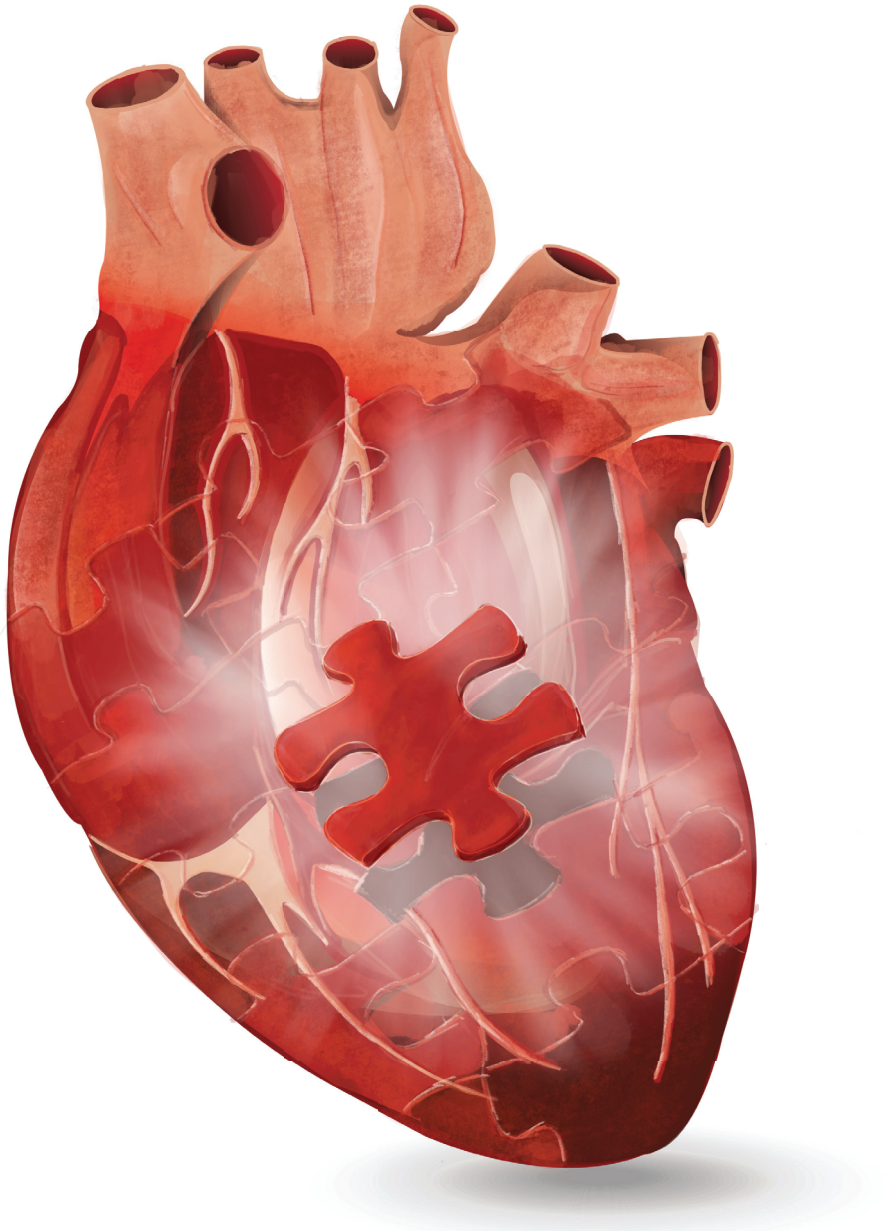
IMPROVING RISK ASSESSMENT IN ACQUIRED HEART DISEASE:

biomarkers and beyond



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Improving Risk Assessment in Acquired Heart Disease: Biomarkers and Beyond

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CONTENTS

Chapter 1

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality in developed countries.[1] The 2010 Global Burden of Disease study estimated that CVD caused 15.6 million deaths worldwide, which is 29.6% of all deaths. As longevity increases, mortality rates due to cardiovascular disease are expected to rise even further. Heart failure (HF) plays a particularly important part in these developments. From 1994 to 2004, deaths from HF increased by 28%, while the overall death rate decreased by 2%.[2] In the western world, approximately 1-2% of the adult population and >10% of patients of 70 years of age or older suffers from HF.[3] Thus, HF has become one of the key contributors to the burden of chronic disease in the elderly in terms of mortality, morbidity, and cost.

The form of HF whose pathophysiology is best understood is HF with reduced ejection fraction (HFREF; sometimes also referred to as 'systolic HF'), which is also the main focus of the European Society of Cardiology (ESC) guidelines.[4] In >60% of all cases, HFREF is caused by coronary artery disease (CAD), even though hypertension and diabetes are often contributing factors (ref). After myocardial injury (e.g. myocardial infarction), systolic dysfunction may occur, which is characterized by progressive worsening over time due to maladaptive changes that cause increased enlargement of the left ventricle and decline in ejection fraction, called 'remodeling'. Reduction of the cardiac function due to remodeling causes systemic responses, including systemic neurohormonal activation. This subsequently causes further myocardial injury, creating a pathophysiological 'vicious cycle', eventually leading to the syndrome of HF. The syndrome of HF includes pump-failure, that may cause decompensation and may eventually lead to cardiac death, as well as myocardial electrical instability that may lead to life-threatening arrhythmias.

Installing appropriate secondary prevention measures in HF patients, e.g. interventional, medicinal or artificial in the form of electrical devices, requires adequate identification of individuals at highest risk. Nowadays, prognosis of HF patients is usually assessed by means of physical examination and a limited number of standard imaging and laboratory tests. For individual patients, this approach falls short; although we know that overall, 50% of patients will die in 5 years, we still cannot foresee to which *individual* patients this pertains. Thus, improved methods for risk stratification are urgently needed.

The main purpose of this thesis was to provide knowledge on patient and procedural characteristics associated with adverse clinical outcome and to present novel prediction models that may aid in risk stratification of patients at different stages of CVD. First we focused on patients with CAD, with or without left ventricular dysfunction. Specifically, we examined patients undergoing percutaneous coronary intervention (PCI), and studied outcome after initial intervention. Then we focused on the disease course of patients with HF. Finally, we examined HF patients carrying implantable cardioverter-defibrillators (ICDs) to protect them from potentially life-threatening arrhythmias due to myocardial electrical instability

The research questions investigated in this thesis were as follows:

1. *First part of this thesis: patients with CAD, with or without left ventricular dysfunction:*
   a. What are the limitations of current mortality risk scores and prediction tools after PCI and can we provide a better tool?
   b. Different generations of drug-eluting stents (DES) carry different prognosis, but do these results apply to all patient-categories, in particular, to both patients with and without established cardiac failure?
   c. What (angiographic) factors are related to presentation with an out of hospital cardiac arrest (OHCA) in ST-elevation myocardial infarction (STEMI) patients?
2. *Second part of this thesis: patients with established HF. Specifically, we investigated methods that may aid in individual risk assessment of patients with acute or chronic HF (CHF):*
   a. What is the incremental value of serially measuring blood biomarkers, to clinical assessment in terms of serial NYHA class measurements, for monitoring stable CHF outpatients?
   b. Which biomarkers or combinations of biomarkers are best suited for prognostication in (C)HF patients?
3. *Third part of this thesis: patients with a poor cardiac function, which mandates ICD implantation with or without cardiac resynchronization therapy (CRT) for protection from potentially life-threatening arrhythmias:*
   a. Which patient characteristics favor or disfavor defibrillation therapy, with or without cardiac resynchronization therapy CRT?
   b. Which patient characteristics are associated with response to CRT and with clinical outcome after installment of CRT?
   c. What are the limitations of current CRT risk scores and are we able to provide a better tool for predicting mortality?

The outline of this thesis is as follows.

Part I : The influence of patient baseline and procedural characteristics on outcome after percutaneous coronary interventions.

Multiple mortality risk scores and prediction tools for patients that have undergone PCI have been developed, but nearly all of them focus on short term outcome, i.e. 30-day mortality.[5-17]. Risk prediction instruments with a prolonged time horizon are becoming increasingly important, as decisions during PCI have long-term consequences for patient management, and impact long-term outcome. In **Chapter 2** we aimed to develop a model to predict long-term, e.g. 5-year, mortality after PCI, which may also aid in selecting patients suitable for bioresorbable scaffolds, specifically designed to eliminate the late complications of permanent metallic implants, and to improve vessel physiology.

Furthermore, it is known that newer generation DES are superior to early generation DES,[18-20]; on the other hand, it is known that patients with a reduced left ventricular ejection fraction (LVEF) are more prone to stent thrombosis (ST) than those with a normal LVEF.[21-23] In **Chapter 3** we aimed to investigate the difference in clinical outcome of patients with or without LV dysfunction using newer generation DES vs. early generation DES.

Chapter 4 describes factors related to the occurrence of out-of-hospital cardiac arrest (OHCA) in ST-elevation myocardial infarction (STEMI), especially the differences in angiographic factors of STEMI patients presenting with and without OHCA.

Part II : novel methods and markers for risk assessment of patients with heart failure.

Blood biomarkers are capable of monitoring subtle (patho)physiological processes that reflect and possibly predict adverse changes before they become clinically apparent.[24,25] B-type natriuretic peptides (BNP) and N-terminal proBNP (NT-proBNP), cardiac troponin T and I and C- reactive protein (CRP) have been related to adverse clinical outcomes in HF patients in several large studies.[24,26-35] The majority of these studies have examined single, baseline measurements. However, temporal biomarker patterns may improve risk assessment. So far, studies that have examined changing biomarker patterns over time have mostly focused on natriuretic peptides, generally used only few repeated biomarker measurements, and have utilized simplified representations of temporal biomarker evolution. In **chapters 5 and 6** we present the first results of the Bio-SHiFT study. In this study, we performed frequent, longitudinal blood sampling and assessed multiple biomarkers simultaneously,[36] and thereby aimed to provide a basis for improved, personalized risk assessment in patients with CHF. First we examined the associations between serially measured biomarkers (i.e. NT-proBNP, high-sensitive cardiac troponin T (HsTnT) and CRP), serially scored New York Heart Association (NYHA) functional class, as well as the associations of their temporal patterns with adverse clinical outcome (chapter 3.1). Based on this, we evaluated the incremental value of serially measuring blood biomarkers to clinical assessment for monitoring stable CHF outpatients. Second we applied joint modelling to examine whether individual, temporal trajectories of NT-proBNP, HsTnT and CRP are associated with longer-term prognosis (chapter 3.2).

MicroRNAs (miRs) are promising, upcoming novel biomarkers. MiRs are non-coding, ~22 nucleotide long RNA sequences, which target mRNAs for cleavage or translational repression and thereby influence a great variety of biological processes.[37] The stability of miRs in plasma, and consequently their reliable assessment in easily accessible samples, potentially makes them attractive biomarkers for a wide range of diseases.[38] Several studies have shown associations between miRs and myocardial infarction (MI)[39-42] and HF,[43-48], as well as associations with diabetes mellitus,[49] However, most studies pertain-

ing to HF were performed in case-control settings and used limited study sample size. Furthermore, these studies usually assessed miRs only once. In **chapter 7** we have performed frequent repeated measurements of multiple miRs (miR-1254a, miR-22-3p, miR-345-5p, miR-378, miR-423-5p, miR-486-5p and miR320) in patients with CHF from the Bio-SHiFT study, and have investigated the associations of the thus obtained temporal patterns with adverse clinical outcome. Finally, in **Chapter 8** we performed an analysis similar to the analysis presented in chapter 7. We used RNA sequencing to identify novel candidate miRs and compared this to previously identified miRs (miR-1254, miR-22-3p, miR-345-5p, miR-378a-3p, miR-423-5p, miR-320a, miR-133a-3p, miR-133b, miR-499a-5p, miR-622, and miR-208a-3p) in patients with acute HF that were included in the TRIUMPH study.

Part III: Novel insights in characteristics associated with favorable outcome in defibrillation therapy and response to cardiac resynchronization therapy.

Patients are eligible for ICD therapy if they have a LVEF ≤35%.[50] Patients who have a LVEF ≤35% and cardiac dyssynchrony, are eligible for CRT-D.[51] Data from randomized and observational studies have shown beneficial effect of CRT in selected patients with drug refractory HF, reduced left ventricular ejection fraction (LVEF), and electrical dyssynchrony: it improves clinical symptoms, reduces hospitalizations and lowers mortality in a considerable proportion of patients.[52-55] Theoretically, all patients who meet the indication criteria for CRT also qualify to have an ICD for primary prevention of sudden cardiac death. Consequently, ICDs combined with CRT (CRT-D) are part of the standard management of HF patients with reduced LVEF.[4,56] Some patients respond well to CRT and reach a LVEF of >35%. In **Chapter 9** we referred to these patients as 'functional responders' and we assessed characteristics associated with functional response and the necessity of defibrillation therapy in these patients. In **chapter 10** we investigated the influence of atrial fibrillation on outcome of CRT-D patients. **Chapter 11** describes the difference in outcome of patients with ICD and CRT-D that have ischemic versus non-ischemic heart disease. Finally, since mortality risk among HF patients is highly heterogeneous and only a minority of patients will experience ventricular arrhythmias, appropriate risk prediction in CHF patients carrying a CRT-D device is of paramount importance. However, adequate risk estimation models to predict mortality in this specific patient population are lacking. In **chapter 12** we constructed a risk model for predicting mortality in CHF patients with a CRT-D device.

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Part I

The influence of patient baseline and procedural characteristics on outcome after percutaneous coronary interventions

Chapter 2

Development and validation of a risk model for long-term mortality after percutaneous coronary intervention: the IDEA-BIO study

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ABSTRACT

Background: Clinical trials are currently designed to demonstrate superiority of bioabsorbable scaffolds over metal devices up to 5 years after implantation. We aimed to develop a model to predict long-term mortality after percutaneous coronary intervention (PCI), to aid in selecting patients with sufficient life expectancy to benefit from bioabsorbable scaffolds.

Methods: From 2000-2011, 19.532 consecutive patients underwent PCI in a tertiary referral hospital. Patients were randomly (2:1) divided into a training (N=13,090) and validation (N=6,442) set. Cox regression was used to identify determinants of long-term mortality in the training set and used to develop a risk model. Model performance was studied in the training and validation dataset.

Results: Median age was 63 years (IQR 54 - 72) and 72% were men. Median follow-up was 3.6 years (interquartile range [IQR] 2.4-6.8). The ratio elective vs. non-elective PCIs was 42/58. During 88620 patient-years of follow-up 3156 deaths occurred, implying an incidence rate of 35.6 per 1000. Estimated 5-year mortality was 12.9%.Regression analysis revealed age, body mass index, diabetes mellitus, renal insufficiency, prior myocardial infarction, PCI indication, lesion location, number of diseased vessels and cardiogenic shock at presentation as determinants of mortality. The long-term risk model showed good discrimination in the training and validation sets (c-indices 0.76 and 0.74), whereas calibration was appropriate.

Conclusions: A simple risk model, containing 9 baseline clinical and angiographic variables effectively predicts long-term mortality after PCI and may possibly be used to select suitable patients for bioabsorbable scaffolds.

INTRODUCTION

Percutaneous coronary intervention (PCI) has become one of the most important and widely used treatments in patients suffering from coronary artery disease (CAD).[1] In general, clinical outcome after PCI is good, but baseline risk factors as diabetes mellitus, renal impairment and cardiogenic shock at presentation proved to significantly impact both short and long-term outcomes. In the past decade, several mortality risk models have been developed in order to quantify clinical prognosis after PCI.[2-14] The prediction horizon of the existing models, however, is limited and usually only covers the 30 day post-PCI period. Risk prediction instruments with a prolonged time horizon are becoming increasingly important, as decisions during PCI have long-term consequences for patient management, and impact long-term outcome. For example, the benefit of second generation drug eluting stent (DES) over first generation DES on the incidence of stent thrombosis, myocardial infarction (MI) and mortality appears beyond 3 years.[15] The potential benefits of bioresorbable scaffolds potential benefit is anticipated at even later time points.[16] Few tools are available to predict longer-term outcome.[17,18] Syntax score was created using a dataset consisting of study patients with three-vessel or left main CAD and was mainly designed to aid in decision making between coronary artery bypass grafting (CABG) and PCI.[17] The other score was based on a dataset with follow-up up to 2008 and consequently consisted of less up-to-date characteristics, such as a lower number of DES.[18]

Bioresorbable scaffolds are specifically designed to eliminate the late complications of permanent metallic implants, and improve vessel physiology. Based on the excellent early outcome of recent studies on bioresorbable scaffolds,[19,20] large-scale clinical trials are currently designed to demonstrate the superiority of these scaffolds over metal devices up to 5 years after implantation. Then, obviously, instruments are needed to help select patients with sufficient life expectancy, who will potentially benefit most from these more expensive stents.

The main objective of the Improved Drug Eluting Artificial BIOcompatable Coronary Implants: IDEA-BIO study was to create a risk model to predict long-term mortality after PCI, using simple baseline clinical and procedural variables. We explored the dataset of the 19,532 patients who underwent PCI during 2000-2011 in our institution, with up to 12 years follow-up.

METHODS

Patients and treatment

The IDEA-BIO study contains information of a total of 19,996 consecutive patients, who underwent PCI with stent placement at the Erasmus MC, Rotterdam, The Netherlands

from January 2000 to December 2011.The preferred stent type changed during the study period: bare metal stents (BMS) were used until April 2002, sirolimus-eluting stents (SES) between April 2002 and March 2003, paclitaxel-eluting stents (PES) between March 2003 and March 2007, and everolimus-eluting stents (EES) between March 2007 and December 2011. The preferred stent was almost exclusively used in all patients within these subsequent periods, except for (the small number of) patients who participated in trials comparing different stents.

Patient management was in accordance with the applicable guidelines of the European Society of Cardiology, which changed over time.[21-29] In summary, patients received antiplatelet therapy by aspirin and clopidogrel (until 2008) or prasugrel (since 2008) around the PCI procedure. Patients presenting with ST-elevation MI (STEMI) received both drugs preferably in the ambulance during transportation to the cathlab. Glycoprotein IIb/IIIa antagonists were used at the discretion of the clinician. All patients were advised to remain on a lifetime dose of aspirin, whereas clopidogrel or prasugrel was advised to be continued for at least 1, 3 or 6 months in patients with BMS, SES, PES and EES, respectively.

Baseline data Collection

At baseline, data were collected on age, gender, CAD risk factors, the broad history of CAD, renal function, indication for the index PCI (emergent, urgent or elective) and hemodynamic status at presentation. Furthermore, angiographic and procedural characteristics were collected, including the location of the culprit lesion, the number of diseased vessels, and the type, number, length en diameter of the implanted stents. Renal failure was defined as an estimated glomerular filtration rate (eGFR) <60ml/min/1.73m^2 (eGFR was calculated using the modification of diet in renal disease (MDRD) formula).

Study endpoint

The primary endpoint of this study was 5-year all-cause mortality. Patients were actively followed up on this endpoint by reviewing hospital medical records and municipal civil registries. The latest follow-up was performed in December 2012. A total of 464 (2.3%) patients were lost-to-follow-up. We report on the 19,532 patients with complete data on the primary endpoint, with a median follow-up of 3.6 years (interquartile range [IQR] 2.4 - 6.8; maximum 12 years).

Statistical analysis

Most clinical and angiographical variables that we considered had complete information in 95% of all patients. However, for body mass index (BMI), information was missing in a larger portion of research subjects (24% missing). We considered variables with up to 25% missing values and applied multiple imputation to evaluate the relation between

patient characteristics and the primary endpoint. Since the efficiency of an estimate based on m imputations is approximately $(1+y/m)^{-1}$ where y is the rate of missing information.[30] Five imputed datasets sufficed, since $(1+0.25/5)^{-1}$ provided 95.2% efficiency.

The associations between continuous variables and the primary endpoint were tested for linearity using splines and cut-off values were based on the shape of the curve. In case of linearity, cut-off values were based on clinical grounds or practical use. In case of non-linearity or u-shaped curves, multiple cut-off values were tested in the multivariate Cox model.

We randomly (2:1 ratio) divided the available patients in a training (N=13090) and validation (N=6442) dataset.[31] Multivariate Cox proportional hazard regression analyses were applied on the training set to develop a model for the prediction of long-term mortality. We used the broad range of characteristics that are listed in table 1 as potential determinants. Initially, all variables entered the model, whereas backward elimination of the least significant variables was applied, until all variables had a significance level of $p<0.05$. Based on the results of these analyses, a risk score was developed to predict long-term (i.e. 5-year) mortality post-PCI, which included the variables that comprised the final regression model, weighed according to the corresponding regression coefficient. The performance of the risk score was studied with respect to discrimination (c-index) in the training and validation data sets. For this purpose we calculated the area under the receiver characteristic curve using SPSS. However, routine tests for assessing c-indices are not designed for survival analysis. Therefore, we performed a sensitivity analysis of the calculated c-indices, by determining Harrell's c-index, which is more suitable for survival analysis,[32] using R statistical software (package "dynpred"). Performance was also analysed with respect to calibration (difference between predicted and observed mortality).

Information on left ventricular function (LVF) was lacking in 56% of patients, and therefore we decided to not consider this variable in our main analyses. Still, LVF is an important prognostic factor in PCI patients. Consequently, we decided to perform a sensitivity analysis on the subgroup of patients with complete data on LVF. We also performed a sensitivity analysis in the 4.7% of patients presenting with cardiogenic shock.

All analyses were performed by using SPSS 22.0 (SPSS Inc., Chicago, IL).

Ethics

This is an observational study. For the purpose of this study patients were not subject to acts, neither was any mode of behaviour imposed, otherwise than as part of their regular treatment. Therefore, according to Dutch law, written informed consent for a patient to be enrolled in this study was not required. This study was conducted according to the Privacy Policy of the Erasmus MC, and according to the Erasmus MC regulations for the appropriate use of data in patient oriented research.

Table 1 - Baseline characteristics

	Training cohort N=13090	Validation cohort N=6442	P
Age	63 (54-71)	63 (54-72)	0.76
Male gender	72	72	0.80
Body mass index	27 (24-30)	27 (24-30)	0.54
Hypertension	48	48	0.98
Hypercholesterolemia	55	56	0.26
Insulin dependent diabetes mellitus	5.4	5.4	0.93
Familial history of cardiovascular disease	33	34	0.23
Diabetes mellitus	19	19	0.64
Current smoker	26	26	0.66
Ever smoker	41	40	0.36
Prior myocardial infarction	29	31	0.09
Prior PCI	74	73	0.40
Prior CABG	9.3	9.8	0.30
Renal insufficiency	5.9	5.8	0.70
Indication			
Emergent PCI	32	32	0.07
Urgent PCI	28	28	0.41
Elective PCI	42	42	0.27
Number of diseased vessels			
1	50	49	0.38
2	31	32	0.28
3	19	19	0.97
Culprit vessel			
Left main	3.2	3.4	0.43
Proximal Left anterior descending	21	21	0.40
Left anterior descending	41	41	0.79
Right coronary artery	36	35	0.33
Left circumflex	23	23	0.89
Cardiogenic shock	4.6	4.8	0.52
Left ventricular function*			
Normal	77	76	0.66
Moderate	18	18	0.41
Poor	5.9	5.6	0.58
Bare metal stent	18	19	0.41
Drug eluting stent			
Paclitaxel eluting stent	36	36	0.88
Sirolimus eluting stent	7.7	7.3	0.28
Everolimus eluting stent	38	38	0.83

Continuous data are presented as median values (25th–75th percentile); nominal data are presented as percentages. CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention.
*Available for 44% of all patients

RESULTS

Patient Characteristics

Median age of the study cohort was 63 years (interquartile range 54-72) and 72% were men. The majority of patients underwent an elective procedure (42%) in hemodynamically stable condition (4.7% of patients had cardiogenic shock). Most patients received a drug eluting stent (82%). Baseline clinical and procedural characteristics were similar in the patients who composed the training and validation datasets (table 1).

Mortality risk score

A total of 3156 deaths occurred during 88620 patient-years of follow-up, implying an incidence rate of 35.6 per 1000. Overall mortality at 5 year was 12.9% and the estimated survival time of the total study population was 9.6 (CI 9.6-9.7) years. Mortality in the training and validation sets was similar. Estimated survival times assorted by age are presented in figure 1.

Variables associated with the primary endpoint in the training dataset were age, BMI <25, diabetes mellitus, renal insufficiency (glomerular filtration rate <60 mL/min) , prior myocardial infarction, indication for PCI, culprit left main, number of diseased vessels and cardiogenic shock (table 2). An analysis on the patients presenting without cardiogenic shock revealed the same factors.

The association between age and the primary endpoint was linear and the association between BMI and the primary endpoint was U-shaped, but only a BMI <25 was significantly associated with the primary endpoint when using multiple cut-off values.

Figure 1 – Kaplan-Meier curves of survival assorted by age

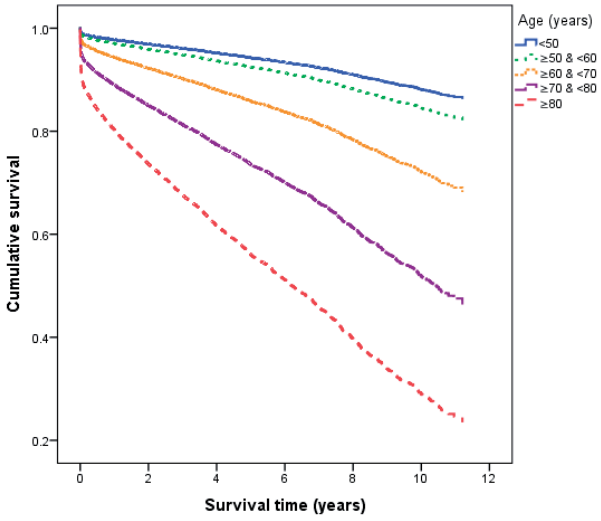


Table 2 – Risk scores for long term mortality after PCI

Risk factor	Score	Hazard ratio (95% confidence interval)
Age		
<50 years	0	-
≥50 - 60 years	2	1.66 (1.28-2.15)
≥60 - 70 years	4	2.98 (2.33-3.81)
≥70 - 80 years	6	5.14 (4.04-6.55)
≥80 years	9	9.11 (7.05-11.77)
Body mass index <25	1	1.25 (1.12-1.39)
Diabetes mellitus	2	1.64 (1.47-1.83)
Renal insufficiency	3	2.08 (1.77-2.45)
Prior myocardial infarction	1	1.25 (1.13-1.39)
Indication of PCI		
Elective	0	-
Urgent	1	1.37 (1.19-1.57)
Emergent	3	2.07 (1.77-2.41)
Culprit left main	2	1.55 (1.23-1.97)
Number of diseased vessels		
1	0	-
2	1	1.23 (1.09-1.39)
3	3	1.91 (1.69-2.16)
Cardiogenic shock	4	2.89 (2.12-3.94)

PCI, percutaneous coronary intervention.

Figure 2 – The long-term mortality after percutaneous coronary intervention prediction model with scores assigned in training cohort

Risk factor	Score
Age	
<50 years	0
≥50 - 60 years	2
≥60 - 70 years	4
≥70 - 80 years	6
≥80 years	9
Body mass index <25	1
Diabetes mellitus	2
Renal insufficiency	3
Prior myocardial infarction	1
Indication of PCI	
Elective	0
Urgent	1
Emergent	3
Culprit left main	2
Number of diseased vessels	
1	0
2	1
3	3
Cardiogenic shock	4

Total risk score	Patients %	Predicted risk % (mean)
0 - 5	35.9	1.7 – 6.0 (4.2)
6 - 10	46.8	7.6 – 18.6 (12.1)
11 - 15	15.1	22.8 – 45.2 (30.4)
15 - 25	2.2	51.6 – 91.4 (59.1)

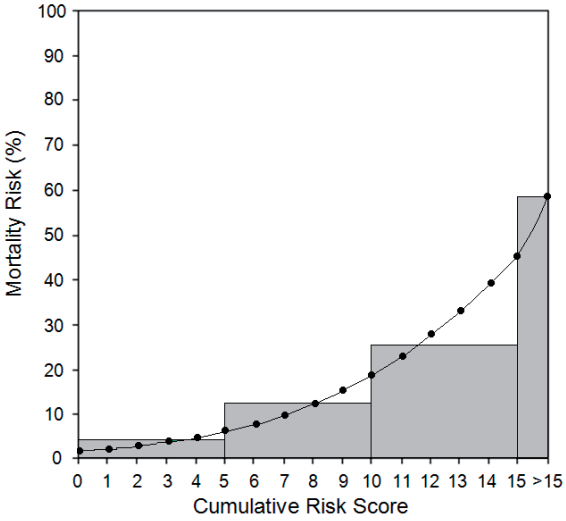


Figure 2 shows the prediction model for long-term mortality after PCI based on the training set. Theoretically, the risk score ranges between 0 and 25 points. Actually, the majority of patients had a low or intermediate mortality risk score: the 5th and 95th percentiles were 2 and 14, respectively (table 3).

Table 3 - Total risk scores

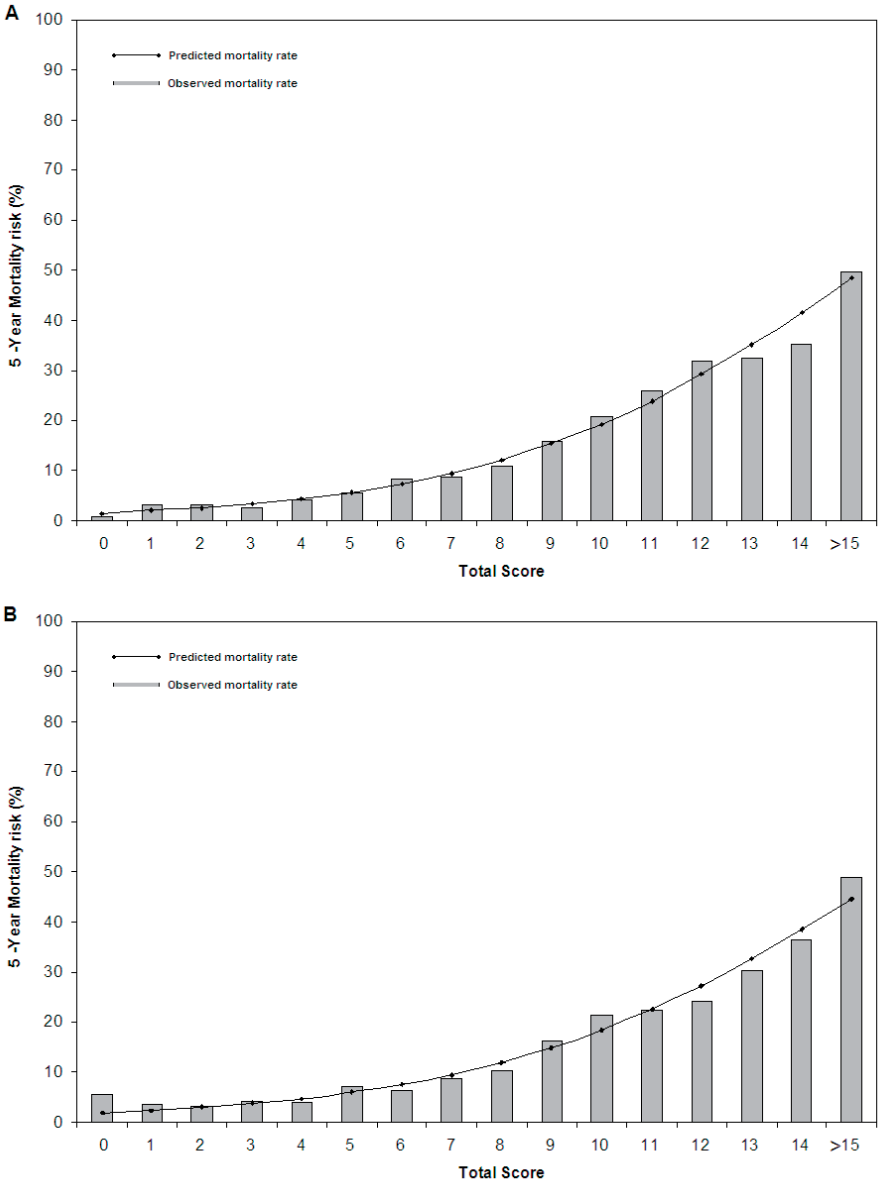
Total risk score	Patients (%)	Predicted risk (%)	
0	1.01	1.7	
1	2.23	2.2	
2	4.28	2.9	4.2
3	8.28	3.7	
4	9.02	4.7	
5	11.07	6.0	
6	10.89	7.6	
7	11.45	9.6	
8	9.60	12.1	12.1
9	8.01	15.1	
10	6.86	18.6	
11	4.91	22.8	
12	3.48	27.7	
13	3.24	33.1	30.4
14	2.11	39.0	
15	1.38	45.2	
16	0.88	51.6	
17	0.60	57.9	
18	0.34	64.0	58.1
19	0.15	69.6	
20	0.12	74.8	
21	0.04	79.3	
22	0.04	83.2	
23	0.01	86.5	81.5
24	0.00	89.2	
25	0.00	91.4	

Model performance

The performance of the long-term mortality risk prediction model in terms of discrimination was fair in the training cohort, with a median c-index 0.76 (range 0.75-0.76 in the 5 times multiple imputed datasets), as well as in the validation cohort (c-index 0.74 in all of the 5 times multiple imputed datasets) using routine tests. The performance of the model in terms of discrimination using Harrell's C-index displayed the same c-indices in

the training cohort (Harrell's c-index 0.76 in all of the 5 times multiple imputed datasets), as well as in the validation cohort (Harrell's c-index 0.74 in all of the 5 times multiple imputed datasets). Furthermore, as shown in figure 3, the model has a fair calibration in both the training and validation cohort. H-L-value of the complete risk score was signifi-

Figure 3 – Observed and predicted mortality risk by total risk score for all patients in the a) training (n=13090) and b) validation (n=6442) cohort



cant in the training cohort (p-value 0.025 (range 0.016 to 0.061)), but it has to be noted that our power was very low for patients with risk scores >15 (±2%, as shown in table 3). If the patients with scores >15 were pooled, as shown in figures 2 and 3, H-L-value of the training cohort was 0.086 (range 0.085-0.27). H-L-value of the complete risk score was 0.065 (range 0.041-0.37) in the validation cohort and 0.12 (range 0.057-0.45) if the patients with scores >15 were pooled, as described before.

Table 4 shows the model performance in clinically relevant subgroups.

Table 4 - Subgroup validation in validation cohort

Subgroup	Sample (mortality percentage)	C-index [range]	Hosmer Lemeshow p-value [range]
Age			
<50 years	967 (7.0)	0.69 [0.68-0.70]	0.13 [0.07-0.33]
≥50 - 60 years	1666 (5.8)	0.65 [0.64-0.65]	0.05 [0.05-0.06]
≥60 - 70 years	1882 (11)	0.67 [0.67-0.68]	0.09 [0.08-0.09]
≥70 - 80 years	1490 (20)	0.69 [0.69-0.69]	0.92 [0.62-0.97]
≥80 years	438 (30)	0.64 [0.64-0.65]	0.88 [0.73-0.93]
Body mass index <25	2090 (16)	0.74 [0.72-0.74]	0.34 [0.19-0.51]
Diabetes mellitus	1207 (18)	0.74 [0.74-0.75]	0.47 [0.20-0.62]
Renal insufficiency	371 (29)	0.70 [0.69-0.71]	0.64 [0.26-0.95]
Prior myocardial infarction	1968 (15)	0.76 [0.76-0.76]	0.35 [0.28-0.75]
Indication of PCI			
Elective	2795 (9.1)	0.70 [0.69-0.70]	0.48 [0.38-0.80]
Urgent	1858 (15)	0.72 [0.71-0.72]	0.44 [0.34-0.82]
Emergent	1953 (16.1)	0.76 [0.76-0.76]	0.07 [0.01-0.31]
Culprit left main	221 (29)	0.74 [0.71-0.76]	0.61 [0.36-0.99]
Number of diseased vessels			
1	3094 (8.3)	0.70 [0.70-0.70]	0.63 [0.56-0.93]
2	1919 (12)	0.72 [0.72-0.73]	0.12 [0.03-0.20]
3	1168 (23)	0.69 [0.69-0.70]	0.92 [0.58-0.95]
Cardiogenic shock	311 (31)	0.70 [0.69-0.71]	0.15 [0.12-0.16]

C-index and Hosmer Lemeshow p-value are displayed as median [range] of the 5 times multiple imputated datasets. PCI, percutaneous coronary intervention.

Subgroup analyses on patients with available data on LVF

In the patients with complete data on LVF, 77% had a normal LVF, whereas 18% and 6% had a moderate or poor LVF, respectively. After adjustment for all variables that compose the main risk model, LVF appeared a significant determinant of long-term mortality (moderate LVF HR 1.80, CI 1.49-2.16; poor LVF HR 4.25, CI 3.44-4.25). Except for culprit left main and prior myocardial infarction, all other variables remained significant. Adding

LVF to the main model did not result in an improvement of performance measures: the c-index in the training and validation sets was 0.74 and 0.78, respectively.

DISCUSSION

We succeeded in the objective of the IDEA-BIO study to create an easy to use long-term mortality risk score based on nine simple clinical and angiographic baseline variables (Table 2). A score of >10 resulted in a 20% mortality risk at 5 years, while a score of >15 resulted in 50% mortality. The score included age, BMI, diabetes mellitus, renal insufficiency, prior myocardial infarction, indication for PCI, culprit left main, number of diseased vessels and cardiogenic shock as key predictors. The risk score showed good performance in terms of discrimination and calibration. The strengths of our risk-model are the large sample size and the long follow-up, which could make it a useful tool to predict long-term mortality in PCI patients. The risk model may possibly aid in selecting patients who might benefit from more expensive treatment options, such as biodegradable scaffolds, in PCI if long-term benefit will be demonstrated by current studies.

Wilson et al described "long-term" predictors of patients undergoing PCI and revealed associations between long-term mortality after PCI and age, diabetes, cardiogenic shock, renal failure, multivessel disease and indication for PCI, but they did not create a risk score, nor did they use a specific time frame.[33] Wu et al also created a risk score for prediction of long-term mortality.[18] However, their model was based on a smaller data set with fewer DES used. Our objective was to create an easy to use risk score based on simple baseline and angiographic variables, which could be used as an aid for patients and clinicians to predict long-term survival. Our model containing only 9 variables, achieved a predictive value comparable to the 11 variable based model of Wu et al.

The original anatomical Syntax Score was developed for optimal selection of patients for multivessel disease or left main PCI or which should be referred for CABG and is based on 12 parameters assigned for all 15 major coronary artery segments.[34] Although a web based algorithm is available the score is considered cumbersome and has a poor correlation coefficient of r=0.49 and wide limits of agreement when scores of interventional cardiologist are compared to technicians of a trained corelab.[35] More important it did not include any patient or clinical parameters. The logistic clinical Syntax score based on 7 randomized trials introduced these parameters but as for most clinical trial included mostly a relative low-risk patient group (3-year mortality of pooled analysis 6.3%). Iqbal et al build a prediction model adding 4 clinical parameters to the original Syntax Score for predicting 3-year mortality.[36] The final predictive value of this model showed a c-index of 0.71, which does not reflect a potential beneficial effect of including a more extensive coronary anatomy model (Syntax Score) vs. our model with only

2 coronary artery anatomy related parameters. Their most extensive model showed comparable predictive value. The addition of an extensive anatomical model did not provide improvement of predictive value beyond our model. Finally, Farooq et al created the Syntax score 2, which may be used to predict long-term (4-year) mortality after PCI, but this score was designed for decision making for PCI versus CABG, in a study population of patients with three-vessel or left main CAD.[17] Our model was specifically created to identify PCI patients with a longer life expectancy. These patients with a longer life expectancy might benefit from new developments in PCI like more expensive stents with bioresorbable coatings or fully bioresorbable scaffolds. These stents have shown similar outcomes at one year in the selected patients,[20,37,38] and (ABSORB IV; ClinicalTrials.gov number, NCT02173379) but are designed to demonstrate superiority at 5 years. The COMPARE-ABSORB (NCT02486068) trial will include more real-world patients at higher risk of target vessel failure but with an expected life expectancy of >5 years.

LVF was a significant predictor of long-term mortality, but adding LVF to the main model did not improve its performance. This could be due to the fact that adding LVF to the model was at the expense of removing culprit left main disease and prior myocardial infarction as significant predictors. Also, the presence of cardiogenic shock, which is included in our model, often identifies patients with a poor LVF. Furthermore, the relatively great number of missing information on LVF directly reflects clinical practise since LVF is not frequently measured during or before emergent or urgent PCIs. This is also shown by the fact data on LVF is often missing in PCI risk models, created by real world registries of PCI patients.

Limitations

Several issues concerning this study warrant further consideration. First of all, PCI strategies have improved over time, which could lead to better outcomes in patients who underwent PCI more recently than patients receiving a PCI over 10 years ago. Despite all developments in PCIs over time, we did not find significant differences in 30-day mortality rates between patients who underwent a PCI between 2000-2002, 2003-2005, 2006-2008 and 2009-2011, but we cannot compare long-term differences in mortality rates, due to the differences in follow-up time inherent to the time-period of inclusion.

Moreover, some patients have very long-term follow-up data (i.e. 12 years), but the data is not robust at this range of follow-up. Since median follow-up was 3.6 years, the model should be useful for PCI patients with a longer life expectancy in general, but future studies should study its usefulness in bioabsorbable scaffolds recipients.

Additionally, despite the fact that our registry includes multiple important characteristics, it is possible that several potentially important factors were not recorded, such as atrial fibrillation, haemoglobin level (which is a known risk factor for PCI patients, as described by Nikolsy et al),[39] or other co morbidity burden or biomarkers, could be

prognostically important. Addition of biomarkers associated with adverse outcomes after PCI, may further enhance long-term mortality risk prediction after PCI. Medicinal treatment such as the use of beta-blockers, diuretics, angiotensin-converting enzyme, platelet inhibitors, etc., was deliberately not entered into the models, because their estimates could lead to confounding by indication. Furthermore, renal failure was calculated using the MDRD formula, but recent data suggests using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation for risk stratification among patients undergoing PCI.[40]

Finally, our results are based on a single centre experience, which could limit external validity. Yet, the Thoraxcentre in Rotterdam can be considered representative for larger tertiary referring and teaching or academic hospitals.

Conclusion

We developed and validated a risk score to predict long-term mortality after PCI, based on a large sample of heterogeneous patients with long-term follow-up, up to 12 years. The tool is easy to use, contains solely simple baseline clinical and angiographic variables and adequately predicts long-term mortality after PCI. The model may be a useful tool to select patients who might benefit from more expensive PCI treatment options, such as biodegradable scaffolds, if long-term benefit will be demonstrated by current studies. A score of 10 or more selects patients with a predicted 5 year mortality of more than 20 percent.

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Chapter 3

Stent thrombosis in early-generation drug-eluting stents versus newer-generation everolimus-eluting stent assorted by left ventricular ejection fraction

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ABSTRACT

Background: Everolimus drug-eluting stents (EES) are superior to early generation drug-eluting stents (DES), releasing sirolimus (SES) or paclitaxel (PES) in preventing stent thrombosis (ST). Since an impaired left ventricular ejection fraction (LVEF) seems to increase the risk of ST, we aimed to investigate the difference in outcome of patients with varying LVEF using EES versus early generation DES.

Methods: In a prospective cohort study we compared the risk of ST in patients in 3 LVEF subgroups: normal (LVEF >50%), mildly impaired (LVEF >40%&≤50%) and moderate-severely impaired (LVEF ≤40%). Within these various LVEF groups we compared EES to SES and PES after adjustment for baseline differences.

Results: We assessed a cohort of 5363 patients, with follow-up of up to 4 years and available LVEF. Overall definite ST occurred in 123 (2.3%) patients. ST rates were higher in the LVEF moderate-severely impaired group as compared to the normal LVEF group (2.8% vs. 2.1%; Hazard radio (HR) 1.82; confidence interval (CI) 1.10-3.00). Especially early ST (EST) was more frequent in the moderate-severely impaired LVEF group (HR 2.20; CI 1.06-4.53). Overall rates of definite ST were lower in patients using EES, as compared to patients using SES or PES in all LVEF groups. Interaction terms were not statistically significant. ST rates were higher in the moderate-severely impaired LVEF group compared to the normal LVEF group when using SES or PES, but not significantly different when using EES.

Conclusions: EES was associated with a lower risk of definite ST compared to early generation DES. This lower risk was independent of LVEF, even though ST rates were higher in patients with a moderate-severely impaired LVEF.

INTRODUCTION

Early-generation drug-eluting stents (DES), sirolimus eluting stents (SES) and paclitaxel eluting stents (PES) appeared to be very effective at reducing the rates of restenosis and target lesion revascularization compared to bare metal stents.[1-4] However, stent thrombosis (ST), which causes acute coronary obstruction and could lead to myocardial infarction (MI) or sudden cardiac death, was found to be an infrequent, but devastating complication of DES. Multiple randomized trials showed a comparable incidence of ST in DES compared to bare metal stents up to 1 year after the percutaneous coronary intervention (PCI).[5-7] However, ST occurring more than 1 year after the index PCI, which is mainly caused by delayed healing in combination with other clinical and procedural risk factors of the stented coronary segment,[8-11] appeared as a serious complication of DES stents. More recently, several randomized controlled trials have shown a reduction in the incidence of ST occurring more than 1 year after the index PCI with newer generation DES, releasing everolimus (i.e. everolimus-eluting stents - EES),[12-14] which may have a favourable effect on cardiac death and incident MI. In these stents, everolimus, a sirolimus analogue, is released from a thin coating with more biocompatible polymers and reduced drug dose.

Several studies have identified impaired left ventricular function as a risk factor for mortality,[15-19] MI,[15,17], ST.[17,20,21] and target lesion revascularization,[22] in patients undergoing PCI. It has been suggested that these patients may benefit from drug-eluting stents,[22] especially since patients with impaired left ventricular ejection fraction (LVEF) more frequently present with an acute coronary syndrome in case of in-stent restenosis.[23] Since newer generation EES stents seem to be superior to early generation DES in an overall population of patients undergoing PCI,[12-14] it would be useful to provide knowledge about the long-term safety and benefits of newer generation EES in patients with a reduced cardiac function.

Against this background, we evaluated the long-term incidence of ST and clinical outcomes after PCI with DES in relation to LVEF in the Bern part of the Bern-Rotterdam registry. Secondly, we studied the performance of the newer generation DES (EES) compared with the early-generation SES and PES in relation to left ventricular function.

METHODS

Study Design, Patient Population and Procedures

For the purpose of this study, we used the Bern (Bern University Hospital, Bern) part of the Bern-Rotterdam registry, which has been described before.[12] We only used the Bern part of the registry, since systematic data on the LVEF was not available in the Rot-

terdam part of the study population. The study population we used, consisted of 5761 consecutive patients, who underwent PCI with PES, SES or EES, between April 2002 and March 2009. Between April 16, 2002, and December 31, 2005, a total of 2774 consecutive patients underwent coronary intervention with SES (Cypher, Cordis Corp, Johnson & Johnson, Warren, NJ) and 1365 were treated with PES (TAXUS, Express, or Liberte´, Boston Scientific) in the Swiss centre. The individual use of both stent types has been described in detail elsewhere.[24] A total of 1622 patients were treated with EES (XIENCE V, Abbott Vascular, Santa Clara, CA; or PROMUS, Boston Scientific, Natick, MA), which has been part of the usual care since November 1, 2006 and implanted on a daily basis alternating with biolimus-eluting stents and zotarolimus-eluting stents. Patients with EES were included until March 31, 2009. Patients were excluded from the registry if more that one stent type was implanted during PCI. The nature of this study was observational and the procedure, periprocedural and postprocedural medication regimen, were performed according to current practice guidelines. The diameter of EES and PES was 2.25 to 4.0mm and the diameter of SES was 2.25 to 3.5mm. The length of all stents started at 8 mm, with a maximum length of 28mm of EES, 33mm of SES and PES had a maximum length of 32mm. All patients received a loading dose of clopidogrel 300 to 600mg during or immediately after PCI and a lifelong prescription of daily aspirin. Clopidogrel was prescribed for duration of at least 12 months. Glycoprotein IIb/IIIa antagonists were used at the discretion of the clinician.

The registry was approved by the local ethics committees, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

Left ventricular function

Baseline LVEF was routinely assessed by left ventricular angiography at the time of coronary angiography and determined by visual estimate. The visual estimate was performed by 2 experienced cardiologists. First by a fellow interventional cardiology and this was subsequently verified or overruled by an attending senior invasive cardiologist. The estimate was based on the average of the right anterior oblique and left anterior oblique projection. Cineangiography was usually performed at 15 frames per second during injection of at least 30 ml contrast agent at a rate of 10 ml per second. Attention was paid to left ventricular size, overall contractility, any wall motion abnormalities and presence of mitral regurgitation. All senior invasive cardiologists perform yearly more than 500 coronary angiographies and the experience of a fellow interventional cardiology evolves over time. On average, a fellow interventional cardiology performs more than 800 cases in one year.

We divided patients in 3 different categories: 1) normal LVEF (>50%), 2) mildly impaired LVEF (>40-≤50%) or 3) moderate-severely impaired LVEF (≤40%), which corresponds

with generally accepted thresholds.[15,19,25] LVEF was available in 5446 (94.5%) patients in the Swiss centre. Further analysis was performed only on patients with available LVEF.

Study endpoints

In Bern, follow-up lasted until February 1, 2007 in patients with SES or PES implantation and patients who received EES were followed-up until February 1, 2010. Thus, the follow-up observation was up to 4 years in these subjects. Survival status was obtained from hospital records and municipal civil registries. Patients were actively followed up on adverse cardiac events by questionnaires that were sent to patients including questions on rehospitalization and major adverse cardiac events. Medical records, discharge letters and coronary angiography documentation were collected and reviewed in patients with suspected events.

Primary endpoint: definite stent thrombosis

Definite ST was the primary endpoint of our study, which was defined in agreement with the definitions of the Academic Research Consortium (ARC).[26] We further classified the endpoint according to its timing (again in agreement with ARC): early ST (EST) (0-30 days after stent implantation) and late ST (LST) (>30 days). An independent clinical event committee adjudicated all suspect ST events. The committee members were not informed on the type of stent implanted.

Secondary endpoints

Secondary endpoints comprised adverse clinical events, including death from any cause, cardiac death, MI, as well as definite or probable ST.

Cardiac death was defined as any death from an immediate cardiac cause, procedure-related death, unwitnessed death, and death with an unknown cause. Myocardial infarction (MI) was defined as increased creatine kinase >2 times the upper limit of the normal value and >3 times the upper limit of the normal value of creatine kinase-MB in combination with ischemic changes on ECG. All suspect clinical events were adjudicated by the cardiologists who were affiliated with the institutions in which the patients were treated.

Statistical Analysis

Categorical baseline and procedural variables are presented as counts and percentages, whereas differences between the three LVEF groups are evaluated by Pearson's χ^2 test, or Fisher's exact test, as appropriate. Continuous variables are presented as mean ± one standard deviation (SD), in case of a normal distribution, or as median, 1st and 3rd quartiles, in case of a non-normal distribution. Differences between the LVEF groups

were evaluated by applying analysis of variance (ANOVA), Student t tests, Kruskall-Wallis tests and Mann-Whitney U tests, as appropriate.

The incidence of the primary and secondary endpoints during follow-up in relation to left ventricular function was evaluated according to the method of Kaplan-Meier in combination with log-rank tests, and by Cox proportional hazard regression analysis. Multivariable Cox models were developed to adjust the relation between LVEF and outcome for potential confounders. Baseline (clinical and procedural) characteristics that had a statistically significant ($p<0.05$) relation with the endpoint were entered into the model. No model-reduction strategies were applied. We report adjusted hazard ratios (aHR) and their 95% confidence intervals (CI). All events were only counted once.

Subsequently, we studied the relation between DES types and the study endpoints in relation to left ventricular function. For this purpose we used a method that our research group applied previously.[12] We estimated propensity scores for receiving EES using a logit model that included age, sex, and pre-treatment variables associated with stent selection at $p<0.10$: family history of coronary artery disease, acute coronary syndrome, cardiogenic shock and body mass index. Propensity scores were used to derive the inverse probability of treatment weights, with the inverse of the propensity score as analytic weights in EES patients and the inverse of 1 minus the propensity score in early-generation DES patients. Comparisons between DES types were performed with the Cox model. Adjusted HRs were calculated with the inverse probability of treatment weights as analytical weighing factors. All Cox proportional hazard assumptions were visualized using the graph of the log(-log(survival)) versus log of survival time graph and tested with Schoenfeld residuals. Interaction terms between LVEF and DES type were added to evaluate homogeneity of the effect of DES type on the study endpoints in relation to left ventricular function.

RESULTS

A total of 5761 consecutive patients underwent PCI with PES (1365), SES (2774) or EES (1622) between April 16, 2002, and March 31 2009. All analyses were performed on 5363 patients with PES (1298), SES (2599) or EES (1466), because LVEF was unavailable in 315 patients and 83 patients were lost to follow-up. Median follow-up was 2.4 years (interquartile range (IQR) 1.9-3.0 years) in patients treated with EES, 3.6 years (IQR 2.8-4.0 years) in patients treated with SES and 4.0 (IQR 3.4-4.0 years) in patients treated with PES.

