical practice

Adding up all currently existing data and evidence considering blood biomarkers in ACHD, NT-proBNP should be recommended in future guidelines for the management of ACHD in the regular follow-up. Besides NT-proBNP, other blood biomarkers can be valuable for risk

stratification in patients with ACHD, and should therefore be considered in the regular follow-up of ACHD patients. For instance, in patients in who the clinical picture may not give a clear-cut view on the disease status and subsequent prognosis of the patient. It is important to stress that blood biomarkers do not replace the need of other modalities, such as echocardiography, to assess prognosis in ACHD. However, the value of blood biomarkers in for instance patients with complex anatomy in who echocardiographic imaging is challenging, should not be underestimated. As blood biomarker measurements are subject to biological variation, measurement error and sometimes extra-cardiac diseases, it is of key importance to always interpret blood biomarker levels in the broader context of the patient.

Although more evidence is first needed to further support the prognostic value of relatively novel blood biomarkers in ACHD, the results of this thesis show that blood biomarkers are a very promising modality to assess prognosis in ACHD and this topic deserves more attention by both researchers and clinicians in the coming years. Online applications helping clinicians with the interpretation of repeated biomarker measurements may be key to aid in the interpretation of what a clinical meaningful change is, so repeated blood biomarker measurements easily and accessibly be used by clinicians.

As risk stratification should never be solely based on a single parameter, incorporation of blood biomarkers in risk prediction models including other measurements based on for instance echocardiography and patients' symptoms, are of paramount importance. Application of models that can help clinicians to combine patient information into individualized risk estimations are important for further adjust personalized follow-up and treatment strategies.

Pulmonary hypertension

This thesis mainly focused on cardiac-related biomarkers and their association with prognosis in adults with pulmonary hypertension (PH). In Chapters 8-10, we investigated baseline blood biomarker levels in PH patients, and plotted the biomarker levels according to the subgroups of PH. Although sample sizes of some subgroups were tiny and this observation should therefore be interpreted with caution, different etiologies of PH seem to have different biomarker expressions. The downside of studying biomarker levels on patient-level, is that the origin of the biomarker release cannot be detected. Therefore, the biomarker differences that we observed can have multiple explanations. One reason could have been the different underlying etiology leading to differences in biomarker releases, but the differences can also be reflections of disease severity and therefore higher or lower biomarker expressions, as the prognosis strongly varies among the different types of PH[58]. While all biomarkers discussed in Chapters 8-10 have been related to prognosis in cardiovascular disease, this does not preclude that these biomarkers always reflect cardiac burden. In Chapter 10, we studied the prognostic value of GDF-15 in PH patients of mixed etiologies. GDF-15 is a nonspecific biomarker that plays a role in cell growth and differentiation, and has been associated with mortality in cardiovascular diseases as well

as in other diseases among which cancer[59, 60]. We hypothesized that the non-specific nature could be of help to overcome the heterogeneity and comorbidities that influence prognosis in PH. The results of this study showed that GDF-15 was able to identify patients with a very low mortality risk in the first two years after their diagnosis, independent of NT-proBNP. GDF-15 requires further research to investigate its potential role besides the regular assessment of NT-proBNP in PH.

The current results included in this thesis were based on the first 106 patients that were enrolled in the BioPulse study. Continuation of patient enrollment over the coming years will result in a larger sample size, seizing opportunities to perform subgroup-specific analysis. Currently, risk stratification in PH often focuses on pulmonary arterial hypertension (PAH) in particular. Our studies suggest that the value of blood biomarkers for risk stratification is not restricted to PAH, but can be useful in other subgroups as well. However, attention should be given to differences in prognostic value among the different PH subtypes.

How biomarker levels evolve over time in adults with pulmonary hypertension in the BioPulse study remains to be answered. The study protocol includes half-yearly blood sampling during the first 4 years after study enrollment. These data are thus available, but should be analyzed in future studies. One of the primary aims should be how biomarkers respond to treatment initiation in CTEPH and PAH patients, and whether blood biomarkers can guide treatment in terms of timing of uptritration with specific PH medication as well as heart-failure targeted therapy with diuretics.

Future perspectives

The role of (inter)national biobank studies

In contrast to the studies described in this thesis in which biomarkers were measured with individual assays, nowadays new assay techniques allow to measure a whole panel of biomarkers at once. These biomarker panels help to seek for novel biomarkers on a large scale and may even further increase the influx of blood biomarkers in medical research. As BioBanks are taking care of storage of blood samples on a large scale, this gives opportunities for postponement of biomarker measurements. Storage of these blood samples have proved very useful for newly detected biomarkers, but also facilitate collaborations between studies accompanied by blood sample storage for the determination of novel markers. In the future, it can be expected that these large BioBank studies including an extensive collection of blood samples, will continue to contribute to important blood biomarker research. Both the BioPulse and BioCon study attained the large collection of blood samples in a BioBank, which seizes new opportunities for blood biomarker research in the near future.

Is machine learning going to change risk prediction?

With the introduction of electronic patient records, a growing volume of individual patient and medical-related data, machine learning is starting to find its way to the field of medicine. The big advantage of machine learning is that it does not require structured data and therefore is not depending on manual collection of data, and so is less time consuming. The downside of machine learning is sometimes its lack of transparency in the creation of the networks and obtainment of results, specifically for clinicians who lack knowledge in machine learning but at the same time are the target audience of these studies. Artificial intelligence, the use of computer systems to fulfill tasks which normally requires human knowledge, has recently entered the field of ACHD. In 2019, Diller et al. were the first to provide evidence for the clinical usefulness of machine learning algorithms in ACHD [61, 62]. Diller and his colleagues used data from over 10,000 patients to develop a machine learning algorithm to predict drug therapies in ACHD based on symptoms, clinical status, and drugs utilization[61]. To put in perspective; the results of this thesis are mainly based on data of 602 ACHD and 106 pulmonary hypertension patients that were manually collected, and can be regarded relatively large sample sizes.