Baseline and procedural characteristics

We observed several differences in clinical baseline and procedural characteristics between the three LVEF groups (table 1). Most notable were the differences in age, hy-

Table 1 – Baseline and Procedural Characteristics

Variable	LVEF >50% (n=3106)	LVEF >40%-≤50% (n=1244)	LVEF ≤40% (n=1013)	P
Age (years)	63.4±11.2	62.5±12.2	65.6±12.0	<0.0001
Male sex	2343 (75.4)	982 (78.9)	759 (74.9)	0.03
BMI	27.4±4.2	27.3±4.3	26.6±4.2	<0.0001
Hypertension	1897 (61.2)	640 (51.6)	490 (49.0)	<0.0001
Family history of CAD	960 (30.9)	323 (26.0)	219 (21.9)	<0.0001
Current smoking	1646 (53.1)	666 (53.7)	466 (46.6)	0.0007
Dyslipidemia	1827 (58.9)	609 (49.1)	444 (44.4)	<0.0001
Diabetes Mellitus	529 (17.1)	203 (16.4)	195 (19.5)	0.12
Renal failure (GFR<60mL/min)	291 (12.1)	107 (12.0)	180 (21.8)	<0.0001
Acute coronary syndrome	1276 (41.1)	921 (74.0)	757 (74.7)	<0.0001
Unstable angina	222 (17.4)	55 (6.0)	27 (3.6)	
Non-ST-segment-elevation MI	686 (53.8)	343 (37.2)	267 (35.3)	
ST-segment-elevation MI	368 (28.8)	523 (56.8)	463 (61.1)	
Cardiogenic shock	0	11 (0.9)	59 (5.8)	<0.0001
Paclitaxel eluting stent	804 (25.9)	300 (24.1)	194 (19.2)	<0.0001
Sirolimus eluting stent	1566(50.4)	584 (46.9)	449 (44.3)	0.002
Everolimus eluting stent	736 (23.7)	360 (28.9)	370 (36.5)	<0.0001
Multivessel treatment	609 (19.7)	177 (14.3)	181 (17.9)	0.0001
Lesions treated per patient	1.6±0.8	1.5±0.8	1.6±0.9	0.23
Culprit left main coronary artery	59 (1.8)	22 (1.8)	62 (6.1)	<0.0001
Arterial bypass graft	8 (0.3)	2 (0.2)	3 (0.3)	0.78
Saphenous vein graft	80 (2.6)	33 (2.7)	44 (4.3)	0.01
Multistent treatment	1492 (48.0)	632 (50.8)	549 (54.2)	0.03
Average stent diameter	2.9±0.6	2.9±0.4	3.0±0.4	0.004
Total stent length per patient	28.6±18.0	30.8±18.1	32.2±18.8	<0.0001
Glycoprotein IIb/IIIa antagonist	441 (14.2)	390 (31.4)	353 (34.8)	<0.0001
Aspirin at discharge	3176 (99.1)	1225 (98.5)	963 (95.1)	<0.0001
Clopidogrel at discharge	3098 (99.7)	1227 (98.6)	963 (95.1)	<0.0001
Oral anticoagulation at discharge	37 (1.2)	18 (1.5)	38 (3.8)	<0.0001

Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. Continuous variables are expressed as mean ±SD when appropriate. BMI indicates body mass index; CAD, coronary artery disease; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; MI, myocardial infarction.

pertension, body mass index, family history of coronary artery disease, smoking status, dyslipidemia and renal failure. Patients with a moderate-severely impaired or mildly impaired LVEF presented more often with an acute coronary syndrome (74.7% and 74.0%

respectively) than patients with a normal LVEF (41.1%). Only patients with an impaired left ventricular function presented with cardiogenic shock (0.9% in the mildly impaired LVEF group and 5.8% in the moderate-severely impaired LVEF group).

Remarkable were the differences in number of patients undergoing multivessel treatment, stent diameter and length, number of stents implanted, use of glycoprotein IIb/IIIa and discharge medication. Normal LVEF patients more frequently had PES or SES implanted, compared to more EES use in moderate-severely impaired LVEF patients. In the moderate-severely impaired LVEF group, the culprit vessel was more frequently the left main or left anterior descending artery, whereas relatively more normal LVEF patients culprit lesions in the left circumflex or right coronary artery.

Study endpoints in relation to left ventricular function

The study endpoints are presented in table 2. Definite ST occurred in 123 (2.3%) patients and definite/probable ST occurred in 331 (6.2%) subjects. No patients had multiple ST's. In general, patients with impaired left ventricular function had a higher incidence of ST. In particular, patients with a moderate-severely impaired LVEF had a higher incidence of definite ST (aHR 1.82; 95% CI 1.10-3.00; p-value 0.02) and definite/probable ST (aHR 1.86; 95% CI 1.30-2.66; p-value 0.001) than patients with a normal LVEF. This difference was based on higher EST rates in moderate-severely impaired LVEF patients (aHR 2.20; CI 1.06-4.53), whereas LST rates did not significantly differ (aHR 1.34; CI 0.72-2.50).

Table 2 - Clinical Outcomes

				Adjusted hazard ratio			
Variable	**LVEF >50% (n=3106)**	**LVEF >40%-≤50% (n=1244)**	**LVEF ≤40% (n=1013)**	**LVEF >40%-≤50% vs LVEF >50%**	**P**	**LVEF ≤40% vs LVEF >50%**	**P**
Definite ST	64 (2.1)	31 (2.5)	28 (2.8)	1.22 (0.75-1.99)	0.42	1.82 (1.10-3.00)	0.02
Early	18 (0.6)	10 (0.8)	14 (1.4)	1.24 (0.56-2.73)	0.60	2.20 (1.06-4.53)	0.03
Late	46 (1.5)	21 (1.7)	14 (1.4)	1.09 (0.63-1.86)	0.77	1.34 (0.72-2.50)	0.35
Definite/ probable ST	143 (4.6)	75 (6.0)	113 (11.2)	1.28 (0.88-1.86)	0.20	1.86 (1.30-2.66)	0.001
Early	50 (1.6)	38 (3.1)	82 (8.1)	1.39 (0.80-2.41)	0.24	2.09 (1.27-3.41)	0.004
Late	93 (3.0)	37 (3.0)	31 (3.1)	1.17 (0.69-1.97)	0.56	1.75 (1.03-2.98)	0.04
Death	177 (5.7)	109 (8.8)	194 (19.2)	1.46 (1.09-1.95)	0.01	2.09 (1.60-2.73)	<0.0001
Cardiac death	104 (3.4)	82 (6.6)	150 (14.8)	1.88 (1.32-2.66)	<0.0001	2.58 (1.86-3.58)	<0.0001
MI	138 (4.4)	53 (4.2)	49 (4.8)	1.05 (0.76-1.45)	0.77	1.37 (0.98-1.92)	0.07

LVEF indicates left ventricular ejection fraction; ST, stent thrombosis; MI, myocardial infarction. Clinical outcome numbers are expressed as counts (percentage). Adjusted HR's were calculated using multivariate analysis. All baseline and procedural characteristics with P<0.05 in univariate analysis were entered into the multivariate analysis to calculate adjusted HR's.

The incidence of clinical endpoints was also clearly associated with left ventricular function. Specifically, more patients in the moderate-severely impaired LVEF group died (aHR 2.09; CI1.60-2.73). A Similar difference in clinical outcome was found in the incidence of cardiac death (aHR 2.58; CI 1.85-3.58). The occurrence of MI between was equal in the various LVEF groups.

Left ventricular function, stent type and study endpoints

Table 3 and figure 1 show the relationship between DES type and study endpoints. In general, patients who received EES had a lower incidence of ST than patients receiving PES or SES, although statistical significance could not be demonstrated for all endpoints. Differences between EES and PES were most pronounced. These observations were consistent in the three groups of patients according to LVEF. In particular, patients treated with EES had a lower incidence of definite ST than those treated with PES (aHRs 0.15, 0.13

Table 3 – Clinical Outcomes, Sorted by Drug-Eluting Stent Type

					Adjusted hazard ratio			
Variable	LVEF	EES	SES	PES	EES vs SES	P	EES vs PES	P
Overall definite ST	**≤40%**	3 (0.03)	14 (0.10)	11 (0.16)	0.28 (0.08-0.98)	0.04	0.15 (0.04-0.55)	0.004
	>40%-≤50%	1(0.01)	22 (0.10)	8 (0.07)	0.11 (0.02-0.78)	0.03	0.13 (0.02-0.99)	0.049
	>50%	3 (0.01)	35 (0.06)	26 (0.08)	0.20 (0.06-0.71)	0.01	0.12 (0.03-0.44)	0.001
Early definite ST	**≤40%**	1 (0.29)	7 (1.68)	6 (3.31)	0.21 (0.03-1.71)	0.14	0.11 (0.01-0.89)	0.04
	>40%-≤50%	1(0.28)	5 (0.88)	4 (1.37)	0.44 (0.05-3.75)	0.45	0.30 (0.02-2.83)	0.15
	>50%	2 (0.27)	11 (0.71)	5 (0.63)	0.42 (0.09-1.91)	0.26	0. (0.09-2.34)	0.35
Late definite ST	**≤40%**	2 (0.02)	7 (0.05)	5 (0.08)	0.37 (0.08-1.69)	0.20	0.21 (0.04-1.07)	0.06
	>40%-≤50%	0	17 (0.08)	4 (0.03)	N.A.	N.A.	N.A.	N.A.
	>50%	1 (0.00)	24 (0.04)	21 (0.06)	0.05 (0.01-0.36)	0.03	0.02 (0.00-0.18)	<0.0001
Overall definite/ probable ST	**≤40%**	31 (0.31)	53 (0.37)	29 (0.44)	0.70 (0.45-1.09)	0.12	0.52 (0.31-0.88)	0.02
	>40%-≤50%	15 (0.15)	38 (0.18)	22 (0.18)	0.67 (0.36-1.25)	0.21	0.65 (0.33-1.30)	0.22
	>50%	19 (0.09)	77 (0.13)	47 (0.14)	0.73 (0.44-1.20)	0.21	0.47 (0.27-0.81)	0.01

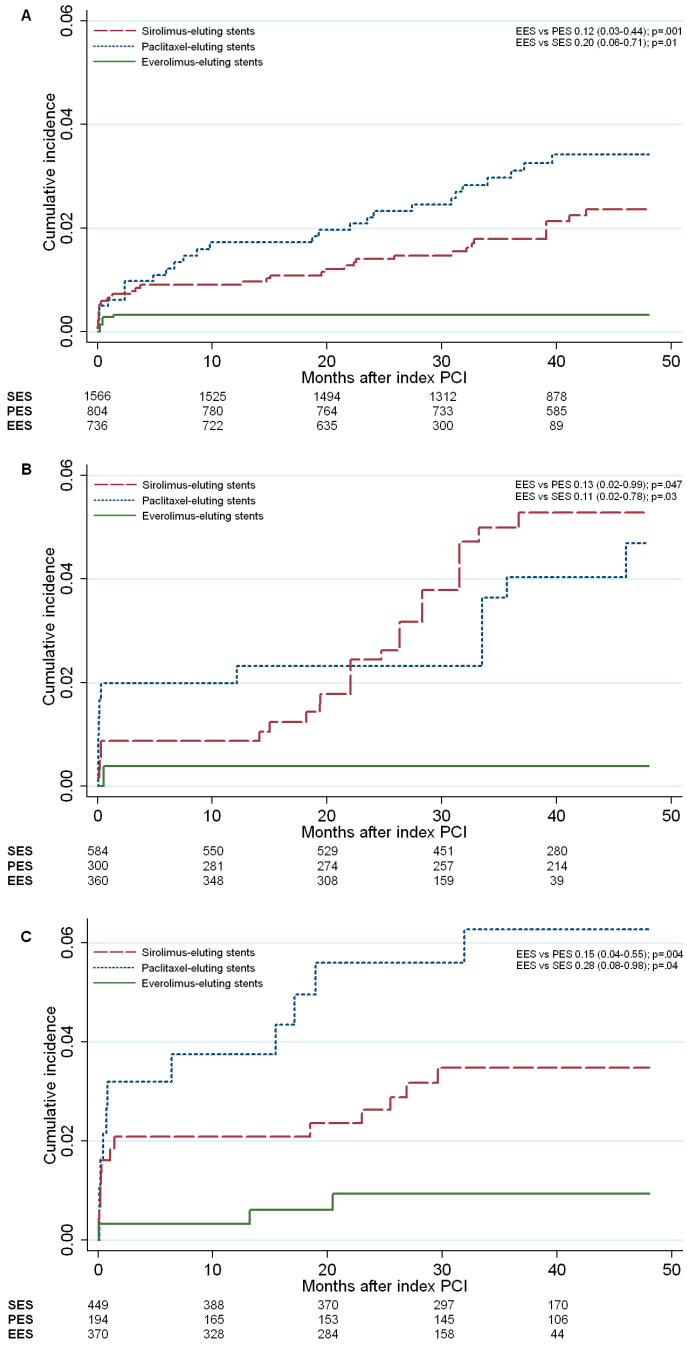
Table 3 – Clinical Outcomes, Sorted by Drug-Eluting Stent Type (continued)

Variable	LVEF	EES	SES	PES	Adjusted hazard ratio: EES vs SES	P	EES vs PES	P
Early definite/ probable ST	**≤40%**	26 (7.49)	38 (9.14)	18 (9.94)	0.77 (0.47-1.28)	0.32	0.73 (0.40-1.33)	0.30
	>40%-≤50%	11 (3.14)	16 (2.81)	11 (3.79)	1.00 (0.45-2.21)	0.99	0.71 (0.29-1.72)	0.44
	>50%	12 (1.65)	23 (1.49)	15 (1.90)	1.18 (0.58-2.38)	0.65	0.89 (0.41-1.90)	0.75
Late definite/ probable ST	**≤40%**	5 (0.05)	15 (0.11)	11 (0.17)	0.48 (0.17-1.33)	0.16	0.22 (0.07-0.71)	0.01
	>40%-≤50%	4 (0.04)	22 (0.11)	11 (0.09)	0.41 (0.13-1.23)	0.11	0.58 (0.19-1.78)	0.34
	>50%	7 (0.03)	54 (0.09)	32 (0.10)	0.45 (0.20-.0.98)	0.045	0.26 (0.11-0.61)	0.002
Death	**≤40%**	59 (0.59)	92 (0.61)	43 (0.61)	0.81 (0.58-1.13)	0.22	0.78 (0.52-1.18)	0.24
	>40%-≤50%	27 (0.26)	50 (0.23)	32 (0.26)	1.01 (0.61-1.67)	0.96	0.93 (0.53-1.62)	0.80
	>50%	28 (0.13)	91 (0.15)	58 (0.17)	0.84 (0.55-1.28)	0.42	0.71 (0.45-1.11)	0.13
Cardiac Death	**≤40%**	47 (0.47)	68 (0.45)	35 (0.49)	0.85 (0.58-1.25)	0.42	0.75 (0.48-1.18)	0.21
	>40%-≤50%	21 (0.20)	35 (0.16)	26 (0.21)	1.12 (0.64-1.98)	0.69	0.83 (0.45-4.53)	0.55
	>50%	16 (0.07)	50 (0.08)	38 (0.11)	0.85 (0.49-1.49)	0.57	0.62 (0.35-1.11)	0.11
MI	**≤40%**	6 (0.06)	27 (0.19)	16 (0.24)	0.29 (0.12-0.70)	0.01	0.19 (0.07-0.54)	0.002
	>40%-≤50%	11 (0.11)	25 (0.12)	17 (0.14)	0.65 (0.35-1.57)	0.44	0.65 (0.29-1.43)	0.28
	>50%	19 (0.09)	74 (0.12)	45 (0.14)	0.77 (0.47-1.29)	0.32	0.50 (0.29-0.87)	0.01

EES indicates everolimus-eluting stent; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PES, paclitaxel-eluting stent; SES, sirolimus-eluting stent; ST, stent thrombosis. Clinical outcome numbers are expressed as counts (number of events/ month/ 100 patients). Adjusted risk ratios were calculated with the inverse probability of treatment weights as analytical weighting in Cox proportional hazards models.

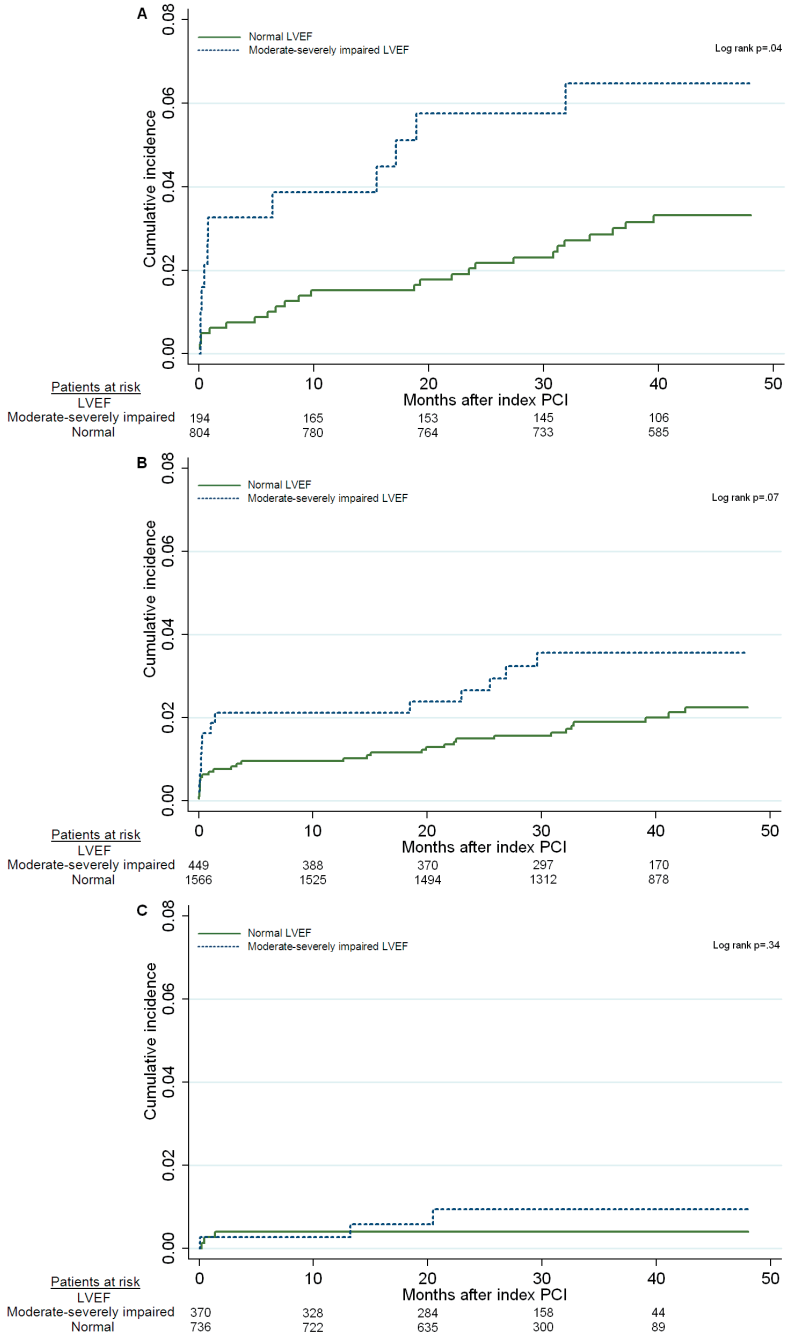
and 0.12 for those with a normal, mildly impaired and moderate-severely impaired LVEF respectively) and a lower incidence of definite/probable ST (aHRs 0.47, 0.65 and 0.52). Moderate-severely impaired LVEF patients had significantly higher rates of definite ST when using PES (P=0.04) and a trend towards a higher incidence of ST when using SES

Figure 1 - Kaplan-Meier Hazard Curve of Overall Definite Stent Thrombosis



Overall definite stent thrombosis in patients using different drug-eluting stents of patients with a a) moderate-severely impaired left ventricular ejection fraction, b) mildly impaired left ventricular ejection fraction and c) normal left ventricular ejection fraction.

Figure 2 - Kaplan-Meier Hazard Curve of Overall Definite Stent Thrombosis



Overall definite stent thrombosis in patients with a moderate-severely impaired vs. normal left ventricular ejection fraction of patients with a) paclitaxel-eluting stents (PES), b) sirolimus-eluting stents (SES) and c) everolimus-eluting stents (EES).

(P=0.07), whereas ST rates were similar in both LVEF groups when using EES (P=0.34) (figure 2).

Most noteworthy, concerning the differences in incidence of clinical endpoints between the DES types, is the difference in the incidence of MI between patients treated with EES and those treated with PES (aHR 0.50, 0.65 and 0.19). The graph of the log(-log(survival)) versus log of survival time graph resulted in parallel curves and Schoenfeld residuals indicated Cox proportional hazard assumptions were not violated.

None of the LVEF * DES type interaction terms were significant in the multivariate analyses that we applied. Thus, we did not reveal any indication of heterogeneity in the relation between DES type and the study endpoints according to left ventricular function.

DISCUSSION

This large observational cohort study with long-term follow-up of patients undergoing PCI with early generation DES or newer generation EES shows us that: 1) impaired left ventricular function was associated with increased risk of ST; 2) newer generation EES was associated with a reduced risk of ST compared to early generation DES; 3) the association between relative reduction of ST and EES was independent of LVEF. 4) EES seemed especially associated with reduced LST rates in patients with a normal LVEF, while the lower ST rates in the mildly impaired and moderate-severely impaired LVEF groups seemed unrelated to the timing of ST.

Our findings, concerning the association between increased risk of ST and patients with impaired left ventricular function are consistent with earlier findings.[17,20,21] Although the exact cause of the higher rate of ST in patients with reduced left ventricular function remains unclear, it has been suggested that a decrease in LVEF is associated with impaired blood flow through the stented coronary artery, increasing the risk of ST.[27] In this study, the overall risk of ST in patients with impaired LVEF was increased by 61% compared with patients with a normal LVEF and by 13% compared to patients with a mildly impaired LVEF. Interestingly, a moderate-severely impaired LVEF did not seem to be associated with LST, but the difference in overall ST rates seems to be due to the strong association between the increased risk of having EST and a a moderate-severely impaired LVEF, compared to patients with a normal LVEF (a moderate-severely impaired LVEF was associated with a 120% increased risk of having EST). Sardi et al found an increase in the risk of ST with a HR of 2.56 (CI 1.44-4.55) when comparing patients with a LVEF of 25-40% to patients with a normal LVEF during 1 year follow-up, which is somewhat higher than the increase in the risk of ST we found in patients with a moderate-severely impaired LVEF of ≤40% at 1 year (HR 1.84; CI 1.01-3.34), but CI's are

overlapping.[17] Van Werkum et al also found considerably higher ST rates in patients with impaired systolic LV function (HR 2.27; CI 1.43-3.60), but they defined impaired cardiac function as LVEF<30%.[21] A LVEF <30% is substantially lower than a LVEF ≤40%. Since a poorer LVEF seems to increase the risk of ST, a higher HR in this study is in line with expectations.

Patients with a moderate-severely impaired LVEF, treated with different kinds of DES also had higher all-cause mortality and cardiac mortality risk compared to normal LVEF patients. A trend towards a higher risk of MI was found in patients with a moderate-severely impaired LVEF. Our findings regarding the relationship between a reduced LVEF and the increased mortality,[15-18] and risk of MI,[15,17] are also consistent with earlier findings. Mortality and cardiac mortality seem to increase as LVEF drops. In the moderate-severely impaired LVEF group, overall one year mortality was 11.8% and 11.3% in patients who had EES implanted. The one year mortality rates in this group are comparable to earlier findings.[15,17] Patients with a mildly impaired LVEF and normal LVEF showed one year mortality rates of 4.4% and 1.8% respectively.

The present study is the first study that specifically compared the outcome of early generation DES stents, SES and PES, compared to newer generation EES in patients with a varying systolic cardiac function, in a registry with long-term follow-up observation to 4 years. This registry has the advantage that it consists of consecutive patients, has long-term follow-up and is not a post hoc analysis of a large randomized controlled trial, which apply specific inclusion criteria that complicates extrapolation of their results to the more diverse, real-life population of patients.

The study shows that EES is associated with lower ST rates compared to early generation DES, irrespective of left ventricular function. Interestingly, when comparing the difference in definite overall ST rates between patients with a moderate-severely impaired LVEF versus normal LVEF per stent, we found statistically higher ST rates in patients using PES and a trend towards higher ST rates when using SES, but no differences in the incidence of ST when found when using EES. On the other hand, we found no interaction when comparing EES to early generation DES in various LVEF groups. It could be possible that the event rate in our study population of patients using EES was to low to show interaction. Another explanation could be the fact that a moderate-severely impaired LVEF seemed to be especially associated with increased risk of EST, whereas EES, a sirolimus derivate, with more biocompatible polymers and reduced drug dose, seemed predominantly associated with lower LST and very late ST rates compared to early generation DES.[12-14] Although EES also seems superior in preventing EST compared to early generation DES.[12,13]

The findings of this study can be used in clinical practice when performing a PCI in patients with systolic dysfunction. The study emphasizes the known fact that EES is superior to early generation DES, but also shows that whether systolic dysfunction is pres-

ent or not, EES remains superior. Even after 4 years. When performing PCI on patients with a reduced LVEF, the executive physician should keep in mind that these patients have higher ST rates, in particular EST, higher incidence of MI and higher mortality rates. Despite these improved lower ST rates achieved by using EES, more efforts and research to improve ST and mortality rates is mandated to improve the outcome of patients with a reduced systolic cardiac function, undergoing PCI.

Limitations

Several issues concerning this study warrant further consideration. First of all, the ST event-rate was quite low, especially in EES patients. This low event-rate may have caused a lack of power to show significant differences.

The use of LVEF, which was performed angiographically, using biplane assessment by visual estimate, is inherently an issue, since reproducibility of this method may be questioned. To accommodate this limitation, we divided all study patients in 3 different LVEF categories, to reduce the effect of measurement discrepancies in LVEF assessment.

Furthermore, there were differences in baseline characteristics between patients who had been stented with EES, SES and PES after PCI and no randomization was performed. These differences in baseline characteristics had to be adjusted for using the inverse probability of treatment weighting, as we did before,[12] to minimize a potential bias. Also, follow-up at 4 years was not complete in patients treated with EES and PES.

Another issue that should be taken into account, is the fact that a primary arrhythmia causing death within 30 days would full the ARC criteria for probable ST,[26] which withholds us from distinguishing between sudden death due to ST and a primary arrhythmia. This may consequently have influenced our results.

PCI strategies have improved over time, which may have contributed to an improved outcome among patients with EES, compared to patients with early generation DES.

Other limitations of this study are related to the database and these limitations have been reported before.[12]

Conclusion

Newer generation EES was associated with a reduced risk of ST compared to early generation DES, even after long-term follow-up of up to 4 years, regardless of LVEF. The relative reduction found, was independent of LVEF, even though ST rates were higher in patients with a moderate-severely impaired LVEF. This association between reduced ST rates and a normal LVEF using EES was predominantly based on lower LST rates, whereas the lower occurrence of ST achieved by EES in patients with a reduced LVEF seemed unrelated to the timing of ST. Finally, all-cause mortality and cardiac mortality rates seems inversely related to left ventricular function.

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Supplemental Table – Baseline and Procedural Characteristics of all Patients, Sorted by Stent Type

Variable	Paclitaxel eluting stent (n=1298)	Sirolimus eluting stent (n=2599)	Everolimus eluting stent (n=1466)	P
Age (years)	63.9±11.6	62.7±11.5	64.9±11.7	<0.0001
Male sex	990 (76.3)	1976 (76.0)	1118 (76.3)	0.98
BMI	27.0±4.1	27.2±4.3	27.2±4.4	0.12
Hypertension	748 (57.6)	1422 (54.7)	857 (59.3)	0.01
Family history of CAD	334 (26.5)	738 (28.4)	420 (29)	0.30
Current smoking	671 (51.7)	1371 (52.8)	736 (50.9)	0.51
Dyslipidemia	683 (52.6)	1403 (54.0)	794 (54.9)	0.48
Diabetes Mellitus	210 (16.2)	471 (18.1)	246 (17.0)	0.30
Renal failure (GFR<60mL/min)	138 (14.2)	294 (15.6)	146 (11.6)	0.005
Acute coronary syndrome	714 (55.1)	1385 (53.3)	855 (58.3)	0.009
Unstable angina	67 (9.4)	129 (9.3)	108 (12.6)	
Non-ST-segment-elevation MI	292 (40.9)	622 (44.9)	382 (44.7)	
ST-segment-elevation MI	355 (49.7)	634 (45.8)	365 (42.7)	
Cardiogenic shock	9 (0.7)	28 (1.1)	33 (2.3)	0.001
LVEF	55.3±11.5	54.3±11.9	51.5±12.7	<0.0001
Moderate-severely impaired LVEF	194 (14.9)	449 (17.3)	370 (25.2)	<0.0001
Mildly impaired LVEF	300 (23.0)	584 (22.4)	360 (24.6)	
Normal LVEF	804 (61.9)	1566 (60.3)	736 (50.2)	
Multivessel treatment	218 (16.8)	407 (15.7)	342 (23.3)	<0.0001
Lesions treated per patient	1.5±0.7	1.5±0.7	1.8±1.0	<0.0001
Culprit left main coronary artery	30 (2.3)	46 (1.8)	67 (4.6)	<0.0001
Arterial bypass graft	5 (0.4)	5 (0.2)	3 (0.2)	0.49
Saphenous vein graft	33 (2.5)	77 (3.0)	47 (3.2)	0.58
Multistent treatment	614 (47.3)	1215 (46.7)	844 (57.6)	<0.0001
Average stent diameter	2.9±0.4	2.9±0.6	2.9±0.4	0.21
Total stent length per patient	29.1±16.9	31.9±18.3	32.8±18.9	<0.0001
Glycoprotein IIb/IIIa antagonist	245 (18.9)	501 (19.3)	438 (29.9)	<0.0001
Aspirin at discharge	1282 (98.8)	2549 (98.1)	1433 (97.7)	0.13
Clopidogrel at discharge	1290 (99.4)	2566 (98.7)	1432 (97.7)	0.001
Oral anticoagulation at discharge	15 (1.2)	51 (2.0)	27 (1.8)	0.17

Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. Continuous variables are expressed as mean ±SD when appropriate. BMI indicates body mass index; CAD, coronary artery disease; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; MI, myocardial infarction.

Chapter 4

Association between angiographic culprit lesion and out-of-hospital cardiac arrest in ST-elevation myocardial infarction patients

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ABSTRACT

Background: Factors related to the occurrence of out-of-hospital cardiac arrest (OHCA) in ST-elevation myocardial infarction (STEMI) are still poorly understood. The current study sought to compare STEMI patients presenting with and without OHCA to identify angiographic factors related to OHCA.

Methods: This multicenter registry consisted of consecutive STEMI patients, including OHCA patients with return-of-spontaneous circulation. Patients were treated with primary percutaneous coronary intervention (PCI) and therapeutic hypothermia when indicated. Outcome consisted of in-hospital neurological recovery, scored using the Cerebral Performance Categories (CPC) scale, and 1-year survival. Logistic regression was used to identify factors associated with OHCA and survival was displayed with Kaplan Meier curves and compared using log rank tests.

Results: In total, 224 patients presented with OHCA and 3259 without OHCA. Average age was 63.3 years and 75% of patients were male. OHCA occurred prior to ambulance arrival in 68% of patients and 48% required intubation. Culprit lesion was associated with OHCA: risk was highest for proximal left coronary lesions and lowest for right coronary lesions. Also, culprit lesion determined the risk of cardiogenic shock and sub-optimal reperfusion after PCI, which were strongly related to survival after OHCA. Neurological recovery was acceptable (CPC≤2) in 77.1% of OHCA patients and did not differ between culprit lesions.

Conclusions: In the present STEMI population, coronary culprit lesion was associated with the occurrence of OHCA. Moreover, culprit lesion influenced the risk of cardiogenic shock and success of reperfusion, both of which were related to prognosis of OHCA patients.

INTRODUCTION

Out-of-hospital cardiac arrest (OHCA) is a common and life threatening condition frequently caused by coronary artery disease.[1] Historically, prognosis of OHCA has been poor.[2] Revascularization techniques in OHCA have been under investigation for some time in an attempt to improve the prognosis of these patients. While thrombolysis during resuscitation failed to proof beneficial, coronary angiography with angioplasty showed promising results.[3,4] Early or primary percutaneous coronary intervention (PPCI) has been shown to improve survival after OHCA due to ST-elevation myocardial infarction (STEMI) and at present, PPCI is readily available in the Netherlands for STEMI patients suffering an OHCA due to extensive nationwide networks of care designed to minimize ischemic times.[5-7]

Although PPCI for OHCA due to STEMI is commonly performed, factors associated with the occurrence of OHCA in setting of STEMI are still poorly understood. The current study sought to compare STEMI patients presenting with and without OHCA to identify angiographic factors related to the occurrence and prognosis of OHCA treated with PPCI and therapeutic hypothermia (TH).

METHODS

Design and patients

The current Dutch registry prospectively included STEMI patients treated in 3 high-volume tertiary centers in the Netherlands. The design of this registry has been described previously.[8] In short, all consecutive STEMI patients undergoing PPCI between January 2006 and December 2009 were included. STEMI was defined as symptoms of angina lasting longer than 30 minutes along with typical electrocardiographical changes (ST-segment elevation ≥0.2 mV in ≥2 contiguous leads in V_1 through V_3 or ≥0.1 mV in other leads or presumed new left bundle branch block) or presumed new regional wall motion abnormalities on echocardiogram when these criteria were unavailable or inconclusive. In case of OHCA, only patients with return-of-spontaneous-circulation (ROSC) on arrival at the catheterization laboratory were included. Patients permanently living outside the Netherlands were excluded to make follow-up through municipality records possible.

Emergency medical services (EMS) were staffed with nurses trained in advanced cardiac life support. Patients were triaged in the field by 12-lead electrocardiogram faxed to the operator on call. In-ambulance medication included aspirin, intravenous heparin bolus and loading dose of clopidogrel. Glycoprotein IIb/IIIa inhibitors were administered up-front in the Leiden University Medical Center and periprocedurally in the other hospitals. Upon arrival at the hospital, unresponsive patients were admitted to the emer-

gency department and following stabilization transferred directly to the catheterization laboratory. Stable patients were transferred directly to the catheterization laboratory. Procedures were performed according to current clinical guidelines. Patients remaining unresponsive (Glasgow Coma Scale <8) after resuscitation were transferred to the intensive care unit, where TH (32-34°C) was induced for 24 hours using ice packs, cooling blankets and intravenous NaCl 0.9% of 4°C, if necessary. After this period, TH was ceased and as body temperature returned to normal values sedation was weaned. Patients remaining unresponsive (Glasgow Coma Scale motor response <5) 24 hours after reaching normothermia and weaning of sedation underwent sensory evoked potentials testing. Severe and permanent neurologic dysfunction was diagnosed if the N20 response was bilaterally absent. Patients with a positive N20 response remaining comatose after 72 hours underwent neurological clinical examination and electro-encephalography after which further treatment strategy was decided.

Patients treated in the Leiden University Medical Center were treated according to the institutional MISSION! protocol, a standardized pre-hospital, in-hospital and outpatient clinical framework for STEMI care.[9] These patients were intensively monitored at the outpatient clinic for 1 year, after which they were referred to the general practitioner or regional cardiology clinic. In the other centers, local residents were managed at the outpatient clinics and patients referred from regional hospitals were referred back for further management by regional cardiologists.

Data collection

All hospitals prospectively registered patients. Close collaboration with regional EMS supplied pre-hospital times and resuscitation characteristics. Vital status was obtained using municipality records. In-hospital outcome was a composite of all-cause mortality and neurological outcome. Neurological outcome was scored retrospectively using the Cerebral Performance Categories (CPC) scale consisting of 5 categories: 1. Conscious, good cerebral performance, able to work; 2. Conscious, moderate cerebral disability, able to work in a sheltered environment; 3. Conscious, severe cerebral disability, dependent on others; 4. Coma or vegetative state; 5. Brain death.[10] Long term outcome consisted of 1-year all-cause mortality. Deaths were considered cardiac unless a clear non-cardiac cause could be identified.

Statistical analyses

Continuous variables are presented as mean ± standard deviation or median (25^{th} to 75^{th} percentile) and were compared using Student's t-test in case of mean and Mann-Whitney U test in case of median. Categorical variables are expressed as counts and percentages and were compared by means of Pearson's χ^2 test. All statistical tests were 2-tailed and a p-value <0.05 was considered statistically significant. Univariable logistic regression was

performed to investigate the association of angiographic factors with OHCA and other prognostic factors. Cumulative incidences of endpoints were displayed visually using Kaplan-Meier plots and compared with log rank tests. Analyses were performed using IBM SPSS Statistics version 20.

RESULTS

Of the 3483 consecutive STEMI patients treated during the inclusion period, 224 (6.4%) presented with OHCA and 3259 (93.6%) without cardiac arrest. Baseline characteristics (Table 1) showed that symptom-to-balloon time was shorter in patients presenting with OHCA. In contrast, door-to-balloon time was longer in OHCA patients compared to non-arrest patients. During angiography, patients presenting with OHCA more frequently

Table 1 - Baseline and procedural characteristics

	OHCA (N=224)	No OHCA (N=3259)	p-Value
Age, years, mean ± standard deviation	62.5 ± 12.1	63.3 ± 12.5	0.365
Male sex	78.6 (176/224)	74.8 (2439/3259)	0.212
Diabetes mellitus	9.1 (20/219)	11.3 (366/3230)	0.318
Previous myocardial infarction	10.9 (24/221)	10.8 (351/3241)	0.989
Previous percutaneous coronary intervention	6.8 (15/221)	8.5 (275/3241)	0.378
Previous coronary artery bypass grafting	3.2 (7/221)	2.4 (78/3246)	0.477
Symptoms-to-balloon time, median minutes	150 (116-192)	181 (131-285)	<0.001
Door-to-balloon time, median minutes	53 (36-79)	46 (33-66)	0.014
Culprit artery			<0.001
Left main	3.1 (7/224)	1.2 (40/3257)	0.017
Left anterior descending	55.8 (125/224)	39.4 (1282/3257)	<0.001
Left circumflex	21.9 (49/224)	15.5 (504/3257)	0.011
Right coronary artery	18.3 (41/224)	42.8 (1393/3257)	<0.001
Bypass graft	0.9 (2/224)	1.2 (38/3257)	0.710
Multivessel disease	50.9 (114/224)	53.1 (1729/3256)	0.522
Stenting	96.9 (217/224)	95.6 (3114/3257)	0.367
Cardiogenic shock during PCI	30.8 (69/224)	4.8 (158/3259)	<0.001
Intra-aortic balloon pump implantation	25.4 (57/224)	2.8 (92/3259)	<0.001
TIMI flow ≤1 pre-procedure	79.9 (179/224)	79.3 (2582/3254)	0.841
TIMI flow 3 post-procedure	91.1 (204/224)	91.5 (2976/3254)	0.842

Values are percentage (n) or median (25^{th} to 75^{th} percentile). TIMI = Thrombolysis in myocardial infarction. *defined as systolic blood pressure lower than 90 mmHg with signs of tissue hypoperfusion requiring treatment in form of inotropic agents or assistant devices.

showed left coronary artery culprit lesions compared to patients without arrest. Also, OHCA patients were more often in cardiogenic shock and were treated more commonly with intra-aortic balloon pumps. Thrombolysis-in-myocardial infarction flow pre- and post-procedure was comparable between the groups.

Table 2 shows the characteristics of the OHCA patients according to moment of arrest. Approximately two thirds of patients suffered a cardiac arrest before arrival of EMS. Most OHCAs were witnessed and delay in basic life support occurred in a quarter of patients with OHCA before EMS arrival. In most cases, the first observed rhythm was ventricular fibrillation or tachycardia. Intubation was performed in 108 patients. Of these patients, 95.4% (103/108) survived PCI and 88.0% (95/108) underwent TH. The rest had no indication for TH due to return of consciousness.

Factors associated with OHCA

Angiographic culprit lesion was associated with risk of OHCA (Figure 1, Table 3). Lesions located in the left coronary artery (with the exception of diagonal branch lesions) were found to result in the highest risk of OHCA. In addition, proximally located left coronary artery lesions displayed a higher risk of OHCA compared to non-proximally located left coronary artery lesions: OR 1.43 (95% CI 1.05-1.95, p=0.025). Separately, proximal vs. non-proximal left anterior descending or left circumflex culprit lesions were not significantly associated with higher risk.

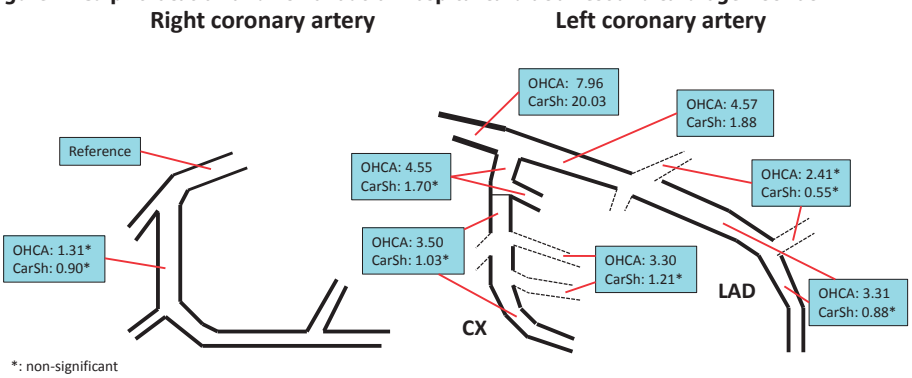
Table 2 - Characteristics and treatment of out-of-hospital cardiac arrest

	OHCA witnessed by EMS (N=71)	OHCA not witnessed by EMS (N=153)
Delay in basic life support >5 minutes*	0.0 (0/71)	24.2 (37/153)
Bystander witnessed arrest	100 (71/71)	90.8 (139/153)
First observed rhythm		
Ventricular fibrillation / tachycardia	95.8 (68/71)	93.4 (142/152)
Bradycardia	2.8 (2/71)	2.0 (3/152)
Asystole / Pulseless electrical activity	1.4 (1/71)	4.6 (7/152)
Treatment performed		
Automatic external defibrillator	0.0 (0/71)	17.6 (27/153)
Defibrillation	95.7 (3/70)	94.1 (143/152)
Average number of shocks	1 (1-2)	2 (1-4)
Chest compressions	51.4 (36/70)	88.2 (135/153)
Intubation	10.0 (7/70)	66.0 (101/153)
Therapeutic hypothermia†	100 (7/7)	87.1 (88/101)

Values are percentage (n) or median (25^{th} to 75^{th} percentile).

*Based on history from bystanders; † The denominator is intubated patients.

Figure 1 - Culprit location and risk of out-of-hospital cardiac arrest and cardiogenic shock



Values are odds ratios. CarSh = cardiogenic shock; CX = circumflex artery; LAD = leftanterior descending artery; OHCA = out-of-hospital cardiac arrest.

Table 3. Association of angiographic culprit location with OHCA, cardiogenic shock and TIMI flow

	OHCA	OR (95% CI)	p-Value	CarSh	OR (95% CI)	p-Value	TIMI <3 after PCI	OR (95% CI)	p-Value
RCA									
Proximal (N=530)	2.4 (12)	Reference	-	5.0 (25)	Reference	-	8.7 (44)	Reference	-
Non-proximal (N=834)	3.1 (29)	1.31 (0.66-2.59)	0.436	4.5 (42)	0.90 (0.54-1.50)	0.685	8.8 (82)	1.00 (0.68-1.47)	0.984
Left main artery (N=43)	16.3 (7)	7.96 (2.95-21.45)	<0.001	51.2 (22)	20.03 (9.74-41.18)	<0.001	18.6 (8)	5.96 (2.37-14.99)	<0.001
LAD									
Proximal (N=816)	10.0 (82)	4.57 (2.47-8.47)	<0.001	8.9 (73)	1.88 (1.18-3.00)	0.008	9.2 (75)	1.06 (0.72-1.56)	0.780
Non-proximal (N=521)	7.5 (39)	3.31 (1.71-6.40)	<0.001	4.4 (23)	0.88 (0.49-1.58)	0.674	7.9 (41)	0.90 (0.57-1.40)	0.624
Side branch (N=72)	5.6 (4)	2.41 (0.76-7.68)	0.138	2.8 (2)	0.55 (0.13-2.36)	0.418	4.2 (3)	0.45 (0.14-1.50)	0.195
Cx									
Proximal (N=220)	10.0 (22)	4.55 (2.21-9.36)	<0.001	8.2 (18)	1.70 (0.91-3.19)	0.096	6.8 (15)	0.76 (0.42-1.40)	0.384
Non-proximal (N=216)	7.9 (17)	3.50 (1.64-7.45)	0.001	5.1 (11)	1.03 (0.50-2.12)	0.945	4.6 (10)	0.51 (0.25-1.03)	0.059
Side branch (N=134)	7.5 (10)	3.30 (1.39-7.81)	0.007	6.0 (8)	1.21 (0.54-2.76)	0.643	9.8 (13)	1.13 (0.59-2.17)	0.712
Bypass graft (N=22)	2 (9.1)	4.09 (0.86-19.52)	0.077	13.6 (3)	3.02 (0.84-10.88)	0.091	8 (36.4)	2.38 (1.04-5.46)	0.040

CarSh = cardiogenic shock; Cx = Circumflex artery; LAD = Left anterior descending artery; OR = odds ratio; RCA = Right coronary artery other abbreviations as in table 1.

Left main and proximal left anterior descending artery lesions were also associated with development of cardiogenic shock (Figure 1, Table 3). Moreover, culprit lesions in the left main and bypass grafts were associated with sub-optimal TIMI flow after PCI (Table 3).

Neurological recovery and outcome during 1-year follow-up

Discharge CPC was known in 218 OHCA patients (97.3%) (Figure 2). The majority of patients had acceptable CPC scores (CPC≤2 in 77.1%, n=168). Thirty-five patients were in CPC 5/dead, of which 21 patients were brain dead and 14 patients suffered cardiac death. Of the 107 patients with a proximal left coronary culprit lesion, 77 recovered (72.0%). This rate was slightly higher for the patients with a non-proximal left culprit lesion (84.1%, 58/69 patients recovered) and the patients with a right culprit lesion (80%, 32/40 patients recovered). Of the 2 patients with a bypass graft culprit lesion, one died due to neurological causes. The p-value for trend between the culprit groups was 0.212.

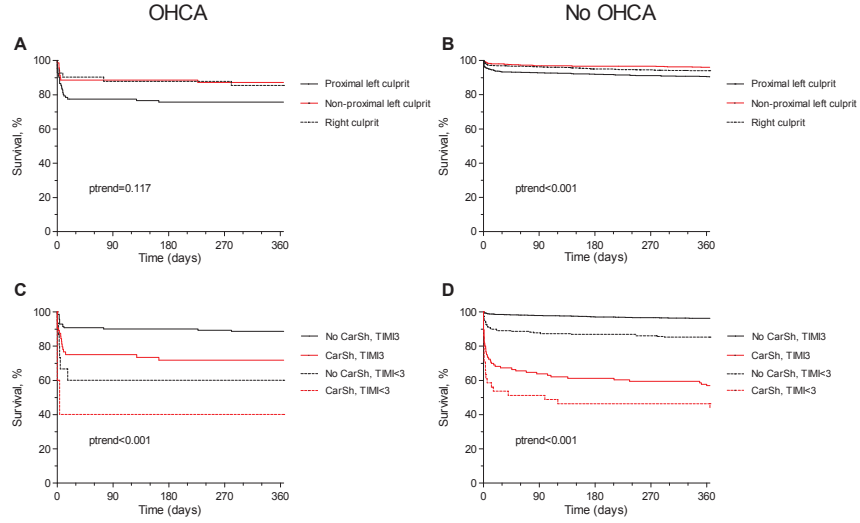
One-year survival status was known in 3479 patients. In-hospital mortality was higher in OHCA patients compared to patients without OHCA (16.5% vs. 3.1%, p<0.001). Also, 1-year mortality (19.2% vs. 6.6%, p<0.001) was higher, which was due to in-hospital

Figure 2 - In-hospital Cerebral Performance Categories scale according to culprit lesion

CPC = cerebral performance categories scale.
Other abbreviations as in figure 1.

mortality as post-discharge survival was similar between patients with and without OHCA (3.2% vs. 3.6%, p=0.774). Figure 3A and 3B show the association between culprit location and mortality during follow-up in patients with and without OHCA. Figure 3C and 3D shows the association of cardiogenic shock and success of reperfusion with survival. OHCA patients with optimal TIMI flow after PCI had a better prognosis than OHCA patients with a sub-optimal TIMI flow after procedure, regardless of cardiogenic shock during PCI. In contrast, presence of cardiogenic shock was more important than success of reperfusion in patients without OHCA.

Figure 3 - Survival in patients with and without OHCA according to A) and C) culprit lesion, B) and D) cardiogenic shock and TIMI flow after PCI



DISCUSSION

The present multicenter registry identified an association between angiographic culprit location and the occurrence of OHCA in STEMI patients: left proximal coronary lesions were associated with the highest risk for OHCA and right coronary lesions with the lowest. Moreover, culprit location was associated with cardiogenic shock and sub-optimal reperfusion after PCI, both of which were driving factors of prognosis after OHCA.

Attempts to improve the historically poor prognosis of OHCA led to investigation of revascularization techniques for OHCA patients.[2] While thrombolysis during resuscitation failed to prove beneficial, coronary angiography with angioplasty showed promise from early on.[3,4] At present, extensive networks of care make PPCI readily available for patients suffering an OHCA due to STEMI. In the current STEMI population, symptom-

to-balloon times were strongly reduced in OHCA patients, a finding also observed by others, possibly reflecting the severity of symptoms leading to early initiation of professional care by either patient or bystanders.[5,6] In contrast, the prolonged door-to-balloon times in OHCA patients were likely related to time needed for in-hospital patient stabilization. Patients presenting with OHCA were more frequently in cardiogenic shock on arrival, due to impaired coronary perfusion during cardiac arrest and culprit lesion location. Culprit location varied between OHCA and non-arrest patients and was found to be associated with occurrence of OHCA. Left coronary lesions resulted in the highest and right coronary artery lesions in the lowest risk for OHCA. Moreover, proximally located culprit lesions within the left coronary artery were associated with higher risk of OHCA compared to non-proximally located lesions. This is likely explained by the larger area of myocardium-at-risk in proximal left lesions, which was supported by the finding that left main and proximal left anterior descending artery culprits were also associated with cardiogenic shock.[11] The lower percentage of right coronary artery culprit lesions is possibly explained by the commonly occurring vagal reaction in inferior MI, which may have a protective effect against VF.[12] However, it cannot be completely ruled out that inferior MI may have caused more severe ischemia, preventing ROSC and thus inclusion in this registry.

In-hospital and 1-year outcome rates stratified according to culprit lesion were similar in OHCA patients. Nevertheless, location of culprit lesions contributed indirectly to mortality due to the association with cardiogenic shock and sub-optimal reperfusion. Left main lesions were the highest risk lesions for STEMI patients, due to the strong association with OHCA, cardiogenic shock and sub-optimal reperfusion, which is supported by other reports.[13] Also, bypass graft culprit lesions were associated with reperfusion failure. The no-reflow phenomenon in setting of STEMI has multiple mechanisms, among which distal embolization and ischemic injury.[14] The occurrence of distal embolization is notorious in PCI of saphenous vein grafts and remains a challenge for operators.[15] The importance of optimal reperfusion in OHCA patients was stressed by the lower survival rates for sub-optimally reperfused OHCA patients without cardiogenic shock during PCI compared to optimally reperfused OHCA patients with cardiogenic shock during PCI. No-reflow possibly reflects the duration of ischemia prior to PCI in OHCA patients and the effect on prognosis may therefore also be explained by a prolonged resuscitation. In contrast, presence of cardiogenic shock was a stronger factor than failed reperfusion in patients without OHCA. This was likely explained by a smaller area of myocardium at risk in these patients due to the different distribution of culprit lesions.

Using a combined treatment strategy of primary PCI and TH, 77% of the OHCA population was discharged with acceptable neurological outcome and 1-year survival was 81%. The combination of PPCI and TH was previously investigated in the PROCAT registry, where the investigators reported an overall in-hospital survival rate of 40%, rising to 54%

in STEMI patients after successful PCI which predicted improved prognosis.[7] Furthermore, the positive influence of both PCI and TH on long term survival was established in a cohort of OHCA patients, which included a large percentage of STEMI patients.[16] Also, a recent smaller study focusing specifically on use of TH in STEMI complicated by OHCA reported a neurological recovery rate comparable to the rate observed in the population treated with TH in the current study, supporting the accuracy of our findings.[17]

Limitations

Our investigation represents one of the largest studies covering outcomes in OHCA patients due to STEMI. However, our study was observational and thus shares the limitations of all observational analyses. Because the registry only included STEMI patients with ROSC, no data was available on OHCA patients without ROSC or patients not referred for PPCI. Data covering these patients may have provided more insight into the full community experience.

Conclusions

Location of angiographic culprit lesion was associated with the occurrence of OHCA in the current STEMI population. Moreover, angiographic culprit location predicted cardiogenic shock and success of reperfusion, both of which were associated with the prognosis of OHCA patients.

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Part II

Novel methods and markers for risk assessment of patients with heart failure

Chapter 5

In search of an efficient strategy to monitor disease status of chronic heart failure outpatients: added value of blood biomarkers to clinical assessment

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ABSTRACT

Background: Blood biomarkers carry potential for monitoring severity of chronic heart failure (CHF). Studies correlating repeated measurements of blood biomarkers with repeatedly assessed NYHA class over a prolonged follow-up period, and concomitantly investigating their associations with clinical endpoints, have not yet been performed.

Methods: In 2011-2013, 263 CHF patients were included. At inclusion and subsequently every 3 months, we measured N-terminal pro-B-type natriuretic (NT-proBNP), high-sensitivity troponin T (Hs-TnT) and C-reactive protein (CRP), and assessed NYHA class. The primary endpoint comprised heart failure hospitalization, cardiovascular mortality, cardiac transplantation or left ventricular assist device implantation. Time-dependent Cox models were used.

Results Mean age was 67±13 years, 72% were men and 27% were in NYHA class III–IV. We obtained 886 repeated measures (median 3[IQR 2-5] per patient). The primary endpoint was reached in 41 patients during a median follow-up of 1.0[0.6-1.4] year. Repeatedly measured NT-proBNP and Hs-TnT were significantly associated with repeatedly assessed NYHA class, whereas CRP was not (NT-proBNP: β[95%CI]:1.56[1.17–2.06]ln(ng/L) increase per point increase in NYHA class, p=0.002; HsTNT: β[95%CI]:1.58[1.21–2.07]. Serially measured NT-proBNP (HR[95%CI]: 2.86 [1.73-4.73], CRP (1.69 [1.21-2.34]) and NYHA class (2.33 [1.51-3.62]) were positively and independently associated with the primary endpoint, whereas Hs-TnT lost statistical significance after multivariable adjustment. A model containing serially measured NYHA-class and NT-proBNP displayed a c-index of 0.84, while serially measured NYHA-class and CRP showed a c-index of 0.82.

Conclusions: Temporal NT-proBNP, CRP and NYHA class patterns are independently associated with adverse clinical outcome. Serially measured NT-proBNP and NYHA-class are best suited for monitoring CHF outpatients.

INTRODUCTION

Adjustment of medicinal treatment for chronic heart failure (CHF) requires considerable clinical acumen and may in some cases cause misjudgement in risk assessment and consequently suboptimal treatment.[1-4] Therefore, several diagnostic tools have been developed over the past decades which aim to objectify disease severity, such as the New York Heart Association (NYHA) Functional Classification,[1,4] which has limited reproducibility and high inter-observer variability.[5] Conversely, circulating blood biomarkers are less subjective to interpretation, and carry potential to monitor subtle changes in the heart that reflect and possibly predict adverse changes before they become clinically apparent.[6] The use of biomarkers, such as B-type natriuretic peptides (BNP), cardiac troponins and C- reactive protein (CRP), for risk stratification of CHF patients has already been demonstrated.[7-13] Moreover, although trials on natriuretic peptide-guided therapy of HF have provided somewhat inconsistent results,[9,14] natriuretic peptide-guided HF therapy has recently been given a class IIa recommendation in US HF guidelines to achieve guideline-directed medical therapy.[15,16]

Several studies have previously examined NYHA class in relation to clinical outcome in CHF patients. However, these studies either used single, baseline assessments or 2 repeated assessments taken in a relatively short time interval.[17,18] Furthermore, studies on the association between blood biomarkers and NYHA class in CHF are scarce, and studies assessing both these properties repeatedly are non-existent. Finally, the predictive value of serially assessed blood biomarkers and NYHA class scores for adverse clinical outcome has never yet been compared in stable CHF patients.

Therefore, the aim of the current investigation, performed in 263 patients with CHF, was to examine the associations between repeatedly measured NT-proBNP, troponin T (Hs-TnT), CRP, and NYHA class, as well as the associations of their temporal patterns with adverse clinical outcome. Based on this, we evaluated the incremental value of serially measuring blood biomarkers, to clinical assessment in terms of serial NYHA class scoring, for monitoring stable CHF outpatients.

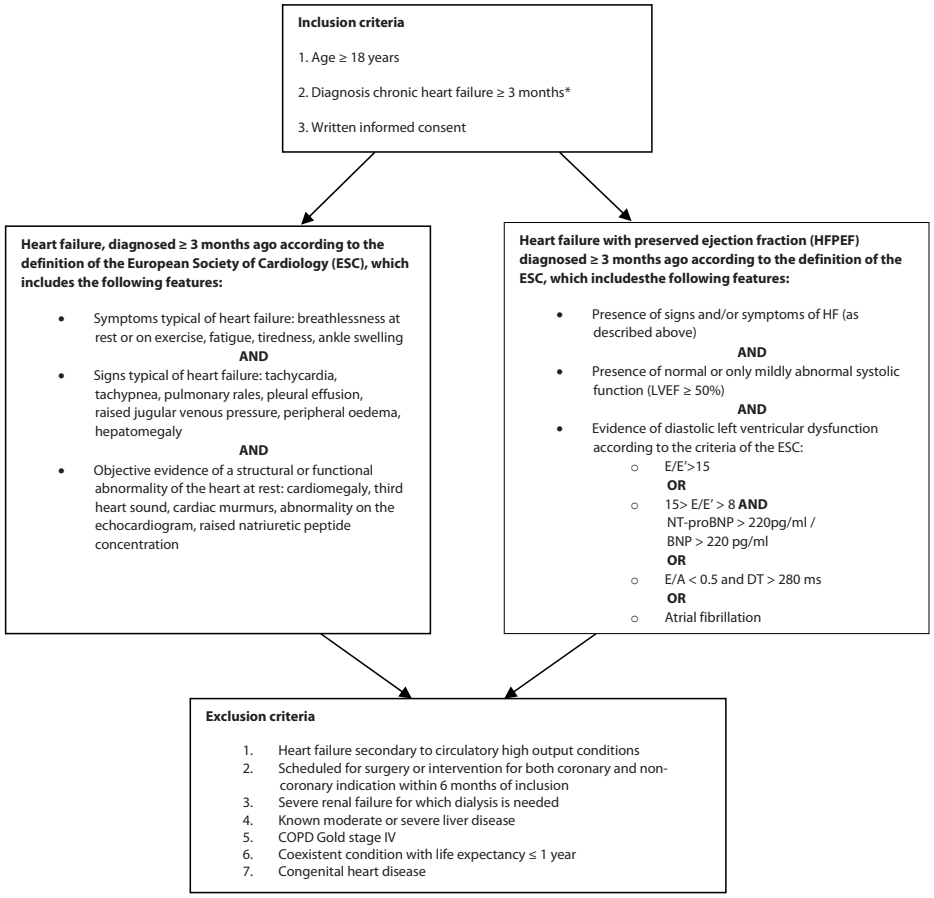
METHODS

Patients

The *Serial biomarker measurements and new echocardiographic techniques in chronic heart failure patients result in tailored prediction of prognosis* (Bio-SHiFT) study was designed to investigate the hypothesis that temporal patterns of biomarkers involved in CHF are associated with prognosis. Bio-SHiFT is an ongoing prospective, observational study of stable outpatients with CHF, conducted in Erasmus MC, Rotterdam, The Netherlands and

Noordwest Ziekenhuisgroep, Alkmaar, The Netherlands. Patients were recruited during their regular outpatient visits and were in clinically stable condition. Patients were eligible for inclusion if aged 18 years or older, capable of understanding and signing informed consent, and if CHF (including HF with preserved ejection fraction) was diagnosed ≥3 months ago according to the guidelines of the European Society of Cardiology (ESC).[1,4,19] Detailed inclusion and exclusion criteria are shown in figure 1. The study was approved by the medical ethics committees of the participating hospitals and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.The study is registered in ClinicalTrials.gov, number NCT01851538. Follow-up for this analysis lasted from October 2011 until November 2013.

Figure 1 - Inclusion and exclusion criteria



Baseline assessment

At baseline, patients were evaluated by trained research physicians, who collected information on HF-related symptoms, including NYHA class.[1,4] History of chronic renal failure was defined as glomerular filtration rate less than 60 mL/min/1.73m^2. Alcohol consumption was defined as drinking ≥1 alcoholic consumption per day. Electrocardiography and echocardiography were performed. Data were entered into electronic case report forms. Non-fasting blood and urine samples were collected.

Follow-up visits

Study follow-up visits were scheduled every 3 months (a window of ±1 month was allowed), for a maximum of 30 months. At each tri-monthly study visit, a short medical evaluation was performed, NYHA functional class was scored and blood and urine samples were collected.

Blood sampling and biomarker measurement

Blood samples were collected at baseline and at each follow-up visit, and were processed and stored locally at a temperature of -80°C within 2 hours after blood collection. When applicable, samples were transported to the central laboratory (Erasmus MC, Rotterdam, The Netherlands) under controlled conditions (at a temperature of -80°C), until batch analysis was performed. Thus, the biomarker measurements performed for this study did not lead to treatment adjustments.

Batch analysis of NT-proBNP, Hs-TnT, and CRP was performed in the Clinical Chemistry Laboratory of the Erasmus MC. Plasma NT–proBNP and Hs-TnT were analysed using electrochemiluminesence immunoassays (Roche Diagnostics, Elecsys 2010, Indianapolis, Indiana, USA). For NT-proBNP, concentrations were measured ranging from 5 to 35.000 pmol/L. Coefficients of variation (CVs) were <5% at mean values ranging from 5.19-274 pmol/L. For Hs-TnT, concentrations were measured ranging from 3-10000 ng/L. CVs were <5% at mean values ranging from 12.7-1819 ng/L. CRP was measured using an immunoturbidimetric assay (Roche Hitachi 912 chemistry analyser, Basel, Switzerland). This system measures concentrations ranging from 0.3 to 350 mg/L, and CVs were <5% at mean values ranging from 0.84-284 mg/L.

Clinical study endpoints

During follow-up, endpoints were recorded in the electronic case report forms by trained research physicians, and associated hospital records and discharge letters were collected. Subsequently, a clinical event committee blinded to the biomarker results reviewed all collected information and adjudicated primary and secondary endpoints.

The primary endpoint comprised the composite of cardiac death, cardiac transplantation, left ventricular assist device implantation, and hospitalization for the management of acute or worsened HF.

Cardiac death was defined as death from myocardial infarction (MI) or other ischemic heart disease (ICD-10: I20-I25), death from other heart disease including HF (I30-I45 and I47-I52), sudden cardiac death (I46), sudden death undefined (R96) or unwitnessed or ill-described death (R98, R99). Hospitalisation for acute or worsened HF was defined as exacerbation of symptoms typical of HF, in combination with 2 of the following: BNP or NT-proBNP >3x the upper limit of normal, signs of worsening HF, such as pulmonary rales, raised jugular venous pressure or peripheral oedema, increased dose or intravenous administration of diuretics, or administration of positive inotropic agents.

Statistical analysis

Statistical methods are described in detail in the supplemental text. In brief, we used linear mixed models to assess the associations between serial biomarker measurements and repeated assessment of NYHA functional class. Associations between serial measurements of biomarkers and NYHA class, and occurrence of the primary endpoint, were examined by entering the serial measurements as time-varying covariates into extended Cox proportional hazards models. First, the models were adjusted for age, gender, systolic blood pressure and estimated glomerular filtration rate (eGFR; calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation). Subsequently, all variables, i.e. NT-proBNP, Hs-TnT, CRP and NYHA functional class, were entered simultaneously into the models to investigate their independence. We calculated time-dependent C-indices based on the extended Cox models. Analyses were performed with R Statistical Software and MedCalc.

RESULTS

Study population

A total of 263 were included from October 2011 to June 2013. Baseline characteristics are displayed in table 1. Mean age was 67 years (SD±13), 72% were men, and 73% were in New York heart association (NYHA) class I or II. Median duration of HF at inclusion was 4.6 years (IQR 1.7–9.9). Median baseline NT-proBNP was 137.3 pmol/L (IQR 51.9–272.9), Hs-TnT:18.0 ng/L (IQR 9.6–33.2) and CRP:2.2 mg/L (IQR 0.9–4.8).

Table 1 – Baseline characteristics

	NYHA class I (n=78)	NYHA class II (n=116)	NYHA class III/IV (n=66)	Total (n=263)
Demographics				
Age	64 (±11)	68 (±13)	72 (±11)	67 (±13)
Male gender	60 (78)	81 (69)	48 (70)	189 (72)
Caucasian ethnicity	71 (92)	107 (92)	66 (94)	244 (94)
Clinical characteristics				
Body mass index kg/m^2	28 (±5)	27 (±4)	28 (±4)	28 (±5)
Heart rate, bpm	63 (±10)	68 (±11)	69 (±13)	67 (±12)
SBP, mmHg	123 (±19)	124 (±20)	120 (±21)	122 (±20)
DBP, mmHg	75 (±11)	72 (±11)	71 (±10)	72 (±11)
Biomarker level				
NT-proBNP (pmol/L)	93 (38-175)	141 (49-583)	225 (120-436)	140 (52-273)
HsTNT (ng/L)	13 (7.8-21)	19 (9.9-37)	24 (16-43)	18 (10 -33)
CRP (mg/L)	1.6 (0.6-3.4)	2.3 (1.0-5.3)	2.7 (1.3-4.9)	2.2 (0.9-4.8)
Creatinine (mg/dL)	1.2 (1.0 – 1.5)	1.1 (1.0 – 1.4)	1.3 (1.0 – 1.6)	1.2 (1.0 – 1.5)
eGFR[a] (ml/min/1.73 m2)	62 (42 – 83)	58 (46 – 78)	53 (38 – 72)	58 (43 – 76)
Medical history				
CAD	29 (39)	57 (50)	56 (84)	142 (46)
ICD	42 (55)	67 (57)	42 (61)	151 (59)
CRT	20 (26)	40 (34)	18 (26)	78 (30)
CVA	8 (10)	18 (15)	15 (22)	41 (16)
Chronic renal failure	34 (44)	61 (52)	41 (59)	136 (53)
Diabetes Mellitus	16 (23)	34 (28)	31 (42)	81 (31)
Hypercholesterolemia	26 (34)	41 (35)	26 (38)	93 (36)
Hypertension	33 (43)	56 (48)	31 (45)	120 (46)
Intoxications				
Alcohol consumption	30 (40)	51 (44)	27 (39)	108 (42)
Ever smoker	58 (75)	79 (68)	48 (70)	185 (71)
Medication use				
ACE-i or ARB	75 (96)	109 (94)	61 (88)	245 (93)
Aldosteron antagonist	44 (56)	81 (70)	53 (80)	178 (68)
Diuretic	64 (82)	107 (92)	66 (96)	237 (90)
Beta-blocker	68 (87)	106 (91)	58 (88)	232 (88)
Aspirin	14 (18)	16 (14)	15 (22)	45 (17)
Vitamin K antagonist	59 (77)	88 (78)	53 (77)	200 (77)

Normally distributed continuous variables are presented as mean (± standard deviation). Non-normally distributed continuous variables are expressed as median (25th – 75th percentile). Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. ACE-i = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CAD = coronary artery disease; CRP = C-reactive protein; CRT = cardiac resynchronisation therapy; CVA = cerebro vascular

accident DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HsTNT = high sensitive cardiac troponin T; ICD = implantable cardioverter defibrillator; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; SBP = systolic blood pressure.
[a]eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

Associations between serial biomarker measurements and serial assessment of NYHA class

During follow-up, we collected 921 blood samples and scored NYHA functional class 1292 times. Of all follow-up visits, 1135 took place before the occurrence of the primary endpoint. During these follow-up visits, 886 blood samples were drawn (median 3; IQR 2-5 per patient), and NYHA functional class was scored 1114 times (median 4; IQR 2-6 per patient).

Repeatedly measured NT-proBNP and Hs-TnT showed strong associations with repeatedly assessed NYHA class, whereas CRP did not (NT-proBNP: β[95%CI]:1.56[1.17–2.06]ln(ng/L) increase per point increase in NYHA class, p=0.002; HsTNT: β[95%CI]:1.58[1.21–2.07], p=0.001; CRP: β[95%CI]:1.22[0.98–1.53], p=0.076)(supplemental table 1).

Clinical endpoints

The composite endpoint was reached by 41 patients (16%), during a median follow-up of 1.0 [0.6-1.4] years: 5 patients died from a cardiovascular cause, 35 patients were re-hospitalized for worsened HF and 1 patient underwent heart transplantation. Of the 35 patients reaching the primary endpoint because of re-hospitalisation for HF, 16 patients died eventually during further follow-up, of whom 12 patients died from a cardiovascular cause. Overall all-cause mortality was 21 (8.0%).

Baseline biomarker measurements and NYHA class and the primary endpoint

NT-proBNP, Hs-TnT, CRP, and NYHA class all displayed strong and positive associations with the primary endpoint (table 2). After multivariable adjustment, NT-proBNP, CRP and NYHA class remained independently associated with the primary endpoint, while, Hs-TnT lost statistical significance. Of all other baseline characteristics, only age was independently associated with the primary endpoint.

Serial measurements of biomarkers and NYHA class, and the primary endpoint

Temporal evolutions of serial biomarker measurements and NYHA class are displayed in figure 2. Serially measured NT-proBNP, Hs-TnT, CRP, and NYHA class all displayed strong and positive associations with the primary endpoint (table 2). After multivariable adjustment for all serially measured variables, NT-proBNP, CRP and NYHA class remained significantly associated with the primary endpoint.

Table 2 – Associations between blood biomarker measurements, NYHA class, and the primary endpoint

	Univariable models[a]		Multivariable model[b]	
Associations between baseline measurements and the primary endpoint				
	HR (95% CI)	**p**	**HR (95% CI)**	**p**
NT-proBNP[c]	3.10 (1.87 – 5.13)	<0.001	2.37 (1.39 – 4.02)	0.003
Hs-TnT[c]	1.94 (1.33 – 2.84)	<0.001	1.50 (0.96 – 2.33)	0.11
CRP[c]	1.55 (1.12 – 2.16)	0.005	1.53 (1.10 – 2.14)	0.013
NYHA class[d]	2.22 (1.41 – 3.50)	<0.001	2.14 (1.33 – 3.45)	0.003
Associations between serial measurements and the primary endpoint				
	Univariable models[a]		**Multivariable model[b]**	
	HR (95% CI)	**p**	**HR (95% CI)**	**p**
NT-proBNP[c]	4.02 (2.50 – 6.47)	<0.001	2.86 (1.73 – 4.73)	<0.001
Hs-TnT[c]	1.95 (1.36 – 2.77)	<0.001	1.26 (0.86 – 1.84)	0.32
CRP[c]	2.08 (1.50 – 2.87)	<0.001	1.69 (1.21 – 2.34)	0.001
NYHA class[d]	2.80 (1.83 – 4.28)	<0.001	2.33 (1.51 – 3.62)	<0.001

HR= hazard ratio; CRP = C-reactive protein; Hs-TnT = high-sensitive cardiac troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association.

[a] Including age and gender

[b] Including age, gender, systolic blood pressure and estimated glomerular filtration rate

[c] HR per standard deviation increase in log transformed level

[d] HR per 1-step increase

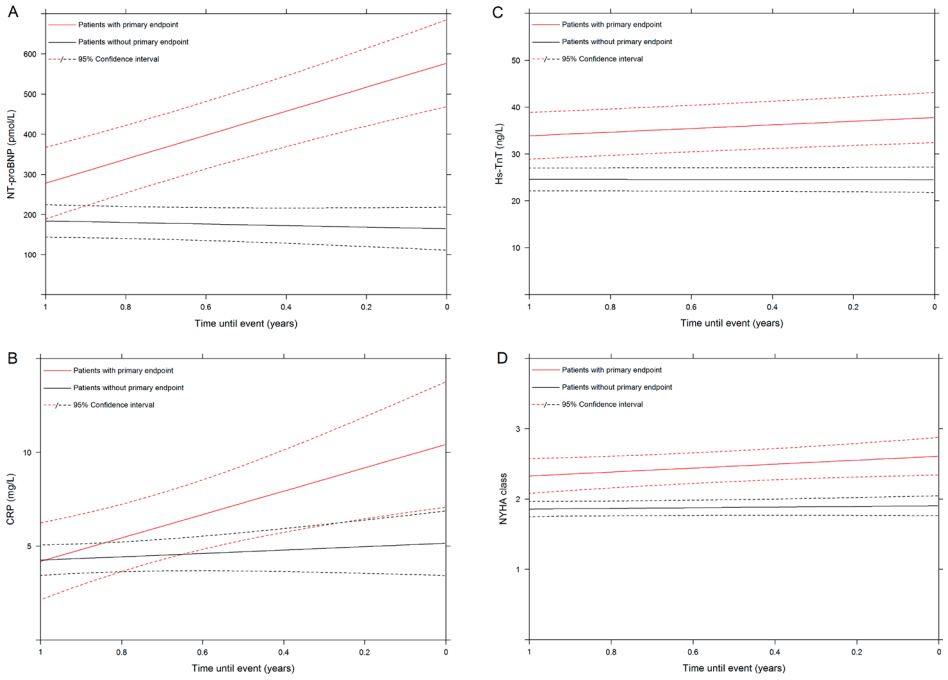
Model performance

The discriminative abilities of the models containing baseline measurements of NT-proBNP, Hs-TnT, CRP and NYHA class are shown in supplemental table 2, and those of serial measurements in table 3. All individual serially assessed c-indices, except for Hs-TnT, were numerically higher than corresponding baseline c-indices. Adding serial NT-proBNP to the model containing age, sex, systolic blood pressure and eGFR and NYHA class, provided a substantial increase in C-index from 0.76(CI 0.66–0.86) to 0.84(0.74 – 0.93), although this did not reach statistical significance (p=0.26). Adding serial CRP instead to this same multivariable model resulted in a C-index of 0.82(0.72 – 0.92), p=0.40. Adding both serial NT-proBNP and CRP to the multivariable model only resulted in a slight improvement compared to addition of NT-proBNP only: 0.85(CI 0.75–0.95), p=0.20.

DISCUSSION

In this prospective, observational cohort of CHF patients, repeatedly measured NT-proBNP and Hs-TnT were positively and significantly associated with repeatedly assessed NYHA class. Serial assessments of NT-proBNP, CRP and NYHA class were independently

Figure 2 - Temporal evolution of serial biomarker measurements and NYHA class



X-axes display the time that is left until occurrence of the clinical endpoint.

associated with adverse clinical outcome. Repeatedly measured NT-proBNP and CRP both added individually to serial NYHA-class assessments in terms of discriminative ability. However, a model combining both of these biomarkers with serially scored NYHA-class, seemed to have little incremental value over serial NYHA-class assessment combined with only one of these blood biomarkers. In particular, adding NT-proBNP only seemed the best suited strategy for monitoring stable CHF outpatients.

Strengths of the current study include simultaneous assessment of multiple biomarkers and NYHA class on the one hand, as well as frequent, repeated assessment of these properties on the other hand. Combined with clinical follow-up on adverse events, this renders insight into temporal evolution and manifestation of CHF. On top of that, using biomarker measurements for monitoring patients with CHF has the appealing feature of being objective, and thus uniform and reproducible.

Serial NT-proBNP and CRP measurements were both independently associated with the endpoint and adding serial NT-proBNP and CRP measurements to a model containing NYHA class assessments greatly increased the c-index, from 0.76 to 0.85. This increase did not reach statistical significance, but recently it has been demonstrated that testing for improvement in prediction performance is actually redundant if a variable

Table 3 – Discriminative ability of models containing serial blood biomarker- and NYHA assessment

Model	C-index (CI)	P-value
Model[a]	0.62 (0.52 – 0.71)	NA
Model[a] + NT-proBNP	0.80 (0.70 – 0.90)	0.010[b]
Model[a] + Hs-TnT	0.72 (0.62 – 0.82)	0.13[b]
Model[a] + CRP	0.76 (0.66 – 0.85)	0.048[b]
Model[a] + NYHA class	0.76 (0.66 – 0.86)	0.040[b]
Model[a] + NYHA class + NT-proBNP	0.84 (0.74 – 0.93)	0.26[c]
Model[a] + NYHA class + Hs-TnT	0.79 (0.69 – 0.89)	0.67[c]
Model[a] + NYHA class + CRP	0.82 (0.72 – 0.92)	0.40[c]
Model[a] + NT-proBNP + CRP	0.82 (0.73 – 0.92)	0.40[c]
Model[a] + NYHA class + NT-proBNP + CRP	0.85 (0.75 – 0.95)	0.20[c]

CI = Confidence interval; CRP = C-reactive protein; Hs-TnT = High-sensitive cardiac troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association.

[a] Including age, gender, systolic blood pressure and estimated glomerular filtration rate

[b] P-value compared to model[a]

[c] P-value compared to model[a] + NYHA class

has already been shown to be an independent risk factor, and that standard testing procedures such as c-indices are very conservative and thus insensitive to improvements in prediction performance.[20] Nevertheless, to provide an impression of the magnitude of the incremental prognostic value, we presented C-indices. Altogether, our results support combining blood biomarkers with clinical assessment for prognostication in CHF patients.

While the prognostic value of blood biomarkers for clinical events has been widely investigated, less is currently known about the association between blood biomarkers and NYHA class in CHF patients. Only two studies have previously assessed this association. These studies measured natriuretic peptide level both at study baseline and at 6±2 weeks of follow-up, and correlated these measurements with, among others, clinical change as categorized by clinicians.[17,18] Studies performing multiple, repeated measurements of biomarkers and NYHA class over a prolonged follow-up period, and concomitantly investigating their association with clinical endpoints have not yet been performed. Although the NYHA functional classification is a common and globally used system,[1,4] its biggest disadvantage is the non-uniformity in its application by individual clinicians. Raphael et al conducted a study to investigate consistency in NYHA functional class assessment and found that inter-observer variability was high, with only 54% concordance between two cardiologists.[5] In this respect, adding biomarker information to clinical patient assessment could be valuable for obtaining a more objective estimate of patient prognosis.

Limitations

Extended Cox models with time-dependent covariates were used to analyse the effects of changes in NYHA class and temporal biomarker patterns on the primary endpoint, because these models are able to accommodate multiple time-varying covariates. However, time-dependent Cox models assume that biomarker levels do not change between measurements.[21] In reality, blood biomarkers are dynamic and continuously change over time, parallel to the condition of the patient. Therefore, we performed a sensitivity analysis by means of a joint modelling approach. Joint models combine a linear mixed-effects model for the serial biomarker measurements with a Cox proportional hazards model for the occurrence of the primary endpoint.[21] We estimated the individual biomarker trajectories and NYHA trajectories using separate joint models, then extracted the fitted trajectories from the joint models, and entered the extracted trajectories simultaneously into one extended Cox model. The results of this analysis were materially the same as those we described in the paper. Furthermore, the majority of the patient population was in NYHA class I or II, and thus at relatively low risk. The results and conclusions should be judged accordingly.

Conclusion

Serial assessments of NT-proBNP and Hs-TnT are positively associated with NYHA class. Temporal patterns of NT-proBNP, CRP and NYHA class are independently associated with adverse clinical outcome. A model containing these serially measured variables displayed good discriminative ability. However, serially measured CRP had only little incremental discriminative value compared to a strategy combining serial assessments of NYHA class and NT-proBNP. Altogether, our findings underscore the incremental value of biomarkers to NYHA class for monitoring stable CHF outpatients.

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SUPPLEMENTAL TEXT

Statistical analysis

Variables with normal distributions are presented as mean±standard deviation (SD). Variables with non-normal distributions are presented as median and interquartile range (IQR). Categorical data are displayed as count and percentage. In case of skewed distributions, continuous variables were logarithmically transformed for further analyses.

To assess the associations between serial biomarker measurements and repeated assessment of NYHA functional class, we used linear mixed models. Time was used as a random effect. NYHA class was used as the independent variable (fixed effect), in order to be able to uniformly display the change in each of the biomarkers per point increase in NYHA class. Each of the biomarkers was consecutively used as the dependent variable. Associations amongst the 3 biomarkers were examined likewise. For these analyses, all samples drawn were used.

Associations between baseline values of NT-proBNP, Hs-TnT, CRP, and NYHA functional class on the one hand, and the primary endpoint on the other hand, were assessed using only the samples drawn before the occurrence of the primary endpoint. Cox proportional hazards models were used. Associations between serial measurements of the aforementioned variables and occurrence of the primary endpoint were examined by entering the serial measurements into extended Cox proportional hazards models as time-varying covariates. First, the models were adjusted for age, gender, systolic blood pressure and estimated glomerular filtration rate (eGFR; calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation). Subsequently, all variables, i.e. NT-proBNP, Hs-TnT, CRP and NYHA functional class, were entered simultaneously into the models to investigate their independence. For serial measurements, this meant that all variables were simultaneously entered as time-varying covariates into the extended Cox analysis. The multivariable models also included age, gender, systolic blood pressure and eGFR.

It has previously been demonstrated that testing for improvement in prediction performance is actually redundant if a variable has already been shown to be an independent risk factor. Independence already proves presence of incremental value [20]. Still, to provide an impression of the *magnitude* of the incremental discriminative ability of the individual and combined serial measurements of the biomarkers and NYHA functional class, we calculated time-dependent C-indices based on the extended Cox models.

Finally, to investigate the incremental value of serial biomarker measurements to baseline assessment only, we simultaneously added the baseline measurements and the series of longitudinal measurements of NT-proBNP, CRP, Hs-TnT and NYHA class into

the models, in order to obtain separate hazard ratios for the baseline measurements and for the series of longitudinal measurements.

Analyses were performed with R Statistical Software using packages 'Survival' and 'nlme'. C-indices were compared using MedCalc. All tests were two-tailed and p-values <0.05 were considered statistically significant.

Supplemental table 1 – Associations between serial blood biomarker measurements and NYHA class

	NT-proBNP[a]					
NT-proBNP[a]	**B (95%CI)**	**p-value**	**Hs-TnT[a]**			
Hs-TnT[a]	1.66 (1.53 – 1.81)	<0.001	**B (95%CI)**	**p-value**	**CRP[a]**	
CRP[a]	1.11 (1.06 – 1.16)	<0.001	1.03 (0.99 – 1.08)	0.11	**B (95%CI)**	**p-value**
NYHA class[b]	1.56 (1.17 – 2.06)	0.002	1.58 (1.21 – 2.07)	0.001	1.22 (0.98 – 1.53)	0.076

CRP = C-reactive protein; Hs-TnT = high sensitive cardiac troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association.

[a] Beta coefficient per standard deviation increase in log transformed level

[b] Beta coefficient per 1-step increase

Supplemental table 2 – Discriminative ability of models containing baseline blood biomarker- and NYHA assessment

Model	C-index (CI)
Model[a]	0.62 (0.52 – 0.71)
Model[a] + NT-proBNP	0.76 (0.67 – 0.86)
Model[a] + Hs-TnT	0.72 (0.62 – 0.81)
Model[a] + CRP	0.68 (0.58 – 0.78)
Model[a] + NYHA class	0.71 (0.60 – 0.81)
Model[a] + NT-proBNP + CRP + Hs-TnT + NYHA class	0.80 (0.71 – 0.91)

CI = Confidence interval; CRP = C-reactive protein; Hs-TnT = High-sensitive cardiac troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association.

[a] Including age, gender, systolic blood pressure and estimated glomerular filtration rate.

Chapter 6

Towards personalized risk assessment in patients with chronic heart failure: detailed temporal patterns of NT-proBNP, troponin T and CRP in the Bio-SHiFT study

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ABSTRACT

Background: We examined the prognostic information of detailed temporal patterns of N-terminal pro B-type natriuretic peptide (NT-proBNP), high-sensitive troponin T (HsTNT) and C-reactive protein (CRP) in patients with chronic heart failure (CHF).

Methods: From 2011-2013, 263 CHF patients were included. NT-proBNP, HsTNT and CRP were measured at baseline and every 3 months. The primary endpoint (PE) comprised heart failure hospitalization, cardiovascular mortality, cardiac transplantation and LVAD-implantation. Associations between temporal biomarker patterns and the PE were investigated by joint modelling, which combines mixed models with Cox regression.

Results: Mean age was 67±12 years and 72% were men. Median follow-up was 2.2 (IQR 1.4–2.5) years. We used 2022 blood samples (median 9(IQR 5-10) per patient) and 70 (27%) patients reached the PE. Temporal patterns of NT-proBNP, HsTNT and CRP level were associated with the PE (multivariable adjusted HR per doubling of biomarker: NT-proBNP 2.28 (95%CI 1.82–2.86), HsTNT 2.05(1.63–2.58), CRP 1.65 (1.30–2.08). A combined 3 biomarker model demonstrated independent associations for the temporal patterns of NT-proBNP and CRP level (HRs 2.06(1.53–2.79) and 1.38(1.01–1.89), respectively). Instantaneous change in biomarker level was also independently associated with the PE for NT-proBNP and CRP. Long-term biomarker elevation showed an association for NT-proBNP.

Conclusions: Temporal patterns representing evolution of level and rate of change in level of NT-proBNP and CRP, and long-term elevation of NT-proBNP are independently associated with adverse prognosis in CHF patients. Individual patterns of change and combining multiple biomarkers could carry value for prognostication and for therapy guidance.

INTRODUCTION

The diagnosis of progression of chronic heart failure (CHF) is primarily based on clinical signs and symptoms and the decision to adjust therapy is usually made once symptoms of progression have become manifest. Blood biomarkers are capable of monitoring subtle (patho)physiological processes that reflect and possibly predict adverse changes before they become clinically apparent.[1,2] B-type natriuretic peptides (BNP) and N-terminal proBNP (NT-proBNP), cardiac troponin T and I and C- reactive protein (CRP) have been unequivocally related to adverse clinical outcomes in heart failure (HF) patients in several large studies.[2-12]

The majority of these studies have examined single, baseline measurements of these blood biomarkers. However, since patients with CHF display large biological heterogeneity, distinguishing patients at different levels of risk of adverse events based on single biomarker measurements only is challenging. Measuring biomarkers repeatedly could contribute to individualized risk assessment. Studies that have assessed changing biomarker patterns over time have mostly focused on natriuretic peptides, generally used only few repeated biomarker measurements, and have utilized simplified representations of temporal biomarker evolution, such as change between two time-points.[3-6,12-14] Results of these studies strongly depend on the statistical approach that was used.[1] Subsequent trials on natriuretic peptide-guided therapy of HF have provided inconsistent results.[8-10,15,16] Although such trials are promising for individual risk assessment and personalized treatment, most existing trials have applied uniform natriuretic peptide cut-off values for all patients, and did not use individualized target levels; nor did they take into consideration multiple biomarkers. Moreover, they did not investigate optimal frequency of the measurements, nor did they adapt this frequency to the patient's individual situation.

The above illustrates that in order to properly install personalized risk assessment that makes use of blood biomarkers, first, more detailed information is needed on temporal biomarker patterns in individual patients. Specifically, having measurements available that are performed closely in time to the moment that the endpoint of interest occurs, would provide further insight into the biomarkers' behaviour as this endpoint nearly approaches. This would enable an adequate investigation of whether, and to which degree, increasing (or decreasing) biomarker levels contribute to an individual's risk, regardless of whether his or her blood levels exceed classic, absolute cut-points at any random point in time (such as 'study baseline'). However, in practice, biomarker measurements performed shortly before the endpoint occurs are difficult to acquire, because they require a high frequency of blood sampling during prolonged follow-up. Therefore, most studies on this topic have performed only two measurements over time and are thus not able to properly investigate the biomarker trajectory shortly before the endpoint occurs.

In the current study, we have performed frequent (up to 11), repeated measurements of multiple blood biomarkers (NT-proBNP, HsTNT and CRP) in 263 patients with CHF, and have investigated the associations of the thus obtained temporal patterns with adverse clinical outcome. These 3 biomarkers were chosen because each of them represents different aspects of heart failure pathophysiology (wall stress, myocyte damage and inflammation) and because a large body of evidence exists for the prognostic value of single measurements of these markers. By performing multiple, longitudinal measurements, assessing multiple biomarkers simultaneously and using appropriate, modern statistical methods, we aimed to provide a basis for improved, personalized risk assessment in patients with CHF.

METHODS

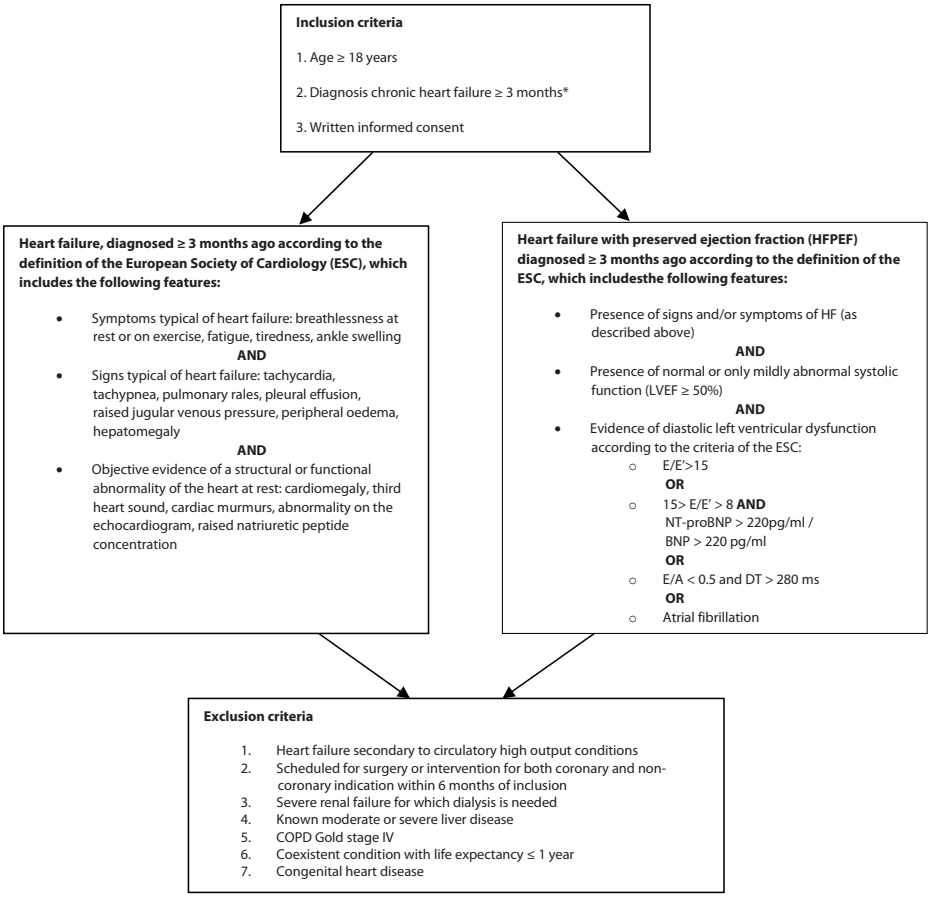
Patients

Bio-SHiFT is a prospective, observational study of stable outpatients with CHF, conducted in Erasmus MC, Rotterdam, The Netherlands and Noordwest Ziekenhuisgroep, Alkmaar, The Netherlands. Patients were recruited during their regular outpatient visits and were in clinically stable condition. Detailed inclusion and exclusion criteria are shown in figure 1. Patients were eligible if CHF (including HF with preserved ejection fraction) was diagnosed ≥ 3 months ago according to the guidelines of the European Society of Cardiology (ESC).[17-19] This study was approved by the medical ethics committee of the Erasmus MC, Rotterdam, The Netherlands and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. The study is registered in ClinicalTrials.gov, number NCT01851538. Estimated enrolment is 400 patients. In this paper, we have performed an interim analysis on the 263 patients who were enrolled during the first inclusion period between October 2011 and June 2013.

Baseline assessment

At baseline, patients were evaluated by trained research physicians, who collected information on HF-related symptoms, including NYHA class,[17,18] and performed physical examination, including blood pressure, heart rate and body mass index. Information on aetiology of heart failure, presence of systolic dysfunction, cardiovascular risk factors, medical history and medical treatment was retrieved primarily from hospital records. History of chronic renal failure was defined as glomerular filtration rate (GFR) less than 60 mL/min/1.73 m^2. Alcohol consumption was defined as drinking ≥1 alcoholic consumption per day. Data were entered into electronic case report forms. Electrocardiography

Figure 1 - Inclusion and exclusion criteria



and echocardiography were performed. Non-fasting blood and urine were collected, as described below.

Follow-up visits

Routine outpatient follow-up by the treating physician continued for all patients during the study. Study follow-up visits were scheduled every 3 months (a window of ±1 month was allowed), to a maximum follow-up duration of 30 months. At each follow-up visit, a short medical evaluation was performed and blood and urine samples were collected. Changes in medication as well as occurrence of adverse cardiovascular events since the previous visit were recorded.

Blood sampling and biomarker measurement

Blood samples were processed and stored at a temperature of -80°C within 2 hours after blood collection. When applicable, samples were transported to the central laboratory (Erasmus MC, Rotterdam, The Netherlands) under controlled conditions (at a temperature of -80°C), until batch analysis was performed. Accordingly, results of the biomarker assays were not available to treating physicians at the time of the outpatient visits. Thus, the biomarker measurements performed for this study did not lead to drug adjustments, and all patients received usual care. This concurs with Bio-SHiFT being a strictly observational study, as described above.

For the purpose of the current analysis, three biomarkers (NT-proBNP, HsTNT, and CRP) were measured in one batch in stored serum samples. Plasma NT–proBNP was analysed using an electrochemiluminesence immunoassay (Roche Diagnostics, Elecsys 2010, Indianapolis, Indiana, USA), which measures concentrations ranging from 5 to 35000 ng/L. Cardiac troponin T was also measured using an electrochemiluminesence immunoassay (Roche Diagnostics, Elecsys 2010 immunoassay analyser, Indianapolis, Indiana, USA), measuring concentrations ranging from 3-10000 ng/L. CRP was measured using an immunoturbidimetric assay (Roche Hitachi 912 chemistry analyser, Basel, Switzerland). This system measures concentrations ranging from 0.3 to 350 mg/L. All coefficients of variation were <5%.

Clinical study endpoints

During follow-up, hospitalizations for HF, myocardial infarction (MI), percutaneous coronary interventions (PCIs), coronary artery bypass grafting (CABG), arrhythmias, and cerebrovascular accidents (CVAs), as well as cardiac transplantation, left ventricular assist device implantation (LVAD) and mortality, were recorded in the electronic case report form by trained research physicians, and associated hospital records and discharge letters were collected.

Subsequently, hospital records and discharge letters were reviewed by a clinical event committee blinded to the biomarker results, and primary and secondary endpoints were adjudicated. The primary endpoint comprised the composite of cardiac death, cardiac transplantation, LVAD- implantation, and hospitalization for HF, whichever occurred first in time. Secondary endpoints included individual components of the primary endpoint, and also MI, PCI, CABG, CVA, and all-cause mortality.

Cardiac death was defined as death from MI or other ischemic heart disease (ICD-10: I20-I25), death from other heart disease including HF (I30-I45 and I47-I52), sudden cardiac death (I46), sudden death undefined (R96) or unwitnessed or ill-described death (R98, R99). Hospitalisation for acute or worsened HF was primarily based on exacerbation of HF symptoms, requiring hospitalization. On top of this, a combination of 2 of the following was required: BNP or NT-proBNP >3x ULN, signs of worsening HF, such as

pulmonary rales, raised jugular venous pressure or peripheral oedema, increased dose or intravenous administration of diuretics, or administration of positive inotropic agents.

Statistical analysis

Distributions of continuous variables, including biomarker concentrations, were tested for normality using the Kolmogorov-Smirnov test. Normally distributed continuous variables are presented as mean ± standard deviation (SD). Non-normally distributed continuous variables are expressed as median and interquartile range (IQR). Categorical data are displayed as count and percentage.

In case of skewed distributions, continuous variables were logarithmically transformed (log base 2) for further analyses. Associations between patient characteristics and baseline biomarker levels were evaluated using univariable linear regression. Associations between baseline patient characteristics, including baseline biomarker levels, and the primary endpoint, were evaluated using Cox proportional hazards models. These analyses were first performed univariably. Subsequently, to evaluate independent associations, all baseline characteristics that showed statistically significant associations (with p-values <0.05) were forced into a multivariable Cox model.

Associations between temporal biomarker patterns of each separate biomarker and the primary endpoint were assessed using a joint modelling approach, which combines a linear mixed-effects (longitudinal) submodel to assess the temporal evolution of the repeatedly measured marker, with a Cox proportional hazards submodel to analyze the association of this temporal evolution with the study endpoint. In line with the logarithmic (base 2) transformation of the biomarker concentrations, the results are presented as hazard ratios (HRs) per doubling of the biomarker concentration at any point in time, along with the corresponding 95% CIs. First, analyses were performed univariably. Subsequently, potential confounders were entered into the joint models. These included all variables that were significantly associated with the primary endpoint in the multivariable 'baseline' Cox proportional hazards model (NYHA class and diabetes mellitus), as well as variables selected from existing literature (age, gender, renal function, body mass index). Covariates were missing in less than 3% of patients. Multiple imputation (5 times) of these covariates was performed in the multivariable analyses.

The above-described analysis assesses the predictive value of repeatedly measured biomarker levels; specifically, it provides hazard ratios that estimate the risk of the endpoint associated with doubling of biomarker level at any point in time. However, in the context of serial marker measurements, there could be additional features of the marker's trajectory that better predict the primary endpoint.[20] Therefore, we investigated the predictive value of: (1) the 'instantaneous slope' of the marker's trajectory, indicating whether a marker is decreasing, increasing, or remains stable; and (2) the area under the curve of the marker's trajectory, indicating the cumulative effect of all the values the

marker has taken in the past (this area under the curve does not provide information on increasing or decreasing biomarker values, which should be derived from the slope).

We chose not to correct for multiple testing, because the selection of the currently investigated 3 biomarkers was based on previous research and thus hypothesis-driven.[2-12]

In order to simultaneously investigate the effect of all 3 biomarkers on the primary endpoint, and thus to assess their independent predictive value, all individual temporal biomarker patterns derived from the adjusted joint models were saved and subsequently entered simultaneously as time-varying covariates into an extended Cox analysis. The same approach was used to investigate the independent predictive value of the slope and the area under the curve of the 3 temporal biomarker patterns. Adjustment for potential baseline confounders was performed as described above. Additionally, these extended Cox models were adjusted for temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone, which were also entered into the models as time-varying covariates.

To illustrate how joint modelling can be applied to estimate prognosis of an individual patient based on his or her repeatedly assessed biomarker values, we plotted the temporal patterns of the biomarkers in several individual patients (i.e., example patients drawn from our dataset), together with their corresponding dynamic, individual probabilities of survival as estimated by the joint model (which we developed on the total study population as described above). As such we graphically demonstrated individual survival probabilities, which are updated each time that an additional measurement is performed in the patient as he or she visits the outpatient clinic.

Finally, to investigate the discriminative ability of models containing serial measurements and models containing baseline measurements only, we calculated c-indices based on extended Cox models containing temporal biomarker patterns derived from the adjusted joint models, as well as c-indices based on Cox models containing baseline biomarker values only.

All analyses were performed with R Statistical Software using package JM.[20] All statistical analyses were two-sided and p-values <0.05 were considered statistically significant.

Power calculation

The current investigation comprised 263 patients, of whom 70 reached the primary endpoint. For baseline measurements, these numbers are sufficient to detect odds ratios around 2 for the upper quintile of a biomarker associated with the endpoint (α-error 0.05, power of 80%) when comparing cases with non-cases. For repeated measurements, power is further enhanced. A median of 9 samples per patient were available. We calculated power for repeated measurements by assuming a linear association and a continuous autoregressive correlation matrix. We used NT-proBNP to derive the measurement error standard deviation (sigma, equal to 463) and the input parameter for the

autoregressive correlation matrix (rho, equal to 0.49). Based on these input parameters, and using 1000 simulations, we calculated that a difference in change of NT-proBNP level over time of 51 ng/L per month can be demonstrated between cases and non-cases (α-error 0.05, power of 80%). This difference is small in clinical terms, demonstrating that the study has high statistical power.

RESULTS

Baseline findings

From October 2011 to August 2015, 263 patients were included. Baseline characteristics are shown in Table 1. Mean age of the study population was 67 years (SD ±12). The majority were men (72%) in New York heart association (NYHA) class I or II (73%). Median duration of HF was 4.6 years (IQR 1.7 – 9.9). Median baseline NT-proBNP was 1161 ng/L (IQR 439 – 2305), HsTNT 18.0 ng/L (IQR 9.6 – 33.2) and CRP 2.2 mg/L (IQR 0.9 – 4.8). Positive associations were found between baseline NT-proBNP level and age (p=0.01), heart rate (p=0.01), NYHA class (p<0.001), and renal failure (p<0.001). Inverse associations were found between NT-proBNP and diastolic blood pressure (p<0.001) and BMI (p<0.001). Baseline HsTNT level was positively associated with age (p<0.001), NYHA class (p<0.001) and renal failure (p<0.001). Baseline CRP level showed positive associations with heart rate (p=0.01) and renal failure (p=0.045), and inverse associations with systolic (p=0.046) and diastolic blood pressure (p<0.001).

Table 1 – Baseline characteristics

	Total (n=263)
	No. (%) / Mean (±SD) / Median (25th – 75th percentile)
Demographics	
Age	67 (±13)
Male gender	189 (72)
Caucasian ethnicity	244 (94)
Clinical characteristics	
Body mass index kg/m^2	28 (±5)
Heart rate, bpm	67 (±12)
Systolic blood pressure, mmHg	122 (±20)
Diastolic blood pressure, mmHg	73 (±11)
Biomarker level	
NT-proBNP (ng/L)	1161 (439 – 2305)
HsTNT (ng/L)	18.0 (9.6 – 33.2)
CRP (mg/L)	2.2 (0.9 – 4.8)

Table 1 – Baseline characteristics (continued)

	Total (n=263)
	No. (%) / Mean (±SD) / Median (25th – 75th percentile)
Features of heart failure	
Duration of heart failure, years	4.6 (1.7 – 9.9)
NYHA class I or II	190 (73)
NYHA class III or IV	69 (27)
Left ventricular function	
Systolic dysfunction	250 (95)
HFPEF	13 (5)
LVEF*	32 (±10)
Etiology of heart failure	
Ischemic heart disease	117 (44)
Hypertension	34 (13)
Secondary to valvular heart disease	12 (5)
Cardiomyopathy	68 (26)
Dilated	49 (19)
Hypertrophic	12 (5)
Non compaction	4 (1)
Unclassified	3 (1)
Unknown	19 (7)
Other	13 (5)
Medical history	
Myocardial infarction	94 (36)
PCI	82 (31)
CABG	43 (16)
Valvular heart disease	136 (53)
Atrial fibrillation	105 (40)
Other arrhythmia	82 (32)
ICD	151 (59)
CRT	78 (30)
Pacemaker	38 (15)
CVA	41 (16)
Chronic renal failure	136 (53)
Diabetes Mellitus	81 (31)
Known hypercholesterolemia	93 (35)
Hypertension	120 (46)
Sleep apnea	26 (10)

Table 1 – Baseline characteristics (continued)

	Total (n=263)
	No. (%) / Mean (±SD) / Median (25th – 75th percentile)
Intoxications	
Alcohol consumption (>1 unit/day)	108 (42)
Smoking	185 (71)
Ever	186 (72)
Current	26 (10)
Medication use	
ACE-i	173 (67)
ARB	75 (29)
Aldosteron antagonist	178 (68)
Diuretic	237 (90)
Beta-blocker	232(88)
Aspirin	45 (17)
Vitamin K antagonist	200 (77)
Nitrates	44 (17)
Digoxin	59 (23)
Antiarrhythmics	39 (15)

Normally distributed continuous variables are presented as mean (± standard deviation). Non-normally distributed continuous variables are expressed as median (25^{th} – 75^{th} percentile). Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. ACE-I = ace inhibitor; ARB = angiotensin II receptor blocker; CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; CRT = cardiac resynchronization therapy; CVA = cerebrovascular accident; HFPEF = heart failure with preserved ejection fraction; HsTNT = high sensitive cardiac troponin T; ICD = implantable cardioverter / defibrillator; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type natriuretic peptide NYHA = New York heart association; PCI = percutaneous coronary intervention; SD = standard deviation.

* Baseline echocardiograms were available in 72% of all patients because of logistic reasons.

Clinical endpoints

During a median follow-up of 2.2 (IQR 1.4–2.5) years, 27 (10%) patients died from a cardiovascular cause, 56 (21%) patients were re-hospitalized for worsened HF, 5 (1.9%) patients underwent heart transplantation and 3 (1.1%) patients received LVAD-implantation (Table 2). Since 21 patients were re-hospitalized for worsened HF before dying from cardiovascular causes eventually during further follow-up, 70 patients (27%) reached the composite primary endpoint. Overall all-cause mortality was 32 (12%).

Associations between baseline characteristics and the primary endpoint are shown in Table 3. After multivariable adjustment, baseline NT-proBNP (HR 1.02; CI 1.01 – 1.02), baseline HsTNT (HR 1.08; CI 1.02 – 1.16), NYHA class (HR 1.61; CI 1.14 – 2.26) and diabetes

Table 2 – Endpoints

Endpoint	N (%)
Primary	
Combined primary endpoint*	70 (27)
Secondary	
Hospitalization for acute or worsening heart failure	56 (21)
All-cause mortality	32 (12)
Cardiovascular mortality	27 (10)
Heart transplantation	5 (1.9)
Left ventricular assist device implantation	3 (1.1)

Variables are displayed as count (percentage).
* The primary endpoint comprised heart failure hospitalization, cardiovascular mortality, cardiac transplantation and LVAD-implantation

Tabel 3 – Associations between baseline characteristics and the primary endpoint

Variabele	Crude HR (CI)	P	Adjusted HR (CI)‡	P
NT-proBNP (pmol/L)*	1.02 (1.02 – 1.03)	<0.001	1.02 (1.01 – 1.02)	<0.001
HsTNT (pg/mL)*	1.12 (1.08 – 1.16)	<0.001	1.08 (1.02 – 1.16)	0.020
CRP (mg/L)*	1.26 (1.06 – 1.50)	0.016	1.18 (0.96 – 1.45)	0.12
Age†	1.02 (1.01 – 1.05)	0.035	1.00 (0.98 – 1.02)	0.86
Male gender	1.27 (0.80 – 2.19)	0.40		
Systolic blood pressure†	0.99 (0.98 – 0.99)	0.040	0.99 (0.98 – 1.01)	0.26
Diastolic blood pressure†	0.98 (0.96 – 1.00)	0.055		
Heart rate†	1.01 (0.99 – 1.03)	0.24		
Body mass index kg/m^2†	1.00 (0.96 – 1.05)	0.88		
NYHA-class†	2.10 (1.56 – 2.54)	<0.001	1.61 (1.14 – 2.26)	0.006
Chronic renal failure	2.11 (1.28 – 3.50)	0.004	1.25 (0.72 – 2.18)	0.42
Diabetes mellitus	2.06 (1.29 – 3.29)	0.003	1.91 (1.17 – 3.11)	0.010
Hypercholesterolemia	1.37 (0.85 – 2.20)	0.20		
Hypertension	1.31 (0.82 – 2.10)	0.26		
Ever smoker	1.48 (0.84 – 2.62)	0.18		
History of CAD	1.56 (0.96 – 2.53)	0.074		
History of CVA	1.40 (0.78 – 2.51)	0.26		
ICD	1.20 (0.74 – 1.95)	0.47		
CRT	0.80 (0.47 – 1.36)	0.42		

CRP = C-reactive protein; CRT = cardiac resynchronization therapy; CVA = cerebrovascular accident; HsTNT = high sensitive cardiac troponin T; ICD = implantable cardioverter / defibrillator; NT-proBNP = N-terminal pro-B-type natriuretic peptide NYHA = New York heart association.
* HR per 10 units increase
† HR per unit increase
‡ All characteristics univariably associated with the primary endpoint (p<0.05) were entered into the multivariable Cox regression model.

mellitus type 2 (DM) (HR 1.91; CI 1.17 – 3.11) were independently associated with the primary endpoint (Table 3).