Focusing on blood biomarkers, machine learning may be helpful for the analysis of large number of large biomarker panels, and a more data-driven discovery of novel blood biomarkers and their value. In this thesis we mainly tried to combine several biomarkers and assess their prognostic value based on statistical regression methods. It would be of great interest whether machine learning can uncover new blood biomarkers, or even reveal novel pathways or pathophysiologic process. Cluster analyses is also a technique sometimes used to identify novel ways of grouping. For instance in PH this can be valuable to gain insight in the different types of PH. A cluster analysis performed in 252 idiopathic PAH patients based on clinical, hemodynamic, and echocardiographic assessments, identified 4 distinct phenotypes of idiopathic PAH[63]. Identification of these different phenotypes are of importance, as they can contribute to further improvement of therapies and adjustment of patient's management. Machine learning may in the future help to identify or group patients based on a bulk of information. Recently, machine learning was used to identify patients with prevalent atrial fibrillation on the basis of 40 common cardiovascular biomarkers [64]. This data-driven assessment of cardiovascular biomarkers identified five robust biomarkers for the detection of atrial fibrillation. Besides the common biomarkers age, sex, body mass index and BNP, a less common blood biomarker seemed an important predictor; fibroblast growth factor-23. This study supports the usefulness of machine learning to identify novel important biomarkers, which at first may not have been associated with a certain condition or disease.

Besides the discovery of blood biomarkers, there may also be a role for machine learning in the interpretation of blood biomarkers in the greater context of a patient. For instance the development of an algorithm which automatically uses the previous blood assessments and a patients' history to interpret the new laboratory assessment, followed by clinical implications for the specific patient. It is difficult for clinicians to compare multiple previous laboratory measurements to the current laboratory measurements; apart from the challenging in

interpretation, this is very time consuming. Hence, there may be a role for machine learning to support clinical decision making based on repeatedly measured blood biomarkers in specific.

The geriatric ACHD patient

As the distribution of patients with CHD has shifted from children to adults in the past decades, and the survival prospective of ACHD patients may continue to increase, the shift of the epidemiology of the CHD population has not come to an end. In the future we can expect more patients with CHD that will be aged over 60 years old, demanding increasing care for the geriatric ACHD patients[65]. The prevalence of acquired heart diseases, such as coronary artery disease, will likely rise, and so will other diseases which are associated with increased age.

However, besides these more predictable diseases, we may not foresee all problems that can become prevalent in the older ACHD patient[66]. An example from the last decade is the increasing prevalence of liver diseases in patients with a Fontan circulation, now also known as Fontan Associated Liver Disease (FALD)[67]. While these patients are relatively young and therefore not really qualifying as 'geriatric', their increased life expectancy has uncovered other extra-cardiac frailties. The impact of long-term sustained abnormal cardiac circulation, may cause concomitant pathologies that may currently be unknown and will manifest. This shows that the field of ACHD is dynamic and demands ongoing research to keep up with this fast-changing field.

Main conclusions

The aim of this thesis was to address the prognostic value of blood biomarkers in patients with ACHD and in adults with PH, and how these blood biomarkers can contribute to improved strategies for risk stratification. The ideal biomarker should be low in costs, easily and accurately measurable, have a low biological variation, and should show strong and consistent associations with clinical events. Blood biomarkers should be used and interpreted in clinical practice accompanied by other modalities to reach the most adequate prognostication, and risk prediction models can be an easy tool to guide clinicians in the use of these modalities. The interpretation of repeatedly measured blood biomarkers that clinicians may be increasingly facing as biomarkers are making their entrance in the regular follow-up of patients, demands attention and further development of tools to aid interpretation.

In ACHD, we advise to measure NT-proBNP at least once in all moderately to severely complex ACHD patients, and include NT-proBNP in the regular follow-up of these patients. In particular for patients with high NT-proBNP levels (>14 pmol/L), it is desirable to measure NT-proBNP frequently during the regular follow-up of these patients. More research and longitudinal data are needed to establish the role of NT-proBNP in ACHD of mild complexity, such as patients with a bicuspid aortic valve. Besides NT-proBNP, this thesis also provided evidence for novel blood biomarkers that have potential to optimize risk stratification in

ACHD, however this will require further research and replication of these findings by other study cohorts to establish firm evidence whether these blood biomarkers can contribute to prognostication. It should be kept in mind that the ACHD population is heterogeneous; blood biomarkers seem to be relevant in most of the ACHD diagnoses, but there may be differences in their precise prognostic value. Unlike echocardiography, blood biomarkers are not explicitly hindered by the anatomy of a patient, this is advantageous for patients with an anatomically complex heart in who echocardiographic analysis can be challenging. The risk prediction model showed that together with four other readily available clinical variables, NT-proBNP can provide reliable estimations to determine prognosis in ACHD patients. Development of diagnosis-specific and outcome-specific models deserve attention, to improve the clinical applicability of the model and its usefulness for clinical decision making. Recapitulatory, biomarkers deserve a more important role in the follow-up of ACHD patients.

The first results of the BioPulse study showed that high levels of cardiac biomarkers are found in adults with PH at the time of their diagnosis. In patients with the worst prognosis, blood biomarkers more often seem to be elevated. Besides NT-proBNP and hs-TnT, novel blood biomarkers like GDF-15 and RDW, show potential to aid the risk stratification and need to be investigated in the currently available risk stratification approaches in PH to further assess their relevance. As the heterogeneity in PH limits and challenges the applicability of a universal approach for risk stratification, subgroup analysis for the prognostic value of blood biomarkers are important. On the other hand, focusing on a non-specific blood biomarker such as GDF-15 to identify patients with a good prognosis may be another approach to overcome the problem of heterogeneity.

As a final point to end this thesis with, blood biomarkers thus far seem to be a promising modality to reflect prognosis and disease severance in a wide variety of patients. As the life expectancy of humans continues to increase, with more and more people suffering from chronic diseases worldwide, accessible and adequate modalities are needed to allow monitoring of all these patients in the future. Blood biomarkers have great potential to fulfill this need. The successes of finding the ideal blood biomarkers to fulfill this job, will lie within the strength of contributing biobanks from all over the world, in conjunction with the increasing technological advances, like artificial intelligence.

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EPILOGUE

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Dankzij de ontwikkelingen in de kindercardiologie en de chirurgie, is de levensverwachting van kinderen die geboren worden met een aangeboren hartafwijking de afgelopen decennia sterk verbeterd. De toegenomen levensverwachting van patiënten met een aangeboren hartafwijking heeft ertoe geleid dat er binnen de cardiologie een nieuw specialisme is ontstaan welke betrekking heeft op volwassenen met een aangeboren hartafwijking. Dit specialisme wordt gekenmerkt door een unieke patiëntengroep bestaande uit een relatief groot aandeel aan jongvolwassenen met een hoog risico op hartfalen, ritmestoornissen, heringrepen en vroegtijdig overlijden. Veel van de problematiek van volwassenen met een aangeboren hartafwijkingen zien we niet expliciet bij verworven hartziekten, waardoor deze patiënten andere en dus gespecialiseerde zorg nodig hebben. Het cardiologische vakgebied 'volwassenen met een aangeboren hartafwijking', ook wel congenitale cardiologie genoemd, heeft door de toename van het aantal patiënten steeds meer aandacht gekregen waardoor de expertise op dit gebied steeds verder is toegenomen.

Een aangeboren hartafwijking is in veel gevallen een chronische aandoening en daardoor is het merendeel van deze patiënten genoodzaakt tot levenslange ziekenhuiscontroles door een cardioloog. Ondanks dat zij gedurende hun hele leven geconfronteerd worden met hun ziekte, hebben volwassenen met een aangeboren hartafwijking vaak dezelfde levensdoelen en wensen als hun leeftijdsgenoten. Hierbij valt te denken aan: een kinderwens, arbeidsparticipatie, meedoen aan (extreme) sporten en het maken verre reizen. Door beter inzicht te krijgen in het ziekteproces en daarnaast beter onderscheid te kunnen maken in patiënten met een goede en slechte prognose, hopen we patiënten beter te kunnen begeleiden in hun klinische zorg. Daarnaast kan een betere informatieverstrekking aan patiënten over hun prognose ook bijdragen aan een beter verwachtingsmanagement en levensplanning. Manieren waarop we deze patiënten makkelijk kunnen monitoren zijn belangrijk om niet alleen betere zorg te kunnen leveren, maar ook om de kwaliteit van leven te verbeteren.

Naast volwassenen met een aangeboren hartafwijking, wordt er in dit proefschrift ook onderzoek gedaan naar volwassenen met pulmonale hypertensie. Pulmonale hypertensie betekent letterlijk 'een verhoogde bloeddruk in de longen'. Deze zeldzame chronische ziekte kan zich op elke leeftijd uiten en is progressief. Voor de patiënten betekent dit dat zij veelal een sombere prognose hebben. Ondanks dat pulmonale hypertensie in eerste instantie met name een ziekte is die de longen aantast, zijn op de langere termijn de gevolgen voor het hart het grootst. De meeste patiënten ontwikkelen hartfalen ten gevolgen van de verhoogde druk in de longen en ervaren klachten van vermoeidheid, benauwdheid tijdens inspanning en pijnklachten op de borst. De oorzaak van pulmonale hypertensie is niet eenduidig. Uiteenlopende ziektes kunnen pulmonale hypertensie veroorzaken, waaronder verschillende bindweefselziektes en sommige aangeboren hartafwijkingen. Doordat de klachten aspecifiek

zijn en pulmonale hypertensie niet veel voorkomt, zit er vaak een lange tijd tussen het optreden van de eerste symptomen en het stellen van de diagnose. Pulmonale hypertensie is in de meeste gevallen niet te genezen, maar er bestaan wel behandelingen die tijdelijk de ziekte progressie kunnen remmen en klachten kunnen verminderen. De behandeling van patiënten met pulmonale hypertensie wordt in Nederland alleen in academische ziekenhuizen met expertise op dit gebied uitgevoerd. De zorg voor deze patiënten vereist een multidisciplinaire aanpak, waarin zowel longartsen, cardiologen en andere betrokken specialismes, afhankelijk van de onderliggende oorzaak van de pulmonale hypertensie, een rol spelen.

Dit proefschrift richt zich met name op bloedbiomarkers in patiënten met een aangeboren hartafwijking en patiënten met pulmonale hypertensie op de volwassen leeftijd. Bloedbiomarkers zijn signaalstofjes die worden uitgescheiden in het bloed van de patiënt. Deze stofjes zijn hierdoor relatief makkelijk te meten door bloed af te nemen bij de patiënt. Het doel van dit proefschrift was met name om te kijken of we door middel van deze signaalstoffen in het bloed een betere indeling kunnen maken tussen patiënten met een laag- en hoog risico op complicaties.

DEEL I Bloedbiomarkers in volwassenen met een aangeboren hartafwijking

In deel I beschrijven we de prognostische waarde van zowel nieuwe als meer bekende bloedbiomarkers in een groep van volwassenen met een matig tot ernstig complexe aangeboren hartafwijking. Ook beschrijven we de prognostische waarde van sommige bloedbiomarkers in specifieke aangeboren hartafwijkingen, waaronder: systeem rechterkamer ten gevolge van een transpositie van de grote vaten, atrium septum defect en bicuspide aorta klep. Een groot deel van de resultaten die in deel I aan bod komen, zijn afkomstig uit de BioCon-studie. Dit is een groot project waarbij 602 volwassenen met een matig tot ernstig complexe aangeboren hartafwijking zijn gevolgd gedurende een periode van ongeveer 5 jaar, waarbij zij jaarlijks werden teruggezien op de Cardiologie polikliniek in het Erasmus MC. De patiënten ondergingen verschillende onderzoeken, waaronder een bloedafname, hartfilmpje en echo van het hart.