Temporal biomarker patterns and the primary endpoint

During follow-up, we collected 2193 blood samples, of which 2022 were drawn before the occurrence of the primary endpoint (median of 9 (IQR 5-10) samples per patient). The associations between the temporal biomarker patterns and the primary endpoint are shown in Table 4.

Table 4 - Association between temporal patterns of logarithmically transformed NT-proBNP, hsTNT and CRP and the primary endpoint

	NT-proBNP		HsTNT		CRP	
	HR* (95% CI)	p-value	HR* (95% CI)	p-value	HR* (95% CI)	p-value
Temporal pattern of biomarker level						
Adjusted for age and gender	2.20 (1.83–2.65)	<0.001	2.21 (1.79–2.72)	<0.001	1.80 (1.43–2.26)	<0.001
Multivariable adjusted†	2.28 (1.82–2.86)	<0.001	2.05 (1.63–2.58)	<0.001	1.65 (1.30–2.08)	<0.001
Instantaneous slope of temporal pattern						
Adjusted for age and gender	2.16 (1.79–2.62)	<0.001	2.14 (1.71–2.66)	<0.001	1.85 (1.43–2.41)	<0.001
Multivariable adjusted†	2.15 (1.71–2.68)	<0.001	2.02 (1.58–2.58)	<0.001	1.69 (1.32–2.18)	<0.001
Area under the curve of temporal pattern						
Adjusted for age and gender	1.68 (1.45–1.96)	<0.001	1.74 (1.46–2.07)	<0.001	1.32 (1.13–1.55)	<0.001
Multivariable adjusted†	1.54 (1.30–1.83)	<0.001	1.55 (1.29–1.86)	<0.001	1.28 (1.09–1.51)	0.003
Three biomarkers combined						
Level, multivariable adjusted†	2.06 (1.53–2.79)	<0.001	1.41 (0.93–2.13)	0.104	1.38 (1.01–1.89)	0.047
Level, medication adjusted‡	2.08 (1.54–2.80)	<0.001	1.46 (0.95–2.23)	0.083	1.38 (1.01–1.90)	0.044
Slope, multivariable adjusted†	2.04 (1.51–2.78)	<0.001	1.47 (0.94–2.16)	0.093	1.41 (1.02–1.94)	0.036
Slope, medication adjusted‡	2.06 (1.51–2.80)	<0.001	1.44 (0.94–2.21)	0.089	1.42 (1.02–1.99)	0.040
Area, multivariable adjusted†	1.99 (1.49–2.66)	<0.001	1.42 (0.94–2.13)	0.092	1.32 (0.96–1.80)	0.084
Area, medication adjusted‡	2.01 (1.49–2.73)	<0.001	1.42 (0.94–2.15)	0.098	1.26 (0.91–1.75)	0.16

HR = hazard ratio.

* Hazard ratios are given per doubling of level, slope or area under the curve at any point in time.

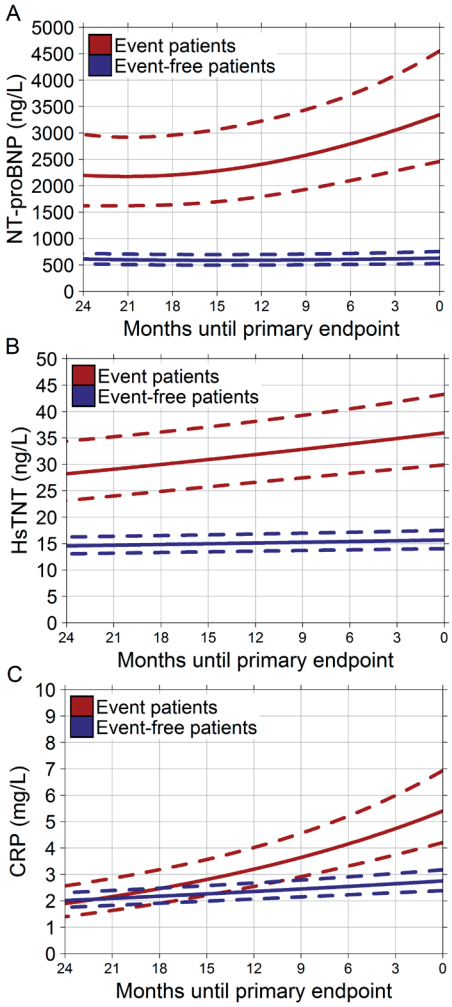
† Adjusted for age, gender, BMI, renal function, NYHA class and diabetes mellitus type 2.

‡ Adjusted for age, gender and temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone.

The temporal NT-proBNP pattern derived from the repeated measurements was a significant predictor of the primary endpoint after adjustment for age, gender, BMI, renal function, NYHA class and DM (HR per doubling of NT-proBNP: 2.28; CI 1.82 – 2.86; p<0.001). Figure 2a displays the curves depicting the temporal NT-proBNP pattern of patients who reached the primary endpoint versus those who did not.

Figure 2b depicts temporal HsTNT patterns of patients who reached the primary endpoint and those who did not. We found an association between the temporal HsTNT

Figure 2 - Temporal patterns



The temporal patterns, displayed as time until event, of A) N-terminal pro B-type natriuretic peptide (NT-proBNP), B) high-sensitive troponin T (HsTNT) and C) C-reactive protein (CRP) of patients who reached the primary endpoint versus those who did not.

pattern and the primary endpoint, which remained present after multivariable adjustment (HR per doubling of biomarker: 2.05; CI 1.63 – 2.58; p<0.001).

As shown in figure 2c, the temporal CRP pattern was also a significant predictor of the primary endpoint (HR per doubling of CRP after multivariable adjustment: 1.65; CI 1.30 – 2.08; p<0.001).

NT-proBNP, HsTNT and CRP patterns and the primary endpoint using a combined 3-biomarker model

When we combined temporal patterns of all 3 biomarkers in one model, we found independent associations of NT-proBNP (HR per doubling of NT-proBNP level at any given time point: 2.06; CI 1.53 – 2.79; p<0.001) and CRP (HR per doubling of CRP level: 1.38; CI 1.01 – 1.89; p=0.047) with the primary endpoint. These associations were also independent of temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone (table 4). However, HsTNT was no longer associated with the primary endpoint in this model (HR per doubling of HsTNT: 1.41; CI 0.93 – 2.13; p=0.10), illustrating that its predictive value was not independent of NT-proBNP and CRP.

Slopes and areas under the curve of temporal patterns

Table 4 displays hazard ratios for the doubling of the instantaneous slopes and areas under the curve of the temporal biomarker patterns. The instantaneous slopes of the temporal patterns as well as the areas under the curve of NT-proBNP, HsTNT and CRP were all associated with the primary endpoint after multivariable adjustment, including adjustment for temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone.

When we entered the instantaneous slopes of the temporal biomarker patterns of the 3 biomarkers into 1 model, they remained independent predictors for NT-proBNP and CRP, but not for HsTNT. Simultaneously entering the areas under the curve of the 3 temporal biomarker patterns into 1 model showed that only NT-proBNP was independently associated with the primary endpoint.

Personalized prediction: individual, dynamic risk estimation

Figure 3 shows the temporal patterns of the biomarkers in several individual patients from our dataset, together with their corresponding individual probabilities of survival as estimated by the joint model. The figure shows that each time an additional measurement is performed in the patient, the individual probability of survival is updated. Specifically, rising marker levels and worsening prognosis can be seen in the example patients who ultimately reached the composite endpoint, versus stable or decreasing marker levels and more favorable prognosis in the example patients who stayed event-

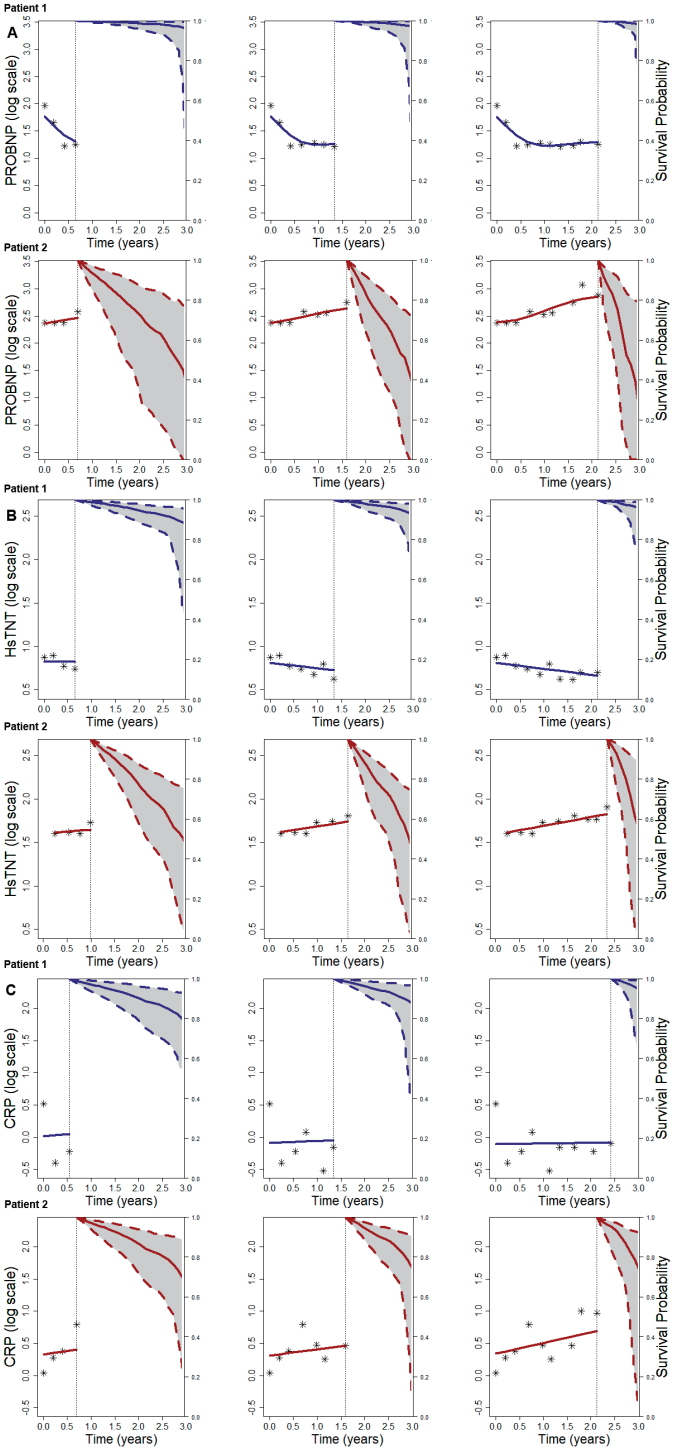

Patient 1
A
PROBNP (log scale)
Time (years)
Survival Probability
Patient 2
PROBNP (log scale)
Time (years)
Survival Probability
Patient 1
B
HsTNT (log scale)
Time (years)
Survival Probability
Patient 2
HsTNT (log scale)
Time (years)
Survival Probability
Patient 1
C
CRP (log scale)
Time (years)
Survival Probability
Patient 2
CRP (log scale)
Time (years)
Survival Probability

Figure 3 - Dynamic profiling of an individual patient's risk using patient-specific temporal trajectories

The solid red lines depict patients who experienced the study endpoint, and the solid blue lines depict patients who did not. The X-axis depicts follow-up time starting from baseline. Biomarker levels (on the log scale) are displayed on the left Y-axis and survival probability (%) on the right Y-axis. Patient-specific temporal biomarker trajectories are displayed left of the vertical dotted black line. To the right of this line, the corresponding conditional survival probability curve is displayed with 95% confidence intervals (grey area). To show how this conditional survival probability curve is dynamically updated every time an extra measurement is recorded, we provide the curves for three time-points at which risk was updated.

free. These individual estimates of prognosis can be obtained by clinicians in an easy, user-friendly manner. Joint models, like those we have constructed, can be uploaded into an app (http://shiny.rstudio.com/) that creates an interface into which a clinician can add the characteristics, and consecutive biomarker measurements, of an individual patient. Subsequently, the app returns the curve depicting individual prognosis (supplemental figure 1).

Model performance

Discriminative ability of models containing the temporal patterns of the biomarker levels and baseline measurements only is shown in table 5. For all 3 biomarkers, models containing temporal biomarker patterns showed higher c-indices than those containing baseline measurements only. The highest c-index resulted from the multivariable model containing all 3 temporal biomarker patterns as well as age, gender, BMI, renal function, NYHA class and DM (c-index 0.84).

Table 5 – Discriminative ability of models containing the temporal patterns of NT-proBNP, HsTNT and CRP level, as well as models containing baseline measurements only

	Baseline measurements	Temporal patterns
	c-index; multivariable model*	c-index; multivariable model*
NT-proBNP	0.78	0.83
HsTNT	0.73	0.75
CRP	0.67	0.69
Combined model†	0.79	0.84

* The multivariable models were corrected for: age, gender, BMI, renal function, NYHA class and diabetes mellitus type 2.

† NT-proBNP, HsTNT and CRP are all included in the combined model.

Temporal patterns of NT-proBNP, HsTNT and CRP in relation to hospitalisation for acute or worsening HF (secondary endpoint)

All three biomarker patterns were strong individual predictors of HF hospitalisations (age- and gender adjusted HRs per doubling of biomarker: NT-proBNP, 2.17; CI 1.57 –

2.66; p<0.001; HsTNT, 2.18; CI 1.72 – 2.76; p<0.001 and CRP, 1.99; CI 1.53 – 2.59; p<0.001). These associations remained statistically significant after multivariable adjustment (HRs per doubling of biomarker: NT-proBNP, 2.31; CI 1.77 – 3.01; p<0.001; HsTNT, 1.95; CI 1.49 – 2.55; p<0.001 and CRP, 1.80; CI 1.37 – 2.35; p<0.001). After creating a time-dependent Cox model using all 3 temporal biomarkers patterns, derived from the individual joint models, we found that each of the 3 biomarkers remained independent predictors of HF hospitalizations (HR per doubling of biomarker: NT-proBNP, 1.51; CI 1.26 – 1.80; p<0.001; HsTNT, 1.57; CI 1.24 – 2.00; p=0.001 and CRP, 1.41; CI 1.15 – 1.74; p<0.001). These associations persisted after adjusting for temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone (HR per doubling of biomarker: NT-proBNP, 1.49; CI 1.23 – 1.80; p<0.001; HsTNT, 1.50; CI 1.18 – 1.91; p=0.001 and CRP, 1.39; CI 1.11 – 1.74; p=0.004.

DISCUSSION

In this prospective, observational study we demonstrate that the dynamic, temporal patterns of serially-measured NT-proBNP and CRP levels are strong and independent predictors of adverse clinical events in CHF patients. Moreover, instantaneous slope of these biomarkers' temporal trajectories, as well as the area under the curve of their temporal trajectories, are associated with adverse events. The temporal patterns of HsTNT also significantly predict adverse events, but lose their predictive capability when combined with temporal NT-proBNP and CRP patterns. We also demonstrate, based on these dynamic models, how individual, temporal biomarker trajectories can be used for calculating patient-specific risk estimates, which are dynamically updated every time a patient has a new measurement performed.

Studies on the prognostic value of repeated natriuretic peptide measurements have mostly been performed in trial participants,[6,13] and studies on the prognostic value of repeated biomarker measurements other than natriuretic peptides, are scarce.[3,4,12] Altogether, these existing studies describing temporal changes in biomarkers in relation to patient prognosis have three major limitations. Firstly, changes are often presented as a difference between just two measurements that are separated in time. Such an approach fails to fully capture the true biomarker pattern of the dynamic disease. Moreover, it fails to expose changes in biomarker level prior to clinically relevant endpoints, because on average, a long period time period lies between the last (i.e., second) biomarker measurement and the incident endpoint. To properly investigate whether an increase in biomarker level is present at the time an endpoint is approaching, and whether this increase truly contributes to an individual's risk, the time period between the last measurement and the endpoint should be kept as brief as possible.

This implies that a high frequency of blood sampling during prolonged follow-up is needed. Secondly, biomarkers are often studied in isolation, thus actually ignoring the different underlying aetiologies that converge to adverse cardiac remodelling and HF progression. The third limitation of existing studies is related to the applied methods of data analysis. Often, absolute or relative differences between two measurements are calculated, or categorical changes across a threshold value are assessed. These various approaches to temporal change all render different estimates for associations between changes in biomarker level and outcome,[1] which is an illustration of their shortcomings. At best, Cox models with so called time-dependent covariates are used to analyse the effects of temporal biomarker patterns. While time-dependent Cox models assume that biomarker levels do not change between measurements, it is known that biomarker patterns are dynamic and continuously change over time, parallel to the condition of the patient. All these limitations are overcome in Bio-SHiFT: we have performed a large number of frequent, repeated measurements (up to 11 trimonthly samples per patient), we have studied multiple biomarkers, and we have applied modern statistical methods ('joint modelling'), which, as stated above, take into account the continuous, dynamic changes in biomarker patterns and thus result in less bias.[21]

Several randomized trials have been performed to investigate whether using serial natriuretic peptide measurements to titrate medical therapy can improve clinical outcome of HF patients. However, since the results of these trials were not fully consistent, natriuretic peptide guided therapy remains controversial.[8-10,15] It should be noted that most of these trials were based on protocols that used uniform natriuretic peptide targets in the intervention groups.[22-26] Existing trials that used individualized treatment targets are in the minority, and often based their targets on natriuretic peptide levels that were measured briefly after the index episode of decompensation, when titration of therapy was still ongoing.[27-29] Conversely, our study describes in detail the temporal biomarker patterns in stable CHF patients, and reveals significant associations between temporal patterns of biomarker levels and adverse events. CHF patients who did not experience adverse cardiac events during prolonged follow-up were shown to have lower levels of NT-proBNP, HsTNT and CRP at any moment in time compared to patients who did experience adverse cardiac events during follow-up. Additionally, the instantaneous rate of change in biomarker levels (represented by the slope of the temporal biomarker patterns), as well as the cumulative values the marker has taken in the past (represented by the area under the temporal biomarker patterns), were associated with adverse outcome. These findings support the concept of an individualized biomarker target level, instead of a generally applicable uniform cut-off value for all patients. On top of this, they suggest that rate of change in biomarker level as well as duration of biomarker level elevation, merit attention in order to provide appropriate individual treatment targets

as well as correct estimates of prognosis. Our study also demonstrates that temporal patterns of CRP predict adverse clinical outcome independently of NT-proBNP.

Future trials on biomarker-guided therapy may benefit from incorporating these findings. Firstly, future trials should use personalized biomarker cut-off values, i.e. interpret a patient's biomarkers level in the context of his or her previous series of levels. This means they should not only take into account the absolute biomarker level, but also incorporate the instantaneous slope of the marker's trajectory. Secondly, upcoming trials should use a combination of multiple biomarkers, representing different pathophysiological pathways, to guide HF therapy. Finally, additional research should be performed on the frequency of biomarker measurement and tailoring thereof to individual patients; subsequently these findings should be incorporated into biomarker-guided trials as well.

Miller et al. published a study that might be considered comparable to Bio-SHiFT to a certain extent, as they evaluated serial measurements of cardiac troponine T and BNP in 190 ambulant CHF patients.[12] Again, an important limitation of this study is the use of time-dependent Cox models. Still, Miller et al. found that cardiac troponine T and BNP were both independent predictors of cardiac mortality or cardiac transplantation, and that combined elevation of these biomarkers substantially adds to risk. We could only partly confirm these results. In Bio-SHiFT, although predictive as a separate marker, the HsTNT pattern appeared no longer significantly associated with the primary study endpoint after adjustment for the NT-proBNP and CRP patterns (and also after adjustment for NT-proBNP alone; data not shown). This may (at least in part) be due to the above-described differences in data analysis.

Some aspects of this study warrant consideration. With 263 patients, sample size is limited; and the majority of the patient population was in NYHA class I or II, and had systolic dysfunction. Also, a large proportion had concomitant valvular heart disease. The results and conclusions should be judged accordingly. Nevertheless, given the repeated measures design, over 2000 blood samples were available; and all 3 investigated biomarkers, each having different pathophysiological properties, showed the hypothesised rising temporal pattern. This strengthens our findings and makes them less likely attributable to bias or chance. Further to this, the current investigation was an interim analysis of the patients enrolled in the first inclusion round. The full Bio-SHiFT cohort was designed to enrol 400 patients and to have sufficient statistical power to perform large-scale, hypothesis-free research on novel, lesser known biomarkers. In such cases, correction for multiple testing is warranted. The current investigation, however, examines 3 well-established biomarkers, which have been extensively implicated in heart failure in previous studies and which were chosen based on pathophysiological considerations, rendering correction for multiple testing redundant. Furthermore, additional investigations are needed to estimate the most efficient frequency of biomarker measurement, so that optimal prognostic information can be gained without superflu-

ous blood sampling. In our study, patients were monitored every 3 months, in order to construct a data framework for our joint models. An extension to joint modelling is currently being developed to define optimal timeframes for individual patients to return for consecutive measurements. In this context, the optimal frequency for biomarker measurement is expected to vary from patient to patient; it is likely that once a stable biomarker value is found in a patient, this patient could be re-examined after a longer time-period, while if for example a biomarker value is found to have risen and thus prognosis is worsening, the patients may need to return more quickly. Finally, in our study, repeatedly measured NT-proBNP and CRP were both independently associated with the primary endpoint. This implies a multimarker model would benefit monitoring of CHF patients (even though a model combining both of these biomarkers seemed to have little incremental discriminative value over serial NT-proBNP assessment only as suggested by the C-index; the C-index is known to be rather insensitive to improvements in prediction performance, and it has been demonstrated previously that testing for improvement in prediction performance is actually redundant if a variable has already been shown to be an independent risk factor.[30] Future studies should investigate a broader spectrum of biomarkers to further improve risk assessment.

In conclusion, detailed temporal patterns of NT-proBNP and CRP are strong, independent predictors of adverse clinical events in patients with stable CHF. Not only evolution of biomarker level, but also instantaneous rate of change in level of NT-proBNP and CRP as well as the area under the curve of the trajectory of NT-proBNP, were associated with adverse outcome. These findings suggest that individual patterns of change of biomarkers, as well as combinations of multiple biomarkers, should be taken into consideration for prognostication in patients with stable CHF Overall, our study illustrates that several aspects of biomarker –guided risk stratification have been incompletely addressed so far, and that there still seems to be room for improvement with regard to personalized risk assessment. Future steps could potentially include determining optimum timing of blood sampling, determining optimum combinations of biomarkers, and eventually a biomarker guided trial that is based on personalized temporal patterns of multiple biomarkers.

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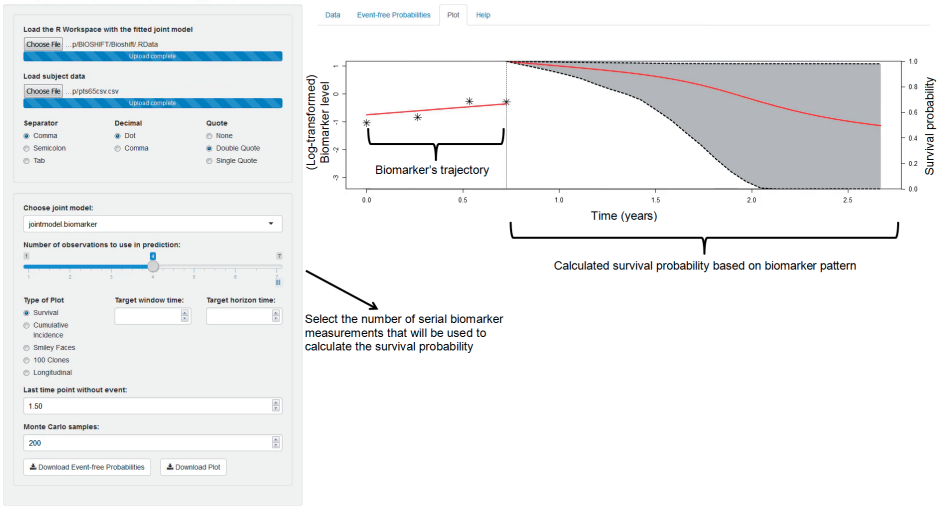
Supplemental figure 1 - Example of the Shiny app



A clinician can add individual, consecutive biomarker measurements of a patient into the Shiny app to depict the curve of this patient's individual prognosis.

Chapter 7

Serially measured circulating miR-22-3p is a biomarker for adverse clinical outcome in patients with chronic heart failure: the Bio-SHiFT study

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ABSTRACT

Background: Several studies have suggested circulating microRNAs (miRs) are associated with heart failure, but these studies were small, and limited to single miR measurements.

We examined 7 miRs which were previously linked to heart failure, and tested whether their temporal expression level predicts prognosis in a prospective cohort of chronic heart failure (CHF) patients.

Methods: In 2011-2013, 263 CHF patients were included. At inclusion and subsequently every 3 months, we measured 7 miRs. The primary endpoint (PE) comprised heart failure hospitalization, cardiovascular mortality, cardiac transplantation and LVAD implantation. Associations between temporal miR patterns and the PE were investigated by joint modelling, which combines mixed models with Cox regression.

Results: Mean age was 67±13 years, 72% were men and 27% NYHA class III–IV. We obtained 873 blood samples (median 3 [IQR 2-5] per patient). The PE was reached in 41 patients (16%) during a median follow-up of 0.9 [0.6-1.4] years. The temporal pattern of miR-22-3p was independently associated with the PE (HR [95% CI] per doubling of level: 0.64 [0.47 - 0.77]). The instantaneous change in level (slope of the temporal miR pattern) of miR-22-3p was also independently associated with the PE (HR [95% CI] per doubling of slope: 0.33 [0.20–0.51]). These associations remained statistically significant after adjustment for temporal patterns of NT-proBNP, Troponin T and CRP.

Conclusions: The temporal pattern of circulating miR-22-3p contains important prognostic and independent information in CHF patients. This concept warrants further investigation in larger series with extended follow-up.

INTRODUCTION

Contemporary treatment of chronic heart failure ((C)HF) generally aims to stabilize or at least decelerate disease progression. Adjustment of pharmacotherapy is largely based on clinical judgement and thus mostly relies on the worsening of symptoms or signs.[1,2] However, in the context of the complexity of HF therapy, considerable clinical skills, as well as cooperation of the patients, are required to recognize opportunities to titrate therapies and to implement such changes intervene early and timely.[3] Consequently, higher-risk patients may be undertreated.[3] Blood biomarkers have the potential to monitor subtle changes in the heart that reflect and possibly predict adverse changes before they become clinically apparent or reported.[4] The value of biomarkers, such as B-type natriuretic peptides (BNP), cardiac troponins and C- reactive protein (CRP), for risk stratification of CHF patients has already been demonstrated.[5-8] Moreover, natriuretic peptide-guided HF therapy has recently been given a class IIa recommendation in US HF guidelines to achieve guideline-directed medical therapy.[2,9]

Nevertheless, the predictive capability of the aforementioned biomarkers for worsening of CHF still leaves room for improvement. MicroRNAs (miRs) are upcoming novel biomarkers that seem promising for early diagnosis and treatment of HF. MiRs are non-coding, ~22 nucleotide long RNA sequences, which target messenger RNAs for cleavage or translational repression and thereby influence a great variety of biological processes.[10] The stability of miRs in plasma, and consequently their reliable assessment in easily accessible samples, potentially makes them attractive biomarkers for a wide range of diseases.[11] Studies revealing that deletion of Dicer, a gene encoding an RNase III endonuclease essential for miR processing, leads to cardiac remodelling and dilation, were the first to show involvement of miRs in HF[12,13] and to suggest that miRs might be used as biomarkers for cardiovascular diseases.[14] Several other studies have subsequently shown associations between miRs and myocardial infarction[15-18] and HF.[19-24] However, most studies pertaining to HF were performed in case-control settings and had a limited sample size. Furthermore, these studies usually assessed miRs only once. Repeated, longitudinal miR assessment in CHF patients may, however, provide insight into individual, temporal patterns and the patient's ensuing risk of disease progression. The temporal patterns of miRs in patients with known CHF have not yet been investigated.

In the current prospective, observational study, we have performed frequent (up to 8), repeated measurements of multiple miRs that were previously linked to HF (miR-1254, miR-22-3p, miR-423-5p, miR-486-5p and miR-320a) or have been shown to be cardiac-enriched (miR-345-5p, miR-378a-3p) in a cohort of 263 outpatients with CHF, and have subsequently investigated the associations of the obtained temporal patterns with adverse clinical outcome during follow-up.

METHODS

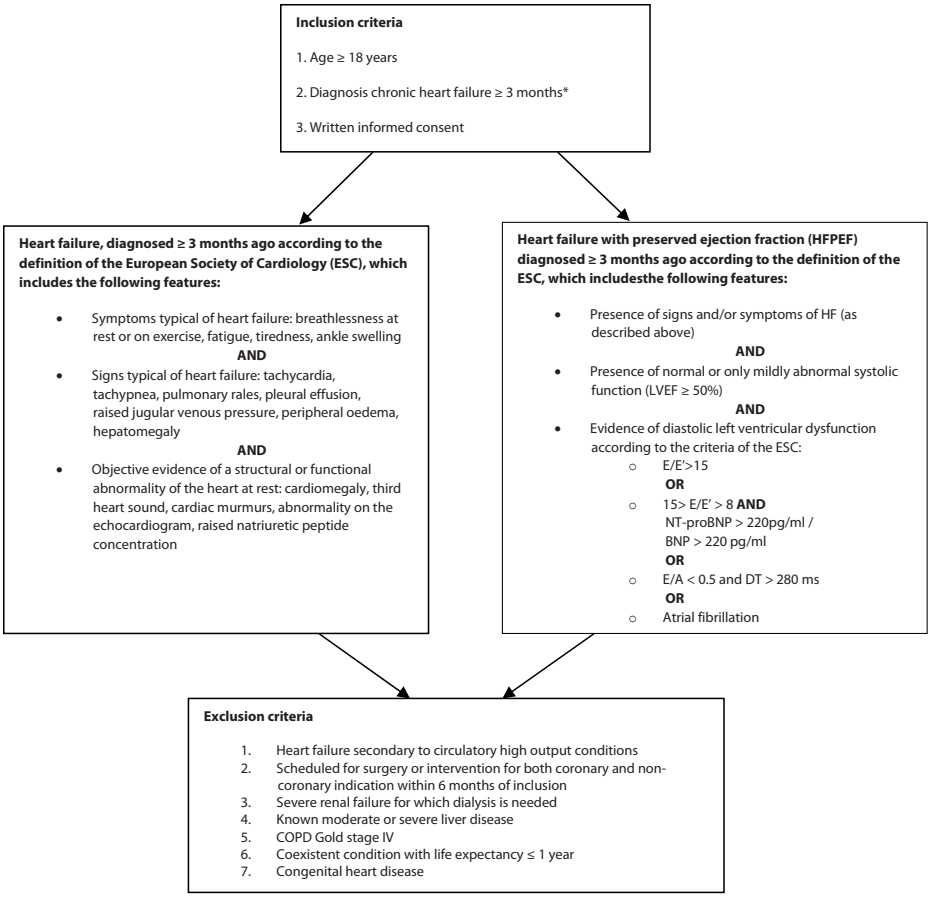
Patients

The *Serial biomarker measurements and new echocardiographic techniques in chronic heart failure patients result in tailored prediction of prognosis* (Bio-SHiFT) study was designed to investigate the relationship between temporal patterns of biomarkers involved in CHF and prognosis. Bio-SHiFT is an ongoing prospective, observational study of stable outpatients with CHF, conducted in Erasmus MC, Rotterdam, The Netherlands and Medical Centre Alkmaar, The Netherlands. Patients were recruited during their regular outpatient visits and were in clinically stable condition. Patients were eligible for inclusion if aged 18 years or older, capable of understanding and signing informed consent, and if CHF (including HF with preserved ejection fraction (HFPEF)) was diagnosed ≥ 3 months ago according to the guidelines of the European Society of Cardiology (ESC).[25-27] Detailed inclusion and exclusion criteria are shown in figure 1. The study was approved by the medical ethics committees of the participating hospitals and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.The study is registered in ClinicalTrials.gov, number NCT01851538. In the current paper, we have performed an analysis on the 263 patients who were enrolled during the first inclusion period between October 2011 and June 2013. Follow-up for this analysis lasted from October 2011 until November 2013.

Baseline assessment

At baseline patients were evaluated by trained research physicians, who collected information on HF related symptoms including NYHA class[25,26] and performed a physical examination, including blood pressure, heart rate and body mass index. Information on aetiology of heart failure, presence of systolic dysfunction, cardiovascular risk factors, medical history and medical treatment was retrieved primarily from hospital records and was completed by anamnesis in case of ambiguities. History of myocardial infarction, percutaneous coronary intervention (PCI), coronary artery bypass grafting, valvular heart disease, atrial fibrillation or other arrhythmias, device implantation, cerebrovascular accident, diabetes mellitus, hypercholesterolemia, hypertension, and sleep apnea were defined as a clinical diagnosis of these conditions, as reported by the treating physician in the medical chart. History of chronic renal failure was defined as glomerular filtration rate less than 60 mL/min/1.73 m^2. Alcohol consumption was defined as drinking ≥1 alcoholic consumption per day. Electrocardiography and echocardiography were performed. Data were entered into electronic case report forms. Non-fasting blood and urine samples were collected.

Figure 1 - Inclusion and exclusion criteria



Follow-up visits

All patients continued routine outpatient visits during the study. Study follow-up visits were scheduled every 3 months (a window of ±1 month was allowed), for a maximum of 30 months. For patients' convenience, study visits and routine outpatient visits were combined when possible. At each tri-monthly study visit, a short medical evaluation was performed and blood and urine samples were collected. Adverse cardiovascular events and changes in medication were recorded in electronic case report forms.

Blood sampling and miR measurement

Blood samples were collected at baseline and at each follow-up visit, and were processed and stored locally at a temperature of -80°C within 2 hours after blood collection. When applicable, samples were transported to the central laboratory (Erasmus MC, Rotterdam, The Netherlands) under controlled conditions (at a temperature of -80°C), until batch

analysis took place. Accordingly, results of the biomarker assays were not available to treating physicians at the time of the outpatient visits. Thus, the biomarker measurements did not lead to treatment adjustments, and all patients received usual care based on European guidelines.[25,26]

For the purpose of the current investigation, stored plasma samples were transported under controlled conditions to ACS Biomarker, Amsterdam, The Netherlands, and seven miRs were measured in one batch: miR-1254, miR-22-3p, miR-345-5p, miR-378a-3p, miR-423-5p, miR-486-5p and miR320a. MiR-1254, miR-22-3p, miR-423-5p, miR-486-5p and miR-320a were selected because they were associated with HF in previous studies.[11,21,24] MiR-378a-3p and miR-345-5p were selected because of their presence in cardiomyocytes.[28] Plasma was thawed on ice and RNA isolation was performed using the TRIZOL LS reagent (Life Technology) according to the manufacturer's protocol, with a starting volume of 200ul plasma. Subsequently, 8ul of the eluate from the RNA isolation was used for the reverse transcription reaction. The transcription followed the manufacturer's protocol of the miScript Reverse Transcription Kit (Qiagen). For real-time PCR, 2ul of 10x diluted cDNA was used in a total volume of 10ul. The real-time PCR reaction was performed on a LightCycler 480 using the following program: 5 min of pre-incubation at 95°C; 10 sec of denaturation at 95°C, 20 sec of annealing at 58°C and 30 sec of elongation at 72°C, in a total of 45 cycles. Data were analysed with LinRegPCR quantitative PCR data analysis software. MiR values were normalized using exogenous C. elegans miR-39 as a spike-in control, which was applied prior to the RNA isolation step. The forward primers used were: miR-423-5p: TGAGGGGCAGAGAGCGAGACTTT; miR-22-3p: AAGCTGCCAGTTGAAGAACTGT; miR-378a-3p: ACTGGACTTGGAGTCAGAAGG; miR-1254: CTGGAAGCTGGAGCCTGC; miR-345-5p: GCTGACTCCTAGTCC; miR-486-5p: TCCTGTACTGAGCTG; miR-320a: AAAAGCTGGGTTGAGAGGGCGA.

Batch analysis of N-terminal pro B-type natriuretic peptide (NT-proBNP), high-sensitive cardiac troponin T (HsTNT) and C-reactive protein was performed in the Clinical Chemistry Laboratory of the Erasmus MC, Plasma NT–proBNP and cardiac Troponin T were analysed using electrochemiluminesence immunoassays (Roche Diagnostics, Elecsys 2010, Indianapolis, Indiana, USA), measuring concentrations ranging from 5 to 35000 ng/L and 3 to 10000 ng/L, respectively. CRP was measured using an immunoturbidimetric assay (Roche Hitachi 912 chemistry analyser, Basel, Switzerland), which measures concentrations ranging from 0.3 to 350 mg/L.

Clinical study endpoints

During follow-up, endpoints were recorded in the electronic case report forms by trained research physicians, and associated hospital records and discharge letters were collected. A clinical event committee blinded to the biomarker results subsequently reviewed all collected information and adjudicated primary and secondary endpoints.

The primary endpoint comprised the composite of cardiac death, cardiac transplantation, left ventricular assist device implantation, and hospitalization for the management of acute or worsened HF. Secondary endpoints included individual components of the primary endpoint, as well as myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, cerebrovascular accident, and all-cause mortality.

Cardiac death was defined as death from myocardial infarction or other ischemic heart disease (ICD-10: I20-I25), death from other heart disease including HF (I30-I45 and I47-I52), sudden cardiac death (I46), sudden death undefined (R96) or unwitnessed or ill-described death (R98, R99). Hospitalisation for acute or worsened HF was defined as exacerbation of symptoms typical of HF, in combination with 2 of the following: BNP or NT-proBNP >3x ULN, signs of worsening HF, such as pulmonary rales, raised jugular venous pressure or peripheral oedema, increased dose or intravenous administration of diuretics, or administration of positive inotropic agents.

Statistical analysis

Distributions of continuous variables, including biomarker and miR concentrations, were tested for normality using the Kolmogorov-Smirnov test. Variables with normal distributions are presented as mean±SD. Variables with non-normal distributions are presented as median and interquartile range (IQR). Categorical data are displayed as count and percentage.

In case of skewed distributions, continuous variables were logarithmically transformed for further analyses. Associations between temporal patterns of each separate miR and the primary endpoint were assessed using a joint modelling approach, which combines a linear mixed-effects model for the serial miR measurements with a Cox proportional hazards model for the occurrence of the primary endpoint. Results are presented as hazard ratios (HRs) per doubling of the miR concentration at any point in time, along with the corresponding 95% CIs. All analyses were adjusted for age and gender. Subsequently, additional multivariable adjustment for potential confounding variables was performed. For this purpose, associations between patient characteristics and baseline miR levels, as well as associations between patient characteristics and the primary endpoint, were identified using univariable linear- or Cox regression analyses, when appropriate. All characteristics associated with the miRs or the primary endpoint were subsequently used as covariates in the mixed-effects and Cox models used to construct the joint models. Adjustment for repeatedly assessed NYHA class was performed by entering NYHA class as a time-dependent variable into the joint model.

The above-described analysis assesses the predictive value of the temporal pattern of the actual miR measurements. However, additional features of the miR's trajectory may better predict the primary endpoint.[29,30] Therefore, we investigated whether the instantaneous rate of change in miR (slope of the miR trajectory) is associated with the

risk of the primary endpoint. Furthermore, to assess the predictive value of long-term elevation of miR levels, we investigated the associations between the area under the miRs' trajectory and the primary endpoint. This area can be considered as a weighted average of the total longitudinal profile of each patient. The results are presented as HRs per doubling of the area at any point in time, with 95% CIs.

Subsequently, we assessed whether the predictive value of the temporal miR patterns was independent of temporal patterns of NT-proBNP, HsTNT and CRP. For this purpose, all individual temporal miR patterns (of miRs significantly associated with the primary endpoint), as well as NT-proBNP, HsTNT and CRP patterns, derived from the adjusted joint models were saved and subsequently entered simultaneously as time-varying covariates into an extended Cox analysis. The same approach was used to investigate the independent predictive value of the slope and the area under the curve of the temporal miR patterns associated with the primary endpoint.

To investigate the associations between repeated miR measurements and repeated NT-proBNP, HsTNT and CRP measurements, we used generalised estimating equations. Herewith, we examined whether the miR level at a certain time-point is associated with the level of the other biomarkers at that same time-point.

All analyses were performed with R Statistical Software using package JM.[29,30] All tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

From October 2011 to June 2013, 263 patients were included. Mean age was 67 years (SD ±13), 72% were men, and 73% were in New York Heart Association (NYHA) class I or II (Table 1). Median baseline NT-proBNP was 139.6 pmol/L (IQR 51.9 – 272.9), HsTNT 18.0 ng/L (IQR 9.6 – 33.2) and CRP 2.2 mg/L (IQR 0.9 – 4.8). Median duration of HF at inclusion was 4.6 years (IQR 1.7 – 9.9). Associations between patient characteristics and baseline miR measurements are presented in the supplementary table. Of note is the presence of a significant association between ischemic cardiomyopathy (ICM) and several miRs. This is further illustrated in figure 2. No significant associations were present with other baseline characteristics.

Clinical endpoints

The primary, composite endpoint was reached by 41 patients (16%), during a median follow-up of 0.9 [0.6-1.4] years: 5 patients died from a cardiovascular cause, 35 patients were re-hospitalized for worsened HF and 1 patient underwent heart transplantation. Of the 35 patients reaching the primary endpoint because of re-hospitalisation for HF, 16

Table 1 – Baseline characteristics

	Total (n=263)
Demographics	
Age	67 (±13)
Male gender	189 (72)
Caucasian ethnicity	244 (94)
Clinical characteristics	
Body mass index kg/m^2	28 (±5)
Heart rate, bpm	67 (±12)
Systolic blood pressure, mmHg	122 (±20)
Diastolic blood pressure, mmHg	73 (±11)
Biomarker level	
NT-proBNP (pmol/L)	139.6 (51.9 – 272.9)
HsTNT (ng/L)	18.0 (9.6 – 33.2)
CRP (mg/L)	2.2 (0.9 – 4.8)
Features of heart failure	
Duration of heart failure, years	4.6 (1.7 – 9.9)
NYHA class I	75 (29)
NYHA class II	115 (44)
NYHA class III or IV	69 (27)
Left ventricular function	
Systolic dysfunction	250 (95)
HFPEF	13 (5)
LVEF*	32 (±10)
Etiology of heart failure	
Ischemic heart disease	117 (44)
Hypertension	34 (13)
Secondary to valvular heart disease	12 (5)
Cardiomyopathy	68 (26)
Dilated	49 (19)
Hypertrophic	12 (5)
Non compaction	4 (1)
Unclassified	3 (1)
Unknown	19 (7)
Other	13 (5)
Medical history	
Myocardial infarction	94 (36)
PCI	82 (31)
CABG	43 (16)
Valvular heart disease	136 (53)
Atrial fibrillation	105 (40)

Table 1 – Baseline characteristics (continued)

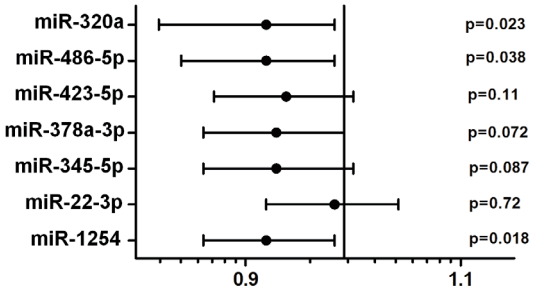
	Total (n=263)
Other arrhythmia	82 (32)
ICD	151 (59)
CRT	78 (30)
Pacemaker	38 (15)
CVA	41 (16)
Chronic renal failure	136 (53)
Diabetes Mellitus	81 (31)
Known hypercholesterolemia	93 (35)
Hypertension	120 (46)
Sleep apnea	26 (10)
Intoxications	
Alcohol consumption (>1 unit/day)	108 (42)
Smoking	185 (71)
Ever	186 (72)
Current	26 (10)
Medication use	
ACE-i	173 (67)
ARB	75 (29)
Aldosteron antagonist	178 (68)
Diuretic	237 (90)
Beta-blocker	232(88)
Aspirin	45 (17)
Vitamin K antagonist	200 (77)
Nitrates	44 (17)
Digoxin	59 (23)
Antiarrhythmics	39 (15)

Normally distributed continuous variables are presented as mean (± standard deviation). Non-normally distributed continuous variables are expressed as median (25^{th} – 75^{th} percentile). Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. ACE-I = ace inhibitor; ARB = angiotensin II receptor blocker; CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; CRT = cardiac resynchronization therapy; CVA = cerebrovascular accident; HFPEF = heart failure with preserved ejection fraction; HsTNT = high sensitive cardiac troponin T; ICD = implantable cardioverter / defibrillator; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type natriuretic peptide NYHA = New York heart association; PCI = percutaneous coronary intervention.

* Baseline echocardiograms were available in 72% of all patients because of logistic reasons.

patients died eventually during further follow-up, of whom 12 died from a cardiovascular cause. Overall all-cause mortality was thus 21 (8.0%).

Figure 2 - Associations between baseline MicroRNA levels and presence of ischemic cardiomyopathy



Odds ratios for doubling of microRNA (miR) expression level

Of all baseline variables, NYHA class (HR 1.98; CI 1.24 – 3.16 for III/IV vs I/II), baseline NT-proBNP (HR per two-fold difference of baseline NT-proBNP 1.42; CI 1.09 – 1.86), and baseline CRP (HR per two-fold difference of baseline CRP 1.29; CI 1.05 – 1.60) were independently associated with the primary endpoint. As shown in table 2, none of the baseline miR values were associated with the primary endpoint, or the secondary endpoint comprising HF hospitalisations only.

Table 2 – Associations between baseline miRs and the primary endpoint and secondary endpoint

	Primary endpoint		Secondary endpoint	
	HR* (95% CI)	**P**	**HR (95% CI)**	**P**
miR-1254	1.01 (0.92–1.09)	0.91	1.00 (0.91–1.09)	0.99
miR-22-3p	1.02 (0.93–1.12)	0.61	1.00 (0.90–1.09)	0.85
miR-345-5p	0.99 (0.89–1.09)	0.81	0.95 (0.86–1.06)	0.38
miR-378a-3p	1.00 (0.91–1.11)	0.93	0.99 (0.89–1.09)	0.77
miR-423-5p	1.01 (0.91–1.11)	0.92	0.97 (0.87–1.07)	0.53
miR-486-5p	1.04 (0.94–1.15)	0.47	1.00 (0.89–1.11)	0.93
miR-320a	1.04 (0.95–1.13)	0.44	1.02 (0.93–1.12)	0.71

miR = microRNA.

Primary endpoint: cardiac death, cardiac transplantation, left ventricular assist device implantation, and hospitalization for the management of acute or worsened heart failure; secondary endpoint: hospitalization for the management of acute or worsened heart failure.

* Hazard ratios (HRs) and corresponding confidence intervals (CIs) were calculated using Cox regression analyses and are given per doubling of level of miR.

Temporal miR patterns and the primary endpoint

During follow-up, we collected 923 blood samples, of which 885 were drawn before the occurrence of the primary endpoint (median of 3 (IQR 2-5) samples per patient). MiRs were successfully determined in 873 of these samples. The temporal pattern of miR-22-3p was inversely associated with the primary endpoint (age and gender adjusted HR

per doubling of miR-22-3p level, 0.64; CI 0.47 - 0.77; p<0.001), i.e. higher miR-22-3p was associated with lower risk of events (table 3). As described above, baseline measurements of several miRs were associated with ICM. On the other hand, NYHA class was independently associated with the primary endpoint. Therefore, the baseline variables ICM and NYHA class were added to the models. After adjustment for age, gender, NYHA class and ICM, the association between the temporal pattern of miR-22-3p and the primary endpoint remained present (HR per doubling of miR-22-3p level 0.71; CI 0.44 - 0.94; p=0.005). When serial NYHA class assessments were added to the model instead of baseline NYHA assessments only, the association between miR miR-22-3p and the primary endpoint also remained present (HR per doubling of miR-22-3p level, 0.52; CI 0.44 - 0.61; p<0.001). The temporal patterns of the other 6 miRs were not significantly associated with the primary endpoint.

Baseline echocardiograms were available in 72% of all patients due to logistic reasons . After correction for left ventricular ejection fraction in this subgroup, miR-22-3p remained significantly associated with the primary endpoint (HR per doubling of miR-22-3p level, 0.91; CI 0.85 - 0.95; p<0.001).

Figure 3 shows that in the patients who reached the endpoint, miR-22-3p values declined as this endpoint approached, while in the patients who did not reach the endpoint, miR-22-3p values remained stable.

Slope and area under the curve of the temporal miR patterns and the primary endpoint

The hazard ratios for the doubling of the slopes and areas under the curve of the temporal miR patterns are displayed in table 3. For 4 of the miRs (miR-345-5p, miR-378a-3p, miR-423-5p, and miR-486-5p), slopes remained virtually constant over time, and hazard ratios were not calculable. The slope of the temporal pattern of miR-22-3p was inversely associated with the primary endpoint after multivariable adjustment (HR per doubling of miR-22-3p slope at any given time point, 0.33; CI 0.20–0.51; p<0.001), while the slopes of the patterns of miR-1254 and miR-320a were not. None of the areas under the curves of the temporal miR patterns were associated with the primary endpoint.

Temporal patterns of miRs, NT-proBNP, Hs-TNT and CRP combined in a multiple-marker model

After constructing the multivariable models that corrected for age, sex, ICM and NYHA class, we proceeded to additionally adjust the association between the temporal miR-22-3p pattern and the primary endpoint for temporal patterns of the other biomarkers which were measured in this study (NT-proBNP, HsTNT and CRP). We did this by entering the multivariably adjusted temporal pattern of miR-22-3p, as well as the multivariably adjusted temporal patterns of NT-proBNP, HsTNT and CRP simultaneously into one

Table 3 - Associations between temporal miR patterns and the primary endpoint

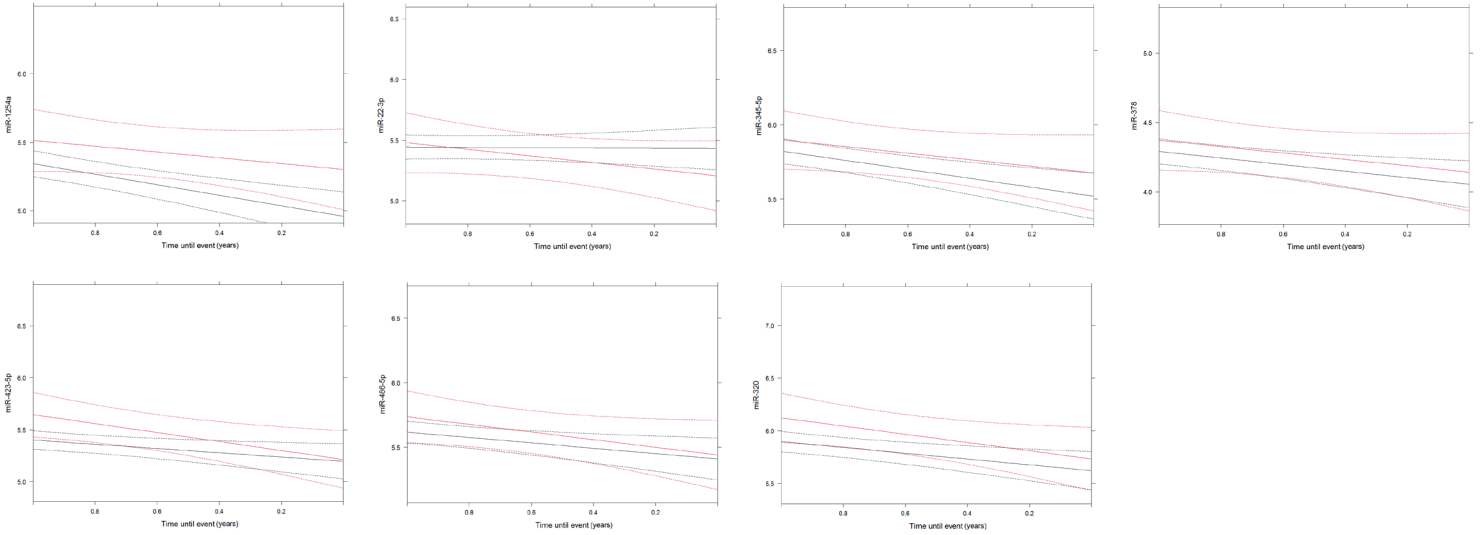
	miR-1254		miR-22-3p		miR-345-5p		miR-378a-3p		miR-423-5p		miR-486-5p		miR-320a	
	HR* (95% CI)	p	HR* (95% CI)	p	HR* (95% CI)	p	HR* (95% CI)	p	HR* (95% CI)	p	HR* (95% CI)	p	HR* (95% CI)	p
Temporal pattern of miR value														
Adjusted for age and gender	1.02 (0.71–1.26)	0.782	0.64 (0.47–0.77)	<0.001	1.01 (0.97–1.04)	0.624	0.84 (0.69–1.03)	0.085	1.01 (0.97–1.05)	0.800	1.00 (1.00–1.01)	0.069	0.92 (0.74–1.19)	0.516
Multivariable adjusted [y]	0.97 (0.78–1.22)	0.757	0.71 (0.44–0.94)	0.005	1.00 (0.97–1.03)	0.921	0.88 (0.71–1.06)	0.148	1.00 (0.95–1.08)	0.845	1.00 0.99–1.01)	0.380	1.01 (0.78–1.22)	0.896
Slope of temporal pattern														
Adjusted for age and gender	1.29 (0.75–2.49)	0.456	0.20 (0.09–0.50)	<0.001	NA	NA	NA	NA	NA	NA	NA	NA	0.51 (0.29–0.90)	0.012
Multivariable adjusted [y]	1.30 (0.79-2.35)	0.288	0.33 (0.20–0.51)	<0.001	NA	NA	NA	NA	NA	NA	NA	NA	0.59 (0.33–1.12)	0.102
Area under the curve of temporal pattern														
Adjusted for age and gender	1.09 (0.97–1.27)	0.206	1.00 (0.80–1.14)	0.898	NA	NA	NA	NA	NA	NA	NA	NA	1.16 (0.94–1.42)	0.257
Multivariable adjusted [y]	1.26 (0.95–1.53)	0.100	0.99 (0.88–0.17)	0.846	NA	NA	NA	NA	NA	NA	NA	NA	1.19 (0.88–1.44)	0.330

miR = microRNA.

* Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) are given per doubling of value, slope or area under the curve at any point in time.

[y] Adjusted for age, gender, New York Heart Association class and ischemic cardiomyopathy.

Figure 3 - Temporal evolution of microRNA levels



Temporal evolution was estimated by using mixed models. Y-axis: arbitrary units [a.u.] representing relative miRNA expression levels. X-axis: time in years as the endpoint approaches (time=0 denotes the moment that the endpoint occurs for the patients reaching the endpoint, and the moment of right-censoring for the patients not reaching the endpoint). Red solid line: miR expression level in patients reaching the endpoint. Red dotted lines: 95% confidence interval. Black solid line: miR expression level in patients not reaching the endpoint. Black dotted line: 95% confidence interval.

model. We found an independent association of miR-22-3p (HR per doubling of miR-22-3p level at any given time point, 0.61; CI 0.51–0.73; p<0.001) with the primary endpoint. Temporal patterns of NT-proBNP and CRP level were also independently associated with the primary endpoint in the multiple-marker model (HRs: 1.34 (1.04–1.71) and 2.14 (1.59–2.88), respectively), while the temporal pattern of HsTNT was not (HR: 1.30 (0.91–1.84)). The slope of the temporal pattern of miR-22-3p also remained significantly associated with the primary endpoint after adjustment for the slopes of the temporal patterns of NT-proBNP, Troponin T and CRP (HR per doubling of miR-22-3p slope at any given time point, 0.55 (0.46–0.65); p<0.001).

Repeated miR measurements and other biomarkers

Associations between repeatedly measured miR and repeatedly measured NT-proBNP, HsTNT and CRP, calculated using generalised estimating equations, are displayed in table 4. Of note are the significant inverse associations between miR-345-5p and CRP (β-coefficient [95%CI]: -0.04 [-0.07 – -0.01] 10log(mg/L) per 10log increase in miR-345-5p level; p=0.012) and miR-22-3p and HsTNT (β-coefficient [95%CI]: -0.01 [-0.02 – -0.00]

Table 4 – Associations between repeatedly measured miRs and B-type natriuretic peptide, high sensitive cardiac troponin T and C- reactive protein

	NT-proBNP		HsTNT		CRP	
	Beta coefficient (95% CI)	**P**	**Beta coefficient (95% CI)**	**P**	**Beta coefficient (95% CI)**	**P**
miR-1254	0.07 (-0.05 – 0.02)	0.27	0.00 (-0.01 – 0.01)	0.59	0.00 (-0.02 – 0.02)	0.92
miR-22-3p	-0.01 (-0.022 – -0.00)	0.14	-0.01 (-0.02 – -0.00)	0.014	-0.03 (-0.06 – 0.00)	0.051
miR-345-5p	-0.04 (-0.02 – 0.01)	0.57	-0.01 (-0.02 – 0.01)	0.35	-0.04 (-0.07 – -0.01)	0.012
miR-378a-3p	-0.01 (-0.02 – 0.00)	0.12	0.00 (-0.01 – 0.01)	0.91	-0.02 (-0.04 – 0.01)	0.27
miR-423-5p	0.00 (-0.02 – 0.01)	0.81	-0.01 (-0.02 – 0.00)	0.17	-0.01 (-0.04 – 0.03)	0.63
miR-486-5p	-0.01 (-0.02 – 0.01)	0.43	-0.01 (-0.02 – 0.01)	0.37	-0.03 (-0.06 – 0.00)	0.070
miR-320a	0.00 (-0.01 – 0.02)	0.69	0.00 (-0.01 – 0.01)	0.74	0.01 (-0.02 – 0.03)	0.71

Beta coefficients and corresponding 95% confidence intervals (CIs) were calculated using generalised estimating equations with 10log transformed microRNAs (miRs) as independent variables and 10log transformed N-terminal pro-B-type natriuretic peptide (NT-proBNP), high sensitive cardiac troponin T (HsTNT) and C- reactive protein (CRP) as dependent variables.

10log(mg/L) per 10log increase in miR-22-3p level; p=0.014; as well as the borderline significant inverse association between miR-22-3p and CRP (β-coefficient [95%CI]: -0.02 [-0.04 – 0.00] 10log(mg/L) per 10log increase in miR-22-3p level; p=0.051). No further associations between repeatedly measured miR and repeatedly measured NT-proBNP, HsTNT and CRP were found.

DISCUSSION

In this prospective, observational cohort of CHF patients, temporal miR-22-3p patterns were inversely and independently associated with adverse outcome. The association was independent of temporal NT-proBNP, HsTNT and CRP patterns. The instant rate of change in miR-22-3p level (in terms of the slope of the temporal pattern) was also inversely associated with adverse outcome. Moreover, we found inverse associations of temporal patterns of miR-22-3p with temporal patterns of HsTNT and CRP. An additional finding of this study was the significant, inverse association between ICM and miR-1254, miR486-5p and miR-320a.

Our study has several strengths. Although the number of incident endpoints was limited, this is the largest study to date that has examined miRs in a prospective cohort of patients with chronic HF. NT-proBNP, HsTNT and CRP were measured concomitantly, and, in addition, frequent repeated measurements were performed during follow-up, both of the miRs and of the established blood biomarkers. Statistical models containing all these, repeatedly measured, biomarkers were constructed and modern statistical methods were applied ('joint modelling'), which take into account the continuous, dynamic changes in biomarker patterns and thus result in less bias than simpler analyses such as delta's between two measurements. Herewith, the study expands existing evidence with regard to miRs and cardiovascular disease in several respects.

MiR-22-3p has previously received attention in the cancer field; it has been implicated in the regulation of various cellular processes, including cell growth, apoptosis, motility, and the cell cycle.[31] With regard to cardiovascular disease, fundamental experiments have demonstrated that expression of miR-22-3p is enriched in striated muscle tissues (i.e. cardiac and skeletal muscles); and miR-22-3p is thought to be required for normal cardiac remodelling in response to stresses.[32] A recent study in miR-22 deficient mice demonstrated that miR-22 depleted hearts quickly progress to adverse cardiac remodelling and develop cardiac dilation, with increased cardiomyocyte loss and deposition of fibrotic tissue, and signs of heart failure.[33] Another study also found that mice without miR-22 develop dilatation of myocardium more quickly.[34] Interestingly, several animal models have shown that miR-22 may potentially serve as a therapeutic target in cardiac hypertrophy.[33] Recently, Gupta et al found that pharmacological inhibition of miR-22 post-infarction activated cardiac autophagy, led to improved cardiac function, and inhibited cardiac remodelling in older mice.[35] They also found that circulating miR-22 provides prognostic information in HF patients. Specifically, they demonstrated an association between higher baseline levels of circulating miR-22 and mortality in154 HF patients. Repeated measurements were not performed. In the present study, although we saw a temporal decline of miR-22-3p levels in patients with adverse clinical outcome, we could not demonstrate a significant association between higher baseline miR-22-3p

and clinical outcome. The shorter follow-up duration of our study (median of approx. 1 year, versus 3 years follow-up in the study of Gupta et al) may have contributed to this. Furthermore, in an earlier study, Goren et al, demonstrated that elevated serum levels of miR-22-3p identify patients with systolic heart failure using 30 stable chronic systolic heart failure patients and 30 controls.[24] This seeming discrepancy with our study could possibly be explained by differences in study design. While Goren et al focused on diagnostic properties of miR-22-3p by comparing cases and controls, we focused on prognostic value of miR-22-3p in patients with established CHF. To the best of our knowledge, other cardiovascular studies on miR-22-3p in human subjects are lacking. Altogether, our study is in line with previous fundamental experiments which demonstrated that loss of miR-22-3p leads to dilatation of myocardium and progressive HF. Our study confirms that lower levels of miR-22-3p are associated with worsening of CHF, and that miR-22-3p may complement established HF biomarkers in prognostication.

Previously, several studies using animal models have shown decreased levels of miR-378a-3p during cardiac remodelling[36] and in HF due to cardiac hypertrophy.[37-39] Furthermore, a significant down-regulation of miR-378a-3p in hearts with dilated cardiomyopathy compared to non-failing control hearts has been observed.[23] Since the presence of miR-378a-3p is required to resist ventricular remodelling during cardiac stress, it may offer therapeutic potential for the management of HF.[38] It has been suggested, that up-regulation of miR-378a-3p could serve as a potential novel treatment for apoptosis and ischemic heart disease, since up-regulation of this miR enhanced cell viability, and inhibited apoptosis and necrosis in myocardium of rats.[40] In the current study, we also observed a tendency towards an inverse association between the temporal pattern of miR378a-3p and the primary endpoint in univariable models. These results concur with the aforementioned studies.

Another miR that has previously been associated with HF, is miR-423-5p.[19,21,22,24] However, in our study, there was no association between the temporal pattern of miR-423-5p and the primary endpoint. Existing studies on miR-423-5p in humans all compared single measurements in (C)HF patients with measurements in healthy controls, and imply that this miR has diagnostic value for HF.[19,21,24] We did not evaluate the diagnostic value of miRs, but the prognostic value of serial miR measurements in patients with known CHF. Although miR-423-5p may discriminate between HF patients and healthy controls, it may lack prognostic value in patients with longstanding CHF. Earlier findings support this hypothesis.[20] Bauters et al serially measured serum levels of circulating miR-423-5p in 246 patients with a first anterior Q-wave myocardial infarction, at discharge and subsequently at 3 months and 1 year. They found no association between miR-423-5p levels and indices of left ventricular function and left ventricular remodelling, which were serially assessed during a 1 year period after the index myocardial infarction, nor with

B-type natriuretic peptide. They concluded that miR-423-5p is not a useful biomarker of left ventricular remodelling after myocardial infarction.[20]

The selection of the miRs for the current investigation was based on previous studies, which used animal models and relatively small patient groups. Some of these studies have shown associations between miR-1254, miR-345-5p, miR-486-5p and

miR-320a and heart failure.[19,21,24,41,42] Again, these were mostly studies that compared HF patients to healthy controls. In our study, we did not find any associations between these miRs and poor outcome in CHF. Multiple other miRs, such as miR-133a,[43] have previously been associated with HF.[44] Serial measurements of these other miRs, which were not investigated in this study, warrant further investigation. This could lead to further improvements in CHF prognostication, as well as tailored adjustment of treatment.

Some aspects of this study warrant consideration. For the current investigation we only used the first round of inclusions of the ongoing Bio-SHiFT study (n=263). It should be noted that the full Bio-SHiFT cohort is designed to have sufficient statistical power to enable correction for multiple testing in case of application of modern, large-scale biomarker measurement methods. However, the current investigation examines only 7 miRs, which were chosen based on pathophysiological considerations. Thus, the current investigation was hypothesis-driven and not data-driven, rendering correction for multiple testing unnecessary in this particular case. Furthermore, with an average follow-up ~1 year, the number of primary endpoints in the current investigation (n=41) was limited, and consequently so was the number of covariables that could be entered into the models. The lack of a replication population is another limitation of the study. Finally, the majority of the patient population was in NYHA functional class I or II, and thus at relatively low risk. Accordingly, the primary outcome was predominantly composed of heart failure hospitalizations. This may have hampered the prognostic information of the other miRs tested in this study. Future studies using high-risk patient populations should therefore re-investigate the prognostic information carried by repeated assessment of these miRs.

Remarkably, in the present study, the rate of HFPEF was very low compared to other epidemiological studies. This can most likely be attributed to the fact that in the Netherlands, most HFPEF patients are treated by the general practitioner or in secondary referral centres, while the current study was performed in two centres which were both tertiary referral centres. Potential inclusion bias is not a likely reason for the low HPEF rate, because consecutive patients were screened in both participating centres. Moreover, the number of patients on vitamin K antagonists was quite high in this study, most likely due to the high number of patients with left ventricular aneurysms and thrombi, as well as the high number of patients with atrial fibrillation.

In conclusion, in patients with stable CHF, the temporal pattern of circulating

miR-22-3p is a strong and independent predictor of prognosis. Thus, the use of individual patterns of change of circulating miR-22-3p for CHF prognostication, as well as for tailored adjustment of treatment, warrants further investigation.

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Supplemental table 1 – Associations between baseline characteristics and miRNAs measured at baseline

	miR1254a		miR22-3p		miR345-5p		miR378		miR423-5p		miR486-5p		miR320	
	Beta coefficient (95% CI)	P	Beta coefficient (95% CI)	P	Beta coefficient (95% CI)	P	Beta coefficient (95% CI)	P	Beta coefficient (95% CI)	P	Beta coefficient (95% CI)	P	Beta coefficient (95% CI)	P
Age*	0.02 (-0.03 – 0.08)	0.44	-0.04 (-0.09 – 0.01)	0.16	0.00 (-0.04 – 0.05)	0.88	-0.02 (-0.07 – 0,03)	0.34	-0.03 (-0.08 – 0.02)	0.31	-0.02 (-0.06 – 0.03)	0.52	0.00 (-0.04 – 0.06)	0.87
Male gender	-0,10 (-0.40 – 0.21)	0.53	0.11 (-0.19 – 0.41)	0.46	0.01 (-0.25 – 0.27)	0.92	0.11 (-0.17 – 0.40)	0.42	0.12 (-0.17 – 0.40)	0.42	0.13 (-0.14 – 0.39)	0.35	0.13 (-0.18 – 0.43)	0.41
BMI	0.00 (-0.03 – 0.03)	0.82	0.02 (-0.01 – 0.05)	0.12	0.01 (-0.02 – 0.03)	0.45	0.01 (-0.02 – 0.04)	0.49	0.00 (-0.03 – 0.03)	0.92	0.01 (-0.02 – 0.03)	0.44	0.00 (-0.02 – 0.03)	0.76
HR*	0.05 (-0.01 – 0.11)	0.10	0.00 (-0.06 – 0.06)	0.93	0.02 (-0.03 – 0.07)	0.45	0.03 (-0.03 – 0.08)	0.37	0.02 (-0.04 – 0.08)	0.48	0.03 (-0.02 – 0.09)	0.22	0.03 (-0.03 – 0.09)	0.30
SBP*	-0.01 (-0.05 – 0.02)	0.44	-0.01 (-0.04 – 0.03)	0.62	-0.01 (-0.04 – 0.01)	0.33	-0.02 (-0.06 – 0.01)	0.14	-0.02 (-0.05 – 0.01)	0.30	-0.02 (-0.05 – 0.01)	0.28	-0.01 (-0.05 – 0.02)	0.42
DBP*	-0.01 (-0.08 – 0.06)	0.79	0.00 (-0.07 – 0.06)	0.91	0.00 (-0.06 – 0.05)	0.96	0.01 (-0.05 – 0.07)	0.71	-0.01 (-0.07 – 0.05)	0.64	0.00 (-0.06 – 0.06)	0.96	-0.01 (-0.08 – 0.05)	0.68
NYHA III/V	-0.10 (-0.41 – 0.21)	0.54	-0.27 (-0.57 – 0.04)	0.086	-0.14 (-0.40 – 0.13)	0.32	-0.24 (-0.53 – 0.04)	0.093	-0.09 (-0.38 – 0.20)	0.54	-0.09 (-0.36 – 0.18)	0.52	-0.02 (-0.33 –0.29)	0.91
ICMP	-0.34 (-0.61 – -0.06)	0.017	-0.05 -0.32 – 0.22)	0.73	-21 (-0.44 – 0.03)	0.085	-0.23 (-0.49 – 0.02)	0.070	-0.21 (-0.46 – 0.05)	0.11	-0.26 (-0.50 – -0.02)	0.035	-0.32 (-0.60 – -0.05)	0.021
HCMP	0.31 (-0.09 – 0.72)	0.13	-0.25 (-0.65 – 0.14)	0.21	-0.03 (-0.38 – 0.32)	0.86	0.01 (-0.36 – 0.39)	0.95	-0.10 (-0.47 – 0.28)	0.61	0.17 (-0.18 – 0.53)	0.33	0.17 (-0.23 – 0.57)	0.41
Renal failure	0.12 (-0.16 – 0.40	0.39	-0.13 (-0.40 – 0.14)	0.34	-0.12 (-0.37 – 0.11)	0.30	-0.10 (-0.36 – 0.16)	0.45	-0.05 (-0.31 – 0.20)	0.69	-0.13 (-0.37 – 0.11)	0.30	0.15 (-0.13 – 0.43)	0.30
DM	0.00 (-0.30 – 0.29)	0.97	-0.01 (-0.31 – 0.28)	0.93	-0.04 (-0.29 – 0.22)	0.76	0.03 (-0.24 – 0.31)	0.82	-0.03 (-0.30 – 0.25)	0.84	0.03 (-0.23 – 0.29)	0.84	-0.11 (-0.41 – 0.19)	0.49

Supplemental table 1 – Associations between baseline characteristics and miRNAs measured at baseline (continued)

	miR1254a		miR22-3p		miR345-5p		miR378		miR423-5p		miR486-5p		miR320	
	Beta coefficient (95% CI)	**P**	**Beta coefficient (95% CI)**	**P**	**Beta coefficient (95% CI)**	**P**	**Beta coefficient (95% CI)**	**P**	**Beta coefficient (95% CI)**	**P**	**Beta coefficient (95% CI)**	**P**	**Beta coefficient (95% CI)**	**P**
HT	0.29 (0.02 – 0.57)	0.038	-0.02 (-0.29 – 0.25)	0.90	0.09 (-0.15 – 0.33)	0.47	0.08 (-0.18 – 0.34)	0.54	0.08 (-0.17 – 0.34)	0.53	0.00 (-0.24 – 0.24)	0.98	0.12 (-0.16 –0.39)	0.42
Ever-smoker	0.13 (-0.14 – 0.44)	0.44	-0.16 (-0.46 – 0.14)	0.29	-0.06 (-0.31 –0.20)	0.67	-0.04 (-0.32 – 0.25)	0.80	-0.07 (-0.35 – 0.22)	0.65	0.00 (-0.27 – 0.27)	0.99	0.08 (-0.22 – 0.39)	0.60

Beta coefficients and corresponding 95 % confidence intervals (CIs) were calculated using linear regression with the 10log transformed microRNAs (MIRs) as dependent variables.

BMI = body mass index; COPD = chronic obstructive pulmonary disease; DBP = diastolic blood pressure; DM = diabetes mellitus; HCMP = hypertensive cardiomyopathy; HR = heart rate; HT = hypertension; ICMP = ischemic cardiomyopathy; NYHA = New York heart association class; SBP = systolic blood pressure;

* Per 5 points increase

Chapter 8

Serially Measured Circulating MicroRNAs and Adverse Clinical Outcome in Patients with Acute Heart Failure

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Eur J Heart Fail. 2017 Sep 25

ABSTRACT

Background: Previous studies have identified candidate circulating microRNAs (circmiRs) as biomarkers for heart failure (HF) by relatively insensitive arrays, validated in small cohorts. We used RNA sequencing to identify novel candidate circmiRs and compared this to previously identified circmiRs in a large, prospective cohort of acute HF (AHF) patients.

Methods: RNA sequencing of plasma from instrumented pigs was used to identify circmiRS produced by myocardium, and found production of known myomirs and microRNA(miR)-1306-5p. We next tested the prognostic value of this and 11 other circmiRs in a prospective cohort of 496 AHF patients, from whom blood samples were collected at several time points (max 7) during the study's 1-year follow-up. The primary endpoint (PE) was the composite of all-cause mortality and HF rehospitalization.

Results: In the prospective AHF cohort, 188 patients reached the PE, and higher values of repeatedly measured miR-1306-5p were positively associated with the risk of the PE at that same time-point (HR(95%CI):4.69(2.18–10.06)), independent of clinical characteristics and NT-proBNP. Baseline miR-1306-5p did not improve model discrimination/reclassification significantly compared to NT-proBNP. For miR-320a, miR-378a-5p, miR-423-5p and miR-1254 associations with the PE were present after adjustment for age and sex (HRs(95%CI):1.38(1.12–1.70), 1.35(1.04–1.74), 1.45(1.10–1.92),1.22(1.00–1.50), respectively). Detection rate of myomiRs miR208a-3p and miR499a-5p was very low.

Conclusions: Repeatedly-measured miR-1306-5p was positively associated with adverse clinical outcome in AHF, even after multivariable adjustment including NT-proBNP. Yet, baseline miR-1306-5p did not add significant discriminatory value to NT-proBNP. Low-abundant, heart-enriched myomiRs are often undetectable which mandates more sensitive assays.

INTRODUCTION

To date, natriuretic peptides are the only circulating biomarkers which are routinely used for diagnosis and prognostication of heart failure (HF).[1] Improved HF prognostication may identify patients that could benefit from closer follow-up and from more aggressive treatment. Therefore, exploration of novel prognostic markers of HF can improve clinical management.

Circulating microRNAs (circmiRs) have been proposed as an attractive new class of biomarkers because of their stability in the circulation, and their ensuing reliable assessment in easily accessible samples.[2] However, most published studies to date involve relatively small numbers of HF patients with most often discrepant findings between separate studies.[3-7] Larger studies are scarce and have not investigated the temporal patterns of microRNAs (miRs) in patients with HF.[8] Importantly, longitudinal circmiR measurements in HF patients may provide further insight into individual, temporal patterns and the patient's ensuing risk of disease progression and adverse outcome.

In the present study, we used an RNA sequencing discovery experiment in pigs to identify circmiRs produced by the myocardium. Subsequently, we tested the potential for prognostication of the most promising novel circmiR (miR-1306-5p) in a set of 475 patients who were prospectively included for serial sampling after an AHF admission and compared it to multiple miRs known to be cardiac-enriched or already previously linked to HF (miR-1254, miR-22-3p, miR-345-5p, miR-378a-3p, miR-423-5p, miR-320a, miR-133a-3p, miR-133b, miR-499a-5p, miR-622, and miR-208a-3p).

METHODS

Part I: Preclinical study design

Aortic Banding and plasma and tissue harvesting

Experiments were performed in Aortic Banding (AoB)-treated (n=29) and sham-operated (n=21) Yorkshire x Landrace swine (see Supplemental Material for details, including surgical procedures and sacrifice of the animals). Briefly, following thoracotomy, the proximal ascending aorta was dissected free and, in AoB animals a band was placed.[9] Up to eight weeks later, swine were instrumented for simultaneous arterial and coronary venous blood sampling, followed by excision of the heart and harvesting of myocardial tissue samples from the left ventricular anterior wall.

RNA Sequencing

RNA was isolated from myocardial tissue and from arterial and coronary venous plasma samples of AoB-treated (n=4) and sham-operated (n=4) swine at 8 weeks follow-up after sham and AoB. For subsequent sequencing, RNA was pooled from myocardial tissue samples and from plasma obtained from arterial and coronary venous samples from AoB-treated and sham-operated samples, respectively. Pooled RNA from each sample was then divided into two, to have 2 technical replicates per sample. This resulted in a total of 16 samples, which were sent to BGI Shenzhen (China) for sequencing of small RNAs. At the BGI, libraries were prepared using the NEBNext® Multiplex Small RNA Library Prep Set for Illumina® kit. Samples were sequenced on an Illumina NextSeq 500 platform and base-calling was performed using the bcl2fastq 2.0 Conversion Software from Illumina.

Quality control of fastq files was performed using FASTQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/). Trimmomatic version 0.32 was used to carry out 3´ adapter clipping of reads, using a phred score cut-off of 30 in order to trim low quality bases whilst ensuring that reads with a length below 18 bases were discarded.[10]

Differential miR expression analysis

We analyzed differential expression in the RNA sequencing data using the R Bioconductor package, DESeq2.[11] MiRs were selected based on next-generation sequencing results. Only miRs that were differentially expressed or had a high abundance in heart tissue were analyzed. We used quantitative polymerase chain reaction (PCR) to analyze expression levels of selected miRs in coronary venous and arterial plasma samples from 21 sham pigs and 29 AoB pigs. Plasma samples were analyzed to obtain a trans-coronary gradient in a comparable fashion; sham arterial plasma vs. coronary venous plasma, and AoB arterial plasma vs. coronary venous plasma. Owing to the availability of replicates, the dispersion method "pooled" from DESeq2 was used to accurately estimate dispersion between each comparison. DESeq2's negative binomial model was used to estimate differentially expressed miRs for each analysis. At the end, only those miRs passing a fold-change (log2) cut-off of 1.0 together with a False Discovery Rate cut-off of 0.05 were deemed significantly differentially expressed.

Part II: Clinical study design

TRIUMPH was an observational, prospective study enrolling patients admitted with acute HF in 14 hospitals in The Netherlands, between September 2009 and December 2013. The study was designed to allow analysis of novel potential biomarkers for prognostication of HF patients, with a particular interest directed towards changes in blood-biomarker patterns over time and their value for prognostication in HF patients.

The study was approved by the medical ethics committee at all participating centers. All patients provided written informed consent.

Patients

Patients were eligible if ≥18 years old and hospitalized for acute HF, resulting from decompensation of known, chronic HF or newly diagnosed HF, and all three of the following criteria were met: (1) natriuretic peptide levels elevated to ≥3 times the upper limit of normal (determined in each individual hospital); (2) evidence of sustained left ventricular dysfunction, defined as moderate to poor systolic function or grade II (pseudonormal) to grade IV (fixed restrictive) diastolic dysfunction on echocardiography during hospitalization; and (3) treatment with intravenous diuretics. Patients were excluded in case they suffered from HF precipitated by a non-cardiac condition, by an acute ST-segment elevation myocardial infarction or by severe valvular dysfunction without sustained left ventricular dysfunction. Furthermore, patients were excluded if they were scheduled for coronary revascularization, listed for heart transplantation, suffered from severe renal failure for which dialyses was needed, or had a coexistent condition with a life expectancy <1 year.

Patient management

Patient management was at the discretion of the treating clinician, in accordance with the guidelines of the European Society of Cardiology.[12] Of note, biomarker data obtained in the context of this study were unknown to the treating physicians and thus were not used for clinical decisions.

Study procedures

Blood samples were obtained from all patients during hospitalization at admission (day 1), once during days 2 to 4 and subsequently at discharge; thus, 3 samples per patient were drawn during hospitalization. Additionally, blood samples were obtained at outpatient clinic follow-up visits, planned 2 to 4 weeks, 3 months, 6 months, and 9 to 12 months after discharge; thus, 4 samples were drawn during follow-up. As such, a total of 7 samples were obtained for each patient, unless a patient was censored or died before all samples could be taken. A short medical evaluation was performed and blood samples were collected at every follow-up visit. Adverse cardiovascular events and changes in medication were recorded in electronic case report forms.

MiR- and NT-proBNP measurements

MiRNAs were measured in all separate plasma samples as described in detail in the Supplemental Material. MiR-1254, miR-22-3p, 423-5p, miR-320a and miR-622 were selected because they were associated with HF in previous studies,[5,7,13] miR-378a-3p and

miR-345-5p because of their enrichment in cardiomyocytes,[14] and miR133a-3p, miR133b, miR208a-3p and miR499a-5p are muscle specific miRs (so-called 'myomiRs'), of which the latter two are heart specific and are released during myocardial injury.[15,16] MiR486-5p was used for normalization of the other miRs, because endogenous miRs have been shown to carry advantages for normalization compared to spike-in (e.g. Cel39) or small RNAs.[17] In the RNA-sequencing experiment we noticed that miR486-5p is exceptionally abundant (representing the vast majority of all detected miRs in the circulation, see Results below) and stable compared to other miRs, making it a suitable candidate to use as a normalizer (details of normalization are described in the Supplementary Material NT-proBNP measurements are also described in the Supplemental Material.

Quality control of human miR measurements

PCR of circulating miRs is sensitive to false or inaccurate signals, which may result in missing values.[18] Missing values may result from technical errors, but are most often due to template levels that are too low to measure reliably with qPCR. Therefore, we used a quality assessment algorithm to ensure the validity of each measurement. This algorithm is described more extensively elsewhere.[19] In brief, we distinguished three groups of measurements: 'detectable', 'non-detectable' (signal too low) and 'invalid'. If the measurement passed all the quality checks, it was considered valid and was marked 'detectable'. In case of a 'non-detectable' signal, the measurement was set to a low value, which was based on the PCR experiment parameters. If the measurement did not pass the quality controls of the algorithm, it was defined as 'invalid'. Such measurements were not used in further analyses.

Endpoints

The primary endpoint comprised the composite of all-cause mortality and readmission for HF. The latter was defined as an unplanned rehospitalization due to acute HF, with at least two of the following three criteria: (1) elevated natriuretic peptide levels ≥3 times the upper limit of normal, (2) symptoms of cardiac decompensation (e.g. rales, edema or elevated central venous pressure), and (3) administration of intravenous diuretics. Secondary endpoints included the individual components of the primary endpoint and additionally cardiovascular mortality.

During follow-up, information on vital status and hospital readmissions was obtained until at least 9 months with a maximum of 400 days after the index hospital admission. We approached the civil registry, screened all medical records, and asked patients for information during their follow-up visits. A clinical event committee blinded to the biomarker results subsequently reviewed all collected information and adjudicated primary and secondary endpoints.

Statistical analysis

The associations between the baseline miR measurements and the risk of a study endpoint were assessed using Cox proportional hazards models. Abundant miRs were examined as continuous variables, while low-abundance miRs were entered into the models as dichotomous variables (detectable versus non-detectable, as defined by the algorithm described above), For repeated miR measurements, associations between the current level of each separate miR at a particular time point and the risk of an endpoint at that same time point were assessed using a joint modeling approach, which combines a linear mixed-effects model for the repeated miR measurements with a Cox proportional hazards model for the risk of experiencing the event of interest.[20] A detailed description of the statistical analysis is provided in the Supplemental Material.

RESULTS

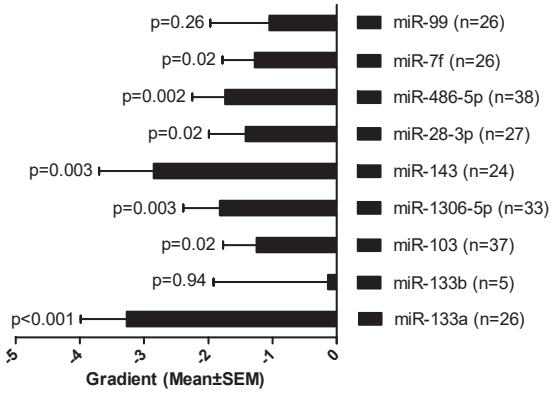
RNA sequencing in pigs samples

Post-quality control, the total number of reads per sample successfully aligned to pig-specific hairpin sequences ranged from 83.7 to 97.3 %. Combining all reads together, followed by discarding sequences longer than 25 nucleotides and those with low abundance (< 4 reads per sample) resulted in 373 x 10^6 reads that were successfully mapped to pig hairpin sequences. Aligning unmapped reads to hairpin sequences of other species increased the alignment rate by a negligible fraction (0.46%), suggesting that known hairpin sequences of Sus Scrofa were close to complete. We therefore, only used those sequences that were mapped to Sus scrofa hairpins.

Whilst calculating the number of reads aligned to each hairpin and mature miR sequence, a high abundance of miR-486-5p was observed in plasma samples (constituting 92.5-97% of all reads). There were a number of circmirs with a positive and significant trans-coronary gradient (figure 1). Among these were also known myomirs like miR-133a. In addition, less known circmirs like miR-1306 also showed a positive gradient. A comparison of next-generation sequencing based miR expression across tissue samples revealed a total of 16 miRs differentially expressed in sham-operated tissue compared to AoB-treated tissue (Table 1) among which miR-1306-5p was also significantly upregulated.

Given the positive trans-coronary gradient of miR-1306-5p and its significant up-regulation in myocardial tissue of AoB compared to Sham pigs, we further evaluated the potential role of miR-1306-5p as a circulating biomarker. We compared the values obtained for miR-1306 in the control samples that are routinely taken along on the qPCR plates with the measurement of the HF samples, which showed that levels of circulating miR-1306-5p were significantly higher in the HF patients OR [95%CI] = 1.43 (1.033 – 1.98)

Figure 1 - Trans-coronary gradients in plasma microRNAs



The number indicates the number of pigs (out of a total of 44 pigs) with both a detectable venous and arterial microRNA value. The gradient is calculated as arterial minus venous Ct value of the microRNA, and shown as Mean±SEM. A negative value indicates release of the microRNA by the myocardium, and a positive value indicates uptake. The p-value is calculated using a paired samples T-test, and indicates the difference between arterial and venous Ct value of the microRNA.

in arbitrary unit)/ln(pg/ml), $p<0.05$), further increasing the probability that circulating miR-1306-5p could serve as a novel biomarker for HF.

Prospective Clinical study: Baseline characteristics

A total of 496 patients were enrolled in the TRIUMPH clinical cohort and provided written informed consent. Three patients withdrew their informed consent. Eighteen patients were withdrawn from statistical analyses due to inclusion violation. These patients had no evidence of sustained systolic or diastolic left ventricular dysfunction on echocardiography. Accordingly, 475 patients compose the analysis set. Median age was 74 years (interquartile range (IQR) 65-80), 63% were men and median left ventricular ejection fraction was 30% (IQR 21-42) (Table 2). Median baseline NT-proBNP level was 4135 pg/mL (IQR 2123–9328).

Clinical endpoints

The composite primary endpoint was reached by 188 patients (40%) during a median follow-up of 325 (IQR 85–401) days. A total of 113 patients died, of which 77 were confirmed to die from a cardiovascular cause, and 123 patients were re-hospitalized for decompensated HF.

Table 1 – Differentially expressed microRNAs across tissue samples

MiR	Fold change*	Adjusted p-value
306-5p	1,354	0.002
132	1,554	0.013
133a-3p	1,107	0.004
142-5p	1,992	<0.001
144	1,457	0.004
144-5p	2,621	<0.001
150	1,767	0.006
15b	1,996	<0.001
15b-5p	1,922	<0.001
342	1,932	<0.001
365-3p	1,507	<0.001
451	3,015	<0.001
532-3p	1,956	0.001
7139-3p	1,889	<0.001
92b-3p	1,04	0.015
99b-3p	-1,225	0.023
133b	0,69	0,07
103	-0,198	0,72
143-3p	-0,251	0,75
143-5p	-0,297	0,755
28-3p	-0,347	0,53
486-5p	0,166	0,77
7f	0,472	0,51
99	-0,53	0,11

Myocardial samples were obtained from the left ventricular free wall and compared between sham-operated and TAC-treated swine. P-values were calculated using the negative binomial model from DESeq. MiR = microRNA.

* Log2 fold change

Circulating miR measurements

A total of 2214 blood samples were available for the current investigation. Median (IQR) number of miR measurements per patient was 3 (IQR 2–5). Supplemental table 1 displays the number of measurements that were detectable per miR. MiRs that were detectable in less than 700 out of 2214 samples were not used as continuous variables in further analyses but were dichotomized (detectable vs. non-detectable) as described above. MiRs that were examined as continuous variables were: miR-320a, miR-1254, miR-22-3p, miR-378a-3p, miR-423-5p, miR-345-5p and miR-1306-5p. MiRs that were dichotomized were: miR-133a-3p, miR-133b, and miR-499a-5p. MiR-486-5p was used for normalization of these miR levels. MiR-622 and miR-208a-3p were only detectable in 56 and 6 out of

Table 2 – Baseline characteristics

Variables	Overall sample (n=475)
Demographic characteristics, median [IQR] or number (%)	
Age, years	73 [64 - 80]
Female, %	36.6 (167)
Caucasian, %	94.3 (430)
Measurements at baseline, median [IQR] or number (%)	
Body mass index, kg/m2	27.5 [24.7 - 31.1]
Systolic blood pressure, mmHg	125 [110 - 147]
Diastolic blood pressure, mmHg	75 [65 - 85]
Heart rate, bpm	85 [72 - 100]
eGFR	46 [34.4 - 61.7]
Left ventricular ejection fraction, %	30 [21 - 42]
Heart failure with reduced ejection fraction, %	79.8 (289)
NT-proBNP (pg/ml)	4143.7 [2097.5 - 9053.2]
Medical history, number (%)	
Previous heart failure admission within 6 months	19.8 (90)
Ischemic heart failure	48.1 (219)
Myocardial infarction	40.4 (184)
Hypertension	50 (228)
Atrial fibrillation	42.5 (194)
Diabetes Mellitus	36.5 (166)
Stroke	17.5 (80)

IQR = Inter-quartile range, eGFR = estimated glomerular filtration rate.

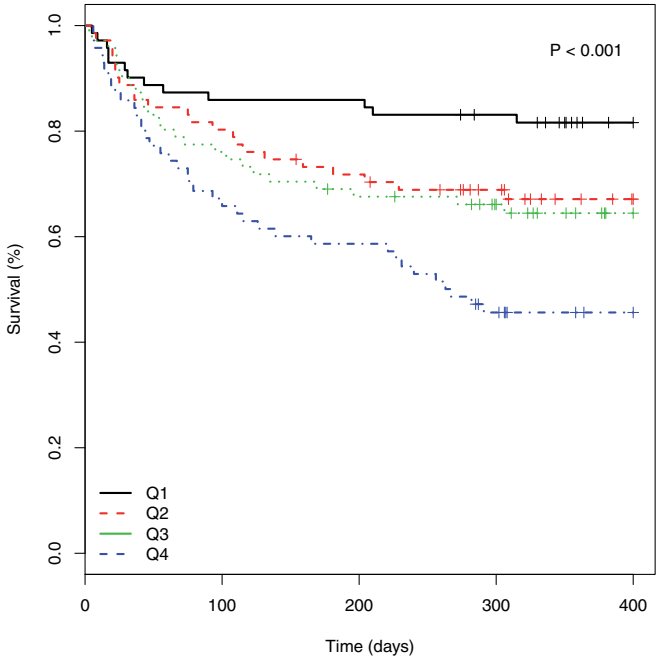
2214 samples, respectively. This low expression did not allow for meaningful statistical analysis of these miRs. Additionally, supplemental table 2 shows the baseline characteristics stratified by invalid versus valid measurement of baseline miR-1306-5p.

Finally, miR expression levels in patients with HF with reduced ejection fraction (HFrEF) vs. HF with preserved ejection fraction (HFpEF) are presented in supplemental table 3.

Associations between baseline miR levels and clinical endpoints

Figure 2 shows the difference in the risk of experiencing the primary endpoint for patients in different quartiles of baseline miR1306-5p levels ($p< 0.001$). This was confirmed in the subsequently fitted Cox models, where baseline miR1306-5p levels were significantly and independently associated with the primary endpoint (hazard ratios (HRs) (95%CI)): 1.13(1.03-1.23) (Table 3). From the other known miRs, only the baseline levels of miR-320a were significantly and independently associated with the primary endpoint (HRs(95%CI): 1.10(1.00-1.21)). Associations with secondary endpoints are shown in Supplemental Table 4. A sensitivity analysis on the subgroup of HFrEF patients, rendered

Figure 2 - Kaplan-Meier survival curves for the primary endpoint of death or readmission for HF in the four quartiles of baseline miR-1306-5p levels



Q1 lowest quartile, Q4 highest quartile.

Table 3 - Associations between baseline microRNA levels and primary endpoint

miR	Hazard ratio (95% CI)		
	Model 1	**Model 2**	**Model 3**
320a*	1.19 (1.09 – 1.30)	1.18 (1.08-1.29)	1.10 (1.00 – 1.21)
1254*	1.06 (0.98 – 1.14)	1.05 (0.97 – 1.14)	1.00 (0.92 – 1.08)
22-3p*	1.04 (0.96 – 1.12)	1.05 (0.97 – 1.14)	1.02 (0.94 – 1.10)
378a-3p*	1.08 (0.98 – 1.18)	1.07 (0.97 – 1.18)	1.03 (0.93 – 1.14)
423-5p*	1.08 (0.98 – 1.18)	1.09 (0.98 – 1.20)	1.05 (0.95 – 1.16)
345-5p*	1.03 (0.97 – 1.11)	1.03 (0.96 – 1.10)	0.99 (0.93 – 1.07)
1306-5p*	1.19 (1.09 – 1.30)	1.18 (1.09 – 1.29)	1.13 (1.03 – 1.23)
133a-3p†	0.84 (0.56 – 1.24)	0.89 (0.60 – 1.34)	1.00 (0.66 – 1.53)
499a-5p†	1.49 (0.79 2.84)	1.53 (0.81 – 2.92)	1.25 (0.64 – 2.42)
133b†	0.97 (0.40 – 2.36)	0.97 (0.40 – 2.36)	1.07 (0.43 – 2.67)
NT-proBNP	1.47 (1.27 – 1.71)	1.46 (1.25 – 1.69)	1.36 (1.15 – 1.60)

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, baseline eGFR, and baseline NT-proBNP level. BMI = Body mass index, HF = Heart failure, miR = MicroRNA. Primary endpoint: composite of all-cause mortality and readmission for heart failure

* Hazard ratio per per ln[arbitrary unit] of miR level

† Hazard ratio of detectable vs. non-detectable miR level

a HR for baseline miR1306-5p in relation to the primary endpoint that was similar to the HR in the total group, but with a wider CI ((HR(95%CI): 1.09(0.95–1.25) (supplemental table 5). This was most likely caused by a decrease in statistical power in this subgroup.

Associations between temporal miR patterns and clinical endpoints

Repeatedly measured miR1306-5p level was positively and independently associated with the primary endpoint (HR(95%CI): (4.69(2.18–10.06)), p< 0.001 (Table 4). The temporal patterns of miR-320a, miR-378a-3p and miR-423-5p were positively associated with the primary endpoint after adjustment for age and sex. However, these associations disappeared after multivariable adjustment. The temporal pattern of miR-1254 displayed a borderline significant association with the primary endpoint after adjustment for age and sex (HR(95%CI): 1.22(1.00-1.50). Associations of temporal patterns with secondary endpoints are shown in Supplemental Table 6.

Table 4 - Associations between repeated microRNA measurements and primary endpoint

	Hazard ratio (95% CI)		
miR	**Model 1**	**Model 2**	**Model 3**
320a	1.40 (1.14 – 1.72)	1.38 (1.12 – 1.70)	1.13 (0.91 – 1.40)
1254	1.26 (1.03 – 1.55)	1.22 (1.00 – 1.50)	1.00 (0.82 – 1.22)
22-3p	1.25 (0.97 – 1.63)	1.27 (0.99 – 1.64)	1.18 (0.91 – 1.52)
378a-3p	1.39 (1.07 – 1.80)	1.35 (1.04 – 1.74)	1.01 (0.76 – 1.34)
423-5p	1.45 (1.10 – 1.90)	1.45 (1.10 – 1.92)	1.08 (0.81 – 1.44)
345-5p	1.11 (0.93 – 1.34)	1.10 (0.92 – 1.32)	1.00 (0.89 – 1.12)
1306-5p	5.16 (2.58 – 10.31)	3.05 (1.58 – 5.88)	4.69 (2.18 – 10.06)

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, baseline eGFR, and baseline NT-proBNP level. BMI = Body mass index, HF = Heart failure, miR = MicroRNA. Primary endpoint: composite of all-cause mortality and readmission for heart failure
Hazard ratios per ln[arbitrary unit] of miR level

Incremental prognostic value of miR-1306-5p

Adding miR-1306-5p to a model containing NT-proBNP age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, and baseline eGFR, we found a change in C-statistic of 0.012 (95%CI: -0.006–0.029), a continuous net reclassification (cNRI) improvement of 0.125(-0.016–0.267), and an integrated discrimination index (IDI) improvement of 0.020(-0.013–0.053), as shown in supplemental table 7. Thus, the incremental prognostic value of miR1306-5p on top of NT-proBNP did not reach statistical significance.

DISCUSSION

Direct RNA sequencing of plasma from instrumented pigs revealed a number of circmiRs to be produced by the pig myocardium, including miR-1306-5p which had not yet been identified as a miR related to the heart. Subsequently, we found in a prospective AHF cohort that repeatedly-assessed circulating miR-1306-5p is positively and independently associated with all-cause mortality and HF hospitalization. This association was independent of NT-proBNP. However, a model containing baseline miR-1306-5p measurements did not significantly improve model discrimination or reclassification when compared to NT-proBNP. Repeatedly-assessed circulating miR-320a, miR-378a-3p, miR-423-5p and miR-1254 were associated with the primary endpoint after adjustment for age and sex (albeit borderline for miR-1254), but not after further multivariable adjustment for clinical characteristics. Furthermore, an independent association was found between baseline values of miR-1306-5p and miR-320a and the primary endpoint.

Importantly, our findings are in line with those described in a manuscript where two large cohorts have been studied (Bayes-Genis et al, submitted back-to-back). In those two independent cohorts, miR-1306-5p was also positively and significantly associated with the risk of all-cause mortality or HF hospitalization. This further strengthens our findings and for the first time we see reproducible results on circulating miRs across three large cohorts. This contrasts with previous studies where usually one, mostly smaller cohort was analyzed,[21] and results have most often been discrepant between separate studies. To the best of our knowledge, the association between miR-1306-5p and cardiovascular disease has not been previously investigated in other studies, and further research is warranted on its expected targets.

RNA sequencing using plasma-derived RNA led to the discovery of miR-1306-5p produced by the heart. Akat et al also used RNA sequencing to analyze miRs potentially produced by the human heart.[22] However, their study was not designed to assess the clinical value of circmiRs as biomarkers. A word of caution concerns the large proportion of invalid and undetectable miR-1306-5p measurements which reduces power and illustrates the need for more sensitive methods of miR assessment to enable optimal use of this marker for clinical prognostication. Nevertheless, the current study carried sufficient statistical power to demonstrate a significant association between repeatedly measured miR-1306-5p and the primary and secondary endpoints in spite of the proportion of invalid and undetectable measurements.

In line with our results, the study by Bayes-Genis et al. also found an association between miR-1254 and clinical outcome. Other existing data on miR-1254 are limited; of note is that Tijsen et al demonstrated upregulation of miR-1254 in HF cases compared to healthy controls.[5] An association between higher baseline miR423-5p levels and signs of progressive HF has been demonstrated in animal models,[6] and human studies with

limited sample size.[3,5] Rising miR423-5p has also been related to worsening left ventricular function and has been shown to be upregulated in non-ST elevation myocardial infarction patients.[23] Our results agree with the findings of the aforementioned studies. Conversely, in recent a study in 236 acute HF patients, an inverse association was observed between miR423-5p and hospital readmission.[8] However, this finding could not be reproduced in the validation cohort which was examined.[8] Smaller studies have previously demonstrated higher circulating levels of miR-320a in HF patients compared to healthy individuals.[7,24] In addition, rat models have proven that overexpression of miR-320a leads to a greater loss of cardiomyocytes during infarction and that inhibition of miR-320a leads to reduced infarction size.[25] Furthermore, miR-320a showed a protective effect on left ventricular remodeling after myocardial ischemia-reperfusion injury in a rat model.[26] The results of the current study are in line with these previous studies, and further expand the evidence concerning miR-320a by showing that baseline measurements are independently associated with adverse prognosis in patients with HF, and that repetitively-measured miR-320a is independently associated with heart failure hospitalization in particular. The temporal pattern of miR-378a-3p was also associated with the primary endpoint. Naga Prasad et al showed downregulation of miR-378a-3p in left ventricular free wall tissue of HF patients with dilated cardiomyopathy.[4] In contrast, in the current study we examined circulating levels of miR-378a-3p. In addition, Weber et al found higher levels of circulating miR-378a-3p in 5 patients with coronary artery disease, compared to 5 healthy controls.[27] However, studies other than ours on the prognostic value of miR-378a-3p in patients with HF are lacking.

Repeatedly measured, highly-abundant miRs only showed age-and sex-adjusted significant associations with the primary endpoint, and associations disappeared after multivariable adjustment. Possibly, prognostic information of these circmiRs, which are probably not produced by the heart, can be easily diluted. Conversely, myomiRs, i.e. miRs which are skeletal- and cardiac-muscle specific, carry potential to provide prognostic information that is incremental to clinical characteristics. Such myomiRs play a central role in myogenesis regulation and muscle remodeling.[28,29] Although the main sources of circulating myomiRs, and in particular the relationship between myomiRs in tissue and plasma have yet to be fully elucidated, an association between cardiac damage (caused by myocardial infarction or myocarditis) and upregulation of circulating myomiRs has been previously demonstrated.[15] Moreover, circulating myomiR levels have been associated with skeletal muscle wasting.[30] We examined several myomiRs in the current investigation (miR133a-3p, miR133b, miR208a-3p and miR499a-5p). However, myomiRs are lowly expressed in the circulation, as illustrated by the fact that they were non-detectable in a large proportion of the samples available in our study. Thus, we were forced to perform a simplified analysis and examined the association between presence of detectable myomiR levels at baseline and occurrence adverse events. The loss of

information inherent to such an analysis may have obscured potential associations with the outcome. Therefore, more sensitive assays are needed to properly examine the roles of myomiRs in HF.

To remove noise by less robust QPCR results we designed and implemented a strict and conservative algorithm to remove unreliable QPCR data, and at the same time retain reliable assessment of 'too low to detect' signals. Furthermore, we used miR486-5p to normalize our data, as using such endogenous miRs for this purpose has been shown to carry advantages.[17] We have separately described our quality control algorithm we used here (provided for review purposes) and given the strong concordance between three large cohorts we have thus measured strengthens the point of view that such algorithms help to remove noise and improve reproducibility.

Some aspects of this study warrant consideration. First, aortic banding has been used to model heart failure. This is a model that shows strong similarity to the TAC model in mice and has previously been used in multiple studies as a model for pressure-overload hypertrophy.[31-34] This model may not be fully representative of human left ventricular dysfunction. However, our observation that miR 1306-5p, identified in our swine model, does provide prognostic potential in the clinic, underscores the validity of our approach. Second, we did not adjust our analyses for multiple comparisons, because the miRs we examined were not selected in a hypothesis-free manner but had resulted from previous fundamental and clinical studies. Nevertheless, if we applied Bonferroni correction, the results would remain statistically significant. The association between repeated miR1306-5p and the primary endpoint rendered a HR(95%CI) of 4.69(2.18–10.06) and a p-value < 0.0001; since we examined 7 repeatedly measured miRs, the Bonferroni threshold for the p-value would be 0.05/7=0.007. Furthermore, we focused on patients with known heart failure. Studies using a healthy control group may provide insights into temporal miR patterns in healthy persons.

In conclusion, in patients hospitalized for AHF, baseline and repeatedly-assessed miR-1306-5p was independently associated with adverse clinical outcome. Associations of temporal patterns of miR-320a, miR-378a-5p, miR-423-5p and miR-1254 with adverse clinical outcome were not independent of clinical characteristics. Myocyte-specific miRs were non-detectable in a large proportion of the samples. More sensitive myomiR assays are needed in order to precisely estimate the risk associated with elevated levels of miRs such as miR1306-5p, and to investigate whether cardiac specific myomiRs on their part are capable of providing additional information to established, clinical risk predictors.

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SUPPLEMENTAL METHODS

Preclinical study in pigs

Aortic banding and plasma and tissue harvesting

Experiments were performed in AoB-treated (n=29) and sham-operated (n=21) Yorkshire x Landrace swine of either sex weighing 25-30 kg. All procedures were performed in compliance with the *"Guiding principles in the care and use of animals"* as approved by the Council of the American Physiological Society and under the regulations of the Animal Care Committee of the Erasmus University Medical Center. Swine were sedated with ketamine (20 mg/kg, i.m.) and midazolam (1 mg/kg, i.m.), Under isoflurane anesthesia, a left thoracotomy was performed and the proximal ascending aorta was dissected free, and, in AoB-animals a band was placed, resulting in a systolic pressure gradient of 68±3 mmHg. Subsequently, the chest was closed and the animals were allowed to recover. Up to eight weeks later, swine we re-anesthetized with sodium pentobarbital (15 mg/kg, i.v.), intubated, and placed on a positive-pressure ventilator (O_2:N_2=1:3 v/v). Catheters were inserted into the right external jugular vein for infusion of physiological saline and sodium pentobarbital (10-15 mg/kg/h) to maintain anesthesia. Following sternotomy, fluid-filled catheters were surgically inserted into the aorta for measurement of aortic blood pressure and sampling of arterial blood. The anterior interventricular vein was cannulated with a 20-gauge catheter for coronary venous blood sampling. Subsequently, arterial and coronary venous blood samples were simultaneously obtained, followed by arresting and excision of the heart and harvesting of myocardial tissue samples from the left ventricular anterior wall.

Swine were sacrificed at three time-points, being 1, 3 and 8 weeks after AoB. The pressure gradient across the aortic banding did not result in overt heart failure at any of these time-points in the present study. Retrospective analysis did not show differences in miR expression between the different time point (although our study may not have been sufficiently powered to detect such differences).