Vrij ST2 (sST2) is onlangs ontdekt als een nieuwe bloedbiomarker voor hartfalen. Het is bekend dat afgifte van sST2 in de bloedbaan wordt gestimuleerd door cardiomyocyten (hartspiercellen) als reactie op beschadiging en stress van het hart. In Hoofdstuk 2 hebben we sST2 gemeten in ontdooide serummonsters van patiënten met een matig tot ernstig complexe aangeboren hartafwijking uit de BioCon-studie. Bovendien werd sST2 gemeten in monsters van gezonde proefpersonen die onderdeel uitmaakten van de desbetreffende "Navigator-studie" om referentiewaarden vast te stellen en de reproduceerbaarheid van de sST2-assay te beoordelen. In totaal werd sST2 gemeten in 142 gezonde personen en 590 patiënten met een aangeboren hartafwijking. Bij mannen werden significant lagere waardes

van sST2 gevonden dan bij vrouwen. Dit zagen we zowel bij de gezonde personen als bij de patiënten met een aangeboren hartafwijking. Gebaseerd op het 97.5e percentiel van de sST2 distributie bij proefpersonen uit de Navigator-studie, was een verhoogde sST2 aanwezig bij 7 vrouwen (2,8%) en 15 mannen (4,4%) met een aangeboren hartafwijking. Hoewel een hogere sST2 waarde geassocieerd was met een hoger risico op cardiovasculaire gebeurtenissen in het algemeen, toonde een gestratificeerde analyse aan dat sST2 vooral van prognostische waarde was bij vrouwen en bij patiënten met een ernstig complexe aangeboren hartafwijking. De geslachtsspecifieke verschillen van sST2 die in deze studie werden gevonden, waren niet eerder in de literatuur beschreven en toekomstig onderzoek zou zich moeten richten op de invloed van hormonen op sST2 waardes.

Van troponine T is bekend dat het door het hart in de bloedstroom wordt afgegeven als reactie op de beschadiging van hartspiercellen. Om deze reden is troponine T met een hoge sensitiviteit (hs-TnT) een gevestigde biomarker voor de diagnose van een acuut coronair syndroom. Gezien het feit dat patiënten met een aangeboren hartafwijking relatief jong zijn en coronaire hartziekten in hun situatie niet heel voor de hand liggend zijn, is het opmerkelijk dat het hs-TnT verhoogd is bij een aanzienlijk aantal patiënten met een aangeboren hartafwijking. Dit werd eerder aangetoond in een studie door Eindhoven et al. In Hoofdstuk 3 hebben we het verloop van hs-TnT over de tijd bestudeerd en dit gerelateerd aan het risico op cardiovasculaire complicaties bij patiënten die deelnamen aan de BioCon-studie. Hs-TnT werd jaarlijks gemeten tot 4 jaar na inclusie in de studie. We ontdekten dat de hs-TnT waardes gemiddeld hoger waren bij patiënten waarbij een cardiovasculaire complicatie optrad tijdens de follow-up en dat hs-TnT de neiging had te stijgen voorafgaand aan het optreden van een cardiovasculaire complicatie. Echter, ook bij patiënten zonder cardiovasculaire complicaties, nam hs-TnT geleidelijk toe over de tijd. Dit kan duiden op subklinisch verlies van hartspiercellen in patiënten met een aangeboren hartafwijking. Het is echter onwaarschijnlijk dat de verhoging van het hs-TnT onderliggend is geweest aan coronairlijden in deze patiënten. Wat betreft risicostratificatie ondersteunden gegevens van ons onderzoek dat het herhaaldelijk meten van hs-TnT extra prognostische waarde oplevert ten opzichte van een enkele hs-TnT-meting. Echter levert het herhaald meten van hs-TnT geen extra prognostische informatie op naast het meten van herhaald NT-proBNP. We hebben ook waargenomen dat patiënten met stabiele, niet-detecteerbare hs-TnT-spiegels een zeer laag risico liepen op het ontwikkelen van ernstige cardiale complicaties. Dit gegeven kan van nut zijn om patiënten met een laag risico te identificeren.

C-reactive protein (CRP) is een inflammatoire marker die algemeen verkrijgbaar is en regelmatig wordt gebruikt in de dagelijkse klinische praktijk om infecties op te sporen of juist uit te sluiten. Sinds de ontwikkeling van een gevoeligere CRP meting (hs-CRP), kunnen ook laaggradige ontstekingsniveaus worden gemeten. In Hoofdstuk 4 hebben we de rol van hs-CRP in patiënten met aan aangeboren hartafwijking onderzocht met behulp van gegevens uit de BioCon-studie. We onderzochten eerst de prognostische waarde van een enkele baseline hs-CRP-meting en ontdekten dat patiënten met een verhoogd hs-CRP (> 3 mg/L) in combinatie met een verhoogd NT-proBNP (> 14 pmol/L) een hartfalenvrije overleving van slechts 71,6

procent na 6 jaar hadden, terwijl patiënten met normale waarde van beiden biomarkers een 6-jaars hartfalenvrije overleving hadden van 99,6 procent. Hs-CRP bleef een onafhankelijke voorspeller voor overlijden of hartfalen na het corrigeren voor een breed scala aan klinische variabelen en de bloedbiomarkers NT-proBNP en hs-TnT. Bovendien lieten longitudinale patronen van hs-CRP zien dat hs-CRP gemiddeld genomen toenam vóór het overlijden van een patiënt of het optreden van hartfalen, terwijl stabiele niveaus werden waargenomen bij patiënten die niet overleden of een hartfalen episode doormaakten. Herhaalde hs-CRP-metingen waren geassocieerd met het risico op overlijden of hartfalen, onafhankelijk van herhaalde NT-proBNP-metingen. Hoge en stijgende hs-CRP-niveaus lijken patiënten met een slechte prognose te identificeren en daarom kan het meten van hs-CRP worden overwogen naast een NT-proBNP-meting in de reguliere follow-up van patiënten met een aangeboren hartafwijking. Of ontsteking een causale rol kan spelen bij de achteruitgang van patiënten met een aangeboren hartafwijking is een vraag die in toekomstig onderzoek beantwoord dient te worden.

Een atrium septum defect (ASD) kan worden gediagnosticeerd op volwassen leeftijd en kan vervolgens chirurgische of percutane sluiting vereisen. Als de anatomie en de grootte van het defect een percutane sluiting toelaat, is dit vaak de procedure van eerste keuze, omdat dit minder invasief is voor de patiënt. In Hoofdstuk 5 bestudeerden we het verloop van bloedbiomarkers ten tijden van het percutaan sluiten van een ASD in volwassenen. Bloedafnames werden uitgevoerd voorafgaand aan de ASD-sluiting en 1 dag, 3 maanden en 1 jaar na ASD-sluiting bij 50 volwassenen die in het Erasmus MC een percutane ASD-sluiting ondergingen. In deze studie werden zes verschillende biomarkers geanalyseerd: NT-proBNP, hs-TnT, hs-CRP, RDW, GDF-15 en galectine-3. We ontdekten dat bij een substantieel aantal patiënten ten minste één van deze zes onderzochte biomarkers verhoogd was vóór sluiting van het ASD. Een dag na sluiting van het ASD werd een toename in de meeste biomarkers waargenomen. Dit resulteerde er in dat 92% van de patiënten minstens één abnormale biomarker waarde had 1 dag na sluiting. Drie maanden na sluiting van het ASD waren de biomarker waardes terug naar waardes die aanwezig waren voor sluiting, en deze bleven stabiel tot 1 jaar. De biomarker patronen die in deze studie werden gevonden, suggereren dat een ASD-sluiting direct wordt gevolgd door een uitgebreide cardiale respons op moleculair en cellulair niveau. Echocardiografie 1 jaar na sluiting vertoonde tekenen van normalisering van het hart (reverse remodeling), hoewel een verlaging van de bloedbiomarker waardes op 1 jaar ten opzichte van waardes vóór sluiting van ASD, niet werden waargenomen.

Patiënten met een bicuspide aortaklep hebben een aortaklep met twee in plaats van drie (functionele) klepbladen. De resultaten van een cross-sectionele studie om te onderzoeken of bloedbiomarkers geassocieerd zijn met de ernst van de aortapathologie, worden besproken in Hoofdstuk 6. We onderzochten NT-proBNP, hs-CRP, hs-TnT en TGF- β1. TGF-β1 werd ook gemeten bij de gezonde proefpersonen van de Navigator-studie. Lagere TGF-β1 waardes werden gevonden in patiënten met een bicuspide aortaklep ten opzichte van de gezonde proefpersonen. Hogere NT-proBNP en hs-TnT spiegels waren geassocieerd

met een ernstigere aortaklepregurgitatie en een hoger NT-proBNP was geassocieerd met een ernstigere aortaklepstenose. Hs-CRP en TGF-β1 waren niet geassocieerd met de ernst van de aortakleppathologie, noch met de aortadiameter of linkerventrikel ejectiefractie. Toekomstige studies zouden moeten onderzoeken of biomarkers geassocieerd zijn met complicaties op de lange termijn, zoals aortadissectie of verergering van aortastenose bij deze patiëntengroep.

In een anatomisch normaal hart pompt de linkerhartkamer (ventrikel) bloed de aorta in en zorgt hiermee voor de systemische bloedcirculatie. Voordat de arteriële switch operatie werd uitgevoerd, werden patiënten geboren met een transpositie van de grote vaten (TGA) geopereerd volgens de Musterd- of Senning-procedure. Dit resulteerde in een rechterhartkamer, in plaats van linkerhartkamer, die de systemische circulatie van bloed voorziet. Bij patiënten met congenitaal gecorrigeerde transpositie van de grote vaten, dient de rechterkamer ook als systemische hartkamer. In Hoofdstuk 7 hebben we de prognostische waarde van nieuwe echocardiografische metingen en bloedbiomarkers onderzocht voor hun rol in de risicostratificatie van volwassenen met een systeem rechterhartkamer. In totaal werden 86 patiënten met een systeem rechterhartkamer uit de BioCon-studie in deze analyse meegenomen. We vonden een hoge incidentie van hartfalen, ritmestoornissen en overlijden in deze groep, wijzend op de noodzaak voor een goede aanpak om hoog- en laag risico patiënten te identificeren. Verschillende klinische kenmerken, bloedbiomarkers en conventionele echocardiografische metingen werden in verband gebracht met klinische uitkomsten. Desalniettemin waren bloedbiomarkers betere voorspellers voor klinische uitkomsten bij volwassenen met een systeem rechterhartkamer dan nieuwe echocardiografische strain metingen. Met name GDF-15 toonde een sterke associatie met het risico op overlijden of hartfalen, zelfs sterker dan conventionelere biomarker NT-proBNP. De resultaten van deze studie suggereren dat de achteruitgang van de hartfunctie van patiënten met een systeem rechterhartkamer complex is en dat risicostratificatie hierdoor uitdagend is. Op basis van deze studie suggereerden we dat een alomvattende klinische benadering nodig is, waarin er een belangrijke rol weggelegd is voor bloedbiomarkers.