Alignment of RNA-seq reads to the genome

All known hairpin sequences belonging to *Sus Scrofa* (pig), *Homo Sapiens* (human), *Bos Taurus* (cow) and *Equus Caballus* (horse) were downloaded from release 20 of miRBase. A blast database containing these species-specific hairpin sequences was generated. First, reads from all RNA-seq fastq files were aligned to pig hairpin sequences using BLASTN. Reads failing to map to pig hairpins were then aligned to human, cow and horse hairpin sequences using BLASTN. To further increase alignment efficiency, reads that remained unaligned were mapped to version 10.2 of the Sus Scrofa ncRNA and cDNA database,

downloaded from Ensembl. In cases where a sequence was mapped to multiple hairpins, the one with the higest bitscore, i.e. the best alignment, was chosen.

QPCR measurement of selected miRs for assessment of trans-coronary gradient

RNA was extracted from 200µl plasma using 750µl TRIzol LS reagent (Invitrogen Corp., Carlsbad, CA) and was incubated for 10 minutes at room temperature followed by 200µl chloroform. The mixture was centrifuged at 12,000 g for 10 minutes, and the aqueous layer was transferred to a new tube. RNA was precipitated by isopropanol and washed with 75% ETOH subsequently. RNA pellet was dissolved in 50 µl RNAse free water.

The primers used for qPCR were: 133b: TTTGGTCCCCTTCAACCAGCTAT; miR-28-3p: CACTAGATTGTGAGCTCCTGGA; miR-99: AACCCGTAGATCCGATCTTGTG; miR-486-5p: TCCTGTACTGAGCTGCCC CGAG; miR-133a: TTGGTCCCCTTCAACCAGCTG; miR-103: AGCAGCATTGTACAGGGCTATGA; miR-1306-5p: CCACCTCCCCTGCAAACGTCC A; miR-7f: TGAGGTAGTAGATTGTATAGTT; miR-143: TGAGATGAAGCACTGTAGCTC.

Number of animals sacrificed: considerations

A total of 8 swine were used for next generation sequencing (NGS), and a total of 50 swine for qPCR. The number of animals used may thus seem quite large for identifying a single novel miR. However, with NGS, we detected a much larger number of miRs that were either differentially regulated or highly expressed in the myocardium, of which a smaller number was tested with qPCR, to identify a transcoronary gradient. Only the most promising one was subsequently tested as a clinical biomarker. Furthermore, it should be noted that the swine material used in the present study was not specifically collected for this study alone, but is part of a biobank that was developed as part of a larger study aimed at correlating changes in tissue morphology, proteomics, metabolomics and genomics to well-characterized hemodynamics in an animal model of pressure-overload hypertrophy. This means that a large number of samples was available, and only part of these samples were used for validation of our NGS results.

Clinical study

Blood collection

Non-fasting blood samples were obtained by venipuncture and transported to the clinical chemistry laboratory of each participating hospital for further processing according to a standardized protocol. Blood aliquots were subsequently stored at a temperature of -80ºC within 2 hours after venipuncture. Subsequently, stored EDTA plasma samples were transported under controlled conditions to ACS Biomarker BV, Amsterdam, The Netherlands, where a selection of miRs was measured batch-wise.

MiR measurements

Reverse transcriptase of miRs

cDNA was obtained from high abundant miRNAs (miR-1254, -378a-3p, -423-5p, -320a, -345-5p, -22-3p, -486-5p) using the miScript reverse transcription kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instruction. More specifically, the RT reaction consisting of 7.5µl RNA from the isolation, 0.5µl miscript RT and 2µl of 5x RT Buffer was incubated at 37 °C for 60 minutes and at 95°C for 5 minutes and the held at 4°C for 5 minutes.

For less abundant miRNAs (miR-133a-3p, -133b, -208a-3p, -499a-5p, -622, -1306-5p), qScript™ microRNA cDNA Synthesis Kit (Quanta BioSciences, Gaithersburg, USA) was used, according to the manufacturer's protocol. Specifically, first, a poly(A) tailing reaction was performed using 3 µl of RNA, 2µl of poly(A) tailing Buffer (5x) 4µl of nuclease-free water and 1µl Poly(A) polymerase. This was incubated for 60 minutes at 37 °C followed by 5 minutes on 70 °C. Subsequently, 10 µl of this poly(A) tailing reaction, 9 µl of miRNA cDNA reaction mix and 1 µl of qscript RT were incubated for 20 minutes at 42 °C followed by 5 minutes at 85 °C. Both a non-template control and a no-RT control were included in the sample tot ensure that products were not the results of genomic DNA of RNA.

Quantification of miRNA expression by RT-qPCR

Expression levels of miRNAs of each miRNA were quantified by RT-qPCR using Sybr Green (Roche, Basel, Switzerland) and miRNA primers (Eurofins, Ebersberg, Germany) in a total mix of 10µl according to the manufacturer's instruction. This mix contained 5 µl of SybrGreen dye, 0.5 µl of forward primer, 0.5 µl of reverser primer, 2 µl of RNase-free water and 2 µl of template DNA. RT-qPCR reactions were run in duplicates on the Light cycler 480. The reaction mixture was pre-incubated at 5 °C for 10 seconds, followed by 45 cycles of 95 °C for 10 seconds, 58 °C or 55 °C for 20 seconds (dependent on the primer character) and 72 °C for 30 seconds . Melting curve analysis was done by hand and melting curves were marked as bad when the melting curve deviated from the tissue control or showed multiple peaks that could not be distinguished from the amplicon. Data were analyzed using LinRegPCR quantitative qPCR data analysis software version 2014.61. The primers used were: miR-133b: TTGGTCCCCTTCAACCAGCTA; miR-1254: CTGGAAGCTGGAGCCTGC; miR-378a-3p: ACTGGACTTGGAGTCAGAAGG; miR-423-5p: TGAGGGGCAGAGAGCGAGACTTT; miR-320a: AAAAGCTGGGTTGAGAGGGCGA; miR-345-5p: GCTGACTCCTAGTCC; miR22-3p: AAGCTGCCAGTTGAAGAACTGT; miR-1306-5p: CCACCTCCCCTGCAA ACGTCCA; miR-133a-3p: TTGGTCCCCTTCAACCAGCTG; miR-622: ACAGTCTGCTGAGGTTG; miR-499a-5p: GACTTGCAGTGATGTT; miR-208a-3p: ATAAGACGAGCAAAAAGCTTGT; miR-486-5p: TCCTGTACTGAGCTG.

Normalization using miR-486-5p

In a previous study, we showed that for normalization endogenous miRNAs are preferred over normalization with a spike-in (e.g. Cel39 spike-in) or small RNAs (e.g. RNU6B).[1] To date, however no plasma normalization panel with endogenous miRNAs has been described in the literature. To function as a good normalizer, an endogenous miRNA must be stably expressed and abundant in plasma.[2] In the current study, RNA sequencing of plasma samples revealed that miR-486-5p was the most abundant miRNA (>90% of all measured miRNAs) in plasma. Next, we used the geNorm algorithm[3] to calculate the M-value and coefficient of variation and used these characteristics to assess which miRNAs were most stable and suitable for normalization in 2 clinical cohorts (Bayes-Genis et al, manuscript submitted back-to-back). Among the measured miRNAs, miR-486-5p, displayed highest stability (see supplemental table 8). We compared its stability to miR-320a as in these large cohorts miR-320 appeared also as very stable. It should be noted that miR-320a has been identified as a putative biomarker for HF so that we did not choose this microRNA for normalization, but merely to assess the effect of using a different normalizer. Therefore, miR-486-5p (mean M-value 0.68) was used as the primary normalizer.

NT-proBNP measurements

For batch analysis of NT-proBNP, heparin plasma samples were transported under controlled conditions to the Canisius Wilhelmina Hospital, Nijmegen, The Netherlands, where measurements were conducted using the Elecsys NT-proBNP assay on a Cobas 8000 analyzer (Roche Diagnostics Limited, Rotkreuz, Switzerland)

Statistical analysis

Normally distributed continuous variables are presented as mean ± standard deviation (SD). Non-normally distributed continuous variables are expressed as median and interquartile range (IQR). Categorical data are displayed as count and percentage.

The associations between the baseline miR measurements and the risk of a study endpoint were assessed using Cox proportional hazards models. First, analyses were performed unadjusted. Subsequently, we corrected for age and gender. Finally, additional multivariable adjustment was performed. Potential confounders were selected based on previous literature and included systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, baseline eGFR, and baseline NT-proBNP level. Individual covariates each contained less than 7% missing values. Data on all covariates were complete in 87% of the patients. Multiple imputation was applied to account for missing covariates. For abundant miRs (miR-1254, miR-22-3p, miR-345-5p, miR-378a-3p, miR-423-5p, miR-320a, miR-1306-5p), the results are presented as hazard ratios (HR) per ln[arbitrary unit] of miR level with

95% confidence intervals (CI). For low-abundance miRs, a different approach had to be chosen because their low expression levels did not allow for these miRs to be entered into the models as continuous variables. Thus, they were entered into the models as dichotomous variables (detectable versus non-detectable, as defined by the algorithm described above), and HRs were presented accordingly. First, analyses were performed in the full cohort. Subsequently, a sensitivity analysis was performed in the patients with HF with reduced ejection fraction.

Subsequently, repeated miR measurements were examined in relation to the risk of a study endpoint. The primary endpoint consisted of all-cause mortality and readmission for HF, whichever occurred first. Thus, all measurements drawn up to the moment of the first readmission for HF, or mortality, were used for these analyses. Specifically, associations between the current level of each separate miR at a particular time point and the risk of an endpoint at that same time point were assessed using a joint modeling approach, which combines a linear mixed-effects model for the repeated miR measurements with a Cox proportional hazards model for the risk of experiencing the event of interest. For the mixed model, we used cubic splines, with knots set at 1 week and 1 month after initial hospitalization. Analyses were first performed without adjustment, and were subsequently adjusted for the potential confounding variables listed above. The results are presented as hazard ratios (HRs) per ln[arbitrary unit] miR concentration at any point in time, along with the corresponding 95% CIs. These analyses were not performed for low-abundance miRs, because their low expression levels did not allow so.

To assess incremental predictive value of baseline miR-1306-5p and baseline NT-proBNP levels for the primary endpoint, C-statistics, continuous Net Reclassification Indices (NRIs) and Integrated Discrimination Indices (IDIs) were calculated for subsequent addition of these biomarkers to a model containing age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, and baseline eGFR.

All analyses were performed with R Statistical Software using package JM.[4,5] All tests were two-tailed and p-values <0.05 were considered statistically significant.

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Supplemental table 1 – Number of detectable, non-detectable and missing miR measurements

N = 2214	Detectable	Non-detectable	Invalid
miR-486-5p	1677	99	438
miR-320a	1599	20	595
miR-1254	1574	31	609
miR-22-3p	1444	319	451
miR-378a-3p	1409	363	442
miR-423-5p	1408	205	601
miR-345-5p	884	1066	264
miR-1306-5p	727	650	837
miR-133a-3p	494	1488	232
miR-499a-5p	103	1946	165
miR-133b	85	1959	170
miR-622	56	1848	310
miR-208a-3p	6	2100	108

MiR = MicroRNA.

Supplemental table 2 – Baseline characteristics stratified by invalid versus valid for baseline miR-1306-5p

Variable	Invalid (n=166)	Valid (n=290)	p-value
Demographic characteristics [IQR] or (%)			
Age	73 [63 - 78]	74 [65 - 80]	0.12
Female	38.6 (64)	35.5 (103)	0.52
Caucasian	92.2 (153)	95.5 (277)	0.14
Measurements at baseline [IQR] or (%)			
Body mass index	27.6 [24.6 - 31.8]	27.5 [24.8 - 30.9]	0.68
Systolic blood pressure	130 [110 - 150]	124 [110 - 145]	0.22
Diastolic blood pressure	75 [66 - 90]	73 [64 - 85]	0.09
Heart rate	85 [72 - 101]	85 [71 - 100]	0.59
eGFR	45 [31.9 - 61.1]	46.7 [35.7 - 61.9]	0.29
Left ventricular ejection fraction	31 [23 - 45]	30 [21 - 40]	0.54
Heart failure with reduced ejection fraction	79.2 (103)	80.2 (186)	0.83
NT-proBNP	4046.5 [1943.2 - 9835]	4165 [2173 - 8556.8]	0.78
Medical history (%)			
Previous heart failure admission within 6 months	26.5 (44)	15.9 (46)	0.006
Ischemic heart disease	48.2 (80)	48.1 (139)	0.98
Myocardial infarction	40.4 (67)	40.3 (117)	1
Hypertension	51.2 (85)	49.3 (143)	0.7
Atrial fibrillation	41 (68)	43.4 (126)	0.61
Diabetes Mellitus	39.2 (65)	34.9 (101)	0.37
Stroke	19.3 (32)	16.6 (48)	0.46

IQR = Inter-quartile range, eGFR = estimated glomerular filtration rate.

Supplemental table 3 – miR expression levels in patients with HFrEF versus patients with HFpEF

miR	Expression values of HFpEF patients (n=352) median [IQR]	Expression values of HFrEF patients (n=1457) median [IQR]	p-value
1254	2.6 [1.4 – 4.9]	2.3 [1.3 - 4.6]	0.3
486.5p	37 [98 - 920]	20 [13 - 150]	<0.001
423.5p	1.5 [0.48 - 5.5]	2.0 [0.55 – 7.0]	0.044
378a-3p	0.18 [0.046 – 0.49]	0.21 [0.065- 0.67]	0.031
345.5P	0.12 [0.12 – 1.4]	0.12 [0.12 – 3.1]	<0.001
320a	19 [6.6 - 49]	27 [8.7 – 72]	0.0019
22.3p	2.9 [0.19 – 21]	5.7 [0.42 - 36]	0.017
208a	0.02 [0.02 – 0.02]	0.02 [0.02 – 0.02]	0.27
499a.5p	0.009 [0.009 – 0.009]	0.009 [0.009 – 0.009]	0.95
1306-5p	0.04 [0.012 – 0.19]	0.04 [0.012 – 0.19]	0.7
622	0.012 [0.012 -0.012]	0.012 [0.012 -0.012]	0.43
133b	0.014 [0.014 – 0.014]	0.014 [0.014 – 0.014]	0.88
133a-3p	1.0 [1.0 – 1.0]	1.0 [1.0 – 1.0]	0.67

All expression values were multiplied by 10^8.
HFrEF = Heart failure with preserved ejection fraction, HFrEF = Heart failure with reduced ejection fraction, IQR = Inter-quartile range, miR = microRNA

Supplemental table 4 - Associations between baseline microRNA levels and secondary endpoints

	miR	Hazard ratio (95% CI)		
		Model 1	Model 2	Model 3
All-cause mortality	320a*	1.10 (0.98 - 1.23)	1.08 (0.96 – 1.21)	0.98 (-0.87 – 1.10)
	1254*	0.99 (0.90 – 1.10)	0.98 (0.88 – 1.08)	0.93 (0.84 – 1.04)
	22-3p*	1.00 (0.90 – 1.11)	1.01 (0.91 – 1.12)	0.99 (0.89 – 1.11)
	378a-3p*	0.98 (0.86 – 1.11)	0.97 (0.85 – 1.10)	0.92 (0.80 – 1.06
	423-5p*	1.00 (0.88 – 1.13)	1.01 (0.89 – 1.15)	0.98 (0.87 – 1.10)
	345-5p*	0.97 (0.89 – 1.06)	0.97 (0.88 – 1.06)	0.92 (0.83 – 1.02)
	1306-5p*	1.10 (0.98 – 1.25)	1.09 (0.97 – 1.23)	1.03 (0.90 – 1.17)
	133a-3p†	0.82 (0.49 – 1.39)	0.92 (0.54 – 1.57)	1.11 (0.64 – 1.92)
	499a-5p†	2.25 (1.09 – 4.66)	0.92 (0.54 – 1.57)	2.04 (0.96 – 4.43)
	133b†	1.40 (0.52 – 3.82)	1.40 (0.51 – 3.82)	1.55 (0.54 – 4.43)

Supplemental table 4 - Associations between baseline microRNA levels and secondary endpoints (continued)

	miR	Hazard ratio (95% CI)		
		Model 1	Model 2	Model 3
Heart failure hospitalizations	320a*	1.26 (1.13 – 1.40)	1.27 (1.14 – 1.41)	1.19 (1.06 – 1.33)
	1254*	1.12 (1.03 – 1.23)	1.12 (1.03 – 1.29)	1.06 (0.97 – 1.17)
	22-3p*	1.05 (0.96 – 1.15)	1.06 (0.97 – 1.16)	1.02 (0.93 – 1.12)
	378a-3p*	1.15 (1.03 – 1.29)	1.15 (1.03 – 1.29)	1.13 (1.00 – 1.28)
	423-5p*	1.20 (1.06 – 1.35)	1.20 (1.06 – 1.36)	1.16 (1.02 – 1.31)
	345-5p*	1.06 (0.98 – 1.15)	1.06 (0.98 – 1.15)	1.03 (0.94 – 1.12)
	1306-5p*	1.27 (1.15 – 1.40)	1.27 (1.15 – 1.40)	1.22 (1.10 – 1.36)
	133a-3p†	0.73 (0.44 – 1.21)	0.73 (0.44 – 1.22)	0.75 (0.44 – 1.29)
	499a-5p†	1.09 (0.45 – 2.68)	1.09 (0.44 – 2.69)	0.89 (0.35 – 2.25)
	133b†	0.56 (0.14 – 2.29)	0.56 (0.14 – 2.28)	0.71 (0.17 – 2.99)

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, baseline eGFR, and baseline NT-proBNP level. BMI = Body mass index, HF = Heart failure, miR = MicroRNA. Primary endpoint: composite of all-cause mortality and readmission for heart failure

* Hazard ratio per per ln[arbitrary unit] of miR level

† Hazard ratio of detectable vs. non-detectable miR level

Supplemental table 5 - Associations between baseline microRNA levels and primary endpoint in the subgroup of HFrEF fraction patients

miR	**Hazard ratio (95% CI)**		
	Model 1	**Model 2**	**Model 3**
320a*	1.14 (1.01 – 1.29)	1.13 (1.00 – 1.29)	1.08 (0.95 – 1.24)
1254*	1.01 (0.91 – 1.11)	0.99 (0.89 – 1.10)	0.96 (0.86 – 1.07)
22-3p*	1.00 (0.91 – 1.11)	1.01 (0.92 – 1.12)	0.95 (0.85 – 1.06)
378a-3p*	1.01 (0.88 – 1.15)	1.00 (0.87 – 1.14)	0.96 (0.83 – 1.11)
423-5p*	0.99 (0.87 – 1.13)	1.00 (0.88 – 1.15)	1.01 (0.89 – 1.16)
345-5p*	0.98 (0.89 – 1.07)	0.96 (0.88 – 1.05)	0.95 (0.86 – 1.04)
1306-5p*	1.13 (1.00 – 1.27)	1.12 (0.99 – 1.27)	1.09 (0.95 – 1.25)
133a-3p†	0.64 (0.36 – 1.15)	0.71 (0.39 – 1.29)	0.94 (0.51 – 1.77)
499a-5p†	1.34 (0.54 – 3.29)	1.41 (0.57 – 3.47)	1.20 (0.47 – 3.07)
133b†	0.63 (0.15 – 2.55)	0.63 (0.16 – 2.57)	1.04 (0.24 – 4.59)

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic heart failure, baseline eGFR, and baseline NT-proBNP level. BMI = Body mass index, HFrEF = Heart failure with reduced ejection fraction, miR = MicroRNA. Primary endpoint: composite of all-cause mortality and readmission for heart failure

* Hazard ratio per ln[arbitrary unit] of miR level

† Hazard ratio of detectable vs. non-detectable miR level

Supplemental table 6 - Associations between repeated microRNA measurements and secondary endpoints

Endpoint	MiR	Hazard ratio (95% CI)		
		Model 1	Model 2	Model 3
All-cause mortality	320a	1.20 (0.93 – 1.54)	1.16 (0.90 – 1.50)	0.89 (0.69 – 1.15)
	1254	1.21 (0.94 – 1.56)	1.17 (0.91 – 1.52)	0.87 (0.66 – 1.15)
	22-3p	1.17 (0.82 – 1.67)	1.24 (0.88 – 1.76)	1.16 (0.81 – 1.65)
	378a-3p	1.28 (0.89 – 1.75)	1.22 (0.90 – 1.66)	0.86 (0.61 – 1.22)
	423-5p	1.25 (0.89 – 1.75)	1.21 (0.87 – 1.68)	0.83 (0.57 – 1.19)
	345-5p	0.99 (0.79 – 1.24)	0.94 (0.75 – 1.18)	0.82 (0.64 – 1.05)
	1306-5p	2.78 (1.43 – 5.38)	1.83 (1.15 – 2.90)	2.31 (1.15 – 4.64)[$]
Heart failure hospitalizations	320a	1.54 (1.20 – 1.98)	1.54 (1.20 – 1.98)	1.30 (1.00 – 1.69)
	1254	1.30 (1.02 – 1.66)	1.29 (1.01 – 1.66)	1.06 (0.83 – 1.35)
	22-3p	1.27 (0.93 – 1.73)	1.26 (0.93 – 1.70)	1.14 (0.83 – 1.55)
	378a-3p	1.43 (1.05 – 1.95)	1.43 (1.05 – 1.95)	1.06 (0.77 – 1.47)
	423-5p	1.71 (1.22 – 2.41)	1.71 (1.21 – 2.40)	1.30 (0.92 – 1.84)
	345-5p	1.18 (0.95 – 1.47)	1.19 (0.95 – 1.48)	1.10 (0.88 – 1.37)
	1306-5p	5.50 (2.21 – 13.69)	3.95 (1.89 – 8.26)	4.95 (2.02 – 12.12)

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, baseline eGFR, and baseline NT-proBNP level. BMI = Body mass index, HF = Heart failure, miR = MicroRNA. Primary endpoint: composite of all-cause mortality and readmission for heart failure

* Hazard ratio per per ln[arbitrary unit] of miR level

† Hazard ratio of detectable vs. non-detectable miR level

Supplemental table 7 - Added predictive value of baseline miR-1306-5p and baseline NT-proBNP levels for the primary endpoint

	C -statistic (95% CI)	Change in C-statistic (95% CI)	cNRI (95% CI)	IDI (95% CI)
miR-1306-5p	0.619 (0.562 – 0.677)			
NT-proBNP	0.607 (0.566 – 0.648)			
Model	0.677 (0.634 – 0.721)			
Model + miR-1306-5p	0.694 (0.649 – 0.739)	0.017 (-0.004, 0.038)	0.138 (-0.004 – 0.280)	0.024 (-0.012 – 0.059)
Model + NT-proBNP	0.698 (0.655 – 0.740)	0.020 (0 – 0.041)	0.074 (-0.053 – 0.201)	0.027 (-0.005 – 0.058)
Model + NT-proBNP + miR-1306-5p	0.709 (0.665 – 0.754)	0.012 (-0.006 – 0.029)	0.125 (-0.016 – 0.267)	0.020 (-0.013 – 0.053)

Model: age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, and baseline eGFR

BMI = Body mass index, cNRI = continuous net reclassification improvement, HF = Heart failure, IDI = integrated discrimination improvement, miR = MicroRNA

Supplemental table 8 - Normalization: M-value and coefficient of measured microRNAs*

	Barcelona	Detroit	Mean	Rank CV	Barcelona	Detroit	Mean	Rank M-value	Rank CV	Rank M value	Mean rank	Final rank
	CV (%)	CV (%)	CV (%)		Final M-value	Final M-value	Final M-value					
miR-133b	2828	321	1575	10	0.65	0.83	0.74	4	10	4	7	6
miR-1254	298	576	437	3	0.31	1.21	0.76	5	3	5	4	4
miR-378a-3p	2226	526	1376	7	1.71	1.07	1.39	13	7	13	10	9
miR-423-5p	2888	207	1547	9	1.53	0.44	0.99	6	9	6	7.5	7
miR-320a	172	122	147	1	0.83	0.44	0.64	2	1	2	1.5	1
miR-345-5p	2357	544	1451	8	1.56	0.92	1.24	12	8	12	10	9
miR22-3p	231	228	229	2	0.86	1.15	1.01	8	2	8	5	5
miR-133a-3p	1437	691	1064	6	0.74	1.28	1.01	9	6	9	7.5	7
miR-1306-5p	2291	1200	1746	11	0.91	1.43	1.17	11	11	11	11	10
miR-622	120	3715	1917	12	0.44	1.56	1.00	7	12	7	9.5	8
miR-499a-5p	2722	3715	3218	13	0.79	1.33	1.06	10	13	10	11.5	11
miR-208a-3p	1047	649	848	5	0.31	0.84	0.58	1	5	1	3	2
miR-486-5p	726	159	442	4	0.7	0.65	0.68	3	4	3	3.5	3

* Using geNorm, an M-value and a coefficient of variation was calculated for all measured miRNAs. Top candidates for normalization were miR-320a, miR-208a-3p and miR-486-5p. MiR = MicroRNA.

Part III

Novel insights in characteristics associated with favorable outcome in defibrillation therapy and response to cardiac resynchronization therapy

Chapter 9

Functional response to cardiac resynchronization therapy is associated with improved clinical outcome and absence of appropriate shocks

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ABSTRACT

Background: We evaluated clinical outcome and incidence of (in)appropriate shocks in consecutive chronic heart failure (CHF) patients treated with CRT with a defibrillator (CRT-D) according to functional response status. Furthermore, we investigated which factors predict such functional response.

Methods: In a large teaching hospital, 179 consecutive CHF patients received CRT-D in 2005-2010. Patients were considered functional responders if left ventricular ejection fraction (LVEF) increased to ≥35% post-implantation. Analysis was performed on 142 patients, who had CRT-D as primary prevention, complete data and a baseline LVEF <35%. Endpoints consisted of all-cause mortality, heart failure (HF) hospitalizations, appropriate shocks and inappropriate shocks.

Results: Median follow-up was 3.0 years (interquartile range (IQR)1.6 – 4.4) and median baseline LVEF was 20% (IQR 18 – 25%). The functional response-group consisted of 42 patients. In this group no patients died, none were hospitalized for HF, none received appropriate shocks and 3 patients (7.1%) received ≥1 inappropriate shocks. In comparison, the functional non-response-group consisted of 100 patients, of whom 22 (22%) died (p=0.003), 17 (17%) were hospitalized for HF (p=0.007), 17 (17%) had ≥1 appropriate shocks (p=0.003) and 8 (8.1%) received ≥1 inappropriate shocks (p=0.78). Multivariable analysis showed that left bundle-branch block (LBBB), QRS duration ≥150ms and no need for diuretics at baseline are independent predictors of functional response.

Conclusions: Functional responders to CRT have a good prognosis and rarely need ICD therapy. LBBB, QRS duration ≥150ms and lack of chronic diuretic use predict functional response.

INTRODUCTION

Several large studies have demonstrated that cardiac resynchronization therapy (CRT) improves overall survival, reduces heart failure (HF) hospitalizations and induces cardiac reversed remodeling in patients suffering from chronic heart failure (CHF) and wide QRS-complexes.[1-5] Some patients respond very well to CRT and reach a left ventricular ejection fraction (LVEF) of ≥35% after implantation. In this paper we refer to these patients as functional responders. Not much is known about differences in prognosis between functional responders and functional non-responders.

Most patients are assigned to a CRT device with a defibrillator (CRT-D) as a backup, which increases survival by means of appropriate defibrillator-shocks, but also carries the negative effects of inappropriate shocks. If functional responders could be shown to have an excellent prognosis in terms of clinical outcomes, patients could be assigned to CRT-P instead of CRT-D once they have reached functional response and a device change is needed. Furthermore, if functional response to CRT could be predicted beforehand, one may even consider assigning patients likely to functionally respond to CRT-P primarily.

In this paper, we investigated whether functional response to CRT-D therapy is associated with a favorable outcome. For, this purpose, we retrospectively studied a cohort of consecutive patients treated with CRT-D in a large teaching hospital. In this cohort we evaluated the difference in clinical outcome (mortality and heart failure hospitalizations) and incident (in)appropriate shocks between functional responders and functional non-responders. Secondly, we identified patient characteristics associated with a functional response.

METHODS

Study design, patients and baseline characteristics

The population of this retrospective, single-center, follow-up study consisted of 179 patients that were consecutively assigned to CRT-D in a large teaching hospital between February 2005 and December 2010. Therapy assignment was based on the ESC guidelines for cardiac pacing and cardiac resynchronization therapy.[6] All patients suffered from chronic heart failure for at least 3 months and were receiving optimal pharmacologic therapy. The pharmacologic treatment consisted of diuretics, angiotensin-converting–enzyme inhibitors or Angiotensin II antagonists, beta-blockers, and spironolactone, unless one of these was not tolerated or contraindicated. Diuretics were only prescribed if needed. Coronary angiography was performed on all patients before CRT-D implantation and patients underwent revascularization if needed and possible.

Baseline characteristics of all patients were collected by reviewing hospital records and included demographics, medical history, medication use, cardiovascular risk factors and pacing and electrocardiographic characteristics. Patients were only considered users of specific medication if they used the drug chronically.

LVEF was measured with the biplane Simpson's method in the apical 4- and 2-chamber views and averaged. Baseline echocardiograms used were generally performed 2-3 months (with a maximum of 1 year) prior to CRT-D implantation. After implantation, echocardiographic optimization of atrioventricular (AV) delay was performed at 4 weeks follow-up. Subsequently, echocardiographic assessment of LV-function was performed 3 months after this AV optimization according to protocols used in literature.[2,7,8] This last echocardiogram, performed 3 months after AV optimization, was used to measure follow-up LVEF and used to consider patients as functional responders or functional non-responders.

Intraventricular conduction disorders (IVCD) were defined according to the AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram.[9] Left bundle branch block (LBBB) criteria are: QRS duration ≥120 ms; QS or rS in lead V1; wide R (frequently notched) waves in leads I, aVL, V5, or V6; and absent q waves in leads V5 and V6. Right bundle branch block (RBBB) required QRS duration ≥120 ms; rsr′, rsR′, rSR′, or qR in leads V1 or V2; and occasionally, a wide and notched R wave and wide S waves in leads I, V5, and V6. Not specific IVCD (NS-IVCD) is defined as: QRS duration >120ms, not meeting criteria for either LBBB or RBBB. Patient ECG's with QRS duration<120 ms 1 day before implantation were counted as no-IVCD.

Placement procedure and ICD follow-up

All patients received a CRT-D device (Medtronic inc. or Boston Scientific inc). First, the right ventricular (shock-) lead was positioned, mostly in the right ventricular apex using a cephalic cut down technique. Next, the LV pacing lead was inserted through the coronary sinus and positioned in the venous system, preferably in the (postero-)lateral vein. Finally, the atrial lead was positioned in the right atrial appendage. If transvenous LV pacing lead placement failed, an epicardial procedure was scheduled and performed. No major complications occurred. For each patient, both the atrioventricular interval to maximize mitral inflow duration and the interventricular mechanical delay, defined as the time interval between the onset of antegrade blood flow in the right ventricular outflow tract and the onset of antegrade blood flow in the LV outflow tract, were optimized by echocardiography 2 to 4 months after implantation.

As part of usual care, during follow-up, ICD printouts were obtained every 3 months to determine the number and type of arrhythmias and the number of appropriate and inappropriate shocks.

Follow-up and definition of endpoints

Follow-up on clinical events lasted until May 2011 and was performed by reviewing hospital records and by contacting general practitioners. Endpoints comprised death from any cause, heart failure (HF) leading to hospitalization, appropriate shocks and inappropriate shocks. Heart failure was defined as signs and symptoms consistent with congestive heart failure leading to hospitalization and requiring either intravenous or a (temporary) dose increase of oral diuretics. All heart failure events and therapy that followed were assessed by the attending cardiologist.

Patients were considered functional responders if LVEF improved to ≥35% post-implantation. This definition was based on the inclusion criteria of several large CRT-D and ICD trials.[1,1,10-12] In these trials, patients with an LVEF ranging from ≤30% to ≤35% were included.

Patients were excluded from the analyses if: 1) defibrillator therapy was assigned for secondary prevention, 2) pre-implantation LVEF was ≥35% according to echocardiographic data, or 3) echocardiographic follow-up data were missing.

Statistical analysis

Continuous patient characteristics were tested for normality by using the Kolmogorov-Smirnov test. Subsequently, if skewed, continuous patient characteristics of functional responders and functional non-responders were compared using Mann-Whitney's test. Categorical characteristics were compared by using the χ^2 test.

Univariable and multivariable logistic regression were used to examine the association between patient baseline characteristics and functional response to CRT. Characteristics were entered into the multivariable model if they showed a statistically significant association with the outcome during univariable analysis (P value <0.05). We investigated the performance of the combination of independent risk factors, resulting from the multivariable analysis, in the prediction of functional response. For this purpose a C-index was calculated using the area under the receiver operating characteristic (ROC) curve.

Kaplan-Meiers log-rank test was used to compare the differences in outcomes (all-cause mortality, HF hospitalizations, appropriate shocks and inappropriate shocks) between functional responders and functional non-responders.

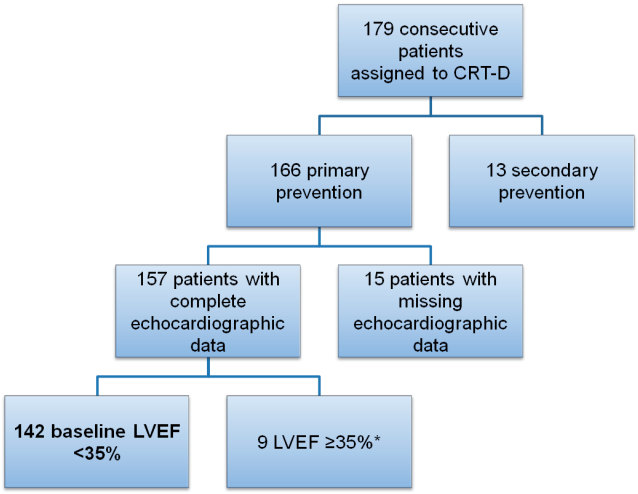
Overall statistical significance was set at a 2-tailed P value <0.05. SPSS 20.0 (SPSS Inc, Chicago, IL) was used for the statistical analysis.

RESULTS

Of the 179 patients that were consecutively assigned to CRT-D, 13 were excluded, because they had backup defibrillation therapy as secondary prevention, 9 because LVEF

at baseline was ≥35% and 15 because of missing echocardiographic data (baseline or outcome) (figure 1). Functional response to CRT occurred in 42 patients and 100 patients were considered functional non-responders. Median overall improvement of LVEF of all 142 study patients was 7.0% (IQR 0.0 – 15%). In the functional non-responders group the median improvement in LVEF was 3.0% (IQR -1.8 – 7.0%), compared to a median improvement of 20% (IQR 15 – 23%) in the functional responders group, P<0.001.

Figure 1 - Inclusion and exclusion of patients



* Patients who had a LVEF of ≥35% or very close to 35% during revision of the echocardiogram.

Comparison of baseline characteristics between functional responders and functional non-responders

Baseline characteristics of the study population are shown in table 1. Median follow-up of the 142 consecutive patients was 3.0 (interquartile range (IQR) 1.6 – 4.4) years, and did not differ between both groups (P=0.64). Median age was 69 (IQR 61 – 74) years (P=0.40), and 70% was male (P=0.15).

In the functional responders group, non-ischemic cardiomyopathy (non-ICM) was more frequent, 41% vs. 62%, P=0.02, chronic diuretics use before CRT was less frequent, 96% vs 71%, P<0.001, and NYHA class was lower, P=0.03. In the functional non-responders group, median urea was 8.3 mmol/l and median creatinine 110 µmol/l. In comparison, median urea in the functional responders group was 6.1 mmol/l, P=0.001 and median creatinine was 92 µmol/l, P=0.001. Finally, median baseline LVEF in the functional responders group was 23%, compared to 20% in the functional non-responders group, P=0.003.

Table 1 - Baseline characteristics

Variable	Total	Functional non-responders (n=100)	Functional responders (n=42)	P value
Age (years)	69 (61 – 74)	70 (61 – 74)	68 (61 – 73)	0.40
Male gender	100 (70)	74 (75)	26 (62)	0.15
NYHA class I	15 (13)	6 (7,5)	9 (25)	0.03
II	45 (39)	30 (38)	15 (42)	
III	52 (45)	40 (50)	12 (33)	
IV	4 (3.4)	4 (5.0)	0 (0)	
Non-ICM	67 (47)	41 (41)	26 (62)	0.02
Follow-up	3.0 (1.6 – 4.4)	3.1 (1.7 – 4.4)	2.9 (1.4 – 4.4)	0.64
Hypertension	73 (51)	48 (48)	25 (60)	0.21
Diabetes mellitus	30 (21)	21 (21)	9 (21)	0.95
Smoking	68 (48)	50 (50)	18 (43)	0.44
ACE-i/ATII-a	139 (98)	97 (97)	42 (100)	0.56
Diuretics	126 (89)	96 (96)	30 (71)	<0.001
B-Blocker	129 (91)	92 (92)	37 (88)	0.46
Serum urea (mmol/l)	8.1 (6.1 – 11)	8.3 (6.7 – 11)	6.1 (4.7 – 8.9)	0.001
Serum creatinine (µmol/l)	105 (88 – 129)	110 (95 – 131)	92 (81 – 105)	0.001
Serum BNP(ng/l)	402 (128 – 690)	467 (153 – 852)	219 (112 – 517)	0.17
LVEF	20 (18 – 25)	20 (16 – 25)	23 (20 – 28)	0.003

Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. Continuous variables are expressed as median (first quartile - third quartile). Non-ICM = non-ischemic cardiomyopathy; ACE-I = Ace inhibitor; ATII-a = Angiotensin II antagonist; LVEF = left ventricular ejection fraction.

Comparison of electrocardiographic baseline characteristics between functional responders and functional non-responders

Table 2 displays the pacing and electrocardiographic baseline characteristics. The number of patients with an LBBB conduction disorder was significantly higher in the functional responders group, 93% vs. 62%, P<0.001. Another significant difference was found in the number of patients with a QRS duration ≥150ms, which was 88% in the functional responders group vs. 64% in the functional non-responders group, P=0.01.

A history of atrial fibrillation was found in 26% of all patients, which did not differ significantly between both groups, P=0.42. An upgrade from CRT-P to CRT-D was found in 3.5% and another 3.5% of all patients received an epicardial lead, because transvenous placement of the LV lead was considered sub-optimal. All patients with a NS-IVCD had wide left anterior fascicular blocks.

Table 2 – Pacing and electrocardiographic baseline characteristics

V Variable	Total	Functional non-responders (n=100)	Functional responders (n=42)	P value
History of AF	37 (26)	28 (28)	9 (21)	0.42
LBBB	98 (71)	61 (62)	37 (93)	<0.001
RBBB	7 (5.1)	7 (7.1)	0 (0)	0.08
NS-IVCD	12 (8.7)	11 (11)	1 (2.5)	0.10
Pacemaker rhythm	18 (13)	16 (16)	2 (5.0)	0.07
No IVCD	3 (2.2)	3 (3.1)	0 (0)	0.56
QRS >150ms	96 (71)	61 (64)	35 (88)	0.01
Epicardial lead	5 (3.5)	3 (3.0)	2 (4.8)	0.63
CRT-P	5 (3.5)	5 (5.0)	0 (0)	0.14

Variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. AF = Atrial fibrillation; LBBB = left bundle branch block; RBBB = right bundle branch block; NS-IVCD = not specific intraventricular conduction disorder; IVCD = intraventricular conduction disorder; CRT-P = Cardiac resynchronization therapy with a pacemaker only, upgraded to cardiac resynchronization therapy with a defibrillator.

Difference in clinical outcome

The difference in clinical outcome between functional responders and functional non-responders is shown in figure 2. In the functional responders group no patients died, no patients were hospitalized for HF, no patients had appropriate shocks and 3 patients (7.1%) had ≥1 inappropriate shocks. In comparison, in the functional non-response-group 22 (22%) patients died, P=0.003, 15 (15%) of these were cardiac deaths (related to a cardiac cause, e.g. MI, low-output failure, fatal arrhythmia), 17 patients (17%) were hospitalized for HF, P=0.007, 17 patients (17%) had ≥1 appropriate shocks, P=0.007, and 9 (9.0%) received ≥1 inappropriate shocks, P=0.77.

Logistic regression analyses

The association between baseline characteristics and cumulative probability of functional response is shown in table 3. Although all baseline variables were analyzed, only the significant univariable predictors of functional response, which were subsequently also entered into the multivariable analysis, are displayed in this table.

Multivariable analysis showed that a LBBB conduction disorder (OR 14.7; CI 1.55 – 140), no need for chronic diuretics at baseline (OR 0.05; CI 0.01 – 0.52) and QRS duration ≥150ms (OR 8.04; CI 1.15 – 56.4), were independently associated with functional response. A model containing these 3 independent predictors of functional response displayed discriminative ability, with a C-index of 0.88 (figure 3).

Given that LBBB and QRS duration ≥150ms often occur concurrently, and both characteristics were found to be significant predictors, we performed an additional analysis

Figure 2 - Kaplan-Meier hazard curves of A) All-cause mortality; B) Heart failure hospitalizations; C) Appropriate shocks; D) Inappropriate Shocks

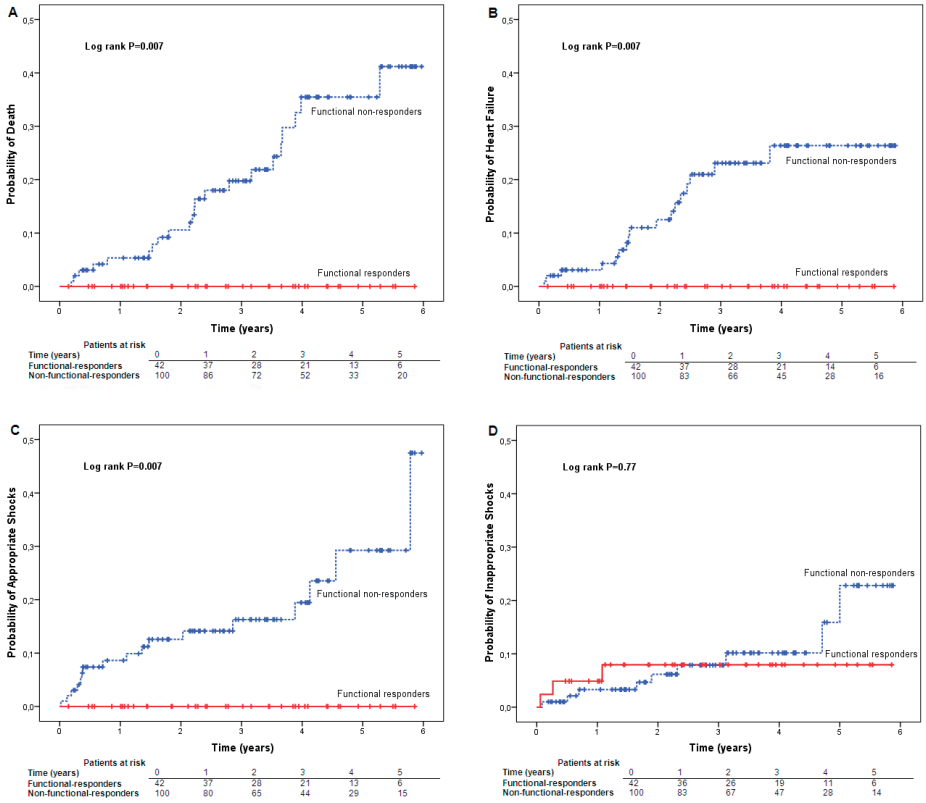
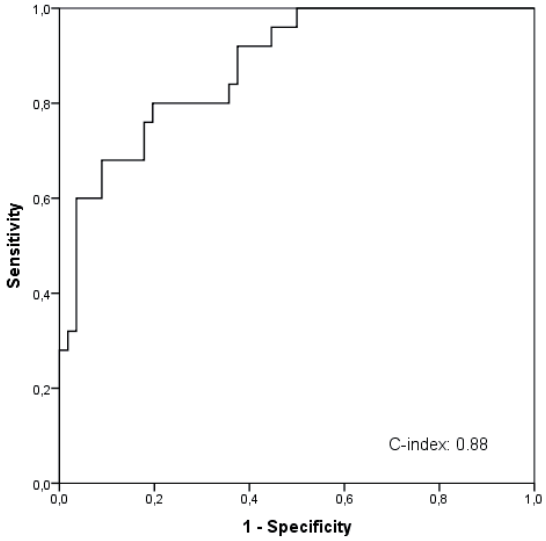


Table 3 – Association between patient characteristics and functional response

Variable	Crude OR (95% CI)	Adjusted OR (95% CI)	P-value
NYHA class	0.45 (0.26 – 0.78)	0.73 (0.32 – 1.67)	0.46
Non-ICM	2.34 (1.12 – 4.90)	1.16 (0.32 – 4.18)	0.83
Diuretics	0.10 (0.03 – 0.35)	0.05 (0.01 – 0.52)	0.01
Serum urea (mmol/l)	0.81 (0.69 – 0.95)	0.97 (0.72 – 1.30)	0.84
Serum creatinine (µmol/l)	0.82 (0.70 – 0.96)	0.83 (0.62 – 1.09)	0.18
LVEF	1.10 (1.03 – 1.18)	1.05 (0.93 – 1.17)	0.45
LBBB	6.86 (1.55 – 30.5)	14.7 (1.55 – 140)	0.02
QRS >150ms	3.90 (1.40 – 10.9)	8.04 (1.15 – 56.4)	0.04

Functional response occurred in 42 out of 142 patients. HR = hazard ratio; CI = confidence interval; Non-ICM = non-ischemic cardiomyopathy; LVEF = left ventricular ejection fraction; AF = Atrial fibrillation; LBBB = left bundle branch block; CRT-P = Cardiac resynchronization therapy with a pacemaker only upgraded to cardiac resynchronization therapy with a defibrillator. Patients, who had no AF, had a sinus rhythm.
*Odds ratio for every 10 units increase.

Figure 3 - ROC curve depicting discriminative value of the multivariable model for functional response



The multivariable model contains LBBB, QRS duration ≥150ms and no need for chronic diuretics at baseline.

to further evaluate the role of their simultaneous presence. An LBBB combined with QRS≥150ms was observed in 73 patients, of whom 33 showed functional response. With univariable analysis, the functional non-responders with a LBBB and QRS≥150ms were found to be older (OR 1.08; CI 1.01 – 1.15), to more often have ischemic cardiomyopathy (OR 2.63; CI 1.01 – 6.80), and to have worse renal function (OR 1.23; CI 1.00 – 1.50).

In the population of patients with a LBBB, QRS≥150ms was a significant predictor of response (p=0.01), in contrast to the population without a LBBB (p=0.87), where QRS≥150ms was not a significant predictor of response. It should be noted that the non-LBBB group was relatively small (n=37).

DISCUSSION

In this retrospective study, performed on a population of CHF patients treated with CRT-D, we found that 30% of heart failure patients with CRT-D therapy appeared to achieve a LVEF ≥35% after receiving CRT. During a median follow-up period of 3.0 years, these CRT functional responders were found to have a good prognosis, as none of them died and none of them was hospitalized for HF. Furthermore, none of these functional responders received appropriate shocks, while 7.1% received ≥1 inappropriate shocks. Secondly, we found that LBBB conduction disorder, QRS duration ≥150ms and no need for chronic

diuretics at baseline are independent predictors of functional response. A model containing these 3 independent predictors of functional response has discriminative ability, with a C-index of 0.88.

Clinical implications

The above-mentioned findings from our study suggest that CRT functional responders may not need backup defibrillation therapy in terms of clinical outcomes during the first 3 years after device implantation. Incidence of inappropriate shocks was similar among functional responders and functional non-responders. Defibrillator-shocks, whether appropriate or not, have adverse consequences for patients and lead to more frequent hospitalization and thereby higher costs. Altogether, our findings thus raise the hypothesis that functional responders may be assigned to CRT-P when a device change is needed, or that ICD therapy may be switched off to prevent unnecessary inappropriate shocks without compromising clinical outcome. However, to confirm this hypothesis, large, prospective, randomized studies with longer term follow-up are warranted.

Furthermore, it should be noted that the implication that a return in LVEF to ≥35% constitutes subsequent protection from arrhythmia is not well established in the literature. Nevertheless, there is growing evidence that anatomic remodeling due to CRT is accompanied by electrical remodeling and could thereby reduce the risk of ventricular arrhythmias.[13-17] Our study contributes to the evidence that remodeling achieved by CRT leads to a reduced risk of life threatening arrhythmias.

Moreover, although there is a growing body of evidence on predictors of response to CRT, accurate response prediction remains challenging. Should functional response prediction be improved in the future, then assignment of patients likely to achieve functional response to CRT-P, without backup defibrillation therapy, may be considered. Schaer et al have previously described this issue, and have also stated that patients who reach a LVEF >35% could be assigned to CRT-P, when a device change is needed.[13] Unlike Schaer et al, we focused on primary prevention patients and chose to examine appropriate shocks and mortality as endpoints, instead of all ICD-therapy.

Prediction of functional response

We showed that cardiomyopathy etiology, diuretics use, serum urea, serum creatinine, LVEF, LBBB and QRS duration ≥150ms were factors univariably associated with functional response. Multivariable analysis showed that only LBBB, QRS duration ≥150ms and no need for chronic diuretics at baseline were independently associated with functional response. This is in line with previous findings. Patients with a LBBB are more likely to have left ventricular dyssynchrony and patients with wide QRS complexes, i.e. QRS complexes of ≥150ms, probably have more intraventricular dyssynchrony. Since CRT corrects intraventricular dyssynchrony, patients with both an LBBB and QRS duration ≥150ms

are more likely to benefit from CRT in terms of LVEF and are consequently more likely to become functional responders. LBBB is a known predictor of response to CRT-D[18-20] and our study contributes to the evidence, although the confidence interval is large.[18-20] Moreover, Goldenberg et al recently identified QRS duration ≥150ms as a predictor of response.[19] Not all patients with a LBBB and QRS duration ≥150ms showed functional response. In our study population, posterolateral scarring by ischemic heart disease was the main cause of functional non-response in these patients.

Patients not requiring chronic diuretics use before receiving CRT probably had less signs of HF, possibly because of a better cardiac function at baseline. More remarkable is the fact that baseline LVEF was not independently associated with functional response. A possible explanation for this is that in this population, patients with a very poor baseline LVEF are just as likely to become functional responders as patients with a moderately reduced baseline LVEF. Alternatively, the reliability of LVEF as a marker of outcome may be questioned; however, since the inter-observer variability of the LVEF measurements made by our echocardiography laboratory is 5%, we believe use of this marker is justified.

Further specification of clinical outcomes

In the functional non-response group, 22 patients died. Of these, 7 were non-cardiac deaths. Occurrence of all of these 7 non-cardiac deaths in the functional non-response group is probably due to chance.

Of the patients in the functional response group, 7.1% received inappropriate shocks, and this was similar in both groups. Most of the inappropriate shocks in both groups were due to atrial fibrillation or lead crushes.

Limitations

Several issues concerning this study warrant further consideration. The study population of 142 patients is relatively small, retrospective and derived from a single center. Larger, prospective, multicenter studies are needed to confirm our exploratory findings and to provide further grounds for statements about prognosis of functional responders and about prediction of functional response.

The use of LVEF as a marker of response is inherently an issue, given the known limitations in reproducibility of this method. Nevertheless, the marker of response we use in this exploratory study is comparable to other CRT trials using echocardiographic markers. Furthermore, since the inter-observer variability of the LVEF measurements made by our echocardiography laboratory is only 5%, which justifies the use of an echocardiographic marker. Our criterion of an LVEF ≥35% post implantation appears to discriminate better between patients with a high or low risk for arrhythmias, than criteria such as an improvement in LVEF of ≥10%. Echocardiograms to assess the effect of CRT

were performed 3 months after AV optimization. Most remodeling and hemodynamic changes must have been achieved, but occasionally remodeling takes more time.

Although the current study tentatively suggests that functional responders may be assigned to CRT-P when a device change is needed, this statement should be interpreted with caution. The battery life of a typical CRT-D device is approximately 4-5 years, and the median follow-up of our study population was 3 years. Still, we expect this follow-up period to cover the timeframe in which risk of arrhythmias is highest. Cardiac reversed remodeling for the greatest part takes place in the first few months after implantation, lasting generally until 1 year after implantation which is congruent with the frequency of occurrence of the arrhythmias (75% in the first year after CRT-D implantation).[13]

Conclusion

In our study population, functional responders displayed a good prognosis during a median follow-up of 3 years, and did not need backup from defibrillation therapy. As such, our study suggests it may be worthwhile to further investigate whether functional responders could be assigned to CRT-P when a device change is needed and whether ICD therapy could be switched off in these patients. This would require large, prospective, randomized studies with longer-term follow-up.

Furthermore, our study shows that LBBB conduction disorder, QRS duration ≥150ms and no need for chronic diuretics at baseline are independent predictors of functional response. Response prediction to CRT currently remains challenging, but if response prediction becomes more precise in the future, one may consider primarily assigning probable functional responders to CRT-P.

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Chapter 10

Atrial fibrillation in cardiac resynchronization therapy with a defibrillator: a risk factor for mortality, appropriate and inappropriate shocks

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ABSTRACT

Background: Knowledge about predictive factors for mortality and (in)appropriate shocks in cardiac resynchronization therapy with a defibrillator (CRT-D) should be available and updated to predict clinical outcome.

Methods: We retrospectively analyzed 550 consecutive patients assigned to CRT-D in 2 tertiary medical centers. The aim of this study was to assess risk factors for all-cause mortality, appropriate and inappropriate shocks.