DEEL II Bloedbiomarkers in volwassenen met pulmonale hypertensie

In deel II wordt de prognostische waarde van verschillende gevestigde en nieuwe bloedbiomarkers in volwassenen met pulmonale hypertensie behandeld. Deze resultaten zijn gebaseerd op de BioPulse-studie, een project waarin we patiënten met pulmonale hypertensie vanaf hun diagnose hebben gevolgd over de tijd waarbij we halfjaarlijks bloed hebben afgenomen. Daarnaast wordt er middels een systematische review van de literatuur een schatting gegeven van de prevalentie van pulmonale arteriële hypertensie (PAH) voor en na ASD-sluiting bij volwassenen.

In Hoofdstuk 8 worden de eerste resultaten van de BioPulse studie gepresenteerd. In totaal werden 106 volwassenen met pulmonale hypertensie geïncludeerd in de studie. Tijdens de diagnostische rechterhartkatheterisatie werden zes verschillende biomarkers gemeten. We hebben de waardes van deze zes biomarkers beschreven en in verband gebracht met het risico op overlijden, longtransplantatie en ziekenhuisopnames voor hartfalen. NT-proBNP, hs-TnT, hs-CRP, galectine-3, RDW en eGFR werden gemeten en alle biomarkers, met uitzondering van eGFR, waren geassocieerd met het risico op overlijden of longtransplantatie. NT-proBNP leverde de sterkste associatie op van alle biomarkers. Een multi-biomarker benadering toonde aan dat patiënten een slechtere prognose hadden als er meer biomarkers waren verhoogd ten tijden van de diagnostische rechterhartkatheterisatie. Opvallend was dat slechts elf patiënten (11%) geen enkele abnormale biomarker waarde had op het moment dat de diagnose pulmonale hypertensie werd gesteld, en dat al deze patiënten nog in leven waren 40 maanden na de diagnose. De zogenoemde "REVEAL Risk Score" is een ontwikkelde risicoscore die wordt gebruikt om patiënten met PAH te stratificeren. In deze studie hebben we de REVEAL-risicoscore voor elke patiënt berekend en beoordeeld of een van de zes biomarkers een extra prognostische waarde had bovenop deze risicoscore. De resultaten toonden aan dat zowel een combinatie van biomarkers, evenals een enkele individuele biomarker, geen extra prognostische waarde kan bieden bovenop de REVEAL-risicoscore. Daarom lijkt risicostratificatie op basis van uitsluitend bloedbiomarkers ontoereikend bij patiënten met pulmonale hypertensie. Desalniettemin suggereert deze studie dat het combineren van meerdere biomarkers gunstig kan zijn bij het detecteren van pulmonale hypertensie patiënten met een laag en hoog risico. Grotere studies, met bij voorkeur continue biomarker waardes in plaats van gedichotomizeerde waardes, zullen dit verder moeten onderzoeken.

Vrij ST2 werd ook gemeten in de BioPulse-studie met dezelfde assay en referentiewaarden zoals beschreven in Hoofdstuk 2 van dit proefschrift. De resultaten van sST2 bij volwassenen met PH worden beschreven in Hoofdstuk 9. In deze studie vonden we dat sST2 verhoogd was in 20% van de patiënten en dat een hogere sST2 waarde was geassocieerd met hogere invasief gemeten drukken van de rechterkant van het hart en met de rechterventrikelfunctie gemeten middels echocardiografie. Bovendien hadden patiënten met een verhoogde sST2 een significant lagere transplantatievrije overleving 6 jaar na hun diagnose. Echter, sST2 had geen onafhankelijke voorspellende waarde bovenop NT-proBNP, waardoor het nut ervan als prognostische biomarker voor risicostratificatie bij volwassenen met pulmonale hypertensie beperkt is. Wat het exacte onderliggende mechanisme is van het vrijkomen van sST2 in pulmonale hypertensie patiënten is niet geheel duidelijk en is een interessant onderwerp voor toekomstige studies.

Groeidifferentiatiefactor-15 (GDF-15) is een niet-specifieke biomarker die betrokken is bij celgroei, differentiatie en apoptose. We evalueerden GDF-15 als voorspeller voor mortaliteit bij volwassenen met pulmonale hypertensie en veronderstelden dat GDF-15 vanwege zijn aspecifieke aard een sterke voorspeller zou kunnen zijn in patiënten met pulmonale hypertensie met uiteenlopende etiologie. De resultaten van deze studie zijn beschreven in Hoofdstuk 10.

GDF-15 werd gemeten in plasmamonsters van patiënten met pulmonale hypertensie en in gezonde proefpersonen uit de Navigator-studie. Er werden duidelijke verschillen waargenomen in de verdeling van GDF-15-waardes tussen patiënten met pulmonale hypertensie en de gezonde proefpersonen. GDF-15-waardes waren significant hoger in patiënten met pulmonale hypertensie en op basis van het 97,5^{e} percentiel van de GDF-15 distributie in de gezonde controles, identificeerden we abnormale GDF-15 waardes bij 74% van de patiënten met pulmonale hypertensie. Opgemerkt werd dat vooral patiënten met normale GDF-15-spiegels een zeer goede prognose hadden. Deze patiënten waren allemaal in leven en vrij van longtransplantatie twee jaar na hun diagnose, in tegenstelling tot slechts 72% van de patiënten met een verhoogde GDF-15 waarde. GDF-15 bleef ook geassocieerd met het risico op overlijden of longtransplantatie wanneer gecorrigeerd voor NT-proBNP. Deze studie toonde aan dat een normale GDF-15-meting patiënten met een laag risico kan identificeren en het meten van GDF-15 zou daarom moeten worden overwogen bij alle patiënten op het moment van de diagnose van pulmonale hypertensie.

Tot slot worden in Hoofdstuk 11 de resultaten gepresenteerd van een systematisch literatuuronderzoek waarin de prevalentie van PAH voor en na het afsluiten van een ASD in volwassenen werd onderzocht. In totaal waren er 30 artikelen geïncludeerd en de prevalentie van PAH varieerde van 29% tot 73% vóór sluiting van ASD. In alle onderzoeken werd een afname van de prevalentie van PAH en de gemiddelde pulmonale arteriële druk waargenomen na het sluiting van het ASD, onafhankelijk van de leeftijd of de pulmonale druk voor de ASD-sluiting. De prevalentie van PAH na het afsluiten van het ASD varieerde echter nog steeds van 5% tot 50%. Deze resultaten rechtvaardigen het uitvoeren van langdurige follow-upstrategieën in volwassenen die een ASD sluiting hebben ondergaan.

DEEL III Risicostratificatie en risicovoorspelling

Deel III beschrijft het gebruik van andere modaliteiten die nuttig zijn om de prognose te bepalen in patiënten met een aangeboren hartafwijking. Naast bloedbiomarkers kunnen klinische kenmerken, demografische gegevens en andere modaliteiten, zoals elektrocardiografie, beeldvorming en cardiopulmonale inspanningstesten, bijdragen aan de inschatting van het risico op een bepaalde cardiovasculaire complicatie.

Gevalideerde instrumenten voor risicovoorspelling zijn schaars in de populatie van volwassenen met een aangeboren hartafwijking en risicostratificatie in deze groep patiënten blijft daarom grotendeels gebaseerd op de mening van experts in het vakgebied. In Hoofdstuk 12 hebben we de ontwikkeling en externe validatie beschreven van een risicovoorspellingsmodel voor volwassenen met een aangeboren hartafwijking. Hiervoor hebben we gebruik gemaakt van gegevens van twee grote prospectieve cohortstudies bestaande uit in totaal 1.351 patiënten met een aangeboren hartafwijking. De BioCon-studie werd gebruikt om het voorspellingsmodel te ontwikkelen en een extern cohort van volwassenen met aangeboren

hartafwijkingen uit Boston Childrens en Brigham and Women's Hospital werd gebruikt om het model te valideren. Het uiteindelijke model bestond uit 5 makkelijk te verkrijgen klinische variabelen: NYHA-klasse, gebruik van cardiale medicatie, heringrepen uitgevoerd na eerdere correctieve operatie, body mass index en NT-proBNP. De risicocalculator leverde betrouwbare schattingen op voor het 1-jaars risico op overlijden, hartfalen of een ritmestoornis op basis van deze 5 variabelen, ondersteund door een goede kalibratie en discriminatie in het externe cohort. Deze studie laat zien dat dit simpele risicomodel valide is en mogelijk kan helpen om passende zorg te leveren aan volwassenen met een aangeboren hartafwijking. Er is een online webapplicatie gebouwd om een eenvoudig gebruik van het risicovoorspellingsmodel in de dagelijkse klinische praktijk te ondersteunen.

Hartfalen is een complex klinisch syndroom en is een van de grootste problemen bij volwassenen met een aangeboren hartafwijking. In Hoofdstuk 13 hebben we de literatuur samengevat betreffende voorspellende factoren voor hartfalen en andere late complicaties in de gehele populatie van volwassenen met een aangeboren hartafwijking. De bijdrage van een breed scala aan factoren voor risicostratificatie werd besproken op basis van de huidige literatuur. Het type aangeboren hartafwijking, genetica, echocardiografie, cardiale magnetische resonantie beeldvorming, cardiopulmonale inspanningstesten en bloedbiomarkers werden geïdentificeerd als belangrijke factoren met betrekking tot de risicostratificatie bij volwassenen met een aangeboren hartafwijking.

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3. **Geenen LW***, Opotowsky AR*, Lachtrupp C, Baggen VJM, Brainard S, Landzberg MJ, et al. Tuning and External Validation of an Adult Congenital Heart Disease Risk Prediction Model. Eur Heart J Qual Care Clin Outcomes. 2020. (**Equal contributions)*
4. **Geenen LW**, Baggen VJM, van den Bosch AE, Eindhoven JA, Kauling RM, Cuypers J, et al. Prognostic value of C-reactive protein in adults with congenital heart disease. Heart. 2020;107(6):474-81.
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7. Jancauskaite D, Rudiene V, Jakutis G, **Geenen LW**, Roos-Hesselink JW, Gumbiene L. Residual Pulmonary Hypertension more than 20 Years after Repair of Shunt Lesions. Medicina (Kaunas). 2020;56(6).
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12. **Geenen LW**, Baggen VJM, van den Bosch AE, Eindhoven JA, Kauling RM, Cuypers J, et al. Prognostic Value of Serial High-Sensitivity Troponin T Measurements in Adults With Congenital Heart Disease. Can J Cardiol. 2019.

13. **Geenen LW**, Baggen VJM, Kauling RM, Koudstaal T, Boomars KA, Boersma E, et al. The Prognostic Value of Soluble ST2 in Adults with Pulmonary Hypertension. J Clin Med. 2019;8(10).
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Book chapters

Baggen VJM, **Geenen LW**, Roos-Hesselink JW. Risk stratification and prognosis. In: Heart failure in Adult Congenital Heart Disease. Springer; Swan L, Frogoudaki A (Editors): 2018.