Results: Mean follow-up was 3.2 (±1.8) years. A total of 112 (20%) patients died, 72 (13%) received ≥1 appropriate shocks, and 33 (6.0%) received ≥1 inappropriate shocks. No patients received a His bundle ablation and biventricular pacing percentage was not analysed. Multivariable Cox regression analysis showed that a history of atrial fibrillation (AF) (HR 1.68 CI 0.98-2.72), higher creatinine (HR 1.11; CI 1.07-1.16) and a poorer left ventricular ejection fraction (LVEF) (HR 0.97; CI 0.94-1.01) independently predict all-cause mortality. In the entire cohort, history of AF and secondary prevention were independent predictors of appropriate shocks and variables associated with inappropriate shocks were history of AF and QRS ≥150ms. In primary prevention patients, history of AF also predicted appropriate shocks as did ischemic cardiomyopathy and poorer LVEF. History of AF, QRS ≥150ms and lower creatinine were associated with inappropriate shocks in this subgroup. Appropriate shocks increased mortality risk, but inappropriate shocks did not.

Conclusions: In symptomatic CHF patients treated with CRT-D, history of AF is an independent risk factor for mortality, but also for appropriate and inappropriate shocks. Further efforts in AF management may optimize the care in CRT-D patients.

INTRODUCTION

Over the past years, several large studies have demonstrated the benefits of cardiac resynchronization therapy (CRT) in patients suffering from chronic heart failure (CHF) and left ventricular dyssynchrony. It reduces mortality and morbidity, and increases left ventricular ejection fraction (LVEF).[1-5] However, uncertainty exists about which pre-implantation patient and particularly rhythm and conduction characteristics are associated with mortality and (in)appropriate shocks. Most data on the survival of CRT patients are limited to post hoc analyses of large randomized controlled trials. These trials have applied specific inclusion criteria with predominance of patients without atrial fibrillation (AF), which complicate extrapolation of their results to the more diverse, real-life population of patients treated with CRT. Patients assigned to CRT, have a poor left ventricular function[6,7], which is also an indication for an implantable cardioverter-defibrillator (ICD).[8-10] Based upon this, patients assigned to CRT receive a cardiac resynchronization defibrillator (CRT-D). This increases survival by means of appropriate defibrillator-shocks, but also carries the negative effects of inappropriate shocks, which consequently increases the number of hospitalizations. These device-related hazards may be reduced by a more appropriate selection of device therapy and optimal care for CRT-D device patients. In addition, CRT-D is an expensive therapy and to provide maximum benefit from the device, optimal care is necessary. Therefore, we conducted a retrospective cohort study in order to assess the effect of baseline demographic variables and rhythm and conduction characteristics on mortality, appropriate and inappropriate defibrillation shocks in a general, unselected group of CHF patients, treated with CRT-D on top of optimal medication.

METHODS

Patients and study design

The population of this retrospective, multi-centre, observational, follow-up study consisted of 550 patients that were consecutively assigned to CRT-D in Erasmus Medical Centre, Rotterdam, the Netherlands and Medical Centre Alkmaar, Alkmaar, the Netherlands, between February 2005 and March 2011. Therapy assignment was based on the ESC guidelines for cardiac pacing and cardiac resynchronization therapy.[11] All patients suffered from chronic heart failure for at least 3 months and were treated with optimal medication. Follow up on mortality lasted until December 2011.

Baseline data collection

Baseline characteristics of all patients were collected by reviewing hospital records and included demographics, medical history, medication use, cardiovascular risk factors and pacing and electrocardiographic characteristics.[12]

Intraventricular conduction disorders (IVCD's) were defined according to the AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram.[13] Left bundle branch block (LBBB) criteria are: QRS duration ≥120 ms; QS or rS in lead V1; wide R (frequently notched) waves in leads I, aVL, V5, or V6; and absent q waves in leads V5 and V6. Left anterior fascicular blocks (Frontal plane axis deviation between -45° and -90°; qR pattern in aVL; and R-peak time in lead aVL of 45ms or more) with QRS duration ≥120ms were also counted as LBBB. Right bundle branch block (RBBB) required: QRS duration ≥120 ms; rsr', rsR', rSR', or qR in leads V1 or V2; and occasionally, a wide and notched R wave and wide S waves in leads I, V5, and V6.

Placement procedure and ICD follow-up

The placement procedure was similar in the two participating Medical Centers.

First, the right ventricular ICD-lead was positioned, mostly in the right ventricular apex using a cephalic cut down technique. Next, the LV pacing lead was inserted through the coronary sinus and positioned in the venous system, preferably in the (postero-)lateral vein. Finally, the atrial lead was positioned in the right atrial appendage. If transvenous LV pacing lead placement failed, an epicardial procedure was scheduled and performed.

During in-hospital and remote ICD follow-up, as part of usual care, ICD printouts were obtained every 3 months to determine the number and type of arrhythmias and the number of appropriate and inappropriate shocks. In Alkmaar, the majority of devices had a 3-zone configuration. The first zone was a monitor zone only, which was set to 160 ±10 bpm, the VT zone was set to 190 ±12 bpm and the VF zone was set to >209 ±15 bpm. In Rotterdam, a two-zone configuration was programmed in 83% of the patients, 16% had a 3-zone configuration. A monitoring zone was activated in the majority of patients with a 3-zone configuration. Mean VT detection rate was 171 ±11 bpm, the mean VF detection rate was set to 216 ±15 bpm. In the Medtronic Inc., Minneapolis, MN, USA, devices, the number of intervals to detect was set to 18/24 episodes in all zones. In the Boston Scientific inc., Indianapolis, IN, USA, devices, the number of intervals to detect was set to 8/10 intervals, with a duration of 8s in the VT zone and 5s in the VF zone. For all patients, ICD programming was intended to avoid inappropriate therapy by activating the available discriminators, e.g. dual-chamber algorithms, onset, stability and morphology. For each patient, programming was tailored according to the clinical presentation.

Definitions

The aim of this study was to assess risk factors for all-cause mortality, appropriate and inappropriate shocks. A shock was considered appropriate if it was delivered on ventricular fibrillation or ventricular tachycardia. ICD interrogations were performed 10 days, 3, 6, 9, and 12 months after implantation and then also every 6 months. Every arrhythmia in the ICD memory was interpreted by two experienced physicians per centre and declared as either appropriate or inappropriate.

Statistical analysis

Normality of distribution was determined by Kolmogorov-Smirnov's test. Continuous data are expressed as mean ± SD, if normally distributed, otherwise by median and interquartile range (IQR). Continuous data were analyzed with Student's *t* test or Mann-Whitney *U* test, when appropriate. Categorical data were compared by using the χ^2 test or Fisher's exact test when appropriate. Multivariable Cox proportional hazards regression was used to analyze the effect of patient baseline characteristics on the outcome measures, eg mortality, appropriate and inappropriate shocks. A backward elimination procedure was used and variables were withdrawn from the model if the corresponding p-value was ≥0.1 until all factors had a p value <0.1. All available baseline characteristics were used for the backward elimination procedure, except for the variable 'CRT-P upgraded to CRT-D', due to the small number of patients in whom this occurred.

The preventive cardioverter/defibrillator function of the device is always indicated in patients, who have CRT-D as secondary prevention for life-threatening arrhythmias.[14] For this reason, we repeated our analysis on appropriate and inappropriate shocks, on solely the group of patients who had received CRT-D as primary prevention.

The influence of appropriate and inappropriate shocks on mortality was investigated by Cox regression analysis. For this purpose, appropriate and inappropriate shocks were treated as two separate time-dependent covariates, which allowed us to take into account the occurrence of multiple shocks that occurred consecutively in time.

We investigated the performance of the combination of independent risk factors, resulting from the multivariable analyses described above. For this purpose a C-index was calculated using the area under the receiver operating characteristic (ROC) curve.

SPSS 14.0 (SPSS Inc, Chicago, IL) was used for statistical analysis.

RESULTS

Baseline characteristics

Baseline characteristics of the patients are shown in table 1. Mean age of the study population was 64 ±11 years and 74% was male. The majority of the patients had re-

Table 1 - Baseline characteristics

Variable	Total study population (n= 550)	Patients with CRT-D for primary prevention (n= 482)
Age (years)	64 ± 11	64 ± 11
Male gender	404 (74)	357 (74)
NYHA class		
I	21 (4.1)	17 (3.8)
II	180 (35)	151 (33)
III	308 (60)	276 (61)
IV	8 (1.5)	8 (1.8)
Ischemic cardiomyopathy	297 (54)	252 (52)
Hypertension	199 (36)	170 (35)
Diabetes mellitus	133 (24)	118 (25)
ACE-i/ATII-a	524 (99)	459 (99)
Diuretics	457 (83)	408 (85)
B-Blocker	456 (83)	396 (82)
Serum urea (mmol/l)	9.8 ± 4.8	9.6 ± 4.8
Serum creatinine (µmol/l)	115 ± 50	113 ± 49
Hb (mmol/l)	8.5 ± 1.1	8.6 ± 1.1
LVEF (%)	24 ± 7.2	24 ± 7.0
History of AF	156 (28)	136 (28)
LBBB	414 (82)	364 (83)
RBBB	27 (5.3)	22 (5.0)
Pacemaker rhythm	57 (10)	49 (10)
IVCD with QRS <120ms	10 (2.0)	9 (2.0)
QRS ≥150ms	387 (72)	340 (72)
LV Lead position		
Posterolateral	305 (57)	270 (58)
Lateral	54 (10)	50 (11)
Posterior	89 (17)	71 (15)
Anterolateral	67 (13)	59 (13)
RVOT	7 (1.3)	7 (1.5)
Epicardial	10 (1.8)	9 (1.9)
CRT-P	14 (2.5)	11 (2.3)

Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing variables. ACE-I = ace inhibitor; ATII-a = angiotensin II antagonist; LVEF = left ventricular ejection fraction; AF = atrial fibrillation; LBBB = left bundle branch block; RBBB = right bundle branch block; IVCD = intraventricular conduction disorder; LV = left ventricular; RVOT = right ventricular outflow tract; CRT-P = Cardiac resynchronization therapy with a pacemaker only, upgraded to cardiac resynchronization therapy with a defibrillator.

ceived CRT-D for primary prevention, while a mere 68 patients (12%) had received CRT-D as secondary prevention for cardiac arrest. Mean LVEF was 24 ±7.2%. A history of AF was found in 156 patients (28%), of whom 60 patients (11%) had permanent AF and 96 patients (17%) had paroxysmal AF. Of all patients, 82% had a LBBB conduction disorder.

A total of 10% of all patients had a dual chamber pacemaker before receiving CRT-D and 2.5% was upgraded from CRT-P to CRT-D. An epicardial lead was placed in 1.8% of all patients, because transvenous placement of the LV lead was considered sub-optimal.

Occurrence of outcome measures

Mean follow-up of the study population of 550 consecutive patients was 3.2 ±1.8 years.

During follow-up, 112 patients (20%) died and the one-year mortality rate was 5.4%.

A total of 72 of all patients (13%) received one or more appropriate shocks, as did 55 (11%) of all 482 primary prevention patients.

A total of 33 (6.0%) patients received one or more inappropriate shocks. Of these, 15 (46%) had a known history of AF at baseline. The trigger for these inappropriate shocks was the occurrence of paroxysmal AF in 5 patients and rapid ventricular response in 10 patients with known permanent AF. In 45%, these inappropriate shocks were not related to AF.

Cox proportional hazards analyses

The association between baseline characteristics and all-cause mortality is shown in figure 1. Three baseline characteristics remained in the multivariable model after the backward elimination procedure, history of AF (HR 1.68; CI 1.03 – 2.72) serum creatinine (HR 1.11;CI 1.07 – 1.16) and LVEF (HR 0.97; CI 0.94 - 1.01),

Factors associated with appropriate shocks were history of AF (HR 2.32; CI 1.29 – 4.18) and secondary prevention (HR 2.61; CI 1.32 – 5.17), as shown in figure 2. In primary prevention patients, history of AF (HR 2.00; CI 1.00 – 3.98), ischemic cardiomyopathy

Figure 1 - Association between patient characteristics and mortality

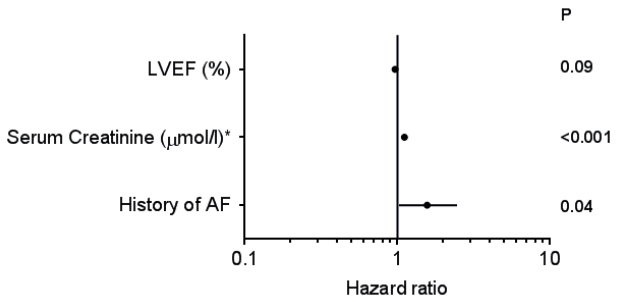
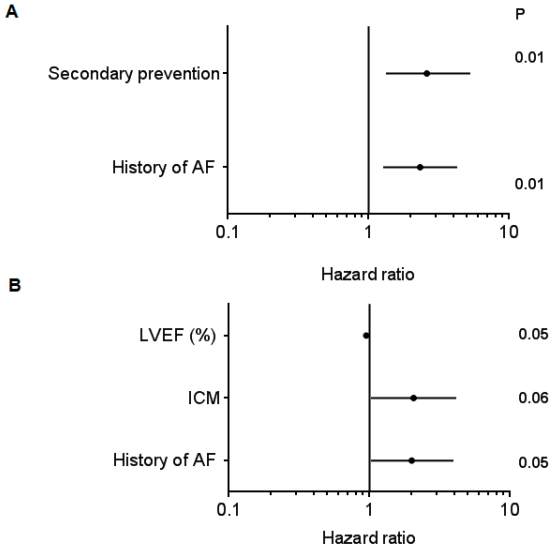


Figure 2 - Association between patient characteristics and appropriate shocks; a) all patients; b) primary prevention patients



(ICM) (HR 2.03; CI 0.98 – 4.20) and LVEF (HR 0.95; CI 0.90 – 1.00) were associated with appropriate shocks.

Variables that were associated with inappropriate shocks are shown in figure 3 and included history of AF (HR 2.29; CI 0.95 – 5.55) and QRS ≥150ms (HR 2.74; CI 1.14 – 6.58). In primary prevention patients, history of AF (HR 3.31; CI 1.29 – 8.49), QRS ≥150ms (HR 2.72; CI 1.08 – 6.86) and serum creatinine (HR 0.84; CI 0.70 – 1.01) were associated with inappropriate shocks.

Finally, an association was present between the occurrence of appropriate shocks and increased mortality (HR 4.15; CI 2,69 – 6,40), even when corrected for baseline variables significantly associated with increased mortality (HR 3.69; CI 2.24 – 6.09). Inappropriate shocks however, were not associated with increased mortality (HR 1.07; CI 0.47 – 2.46).

Discriminative ability

The combination of the 3 independent risk factors for mortality, a history of AF, LVEF and serum creatinine , displayed discriminative ability for mortality, with a C-index of 0.72 (figure 4).

In the full patient cohort, we found that the prognostic model that contained history of AF and CRT-D as secondary prevention discriminated between those with and without appropriate shocks with a C-index of 0.74. In patients who had received CRT-D as primary prevention, the model containing ICM, history of AF and LVEF displayed discriminative ability, with a C-index of 0.75.

Figure 3 - Association between patient characteristics and inappropriate shocks; a) all patients; b) primary prevention patients

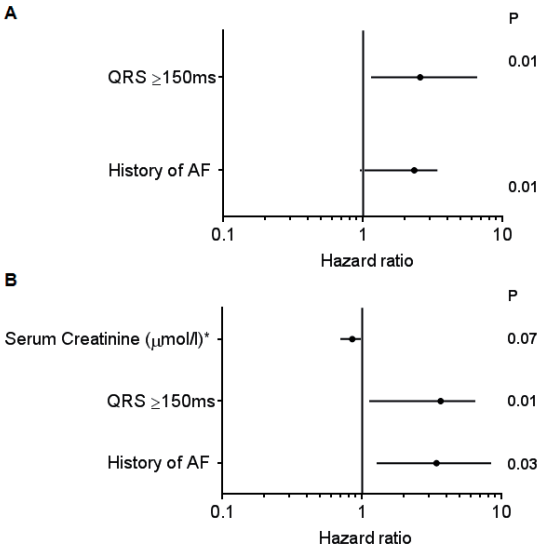
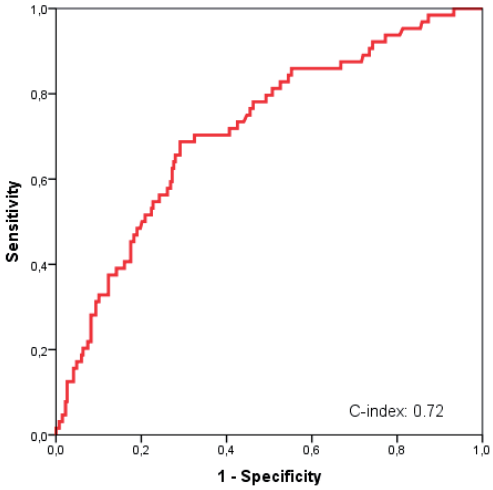


Figure 4 - ROC curve for a model predicting all-cause mortality containing left ventricular ejection fraction, serum creatinine and history of atrial fibrillation



Furthermore, in the full patient cohort, the model containing a history of AF and QRS duration ≥150ms discriminated between those with and without inappropriate shocks, with a C-index of 0.78. In primary prevention CR-D patients , we found a C-index of 0,80, using a prediction model containing a history of AF, QRS duration ≥150ms and serum creatinine for inappropriate shocks.

DISCUSSION

We performed a retrospective, multi-centre, observational, follow-up study, on 550 real-life CHF patients, treated with CRT-D. A history of AF appears to be an important determinant not only for mortality, but also for appropriate and inappropriate shocks, as shown in figure 5. Forty-six percent of all patients receiving inappropriate shocks appeared to have a history of AF at baseline. Other independent predictors of all-cause mortality included higher pre-implantation creatinine and poor LVEF.

Risk stratification for all-cause mortality

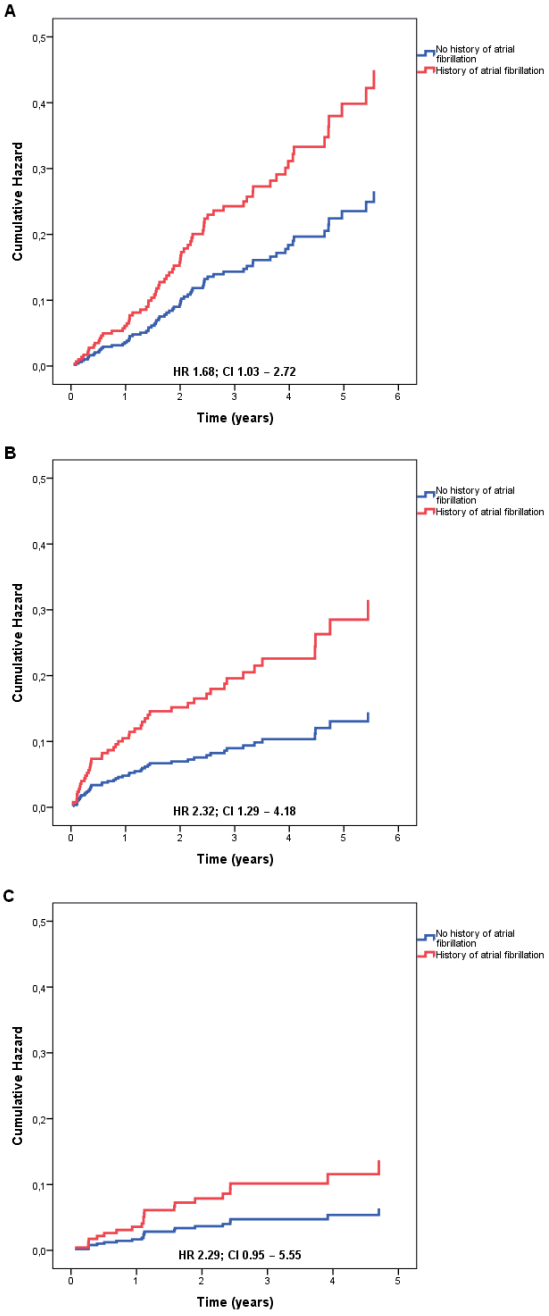
Our findings regarding predictors of mortality may represent the detrimental effects of a reduced cardiac function and progressive heart failure. AF is presumably a sign of generally worse cardiac status, particularly in CHF patients and therefore can be correlated with mortality, which has been shown in ICD trials.[15-19] Recently, Botto et al also found an association between a history of AF and all-cause mortality in CRT-D patients.[20] The fact that AF appears to be a risk factor for mortality in CRT-D patients could also be due to a reduced amount of biventricular pacing in patients with AF which consequently leads to less reversed cardiac remodeling and electrical remodeling.

Mean LVEF in our study population was 24%, which is comparable to the MADIT-CRT trial.[5] LVEF is currently the main criterion for eligibility for a primary preventive ICD or CRT-D device, mainly because a poor LVEF has proven to be one of the primary markers of cardiac death in patients.[16,17,21] We found a trend towards similar results in our real-life population of CHF patients treated with CRT-D.

Renal failure is generally associated with higher mortality, but can also be an expression of a poor cardiac function. Saxon et al also showed in the COMPANION trial that a poor renal function is associated with a higher mortality.[22] We have now extended these findings to a real-life population of patients treated with CRT-D.

Finally, appropriate shocks were independently associated with increased mortality. These shocks may by themselves cause damage to the myocardium, but receiving appropriate shocks is more likely an expression of a poor cardiac function and occurs probably in the sickest patients where clinical deterioration could be expected. The fact that inappropriate shocks were not associated with mortality also adds to the assumption that it is probably not the shock itself that is harmful, but more likely its underlying cause. It is also possible that the number of inappropriate shocks in our study was to low or the follow-up time was to short to find significant differences in mortality, because in contrast, MADIT RIT recently found a decreased mortality in patients receiving less inappropriate therapy.[23]

Figure 5 - Hazard curves of patients with and without atrial fibrillation and a) all-cause mortality; b) appropriate shocks; and c) inappropriate shocks.



Hazard ratios were derived from Cox proportional hazards models. All baseline variables were entered into the model and variables were withdrawn from the model if p≥0.1 until all factors had a p value <0.1.

Risk stratification for appropriate shocks

The occurrence of appropriate shocks may be considered as an important outcome given its effect on patient outcome, its potential detrimental effect on LV function and its socio-economic effects.

Appropriate shocks are related to a history of AF, which may be an expression of a poor cardiac function, but this relation could also be due to a reduced amount of biventricular pacing during periods of paroxysmal or persistent AF with subsequent less electrical remodeling. This could not only lead to mortality, but also result in a higher frequency of ventricular arrhythmias and consequently of appropriate shocks. AF can also initiate ventricular arrhythmias and consequently induce the need for appropriate shocks. The impact of AF on appropriate shocks has been shown before in an ICD study.[15] Also, a recent CRT-D trial displayed an association between recurrent or new atrial arrhythmias and the development of ventricular arrhythmias.[20] We found similar results in a population of CHF patients treated with CRT-D. Antiarrhythmics did not change the risk of having appropriate shocks in AF patients.

Currently, LVEF is the main criterion for assigning patients to CRT-D or an ICD. Several large trials have shown that ICD's improve survival among patients with a depressed LVEF but who have not previously had sustained ventricular arrhythmias.[24,25] This study adds to this information and shows that an even more depressed LVEF increases the risk of life threatening arrhythmias and consequently the need for backup defibrillation therapy.

Cardiac ischemia causes myocardial scarring and greater myocardial scarring tends to be at augmented risk for ventricular arrhythmias.[26] As a result, patients with an ischemic cardiomyopathy are more prone to ventricular arrhythmias, which is shown by the trend we found towards a higher vulnerability to appropriate shocks in patients with ICM.

Inappropriate shocks

Atrial fibrillation and other supraventricular tachycardia's are the leading cause of inappropriate shocks.[15,27-32] The device misdiagnoses AF as a potentially lethal ventricular arrhythmia and subsequently discharges inappropriately. In our series, 15 (46%) of the 33 patients receiving inappropriate shocks had a history of AF at baseline. Further studies are warranted to investigate whether optimal medical AF care may improve the balance of appropriate and inappropriate device therapy in CRT-D patients.

In the current study, multivariate analysis showed a positive correlation between a QRS duration of ≥150ms and the occurrence of inappropriate shocks. Wide QRS complexes express a slow conduction and are a sign of progressive cardiac dysfunction and dilatation of the ventricles and indirectly the atria. Such dilatation of the atria creates a substrate that could lead to AF which may acts as a trigger and subsequently induce inappropriate discharges.

Finally, previous studies have found, apart from AF, that several other variables may be associated with inappropriate shocks. These findings were not always as consistent. For example, both low and high NYHA class have been associated with inappropriate shocks.[29,31] In the current study, we have found an association between lower pre-implantation creatinine and inappropriate shocks. Such an association has not been previously demonstrated. This finding may be due the fact that 33% of all inappropriate shocks were caused by abnormal sensing or sinus tachycardia. Creatinine was lower in patients receiving inappropriate shocks due to these causes compared to the other patients in our cohort. Possibly, patients with a preserved renal function were more physically active, leading to a higher number of shocks from sinus tachycardia or abnormal sensing.[33] These patients were also younger, which has been associated with inappropriate shocks before.[28]

Studies on risk factors of having inappropriate shocks in real-life patients treated with CRT-D are scarce, and further research with larger cohorts and longer follow-up is warranted to provide more information on variables associated with inappropriate shocks to avoid device related hazards.

To the best of our knowledge, such an association have not yet been demonstrated in previous studies.

Clinical implications

Our findings may be helpful in predicting the prognosis of patients treated with CRT-D, and could assist clinicians in more optimal and appropriate patient selection for CRT.

A history of AF was associated with all-cause mortality as well as appropriate and inappropriate shocks. This increased mortality risk together with the greater need for defibrillation therapy implies a definite indication for CRT-D therapy. On the other hand, patients with a history of AF carry a high risk of receiving inappropriate device therapy.

Therefore, these results suggest that benefit may be derived from an optimal medicinal policy to keep patients in sinus rhythm, prevent AF, and possibly a His bundle ablation may be considered. A his bundle ablation would prevent a fast ventricular response rate, which could otherwise trigger the device to discharge inappropriately. An additional advantage of a His bundle ablation, is the fact that it could increase the amount of biventricular pacing in patients with AF and has proven to reduce mortality in CRT patients with AF.[34] None of the patients in our study population had received a His bundle ablation.

Study limitations

The first patient received CRT-D in February 2005 and the last consecutive patient received CRT-D in March 2011. Within this period of time, multiple publications on heart failure treatment and device based therapy have changed the guidelines for selecting

patients for CRT-D.[7,11] This could have caused heterogeneity in the study population, which may have affected the outcome.

A second limitation, is the fact that this study was performed retrospectively, which makes data collection challenging. Nevertheless, all data on primary and secondary outcome could be collected without loss to follow-up.

Even though all debatable ICD events have been double-checked by multiple experts and discussed at a weekly meeting, the absence of a core laboratory adjudicating all ICD events and therapy, may have caused heterogeneity in the interpretation of events. Moreover, despite the quite uniform device programming, it should be stated that variability in configuration could have influenced our outcome. Also, we did not investigate antitachycardia pacing rates, so we can not draw any conclusions regarding the influence of antitachycardia pacing rates on the outcome.

Finally, information on the biventricular pacing percentage was absent, which made it impossible to draw conclusions on the effect of biventricular pacing on outcomes in AF patients.

Conclusion

In symptomatic CHF patients treated with CRT-D, AF is an independent risk factor for mortality and appropriate shocks. However AF also is an independent risk for inappropriate shocks. Further efforts in AF management may optimize the appropriate care in CRT-D device patients.

Appropriate shocks were associated with mortality while inappropriate shocks were not.

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Chapter 11

Defibrillator Therapy: Comparison of Coronary Artery Disease and Dilated Cardiomyopathy

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ABSTRACT

Background: Since several large trials have proven the effectiveness of implantable cardioverter-defibrillators (ICDs) in patients with left ventricular dysfunction, disadvantages have become more apparent. As prognosis of patients with cardiovascular diseases is improving, assessment of ICD patients and re-evaluation the current guidelines, is mandatory. We aimed to evaluate differences in mortality and occurrence of (in)appropriate shocks in ICD patients with coronary artery disease (CAD) or dilated cardiomyopathy (DCM).

Methods: In a large teaching hospital, all consecutive patients with systolic dysfunction due to CAD or DCM who received an ICD with and without resynchronisation therapy, were collected in a database.

Results: A total of 320 consecutive patients (age 67±10 years) were classified as CAD patients and 178 (63±11 years) as DCM patients. Median follow-up was 40 months (interquartile range [IQR] 23 – 57 months). All cause mortality was 14% (CAD 15% vs DCM 13%).

Appropriate shocks occurred in 13% of all patients (CAD 15% vs DCM 11%, p=0,12) and inappropriate shocks occurred in 10% (CAD 8% vs DCM 12%, p=0,27).

Multivariate analysis demonstrated impaired LVEF, QRS>120, age ≥75 years and low eGFR as predictors for all-cause mortality.

Predictors for inappropriate shocks were permanent and paroxysmal atrial fibrillation.

Conclusions: Mortality rates were similar in patients with CAD and DCM, who received an ICD. Furthermore, no differences in the occurrence of appropriate and inappropriate ICD interventions between these patient groups were found.

INTRODUCTION

An implantable cardioverter-defibrillator (ICD) improves survival in patients with an impaired left ventricular function.[1-4] Despite these achievements, disadvantages, i.e. inappropriate therapy or non-benefit,[5-8] of ICDs have become more apparent over the years and cost-effectiveness should be optimized.[9] As prognosis of patients suffering from cardiovascular diseases is improving, current guidelines should be re-evaluated. For this purpose, follow-up data of patients who were assigned to ICD therapy according to the current guidelines are very helpful.

In the current guidelines for device-based therapy and prevention of sudden cardiac death,[10] the indication for ICD-therapy in patients with an impaired left ventricular function due to a dilated cardiomyopathy (DCM) and those for patients with systolic dysfunction due to coronary artery disease (CAD) closely resemble. Therefore, it is of interest to compare the outcome of ICD patients with DCM to ICD patients with CAD, to verify whether the current guidelines are still valid.

The aim of this study was to evaluate mortality and occurrence of both appropriate and inappropriate ICD shocks in patients with an impaired left ventricular function due to CAD and DCM. Furthermore, we assessed predictors for mortality, appropriate and inappropriate shocks.

METHODS

Study population

A database was constructed including all consecutive patients who received an ICD between January 2005 and June 2012 in a large teaching hospital. Follow-up lasted until October 2012.

Therapy assignment was based on the ESC guidelines for device based therapy.[10] Baseline characteristics of all patients were collected by reviewing hospital records and included demographics, medical history, medication, cardiovascular risk factors and electrocardiographic characteristics.

Fifty-two patients who received their ICD for other reasons than coronary artery disease or dilated cardiomyopathy (e.g. idiopathic ventricular fibrillation, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, long QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia) were excluded.

Patients were considered CAD patients if they had a history of myocardial infarction (including Q-wave or enzyme positive), a history of coronary artery disease at coronary angiography or one or more coronary artery bypass grafts (CABG) or percutaneous

coronary interventions (PCI). Renal function was assessed by estimating the baseline glomerular filtration rate (eGFR) using the abbreviated Modification of Diet in Renal Disease (MDRD) Study equation: eGFR (mL/min/1.73 m2 of body surface area) =186× (serum creatinine in mg/dL)−1.154 × (age)−0.203 ×0.742 in female subjects. Renal failure was defined as an eGFR <60 mL/min/1.73 m^2.

ICD follow-up

The majority of devices had a 3-zone configuration. The first zone was a monitor zone only, which was set to 160 ±10 bpm, the VT zone was set to 190 ±12 bpm and the VF zone was set to >209 ±15 bpm. In the Medtronic Inc., Minneapolis, MN, USA, devices, the number of intervals to detect was set to 18/24 episodes in all zones. In the Boston Scientific inc., Indianapolis, IN, USA, devices, the number of intervals to detect was set to 8/10 intervals, with a duration of 8s in the VT zone and 5s in the VF zone. For all patients, ICD programming was intended to avoid inappropriate therapy by activating the available discriminators, e.g. dual-chamber algorithms, onset, stability and morphology. For each patient, programming was tailored according to the clinical presentation.

During in-hospital and remote ICD follow-up, as part of usual care, ICD printouts were obtained every 3 months to determine the number and type of arrhythmias and the number of appropriate and inappropriate shocks. Patients were advised to contact the hospital after experiencing ICD therapy or mandated to visit the hospital if an ICD shock was detected by remote-monitoring. ICD therapy was only considered appropriate when delivered for ventricular tachyarrhythmias. All debatable ICD events were double-checked by multiple experts and discussed at a weekly meeting.

Statistical analysis

Continuous data was analyzed with Student's t test or Mann-Whitney U test, when appropriate. Categorical characteristics were compared by using the χ^2 test.

Kaplan-Meier's log-rank test was used to compare differences in all-cause mortality, appropriate shocks and inappropriate shocks between CAD and DCM patients. Furthermore, multivariable Cox proportional hazards regression was used to examine the association between patient characteristics and outcome (HRs). Characteristics were entered into the multivariable model if they showed a statistically significant association with the outcome during univariable analysis (P value <0.05).

Overall statistical significance was set at a 2-tailed P value <0.05. SPSS 20.0 (SPSS Inc, Chicago, IL) was used for the statistical analysis.

RESULTS

Study population

The study population consisted of 498 consecutive patients who received an ICD between January 2005 and June 2012 in a large teaching hospital. Baseline characteristics are displayed in table 1. The CAD group comprised 320 patients (64%). The DCM group consisted of 178 (36%) patients. The CAD group contained more males than the DCM group (CAD 85% vs. DCM 62%, p<0.001) and were older (CAD 67±10 vs DCM 63±11, p<0.047). Mean left ventricular ejection fraction was 24%±7% and was not significantly lower in one of the groups (25±6 vs 23±8, p<0.131). Permanent atrial fibrillation was more frequent in DCM patients (8% vs 18%, p=0.002).

The use of beta-blockers (CAD 93% vs DCM 93%, p=0.76) and ace-inhibitors/ARBs (CAD 96% vs DCM 97%, p=0.59) did not significantly differ between the groups, but the use of diuretics was higher in patients with DCM (CAD 74% vs DCM 84%, p=0.008). A total of 166 patients (33%) received cardiac resynchronization therapy (CRT) (CAD 28% vs DCM 43%, p<0.001).

Mortality

Figure 1 displays the results of our outcome on mortality, appropriate and inappropriate shocks. Overall mortality of the total study population was 14.5% (72 patients), during a median follow-up of 40 months (IQR 23-57 months) with a median survival time of 31 months (IQR 20 - 44 months). There were no significant differences in mortality between CAD (49 patients, 15%) and DCM (23 patients, 13%) patients (p=0.46) (Figure 2).

Univariable analysis displayed the following predictors for mortality: age≥75 years, LVEF, NYHA class III-IV, permanent AF, QRS>120ms, eGFR and haemoglobin. Multivariate analyses showed that impaired LVEF (HR 0.94, CI 0.90-0.99), age≥75 years (HR 2.18, CI 1.19-3.97), QRS>120ms (HR 2.50, CI 1.21-5.16) and low eGFR (HR 0.98, CI 0.97-0.99) were independent predictors for mortality.

Appropriate ICD therapy

A total of 67 patients (13.5%) received ≥1 appropriate shock and 43 Patients (9%) received >1 appropriate shock during follow-up. The median interval to first appropriate shock after ICD implantation was 21.8 months (IQR 4.9-35.0 months). Cumulative incidence of appropriate shocks was 4.4%, 7.2% and 13.1% at 1, 2 and 5 years follow-up. There were no significant differences in the occurrence of appropriate shocks between CAD patients and DCM patients (CAD 15.0% vs DCM 10.7%, p=0.12)(Figure 3a).

Use of digoxin (15% vs 4%, HR 2.97, CI 1.50-5.88) and a history of smoking (62% vs 48%, HR 2.00, CI 1.77-2.98) predicted appropriate shocks.

Table 1 - Baseline Characteristics

Characteristic	All (n=498)	CAD (n=320)	DCM (n=178)	P Value
Age, years	66±10	67±10	63±11	0,047
Male gender	382(77)	272(85)	110(62)	<0,001
LVEF,	24±7	25±6	23±8	0,13
NYHA classification				
I-II	359(80)	233 (81)	126(79)	0,62
III-IV	87 (20)	54(19)	33(21)	
History of atrial fibrillation				
Permanent	59(12)	27(8)	32(18)	0,002
Paroxysmal	52(10)	39(12)	13(7)	0,09
QRS duration, ms	132±32	130±31	136±35	0,003
QRS > 120ms	254(53)	159(52)	95(57)	0,29
Serum creatinin (µmol/L)	117±79	123±90	105±51	0,06
eGFR (ml/min/1.73m^2)	65±23	63±23	68±22	0,73
Renal Failure	176(40)	121(43)	55(35)	0,13
Hemoglobin (mmol/L)	8,6±1,0	8,6±1,0	8,6±1,1	0,53
Implanted device				
Atrial lead	353(71)	223(70)	130(73)	0.43
CRT	166(33)	89(28)	77(43)	<0,001
Risk factors				
Diabetes	95(19)	69(22)	26(15)	0,06
History of smoking	213(50)	144(53)	69(45)	0,12
Hypertension	147(42)	136(44)	75(44)	0,90
Cardiovascular medication				
Amiodarone	56(11)	42(13)	14(8)	0,08
Beta-blocker	457(93)	294(93)	163(93)	0,95
Digoxin	29(6)	12(4)	17(10)	0,008
ACE inhibitor/ATII antagonist	474(96)	304(96)	170(97)	0,59
Diuretics	382(77)	234(74)	148(84)	0,008

Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. ACE indicates angiotensin-converting enzyme; ATII, angiotensin-II; eGFR, estimated glomerular filtration rate; ICD, Implantable cardioverter-defibrillator; LVEF, Left ventricular ejection fraction; NYHA, New York Heart Association.

Inappropriate ICD therapy

Inappropriate ICD shocks occurred in 48 patients (9.6%). Twenty-four patients (5%) had >1 episode of inappropriate shocks. The median time between implantation and the first inappropriate shock was 6.1 months (IQR 2.3-26.6 months). Cumulative incidence of inappropriate shock therapy was 5.4%, 7.2% and 9.0% at 1, 2 and 5 years follow-up.

Figure 1 - All-cause mortality, appropriate and inappropriate shocks in coronary artery disease (CAD) and dilated cardiomyopathy (DCM) patients

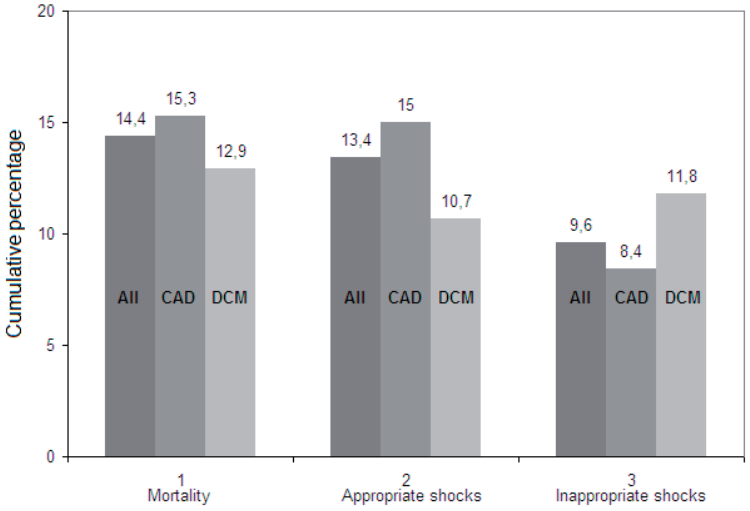
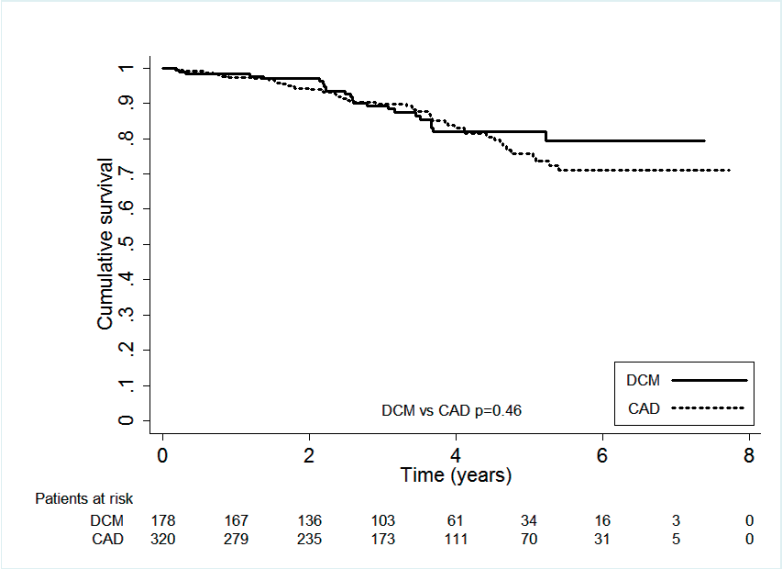


Figure 2 - Kaplan-Meier survival curve of all-cause mortality.

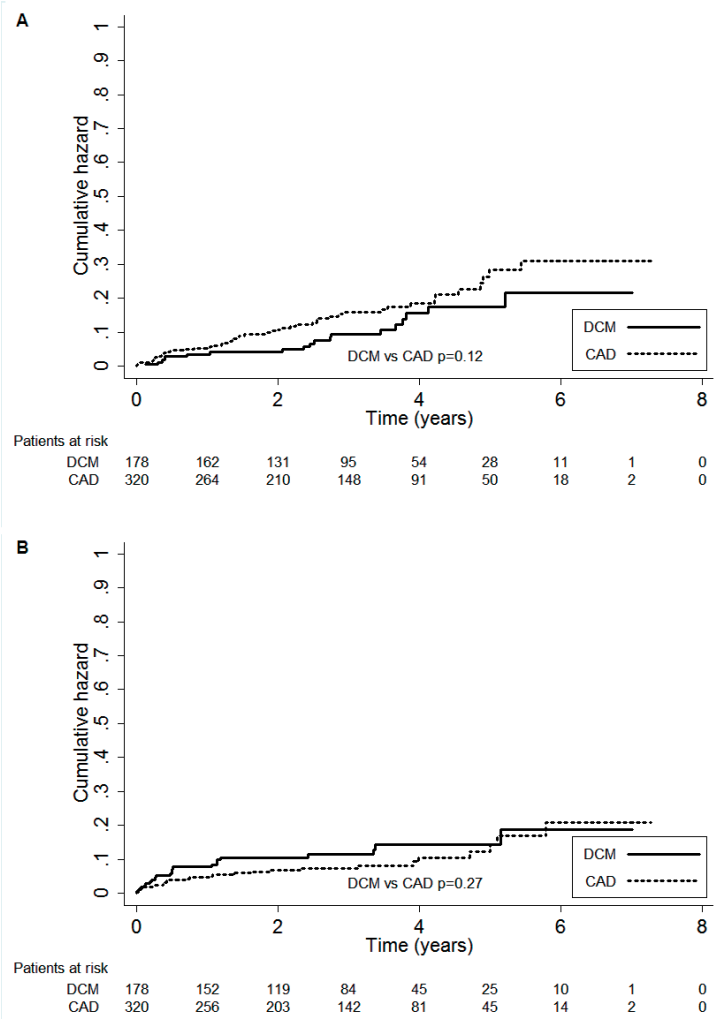


Coronary artery disease (CAD) versus dilated cardiomyopathy (DCM) patients.

There was no significant difference in occurrence of inappropriate shock therapy between CAD patients and DCM patients (CAD 8% vs DCM 12%, p=0.27) (Figure 3b).

Inappropriate shocks occurred significantly more in patients with permanent AF (22% vs 8%, p=0.001), and also in patients with paroxysmal AF (17% vs 9%, p=0.048). Most

Figure 3 - Kaplan-Meier hazard curve of a) appropriate shocks and b) inappropriate shocks.



Coronary artery disease (CAD) versus dilated cardiomyopathy (DCM) patients.

inappropriate shocks in this study were caused by supraventricular tachyarrhythmias (78%), mainly AF. Other causes of inappropriate shocks were shock lead dysfunction (18%) and T wave oversensing (4%).

Multivariate analyses displayed permanent AF (HR 2.85, CI 1.16-7.01) and paroxysmal AF (HR 2.84, CI 1.20-6.74) as independent predictors for inappropriate shocks.

DISCUSSION

We performed a retrospective, observational, follow-up study, on 498 real-life patients, treated with an ICD and evaluated the difference in mortality and occurrence of ICD shocks in patients with left ventricular dysfunction due to CAD versus DCM. All patients received their ICD according to the current guidelines.

The major findings of this study were: (1) Mortality rates are equal in CAD and DCM patients. (2) Incidence of appropriate and inappropriate shocks was similar in both groups. (3) Predictors for mortality in ICD patients were impaired LVEF, age≥75 years, QRS>120ms and low eGFR. (4) Predictors for inappropriate ICD intervention were permanent and paroxysmal AF.

Mortality

Overall cumulative incidence of all-cause mortality of the total study population was 14.5% and at 1, 2 and 5 years, mortality rates were 2.2%, 4.2% and 13.5% respectively. For CAD patients, mortality rates at 1, 2 and 5 years were 2.5%, 5.3% and 14.1% respectively, and for DCM patients 1.7%, 2.8% and 12,4%. Compared to the landmark trails, the cumulative incidence off all-cause mortality was relatively low. The Sudden Cardiac Death in Heart Failure Trial SCD-HeFT found a total mortality rate of 29% at 5 year follow-up and the Multicenter Automatic Defibrillator Implantation Trial II (MADIT-II) reported a mortality-rate of 16% at 2 years follow-up and 52% at 8 years.[2,3,11] This lower mortality could explained by the fact that treatment of patients with systolic dysfunction has improved over time, which may have contributed to a lower mortality in our study population compared to the trials mentioned. Another factor contributing to the lower mortality could be due to the fact that 33% of our study population received CRT, which improves left ventricular function and reduces mortality.[12]

As ICD implantation and follow-up is expensive and as costs in medical practise are rising, a stricter selection of eligible patients is mandatory. The relatively low mortality in our study population calls for reassessment of the indications for an ICD. Therefore, further studies and registries of real-life ICD patients are required to make a more appropriate selection of patients eligible for ICD implantation possible.

Finally, whereas ICDs only act as "safety net" and antiarrhythmic medication has potential side effects and requires close monitoring, the search for other methods to withstand arrhythmias continues. Catheter ablation is an accepted technique and nowadays commonly used in treatment of arrhythmias. Catheter ablation has proven its effectiveness in treatment of VTs in patients with structural heart disease due to CAD or DCM with even higher success rates in CAD patients.[13]

Appropriate intervention

In our study, 13.5% of all patients received appropriate shocks, and this number did not significantly differ between CAD and DCM patients. The SCD-HeFT trial reported a total number of appropriate shocks of 21%, which is higher than the 13% of all patients receiving appropriate shocks we reported. This difference might be clarified by the fact that device programming has improved over time and anti-arrhythmic medication has been enhanced. Also, as stated before, 33% of our study population received CRT, which could also have had a beneficial effect on the number of appropriate shocks by increasing LVEF.

There is some evidence that the number of ventricular tachyarrhythmias are comparable in CAD patients and DCM patients.[14,15] This had also been shown by some small previous studies.[16,17] Our study adds to these findings by showing that the number of ICD shocks are also equivalent in these both groups, even though one third of our patients received CRT, which may have a more beneficial effect on LVEF in DCM patients compared to CAD patients.[18] Our findings confirm the validity of the current guidelines.

Recently, studies have shown that medication indeed reduces appropriate ICD therapy in patients with ischemic heart disease.[19-21] Since ventricular tachyarrhythmias are the major cause of sudden cardiac death, it is important to reduce these arrhythmias and therefore appropriate shocks, which have also proven to be an independent predictor of mortality.[22]

Our study shows history of smoking is a predictor for appropriate shocks, which has been shown before in previous studies.[23] Smokers have increased atherosclerosis, which increases the occurrence of ischemic events, and myocardial scarring, eventually resulting in more tachyarrhythmias and consequently more appropriate shocks.

Finally, most of the patients who received an ICD never received shock therapy, suggesting that a more patient-focused risk stratification could improve clinical benefits and cost-effectiveness. [24]

Inappropriate intervention

In this study, inappropriate shocks occurred in 9.6% of all patients, mostly caused by atrial tachyarrhythmias classified as ventricular tachyarrhythmias, which subsequently caused inappropriate discharges.

Age <75 years was also associated with inappropriate shocks. Younger age is associated with sinus tachycardia and abnormal sensing. This finding has been reported before,[25] and is most likely the explanation for the association between age <75 years and inappropriate shocks. Patients who had an ICD as secondary prevention had a slightly better left ventricular ejection fraction compared to the primary prevention patients and a lower NYHA class. Possibly, these patients were more physically active, which could also lead to a higher number of shocks from sinus tachycardia or abnormal sensing.

Most frequent causes of inappropriate therapy have been studied, and can result in reduced quality of life and even provocation of ventricular arrhythmias.[5,26] An additional phenomenon is phantom shocks - the sensation of an ICD discharge in the absence of an actual discharge – which occur, though not significant, more in patients who received appropriate or inappropriate shocks.[27] Therefore, the occurrence of inappropriate shocks should be minimized as much as possible. Recent studies have shown that enhanced programming algorithms during follow-up reduces inappropriate therapy and even mortality.[28,29]

Limitations

Present study has several limitations. Patients were included in the period between January 2005 and June 2012, so follow-up of the last included patients was only 3 months while some patients have a follow-up of up to 7 years. Within this period of time, multiple publications on treatment of ventricular tachyarrhythmias and device based therapy have changed the selection of eligible patients for ICD treatment. This could have caused heterogeneity in the study population, which may have affected the outcome. This limitation did not influence the aim of the study, since this limitation applies to both CAD and DCM patients.

A second limitation, is the fact that this study was performed retrospectively, which makes data collection challenging. Nevertheless, all data on primary and secondary outcome could be collected without loss to follow-up.

Conclusion

This study shows that mortality and occurrence of appropriate and inappropriate ICD shocks are similar in patients with an ischemic or a dilated cardiomyopathy. An impaired LVEF, age ≥ 75 years, QRS>120ms and low eGFR predicted mortality. Use of digoxin and a history of smoking predicted appropriate shocks. Permanent AF and paroxysmal AF are predictors for inappropriate shocks.

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Chapter 12

Predicting mortality among implantable defibrillator patients treated with cardiac resynchronization therapy: derivation and validation of a risk estimation model

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Submitted

ABSTRACT

Background: The beneficial effects of cardiac resynchronization defibrillator (CRT-D) in patients with heart failure, low LVEF, and wide QRS have clearly been established. Nevertheless, mortality remains high in some patients. The aim of this study was to develop and validate a risk score to identify patients at high risk for early mortality who are implanted with a CRT-D.

Methods: For predictive modeling, 1282 consecutive patients from 5 centers (74% male; median LVEF 25%; NYHA class III-IV 60%; median QRS-width 160 ms) were randomly split into a derivation and validation group (50%/50%). A risk score was developed using logistic regression.

Results: In the total cohort, 181 patients died over 3years of follow-up. After multivariate analysis, a risk score was developed based on myocardial infarction, LVEF ≤ 25%, COPD, chronic kidney disease (CKD), hyponatremia and anemia. At 3 years, mortality was 4.6%, 13.2% and 29.7% by ascending tertile of risk score. Compared with the lowest tertile (T1), mortality was significantly higher in the other tertiles (T2 odds ratio (OR) = 3.1; T3 OR = 8.4; both $P < 0.001$). Discrimination was modest (*C*-statistic 0.73) and the Hosmer-Lemeshow chi-square was 0.95 ($P = 0.33$).

Conclusions: A risk score based on routine, readily available clinical variables can reliably identify patients at high risk for early mortality within 3 years after CRT-D implantation.

INTRODUCTION

Heart failure (HF) is a progressive disease associated with high morbidity and mortality. The prevalence of HF is increasing and the associated costs are rising.[1,2] Data from randomized and observational studies have shown the beneficial effect of cardiac resynchronization therapy (CRT) in selected patients with drug refractory HF, reduced left ventricular ejection fraction (LVEF), and electrical dyssynchrony: it improves clinical symptoms, reduces hospitalizations and lowers mortality in a considerable proportion of patients.[3-6] In addition, HF patients with low LVEF are at increased risk for arrhythmic death. Prophylactic implantable cardioverter-defibrillator (ICD) implantation is indicated for patients with ischemic or non-ischemic cardiomyopathy and LVEF ≤ 35%.[7-9] Theoretically, all patients who meet the indication criteria for CRT also qualify to have an ICD for primary prevention of sudden cardiac death. Consequently, ICDs combined with CRT (CRT-D) are part of the standard management of HF patients with reduced LVEF.[10,11] However, given the heterogeneity in mortality risk among HF patients and the fact that only a minority of patients will experience ventricular arrhythmias, appropriate risk prediction is of paramount importance in maximizing the survival benefit conferred by the CRT-D.[12] Several models have been developed to predict mortality risk in HF patients such as the Seattle Heart Failure Model (SHFM) and the Heart Failure Survival Score (HFSS).[13,14] Despite the fact that the SHFM takes the eventual use of device therapy, such as an ICD or CRT, into account, the model was not designed for HF patients who already had a device implanted. A risk estimation model to predict mortality in HF patients following CRT-D implantation does not exist. Therefore, the purpose of this study was to develop a risk estimation model to predict mortality in primary prevention CRT-D patients.

METHODS

Study population

We used data from prospective ICD registries of the cardiology departments of Erasmus MC (Rotterdam, the Netherlands), the University Hospital of Basel (Basel, Switzerland), Ziekenhuis Oost-Limburg (Genk, Belgium), Medisch Centrum Alkmaar (Alkmaar, the Netherlands), and Medisch Spect Twente (Enschede, the Netherlands). From these registries, all patients (n = 1282) who received a first implantation of a CRT-D device for the primary prevention of sudden cardiac death between January 1st, 2000 and October 31st, 2013 were identified.