PhD portfolio

Name:	Laurie Geenen
Department:	Cardiology
Research school:	COEUR
PhD period:	2017-2021
Promotors:	Prof. Dr. J.W. Roos-Hesselink
	Prof. Dr. E. Boersma
Copromotor:	Dr. A.E. van den Bosch

Year	Activity	Workload (ECTS)
	PhD training	
2016-2018	**Research skills** MSc. Health Sciences, specialization Clinical Epidemiology (NIHES)	*120 (total)*
	Courses	
	Erasmus Summer Program 2016	4.2
	Study design (CC01)	4.3
	Clinical epidemiology (CE02)	5.7
	Biostatistical methods I: Basic principles (CC02)	5.7
	Biostatistical methods II: Classical regression models (EP03)	4.3
	Methodological topics in epidemiologic research (EP02)	1.4
	Advanced topics in decision-making in medicine (EWP02)	2.4
	Course for the quantitative researcher (SC17)	1.4
	Repeated measurements in clinical studies (CE08)	1.4
	Missing values in clinical research (EP16)	0.7
	Cardiovascular epidemiology (EP20)	0.9
	Planning and evaluation of screening (HS05)	1.4
	Joint Models for longitudinal and survival data (ESP72)	0.7
	Cohort studies (ESP39)	0.7
	Pharmaco-epidemiology and drug safety (EWP03)	1.9
	Advanced topics in clinical trials (EWP10)	1.9
	Advanced analysis of prognosis studies (EWP13)	0.9
	Principles of epidemiologic data-analysis (EWP25)	0.7
	Scientific writing in English for publication (SC07)	2.0
	General courses	
2017	Research integrity course	0.3
2017	Photoshop and illustrator workshop	0.3
2018	BROK	1.5
2018	Open Clinica (eCRF)	0.3
	In-depth courses	
2017	Congenital heart disease (COEUR)	0.5
2017	Intensive care research (COEUR)	0.5
2017	Cardiovascular imaging (COEUR)	0.5
2018	Congenital heart disease (COEUR)	0.5
2018	Heart Failure Research (COEUR)	0.5
2019	Vascular Clinical Epidemiology (COEUR)	0.5

Year	Activity	Workload (ECTS)
	Seminars and conferences	
	Seminars	
2017	Seminar: Pulmonary Hypertension, Rotterdam	0.3
2018	Seminar: TI, cause of effect of RV failure, Rotterdam	0.3
2018	ExCOEURsion General Medical Council, the Hague	0.2
2019	Networked Sciences Symposium Erasmus MC, Rotterdam	0.3
	Oral presentations	
2017	NVVC Najaarscongres	0.6
2018	Davos winter Meeting	0.9
2018	NVVC Voorjaarscongres	0.6
2018	ESC congress Munich	0.4
2019	Davos Winter Meeting	0.9
2019	NVVC Voorjaarscongres Lustrum Editie	0.6
2020	Congenitale Cardiologie Werkgroep Nederland	0.4
2020	Davos Winter Meeting *(cancelled)*	-
	Poster presentations	
2017	PAH symposium GSK Leuven	0.4
2018	EuroGUCH Münster	0.4
2018	ACHD conference Toronto	0.4
2018	ESC congress Munich	0.4
2019	EuroGUCH Zagreb	0.4
2019	ACHD conference Skamania/Portland	0.4
2020	EuroGUCH Leuven *(cancelled)*	0.2
	Attended	
2017	NVVC najaarscongres, Papendal (1 day)	0.3
2017	PAH symposium GSK, Leuven (1.5 days)	0.8
2018	Davos Winter Meeting, Davos (4 days)	1.2
2018	NVVC voorjaarscongres, Noordwijkerhout (1 day)	0.3
2018	EuroGUCH congress, Münster (2 days)	0.6
2018	ACHD congress, Toronto (4 days)	1.2
2018	ESC congress, Munich (5 days)	1.5
2018	NVVC najaarscongres, Papendal (1 day)	0.3
2019	Davos Winter Meeting, Davos (4 days)	1.2
2019	EuroGUCH congress, Zagreb (2 days)	0.6
2019	NVVC voorjaarscongres, Rotterdam (1 day)	0.3
2019	ACHD congress, Portland, Oregon (4 days)	1.2
2019	NVVC najaarscongres, Papendal (1 day)	0.3
2020	Davos Winter Meeting, Davos (4 days) *(cancelled)*	-
2020	EuroGUCH congress, Leuven (2 days) *(cancelled)*	-
	Teaching activities	
	Lecturing	
2018	Journal club 'Atopic eczema and CVD'	0.1
2019	Clinical epidemiology research meeting 'The Galton Board'	0.1
2020	Journal club 'Education and coronary heart disease'	0.1
	Supervision	
2018	Supervising systematic review 2nd years medical students	0.2
2018	Supervising master thesis 4th year medical student	1.2
2019	Supervising systematic review 2nd years medical students	0.2
	Other	
2017-2019	Chairman of the COEUR PhD committee and representative COEUR PhD student in the Erasmus MC PhD committee	3.0

About the author

Laurie Willemijn Geenen was born on the 25th of December, 1994 in Goes, the Netherlands. She graduated from secondary school in 2013 (Pontes Goese Lyceum, Goes) and began with her studies in Medicine at the Erasmus University Medical Center Rotterdam. During medical school, she worked as a data manager at the research department of Pulmonary Diseases and conducted a systematic literature review in collaboration with the department of Congenital Cardiology.

After obtaining her Bachelor of Medicine in 2016, she was accepted to participate in the Research Master program Health Sciences, specialization Clinical Epidemiology, at the Netherlands Institute for Health Sciences. She performed her masters' graduation research at the department of Cardiology about blood biomarkers in adult patients with pulmonary hypertension and published her results in an international peer-reviewd journal for which she was awarded the Hippocrates Studiefonds Price. In the meanwhile, she was offered a PhD position at the department of Cardiology at the Erasmus Medical Center under the supervision of Prof. dr. J.W. Roos-Hesselink and Prof. dr. ir. E. Boersma. She interrupted medical school and worked full-time on her PhD thesis from May 2018 till May 2020. During this period she was a committee member of the COEUR PhD student committee and was involved as a volunteer in the Sailing Kids Foundation. In the spring of 2019, she visited Boston Children's Hospital (Boston, MA, the United States of America) for a research period of two months to collaborate on a clinical risk prediction model for adults with congenital heart disease.

As of September 2020, Laurie started with her clinical rotations and she expects to obtain her Medical Degree by the end of 2022. Besides her study, she enjoys travelling, playing field hockey and cycling.

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