Data collection, variable definitions and clinical endpoint

Potential candidate variables associated with mortality in heart failure were identified based on review of the literature, clinical relevance, and their routine availability. They included demographic characteristics, clinical presentation, laboratory data, and preexisting comorbid conditions.

Demographics, clinical data, and medical therapy prior to CRT-D implantation were obtained for all patients by searching the health records of the hospital. If multiple laboratory data were available, values from the date closest to the date of implantation were used; all laboratory values obtained up to 7 days prior to CRT-D implantation were accepted.

Diabetes mellitus was defined as HbA1c > 6.5% or the use of oral hypoglycemic agents or use of parenteral insulin; anemia as a serum hemoglobin concentration of < 12 g/dL (female) or < 13 g/dL (male). The glomerular filtration rate (GFR) was estimated with the formula of Modified Diet in Renal Disease (MDRD).[15] Renal function was stratified according to the KDIGO/KDOQ stages for chronic kidney disease (CKD): stage 1, i.e. ≥ 90 ml/min/1.73m^2; stage 2, i.e. 60-89 ml/min/1.73m^2; stage 3, i.e. < 60 ml/min/1.73m^2 and stage 4, i.e. < 30 ml/min/1.73m^2.[16] Presence of CKD was defined as GFR < 60 ml/min/1.73m^2. Hyponatremia was defined as serum sodium level < 136 mmol/L and sodium levels of 136 - 137 mmol/L as low normal.

The clinical end-point for this study was all-cause mortality; patients who underwent cardiac transplantation were censored at the day of transplantation.

Statistical analysis

For the purpose of this study, half of the patients were randomly selected by use of random integer assignment to form the derivation cohort, and the remainder formed the validation cohort. Summary baseline data are presented as median with 25th and 75th percentiles, and categorical data are presented as percentages and counts. Data were compared by the Kruskal-Wallis *H* test and chi-square test as appropriate. Although most patients had a relatively complete data set, variables with > 5% of missing data were excluded from analysis (body mass index, diastolic and systolic blood pressure, and baseline heart rate). The method of multiple imputation was used to include variables with < 5% of missing data in model selection and regression analysis.

Candidate variables in the derivation cohort that were associated with mortality on univariate analysis ($P \leq 0.1$) were included as covariates in a multiple logistic regression model. Backwards stepwise regression analysis was performed to determine the most predictive variables in the model equation, with a cutoff *P* value of 0.05 for retention in the model. A risk score was calculated for each individual patient as the value of the sum of products of the identified independent variables and their computed coefficients

(β1x1 + β2x2+ ... βnxn| where x1, x2, and xn are the values for the identified variables and β1, β2, and βn are the coefficients).

To assess the prognostic value of the risk score, the population was stratified into tertiles of the continuous risk score. Cumulative mortality rates were calculated according to the Kaplan-Meier method and differences between groups compared with the log-rank test. The discriminative properties of the model were assessed by the Harrell *C* index for Cox models. In addition, the receiver operating characteristic area under the curve (ROC AUC) was assessed. Model discrimination was deemed poor if the *C*-statistic and ROC AUC was between 0.50 and 0.70, modest between 0.70 and 0.80, and good if > 0.80. Calibration of the model was assessed by the Hosmer-Lemeshow goodness-of-fit test. The model was validated externally by assessing model performance in the validation cohort.

Statistical analysis was performed using STATA version 11 SE for Windows (StataCorp, College Station, TX) and SPSS version 21 (IBM Corp., Somers, NY). Statistical significance was defined as $P < 0.05$ (two-tailed).

RESULTS

Description of the derivation and validation cohorts

The derivation cohort consisted of 639 patients and the validation cohort of 643 patients (N = 1282 patients). Demographics and clinical characteristics of both cohorts are presented in Table 1. The derivation and validation cohort were similar with respect to age, gender, etiology of heart failure, comorbid conditions, laboratory values, and medical treatment. The majority of CRT-D recipients were men (76%) with a median age of 66 years. Ischemic etiology of heart failure was present in 50% of the patients.

The mortality rate in the overall cohort was 5.7% and 16.5%, at 1 and 3 years, respectively. In the derivation cohort (n = 639), 1- and 3-year mortality was 5.0% and 15.9%. In the validation cohort (n = 643), 1- and 3-year mortality was 6.4% and 17.0%. The mortality rates were not different between the derivation and the validation cohort ($P = 0.64$).

Predictors of mortality

Univariate logistic regression analysis was performed to identify variables associated with mortality. We found that increased age, the presence of myocardial infarction, diabetes mellitus, COPD, CKD, hyponatremia and anemia were all associated with a higher risk of mortality. In addition, LVEF < 25% was also associated with a higher risk of mortality. Subsequently, a backward stepwise regression analysis was performed to select the most predictive variables for mortality. Model predictors of 3-year mortality included presence of myocardial infarction, COPD, CKD, hyponatremia, anemia, and

Table 1 - Clinical characteristics of the derivation and validation cohorts

	Derivation cohort (n=639)	Validation cohort (n=643)	*P* value
Demographics			
Age, y	67 (58 - 73)	66 (59 - 72)	0.95
Male gender	483 (76%)	469 (73%)	0.31
Clinical characteristics			
NYHA class III-IV	372 (58%)	374 (58%)	0.91
Ejection fraction, %	24 (20 – 30)	25 (20 – 30)	0.40
Ischemic etiology	313 (49%)	326 (51%)	0.64
QRS duration, ms	160 (140 - 180)	160 (140 - 177)	0.07
Comorbid condition			
Atrial fibrillation	128 (20%)	139 (22%)	0.54
Diabetes mellitus	163 (26%)	169 (26%)	0.75
Cerebrovascular disease	54 (8%)	72 (11%)	0.11
Chronic obstructive pulmonary disease	93 (15%)	83 (13%)	0.42
Renal failure	254 (40%)	254 (40%)	0.91
Laboratory values			
Hemoglobin, g/dL	13.7 (12.4 - 14.7)	13.9 (12.7 - 14.9)	0.05
Serum sodium, mmol/L	140 (137 - 142)	140 (137 - 142)	0.79
Serum BUN, mg/dL	8.3 (6.4 - 11.7)	8.1 (6.1 - 10.9)	0.16
Serum creatinine, mg/dL	1.1 (0.9 - 1.4)	1.1 (0.9 - 1.4)	0.23
Medication			
ACE inhibitor	481 (75%)	476 (74%)	0.65
Angiotensin receptor blocker	152 (24%)	160 (25%)	0.65
Amiodarone	82 (13%)	81 (13%)	0.93
Betablocker	521 (82%)	528 (82%)	0.83
Digoxin	104 (16%)	127 (20%)	0.11
Diuretic	513 (80%)	513 (80%)	0.82
Aldosteron antagonist	309 (48%)	284 (44%)	0.15
Allopurinol	48 (8%)	55 (9%)	0.54
Statin	350 (55%)	390 (61%)	0.04

Continuous data are presented as median (interquartile range). Categorical data are presented as n(%).
ACE = angiotensin converting enzyme; BUN = blood urea nitrogen; NYHA = New York Heart Association

LVEF < 25% (Table 2). Age either as continuous variable or as binary variable with different age cutoffs did not reach statistical significance in multivariate analysis.

Risk scores were derived for each individual patient using the obtained β-coefficients.

Risk score *= 0.690 * (MI) + 0.645 * (LVEF25) + 0.632 * (COPD) + 0.890 * (CKD) + 0.712 * (hyponatremia) + 0.495 * (anemia)*

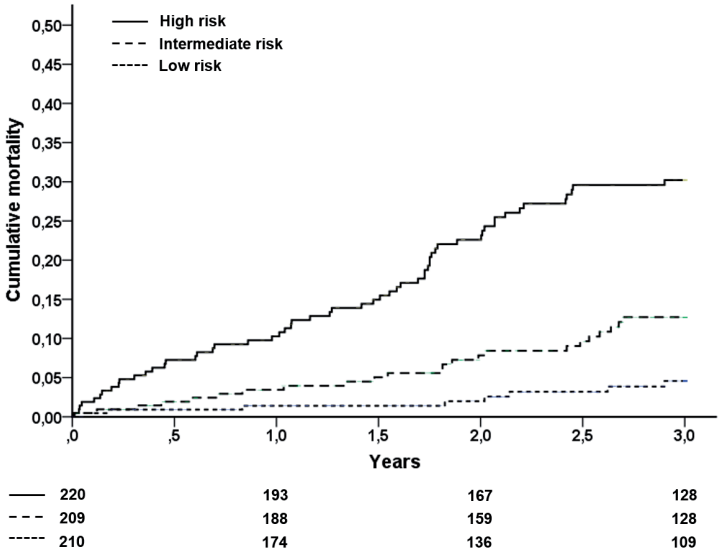
Table 2 - Multivariable analysis of predictors of all-cause mortality

Predictor	OR (95% CI)	*P*-value
Myocardial infarction	1.99 (1.24 - 3.22)	0.005
Ejection fraction 25%	1.91 (1.12 - 3.25)	0.02
Chronic obstructive pulmonary disease	1.88 (1.05 - 3.39)	0.04
Renal failure	2.44 (1.48 - 3.99)	< 0.001
Hyponatremia	2.04 (1.25 - 3.33)	0.004
Anemia	1.64 (1.00 - 2.71)	0.05

where: LVEF25 = LVEF ≤ 25%, 1 if present, otherwise 0; CKD = estimated GFR < 60 ml/min/1.73m^2, 1 if present, otherwise 0; Hyponatremia = serum level of sodium < 138 mmol/L, 1 if present, otherwise 0; Anemia = serum level of hemoglobin < 12 g/dL, 1 if present, otherwise 0; MI, COPD = 1 if present, otherwise 0.

Subsequently, the derivation cohort was stratified by ascending tertiles of the derived risk score (low to high risk). Mortality rates by tertiles of risk are presented in Figure 1. At 3-years follow-up, mortality rates were 4.6% in the low-risk group, 11.0% in the intermediate-risk group, and 29.1% in the high-risk group. Model discrimination as assessed by the *c*-statistic was modest (*c*-index of 0.74 at 3-years follow-up). In addition, the calculated area under the ROC curve was 0.74 (95% CI, 0.68 to 0.79). Calibration of the model was assessed by the Hosmer-Lemeshow test. There was no evidence of lack of fit (chi-square = 0.95; *P* = 0.33).

Figure 1 - Mortality rates in the derivation cohort stratified by risk group

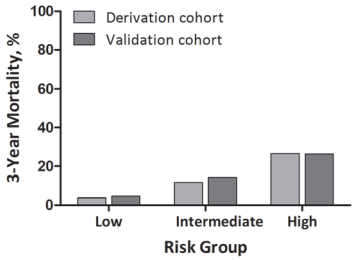


Dotted line = low risk; dashed line = intermediate risk; solid line = high risk.

Model validation

When the 3-year model was applied in the validation cohort (N = 643 patients), the discriminative ability of the model was maintained with an area under the ROC curve of 0.73 (95% CI, 0.68 to 0.79). When comparing the areas under the ROC curve, no difference between the derivation and validation cohort was observed (Figure 2). The predicted and observed mortality rates were in close agreement among the risk groups (Figure 3). Given the good performance of the tentative model in both cohorts, data were merged to develop a final model on the entire cohort of 1282 patients. In Table 3, the mortality risk for the risk groups is presented.

Figure 2 - ROC-curves for the derivation and validation cohort



Solid line = derivation cohort; dashed line = validation cohort.

Figure 3 - The 3-year mortality rates for the derivation and validation cohort stratified by risk group

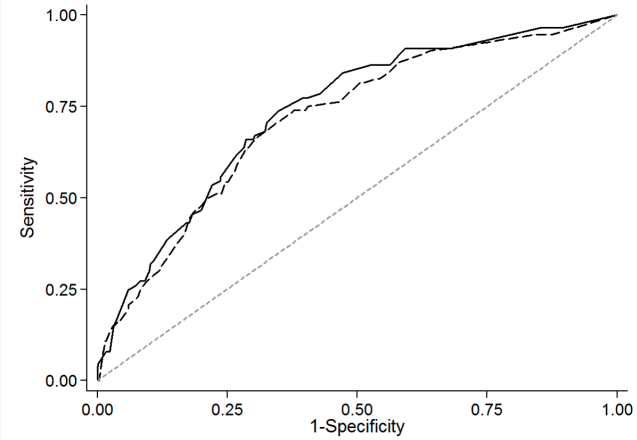


Table 3 - Mortality risk sorted by risk group

Risk groups	1-year mortality, %	3-year mortality, %	OR (95% CI)	*P*-value
Low risk	1.4%	4.6%	1.00	
Intermediate risk	3.4%	12.7%	3.28 (1.43 – 7.50)	0.005
High risk	10.3%	30.2%	9.87 (4.58 – 21.30)	< 0.001

CI = confidence interval; OR = odds ratio.

DISCUSSION

The present international, multicenter, retrospective cohort study of 1282 HF patients demonstrates the feasibility of using a risk score to predict early mortality in a real-world population of CRT-D recipients. The risk score incorporates myocardial infarction, LVEF ≤ 25%, COPD, CKD, hyponatremia, and anemia. These variables are readily available and individually associated with a poor outcome.

In HF patients with reduced LVEF, NYHA class ≥ II, and prolonged QRS duration, CRT improves clinical symptoms, reduces hospitalizations and lowers mortality in a considerable proportion of patients.[5,17] Patients eligible for CRT also qualify for defibrillator therapy as primary prevention of sudden cardiac death. Consequently, implantation of a CRT-D is part of the standard management of HF patients with reduced LVEF. However, the benefit of defibrillator therapy is not uniform and it remains to be determined which patients benefit and whether patients do not benefit from defibrillator therapy.

Better identification of patients who get the highest benefit of the additional defibrillator therapy is desirable to reduce unnecessary implantations and possible complications. Several previous studies have developed risk scores to estimate mortality in ICD recipients.[18-22] Recently, a systematic review and meta-analysis determined older age, poor baseline renal function, history of COPD, diabetes mellitus, peripheral vascular disease, decreased LVEF, and ICD shocks during follow-up as strong predictors of mortality in ICD patients.[23] Some of these predictors were also identified in our study, e.g. poor baseline renal function, COPD and decreased LVEF.

In a sub-analysis of the MADIT-II Trial, a risk score consisting of 5 clinical risk factors (NYHA class > II, atrial fibrillation, QRS duration > 120 ms, age > 70 years, and urea > 26 mg/dL) was developed to differentiate between patients who would benefit from the ICD vs. those who would not.[19] The MADIT-II risk score has recently been evaluated in cohorts of CRT patients showing poor to modest discrimination (*C*-statistic of 0.61 and 0.72).[24,25] Of the 5 risk factors in the MADIT-II score, only poor baseline renal function was a factor in our risk score. Risk factors as QRS width > 120 ms and NYHA > II mostly indicate CRT use.

The results of the present study are in line with those of several previous studies, indicating that renal dysfunction poses a strong and independent risk factor for overall mortality despite CRT-D implantation and optimized medical treatment of congestive heart failure. Besides renal dysfunction, we identified other clinical risk factors such as hyponatremia and anemia. Hyponatremia is a strong determinant of long-term mortality in HF patients, irrespective of LVEF.[26] Sharma *et al.* investigated the prognostic implication of hyponatremia in HF patients receiving CRT.[27] Low baseline serum levels of sodium were associated with poor prognosis. The results of our study confirm the

association between low serum levels of sodium and a higher mortality risk even in a multivariate analysis.

The impact of baseline anemia on all-cause mortality in HF patients with reduced LVEF has been evaluated in the HF-ACTION trial.[28] Over a median follow-up of 30 months, anemia was associated with increased rates of death, hospitalizations, and HF exacerbation. Venkateswaran *et al.* examined the prognostic implication of anemia in CRT patients.[29] Baseline anemia and early post-implantation decline of hemoglobin were associated with a worse 2-year prognosis. In our study, baseline anemia was independently associated with higher mortality.

Taken together, our results confirm that medically complex HF patients, those with low LVEF, anemia, hyponatremia, and comorbidities as COPD and CKD, have an increased risk of mortality. This finding may be explained by an increase in HF and non-arrhythmic mortality as the presence of these clinical variables suggests a more advanced HF status. The decision whether to add ICD therapy is at the discretion of the physician. In this context, Levy *et al.* provided compelling evidence of the heterogeneity of risk among primary prevention ICD patients by applying the SHFM to the SCD-HeFT study cohort.[12] The highest risk group had an increased mortality with no benefit of ICD therapy despite the greatest incidence of appropriate ICD shocks.

Limitations

The current study has several limitations and these should be viewed in its methodological context. First, the design was a retrospective cohort study with prospectively collected data which cannot be compared with that of a randomized clinical trial. In addition, the risk score was not used to decide on implantation of a CRT-D. Second, the study cohort included patients over a 13-year period, during which guidelines for the implantation of defibrillators and treatment of HF changed. In the same period, the programming of devices with respect to detection and treatment of ventricular arrhythmias changed.

Conclusion

A risk score based on routine, readily available clinical variables can reliably identify patients at high risk for early mortality within 3 years after CRT-D implantation.

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Epilogue

Chapter 13

Summary and conclusions

Health care can be divided into preventive, curative, rehabilitative and supportive medicine. Preventive medicine is regarded as the most effective form of medical practise, since good preventive medicine avoids the necessity of curative, rehabilitative and supportive medicine. Preventive medicine does not only aim at preventing diseases from happening (i.e., primary prevention), but also at reducing the impact of a disease that has already occurred and softening the impact of an ongoing illness (i.e., secondary- and tertiary prevention, respectively). Major improvements in cardiovascular medical care, such as percutaneous coronary interventions (PCIs) and implantable cardioverter-defibrillators (ICDs), have significantly reduced mortality, but this improvement in survival has also led to an increase in the number of patients suffering from chronic heart disease. To further improve secondary prevention in these patients, novel methods for risk stratification are urgently needed.

The aim of this thesis was to identify characteristics associated with adverse clinical outcome, and to present novel prediction models that may aid in risk stratification of patients at different stages of cardiovascular disease. First, we focused on patients with coronary artery disease, with or without left ventricular dysfunction. Specifically, we examined patients undergoing PCI, and identified characteristics associated with poor outcome after PCI (Part 1). Secondly, we focused on patients with established (chronic) heart failure ((C)HF), and investigated what strategy or which combination of biomarkers should be used for risk assessment (Part 2). Finally, we examined patients with severely reduced cardiac function, that require prevention of sudden cardiac death by means of an ICD with our without cardiac resynchronization therapy (CRT); here, we aimed to reveal characteristics associated with response and poor clinical outcome (Part 3).

Part I The influence of patient baseline and procedural characteristics on outcome after percutaneous coronary interventions

In **Chapter 2**, we created an easy to use 5-year mortality risk score based on nine simple clinical and angiographic baseline variables. The IDEA-BIO study reveals that age, BMI, diabetes mellitus, renal insufficiency, prior myocardial infarction, indication for PCI, culprit left main, number of diseased vessels and cardiogenic shock are key predictors of mortality in PCI patients. Previous studies that have created mortality risk scores and prediction tools for patients who have undergone PCI have focused on short term outcome (i.e., 30 days). Our study is the first to examine long-term follow-up. Combined with the large sample size, this is an important strength of our study. By providing a tool for long-term risk stratification, we enable selection of patients who might benefit from more expensive PCI treatment options, such as biodegradable scaffolds (provided that long-term benefits of biodegradable scaffolds compared to newer generation drug-eluting stents (DES) will be demonstrated by current studies).

The benefit of newer generation DES, releasing everolimus (i.e. everolimus eluting stents (EES)) compared to early generation DES (i.e. sirolimus eluting stents (SES) and paclitaxel eluting stents (PES)), has been shown by several large trials in patients undergoing PCI. In general, some patient categories are more prone to stent thrombosis (ST) than others; this includes patients with a reduced left ventricular ejection fraction (LVEF). **Chapter 3** showed that newer generation EES are associated with a lower risk of ST compared to early generation DES, independently of LVEF, even though ST rates were higher in patients with a moderate-severely impaired LVEF. This better outcome persisted even after 4 years of follow-up. The long-term safety and benefits of newer generation EES in patients with a reduced cardiac function suggests that EES should be the preferred stent when performing a PCI in patients with established systolic dysfunction.

In **Chapter 4**, we examined prognosis after out-of-hospital cardiac arrest (OHCA). We especially focused on factors related to the occurrence of OHCA in ST-elevation myocardial infarction (STEMI), since these are still poorly understood. The multicenter registry used for this chapter displayed an association between left proximal coronary lesions and high risk of OHCA. Right coronary lesions resulted in the lowest risk of OHCA. Furthermore, culprit location was associated with cardiogenic shock and sub-optimal reperfusion after PCI, both of which were driving factors of prognosis after OHCA. Finally, this study showed that a strict policy consisting of a combined strategy of early start of basic life support, early invasive management with primary PCI and therapeutic hypothermia in patients suffering OHCA due to STEMI, resulted in a relatively large group of patients (77%) that survived one year of follow-up and had reasonable neurological outcome at discharge.

Part II Novel methods and markers for risk assessment of patients with heart failure

Treatment of (C)HF is generally aimed at stabilizing or at decelerating disease progression. Currently, adjustment of therapy is largely based on clinical judgement and thus is usually only performed when symptoms or signs are worsening. Considerable clinical skills are required to recognize opportunities to titrate therapy and to implement interventional changes in a timely manner, which may lead to treatment delay in high-risk patients. Blood biomarkers may reflect subtle pathophysiological and hemodynamical cardiovascular changes, and may herewith predict an adverse disease course before it becomes clinically apparent or before symptoms are reported.

The Serial Biomarker meaSurements and new echocardiographic techniques in chronic Heart Failure patients result in Tailored prediction of prognosis (Bio-SHiFT) study was a prospective, observational study of stable outpatients with CHF, conducted in Erasmus MC, Rotterdam, The Netherlands and Noordwest Ziekenhuisgroep, Alkmaar,

The Netherlands. Patients were recruited during their regular outpatient visits and were in clinically stable condition. Patients were eligible if CHF (including HF with preserved ejection fraction) was diagnosed ≥3 months ago according to the guidelines of the European Society of Cardiology. At baseline and each follow-up visit, a short medical evaluation was performed and blood and urine samples were collected. Study follow-up visits were scheduled every 3 months, to a maximum follow-up duration of 30 months.

In **Chapter 5**, we used the Bio-SHiFT data and showed that serial assessments of

N-terminal pro-brain natriuretic peptide (NT-proBNP), C-reactive protein (CRP) and New York Heart Association (NYHA) Functional Classification class were all independently associated with adverse clinical outcome. Repeatedly measured NT-proBNP and CRP both added individually to serial NYHA-class assessments in terms of discriminative ability. These findings underscore the incremental value of biomarkers to NYHA class for monitoring stable CHF outpatients.

Chapter 6 demonstrated that the dynamic, temporal patterns of serially-measured NT-proBNP and CRP levels are strong and independent predictors of adverse clinical events in CHF patients. On top of that, instantaneous rate of change in level of these biomarkers, as well as their long-term elevation, were also associated with adverse events. While the temporal pattern of high-sensitive troponin T (HsTNT) significantly predicted adverse events, its predictive capability was lost when combined with temporal NT-proBNP and CRP patterns. These results suggest that individual patterns of biomarker change, as well as combinations of multiple biomarkers, should be taken into consideration for prognostication in patients with stable CHF.

Despite these findings, the predictive capabilities of the aforementioned biomarkers for worsening of CHF still leaves room for improvement. MicroRNAs (miRs) are upcoming novel biomarkers that seem promising for early diagnosis and treatment of HF. MiRs are non-coding, ~22 nucleotide long RNA sequences, which target messenger RNAs for cleavage or translational repression and thereby influence a great variety of biological processes. The stability of miRs in plasma, and consequently their reliable assessment in easily accessible samples, potentially makes them attractive biomarkers for a wide range of diseases, such as cardiovascular diseases. In **Chapter 7**, we used the data of the Bio-SHiFT study and investigated the association of frequent repeated measurements of multiple miRs that were previously linked to HF (miR-1254, miR-22-3p, miR-423-5p, miR-486-5p and miR-320a) or have been shown to be cardiac-enriched (miR-345-5p, miR-378a-3p), with adverse clinical events in CHF patients. We discovered an inverse and independent association between temporal miR-22-3p patterns and adverse outcome. The association was independent of clinical characteristics as well as temporal NT-proBNP, HsTNT and CRP patterns. The instantaneous rate of change in miR-22-3p level (i.e., the slope of the temporal pattern) was also inversely associated with adverse outcome. Moreover, we found inverse associations of temporal patterns of miR-22-3p

with temporal patterns of HsTNT and CRP. Therefore, the use of individual patterns of change of circulating miR-22-3p may be beneficial for CHF prognostication. An additional finding of this study was the significant, inverse association between ischemic cardiomyopathy (ICM) and miR1254, miR486-5p and miR320a.

Chapter 8 showed the origination of a number of miRs produced by pig myocardium, including miR-1306-5p which had not yet been identified as a miR related to the heart, using direct RNA sequencing of plasma from instrumented pigs. Subsequently we showed, in a human, prospective acute HF cohort (TRIUMPH), that repeatedly-assessed circulating miR-1306-5p was associated with adverse clinical outcome, and provides prognostic information beyond NT-proBNP. These results imply that measuring miR-1306-5p could aid in risk stratification of patients with HF. Herewith, patients may be identified that could benefit from closer follow-up and from more aggressive treatment.

Part III Novel insights in characteristics associated with favourable outcome in defibrillation therapy and response to cardiac resynchronization therapy

The use of ICDs and CRT has had major impact on survival of patients with reduced cardiac function (i.e. LVEF <35%). CRT has reduced the number of HF hospitalizations and has been shown to induce cardiac reversed remodeling in patients suffering from CHF and wide QRS-complexes. But there are still major unidentified characteristics that favor or disfavor the response to these devices. Some patients respond very well to CRT and reach a LVEF of ≥35% after implantation. In **Chapter 9**, we refered to these patients as functional responders, and we demonstrated that such functional responders have good prognosis. In fact we show that during a median follow-up of 3 years, backup from defibrillation therapy was not used in functional responders. Incidence of inappropriate shocks was similar among functional responders and functional non-responders. Defibrillator-shocks, whether appropriate or not, have adverse consequences for patients and lead to more frequent hospitalization and thereby higher costs. Altogether, our findings thus raise the hypothesis that functional responders may be assigned to CRT-P when a device change is needed, or that ICD therapy may be switched off to prevent unnecessary inappropriate shocks without compromising clinical outcome.

Another major determinant, not only of mortality, but also of appropriate and inappropriate shocks in CHF patients treated with CRT-D, is a history of atrial fibrillation (AF), as described in **Chapter 10**. The retrospective, multi-centre, observational, follow-up study, on 550 real-life CHF patients treated with CRT-D, described in this chapter, shows that a total of forty-six percent of all patients receiving inappropriate shocks appeared to have a history of AF at baseline. These results suggest that benefit may be derived from an optimal medicinal policy to keep patients in sinus rhythm, and prevent AF; and possibly a His bundle ablation may be considered. Furthermore, mortality and occurrence of both appropriate and inappropriate shocks in a population of ICD patients with

an impaired left ventricular function due to coronary artery disease (CAD) vs. dilated cardiomyopathy patients did not seem to differ, as reported in **Chapter 11**. The population described in this chapter consisted of mainly ICD-only patients and only 33% CRT-D patients. Conversely, **Chapter 12** showed that prior myocardial infarction did seem to be associated with (3-year) mortality in a population consisting of all CRT-D patients. Other risk factors associated with mortality were LVEF ≤25%, COPD, chronic kidney disease, hyponatremia and anemia. The results of these studies could be used for prediction of prognosis in patients with CHF and could be used to identify patients in need of closer follow-up.

Conclusions

- Long-term (i.e. 5-year) mortality after PCI can be adequately predicted using a 5-year mortality risk score based on nine simple clinical and angiographic baseline variables.
- Newer generation EES are associated with a lower risk of ST compared to early generation DES, independently of LVEF, even though ST rates are higher in patients with a moderately-to-severely impaired LVEF.
- A strict policy consisting of a combined strategy of early start of basic life support, early invasive management with primary PCI and therapeutic hypothermia in patients suffering OHCA due to STEMI, results in a relatively large group of patients (77%) that survive during one-year follow-up with reasonable neurological outcome at discharge. Left proximal coronary lesions carry higher risk for OHCA after STEMI compared to right coronary lesions.
- Repeatedly measured NT-proBNP and CRP both add individually to serial NYHA-class assessments for monitoring CHF outpatients in terms of discriminative ability, which underscores the value of biomarkers to NYHA class measurements only.
- The dynamic, temporal patterns of serially-measured NT-proBNP and CRP levels are strong and independent predictors of adverse clinical events in CHF patients. On top of evolution of biomarker level, instantaneous rate of change in level of NT-proBNP and CRP and long-term elevation of NT-proBNP are associated with adverse outcome.
- MiR-1306-5p is related to the heart and is associated with adverse clinical outcome in patients with acute HF. In patients with stable CHF, the temporal pattern of circulating miR-22-3p is a strong and independent predictor of prognosis.
- Functional response to CRT (patients who reach a LVEF of ≥35% after implantation) is associated with a good prognosis, and functional responders do not need backup from defibrillation therapy.
- AF is associated with adverse events in CRT-D patients. Furthermore, myocardial infarction, LVEF ≤25%, COPD, chronic kidney disease, hyponatremia and anemia predict mortality in CRT-D patients.

- CAD is associated with poor survival in CRT-D patients, but we did not find an association between mortality and CAD in a population of mainly ICD-only patients, without CRT.

Future directions

This thesis describes additional insights into patient characteristics that facilitate prognostication in patients with cardiovascular diseases. Most importantly, it shows that the individual patterns of change of several circulating biomarkers carry incremental value for prognostication. As such, additional research on the use of blood biomarkers for prognostication, as well as for tailored adjustment of treatment, is warranted. The results of the ongoing Bio-SHiFT study are expected to provide information on individual patterns of change of a wide range of novel biomarker levels and thus carry potential to further improve individual risk prediction. Future studies beyond the Bio-SHiFT study should examine the role of tailored adjustment of treatment using these biomarker patterns and may thereby contribute to personalized medicine.

SAMENVATTING EN CONCLUSIES

Gezondheidszorg kan worden onderverdeel in preventieve, genezingsgerichte, revalidatie-gerichte en ondersteunende geneeskunde. Preventieve geneeskunde wordt beschouwd als de meest effectieve vorm van geneeskunde, omdat preventieve geneeskunde de noodzaak tot genezingsgerichte, revalidatie-gerichte en ondersteunende geneeskunde kan voorkomen. Preventieve geneeskunde richt zich niet alleen maar op het voorkomen van ziektes (oftewel primaire preventie), maar ook op het verminderen van de kans op recidieven en het verlagen van de morbiditeit van een chronische ziekte (secundaire en tertiaire preventie respectievelijk). Belangrijke verbeteringen in de cardiovasculaire gezondheidszorg, zoals percutane coronaire interventies (PCI's) en implanteerbare cardioverter defibrillators (ICD's), hebben de mortaliteit fors verlaagd, maar er wel voor gezorgd dat er een toename is in het aantal patiënten met een chronische ziekte. Om de secundaire preventie in deze patiëntengroep verder te verbeteren, zijn dringend nieuwe methoden voor risicostratificatie nodig.

Het doel van deze dissertatie was het identificeren van factoren geassocieerd met een slechte klinische uitkomst en om nieuwe voorspelmodellen te creëren die kunnen helpen bij het inschatten van de risico's van patiënten in verschillende fasen van cardiovasculaire ziekte. Allereerst hebben wij ons gericht op patiënten met coronarialijden, al dan niet met een verminderde linker kamer functie. Wij hebben specifiek gekeken naar patiënten die een PCI ondergingen en verschillende factoren beschreven die geassocieerd zijn met een slechte klinische uitkomst na PCI (deel 1). Daarna hebben wij ons gericht op patiënten met (chronisch) hartfalen ((C)HF) en hebben gezocht naar de beste strategie en combinatie van biomarkers die zal moeten worden gebruikt voor het bepalen van prognose (deel 2). Tot slot hebben wij patiënten onderzocht met een ernstig verminderde linker kamerfunctie met noodzaak tot bescherming tegen plotse hartdood middels een ICD, al dan niet met cardiale resynchronisatie therapie (CRT). Ook binnen deze patiëntengroep hebben wij ons gericht op karakteristieken geassocieerd met goede reactie op therapie en klinische uitkomst (deel 3).

Deel I De invloed van patiënt karakteristieken en procedurele factoren geassocieerd met klinische uitkomst na percutane coronaire interventies

In **hoofdstuk 2** hebben wij m.b.v. 9 simpele klinische en angiografische karakteristieken een risicomodel gemaakt voor het voorspellen van 5-jaars mortaliteit na PCI. De IDEA-BIO studie liet zien dat leeftijd, BMI, diabetes mellitus, nierfunctiestoornissen, eerdere hartinfarcten, de indicatie voor PCI, culprit hoofdstam, het aantal aangedane coronairen en cardiogene shock voorspellers zijn van 5-jaars mortaliteit na PCI. Eerdere studies hebben risicomodellen gemaakt voor post-PCI patiënten, echter hadden de meeste van deze studies zich gericht op korte termijn uitkomst (30 dagen na PCI). Dit was de eerste

studie waarin lange termijn uitkomst is bekeken. Daarbij is de kracht van deze studie de grote populatie die is onderzocht. Mogelijk maakt dit lange termijn risicomodel het mogelijk om patiënten te selecteren die kunnen profiteren van duurdere interventies, zoals biologisch afbreekbare stents (mits betere overleving door biologisch afbreekbare stents t.o.v. nieuwe generatie 'drug-eluting stents' (DES) op lange termijn kan worden aangetoond in huidige studies).

Het voordeel van nieuwe generatie DES, die everolimus bevatten (oftewel 'everolimus-eluting stents' (EES)) t.o.v. oude generatie DES (oftewel 'sirolimus eluting stents' (SES) en 'paclitaxel eluting stents' (PES)) is reeds in grote studies aangetoond. Sommige patiëntgroepen zijn gevoeliger voor stent trombose (ST) dan anderen. Zo hebben patiënten met een verminderde linker ventrikel ejectie fractie (LVEF) een verhoogd risico op ST. In **hoofdstuk 3** lieten we zien dat nieuwe generatie EES geassocieerd zijn met een lager risico op ST t.o.v. oude generatie DES, onafhankelijk van de LVEF. Dit lagere ST risico zette zich voort tot 4 jaar na follow-up. De lange termijn veiligheid en voordelen van nieuwe generatie EES bij patiënten met een verminderde LVEF suggereert dat EES de stent van keuze is wanneer een PCI moet worden uitgevoerd bij patiënten met een verminderde LVEF.

In **hoofdstuk 4** hebben we de prognose onderzocht van patiënten die een circulatie stilstand buiten het ziekenhuis, oftewel een 'out-of-hospital cardiac arrest' (OHCA), doormaakten. We hebben ons gericht op factoren gerelateerd aan het optreden van OHCA bij patiënten met ST-elevatie myocardinfarcten. De multicenter registratie die wij hebben gebruikt in dit hoofdstuk, liet een associatie zien tussen laesies in de proximale linker coronair en een verhoogd risico op OHCA. Laesies in de rechter coronair waren geassocieerd met het laagste risico op OHCA. Daarbij was de locatie van de culprit geassocieerd met cardiogene shock en suboptimale reperfusie na PCI; beiden belangrijke factoren geassocieerd met prognose na OHCA. Tot slot liet deze studie zien dat een strikt beleid van vroeg starten met basic life support, vroeg invasief handelen middels primaire PCI en therapeutische hypothermie resulteerde in een relatief grote patiënt groep (77%) die langer dan 1 jaar overleefden met een redelijke neurologische uitkomst.

Deel II Nieuwe methoden en markers voor risicostratificatie van patiënten met hartfalen.

De behandeling van (C)HF is voornamelijk gericht op het stabiliseren en vertragen van ziekteprogressie. Op dit moment is het bijstellen van therapie grotendeels gebaseerd op klinische beoordeling, waardoor het bijstellen van therapie normaal gesproken alleen gebeurd als tekenen of symptomen verslechteren. Er zijn aanzienlijke vaardigheden vereist voor de juiste timing voor het bijstellen van therapie, wat zou kunnen lijden tot vertraagde therapie optimalisatie bij hoog risico patiënten. Bloed biomarkers kunnen subtiele pathofysiologische en hemodynamische cardiovasculaire veranderingen laten

zien en mogelijk ziekte verslechtering voorspellen voordat het zich klinisch uit. De ´Serial Biomarker meaSurements and new echocardiographic techniques in chronic Heart Failure patients result in Tailored prediction of prognosis´ (Bio-SHiFT) studie was een prospectieve, observationele studie, opgezet in het Erasmus MC, Rotterdam, Nederland en Noordwest Ziekenhuisgroep, Alkmaar, Nederland, naar stabiele CHF patiënten, die de polikliniek bezochten. Patiënten werden benaderd tijdens regulaire polikliniek bezoeken in stabiele toestanden geschikt bevonden indien zij bekend waren met CHF (al dan niet met behouden kamerfunctie), gediagnosticeerd ≥3 maanden geleden, volgens de richtlijnen van de 'European Society of Cardiology'. Bij start en bij elk follow-up moment werd een korte medische evaluatie verricht en werden bloed en urine monsters verzameld. Studie follow-up werd elke 3 maanden verricht, gedurende maximaal 30 maanden.

In **hoofdstuk 5** werd de data van de Bio-SHiFT studie gebruikt om aan te tonen dat seriële metingen van N-terminal pro-brain natriuretic peptide (NT-proBNP), C-reactive protein (CRP) en New York Heart Association (NYHA) functionele klasse onafhankelijk geassocieerd waren met slechte klinische uitkomst. Herhaalde NT-proBNP en CRP metingen verbeterde de discriminatie t.o.v. een model met alleen seriële NYHA-klasse metingen, hetgeen de waarde van seriële biomarker metingen onderstreept.

Hoofdstuk 6 liet zien dat de dynamische, temporele patronen van serieel gemeten NT-proBNP en CRP levels sterke en onafhankelijke voorspellers zijn van slechte klinische uitkomst bij CHF patiënten. Daarbij zijn directe veranderingen van het level van deze biomarkers en langdurige verhoging ook geassocieerd met slechte klinische uitkomst. Het temporele patroon van high-sensitive troponin T (HsTNT) was ook significant geassocieerd met slechte klinische uitkomst, maar niet onafhankelijk van de temporele patronen van NT-proBNP en CRP. Dit resultaat suggereert dat het gebruik van individuele patronen van verandering van biomarkers en de combinatie van verschillende biomarkers zal moeten worden overwogen bij het bepalen van de prognose van patiënten met stabiel CHF.

Ondanks de genoemde bevindingen is er nog steeds veel ruimte voor verbetering. MicroRNA´s (miRs) zijn biomarkers in opkomst en voorgaande studies hebben veelbelovende resultaten laten zien bij het vroeg diagnosticeren en behandelen van HF. MiRs zijn niet coderende, ~22 nucleotiden lange RNA sequenties, die een rol spelen bij het splijten of bij translationele onderdrukking van messenger RNA en beïnvloeden daarom vele biologische processen. De stabiliteit van miRs in plasma en daarbij hun betrouwbare beoordeling en makkelijke bereikbaarheid, maakt miRs aantrekkelijke biomarkers voor vele ziektebeelden, waaronder cardiovasculaire ziektes. In **hoofdstuk 7** hebben we Bio-SHiFT data gebruikt om de associatie te onderzoeken tussen slechte klinische uitkomst van CHF patiënten en frequente, herhaalde metingen van meerdere miRs die eerder aan HF gerelateerd werden (miR-1254, miR-22-3p, miR-423-5p, miR-486-5p en

miR-320a) of myocard verrijkt zijn (miR-345-5p, miR-378a-3p). Hierbij ontdekten we een omgekeerde en onafhankelijke relatie tussen temporele miR-22-3p patronen en slechte klinische uitkomst. Deze associatie was onafhankelijk van klinische karakteristieken en NT-proBNP, HsTNT en CRP patronen. De acute verandering van miR-22-3p level (oftewel de helling van het temporele patroon) was ook omgekeerd geassocieerd met slechte klinische uitkomst. Verder vonden we dat het temporele patroon van miR-22-3p omgekeerd evenredig was aan de temporele patronen van HsTNT en CRP. De uitkomst suggereert dat de individuele patronen van miR-22-3p zou kunnen worden gebruikt voor het bepalen van de prognose van CHF patiënten. Een bijkomstige bevinding in deze studie was de significant omgekeerde relatie tussen een ischemische cardiomyopathie (ICM) en miR1254, miR486-5p en miR320a.

In **Hoofdstuk 8** lieten we zien hoe we een aantal miRs ontdekten die geproduceerd werden in myocard van varkens. Er werd directe RNA sequencing van plasma van behandelde varkens verricht, waarbij o.a. miR-1306-5p werd gevonden, welke nog niet eerder geassocieerd werd met myocard. Vervolgens hebben we in een prospectief cohort bestaande uit acuut HF patiënten (TRIUMPH) aangetoond dat herhaald gemeten circulerend miR-1306-5p geassocieerd was met slechte klinische uitkomst en prognostische informatie bevat boven NT-proBNP. Deze resultaten impliceren dat het meten van miR-1306-5p kan helpen bij het stratificeren van het risico van HF patiënten. Daarbij zou miR-1306-5p mogelijk kunnen helpen bij het identificeren van patiënten die baat kunnen hebben bij nauwere follow-up en agressievere behandelopties.

Deel III Nieuwe inzichten in karakteristieken geassocieerd met gunstige uitkomst bij defibrillatie therapie en reactie op cardiale resynchronisatie therapie

Het gebruik van ICD's en CRT heeft een grote invloed gehad op de overleving van patiënten met een verminderde kamerfunctie (oftewel een LVEF <35%). CRT heeft het aantal ziekenhuis opnames voor HF verminderd en kan herstel van het hart (reversed remodeling) induceren bij patiënten met CHF en verbrede QRS complexen. Vele karakteristieken geassocieerd met een betere of slechtere respons op CRT moeten nog gevonden worden. Sommige patiënten reageren erg goed op CRT en bereiken een LVEF ≥35% na implantatie. In **hoofdstuk 9** noemen we deze patiënten functionele reactie patiënten en laten zien dat functionele reactie geassocieerd is met een goede prognose. Gedurende een mediane follow-up van 3 jaar hadden functionele reactie patiënten geen schok therapie van hun geïmplanteerde cardioverter-defibrillator nodig. De incidentie van onterechte schokken was gelijk tussen functionele en niet-functionele reactie patiënten. Zulke schokken, terecht of onterecht, hebben nadelige gevolgen voor patiënten en lijden tot frequentere ziekenhuis opnames en daarbij ook hogere kosten. Concluderend suggereren onze bevindingen dat bij functionele reactie patiënten in

geval van noodzaak tot vervanging (bijv. bij het leeg raken van de batterij) het CRT met defibrillator (CRT-D) apparaat kan worden vervangen door een CRT zonder defibrillator apparaat.

Een andere belangrijke determinant bij CHF patiënten behandeld met CRT-D, niet alleen voor sterfte, maar ook voor terechte en onterechte schokken, is het hebben van atrium fibrilleren (AF), zoals beschreven in **hoofdstuk 10**. Het retrospectieve, multicenter, observationele studie, verricht op 550 echte patiënten (dus geen studie patiënten) onderzoek beschreven in dit hoofdstuk laat zien dat 47% van alle patiënten die onterechte schokken kregen, bekend waren met AF. Deze resultaten suggereren dat het bij CRT-D patiënten belangrijk is om ze in sinusritme te houden en AF te voorkomen, mogelijk zelfs door middel van His bundel ablatie.

Mortaliteit en het optreden van (on)terechte schokken in een populatie ICD patiënten met een verminderde LVEF door coronarialijden (oftewel coronary artery disease (CAD)) tegenover patiënten met een dilaterende cardiomyopathie, leek niet te verschillen, zoals te lezen in **hoofdstuk 11**. De populatie waarop het onderzoek beschreven in dit hoofdstuk is verricht, bestond voornamelijk uit patiënten met alleen een ICD en slechts 33% CRT-D patiënten. Omgekeerd laten we in **hoofdstuk 12** zien dat eerdere hartinfarcten geassocieerd waren met (3-jaars) mortaliteit in een populatie bestaande uit alleen maar CRT-D patiënten. Andere risicofactoren geassocieerd met mortaliteit van CRT-D patiënten waren LVEF ≤25%, COPD, chronisch nierfalen, hyponatriëmie en anemie. Het resultaat van deze studie kan worden gebruikt bij het voorspellen van de prognose van CHF patiënten die behandeld worden met CRT-D, die mogelijk nauwere follow-up nodig hebben.

Conclusies

- Lange termijn (5-jaars) sterfte na PCI kan adequaat voorspeld worden middels 5-jaars sterfte risicomodel gebaseerd op 9 eenvoudige klinische en angiografische variabelen.
- Nieuwe generatie EES zijn geassocieerd met een lager risico op ST in vergelijking met oude generatie DES, onafhankelijk van LVEF, ondanks dat ST vaker voorkomt bij patiënten met een matig tot ernstig verminderde LVEF.
- Een strikt beleid, bestaande uit de combinatie van vroeg starten met reanimeren, vroeg invasief handelen middels primaire PCI en therapeutische hypothermie bij patiënten die een circulatie stilstand buiten het ziekenhuis doormaakten, resulteert in een relatief grote groep (77%) patiënten die na het voorval nog in leven waren na 1 jaar en die een redelijke neurologische uitkomst hadden bij ontslag. Laesies proximaal in de linker coronair zijn geassocieerd met een groter risico op een circulatie stilstand buiten het ziekenhuis in vergelijking met laesies in de rechter coronair.

- Herhaaldelijk gemeten NT-proBNP en CRP dragen beiden individueel en onafhankelijk van elkaar bij aan seriële NYHA klasse metingen bij het monitoren van CHF patiënten door het verbeteren van discriminatie. Dit benadrukt de waarde van biomarkers boven het alleen scoren van de NYHA klasse.
- De dynamische, temporele patronen van serieel gemeten NT-proBNP en CRP levels zijn sterke en onafhankelijke voorspellers van slechte klinische uitkomst van CHF patiënten. Naast de voortgang van biomarker levels zijn directe veranderingen van het level van deze biomarkers en langdurige verhoging ook geassocieerd met slechte klinische uitkomst.
- MiR-1306-5p is geassocieerd met het hart en met slechte klinische uitkomst van acuut HF patiënten. Bij patiënten met stabiel CHF is het temporele patroon van circulerend miR-22-3p een sterke en onafhankelijke voorspeller van prognose.
- Functionele reactie op CRT (patiënten die een LVEF ≥35% bereiken na implantatie) is geassocieerd met een goede prognose en bij deze patiënten is er geen noodzaak voor defibrillatie therapie.
- AF is geassocieerd met slechte klinische uitkomst bij CRT-D patiënten. Daarnaast voorspellen een verleden van myocard infarct, LVEF ≤25%, COPD, chronisch nierfalen, hyponatriëmie en anemie mortaliteit bij CRT-D patiënten.
- CAD is geassocieerd met slechte overleving in CRT-D patiënten, maar wij vonden geen associatie tussen sterfte en CAD in een populatie bestaande uit voornamelijk ICD patiënten zonder CRT.

Toekomstige richtingen

Deze dissertatie beschrijft bijdragende inzichten in patiënt karakteristieken die het voorspellen van prognose van patiënten met cardiovasculaire ziekten vergemakkelijkt. Een grote rol is weggelegd voor individuele, veranderende patronen van verschillende circulerende biomarkers, die een grote bijdrage leveren aan het voorspellen van prognose. Om deze reden is het belangrijk dat er meer onderzoek volgt naar het gebruik van biomarkers als voorspellers van prognose, maar ook voor het titreren van therapie. De resultaten van de Bio-SHiFT studie zullen ons naar alle waarschijnlijkheid meer informatie en inzicht in veranderende individuele patronen van het level van een groot aantal nieuwe biomarkers verschaffen. Daarmee zal deze studie bijdragen aan verdere verbetering van individuele risicostratificatie. Toekomstige studies na de Bio-SHiFT studie zullen de rol van het gebruik van deze nieuwe biomarkers bij het titreren van therapie moeten onderzoeken en op die manier mogelijk bijdragen aan persoonlijke geneeskunde.

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CURRICULUM VITAE

Nick van Boven was born on June 10, 1987 in Amsterdam, the Netherlands. In 2005 he graduated from secondary school (Gymnasium, Pascal College, Zaandam). After secondary school he studied medicine at the Vrije Universiteit in Amsterdam. He obtained the degree of medical doctor in 2011. Subsequently he started as PhD student at the department of Cardiology in Noordwest Ziekenhuisgroep in Alkmaar and the Erasmus University in Rotterdam, supervised by Prof. Dr. H. Boersma, Dr. V.A.W.M. Umans and Dr. I. Kardys. He was involved in prospective biomarker studies, primarily to chronic heart failure, but also to acute heart failure and coronary syndromes. On top of that he was involved in epidemiological research to percutaneous coronary interventions and cardiac device therapy. After fulltime work as PhD student, he started in August 2016 as clinical resident (ANIOS) at the department of Cardiology at Noordwest Ziekenhuisgroep in Alkmaar. In January 2016 he worked at the Erasmus MC in Rotterdam, also as clinical resident (ANIOS) at the department of Cardiology. In November 2016 he became cardiologist in training (AIOS) and started his training at the department of Internal Medicine at Franciscus Gasthuis in Rotterdam.

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14. Brankovic M, Akkerhuis KM, **van Boven N**, Anroedh SS, Constantinescu AA, Caliskan K, Manintveld OC, Cornel JH, Baart S, Rizopoulos D, Hillege H, Boersma H, Umans VA, Kardys I. Patient-specific Evolution of Renal function Dynamically Predicts Clinical Outcome in Chronic Heart Failure: Bio-SHiFT Study. *Kidney Int. Accepted.*

PHD PORTFOLIO

Candidate: Nick van Boven	**PhD period:** September 2011 – July 2015
Erasmus MC Department: Cardiology	**Promotor:** Prof. Dr. H. Boersma
Research School: Cardiovascular Research School	**Co-promotors:** Dr. V.A.W.M. Umans and Dr. I. Kardys
Total of ECTS points: 33.1	

Courses from NHS at Papendal	**Year**	**ECTS**
Cardiac Function and Adaption	2013	2.0

Other courses followed	**Year**	**ECTS**
Basic course SPSS	2011	0.6
Advanced course SPSS	2011	0.3
Course writing scientific English	2011	0.9
Pubmed Workshop	2011	0.3
Good Clinical Practice (GCP) course	2011	0.6
Good Clinical Practice (GCP) update	2014	0.3
Course presenting scientific research	2011	0.6
Basiscursus klinisch onderzoeker (BROK)	2011	1.5
Course patient research	2012	0.6
PRONK cursus	2014	0.3
Course introduction to R	2014	0.6
Course Advanced statistics using R	2014	0.3
Open Clinica Training	2014	0.3

Teaching	**Year**	**ECTS**
Training lecture on cardiac resynchronisation therapy at "heart-failure" theme night	2015	0.3
Training lecture on biomarker research	2015	0.3
Nurse training lecture on heart-failure	2015	0.3
Nurse training lecture on interpreting ECGs	2015	0.3

Symposia and congresses	**Year**	**ECTS**
NVVC voorjaarscongres 2012 , Noordwijkerhout, Netherlands	2012	0.6
NVVC najaarscongres 2013, Arnhem, Netherlands	2013	0.6
NVVC voorjaarscongres 2013, Noordwijkerhout, the Netherlands	2012	0.6
NVVC najaarscongres 2013, Arnhem, Netherlands	2013	0.6
NVVC voorjaarscongres 2014, Noordwijkerhout, Netherlands	2013	0.6
NVVC najaarscongres 2014, Arnhem, the Netherlands	2014	0.6
NVVC voorjaarscongres 2015, Noordwijkerhout, the Netherlands	2015	0.6
Netherlands Heart Rhythm Association Congress 2012, Ermelo, Netherlands	2012	0.6
Netherlands Heart Rhythm Association Congress 2013, Ermelo, Netherlands	2013	0.6
Netherlands Heart Rhythm Association Congress 2014, Ermelo, Netherlands	2014	0.6

European Society of Cardiology Congress 2012, Munich, Germany	2012	1.5
European Society of Cardiology Congress 2013 Amsterdam, Netherlands	2013	1.5
European Society of Cardiology Congress 2014, Barcelona, Spain	2014	1.5
European Heart Rhythm Association Europace Congress 2013, Greece, Athens	2013	1.2
Heart Rhythm Society Congress 2012 in Boston, United states	2012	1.2
American Heart Association congress 2014, Chicago, United states	2014	1.5

Awards	**Year**	**ECTS**
Winner Pieter van Foreest poster prize 2015	2015	0.2

Congress presentations	**Year**	**ECTS**
NVVC voorjaarscongres 2012 (1 oral presentation)	2012	0.3
NVVC voorjaarscongres 2013 (1 oral presentation)	2013	0.3
NVVC voorjaarscongres 2014 (1 oral presentation)	2014	0.3
Netherlands Heart Rhythm Association Congress 2012 (1 oral presentation)	2012	0.3
European Society of Cardiology Congress 2012 (1 Oral presentation)	2012	0.3
European Society of Cardiology Congress 2013 (1 poster presentation)	2013	0.3
European Society of Cardiology Congress 2014 (1 oral presentation and 1 poster presentation)	2014	0.6
European Heart Rhythm Association Europace Congress 2013 (1 poster presentation)	2013	0.3
Heart Rhythm Society Congress 2012 (1 poster presentation)	2012	0.3
American Heart Association congress 2014 (2 poster presentations)	2014	0.6

Other	**Year**	**ECTS**
Supervision of medical student during 10 weeks of scientific internship (4 times)	2012-2015	2.0
Chairman of regular research meetings for research personnel and PhD students in Noordwest Ziekenhuisgroep location Alkmaar	2012-2015	2.5

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